THE HERBICIDE GLYPHOSATE: CROP TREE PRODUCTIVITY AND NON-TARGET IMPACTS

A COMPENDIUM OF
REFERENCES AND ABSTRACTS

6th EDITION

2000-2012

INFORMATION REPORT
APPLIED MAMMAL RESEARCH INSTITUTE

RATIONALE AND BACKGROUND

The original concept of a compendium of references and abstracts outlining the "non-target impacts of the herbicide glyphosate" arose from the apparent incomplete and scattered sources of information on this subject. A common complaint from both lay and professional people is: "What research has been done on non-target impacts of glyphosate and how do we access this information?" The digital literature search, resulting in this sixth edition of the compendium, involved several thousand references covering environmental impacts, toxicology, and human health. This edition represents the period 2000-2012. A new section on crop tree productivity was added in this edition and generally covers the period 1990-2012. Thus, there is a considerable ongoing literature base for glyphosate and this compendium was updated to provide, in as complete a manner as possible, a collection of titles and abstracts of articles reporting on the non-target impacts of this herbicide.

ORIGIN AND USE OF COMPENDIUM

This edition of the compendium is designed as an Information Report to provide an objective assessment of the effects of glyphosate on non-target species and ecosystems, as well as crop tree productivity. References are from peer reviewed publications in scientific journals from 2000 to 2012 and were extracted from a computerized literature search via Google Scholar. Titles and abstracts have been reproduced exactly as they appear in the original article or as abstracted by the source system. In the case of a few journal articles without a formal abstract, a summary of the study has been abstracted by the compilers and this is clearly indicated by an asterisk **(*)**.

The compendium is composed of 11 sections: (1) Amphibians, Aquatic Invertebrates and Plants, and Algae, (2) Biodiversity, Conservation, and Habitat Restoration, (3) Birds, (4) Crop Tree Productivity, (5) Fish, (6) Human Health, (7) Mammals, (8) Microbiota and Fungi, (9) Plant and Soil Residues, (10) Terrestrial Invertebrates, and (11) Water Quality. All titles and abstracts of references with author(s) and publication outlet are listed alphabetically in each section. References are identified by country to assist the reader. References which pertain to more than one section are referred to in each section with the abstract appearing in the section deemed most relevant to the article. For complete accuracy, we recommend that users cite from the original references and not from the abstracts presented here.

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Amphibians, Aquatic Invertebrates and Plants, and Algae

1. Battaglin, W.A., K.C. Rice, M.J. Focazio, S. Salmons, and R.X. Barry. 2009. The occurrence of glyphosate, atrazine, and other pesticides in vernal pools and adjacent streams in Washington, DC, Maryland, Iowa, and Wyoming, 2005-2006. *Environmental Monitoring and Assessment* 155(1-4): 281-307.
   See Water Quality Section.

2. Battaglin, W.A., D.W. Kolpin, E.A. Scribner, K.M. Kuivila, and M.W. Sandstrom. 2005. Glyphosate, other herbicides, and transformation products in Midwestern streams, 2002. *Journal of the American Water Resources Association* 41(2): 323-332.
   See Water Quality Section.

3. Beachy, J.R., R. Neville, and C. Arnott. 2011. Successful control of an incipient invasive amphibian: *Eleutherodactylus coqui* on O'ahu, Hawai'i. Pages 140-147 In: Veitch, C. R.; Clout, M. N. and Towns, D. R. (eds.). 2011. *Island invasives: eradication and management.* IUCN, Gland, Switzerland.
   See Biodiversity and Restoration Section.

4. Bernal, M.H., K.R. Solomon and G. Carrasquilla. 2009. Toxicity of formulated glyphosate (Glyphos) and Cosmo-Flux to larval Colombian frogs 1. laboratory acute toxicity. *Journal of Toxicology and Environmental Health,* Part A 72(15): 961-965.
   The spraying of coca (*Erythroxylum coca*) with glyphosate in Colombia has raised concerns about possible impacts on amphibians. There are few toxicity data for species other than those from temperate regions, and these have not been generated with the combination of formulated glyphosate (Glyphos) and the adjuvant, Cosmo-Flux (coca mix) as used in coca control in Colombia. In order to characterize toxicity of the spray mixture to frogs from Colombia, Gosner stage-25 tadpoles of *Scinax ruber, Dendropophus microcephalus, Hypsiboas crepitans, Rhinella granulosa, Rhinella marina, Rhinella typhonius, Centrolene prosoblepon,* and *Engystomops pustulosus* were exposed to the coca mix at concentrations of glyphosate ranging from 1 to 4.2 mg a.e./L diluted in dechlorinated tap water in glass containers. Cosmo-Flux was added to Glyphos in the proportion of 2.3% v/v, as used in aerial application for coca control. Exposures were for 96 h at 23 ± 1.5^{o}C with 12:12-h light/dark cycle. Test solutions were renewed every 24 h. Concentrations, measured within the first hour and at 24 and 96 h using enzyme-linked immunosorbent assay (ELISA) (Abraxis, LLC), ranged from 70 to 130% of nominal values. LC50 values ranged from 1200 to 2780 mg glyphosate acid equivalents (a.e.)/L for the 8 species tested. Data suggest that sensitivity to Roundup-type formulations of glyphosate in these species is similar to that observed in other tropical and temperate species. In addition, sensitivity of larval amphibians to Roundup-type formulations spans a relatively narrow range. Finally, toxicity of the mixture as used to spray coca was likely driven by the surfactant in the glyphosate formulation, as the addition of Cosmo-Flux did not enhance toxicity above those reported for Vision = Roundup®. **(Colombia)**

5. Bernal, M.H., K.R. Solomon and G. Carrasquilla. 2009. Toxicity of formulated glyphosate (Glyphos) and Cosmo-Flux to larval and juvenile Colombian frogs 2. field and laboratory microcosm acute toxicity. *Journal of Toxicology and Environmental Health,* Part A 72(15): 966-973.

   The spraying of coca (*Erythroxylum coca*) with glyphosate (coca mixture, a combination of formulated glyphosate, Glyphos, and an adjuvant, Cosmo-Flux) in Colombia has raised concerns about possible impacts on amphibians. Although acute LC50 for 8 species of Colombian frogs ranged from 1.2 to 2.78 mg acid equivalents (a.e.)/L, these exposures were conducted in the laboratory in the absence of sediments and organic matter such as would occur under realistic field conditions. In order to assess the effects of overspray of frog habitat under field conditions, Gosner stage 25 tadpoles of *Rhinella granulosa*, *R. marina*, *Hypsiboas crepitans*, and *Scinax ruber* were placed in outdoor microcosms made from polyethylene plastic fish ponds (2.07 m in diameter, 37 cm high) in an experimental area in Tolima, Colombia. The bottoms of the microcosms were covered with a 3-cm layer of local soil and they were filled to a depth of 15 cm (above the sediment) with local spring water. After up to 100 tadpoles of each frog species were placed in the microcosms, they were sprayed with the coca mixture at concentrations greater and less than the normal application rate (3.69 kg glyphosate a.e./ha). Mortality at 96 h in the control microcosms was between 0 and 16% and LC50 values were between 8.9 and 10.9 kg glyphosate a.e./ha (equivalent to initial concentrations of 5963 to 7303 µg glyphosate a.e./L). Mortality >LC50 was only observed in the tested species when the application rate was >2- fold the normal application rate. In other experiments, juvenile and adult terrestrial stages of frogs were exposed by direct spraying to a range of concentrations of coca mixture. Juveniles and adults were exposed in plastic food containers (19 x 19 cm). The bottom of the container was filled with moistened soil and leaf litter to a depth of 1 cm and 0.5 cm, respectively. Mortality in the controls was low, from 0 to 10%, and from 0 to 35% at the normal application rate. LC50 values ranged between 4.5 kg a.e./ha and 22.8 kg a.e./ha, 1.5- to 6-fold greater than the normal application rate. Data indicate that, under realistic worst-case exposure conditions, the mixture of Glyphos and Cosmo-Flux as used for control of coca in Colombia exerts a low toxicity to aquatic and terrestrial stages of anurans and that risks to these organisms under field conditions are small. **(Colombia)**

6. Bishop, C.A., S.L. Ashpole, A.M. Edwards, G. van Aggelen, and J.E. Elliott. 2010. Hatching success and pesticide exposures in amphibians living in agricultural habitats of the South Okanagan Valley, British Columbia, Canada (2004–2006). *Environmental Toxicology and Chemistry* 29(7): 1593-1603.

   In 2004 to 2006, in the Okanagan Valley, British Columbia, Canada, we measured pesticides, water chemistry, and hatching success of Great Basin spadefoot *(Spea intermontana*), Pacific treefrog (*Pseudacris regilla*), Western toad (*Bufo boreas*), and Columbia spotted frog (*Rana luteiventris*). Predator-proof cages containing Gosner Stage 4 eggs were placed in ponds in nonagricultural reference sites in conventionally sprayed and organic orchards. Seventeen pesticides were

detected in ponds in sprayed orchards but occurred at low concentrations (ng/L) except for diazinon (1,410 ng/L). Chloride, sulfate, conductivity, nitrate, and phosphorus showed significant differences among sites. Spadefoot mean hatching success ranged from 0 to 92% among sprayed orchards, whereas the range was 48 to 98.6% among organic orchards and 51 to 95.5% among reference sites. Mean hatching success for Pacific treefrog was 22.1 to 76.1% among sprayed orchards, whereas the range was 83.4 to 97.1% among reference sites. Although sample sizes were small and replication was low, we found that trends in hatching success of eggs of Western toad and Columbia spotted frogs were consistent with the other species. Variables that correlated negatively with amphibian hatching success included 12 pesticides and seven water chemistry parameters. However, stepwise regression found that, in 2005, atrazine accounted for 79% of the variation in spadefoot hatching success and, in 2006, atrazine, total nitrate, and chlorpyrifos accounted for 80%. For Pacific treefrog there were no significant correlations with pesticide concentrations; rather, hatching success correlated with water chemistry parameters. The present study also emphasizes the variability in species sensitivity and importance of incorporating water chemistry into the interpretation of water quality for amphibians. **(Canada)**

7. Boone, M.D. and S.M. James. 2003. Interactions of an insecticide, herbicide, and natural stressors in amphibian community mesocosms. *Ecological Applications* 13(3): 829-841.

Amphibians developing in wetlands embedded within or near agricultural lands may frequently encounter chemical mixtures. The objectives of our study were to determine the effects that post-application concentrations of an insecticide (carbaryl) and an herbicide (atrazine) have on body mass, development, and survival of two anuran species (southern leopard frog, *Rana sphenocephala*; American toad, *Bufo americanus*) and two caudate species (spotted salamander, *Ambystoma maculatum*; small-mouthed salamander, *A. texanum*) reared in outdoor cattle tank mesocosms. In one experiment, we manipulated tadpole density (low or high), carbaryl exposure (0, 3.5, 7.0 mg/L), and atrazine exposure (0 or 200 µg/L) to test for effects on development, mass, and survival of larvae. In a second experiment, we manipulated pond hydroperiod (constant or drying), carbaryl exposure (0 or 5 mg/L), and atrazine exposure (0 or 200 µg/L) to test for effects on mass, time, and survival to metamorphosis. Salamanders were virtually eliminated in carbaryl treatments, indicating that at realistic levels, this insecticide could cause population declines for salamanders in contaminated habitats. Carbaryl also had negative effects on toad survival. Exposure to atrazine had negative effects on body size, development, and time to metamorphosis in anuran species, which were associated with reduced chlorophyll levels. Both chemicals interacted significantly with density or hydroperiod, indicating that the environmental conditions could influence the impact of a contaminant. A significant atrazine-bycarbaryl interaction resulted in smaller and less developed spotted salamander larvae than in control ponds. Atrazine exposure, however, appeared to moderate negative effects of carbaryl for spotted salamanders. Our research suggests that important changes in the community's

food web result from chemical exposure, which influence the susceptibility of amphibian species to contaminants. **(U.S.A.)**

8. Borggaard, O.K. and A.L. Gimsing. 2008. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review*. Pest Management Science* 64(4): 441-456.
   See Water Quality Section.

9. Bosch, B., F. Mañas, N. Gorla, and D. Aiassa. 2011. Micronucleus test in post metamorphic *Odontophrynus cordobae* and *Rhinella arenarum* (Amphibia: Anura) for environmental monitoring. *Journal of Toxicology and Environmental Health Sciences* 3(6): 155-163.
   The genotoxic effect of cyclophosphamide and glyphosate in a commercial formulation were determined using the micronucleus test in peripheral blood erythrocytes of *Odontophrynus cordobae* and *Rhinella arenarum*, amphibians widely distributed in the Province of Córdoba, Argentina. For this, the basal frequency of the micronucleated erythrocytes (MNE) was determined by: 0.40±0.18 MNE/1000 erythrocytes in *Odontophrynus cordobae* and 0.30±0.09 MNE/1000 erythrocytes in *Rhinella arenarum*. The frequency of MNE in *Odontophrynus cordobae* increased after 5 days of exposure to glyphosate (100 mg ai/L) and cyclophosphamide. After 2 and 5 days of exposure to glyphosate (200, 400 and 800 mg ai/L), the MNE frequency in *Rhinella arenarum* was higher than the basal frequency, as it occurred in the group exposed to cyclophosphamide. Regarding acute toxicity and genotoxicity, the results show that *Odontophrynus cordobae* is more sensitive to cyclophosphamide and glyphosate exposure than *Rhinella arenarum*. A correlation was detected between exposure concentration and MNE frequency in *Rhinella arenarum*. **(Argentina)**

10. Brain, R.A. and K.R. Solomon. 2009. Comparison of the hazards posed to amphibians by the glyphosate spray control program versus the chemical and physical activities of coca production in Colombia. *Journal of Toxicology and Environmental Health* Part A 72(15): 937-948.
    This study evaluates the cumulative multifactorial physical and chemical impacts resulting from coca production on amphibian populations in comparison with the potential impacts produced by the herbicide glyphosate (Glyphos), which, mixed with the surfactant Cosmo-Flux, is used in the spray control program for illicit crops in Colombia. Using similar worst-case assumptions for exposure, several other pesticides used for coca production, including mancozeb, lambda cyhalothrin, endosulfan, diazinon, malathion, and chlorpyrifos, were up to 10- to 100-fold more toxic to frogs than the Glyphos-Cosmo-Flux mixture. Comparing hazard quotients based on application rates, several of these compounds demonstrated hazards 3-383 times that of formulated glyphosate. Secondary effects, particularly of insecticides, are also a concern, as these agents selectively target the primary food source of amphibians, which may indirectly impact growth and development. Although the potential chemical impacts by other pesticides are considerable, physical activities associated with coca production, particularly deforestation of

primary forests for new coca plots, portend the greatest hazard to amphibian populations. The entire production cycle of cocaine has been linked to ecosystem degradation. The clearing of pristine forests for coca propagation in Colombia is well documented, and some of these regions coincide with those that contain exceptional amphibian biodiversity. This is particularly problematic as coca production encroaches more deeply into more remote areas of tropical rain forest. Transportation of disease, including the chitrid fungus, to these remote regions via human intrusion may also adversely affect amphibian populations. Therefore, the cumulative impacts of coca production, through habitat destruction, application of agrochemicals, and potential transmission of disease, are judged to pose greater risks to amphibian populations in coca-growing regions than the glyphosate spray control program. **(Colombia)**

11. Bringolf, R.B., W.G. Cope, S. Mosher, M.C. Barnhart, and D. Shea. 2007. Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of *Lampsilis siliquoidea* (Unionidae). *Environmental Toxicology and Chemistry* 26(10): 2094-2100.

    Native freshwater mussels (family Unionidae) are among the most imperiled faunal groups in the world. Factors contributing to the decline of mussel populations likely include pesticides and other aquatic contaminants; however, there is a paucity of data regarding the toxicity of even the most globally distributed pesticides, including glyphosate, to mussels. Therefore, the toxicity of several forms of glyphosate, its formulations, and a surfactant (MON 0818) used in several glyphosate formulations was determined for early life stages of *Lampsilis siliquoidea*, a native freshwater mussel. Acute and chronic toxicity tests were performed with a newly established American Society of Testing and Materials (ASTM) standard guide for conducting toxicity tests with freshwater mussels. Roundup®, its active ingredient, the technical-grade isopropylamine (IPA) salt of glyphosate, IPA alone, and MON 0818 (the surfactant in Roundup formulations) were each acutely toxic to *L. siliquoidea* glochidia. MON 0818 was most toxic of the compounds tested and the 48-h median effective concentration (0.5 mg/L) for *L. siliquoidea* glochidia is the lowest reported for any aquatic organism tested to date. Juvenile *L. siliquoidea* were also acutely sensitive to MON 0818, Roundup, glyphosate IPA salt, and IPA alone. Technical-grade glyphosate and Aqua Star® were not acutely toxic to glochidia or juveniles. Ranking of relative chronic toxicity of the glyphosate-related compounds to juvenile mussels was similar to the ranking of relative acute toxicity to juveniles. Growth data from chronic tests was largely inconclusive. In summary, these results indicate that *L. siliquoidea*, a representative of the nearly 300 freshwater mussel taxa in North America, is among the most sensitive aquatic organisms tested to date with glyphosate-based chemicals and the surfactant MON 0818. **(U.S.A.)**

12. Brodeur, J.C., J.V. Candioti, S. Soloneski, M.L. Larramendy, and A.E. Ronco. 2012. Evidence of reduced feeding and oxidative stress in common tree frogs (*Hypsiboas pulchellus*) from an agroecosystem experiencing severe drought. *Journal of Herpetology* 46(1): 72-78.

The intensification of modern agriculture may impact amphibian populations through habitat loss and the direct and indirect effects of the pesticides upon which it relies. The increased homogeneity of modern agricultural landscapes may be detrimental in times of extreme low and high temperatures associated with climate change, as refuge abundance and habitat connectivity decrease. In this study, we evaluated the impacts of the herbicide glyphosate and subsequent intense drought on the Common Tree Frog, *Hypsiboas pulchellus*, inhabiting an agricultural landscape. We examined a series of organismic indices (stomach content index, hepatosomatic index, body fat index, gonadosomatic index, condition factor) as well as biomarkers of oxidative stress (hepatic catalase activity and reduced glutathione [GSH] content), exposure to contaminants (hepatic gluthatione-S-transferase activity), and genotoxicity (frequency of micronuclei). No significant differences were observed in the parameters measured when comparing frogs sampled before, 2, and 15 days after glyphosate exposure. However, anurans sampled in the same site two months later, when a drought was at its peak, presented a decrease in stomach content and hepatosomatic index, as well as an increase in hepatic catalase activity, hepatic GSH content and micronuclei frequency in peripheral circulating erythrocytes. Our findings demonstrate that drought is challenging to these anurans in this environment as evidenced by an apparent reduction in food intake and oxidative stress. **(Argentina)**

13. Brodman, R., W.D. Newman, K. Laurie, S. Osterfeld, and N. Lenzo. 2010. Interaction of an aquatic herbicide and predatory salamander density on wetland communities. *Journal of Herpetology* 44(1): 69-82.

Pesticides can be important conservation tool, but they could have unintended impacts on amphibians. The commercial glyphosate-based herbicide Accord is approved for use in wetlands and ponds because it is designed to be safer to aquatic wildlife than other herbicide formulations (e.g., Roundup or Atrazine); however, field experiments are needed to determine whether there are direct, indirect, or sublethal effects on amphibians or effects on wetland community structure. We conducted a replicated field experiment in constructed ponds to test for both the effects of Accord and predator (Tiger Salamanders, *Ambystoma tigrinum*) density on amphibians and aquatic invertebrates. Herbicide treatment had significant density-dependent effects on Tiger Salamander growth, development, and survival. The survival of anurans and aquatic invertebrates was also affected by herbicide treatment and predator density. At certain Tiger Salamander densities, the community structure was altered such that some species became more common with herbicide treatment, whereas others became less common. Behavior assays of salamander larvae suggest that herbicide treatment alters predator-prey relationships in the experimental pond communities. These results suggest that competition and predation may mediate indirect effects of this herbicide on the aquatic fauna. We conclude that exposure to Accord poses less of a risk to the ecology of amphibians than do other formulations of glyphosate-based herbicides. **(U.S.A.)**

14. Broomhall, S.D. 2005. Measuring chemical impacts on amphibians: Ecotoxicity and behavioural data in governmental regulation. *Applied Herpetology* 2(3): 259-285.

Despite widespread use of agrochemicals globally, surprisingly little is known about their effects on amphibian populations. Regulatory authorities often rely on data from fish and invertebrate species, and data that are available or accepted for amphibians are often short-term, single factor and single-species tests of growth and survival. Investigations that are more biologically realistic, both in terms of the variables considered (e.g. multiple factors, different species, hormone disruption and delayed fitness effects) and in the range of endpoints measured (biochemical, genetic, physiological and behavioural) are essential. Behaviour reflects an integrative measure of physiological, biochemical and ecological processes and mechanisms. Therefore, behavioural indicators, which are sensitive and non-lethal, provide a valuable tool for understanding the effects of contaminants on reproductive success, survival, and competitive and community interactions. In order to translate such understanding into strategies to minimise the impacts of contamination on amphibians, awareness of the requirements and limitations of governmental chemical regulation is also needed. Improved harmonisation of approach and emphasis between ecotoxicology and regulation will focus efforts more effectively on mitigating the risks posed by chemicals to amphibian populations. **(Australia)**

15. Cauble, K. and R.S. Wagner. 2005. Sublethal effects of the herbicide glyphosate on amphibian metamorphosis and development. *Bulletin of Environmental Contamination and Toxicology* 75(3): 429-435.

***** Amphibian populations have experienced dramatic declines attributed to habitat destruction, introduced species, pathogens, acid rain, global climate change, increased UV-B radiation, fertilizers, pesticides, herbicides, as well as synergistic interactions among different factors. Several studies have demonstrated sensitivity of amphibians to herbicides. *Rana cascadae* egg masses collected from Table Mountain in Kittitas County, Washington (USA) were raised for larvae to test the effects of nominal concentrations of glyphosate (Roundup®) (1 ppm, 2 ppm, and a 0 ppm control) on their metamorphosis. Larvae were checked daily for mortality and other activities such as feeding, swimming, limb eruptions, and abnormalities. It was found that low concentrations of Roundup® significantly affect *R. cascadeae* larval development and metamorphosis in static renewal tests. Major effects on larvae include survivability, rate of metamorphosis, and post-metamorphosis mass. At 1.94 ppm no individuals survived until metamorphosis. Metamorphosis was significantly earlier and the metamorphosis mass was significantly smaller in the 1ppm versus the control group. To help mitigate the potential effects of glyphosate applications we suggest its use in aquatic environments occurs at times that do not coincide with amphibian larval development and metamorphosis. **(U.S.A.)**

16. Cavaş, T. and S. Könen. 2007. Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay. *Mutagenesis* 22(4): 263-268.

See Fish Section.

17. Cerdeira, A.L. and S.O. Duke. 2006. The current status and environmental impacts of glyphosate-resistant crops: a review. *Journal of Environmental Quality* 35(5): 1633-1658.

See Biodiversity and Restoration Section.

18. Chen, C.Y., K.M. Hathaway, and C.L. Folt. 2004. Multiple stress effects of Vision® herbicide, pH, and food on zooplankton and larval amphibian species from forest wetlands**.** *Environmental Toxicology and Chemistry* 23(4): 823-831.

As part of a multiple-tier research program, interactions of the herbicide Vision® (glyphosate) with two stressors, pH and food level, were examined. Effects of the formulated product Vision were tested at two test concentrations (0.75 and 1.50 mg acid equivalent/L), two pH levels (pH 5.5 and 7.5), and under high and low food concentrations. Effects of each stressor alone and in combination were examined using two common wetland taxa: Zooplankton, *Simocephalus vetulus*, and tadpoles (Gosner stage 25) of *Rana pipiens.* For *S. vetulus*, survival, reproduction, and development time were measured; survival was measured for *R. pipiens.* For both species, significant effects of the herbicide were measured at concentrations lower than the calculated worst-case value for the expected environmental concentration ([EEC], 1.40 mg acid equivalent/L). Moreover, high pH (7.5) increased the toxic effects of the herbicide on all response variables for both species even though it improved reproductive rate of *S. vetulus* over pH 5.5 in the absence of herbicide. Stress due to low food alone also interacted with pH 5.5 to diminish *S. vetulus* survival. These results support the general postulate that multiple stress interactions may exacerbate chemical effects on aquatic biota in natural systems. **(U.S.A.)**

19. Chen, C.Y., K.M. Hathaway, D.G. Thompson, and C.L. Folt. 2008. Multiple stressor effects of herbicide, pH, and food on wetland zooplankton and a larval amphibian. *Ecotoxicology and Environmental Safety* 71(1): 209-218.

Interactions of herbicides and natural environmental stressors such as pH and food availability are poorly understood. We tested a chemical formulation of triclopyr (Release®) at environmentally relevant test concentrations (0.25 and 0.50 mg L^{-1}) in combination with two levels of pH (pH 5.5 and 7.5), and two levels of food availability (high and low). Population level effects of each stressor alone and in combination with the others were investigated using *Simocephalus vetulus*, a zooplankton species, and *Rana pipiens* tadpoles (Gosner stage 25), both common to forest ponds and wetlands. Herbicide treatments resulted in significant decreases in survival of both test species as well as reproduction and development time for *S. vetulus* at levels 5-10x below predicted worst case environmental concentrations (2.6 mg L^{-1}). This laboratory study demonstrates a probable risk of toxic effects of Release® herbicide which may be significantly increased by low food availability and by low pH at environmentally relevant concentrations. **(U.S.A.)**

20. Comstock, B.A., S.L. Sprinkle, and G.R. Smith. 2007. Acute toxic effects of Round-Up herbicide on wood frog tadpoles (*Rana sylvatica*). *Journal of Freshwater Ecology* 22(4): 705-708.

We examine the toxicity of a commercial formulation of Round-Up to wood frog (*Rana sylvatica*) tadpoles. All tadpoles exposed to nominal concentrations ≥0.00098% glyphosate died within 24 h. However, tadpoles exposed to concentrations ≤0.00049% showed high survivorship. Our results suggest that any direct application of this formulation of Round-Up to aquatic ecosystems could have an impact on amphibian larvae, especially in shallow water. **(U.S.A.)**

21. Contardo-Jara, V., E. Klingelmann, and C. Wiegand. 2009. Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes. *Environmental Pollution* 157(1): 57-63.

The bioaccumulation potential of glyphosate and the formulation Roundup Ultra, as well as possible effects on biotransformation and antioxidant enzymes in *Lumbriculus variegatus* were compared by four days exposure to concentrations between 0.05 and 5 mg L^{-1} pure glyphosate and its formulation. Bioaccumulation was determined using ^{14}C labeled glyphosate. The bioaccumulation factor (BCF) varied between 1.4 and 5.9 for the different concentrations, and was higher than estimated from $logP_{ow}$. Glyphosate and its surfactant POEA caused elevation of biotransformation enzyme soluble glutathione S-transferase at non-toxic concentrations. Membrane bound glutathione S-transferase activity was significantly elevated in Roundup Ultra exposed worms, compared to treatment with equal glyphosate concentrations, but did not significantly differ from the control. Antioxidant enzyme superoxide dismutase was significantly increased by glyphosate but in particular by Roundup Ultra exposure indicating oxidative stress. The results show that the formulation Roundup Ultra is of more ecotoxicological relevance than the glyphosate itself. **(Germany)**

22. Cooman, K., P. Debels, M. Gajardo, R. Urrutia, and R. Barra. 2005. Use of *Daphnia* spp. for the ecotoxicological assessment of water quality in an agricultural watershed in south-central Chile. *Archives of Environmental Contamination and Toxicology* 48(2): 191-200.

See Water Quality Section.

23. Costa, M.J., D.A. Monteiro, A.L. Oliveira-Neto, F.T. Rantin, and A.L. Kalinin. 2008. Oxidative stress biomarkers and heart function in bullfrog tadpoles exposed to Roundup Original®. *Ecotoxicology* 17(3): 153-163.

Oxidative stress biomarkers, in vivo heart rate (f_H), and contraction dynamics of ventricle strips of bullfrog (*Lithobates catesbeiana*) tadpoles were evaluated after 48 h of exposure to a sub-lethal concentration (1 ppm) of the herbicide Roundup Original® (glyphosate 41%). The activities of the antioxidant enzymes superoxide dismutase and catalase were increased in the liver and decreased in muscle, while oxidative damage to lipids increased above control values in both tissues, showing that the generation of reactive oxygen species and oxidative stress are involved in the toxicity induced by Roundup®. Additionally, tadpoles' hyperactivity was associated with tachycardia in vivo, probably due to a stress-induced adrenergic stimulation. Ventricle strips of Roundup®-exposed tadpoles (R-group) presented a

faster relaxation and also a higher cardiac pumping capacity at the in vivo contraction frequency, indicating that bullfrog tadpoles were able to perform cardiac mechanistic adjustments to face Roundup®-exposure. However, the lower maximal in vitro contraction frequency of the R-group could limit its in vivo cardiac performance, when the adrenergic-stimulation is present. The association between the high energetic cost to counteract the harmful effects of this herbicide and the induction of oxidative stress suggest that low and realistic concentrations of Roundup® can have an impact on tadpoles' performance and success, jeopardizing their survival and/or population establishment. **(Brazil)**

24. Davidson, C. 2004. Declining downwind: amphibian population declines in California and historical pesticide use. *Ecological Applications* 14(6): 1892-1902.

Pesticides have long been proposed as a possible cause of amphibian population declines, but due to a number of challenges there has been relatively little ecotoxicological research on pesticides and declines in natural populations. My study examines the association between the spatial patterns of declines for five California amphibian species and historical patterns of pesticide use in California from 1974 to 1991 based on Department of Pesticide Regulation records. Information on declines was derived from maps of historical sites and current population status for the Yosemite toad (*Bufo canorus*), California red-legged frog (*Rana aurora draytonii*), foothill yellow-legged frog (*R. boylii*), Cascades frog (*R. cascadae*), and the mountain yellow-legged frog (*R. muscosa*). Multiple logistic-regression and generalized additive models were used to analyze the relationship between site status (present or absent) and total upwind pesticide use, 64 pesticide classes and groups, and covariates including precipitation, elevation, surrounding urban and agricultural land use, and spatial location. Total pesticide use was a strong, significant variable in logistic-regression models for all species, except *B. canorus*. Total pesticide use was a significant variable even when spatial autocorrelation was accounted for by inclusion of a spatial location covariate. Cholinesterase-inhibiting pesticides (most organophosphates and carbamates) stood out as more strongly associated with population declines than any other class of pesticides. This is the first study in which population declines of multiple declining species have been associated with historical pesticide applications. **(U.S.A.)**

25. Demetrio, P.M., G.D. Bulus Rossini, C.A. Bonetto, and A.E. Ronco. 2012. Effects of pesticide formulations and active ingredients on the coelenterate *Hydra attenuata* (Pallas, 1766). *Bulletin of Environmental Contamination and Toxicology* 88(1): 15-19.

Lethal effects of active ingredients and formulations of widely used soybean pesticides were assessed with the *Hydra attenuata* toxicity test. Studied pesticides were insecticides chlorpyrifos and cypermethrin, and herbicide glyphosate. Results indicate the following toxicity trend: chlorpyrifos > cypermethrin > glyphosate. Tested active ingredients of insecticides and respective formulations did not significantly differ between them. Glyphosate formulation exhibited higher toxicity at low concentrations ($LC_{1–10}$) respect to active ingredient, reversing this behavior at higher concentrations ($LC_{50–90}$). Comparing *H. attenuata* sensitivity with existent toxicity

data for aquatic organisms indicates that this species is poorly sensitive to tested insecticides and highly sensitive to the herbicide. **(Argentina)**

26. Dinehart, S.K., L.M. Smith, S.T. McMurry, T.A. Anderson, P.N. Smith, and D.A. Haukos. 2009. Toxicity of a glufosinate- and several glyphosate-based herbicides to juvenile amphibians from the Southern High Plains, USA. *Science of the Total Environment* 407(3): 1065-1071.

    Pesticide toxicity is often proposed as a contributing factor to the world-wide decline of amphibian populations. We assessed acute toxicity (48 h) of a glufosinate-based herbicide (Ignite® 280 SL) and several glyphosate-based herbicide formulations (Roundup WeatherMAX®, Roundup Weed and Grass Killer Super Concentrate®, Roundup Weed and Grass Killer Ready-To-Use Plus® on two species of amphibians housed on soil or moist paper towels. Survival of juvenile Great Plains toads (*Bufo cognatus*) and New Mexico spadefoots (*Spea multiplicata*) was reduced by exposure to Roundup Weed and Grass Killer Ready-To-Use Plus® on both substrates. Great Plains toad survival was also reduced by exposure to Roundup Weed and Grass Killer Super Concentrate® on paper towels. New Mexico spadefoot and Great Plains toad survival was not affected by exposure to the two agricultural herbicides (Roundup WeatherMAX® and Ignite® 280 SL) on either substrate, suggesting that these herbicides likely do not pose an immediate risk to these species under field conditions. **(U.S.A.)**

27. Dinehart, S.K., L.M. Smith, S.T. McMurry, P.N. Smith, T.A. Anderson, and D.A. Haukos. 2010. Acute and chronic toxicity of Roundup Weathermax® and Ignite® 280 SL to larval *Spea multiplicata* and *S. bombifrons* from the Southern High Plains, USA. *Environmental Pollution* 158(8): 2610-2617.

    Pesticides have been implicated in widespread amphibian declines. We assessed acute and chronic toxicity of two widely used herbicides to larval New Mexico (*Spea multiplicata*) and Plains (*S. bombifrons*) spadefoots from cropland and native grassland playas. Roundup WeatherMAX® (WM) toxicity estimates (48- and 216-h LC_{50}; 48-h LC_1) for both species were similar to environmental concentrations expected from accidental overspray. Chronic (30-day) exposure to WM at predicted environmental concentrations (2.0 and 2.8 mg glyphosate acid equivalents/L) reduced survival of both species. Ignite® 280 SL (IG) toxicity estimates (48-h LC_{50} and LC_1) for both species were above predicted environmental concentrations of 1.0 mg glufosinate/L. Chronic exposure to predicted environmental concentrations of IG did not reduce survival of either species. Toxicity test results suggest that at predicted environmental concentrations IG would not cause extensive mortalities among larval New Mexico and Plains spadefoots. However, WM may cause extensive mortality among larvae of these species. **(U.S.A.)**

28. Domínguez-Cortinas, G., J. Mejía Saavedra, G.E. Santos-Medrano, and R. Rico-Martínez. 2008. Analysis of the toxicity of glyphosate and Faena® using the freshwater invertebrates *Daphnia magna* and *Lecane quadridentata. Toxicological & Environmental Chemistry* 90(2): 377-384.

With the present contribution an evaluation of the toxicity of the pure herbicide glyphosate and its commercial formulation Faena® is reported using the cladoceran *Daphnia magna* Strauss and the rotifer *Lecane quadridentata* Ehrenberg. LC_{50}, EC_{50}, NOEC, and LOEC values for each toxicant and for both test organisms are obtained. Regarding acute toxicity, Faena was 11-fold more toxic to *L. quadridentata* than pure glyphosate and slightly more toxic to *D. magna* (1.7-fold). Inhibition of esterase activity in *L. quadridentata* by glyphosate was the most sensitive end-point; the EC_{50} was 1500-fold smaller than the LC_{50}. The implications of these results and their comparison with established international and national limit values for glyphosate are discussed. **(Mexico)**

29. Dutra, B.K., F.A. Fernandes, D.M. Failace, and G.T. Oliveira. 2011. Effect of roundup® (glyphosate formulation) in the energy metabolism and reproductive traits of *Hyalella castroi* (Crustacea, Amphipoda, Dogielinotidae). *Ecotoxicology* 20(1): 255-263.

    Roundup® (glyphosate formulation) is a nonselective and posts emergent herbicide used for controlling aquatic weeds and different concentrations are used in cultures around the world. The objective of this investigation was to examine the effects of Roundup® (glyphosate formulation) on the biochemical composition, levels of lipoperoxidation, Na+/K+ATPase activity and reproductive traits in the *Hyalella castroi*. Amphipods were collected in summer 2009, in the southern Brazilian highlands. In the laboratory, the animals were kept in aquariums under controlled conditions for 7 days, and after this period they were exposed to 0.36, 0.52, 1.08 and 2.16 mg/l of glyphosate for 7 days. After the period of exposure, the animals were immediately frozen for determination of glycogen, proteins, lipids, triglycerides, cholesterol, levels of lipoperoxidation, and Na+/K+ATPase activity. During each day of the cultivation reproductive traits (number of reproductive pairs, ovigerous females and eggs in the marsupium) were observed. All concentrations of Roundup® induced significant decreases in all biochemical parameters and Na+/K+ATPase activity, and significant increase in lipoperoxidation levels. Showing this form a potentially toxic effect at very low concentrations, this pattern of results can lead to significant changes in trophic structure of limnic environments because these amphipods are important links in food chain in these habitats. **(Brazil)**

30. Edge, C.B., M.K. Gahl, B.D. Pauli, D.G. Thompson, and J.E. Houlahan. 2011. Exposure of juvenile green frogs (*Lithobates clamitans*) in littoral enclosures to a glyphosate-based herbicide. *Ecotoxicology and Environmental Safety* 74(5): 1363-1369.

    The majority of studies on the toxicity of glyphosate-based herbicides to amphibians have focused on larval life stages exposed in aqueous media. However, adult and juvenile amphibians may also be exposed directly or indirectly to herbicides. The potential for such exposures is of particular interest in the littoral zone surrounding wetlands as this is preferred habitat for many amphibian species. Moreover, it may be argued that potential herbicide effects on juvenile or adult amphibians could have comparatively greater influence on overall recruitment, reproductive potential and thus stability of local populations than effects on larvae. In

this experiment, juvenile green frogs (*Lithobates clamitans*) were exposed to two concentrations (2.16 and 4.27 kg a.e./ha) of a glyphosate-based herbicide formulation (VisionMax®), which were based on typical application scenarios in Canadian forestry. The experimental design employed frogs inhabiting in situ enclosures established at the edge of small naturalized wetlands that were split in half using an impermeable plastic barrier. When analyzed using nominal target application rates, exposure to the glyphosate-based herbicide had no significant effect on survival, body condition, liver somatic index or the observed rate of *Batrachochytrium dendrobatidis* infection. However, there were marginal trends in both ANOVA analysis and post-hoc regressions regarding *B. dendrobatidis* infection rates and liver somatic index in relation to measured exposure estimates. Results from this study highlight the importance of field research and the need to include multiple endpoints when examining potential effects of a contaminant on non-target organisms. **(Canada)**

31. Edginton, A.N., P.M. Sheridan, H.J. Boermans, D.G. Thompson, J.D. Holt, and G.R. Stephenson. 2004. A comparison of two factorial designs, a complete 3×3 factorial and a central composite rotatable design, for use in binomial response experiments in aquatic toxicology. *Archives of Environmental Contamination and Toxicology* 46(2): 216-223.

See Water Quality Section.

32. Edginton, A.N., P.M. Sheridan, G.R. Stephenson, D.G. Thompson, and H.J. Boermans. 2004. Comparative effects of pH and Vision® herbicide on two life stages of four anuran amphibian species. *Environmental Toxicology and Chemistry* 23(4): 815-822.

Vision®, a glyphosate-based herbicide containing a 15% (weight:weight) polyethoxylated tallow amine surfactant blend, and the concurrent factor of pH were tested to determine their interactive effects on early life-stage anurans. Ninety-six-hour laboratory static renewal studies, using the embryonic and larval life stages (Gosner 25) of *Rana clamitans, R. pipiens, Bufo americanus*, and *Xenopus laevis*, were performed under a central composite rotatable design. Mortality and the prevalence of malformations were modeled using generalized linear models with a profile deviance approach for obtaining confidence intervals. There was a significant ($p < 0.05$) interaction of pH with Vision concentration in all eight models, such that the toxicity of Vision was amplified by elevated pH. The surfactant is the major toxic component of Vision and is hypothesized, in this study, to be the source of the pH interaction. Larvae of *B. americanus* and *R. clamitans* were 1.5 to 3.8 times more sensitive than their corresponding embryos, whereas *X. laevis* and *R. pipiens* larvae were 6.8 to 8.9 times more sensitive. At pH values above 7.5, the Vision concentrations expected to kill 50% of the test larvae in 96-h (96-h lethal concentration [LC50]) were predicted to be below the expected environmental concentration (EEC) as calculated by Canadian regulatory authorities. The EEC value represents a worst-case scenario for aerial Vision application and is calculated assuming an application of the maximum label rate (2.1 kg acid equivalents [a.e.]/ha) into a pond 15 cm in depth. The EEC of 1.4 mg a.e./L (4.5 mg/L Vision)

was not exceeded by 96-h LC50 values for the embryo test. The larvae of the four species were comparable in sensitivity. Field studies should be completed using the more sensitive larval life stage to test for Vision toxicity at actual environmental concentrations. **(Canada)**

33. Everett, K.D.E and H.W. Dickerson. 2003. *Ichthyophthirius multifiliis* and *Tetrahymena thermophila* tolerate glyphosate but not a commercial herbicidal formulation. *Bulletin of Environmental Contamination and Toxicology* 70(4): 731-738.
    See Fish Section.

34. Favret, K.P. and J.W. Lynn. 2010. Flow-cytometric analyses of viability biomarkers in pesticide-exposed sperm of three aquatic invertebrates. *Archives of Environmental Contamination and Toxicology* 58(4): 973-984.
    Toxicity studies on sperm often use fertilization success as the end point. This type of assay can be affected by sperm density, egg quality, and sperm-egg compatibility. Testing sperm viability biomarkers with flow cytometry is a fast, high-throughput technique for seminal analysis. In this study, we detected sperm viability biomarkers with several fluorescent reporter dyes using flow cytometry in three aquatic invertebrates (*Crassostrea virginica, Dreissena polymorpha*, and *Lytechinus variegatus*) after exposure to a pesticide and herbicide. The pesticide, Bayluscide, appeared to affect mitochondrial membrane potential in the sperm of all three species, as measured with MitoTracker Red CMXRos®. A decrease in the percentage of sperm stained with SYBR®-14 (indicating uncompromised plasma membrane) was observed in *C. virginica* and *D. polymorpha* sperm exposed to Bayluscide, but propidium iodide staining (indicating compromised plasma membranes) appeared to be inhibited by Bayluscide. Acrosome-reacted sperm, as measured by FITC-PNA, decreased after Bayluscide exposure in *C. virginica* and *D. polymorpha* sperm. The herbicide, Roundup Ready To-Use-Plus®, did not affect the overall percentages of sperm stained with MitoTracker but did cause an increase in MitoTracker fluorescence intensity at 16 mg/L in *D. polymorpha*. Roundup also caused significant decreases in SYBR-14 staining, significant increases in propidium iodide staining, and significant increases in FITC-PNA staining in *D. polymorpha* sperm. By not having to rely on egg availability and optimal sperm density, sperm toxicity can be more accurately assessed with flow cytometry as being directly correlated to sperm viability rather than the possibility of altered toxicity results due to sperm-to-egg compatibility. **(U.S.A.)**

35. Frontera, J.L., I. Vatnick, A. Chaulet, and E.M. Rodríguez. 2011. Effects of glyphosate and polyoxyethylenamine on growth and energetic reserves in the freshwater crayfish *Cherax quadricarinatus* (Decapoda, Parastacidae). *Archives of Environmental Contamination and Toxicology* 61: 590-598.
    Freshwater crayfish *Cherax quadricarinatus* have a high commercial value and are cultured in farms where they are potentially exposed to pesticides. Therefore, we examined the sublethal effects of a 50-day exposure to glyphosate acid and polyoxyethylenamine (POEA), both alone and in a 3:1 mixture, on the growth and energetic reserves in muscle, hepatopancreas and hemolymph of growing juvenile

crayfish. Exposure to two different glyphosate and POEA mixtures caused lower somatic growth and decreased muscle protein levels. These effects, caused by both compounds interacting in the mixture, could also be synergistic because they were expressed even at the lowest concentration. The decrease in protein levels could be related to the greater use of other energy reserves. This hypothesis is supported by the decrease in muscle glycogen stores due to glyphosate exposure and the decrease in lipid reserves associated with exposure to POEA. **(Argentina)**

36. Fuentes, L., L.J. Moore, J.H. Rodgers, Jr, W.W. Bowerman, G.K. Yarrow, and W.Y. Chao. 2011. Comparative toxicity of two glyphosate formulations (original formulation of Roundup® and Roundup WeatherMAX®) to six North American larval anurans. *Environmental Toxicology and Chemistry* 30(12): 2756-2761.

    The toxicity of two glyphosate formulations (the original formulation of Roundup® and Roundup WeatherMAX®) to six species of North American larval anurans was evaluated by using 96-h static, nonrenewal aqueous exposures. The 96-h median lethal concentration values (LC50) ranged from 1.80 to 4.22 mg acid equivalent (ae)/L and 1.96 to 3.26 mg ae/L for the original formulation of Roundup and Roundup WeatherMAX, respectively. Judged by LC50 values, four species were more sensitive to Roundup WeatherMAX exposures, and two species were more sensitive to the original formulation. Two of six species, *Bufo fowleri* ($p < 0.05$, $F = 14.89$, degrees of freedom [*df*] = 1) and *Rana clamitans* ($p < 0.05$, $F = 18.46$, $df = 1$), had significantly different responses to the two formulations tested. Increased sensitivity to Roundup WeatherMAX likely was due to differences in the surfactants or relative amounts of the surfactants in the two formulations. Potency slopes for exposures of the original formulation ranged from 24.3 to 92.5% mortality/mg ae/L. Thresholds ranged from 1.31 to 3.68 mg ae/L, showing an approximately three times difference in the initiation of response among species tested. For exposures of Roundup WeatherMAX, slopes ranged from 49.3 to 84.2% mortality/mg ae/L. Thresholds ranged from 0.83 to 2.68 mg ae/L. Margins of safety derived from a simulated direct overspray were above 1, except for one species in exposures of Roundup WeatherMAX. Laboratory data based on aqueous exposures are conservative because of the lack of environmental ligands; however, these tests provide information regarding the relative toxicity between these two Roundup formulations. **(U.S.A.)**

37. Gahl, M.K., B.D. Pauli, and J.E. Houlahan. 2011. Effects of chytrid fungus and a glyphosate-based herbicide on survival and growth of wood frogs (*Lithobates sylvaticus*). *Ecological Applications* 21(7): 2521-2529.

    Anthropogenic-derived stressors in the environment, such as contaminants, are increasingly considered important cofactors that may decrease the immune response of amphibians to pathogens. Few studies, however, have integrated amphibian disease and contaminants to test this multiple-stressor hypothesis for amphibian declines. We examined whether exposure to sublethal concentrations of a glyphosate-based herbicide and two strains of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*) could: (1) sublethally affect wood frogs (*Lithobates sylvaticus*) by altering the time to and size at metamorphosis, and (2)

directly affect survivability of wood frogs after metamorphosis. Neither *Bd* strain nor herbicide exposure alone significantly altered growth or time to metamorphosis. The two *Bd* strains did not differ in their pathogenicity, and both caused mortality in post-metamorphic wood frogs. There was no evidence of an interaction between treatments, indicating a lack of herbicide-induced susceptibility to *Bd*. However, the trends in our data suggest that exposure of wood frogs to a high concentration of glyphosate-based herbicide may reduce *Bd*-caused mortality compared to animals exposed to *Bd* alone. These results exemplify the complexities inherent when populations are coping with multiple stressors. In this case, the perceived stressor, glyphosate-based herbicide, appeared to affect the pathogen more than the host's immune system, relieving the host from disease-caused effects. This suggests caution when invoking multiple stressors as a cause for increased disease susceptibility and indicates that the effects of multiple stressors on disease outcome depend on the interrelationships of stressors to both the pathogen and the host. **(Canada)**

38. Gertzog, B.J., L.J. Kaplan, D. Nichols, G.R. Smith, and J.E. Rettig. 2011. Avoidance of three herbicide formulations by eastern red-backed salamanders (*Plethodon cinereus*). *Herpetological Conservation and Biology* 6(2): 237-241.

    The ability of terrestrial amphibians to avoid chemical pollutants, such as herbicides or fertilizers, is relatively unknown although such chemicals may occur in their habitats. We studied whether the Eastern Red-backed Salamander (*Plethodon cinereus*) would be able to behaviorally avoid three different herbicide formulations (Spectracide Brush Killer, Bayer Advanced Brushkiller Plus, and Roundup Weed and Grass Killer Concentrate Plus). Salamanders avoided all three formulations when encountered at their full recommended application concentration, and were able to detect and avoid the Bayer and Roundup formulations at 10% the full concentration. Salamanders did not avoid any formulation at 1% the full concentration. Our results suggest that salamanders are able to detect these herbicide formulations and behaviorally avoid them, perhaps reducing any negative effects from exposure to some field applications. **(U.S.A.)**

39. Giesy, J. P., S. Dobson, and K. R. Solomon. 2000. Ecotoxicological risk assessment for Roundup® Herbicide. *Reviews of Environmental Contamination and Toxicology* 167: 35-120.

    Roundup® Herbicide (RU) and its active ingredient glyphosate have been extensively investigated in ecological toxicity studies to support registrations in various countries and also in many scientific investigations independent of regulatory considerations. The purpose of this study was to review the current state of knowledge on the ecological toxicity of Roundup and glyphosate and to consider this information in a comprehensive ecological risk assessment. A conservative hazard quotient method was used to evaluate risk. The hazard quotient (HQ) was calculated by dividing the maximum environmental exposure concentration derived from modeling or environmental monitoring data by the greatest level of Roundup or glyphosate found to have no effect on survival, growth, or reproduction of the most sensitive nontarget organisms. Roundup, which contains glyphosate and a

polyethoxylated tallowamine surfactant (POEA), was used for acute HQ determination, because exposure to the total formulation was a reasonable assumption. Because of differential rates of dissipation, the individual components of the formulation (either glyphosate or the surfactant) were considered separately for chronic HQs. The risk assessment evaluated both terrestrial and aquatic uses of Roundup. Conclusions of the analysis are as follows.

1. Roundup® Herbicide contains the isopropylamine salt of glyphosate as the active ingredient and a surfactant (typically polyethoxylated tallowamine) to facilitate plant uptake of the active ingredient. Roundup® is a broad-spectrum herbicide and will control many types of herbaceous and woody plants. Several formulations of Roundup® are used worldwide and may have different amounts of the components.

2. Glyphosate in the environment tends to bind tightly to soil and particulate matter and is essentially unavailable to plants or other soil organisms. As a result, glyphosate has little activity in soil, and the herbicidal effects are limited to foliar contact. In addition, glyphosate is very water soluble and does not partition into animal fats. Consequently, glyphosate does not bioconcentrate in fish or other animals. Less is known about the environmental fates of surfactants, but rates of degradation appear to be similar to glyphosate.

3. The risk assessment described here considered terrestrial and aquatic uses of Roundup®. Acute risk characterization assumed that organisms were potentially exposed to the intact formulation, whereas chronic risk characterization considered potential exposure to the components of Roundup® (glyphosate and the surfactant). This approach allowed the acute assessment to consider acute effects of the surfactant that are greater than glyphosate alone for aquatic animals.

4. For terrestrial uses of Roundup® (agricultural, forestry, rights-of-way, residential, etc.), minimal acute and chronic risk was predicted for potentially exposed nontarget organisms. This conclusion is based on the conservative hazard quotient analysis that resulted in no HQ values greater than 1. The following taxa were evaluated using the hazard quotient method: aquatic microorganisms, aquatic macrophytes, aquatic invertebrates, warm water and cold water fish, amphibians (tadpoles), soil microorganisms, soil invertebrates, birds, and mammals. Honeybees and other beneficial arthropods and nontarget terrestrial plants were not evaluated using the hazard quotient method.

5. Honeybees are not affected by glyphosate formulations, either by ingestion or direct overspray, at maximum use rates. The majority of other beneficial arthropods are unaffected by Roundup®. Although screening tests under extreme exposure conditions indicate toxicity of glyphosate formulations to some beneficial arthropods at the maximum use rates, these effects were reduced or eliminated when more realistic exposure conditions were used. These data demonstrate minimal risk to beneficial arthropods in areas adjacent to treated fields. Within treated fields, vegetation changes resulting from herbicide use can lead to significant changes in beneficial arthropod populations.

6. Nontarget terrestrial plants in areas adjacent to the treated areas may be exposed to Roundup®. From ground applications, small amounts of herbicide may move downwind from treated areas to adjacent non-target areas via spray drift. However, as glyphosate is not active in soil, potential herbicidal effects will be limited

to only those plants very near the treated area that are in a sensitive growth stage at the time of treatment. Aerial applications can result in increased drift relative to ground applications, but recent technological advances have significantly reduced aerial spray drift.

7. Greater exposure of aquatic organisms to Roundup® is likely during use to control emergent aquatic macrophytes compared to terrestrial uses. Acute and chronic HQ values are less than 1.0 (minimal risk) for all taxa for direct addition of Roundup® to 2-m-deep water at the maximum use rate. Acute HQ values can approach or exceed 1.0 in shallow water (0.15 m). Examination of assumptions reveals that degradation, sorption, and interception by target vegetation of greater than 50% will mitigate the potential for effects in shallow waters. Even in shallow waters, the chronic HQ did not exceed 1. Use of Roundup® for aquatic habitat restoration can be safely carried out, but requires consideration of items such as application rate, depth of water, and vegetation coverage. **(Canada)**

40. Hanlon, S.M. and M.J. Parris. 2012. The impact of pesticides on the pathogen *Batrachochytrium dendrobatidis* independent of potential hosts. *Archives of Environmental Contamination and Toxicology* DOI: 10.1007/s00244-011-9744-1. Published online: 07 January 2012

Amphibians around the world are experiencing the greatest organismal decline in recent history. Xenobiotics, such as pesticides, and pathogenic biotic perturbations, including the fungus *Batrachochytrium dendrobatidis* (*Bd*), have played major roles in amphibian decreases. We conducted laboratory culture studies to determine the effects of three pesticides {carbaryl, glyphosate, and thiophanatemethyl [TM; Topsin-M(R) (Cerexagri-Nisso LLC)]} on *Bd* zoospore production and zoosporangia growth. We applied Bd to pesticides mixed in an agar culture to simulate pathogen introduction to a system with pre-existing pesticides (*Bd* addition). Alternatively, pesticides were applied to preestablished *Bd* to simulate pesticide introduction after *Bd* establishment (pesticide addition). We then measured *Bd* zoosporangia and zoospore production. All pesticides significantly inhibited zoospore production; however, glyphosate and TM were more effective at doing so than carbaryl. In addition, only carbaryl and glyphosate inhibited zoosporangia production. Our data suggest that carbaryl and glyphosate are equally effective at inhibiting both zoosporangia and zoospore production; however, TM is selectively toxic to zoospores but not zoosporangia. One possible explanation for this observation could be that TM is toxic to zoospores but not the protective zoosporangia. In the case of pesticides applied to established *Bd* cultures, all pesticides caused significant mortality in both zoosporangia and zoospores, and no differences were found among pesticides. We conclude that examining pesticide and pathogen interactions independent of hosts provides mechanistic understanding of such interactions before and after host infection or contamination. **(U.S.A.)**

41. Hedberg, D. and M. Wallin. 2010. Effects of Roundup and glyphosate formulations on intracellular transport, microtubules and actin filaments in *Xenopus laevis* melanophores. *Toxicology in Vitro* 24(3): 795-802.

Glyphosate containing herbicides, such as Roundup, are commonly used and generally considered to be safe. However, some toxic effects are found on amphibians *in vivo* and human and mouse cells *in vitro*. In this study the effects of Roundup, glyphosate, glyphosateisopropylamine and isopropylamine were studied on intracellular transport by measuring aggregation capacity in *Xenopus laevis* melanophores. The chemicals inhibited retrograde transport of melanosomes in the range of 0.5-5mM. Cellular morphology and localization of microtubules and actin filaments were affected as determined by immunocytochemistry. Both glyphosate and Roundup decreased pH in the media. Acidic pH inhibited melanosome transport and altered microtubule and actin morphology in the absence of chemicals, while transport inhibiting concentrations of glyphosate, Roundup and glyphosateisopropylamine disassembled both microtubules and actin filaments. At physiological pH the effects of Roundup decreased whereas glyphosate failed to inhibit transport. Physiological pH decreases glyphosate lipophilicity and its diffusion into the cytoplasm. The Roundup formulation contains surfactants, such as POEA (polyetylated tallow amine) that increases membrane permeability allowing cellular uptake at physiological pH. Our results show that the effects of glyphosate containing compounds are pH-dependent and that they inhibit intracellular transport through disassembly of the cytoskeleton possibly by interfering with intracellular Ca^{2+}-balance. **(Sweden)**

42. Hewitt, A.J., K.R. Solomon and E.J.P. Marshall. 2009. Spray droplet size, drift potential, and risks to nontarget organisms from aerially applied glyphosate for coca control in Colombia. *Journal of Toxicology and Environmental Health*. Part A 72(15): 921-929.

See Human Health Section.

43. Howe, C.M., M. Berrill, B.D. Pauli, C.C. Helbing, K.Werry, and N. Veldhoen. 2004. Toxicity of glyphosate-based pesticides to four North American frog species. *Environmental Toxicology and Chemistry* 23(8): 1928-1938.

Gyphosate-based herbicides are among the most widely used pesticides in the world. We compared the acute toxicity of the glyphosate end-use formulation Roundup Original® to four North American amphibian species (*Rana clamitans, R. pipiens, R. sylvatica,* and *Bufo americanus*) and the toxicity of glyphosate technical, the polyethoxylated tallowamine surfactant (POEA) commonly used in glyphosate-based herbicides, and five newer glyphosate formulations to *R. clamitans.* For *R. clamitans*, acute toxicity values in order of decreasing toxicity were POEA > Roundup Original > Roundup Transorb® > Glyfos AU®; no significant acute toxicity was observed with glyphosate technical material or the glyphosate formulations Roundup Biactive®, Touchdown®, or Glyfos BIO®. Comparisons between the four amphibian species showed that the toxicity of Roundup Original varied with species and developmental stage. *Rana pipiens* tadpoles chronically exposed to environmentally relevant concentrations of POEA or glyphosate formulations containing POEA showed decreased snout-vent length at metamorphosis and increased time to metamorphosis, tail damage, and gonadal abnormalities. These effects may be caused, in some part, by disruption of hormone signaling, because

thyroid hormone receptor β mRNA transcript levels were elevated by exposure to formulations containing glyphosate and POEA. Taken together, the data suggest that surfactant composition must be considered in the evaluation of toxicity of glyphosate-based herbicides. **(Canada)**

44. Jadhav, A., M. Hill, and M. Byrne. 2008. Identification of a retardant dose of glyphosate with potential for integrated control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach. *Biological Control* 47(2): 154-158.
    See Biodiversity and Restoration Section.

45. Jayawardena, U.A., A.N. Navaratne, P.H. Amerasinghe, and R.S. Rajakaruna. 2011. Acute and chronic toxicity of four commonly used agricultural pesticides on the Asian common toad, *Bufo melanostictus* Schneider. *Journal of the National Science Foundation of Sri Lanka* 39(3): 267-276.
    Laboratory and field studies provide evidence that pesticides may play a role in population declines, range reductions and species extinctions of amphibians. The present study examined the acute and chronic toxicity of four commonly used agricultural pesticides, chlorpyrifos, dimethoate, glyphosate and propanil on the survival, growth and development of malformations in the Asian common toad, *Bufo melanostictus*, under laboratory conditions. The 48 hour LC50 values of the chemicals were within the Pesticide Area Network (PAN) specified limits, except for propanil, which was less than the PAN specified value. Acute exposure to high concentrations of propanil may have a high direct toxic effect on the Asian common toad. The survival of the tadpoles with chronic exposure to ecologically relevant doses of the four pesticides was significantly reduced (survival in chlorpyrifos 39%, dimethoate 41%, glyphosate 36% and propanil 40% in the highest concentration) than in the control group (93%). Exposed tadpoles took more time to metamorphose but were larger in size than the control tadpoles. They also developed malformations at high frequencies (chlorpyrifos 30%, dimethoate 25%, glyphosate 35% and propanil 15% in the highest concentration). Malformations were mainly axial, including kyphosis (hunched back) and scoliosis (curvature) while skin ulcers and oedemas were also observed. Severe limb malformations such as extra or missing limbs as reported for other species of amphibians exposed to pesticides were not observed in the Asian common toad. None of the tadpoles in the control group had any malformations. Glyphosate exposed metamorphs recorded the highest mortality and malformations at high concentrations (1.0 ppm). However, a profound toxic effect was observed in chlorpyrifos exposed group even at low concentrations (0.1 ppm). The study shows that exposure to commonly used agrochemicals poses serious risk to amphibians in Sri Lanka and highlights the importance of investigating the level of agricultural pesticides in freshwater ecosystems and their effect on non-target organisms. **(Sri Lanka)**

46. Jayawardena, U.A., R.S. Rajakaruna, A.N. Navaratne, and P.H. Amerasinghe. 2010. Toxicity of agrochemicals to common hourglass tree frog (*Polypedates cruciger*) in acute and chronic exposure. *International Journal of Agriculture and Biology* 12(5): 641-648.

Direct effect of four common agricultural pesticides viz., chlorpyrifos, dimethoate, glyphosate and propanil, on the survival, growth and development of malformations in common hourglass tree frog, *Polypedates cruciger* (Anura: Ranidae) was studied under laboratory conditions in acute and chronic exposure. Acute exposure to high concentrations was carried out to determine the LC_{50}. The 48 h LC_{50} of the pesticides were within the Pesticide Area Network specified limits, except for propanil. The percentage survival of the tadpoles under chronic exposure to ecologically relevant doses was lower (glyphosate 75%, dimethoate 77.5%, chlorpyrifos 80% & propanil 85%) than the control group (95.5%) and was significantly affected by the concentrations. Exposed tadpoles took more time to metamorphose and were significantly smaller in size than the control tadpoles. They also developed malformations at high frequencies (glyphosate = 69%, dimethoate = 64%, chlorpyrifos = 60%, propanil = 45%). Malformations were mainly kyphosis (hunched back), scoliosis (curvature), skin ulcers and edema. However, severe limb malformations were not observed in the study. Chlorpyrifos had a profound effect even at very low concentrations (0.05 ppm). This study provides the first empirical evidence of a comparative study on the effect of pesticides on an endemic amphibian species in Sri Lanka and underscores the importance of investigation the level of agricultural pesticides in freshwater ecosystems and their effect on non-target organisms. **(Sri Lanka)**

47. Jones, D.K., J.I. Hammond, J.I., and R.A. Relyea. 2011. Competitive stress can make the herbicide Roundup® more deadly to larval amphibians. *Environmental Toxicology and Chemistry* 30(2): 446-454.

Toxicity assessments on nontarget organisms have largely been addressed using short-term, single-species laboratory experiments. Although extremely helpful, these experiments inherently lack many pervasive ecological stressors found in nature. Though a substantial challenge, incorporating these ecological stressors in contaminant studies would shed light on potential synergistic effects. For the world's leading herbicide, glyphosate, we know little about how natural stressors affect the toxicity to nontarget organisms. To explore how the natural stress of competition might interact with a glyphosate-based herbicide, we used outdoor mesocosms containing three tadpole species that were exposed to a factorial combination of three glyphosate concentrations (0, 1, 2, or 3 mg acid equivalent (a.e.)/L of the commercial formulation Roundup Original MAX®) and three tadpole densities (low, medium, or high). We found that increased tadpole density caused declines in tadpole growth, but also made the herbicide significantly more lethal to one species. Whereas the median lethal concentration (LC50) values were similar across all densities for gray treefrogs (*Hyla versicolor*; 1.7–2.3 mg a.e./L) and green frogs (*Rana clamitans*; 2.2–2.6 mg a.e./L), the LC50 values for bullfrogs (*R. catesbeiana*) were 2.1 to 2.2 mg a.e./L at low and medium densities, but declined to 1.6 mg a.e./L at high densities. The large decrease in amphibian survival with increased herbicide concentration was associated with increases in periphyton abundance. We also found evidence that temperature stratification lead to herbicide stratification in the water column, confirming the results of a previous study and raising important questions about exposure risk in natural systems. **(U.S.A.)**

48. Kaiser, K. 2011. Preliminary study of pesticide drift into the Maya mountain protected areas of Belize. *Bulletin of Environmental Contamination and Toxicology* 86(1): 56-59.
    See Water Quality Section.

49. Kelly, D.W., R. Poulin, D.M. Tompkins, and C.R. Townsend. 2010. Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival. *Journal of Applied Ecology* 47(2): 498-504.
    See Fish Section.

50. Kilbride, K.M. and F.L. Paveglio. 2001. Long-term fate of glyphosate associated with repeated rodeo applications to control smooth cordgrass (*Spartina alterniflora*) in Willapa Bay, Washington. *Archives of Environmental Contamination and Toxicology* 40(2): 179-183.
    See Water Quality Section.

51. King, J.J. and R.S. Wagner. 2010. Toxic effects of the herbicide Roundup® Regular on Pacific northwestern amphibians. *Northwestern Naturalist* 91(3): 318-324.
    One of the most widely used herbicides for commercial and home use is glyphosate, the active ingredient in Roundup® Regular. We examined toxicity of the herbicide Roundup® on 6 amphibian species: *Ambystoma gracile, Ambystoma macrodactylum, Anaxyrus [Bufo] boreas, Pseudacris regula, Rana cascadae*, and *Rana luteiventris*. Larvae were exposed to 6 different Roundup® Regular treatments (0 (control), 0.1, 0.5, 1.0, 2.0, and 5.0 mg AI/L dilutions of glyphosate) and monitored for 16 d. Estimated acute lethal concentrations at 24 h (LC50) varied significantly among species (ANOVA, $F_{(3, 56)} = 3.54$, $p < 0.0202$), with concentrations ranging from 0.43 mg AI/L of Roundup® for *P. regilla* to 2.66 mg AI/L for *A. boreas*. Bufonid and ambystomatid larvae were less sensitive than Ranid and Pseudacrid species tested, with no salamander larval mortality occurring at 24 h. Mean time-to-death varied from 1 d for *P. regilla* to 8.3 d for *A. gracile*, respectively (ANOVA, $F_{(5, 971)} = 108$, $p < 0.0001$). For exposure times longer than 24 h, the *A. boreas* was not significantly different than the salamanders for time-to-death, based on Tukey-Kramer comparisons. Results suggest Roundup® Regular is highly toxic to the amphibians at levels below EPA standards for drinking water and at concentrations they may be exposed to during overspray. We recommend the use of less toxic glyphosate-based herbicides in aquatic systems, if applications are necessary, or made during times of year when amphibian larvae are not present. **(U.S.A.)**

52. Kjaer, J., P. Olsen, M. Ullum, and R. Grant. 2005. Leaching of glyphosate and amino-methylphosphonic acid from Danish agricultural field sites. *Journal of Environmental Quality* 34(2): 608-620.
    See Water Quality Section.

53. Kreutz, L.C., L.J.G. Barcellos, A. Marteninghe, E.D. dos Santos, and R. Zanatta. 2010. Exposure to sublethal concentration of glyphosate or atrazine-based

herbicides alters the phagocytic function and increases the susceptibility of silver catfish fingerlings (*Rhamdia quelen*) to *Aeromonas hydrophila* challenge. *Fish & Shellfish Immunology* 29(4): 694-697.

See Fish Section.

54. Lajmanovich, R.C., M.T. Sandoval, and P.M. Peltzer. 2003. Induction of mortality and malformation in *Scinax nasicus* tadpoles exposed to glyphosate formulations. *Bulletin of Environmental Contamination and Toxicology* 70(3): 612-618.

***** Possible causes of amphibian decline include ultraviolet radiation due to ozone depletion, climate change, virulent pathogens and/or toxic effects on larval amphibians of metals and chemicals in insecticides and herbicides. This study looks at induction of mortality and malformation in *Scinax nasicus,* a hylid found in Neotropcal regions specifically agricultural and urban settings, by formulations of glyphosate. Tadpoles were collected from a temporary pond in the Floodpain Paraná River, Argentina. The 96-h acute toxicity tests involved the herbicide GLYFOS® at five concentrations (3.07, 3.84, 4.8, 6 and 7.5 mg GLY-F/L) and were carried out on prometamorphic larvae. At 3.07 mg GL-F/L, tadpole mortality at 48-h was lower than controls but not significantly. Within the range of 3.84 to 7.5 mg GLY-F/L survival was lower at 48 h. An elevated mortality rate was detected at all concentrations at 96-h. The 96-h LC50 was 1.8 times lower than the 24-h LC50, indicating an increase of GLY-F toxicity when exposure time was prolonged. The 48-h LC50 for tadpoles treated in this study with GLY-F/L was3.62 mg GLY/L. Larval maldevelopment occurred in all tests and increased with time and GLY-F concentration. Malformations were minimal at 3.07 mg/L exposed for one day, whereas greater than 90% were malformed at a GLY-F level of 7.5 mg/L. Given the effects of GLY-F on tadpole morphology we suggest that its use in the proximity of temporary pond tadpole habitats should be regulated. **(Argentina)**

55. Lajmanovich, R.C., A.M. Attademo, P.M. Peltzer, C.M. Junges, and M.C. Cabagna. 2011. Toxicity of four herbicide formulations with glyphosate on *Rhinella arenarum* (Anura: Bufonidae) tadpoles: B-esterases and glutathione *S*-transferase inhibitors. *Archives of Environmental Contamination and Toxicology* 60(4): 681-689.

In this study, amphibian tadpoles *Rhinella arenarum* were exposed to different concentrations of Roundup Ultra-Max (ULT), Infosato (INF), Glifoglex, and C-K YUYOS FAV. Tadpoles were exposed to these commercial formulations with glyphosate (CF-GLY) at the following concentrations (acid equivalent [ae]): 0 (control), 1.85, 3.75, 7.5, 15, 30, 60, 120, and 240 mg ae/L for 6–48 h (short-term). Acetylcholinesterase (AChE), butyrylcholinesterase (BChE), carboxylesterase (CbE), and glutathione *S*-transferase (GST) activities were measured among tadpoles sampled from those treatments that displayed survival rates >85%. Forty-eight-hour LC_{50} for *R. arenarum* tadpoles exposed to CF-GLY in the static tests ranged from ULT = 2.42 to FAV = 77.52 mg ae/L. For all CF-GLY, the LC50 values stabilized at 24 h of exposure. Tadpoles exposed to all CF-GLY concentrations at 48 h showed decreases in the activities of AChE (control = 17.50 ± 2.23 nmol/min/mg/protein; maximum inhibition INF 30 mg ae/L, 71.52%), BChE (control = 6.31 ± 0.86 nmol/min/mg/protein; maximum inhibition INF 15 mg ae/L, 78.84%), CbE

(control = 4.39 ± 0.46 nmol/min/mg/protein; maximum inhibition INF 15 mg ae/L, 81.18%), and GST (control = 4.86 ± 0.49 nmol/min/mg/protein; maximum inhibition INF 1.87 mg ae/L, 86.12%). These results indicate that CF-GLY produce a wide range of toxicities and that all enzymatic parameters tested may be good early indicators of herbicide contamination in *R. arenarum* tadpoles. **(Argentina)**

56. Le, T.-H., E.-S. Lim, S.K. Lee, Y.-W. Choi, Y.-H. Kim, and J. Min. 2010. Effects of glyphosate and methidathion on the expression of the *Dhb, Vtg, Arnt, CYP4* and *CYP314* in *Daphnia magna. Chemosphere* 79(1): 67-71.

In this study, the expression of five stress responsive genes was quantified and analyzed using a semi-quantitative RT-PCR to study the changes in their expression in *Daphnia magna* after exposure to known pesticides, glyphosate and methidathion. Hemoglobin (*Dhb*), which was used to show the effect of the oxygen level in the aquatic system, was significantly expressed in *D. magna* after exposure to glyphosate and methidathion. Additionally, aryl hydrocarbon receptor nuclear translocator (*Arnt*), a gene related to the metabolism of aryl hydrocarbons, had lower expression levels in *D. magna* than within the control. *CYP4*, which was used among cytochrome P450s (*CYP*s) to show the effects on the fatty acid and steroids metabolisms, was down-regulated in *D. magna* exposed to glyphosate. However, methidathion affected the expression of *CYP314*, which was used to show effects of ecdysis, not *CYP4* in *D. magna*. Therefore, glyphosate and methidathion probably caused physiological effects with different patterns in *D. magna*, especially metabolisms related to *CYP*s. On the other hand, only vitellogenin (*Vtg*), which was responsive to the estrogenic potency, did not show any differences in *D. magna* after exposure to glyphosate or methidathion. **(South Korea)**

57. Lenkowski, J.R., G. Sanchez-Bravo, and K.A. McLaughlin. 2010. Low concentrations of atrazine, glyphosate, 2,4-dichlorophenoxyacetic acid, and triadimefon exposures have diverse effects on *Xenopus laevis* organ morphogenesis. *Journal of Environmental Sciences* 22(9): 1305-1308.

Many chemicals are released into the environment, and chemical contamination has been suggested as a contributing factor to amphibian declines. To add to a growing body of knowledge about the impact of individual chemicals on non-target organisms, we examined the specificity of deformities induced by exposure to four pesticides (atrazine, 2,4-dichloropheoxyacetic acid (2,4-D), triadimefon, and glyphosate) in the model amphibian species, *Xenopus laevis*. We focused on the period of organ morphogenesis, as it is frequently found to be particularly sensitive to chemical exposure yet also commonly overlooked. We found similar levels of intestine malformations and edemas, as well as disruption of skeletal muscle, in atrazine and triadimefon exposed tadpoles. The effects of 2,4-D were only apparent at the highest concentrations we examined; glyphosate did not induce dramatic malformations at the concentrations tested. While researchers have shown that it is important to understand how chemical mixtures affect non-target organisms, our results suggest that it is first crucial to determine how these chemicals act independently in order to be able to identify consequences of individual pesticide exposure. **(U.S.A.)**

58. Linz, G.M. and H.J. Homan. Use of glyphosate for managing invasive cattail (*Typha* spp.) to disperse blackbird (Icteridae) roosts. *Crop Protection* 30(2): 98-104.
See Biodiversity and Restoration Section.

59. Lipok, J., H. Studnik, and S. Gruyaert. 2010. The toxicity of Roundup® 360 SL formulation and its main constituents: Glyphosate and isopropylamine towards non-target water photoautotrophs. *Ecotoxicology and Environmental Safety* 73(7): 1681-1688.
The toxicity of commercial formulation of Roundup® 360 SL, widely used, nonselective herbicide and its main constituents, glyphosate (PMG), equimolar (1:1) isopropylamine salt of glyphosate (GIPA) and isopropylamine (IPA) was examined towards eight aquatic microphotoautotrophs; seven cyanobacterial strains representing either saline or freshwater communities, and common eukaryotic algae *Chlorella vulgaris* Beijerinck. Autotrophs were cultured 21 days in their appropriate standard media supplemented with various amounts of Roundup®, glyphosate, GIPA and IPA. The determination of the growth of examined photoautotrophs was performed by time-course measurements of total chlorophyll content in experimental cultures. The growth rates related to corresponding concentrations of chemicals, the EC_{50} values and generation doubling time were determined in order to present the toxicity Roundup® 360 SL formulation and its main constituents. Market available formulation of Roundup® was found to possess toxicity significantly higher than this, attributed to its main constituents; however both these compounds, isopropylamine and glyphosate, also inhibited the growth of examined strains in a dose-dependent manner. Notably, the interpretation of toxicity of the examined substances was found to be significantly dependent on the method of EC_{50} calculation. The choice of molar or weight concentration of substances tested separately and in specific formulation was found to be essential in this matter. Due to these findings the EC_{50} values were calculated based either on molar or on weight concentrations. Considering Roundup® 360 SL formulation, these values ranged from 10^{-3} up to 10^{-1} mM and they were one order of magnitude lower than those found for isopropylamine. Quite surprisingly the minimum EC_{50} values found for glyphosate did not reach micromolar concentrations, whereas most of the EC_{50} values revealed to IPA did not exceed this range. Notably, in all the cases except for *Synechocystis aquatilis* Sauvageau, isopropylamine alone was indicated as more toxic than glyphosate. **(Poland)**

60. Lynch, J.D. and S.B. Arroyo. 2009. Risks to Colombian amphibian fauna from cultivation of coca (*Erythroxylum coca*): a geographical analysis. *Journal of Toxicology and Environmental Health* Part A 72(15): 974-985.
The Colombian amphibian fauna is among the richest known in the world, with about 20 species of salamanders (order Caudata), 35 of the limbless caecilians (order Gymnophiona), and more than 700 species of frogs and toads (order Anura) recorded from localities within the country. The potential effects of exposure to glyphosate on amphibians arising from production of illegal crops (coca) were examined. The analysis was based on (1) behavior and ecology of species and (2) proximities of actual museum records to localities in which illegal crops are being

grown and the subset of those that have been sprayed with glyphosate. Based on data on the location of amphibians collected in Colombia, records were obtained for 193 species (28% of the national diversity) of frogs and toads found in localities within 10 km of areas where coca is grown. Further analyses with ARC MAP software allowed for measurement of the direct distance separating collection locations for frogs, known coca fields, and areas where aerial spraying was being conducted. Records in or near coca fields included data for 11 of 13 families of frogs and toads known to be present in Colombia. Only Ceratophryidae and Pipidae were not reported from these locations and appear not to be at risk. For eight species (*Dendrobates truncatus, Craugastor raniformis, Pristimantis gaigeae, Smilisca phaeota, Elachistocleis ovale, Hypsiboas crepitans, Trachycephalus venulosus*, and *Pseudis paradoxa*) selected to represent several habitat preferences and life-cycle strategies, large areas of their distributions lie outside coca production regions and their populations as a whole are at low risk. For a limited number of species that barely enter Colombian territory, the consequences of coca production may be more serious and may have placed several species of frogs at risk. These include *Ameerega bilingua, Dendropsophus bifurcus, Pristimantis colomai, P. degener, P. diadematus, P. quaquaversus, P. variabilis*, and *Trachycephalus jordani*. Other species may also be at risk but exact numbers are unknown since few investigations were undertaken in these areas during the past 30 yr. The main ranges for these species were assumed to be in Ecuador. **(Colombia)**

61. Mann R.M., J.R. Bidwell, and M.J. Tyler. 2003. Toxicity of herbicide formulations to frogs and the implications for product registration: a case study from Western Australia. *Applied Herpetology* 1(1-2): 13-22.

Growing concern about the decline of amphibian populations has highlighted the need to assess the potential impact of agricultural chemicals on these animals. Although the relative sensitivity of amphibians to the toxic effects of pesticides and other environmental contaminants has yet to be established, the perceived vulnerability of amphibians to pesticide effects may actually be attributable to their specific habitat requirements. Shallow temporary ponds, essential to the life cycles of many amphibians, are also areas where pollutants may accumulate without substantial dilution. Research in Western Australia has highlighted the potential risk that agricultural chemicals may pose to fauna that inhabit low dilution environments, and indicates that the data currently required for pre-registration assessment of pesticides may be inadequate to effectively protect these environments. **(Australia)**

62. Mann, R.M., R.V. Hyne, C.B. Choung, and S.P. Wilson. 2009. Amphibians and agricultural chemicals: review of the risks in a complex environment. *Environmental Pollution* 157(11): 2903-2927.

Agricultural landscapes, although often highly altered in nature, provide habitat for many species of amphibian. However, the persistence and health of amphibian populations are likely to be compromised by the escalating use of pesticides and other agricultural chemicals. This review examines some of the issues relating to exposure of amphibian populations to these chemicals and places emphasis on mechanisms of toxicity. Several mechanisms are highlighted, including those that

may disrupt thyroid activity, retinoid pathways, and sexual differentiation. Special emphasis is also placed on the various interactions that may occur between different agro-chemicals and between chemicals and other environmental factors. We also examine the indirect effects on amphibian populations that occur when their surrounding pond communities are altered by chemicals. **(Australia)**

63. McComb, B.C., L. Curtis, C.L. Chambers, M. Newton, and K. Bentson. 2008. Acute toxic hazard evaluations of glyphosate herbicide on terrestrial vertebrates of the Oregon coast range. *Environmental Science and Pollution Research* 15(3): 266-272.

Comparisons of acute toxicity of glyphosate were made with intraperitoneal dosings of technical glyphosate isopropylamine salt to nine species of terrestrial vertebrates (five amphibians, four mammals) and compared with responses in Swiss-Webster laboratory mice. Animals collected from sites that had no recent herbicide application were allowed 7–14 days to equilibrate in captivity before treatment. Median lethal dose ranged from 800 to 1,340 mg kg^{-1} in mammals, and 1,170 to >2,000 mg kg^{-1} in amphibians, with Oregon vole being the most sensitive. White lab mice were in the middle of the mammalian range. Tailed frog, at >2,000 mg $kg^{-1}$was the least sensitive. Calibration of IP sensitivity to oral administration by gavage indicated that roughly four times as much glyphosate must be administered to obtain a comparable estimate of lethality. Administration by gavage in highly concentrated solutions tended to cause physical injury, hence may prove less useful as a relative indicator of toxicity. When sublethal dosages were given to roughskin newts or chipmunks, mobility and use of cover appeared largely unaffected.Direct toxic effects of spraying glyphosate under normal forest management seem unlikely for the nine species examined. Nor could we detect significant indirect effects of exposure on behavior and use of cover features in two species. There may be effects on other aspects of the field biology of these animals, such as reproductive rates, which we did not investigate. Recent field data indicate that changes in habitat quality following herbicide application can result in high reproductive activity in species associated with the grasses and forbs that proliferate following field applications.When compared to field data on body burdens of wild mammals exposed after aerial application of glyphosate at maximum rates in forests, there seems to be a large margin of safety between dosages encountered and those causing either death or limitation of movement, foraging or shelter.Margins of safety for small mammals and amphibians appear to be large under any probable exposure scenarios, however our results indicate high variability in responses among species. Uncertainty introduced into field studies from unknown sources of mortality (e.g, likely predation) must be considered when interpreting our results. **(U.S.A.)**

64. McCoy, K.A., L.J. Bortnick, C.M. Campbell, H.J. Hamlin, L.J. Guillette, C.M. St. Mary. 2008. Agriculture alters gonadal form and function in the toad *Bufo marinus*. *Environmental Health Perspectives* 116(11): 1526-1532.

Background: Many agricultural contaminants disrupt endocrine systems of wildlife. However, evidence of endocrine disruption in wild amphibians living in agricultural areas has been controversial. Typically, studies on the effects of pollutants on wildlife attempt to compare polluted with unpolluted sites. Objectives:

We took a novel approach to address this question by explicitly quantifying the relationship between gonadal abnormalities and habitats characterized by differing degrees of agricultural activity. Methods: We quantified the occurrence of gonadal abnormalities and measures of gonadal function in at least 20 giant toads (*Bufo marinus*) from each of five sites that occur along a gradient of increasing agricultural land use from 0 to 97%. Results: The number of abnormalities and frequency of intersex gonads increased with agriculture in a dose-dependent fashion. These gonadal abnormalities were associated with altered gonadal function. Testosterone, but not 17β-estradiol, concentrations were altered and secondary sexual traits were either feminized (increased skin mottling) or demasculinized (reduced forearm width and nuptial pad number) in intersex toads. Based on the end points we examined, female morphology and physiology did not differ across sites. However, males from agricultural areas had hormone concentrations and secondary sexual traits that were intermediate between intersex toads and non-agricultural male toads. Skin coloration at the most agricultural site was not sexually dimorphic; males had female coloration. Conclusions: Steroid hormone concentrations and secondary sexual traits correlate with reproductive activity and success, so affected toads likely have reduced reproductive success. These reproductive abnormalities could certainly contribute to amphibian population declines occurring in areas exposed to agricultural contaminants. **(U.S.A.)**

65. Moore, L.J., L. Fuentes, J.H. Rodgers, W.W. Bowerman, G.K. Yarrow, W.Y. Chao, and W.C. Bridges. 2011. Relative toxicity of the components of the original formulation of Roundup® to five North American anurans. *Ecotoxicology and Environmental Safety* 78: 128-133.

The responses of five North American frog species that were exposed in an aqueous system to the original formulation of Roundup® were compared. Carefully designed and un-confounded laboratory toxicity tests are crucial for accurate assessment of potential risks from the original formulation of Roundup® to North American amphibians in aquatic environments. The formulated mixture of this herbicide as well as its components, isopropylamine (IPA) salt of glyphosate and the surfactant MON 0818 (containing polyethoxylated tallowamine (POEA)) were separately tested in 96h acute toxicity tests with Gosner stage 25 larval anurans. *Rana pipiens, R. clamitans, R. catesbeiana, Bufo fowleri*, and *Hyla chrysoscelis* were reared from egg masses and exposed to a series of 11 concentrations of the original formulation of Roundup ® herbicide, nine concentrations of MON 0818 and three concentrations of IPA salt of glyphosate in static (non-renewal) aqueous laboratory tests. LC50 values are expressed as glyphosate acid equivalents (ae) or as mg/L for MON 0818 concentrations for comparison between the formulation and components. *R. pipiens* was the most sensitive of five species with 96h-LC50 values for formulation tests, for the five species, ranging from 1.80 to 4.22 mg ae/L, and MON 0818 exposures with 96h-LC50 values ranging from 0.68 to 1.32 mg/L. No significant mortality was observed during exposures of 96 h for any of the five species exposed to glyphosate IPA salt at concentrations up to 100 times the predicted environmental concentration (PEC). These results agree with previous studies which have noted that the surfactant MON 0818 containing POEA

contributes the majority of the toxicity to the herbicide formulations for fish, aquatic invertebrates, and amphibians. These study results suggest that anurans are among the most sensitive species, and emphasize the importance of testing the herbicide formulation in addition to its separate components to accurately characterize the toxicity and potential risk of the formulation. **(U.S.A.)**

66. Morneault, A.E., B.J. Naylor, L.S. Schaeffer, and D.C. Othmer. 2004. The effect of shelterwood harvesting and site preparation on eastern red-backed salamanders in white pine stands. *Forest Ecology and Management* 199(1): 1-10.

We studied the effects of the regeneration cut of the shelterwood system and four site preparation options on populations of eastern red-backed salamanders in 90-100-year-old white pine forests in central Ontario, Canada. We established the study in 1994 using a randomized complete block design with three replicates and five treatments: (1) no harvest, no site preparation; (2) harvest. no site preparation; (3) harvest, mechanical site preparation; (4) harvest, chemical site preparation; (5) harvest, mechanical and chemical site preparation. We applied harvest and site preparation treatments from fall 1995 to fall 1997. We collected pre-treatment data in spring and summer of 1995 and post-treatment data from 1998 to 2002. We monitored salamander abundance using a grid of 20 cover boards surveyed 10 times per year within each of the 15 treatment plots. We also quantified changes in overstory and understory cover, supply of downed woody debris, and disturbance to the forest floor. Our data suggest that shelterwood cutting and site preparation can have immediate negative effects on the abundance of red-backed salamander populations in pine forest. However, effects are relatively short lived (<5 years). Changes in abundance appeared to be related to overstory and understory cover, and forest floor disturbance. **(Canada)**

67. Ortiz-Santaliestra, M.E., M.J. Fernández-Benéitez, M. Lizana, and A. Marco. 2011. Influence of a combination of agricultural chemicals on embryos of the endangered gold-striped salamander (*Chioglossa lusitanica*). *Archives of Environmental Contamination and Toxicology* 60(4): 672-680.

Pollution from agrochemicals may be contributing to the global decline of amphibian populations. Environmentally relevant concentrations of a fertiliser, ammonium nitrate, and a commercial formulation of the herbicide glyphosate Roundup Plus were tested on the embryonic development of *Chioglossa lusitanica*. This study introduces new data at three different levels. First, we provide previously unknown information about hatchling traits of *C. lusitanica*. Second, we present the first ecotoxicological study of this endangered species, to which environmental pollution is considered a major threat. Third, we conduct the first experiment with an amphibian species exposed to a mixture of a glyphosate-based herbicide and a nitrogenous fertiliser. Control individuals hatched with an average (±SD) total length of 18.77 (±2.02) mm and at an average Harrison's developmental stage of 44.58 (±1.24). Mean hatching time among controls was 11.52 (±1.29) weeks. None of the chemicals or their interaction produced lethal effects; however, a significant interaction was found when analysing total length at hatching. Individuals exposed to the herbicide hatched at a larger size than controls, and this effect was especially

clear when the fertiliser was added to the water. The absence of pollutant-related mortality or severe sublethal effects is in agreement with most studies indicating a high tolerance of amphibian embryos to agrochemicals. However, further research considering other life stages and additional natural factors (i.e., predators, food availability) is needed to estimate the ecological impact of chemical mixtures on *C. lusitanica*. **(Spain)**

68. Paganelli, A., V. Gnazzo, H. Acosta, S.L. Lopez, and A.E. Carrasco. 2010. Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. *Chemical Research in Toxicology* 23(10): 1586-1595.

The broad spectrum herbicide glyphosate is widely used in agriculture worldwide. There has been ongoing controversy regarding the possible adverse effects of glyphosate on the environment and on human health. Reports of neural defects and craniofacial malformations from regions where glyphosate-based herbicides (GBH) are used led us to undertake an embryological approach to explore the effects of low doses of glyphosate in development. *Xenopus laevis* embryos were incubated with 1/5000 dilutions of a commercial GBH. The treated embryos were highly abnormal with marked alterations in cephalic and neural crest development and shortening of the anterior-posterior (A-P) axis. Alterations on neural crest markers were later correlated with deformities in the cranial cartilages at tadpole stages. Embryos injected with pure glyphosate showed very similar phenotypes. Moreover, GBH produced similar effects in chicken embryos, showing a gradual loss of rhombomere domains, reduction of the optic vesicles, and microcephaly. This suggests that glyphosate itself was responsible for the phenotypes observed, rather than a surfactant or other component of the commercial formulation. A reporter gene assay revealed that GBH treatment increased endogenous retinoic acid (RA) activity in *Xenopus* embryos and cotreatment with a RA antagonist rescued the teratogenic effects of the GBH. Therefore, we conclude that the phenotypes produced by GBH are mainly a consequence of the increase of endogenous retinoid activity. This is consistent with the decrease of Sonic hedgehog (Shh) signaling from the embryonic dorsal midline, with the inhibition of *otx2* expression and with the disruption of cephalic neural crest development. The direct effect of glyphosate on early mechanisms of morphogenesis in vertebrate embryos opens concerns about the clinical findings from human offspring in populations exposed to GBH in agricultural fields. **(Argentina)**

69. Peltzer, P.M., R.C. Lajmanovich, J.C. Sánchez-Hernandez, M.C. Cabagna, A.M. Attademo, and A. Bassó. 2008. Effects of agricultural pond eutrophication on survival and health status of *Scinax nasicus* tadpoles. *Ecotoxicology and Environmental Safety* 70(1): 185-197.

To test the hypothesis that eutrophication ponds modulate some aspects of the health responses and survival of anuran tadpoles, we conducted field experiments using *Scinax nasicus* as sentinel organism to evaluate the quality of two ponds filled with agricultural runoff in a dominant agricultural landscape of Mid-Western Entre Ríos Province (Argentina). The survival, growth and development rates, erythrocytes

nuclei aberrations, parasite infection, and brain cholinesterase activity were monitored after seven days of exposure. Water samples from the ponds were also analyzed for physico-chemical variables and levels of pesticide residues. Residues of organochlorine pesticides and nutrients were higher in the agricultural ponds with respect to those from the control pond. We suggest that the interactions among washed-off nutrients and pesticides from agriculture and environmental factors account for deleterious effects on *S. nasicus* survival, growth and development rate, thereby compromising their health status. These effects can lead, in turn, to an increase in tadpole vulnerability to opportunistic parasites, erythrocytes nuclei aberrations or hemolysis. **(Argentina)**

70. Peltzer, P.M., R.C. Lajmanovich, L.C. Sanchez, A.M. Attademo, C.M. Junges, C.L. Bionda, A.L. Martino, and A. Bassó. 2011. Morphological abnormalities in amphibian populations from the mid-eastern region of Argentina. *Herpetological Conservation and Biology* 6(3): 432-442.

We present the first compilation and analysis of cases of morphologically abnormal anurans from the mid-eastern region of Argentina (Cordoba, Santa Fe, and Entre Rios Provinces). We sampled for abnormal individuals at 51 sites in agricultural, suburban, and forest settings between January 2000 and December 2009. We recorded 71 abnormal individuals, including 16 types of abnormalities in 15 anuran species. In agricultural sites, we found 12 types of abnormalities, with ectromelia being the most abundant. In suburban sites, we recorded seven types of abnormalities, with brachygnathia and ectromelia being the most common. In forest sites, we found three types of abnormalities, with ectromelia again being the most abundant. This study is the first catalog of anurans with morphological abnormalities in Argentina, thus expanding the geographic range of observed abnormalities in amphibians and illustrating the ubiquity of this phenomenon. **(Argentina)**

71. Pérez, G.L., A. Torremorell, H. Mugni, P. Rodríguez, M.S. Vera, M. do Nascimento, L. Allende, J. Bustingorry, R. Escaray, M. Ferraro, I. Izaguirre, H. Pizarro, C. Bonetto, D.P. Morris, and H. Zagarese. 2007. Effects of the herbicide Roundup on freshwater microbial communities: a mesocosm study. *Ecological Applications* 17(8): 2310-2322.

The impact of the widely used herbicide glyphosate has been mainly studied in terrestrial weed control, laboratory bioassays, and field studies focusing on invertebrates, amphibians, and fishes. Despite the importance of phytoplankton and periphyton communities at the base of the aquatic food webs, fewer studies have investigated the effects of glyphosate on freshwater microbial assemblages. We assessed the effect of the commercial formulation Roundup using artificial earthen mesocosms. The herbicide was added at three doses: a control (without Roundup) and two treatments of 6 and 12 mg/L of the active ingredient (glyphosate). Estimates of the dissipation rate (*k*) were similar in the two treatments (half-lives of 5.77 and 7.37 d, respectively). The only two physicochemical parameters showing statistically significant differences between treatments and controls were the downward vertical spectral attenuation coefficient $k_d(\ )$, where is wavelength, and total phosphorus concentration (TP). At the end of the experiment, the treated mesocosms showed a

significant increase in the ratio k_d(490 nm)/k_d(550 nm) and an eightfold increase in TP. Roundup affected the structure of phytoplankton and periphyton assemblages. Total micro- and nano-phytoplankton decreased in abundance in treated mesocosms. In contrast, the abundance of picocyanobacteria increased by a factor of about 40. Primary production also increased in treated mesocosms (roughly by a factor of two). Similar patterns were observed in the periphytic assemblages, which showed an increased proportion of dead: live individuals and increased abundances of cyanobacteria (about 4.5-fold). Interestingly, the observed changes in the microbial assemblages were captured by the analysis of the pigment composition of the phytoplankton, the phytoplankton absorption spectra, and the analysis of the optical properties of the water. The observed changes in the structure of the microbial assemblages are more consistent with a direct toxicological effect of glyphosate rather than an indirect effect mediated by phosphorus enrichment. **(Argentina)**

72. Peterson, R.K., and A.G. Hulting. 2004. A comparative ecological risk assessment for herbicides used on spring wheat: the effect of glyphosate when used within a glyphosate-tolerant wheat system. *Weed Science* 52: 834-844.

See Birds Section.

73. Piha, H., M. Pekkonen, and J. Merilä. 2006. Morphological abnormalities in amphibians in agricultural habitats: a case study of the common frog *Rana temporaria*. *Copeia* 2006(4): 810-817.

Recent studies suggest that the incidence of morphological abnormalities has increased in many amphibian populations, often exceeding the estimated background deformity frequency of 0-5%. Many chemical contaminants, including agrochemicals, can cause abnormalities in amphibians, but data on the occurrence of morphological abnormalities in wild amphibian populations in Europe is anecdotal at best. In a large scale study covering 264 ha and 26 farmland breeding populations of the Common frog (*Rana temporaria*) in southern Finland, we investigated whether the incidence of morphological abnormalities in metamorphs differed from the background level of 0-5% and among populations along an agrochemical gradient. Abnormalities occurred in a low frequency (1% of the studied individuals; 40/4115), the highest population-specific frequency being 4%. We found no evidence for increased abnormality frequencies in the habitats most likely exposed to agrochemicals. Hence, the data suggest that current Finnish agrochemical practices are not causing increased incidences of morphological abnormalities in Common frog populations breeding in farmland areas. **(Finland)**

74. Poletta, G.L., A. Larriera, E. Kleinsorge, and M.D. Mudry. 2009. Genotoxicity of the herbicide formulation Roundup® (glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and the Micronucleus test. *Mutation Research* 672(2): 95-102.

The genotoxicity of pesticides is an issue of worldwide concern. The present study was undertaken to evaluate the genotoxic potential of a widely used herbicide formulation, Roundup® (glyphosate), in erythrocytes of broad-snouted caiman

(*Caiman latirostris*) after *in ovo* exposure. Caiman embryos were exposed at early embryonic stage to different sub-lethal concentrations of Roundup® (50, 100, 200, 300, 400,500,750,1000,1250 and 1750 µg/egg). At time of hatching, blood samples were obtained from each animal and two short-term tests, the Comet assay and the Micronucleus (MN) test, were performed on erythrocytes to assess DNA damage. A significant increase in DNA damage was observed at a concentration of 500 µg/egg or higher, compared to untreated control animals ($p < 0.05$). Results from both the Comet assay and the MN test revealed a concentration-dependent effect. This study demonstrated adverse effects of Roundup® oil DNA of *C. latirostris* and confirmed that the Cornet assay and the MN test applied on caiman erythrocytes are useful tools in determining potential genotoxicity of pesticides. The identification of sentinel species as well as sensitive biomarkers among the natural biota is imperative to thoroughly evaluate genetic damage, which has significant consequences for short- and long-term survival of the natural species. **(Argentina)**

75. Puértolas, L., J. Damásio, C. Barata, A.M.V.M. Soares, and N. Prat. 2010. Evaluation of side-effects of glyphosate mediated control of giant reed (*Arundo donax*) on the structure and function of a nearby Mediterranean river ecosystem. *Environmental Research* 110(6): 556-564.

    The aim of this study was to evaluate the effect of the application of the herbicide Herbolex (Aragonesas Agro, S.A., Madrid, Spain) to control giant reed (*Arundo donax*), which has glyphosate as active ingredient, on the structure and function of a nearby river ecosystem. Specifically, we assessed glyphosate environmental fate in the surrounding water and its effects on transplanted *Daphnia magna*, field collected caddisfly (*Hydropsyche exocellata*) and on benthic macroinvertebrate structure assemblages. Investigations were conducted in the industrialized and urbanized Mediterranean river Llobregat (NE Spain) before and after a terrestrial spray of glyphosate. Four locations were selected to include an upstream site and three affected ones. Measured glyphosate levels in river water following herbicide application were quite high (20-60 µg/l) with peak values of 137 µg/l after three days. After 12 days of its application, leaching of glyphosate from sprayed riverbanks was quite high in pore water (20-85 µg/l) but not in the river. Closely linked with the measured poor habitat and water physico-chemical conditions, macroinvertebrate communities were dominated by taxa tolerant to pollution and herbicide application did not affect the abundance or number of taxa in any location. Nevertheless, significant specific toxic effects on transplanted *D. magna* and field collected *H. exocellata* were observed. Effects included *D. magna* feeding inhibition and oxidative stress related responses such as increased antioxidant enzyme activities related with the metabolism of glutathione and increased levels of lipid peroxidation. These results emphasize the importance of combined chemical, ecological and specific biological responses to identify ecological effects of pesticides in the field. **(Spain)**

76. Puglis, H.J. and M.D. Boone. 2011. Effects of technical-grade active ingredient vs. commercial formulation of seven pesticides in the presence or absence of UV

radiation on survival of green frog tadpoles. *Archives of Environmental Contamination and Toxicology* 60(1): 145-155.

Commercial formulations of pesticides contain both active and other ingredients. In some instances, the other ingredients have detrimental effects on nontarget species. Other factors such as UV radiation and predator cues have been shown to modify the toxicity of pesticides. In a laboratory study we compared the effects of technical-grade active ingredients to commercial formulations of seven common pesticides in the presence or absence of UV radiation on the survival of *Rana clamitans* (green frog) tadpoles over 96 h. We found a significant difference in the survival of tadpoles in technical-grade active ingredients versus commercial formulations in all of the pesticides tested. We also found that either the presence or the absence of UV radiation affected the survival of tadpoles in five of the seven pesticides tested. These results suggest that there is a need to test the effects of both active ingredients and commercial formulations of pesticides and, also, to include relevant abiotic factors like UV radiation treatments in the testing of pesticides because they can have a dramatic impact on the toxicity of some chemicals. **(U.S.A.)**

77. Quaranta, A., V. Bellantuono, G. Cassano, and C. Lippe. 2009. Why amphibians are more sensitive than mammals to xenobiotics. *PloS ONE* 4(11): e7699 ISSN 1932-6203 doi:10.1371/journal.pone.0007699.

Dramatic declines in amphibian populations have been described all over the world since the 1980s. The evidence that the sensitivity to environmental threats is greater in amphibians than in mammals has been generally linked to the observation that amphibians are characterized by a rather permeable skin. Nevertheless, a numerical comparison of data of percutaneous (through the skin) passage between amphibians and mammals is lacking. Therefore, in this investigation we have measured the percutaneous passage of two test molecules (mannitol and antipyrine) and three heavily used herbicides (atrazine, paraquat and glyphosate) in the skin of the frog *Rana esculenta* (amphibians) and of the pig ear (mammals), by using the same experimental protocol and a simple apparatus which minimizes the edge effect, occurring when the tissue is clamped in the usually used experimental device.The percutaneous passage (P) of each substance is much greater in frog than in pig. LogP is linearly related to $\log K_{ow}$ (logarithm of the octanol-water partition coefficient). The measured P value of atrazine was about 134 times larger than that of glyphosate in frog skin, but only 12 times in pig ear skin. The FoD value (P_{frog}/P_{pig}) was 302 for atrazine, 120 for antipyrine, 66 for mannitol, 29 for paraquat, and 26 for glyphosate.The differences in structure and composition of the skin between amphibians and mammals are discussed. **(Italy)**

78. Quassinti, L., E. Maccari, O. Murri, and M. Bramucci. 2009. Effects of paraquat and glyphosate on steroidogenesis in gonads of the frog *Rana esculenta* in vitro. *Pesticide Biochemistry and Physiology* 93(2): 91- 95.

Herbicides are toxic for amphibians, influencing various developmental stages. in particular, paraquat and glyphosate have teratogenic effects on tadpole development, though little information is available on how they affect reproduction in

amphibians. In the present work, ovarian tissue and testis of the water frog *Rana esculenta* were incubated in vitro in presence of different concentrations of the two herbicides. 17 β-estradiol and testosterone levels were measured in the incubation medium by radioimmunoassay. The data showed that paraquat inhibited testosterone and 17 β-estradiol production, with the lowest-observed-effect concentration (LOEC) of 10^{-4}M, while paraquat inhibition of steroidogenesis in amphibian gonads was reversed by the presence of epigallocatechin gallate in the culture medium. These result indicates that paraquat acts on gonadal steridogenesis through a mechanism involving reactive oxygen species. Glyphosate showed no effect on gonadal steroidogenesis. These results suggest that paraquat may interfere in amphibian reproductive processes. **(Italy)**

79. Raffel, T.R., J.L. Sheingold, and J.R. Rohr. 2009. Lack of pesticide toxicity to *Echinostoma trivolvis* eggs and miracidia. *The Journal of Parasitology* 95(6): 1548-1551.

Pesticides can elevate trematode infections in amphibians. However, direct adverse effects of pesticides on embryos and free-living stages of trematodes have not been thoroughly explored, despite the potential for these effects to reduce amphibian trematode infections. We measured the effects of atrazine, glyphosate, carbaryl, and malathion on embryo and miracidium (free-living stage) survival of *Echinostoma trivolvis*, a common trematode of amphibians. We found no evidence of biologically relevant effects of these pesticides at ecologically relevant concentrations. **(U.S.A.)**

80. Relyea, R.A. 2004. Growth and survival of five amphibian species exposed to combinations of pesticides. *Environmental Toxicology and Chemistry* 23(7): 1737-1742.

The global decline of amphibians has sparked interest in the role that pesticides may play. Pesticides in nature typically exist in combinations, but given the vast number of chemicals used, most toxicological experiments necessarily have examined one pesticide at a time. I examined how four commercial formulations of pesticides (diazinon, carbaryl, malathion, and glyphosate) affected the survival and growth of five larval amphibian species (*Rana pipiens, R. clamitans, R. catesbeiana, Bufo americanus*, and *Hyla versicolor*) when alone (at 1 or 2 mg/L of active ingredient) and in pairwise combinations (1 mg/L of each pesticide). At 1 mg/L, the pesticides reduced survival in 5% of the 20 species-pesticide comparisons and reduced growth in 35% of the comparisons. At 2 mg/L, the pesticides had more widespread effects, reducing survival in 35% of the 20 species-pesticide comparisons and reducing growth in 70% of comparisons. Combined pesticides occasionally caused lower survival and growth than either pesticide alone, but the effects were never larger than the more deadly of the two pesticides alone at 2 mg/L. This suggests that the impact of combining these four pesticides is similar to that predicted by the total concentration of pesticides in the system. **(U.S.A.)**

81. Relyea, R.A. 2005. The lethal impacts of Roundup and predatory stress on six species of North American tadpoles. *Archives of Environmental Contamination and Toxicology* 48(3): 351-357.

The decline in amphibians across the globe has sparked a search for the causes, and recent evidence suggests a connection with pesticides. However, for most pesticides, tests on amphibians are rare and conducted only for short durations (1 to 4 days) and without natural stressors. Recent studies have discovered that the stress of predator cues in the water can make insecticides much more lethal to larval amphibians, but it is unknown whether this phenomenon can be generalized to other types of pesticides. Using six species of North American amphibian larvae (*Rana sylvatica, R. pipiens, R. clamitans, R. catesbeiana, Bufo americanus*, and *Hyla versicolor*), I examined the impact of a globally common herbicide (Roundup) on the survival of tadpoles for 16 days with and without the chemical cues emitted by predatory newts (*Notophthalmus viridescens*). $LC50_{16\text{-}d}$ estimates varied from 0.55 to 2.52 mg of active ingredient (AI)/L, which was considerably lower than the few previous studies using Roundup (1.5 to 15.5 mg AI/L). Moreover, in one of the six species tested (*R. sylvatica*), the addition of predatory stress made Roundup twice as lethal. This discovery suggests that synergistic interactions between predatory stress and pesticides may indeed be a generalizable phenomenon in amphibians that occurs with a wide variety of pesticides. **(U.S.A.)**

82. Relyea, R.A. 2005. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological Applications* 15(2): 618-627.

Pesticides constitute a major anthropogenic addition to natural communities. In aquatic communities, a great majority of pesticide impacts are determined from single-species experiments conducted under laboratory conditions. Although this is an essential protocol to rapidly identify the direct impacts of pesticides on organisms, it prevents an assessment of direct and indirect pesticide effects on organisms embedded in their natural ecological contexts. In this study, I examined the impact of four globally common pesticides (two insecticides, carbaryl [Sevin] and malathion; two herbicides, glyphosate [Roundup] and 2,4-D) on the biodiversity of aquatic communities containing algae and 25 species of animals.

Species richness was reduced by 15% with Sevin, 30% with malathion, and 22% with Roundup, whereas 2,4-D had no effect. Both insecticides reduced zooplankton diversity by eliminating cladocerans but not copepods (the latter increased in abundance). The insecticides also reduced the diversity and biomass of predatory insects and had an apparent indirect positive effect on several species of tadpoles, but had no effect on snails. The two herbicides had no effects on zooplankton, insect predators, or snails. Moreover, the herbicide 2,4-D had no effect on tadpoles. However, Roundup completely eliminated two species of tadpoles and nearly exterminated a third species, resulting in a 70% decline in the species richness of tadpoles. This study represents one of the most extensive experimental investigations of pesticide effects on aquatic communities and offers a comprehensive perspective on the impacts of pesticides when nontarget organisms are examined under ecologically relevant conditions. **(U.S.A.)**

83. Relyea, R.A. 2005. The lethal impact of Roundup on aquatic and terrestrial amphibians. *Ecological Applications* 15(4): 1118-1124.

The global decline in amphibian diversity has become an international environmental problem with a multitude of possible causes. There is evidence that pesticides may play a role, yet few pesticides have been tested on amphibians. For example, Roundup is a globally common herbicide that is conventionally thought to be nonlethal to amphibians. However, Roundup has been tested on few amphibian species, with existing tests conducted mostly under laboratory conditions and on larval amphibians. Recent laboratory studies have indicated that Roundup may be highly lethal to North American tadpoles, but we need to determine whether this effect occurs under more natural conditions and in post-metamorphic amphibians. I assembled communities of three species of North American tadpoles in outdoor pond mesocosms that contained different types of soil (which can absorb the pesticide) and applied Roundup as a direct overspray. After three weeks, Roundup killed 96–100% of larval amphibians (regardless of soil presence). I then exposed three species of juvenile (post-metamorphic) anurans to a direct overspray of Roundup in laboratory containers. After one day, Roundup killed 68–86% of juvenile amphibians. These results suggest that Roundup, a compound designed to kill plants, can cause extremely high rates of mortality to amphibians that could lead to population declines. **(U.S.A.)**

84. Relyea, R.A. 2006. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities – Response. *Ecological Applications* 16(5): 2027-2034.

In their attempt to discredit Relyea (2005a), Thompson et al. (2006) have made a number of missteps. The authors suggest that the application rate used was unusually high. In fact, the rate followed manufacturer recommendations and produced a concentration that was in line with estimated worst-case scenarios and many observed scenarios in ponds and wetlands. The authors offer a list of concentrations observed in nature that is largely composed of second-hand reports, irrelevant well water and stream data, and a subset of wetland data sampled at time points after the herbicide degraded. A complete list reveals that higher concentrations are often found in the environment. The authors propose a number of methodological flaws that are not only without support, but, in many cases, demonstrate a lack of knowledge of aquatic ecology. The authors draw upon past risk assessments that contain little or no amphibian data and also contradict their own published conclusions on the impact of Roundup on tadpoles. When we examine the entire collection of relevant concentration data and experimental data (including those of Thompson and colleagues), it becomes clear that Roundup formulations containing POEA can be highly lethal to tadpoles at environmentally relevant concentrations. It is also evident that we need much more data. There is a critical need to sample natural ponds and wetlands for Roundup at appropriate times across a variety of forest, agriculture, and residential settings. Further, there is a continued need to experimentally evaluate the impact of Roundup on amphibians under a range of experimental conditions. Prior to 2005, members of a single collaborative research group, often in collaboration with Monsanto, have been

involved in all of the risk assessments of Roundup (World Health Organization 1994, Giesy et al. 2000, Solomon and Thompson 2003) and a majority of the experiments (Perkins et al. 2000, Chen et al. 2004, Edginton et al. 2004, Thompson et al. 2004, 2006, Wojtaszek et al. 2004) with a few notable exceptions (Bidwell and Gorrie 1995, Mann and Bidwell 1999, Smith 2001, Lajmanovich et al. 2003). There is a growing movement for other laboratories to independently assess the impacts of Roundup (Howe et al. 2004, Relyea 2004b, 2005a, b, c, Relyea et al. 2005) and we need many more laboratories to conduct Roundup experiments to develop more accurate and relevant risk assessments. In doing so, we can replace unsubstantiated attacks with actual data and arrive at the truth. **(U.S.A.)**

85. Relyea, R.A. 2009. A cocktail of contaminants: how mixtures of pesticides at low concentrations affect aquatic communities. *Oecologia* 159(2): 363- 376.

The ubiquity of anthropogenic chemicals in nature poses a challenge to understanding how ecological communities are impacted by them. While we are rapidly gaining an understanding of how individual contaminants affect communities, communities are exposed to suites of contaminants yet investigations of the effects of diverse contaminant mixtures in aquatic communities are rare. I examined how a single application of five insecticides (malathion, carbaryl, chlorpyrifos, diazinon, and endosulfan) and five herbicides (glyphosate, atrazine, acetochlor, metolachlor, and 2,4-D) at low concentrations (2-16 p. p. b.) affected aquatic communities composed of zooplankton, phytoplankton, periphyton, and larval amphibians (gray tree frogs, Hyla versicolor, and leopard frogs, Rana pipiens). Using outdoor mesocosms, I examined each pesticide alone, a mix of insecticides, a mix of herbicides, and a mix of all ten pesticides. Individual pesticides had a wide range of direct and indirect effects on all trophic groups. For some taxa (i.e., zooplankton and algae), the impact of pesticide mixtures could largely be predicted from the impacts of individual pesticides; for other taxa (i.e., amphibians) it could not. For amphibians, there was an apparent direct toxic effect of endosulfan that caused 84% mortality of leopard frogs and an indirect effect induced by diazinon that caused 24% mortality of leopard frogs. When pesticides were combined, the mix of herbicides had no negative effects on the survival and metamorphosis of amphibians, but the mix of insecticides and the mix of all ten pesticides eliminated 99% of leopard frogs. Interestingly, these mixtures did not cause mortality in the gray tree frogs and, as a result, the gray tree frogs grew nearly twice as large due to reduced competition with leopard frogs. In short, wetland communities can be dramatically impacted by low concentrations of pesticides (both separate and combined) and these results offer important insights for the conservation of wetland communities. **(U.S.A.)**

86. Relyea, R.A. 2011. Amphibians are not ready for Roundup®. *Wildlife Ecotoxicology* 3: 267-300.

The herbicide glyphosate, sold under a variety of commercial names including Roundup® and Vision® , has long been viewed as an environmentally friendly herbicide. In the 1990s, however, after nearly 20 years of use, the first tests were conducted on the herbicide's effects on amphibians in Australia. The researchers found that the herbicide was moderately toxic to Australian amphibians. The leading

manufacturer of glyphosate-based herbicides, Monsanto, declared that the researchers were wrong. Nearly 10 years later, my research group began examining the effects of the herbicides on North American amphibians. Based on an extensive series of experiments, we demonstrated that glyphosate-based herbicides can be highly toxic to larval amphibians. Monsanto declared that we were also wrong. These experiments have formed the basis of a spirited debate between independent, academic researchers, and scientists that either work as consultants for Monsanto or have a vested interest in promoting the application of the herbicide to control undesirable plants in forests and agriculture. The debate also moved into unexpected arenas, including the use of glyphosate-based herbicides in the Colombian drug war in South America where a version of Roundup is being used to kill illegal coca plantations. In 2008, the US EPA completed a risk assessment for the effects of glyphosate-based herbicides on the endangered California red-legged frog (*Rana aurora draytonii*) and concluded that it could adversely affect the long-term persistence of the species. More recent data from Colombia have confirmed that the herbicides not only pose a risk to tadpoles in shallow wetlands, but that typical applications rates also can kill up to 30% of adult frogs. As one reflects over the past decade, it becomes clear that our understanding of the possible effects of glyphosate-based herbicides on amphibians has moved from a position of knowing very little and assuming no harm to a position of more precise understanding of which concentrations and conditions pose a serious risk. **(U.S.A.)**

87. Relyea, R.A. 2012. New effects of Roundup on amphibians: Predators reduce herbicide mortality; herbicides induce antipredator morphology. *Ecological Applications* 22(2): 634-647.

The use of pesticides is important for growing crops and protecting human health by reducing the prevalence of targeted pest species. However, less attention is given to the potential unintended effects on nontarget species, including taxonomic groups that are of current conservation concern. One issue raised in recent years is the potential for pesticides to become more lethal in the presence of predatory cues, a phenomenon observed thus far only in the laboratory. A second issue is whether pesticides can induce unintended trait changes in nontarget species, particularly trait changes that might mimic adaptive responses to natural environmental stressors. Using outdoor mesocosms, I created simple wetland communities containing leaf litter, algae, zooplankton, and three species of tadpoles (wood frogs [*Rana sylvatica* or *Lithobates sylvaticus*], leopard frogs [*R. pipiens* or *L. pipiens*], and American toads [*Bufo americanus* or *Anaxyrus americanus*]). I exposed the communities to a factorial combination of environmentally relevant herbicide concentrations (0, 1, 2, or 3 mg acid equivalents [a.e.]/L of Roundup Original MAX) crossed with three predator-cue treatments (no predators, adult newts [*Notophthalmus viridescens*], or larval dragonflies [*Anax junius*]). Without predator cues, mortality rates from Roundup were consistent with past studies. Combined with cues from the most risky predator (i.e., dragonflies), Roundup became less lethal (in direct contrast to past laboratory studies). This reduction in mortality was likely caused by the herbicide stratifying in the water column and predator cues scaring the tadpoles down to the benthos where herbicide concentrations were lower. Even more striking was the

discovery that Roundup induced morphological changes in the tadpoles. In wood frog and leopard frog tadpoles, Roundup induced relatively deeper tails in the same direction and of the same magnitude as the adaptive changes induced by dragonfly cues. To my knowledge, this is the first study to show that a pesticide can induce morphological changes in a vertebrate. Moreover, the data suggest that the herbicide might be activating the tadpoles' developmental pathways used for antipredator responses. Collectively, these discoveries suggest that the world's most widely applied herbicide may have much further-reaching effects on nontarget species than previous considered. **(U.S.A.)**

88. Relyea, R.A. and D.K. Jones. 2009. The toxicity of Roundup Original Max to 13 species of larval amphibians. *Environmental Toxicology and Chemistry* 28(9): 2004-2008.

With the increased use of glyphosate-based herbicides (marketed under several names, including Roundup and Vision), there has been a concomitant increased concern about the unintended impacts that particular formulations containing the popular surfactant polyethoxylated tallowamine (POEA) might have on amphibians. Published studies have examined a relatively small number of anuran species (primarily from Australia and eastern North America) and, surprisingly, no species of salamanders. Using a popular formulation of glyphosate (Roundup Original Max®), the goal of the present study was to conduct tests of lethal concentrations estimated to kill 50% of a population after 96 h ($LC50_{96\text{-}h}$) on a wider diversity of species from both eastern and western North America. Tests were conducted on nine species of stage 25, larval anurans from three families (*Ranidae: Rana pipiens, R. clamitans, R. sylvatica, R. catesbeiana, R. cascadae; Bufonidae: Bufo americanus, B. boreas;* and *Hylidae: Hyla versicolor, Pseudacris crucifer*) and four species of larval salamanders from two families (*Ambystomatidae: Ambystoma gracile*, *A. maculatum, A. laterale*; and Salamandridae: *Notophthalmus viridescens*). For the nine species of larval anurans, $LC50_{96\text{-}h}$ values ranged from 0.8- to 2.0-mg acid equivalents per liter with relatively little pattern in differential sensitivity among the species or families. The four species of larval salamanders were less sensitive than the anurans, with $LC50_{96\text{-}h}$ values ranging from 2.7- to 3.2-mg acid equivalents per liter and no substantial differences among the species of salamanders. This work substantially increases the available data on amphibian sensitivity to glyphosate formulations that include either POEA surfactants or the equally moderately to highly toxic surfactants of Roundup Original Max and should be useful for improving future risk assessments. **(U.S.A.)**

89. Relyea, R.A., N.M. Schoeppner, and J.T. Hoverman. 2005. Pesticides and amphibians: the importance of community context. *Ecological Applications* 15(4): 1125-1134.

The widespread application of pesticides has attracted the attention of ecologists as we struggle to understand the impacts of these chemicals on natural communities. While we have a large number of laboratory-based, single-species studies of pesticides, such studies can only examine direct effects. However, in natural communities, species can experience both direct and indirect effects. We

conducted an outdoor mesocosm experiment on aquatic communities containing three tadpole species (*Hyla versicolor, Bufo americanus*, and *Rana pipiens*), zooplankton, and algae. We then manipulated a factorial combination of predators (no predators; newts, *Notophthalmus viridescens*; and larval beetles, *Dytiscus* sp.) and pesticides (no pesticides, the insecticide malathion, and the herbicide Roundup). We found that Roundup (1.3 mg of active ingredient/L) had substantial direct negative effects on the tadpoles, reducing total tadpole survival and biomass by 40%. However, Roundup had no indirect effects on the amphibian community via predator survival or algal abundance. Malathion (0.3 mg/L) had few direct effects on the tadpoles. Malathion caused no indirect effects with one of the predators (red-spotted newts) but caused substantial positive effects on amphibians (a five-fold increase in total tadpole survival and biomass) due to the sensitivity of the predatory beetles to the insecticide. Thus, while high concentrations of malathion can directly kill larval anurans, more ecologically relevant concentrations can have large positive effects in mesocosms by removing predatory insects. These results make it clear that pesticides can have both direct and indirect effects in natural communities and that these effects critically depend upon the composition of the community. **(U.S.A.)**

90. Rohr, J.R., T.R. Raffel, S.K. Sessions, and P.J. Hudson. 2008. Understanding the net effects of pesticides on amphibian nematode infections. *Ecological Applications* 18(7): 1743-1753.

Anthropogenic factors can have simultaneous positive and negative effects on parasite transmission, and thus it is important to quantify their net effects on disease risk. Net effects will be a product of changes in the survival and traits (e.g., susceptibility, infectivity) of both hosts and parasites. In separate laboratory experiments, we exposed cercariae of the trematode *Echinostoma trivolvis*, and its first and second intermediate hosts, snails (*Planorbella trivolvis*) and green frog tadpoles (*Rana clamitans*), respectively, to one of four common pesticides (atrazine, glyphosate, carbaryl, and malathion) at standardized, ecologically relevant concentrations (201.0, 3700.0, 33.5, and 9.6 µg/L, respectively). We measured effects of pesticide exposure on six mechanisms important to this host–parasite interaction: (1) survival of *E. trivolvis* cercariae over 26 hours, (2) tadpole survival over two weeks, (3) snail survival over four weeks, (4) snail growth and fecundity, (5) cercarial infectivity, and (6) tadpole susceptibility to a fixed number of cercariae. Pesticides, in general, caused significantly greater mortality of *E. trivolvis* cercariae than did control treatments, but atrazine was the lone chemical to significantly reduce cercarial survival (LC50 value = 267 mg/L) and then only at concentrations greater than commonly found in aquatic ecosystems (≥200 µg/L). None of the pesticides significantly enhanced *E. trivolvis* virulence, decreased tadpole survival, or reduced snail survival, growth, or fecundity. Sublethal exposure of the cercariae to the pesticides (4 h) did not significantly affect trematode encystment in *R. clamitans.* In contrast, sublethal exposure of *R. clamitans* to each of the four pesticides increased their susceptibility as measured by the percentage of cercariae that encysted. The reduction in exposure to trematodes due to pesticide-induced cercarial mortality (a density-mediated effect) was smaller than the pesticide-induced increase in amphibian susceptibility (a trait-mediated effect), suggesting that the net

effect of exposure to environmentally realistic levels of pesticides will be to elevate amphibian trematode infections. These findings highlight the importance of elucidating the lethal and sublethal effects of anthropogenic factors on both hosts and parasites to understand the mechanisms underlying changes in parasite transmission and virulence, an approach that is especially needed for amphibians, a taxon experiencing global disease-related declines. **(U.S.A.)**

91. Schriks, M., M.B. Heringa, M.M.E. van der Kooi, P. de Voogt, and A.P. van Wezel. 2010. Toxicological relevance of emerging contaminants for drinking water quality. *Water Research* 44(2): 461-476.

See Water Quality Section.

92. Smith, G.R. 2001. Effects of acute exposure to a commercial formulation of glyphosate on the tadpoles of two species of anurans. *Bulletin of Environmental Contamination and Toxicology* 67(4): 483-488.

***** The acute toxicity of a commercial formulation of glyphosate (Kleeraway® Grass and Weed Killer RTU) to the tadpoles of the western chorus frog (*Pseudacris triseriata*) and plains leopard frog (*Rana blairi*) was investigated. The results suggest that both species may be at risk from exposure to commercial formulations of glyphosate. Even exposure to relatively low concentrations for a short period of time, as might be experienced in an accidental spill scenario or with an application to clear aquatic vegetation, appears to induce high mortality in these tadpole species. Mortality was slightly higher in western chorus frogs than in plains leopard frogs. Ontogenetic differences in the toxicity of this glyphosate formulation were also found. No effect of acute exposure on subsequent tadpole growth was found in either species. This conclusion is somewhat limited by the total or nearly total mortality in every exposure treatment except for the control and the 0.001 concentration. However, it appears that acute exposure to low levels of this commercial formulation does not affect future performance of these tadpoles. **(U.S.A.)**

93. Solomon, K.R. and D.G. Thompson. 2003. Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. *Journal of Toxicology and Environmental Health.* Part B 6(3): 289-324.

Although the herbicide glyphosate is most widely used in agriculture, some is used for the control of emergent aquatic weeds in ditches, wetlands, and margins of water bodies, largely as the formulation Rodeo. This article presents an ecological risk assessment (ERA) of glyphosate and some of the recommended surfactants as used in or near aquatic systems. Glyphosate does not bioaccumulate, biomagnify, or persist in a biologically available form in the environment. Its mechanism of action is specific to plants and it is relatively nontoxic to animals. As a commercial product, glyphosate may be formulated with surfactants that increased efficacy but, in some cases, are more toxic to aquatic organisms than the parent material. For this risk assessment, three model exposure scenarios--static or low-flow systems such as ponds, flowing waters such as streams, and systems subjected to tidal flows such as estuaries--were chosen and application rates from 1 to 8 kg glyphosate/ha were

modeled. Additional measured exposure data from several field studies were also used. As acute exposures are most likely to occur, acute toxicity data were used as effect measures for the purposes of risk assessment. Toxicity data were obtained from the literature and characterized using probabilistic techniques. Risk assessments based on estimated and measured concentrations of glyphosate that would result from its use for the control of undesirable plants in wetlands and over-water situations showed that the risk to aquatic organisms is negligible or small at application rates less than 4 kg/ha and only slightly greater at application rates of 8 kg/ha. Less is known about the environmental fate and toxicology of the surfactants commonly used in combination with the Rodeo formulation of glyphosate. The surfactants used for this purpose were judged not to be persistent nor bioaccumulative in the environment. Distributional analysis of measured deposition concentrations of LI 700, suggest that this surfactant presents an insignificant acute risk to aquatic organisms. Assuming similar applications rates, significant ecological effects would not be expected from the use of some other surfactants such as Induce or X-77. Risks from the use of glyphosate +MON 0818 (Roundup) were slightly greater than those from glyphosate and surfactants such as LI 700; however, in over-water uses, risks were still considered small. Similar small risks were observed for measured concentrations of glyphosate in surface waters resulting from aerial application of Vision (a formulation equivalent to Roundup) to forestry areas in Canada. Concentrations measured after ground application presented a greater risk, but the data were sparse and the assessment is more uncertain. **(Canada)**

94. Solomon, K.R., A. Anadón, G. Carrasquilla, A.L. Cerdeira, and J. Marshall, and L.-H. Sanin. 2007. Coca and poppy eradication in Colombia: environmental and human health assessment of aerially applied glyphosate. *Reviews of Environmental Contamination and Toxicology* 190: 43-125.

See Human Health Section.

95. Sparling, D.W., C. Matson, J. Bickham, and P. Doelling-Brown. 2006. Toxicity of glyphosate as Glypro® and LI700 to red-eared slider (*Trachemys scripta elegans*) embryos and early hatchlings. *Environmental Toxicology and Chemistry* 25(10): 2768-2774.

More than 8.2 billion ha of cropland, gardens, and forests are treated with the herbicide glyphosate each year. Whereas the toxicity of glyphosate and associated adjuvants has been measured in other vertebrates, few, if any, studies have looked at their effects in reptiles. In some instances, management of turtle habitat requires control of successional stages through application of herbicides. Adults and juvenile turtles may be exposed directly, whereas embryos may contact the chemicals through the soil. In the present study, we exposed eggs of red-eared sliders (*Trachemys scripta elegans*) to single applications of herbicide ranging from 0 to 11,206 ppm wet weight of glyphosate in Glypro® and 0 to 678 ppm of the surfactant, LI700. Hatching success at the highest concentration was significantly lower (73%) than in other treatments (80-100%). At hatch, turtles at the highest concentration weighed less than those at other concentrations. During a 14-d holding period, we observed dose-response relationships in the ability of hatchlings to right themselves

when turned on their backs. At the end of the holding period, hatchlings at the highest dose level were still lighter, and somatic indices were lower, than those in other treatments. Genetic damage, as measured by flow cytometry, increased with treatment concentration except for the highest dose. We conclude that because of the high concentrations needed to produce effects and the protection offered by several centimeters of soil or sediment, glyphosate with LI700 poses low levels of risk to red-eared slider embryos under normal field operations with regards to the endpoints measured in the present study. Carelessness in handling glyphosate or failure to follow label directions may produce adverse effects. There also is a risk that the health of turtle embryos may be affected in ways not measured in the present study. **(U.S.A.)**

96. Struger, J., D. Thompson, B. Staznik, P. Martin, T. McDaniel, and C. Marvin. 2008. Occurrence of glyphosate in surface waters of southern Ontario. *Bulletin of Environmental Contamination and Toxicology* 80(4): 378-384.
See Water Quality Section.

97. Swift, K. and F.W. Bell. 2011. What are the environmental consequences of using silviculturally effective forest vegetation management treatments? *The Forestry Chronicle* 87(2): 201-216.
See Biodiversity and Restoration Section.

98. Takahashi, M. 2007. Oviposition site selection: pesticide avoidance by gray treefrogs. *Environmental Toxicology and Chemistry* 26(7): 1476-1480.
Effects of glyphosate-formulated herbicides on nontarget organisms have received much recent attention. Although previous studies have explored the effects of pesticides on growth, development, and mortality of various amphibian species, no studies have tested the potential effects of herbicides on oviposition site selection by amphibians. Recent studies have found that a combination of pesticide and predatory cues lead to significantly increased mortality of tadpoles of several anuran species relative to that caused by pesticide alone. In the present study, I tested two hypotheses: First, adult gray treefrogs avoid oviposition sites based on the presence of glyphosate formulation (Roundup®). Second, pesticide avoidance is manifested to a greater degree when combined with predatory cues. In the spring of 2006, I conducted an outdoor experiment using artificial ponds by setting up four treatments: Predatory fish cue, Roundup (2.4 mg glyphosate acid equivalent [a.e.]/L), a combination of predatory fish cue + Roundup, and a control. This experiment was designed to assess oviposition site choice among the four treatments by gray treefrogs based on the number of eggs laid in each treatment. Gray treefrogs avoided oviposition in pools contaminated with fish cue and/ or Roundup and placed the significant majority of their eggs in control pools, which suggests that breeding adults may be able to prevent lethal exposure of herbicide to their offspring through oviposition site selection. The present study provided the first evidence that the concentration of herbicide that is expected to be found in the field potentially alters oviposition site choice by amphibians. However, the concentration of 2.4 mg a.e./L is

unlikely ubiquitous in nature. Thus, the further investigation of environmental relevancy of this finding is critical. **(U.S.A.)**

99. Tanguy, A., I. Boutet, J. Laroche, and D. Moraga. 2005. Molecular identification and expression study of differentially regulated genes in the Pacific oyster *Crassostrea gigas* in response to pesticide exposure. *FEBS Journal* 272(2): 390-403.

The effects of pesticide contamination on the metabolism of marine molluscs are poorly documented. We investigated the response of a marine bivalve, the Pacific oyster, *Crassostrea gigas*, using a suppression subtractive hybridization method to identify up- and down-regulated genes after a 30-day exposure period to herbicides (a cocktail of atrazine, diuron and isoproturon, and to the single herbicide glyphosate). A total of 137 unique differentially expressed gene sequences was identified, as well as their associated physiological process. The expression of 18 of these genes was analyzed by RT-PCR under laboratory experimental conditions. The metabolic functions they are associated with include xenobiotic detoxification, energy production, immune system response and transcription. This study provides a preliminary basis for studying the response of marine bivalves to long-term herbicide exposure in terms of regulated gene expression and characterizes new potential genetic markers of herbicide contamination. **(France)**

100. Thompson, D.G., K.R. Solomon, B.F. Wojtaszek, A.N. Edginton, and G.R. Stephenson. 2006. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological Applications* 16(5): 2022-2027.

In summary, given the atypical application rates, unrealistic high aqueous exposure concentrations, and design limitations of this experiment, we do not believe that the resultant data support Relyea's conclusion that Roundup, at current rates of application (i.e., as typically employed in major use sectors), is likely to be lethal to many species of amphibians. Moreover, such a conclusion is certainly not supported by evidence from directly pertinent studies conducted either by ourselves (Edginton et al. 2004, Thompson et al. 2004, Wojtaszek et al. 2004), other researchers (Chen et al. 2004, Howe et al. 2004) nor by several general scientific and regulatory reviews (Giesy et al. 2000, Solomon and Thompson 2003, U.S. Environmental Protection Agency 1993, World Health Organization 1994) all of which consistently conclude that glyphosate-based end-use products, used in accordance with label recommendations, do not pose unacceptable risk to non-target organisms in the environment. Finally, in both this paper and subsequent journal articles (Relyea 2004, Relyea 2005a, b, c) as well as in derivative mass media communications, the author repetitively links experimental results to the global amphibian decline phenomenon with the implication, intended or otherwise, of possible causality. We do not believe that this implication is scientifically defensible based on either Relyea's work per se, or with respect to the available scientific evidence in general. **(Canada)**

101. Thompson, D.G., B.F. Wojtaszek, B. Staznik, D.T. Chartrand, and G.R. Stephenson. 2004. Chemical and biomonitoring to assess potential acute effects of

Vision® herbicide on native amphibian larvae in forest wetlands. *Toxicology and Chemistry* 23(4): 843-849.

In conjunction with operational forest herbicide spray programs in Ontario, Canada, chemical and biological monitoring studies were conducted in 51 different wetlands to quantify the probability and magnitude of contamination by a glyphosate herbicide formulation (Vision®). Wetlands were classified as oversprayed, adjacent, or buffered in relation to the operational target spray blocks. Results show that vegetated buffers significantly mitigated against exposure and thus potential for acute effects. Aqueous concentrations of glyphosate in buffered wetlands were below analytical limits of quantitation (0.02 mg acid equivalent [a.e.]/L) in 14 of 16 cases, with mean concentration (0.03 ± 0.02 mg a.e./L) significantly ($p < 0.05$) less than that of either adjacent (0.18 ± 0.06 mg a.e./L) or oversprayed wetlands (0.33 ± 0.11 mg a.e./L). Biomonitoring with caged amphibian larvae showed no significant differences among mean mortality (48 h) of either *Rana pipiens* ($p = 0.194$) or *Rana clamitans* larvae ($p = 0.129$) exposed in situ to Vision under these various wetland conditions. Percent mortality was not significantly ($p = 0.05$) correlated with exposure concentrations for either amphibian species tested. Results suggest that exposures typically occurring in forest wetlands are insufficient to induce significant acute mortality in native amphibian larvae. **(Canada)**

102. Trumbo, J. 2005. An assessment of the hazard of a mixture of the herbicide Rodeo® and the non-ionic surfactant R-11® to aquatic invertebrates and larval amphibians. *California Fish and Game* 91(1): 38-46.

This study was conducted to determine whether the aquatic herbicide Rodeo® (active ingredient: glyphosate) and the non-ionic surfactant R-11® (active ingredient nonylphenol polyethoxylate or NPE) adversely affect aquatic species including invertebrates and larval amphibians. A Rodeo®/R-11® mixture was applied directly to the surface of a pond in a manner that would produce atypically high concentrations of these compounds in water. Water samples were collected from the treated pond for chemical analyses and toxicity tests with the aquatic invertebrate *Ceriodaphnia dubia*. A toxicity test with the Rodeo®/R-11® mixture was also conducted to determine the LC_{50} value for the larval life stage of the northern leopard frog, *Rana pipiens*. Water samples collected one hour after application contained the following mean concentrations: glyphosate, 1.83 mg/L; NPE, 1.10 mg/L; and 0.02 mg/L of the NPE breakdown product nonylphenol (NP). Concentrations of glyphosate's primary breakdown product, amino methyl phosphonic acid (AMPA), were below the laboratory detection limit of 0.020 mg/L. Water samples collected from the treated pond were not acutely lethal to *Ceriodaphnia dubia*. The 96-h toxicity test with the Rodeo®/R-11® mixture using *Rana pipiens* produced LC_{50} values of 6.5 mg/L for glyphosate and 1.7 mg/L for NPE, indicating that the mixture is moderately toxic to the amphibian. A comparison of toxic units for the herbicide and surfactant in the mixture indicated that the toxicity to larval frogs was likely due to R-11® and not Rodeo®. **(U.S.A.)**

103. Tsui, M.T.K. and L.M. Chu.2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere* 52(7): 1189-1197.

Glyphosate-based herbicides (e.g. Roundup®) are extensively used in the aquatic environment, but there is a paucity of data on the toxicity of the formulated products and the influences by environmental factors. In this study, the acute toxicity of technical-grade glyphosate acid, isopropylamine (IPA) salt of glyphosate, Roundup® and its surfactant polyoxyethylene amine (POEA) to Microtox® bacterium (*Vibrio fischeri*), microalgae (*Selenastrum capricornutum* and *Skeletonema costatum*), protozoa (*Tetrahymena pyriformis* and *Euplotes vannus*) and crustaceans (*Ceriodaphnia dubia* and *Acartia tonsa*) was examined and the relative toxicity contributions of POEA to Roundup® were calculated. The effects of four environmental factors (temperature, pH, suspended sediment and algal food concentrations) on the acute toxicity of Roundup® to *C. dubia* were also examined. Generally, the toxicity order of the chemicals was: POEA>Roundup®>glyphosate acid>IPA salt of glyphosate, while the toxicity of glyphosate acid was mainly due to its high acidity. Microtox® bacterium and protozoa had similar sensitivities towards Roundup® toxicity (i.e. IC50 from 23.5 to 29.5 mg AE/l). In contrast, microalgae and crustaceans were 4-5 folds more sensitive to Roundup® toxicity than bacteria and protozoa. Except photosynthetic microalgae, POEA accounted for more than 86% of Roundup® toxicity and the toxicity contribution of POEA was shown to be species-dependent. Increase in pH (6-9) and increase of suspended sediment concentration (0-200 mg/l) significantly increased the toxicity of Roundup® to *C. dubia*, but there were no significant effects due to temperature change and food addition. **(China)**

104. Tsui, M.T.K and L.M. Chu. 2004. Comparative toxicity of glyphosate-based herbicides: aqueous and sediment porewater exposures. *Archives of Environmental Contamination and Toxicology* 46(3): 316-323.

Glyphosate-based herbicides are widely used for aquatic weed control. However, their aquatic toxicity data, especially those on sediment, are relatively scarce. In this study, the water-only acute toxicity of three formulations based on glyphosate (Rodeo, Roundup Biactive, and Roundup) were compared using a water-column organism (cladoceran: *Ceriodaphnia dubia*) and a benthic organism (amphipod: *Hyalella azteca*). In addition, Roundup Biactive and Roundup were spiked into a clean sediment which was amended with appropriate amounts of peat moss to study the effect of different organic carbon levels (0, 0.4, 1.2, and 2.1%) on their sediment toxicity, with *C. dubia* exposed to overlying water or porewater prepared from the contaminated sediments. Results showed that the toxicity based on 48-h LC50s for the three herbicides in the water-only tests was Roundup (1.5–5.7 mg L^{-1}) > Roundup Biactive (82–120 mg L^{-1}) > Rodeo (225–415 mg L^{-1}), and *H. azteca* was generally more sensitive than *C. dubia* to these herbicides. Toxicity differences between formulations were due to the different surfactant components in these herbicides. From the porewater toxicity tests, Roundup Biactive (340 mg kg^{-1}) and Roundup (244 mg kg^{-1}) were similarly toxic in the sediment tests at 0% organic carbon, indicating that the surfactants in Roundup were considerably more adsorptive than those in Roundup Biactive to the sediment of the same organic

carbon. Also, an increase in organic carbon significantly decreased the toxicity of Roundup in sediment, but not for Roundup Biactive. Sediment–porewater partitioning of glyphosate was found to be influenced by sediment organic carbon (i.e., glyphosate adsorption increased with sediment organic carbon). **(China)**

105. Vendrell, E., D.G. de Barreda Ferraz, C. Sabater, and J.M. Carrasco. 2009. Effect of glyphosate on growth of four freshwater species of phytoplankton: a microplate bioassay. *Bulletin of Environmental Contamination and Toxicology* 82(5): 538-542.

The acute toxicity of glyphosate herbicide was tested on the four species of freshwater phytoplankton, *Scenedesmus acutus, Scenedesmus subspicatus, Chlorella vulgaris* and *Chlorella saccharophila*. Herbicide concentrations eliciting a 50% growth reduction over 72 h (EC_{50}) ranged from 24.5 to 41.7 mg L^{-1}, whilst a 10% growth inhibition is achieved by herbicide concentrations ranging from 1.6 to 3.0 mg L^{-1}, difficult to find neither in paddy fields (it is not used in rice) nor in the lake of the Albufera Natural Park. *Chorella* species are less sensitive to the herbicide than *Scenedesmus* species. It can be concluded that glyphosate has a low potential risk for the tested organisms. **(Spain)**

106. Vera, M.S., L. Lagomarsino, M. Sylvester, G.L. Pérez, P. Rodríguez, H. Mugni, et al. 2010. New evidences of Roundup® (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. *Ecotoxicology* 19(4): 710-721.

Argentina is the second largest world producer of soybeans (after the USA) and along with the increase in planted surface and production in the country, glyphosate consumption has grown in the same way. We investigated the effects of Roundup® (glyphosate formulation) on the periphyton colonization. The experiment was carried out over 42 days in ten outdoor mesocosms of different typology: "clear" waters with aquatic macrophytes and/or metaphyton and "turbid" waters with great occurrence of phytoplankton or suspended inorganic matter. The herbicide was added at 8 mg L^{-1} of the active ingredient (glyphosate) in five mesocosms while five were left as controls (without Roundup® addition). The estimate of the dissipation rate (k) of glyphosate showed a half-life value of 4.2 days. Total phosphorus significantly increased in treated mesocosms due to Roundup degradation what favored eutrophication process. Roundup produced a clear delay in periphytic colonization in treated mesocosms and values of the periphytic mass variables (dry weight, ash-free dry weight and chlorophyll a) were always higher in control mesocosms. Despite the mortality of algae, mainly diatoms, cyanobacteria was favored in treated mesocosms. It was observed that glyphosate produced a long term shift in the typology of mesocosms, "clear" turning to "turbid", which is consistent with the regional trend in shallow lakes in the Pampa plain of Argentina. Based on our findings it is clear that agricultural practices that involve the use of herbicides such as Roundup® affect non-target organisms and the water quality, modifying the structure and functionality of freshwater ecosystems. **(Argentina)**

107. Warren, R.S., P.E. Fell, J.L. Grimsby, E.L. Buck, G.C. Rilling, and R.A. Fertik. 2001. Rates, patterns, and impacts of *Phragmites australis* expansion and effects of

experimental *Phragmites* control on vegetation, macroinvertebrates, and fish within tidelands of the lower Connecticut River. *Estuaries* 24(1): 90-107.

See Fish Section.

108. Wauchope, R.D., T.L. Estes, R. Allen, J.L. Baker, A.G. Hornsby, R.L. Jones, R.P. Richards, and D.I. Gustafson. 2002. Predicted impact of transgenic, herbicide-tolerant corn on drinking water quality in vulnerable watersheds of the mid-western USA. *Pest Management Science* 58(2): 146-160.

See Water Quality Section.

109. Williams, B.K. and R.D. Semlitsch. 2010. Larval responses of three midwestern anurans to chronic, iow-dose exposures of four herbicides. *Archives of Environmental Contamination and Toxicology* 58(3): 819-827.

Low levels of agricultural herbicides often contaminate surface water and might persist throughout the growing season, potentially acting as stressors on aquatic organisms. Although low-dose, chronic exposures to agrochemicals are likely common for many nontarget organisms, studies addressing these effects using end-use herbicide formulations are rare. We exposed three common species of tadpoles to conservative levels of atrazine, S-metolachlor, and glyphosate end-use herbicide formulations throughout the larval period to test for survival differences or life-history trait alterations. Exposure to the glyphosate product Roundup WeatherMax® at 572 ppb glyphosate acid equivalents (a.e.) resulted in 80% mortality of western chorus frog tadpoles, likely as a result of a unique surfactant formulation. Exposure to WeatherMax® or Roundup Original Max® at 572 ppb a.e. also lengthened the larval period for American toads. Chronic atrazine and S-metolachlor exposures induced no significant negative effects on survival, mass at metamorphosis, or larval period length at the levels tested. These results highlight the importance of explicitly tying chronic tests to the natural environment and considering contributions of surfactant/adjuvant components to end-use formulation toxicities, even between very similar products. **(U.S.A.)**

110. Wojtaszek, B.F., B. Staznik, D.T. Chartrand, G.R. Stephenson, and D.G. Thompson. 2004. Effects of Vision® herbicide on mortality, avoidance response, and growth of amphibian larvae in two forest wetlands. *Environmental Toxicology and Chemistry* 23(4): 832-842.

The effects of Vision® (glyphosate, 356 mg acid equivalents (a.e.)/L) on mortality, avoidance response, and growth of larval amphibians (*Rana clamitans* and *Rana pipiens*) were investigated using in situ enclosures deployed in two forest wetlands of northern Ontario, Canada. In addition to untreated controls, Vision was applied to yield initial concentrations ranging from 0.29 to 14.3 mg a.e./L (0.94–46.1 mg/L of Vision). Resultant 96-h median lethal concentration (LC50) values ranged from 2.70 to 11.5 mg a.e./L (8.71-37.1 mg/L of Vision) depending on the species or site involved. Substantial mortality and incidences of abnormal avoidance response occurred only at concentrations exceeding the expected environmental concentrations (EEC) (1.43 mg a.e./L, or 4.61 mg/L of Vision) as calculated by Canadian regulatory authorities. The concentration dependence of larval growth rate

and maximum size varied depending on site and species. Mean growth rates and maximum sizes exposed to 1.43 mg a.e./L (EEC) treatments were the same or greater than controls. Experimental site and biotic/abiotic factors therein, such as pH and suspended sediments, substantially affected the expression of Vision herbicide toxicity in the amphibian larvae tested. Overall, results suggest that the silvicultural use of Vision herbicide in accordance with the product label and standard Canadian environmental regulations should have negligible adverse effects on sensitive larval life stages of native amphibians. **(Canada)**

111. Xi, Y.-L. and L.-K. Feng. 2004. Effects of thiophanate-methyl and glyphosate on asexual and sexual reproduction in the rotifer *Brachionus calyciflorus* Pallas. *Bulletin of Environmental Contamination and Toxicology* 73(4): 644-651.

***** Rotifers, especially *Brachionus calyciflorus* Pallas and *B. plicatilis* Muller, are particularly useful for aquatic toxicology because of their rapid reproduction, short generation time, cosmopolitan distribution and commercial availability of the resting eggs. The main purpose of the present study was to assess the effects of different levels of thiophanate-methyl and glyphosate on asexual and sexual reproduction in the freshwater rotifer *B. calyciflorus*. Based on the 24-hr acute toxicology assays for thiophanate-methyl and glyphosate, the LC50 values of *B. calyciflorus* were 5.02 and 28.0 mg/L, respectively. When the concentrations of glyphosate were 4.0, 6.0 and 8.0 mg/L, the population densities were significantly larger than the control, which might lead to higher mictic rates in the populations. Higher concentrations of thiophanate-methyl inhibited asexual and sexual reproduction of the rotifer *B. calyciflorus*, but the higher concentrations of glyphosate enhanced them. Compared to the controls, the population growth rates were significantly influenced by thiophanate-methyl and glyphosate when their concentrations were higher than 0.2 and 2.0 mg/L, respectively. However, the mictic rates in the populations were markedly affected just when the concentrations of thiophanate-methyl and glyphosate were higher than 0.05 and 0 mg/L, respectively. These results showed that sexual reproduction was more sensitive than asexual reproduction to thiophanate-methyl and glyphosate. Higher mictic rate caused by glyphosate might be one cause of higher resting egg production.Presence of higher concentrations of thiophanage-methyl and glyphosate in freshwater bodies would change the structures and functions of freshwater ecosystems, and the presence of the higher concentrations of thiophanate-methyl would be unfavourable for the survival of the rotifer species at unsuitable environments. For large-scale agriculture use, the final concentrations of less than 0.05 mg/L for thiophanate-methyl or 0.2 mg/L for glyphosate after they entered into freshwater bodies should be required. **(China)**

112. Zhu, G.N., Z.Y. Lou, and J.H. Sun. 2000. Study on toxicity and environmental safety of glyphosate to aquatic organisms. *Journal of Zhejiang University (Agriculture and Life Sciences)* 26(3): 309-312.

See Fish Section.

Biodiversity, Conservation, and Habitat Restoration

1. Ailstock, M.S., C.M. Norman, and P.J. Bushmann. 2001. Common reed *Phragmites australis*: control and effects upon biodiversity in freshwater nontidal wetlands. *Restoration Ecology* 9(1): 49-59.

   Phragmites australis (common reed) has expanded in many wetland habitats. Its ability to exclude other plant species has led to both control and eradication programs. This study examined two control methods—herbicide application or a herbicide-burning combination—for their efficacy and ability to restore plant biodiversity in non-tidal wetlands. Two *Phragmites*-dominated sites received the herbicide glyphosate. One of these sites was burned following herbicide application. Plant and soil macroinvertebrate abundance and diversity were evaluated pre-treatment and every year for four years post-treatment using belt transects. The growth of *Phragmites* propagules—seeds, rhizomes, and rooted shoots—was examined in the greenhouse and under bare, burned, or vegetated soil conditions. Both control programs greatly reduced *Phragmites* abundance and increased plant biodiversity. Plant re-growth was quicker on the herbicide-burn site, with presumably a more rapid return to wetland function. Re-growth at both sites depended upon a pre-existing, diverse soil seed bank. There were no directed changes in soil macroinvertebrate abundance or diversity and they appeared unaffected by changes in the plant community. *Phragmites* seeds survived only on bare soils, while buried rhizomes survived under all soil conditions. This suggests natural seeding of disturbed soils and inadvertent human planting of rhizomes as likely avenues for *Phragmites* colonization. Herbicide control, with or without burning, can reduce *Phragmites* abundance and increase plant biodiversity temporarily. These changes do not necessarily lead to a more diverse animal community. Moreover, unless *Phragmites* is eradicated and further human disturbance is prohibited, it will likely eventually re-establish dominance. **(U.S.A.)**

2. Ammann, K. 2005. Effects of biotechnology on biodiversity: herbicide-tolerant and insect-resistant GM crops. *Trends in Biotechnology* 23(8): 388-394.

   Biodiversity is threatened by agriculture as a whole, and particularly also by traditional methods of agriculture. Knowledge-based agriculture, including GM crops, can reduce this threat in the future. The introduction of no-tillage practices, which are beneficial for soil fertility, has been encouraged by the rapid spread of herbicide-tolerant soybeans in the USA. The replacement of pesticides through Bt crops is advantageous for the non-target insect fauna in test-fields. The results of the British Farm Scale experiment are discussed. Biodiversity differences can mainly be referred to as differences in herbicide application management. **(Switzerland)**

3. Beachy, J.R., R. Neville, and C. Arnott. 2011. Successful control of an incipient invasive amphibian: *Eleutherodactylus coqui* on O‘ahu, Hawai‘i. Pages 140-147 In: Veitch, C. R.; Clout, M. N. and Towns, D. R. (eds.). 2011. *Island invasives: eradication and management.* IUCN, Gland, Switzerland.

   A Puerto Rican icon, the coqui frog (*Eleutherodactylus coqui*), has quickly proliferated across the Hawaiian Islands (Hawai’i) since its introduction in the late

1980s. Shipping of goods, particularly commercial plants, provided coqui with easy passage between islands. Coqui are now firmly established on the Island of Hawai‘i (Big Island) and are the subject of early detection, eradication, and control activities on O‘ahu, Kaua‘i, Maui, and Moloka‘i. Hawai‘i provides an ideal home for coqui; all the benefits of its tropical native range and none of its natural predators. Large coqui populations threaten native arthropods and pose serious problems for the tourism and real estate industries. On O‘ahu, coqui distribution is sparse; only one naturalised population has been documented, in the town of Wahiawā, between a military base and a residential neighbourhood. A multiagency coordinated response resulted in successful eradication. The agencies involved include the O‘ahu Invasive Species Committee, the Hawai‘i State Department of Agriculture, the Department of Land and Natural Resources, and the U.S. Army Garrison Hawai‘i, collectively known as the Coqui Frog Working Group (CWG). Four elements were essential to success: 1) a control method permitted by federal regulatory agencies was known and available; 2) control crews were allowed complete access; 3) there was adequate funding for the operation; and 4) the population was relatively small. After close to a decade of work, the Wahiawā population was eradicated using a combination of habitat modification, nighttime citric acid vegetation sprays, and daytime citric acid ground drenches. Wahiawā is the first such eradication documented in the State. **(U.S.A.)**

4. Bell, F.W. and S.G. Newmaster. 2002. The effects of silvicultural disturbances on the diversity of seed-producing plants in the boreal mixedwood forest. *Canadian Journal of Forest Research* 32(7): 1180-1191.

The practice of clear-cutting, followed by site preparing with mechanical equipment, planting a single tree species, and applying herbicides, has recently been cited as a procedure that creates monocultures in northern forests. Research on a trembling aspen (*Populus tremuloides* Michx.) dominated mixedwood provided an opportunity to examine the potential of silvicultural activities to (*i*) create monocultures, (*ii*) create opportunities for the establishment of exotic plant species, and (*iii*) result in the loss of indigenous plant species. Detailed botanical surveys were conducted for up to 5 years post-treatment in four clearcuts that were mechanically site prepared, planted with a single conifer species, and released with either motor-manual, mechanical, or herbicide treatment. Species richness, abundance (foliar cover), diversity indices, and rank abundance diagrams indicate that the treatments had immediate effects, but none created a monoculture during the period of study. We conclude that the use of clear-cutting, mechanical site preparation, planting a single conifer species, followed by release with motor-manual cutting, mechanical cutting, or herbicide spraying, will not create monocultures in the conditions tested. While 37 exotic species were observed, none of them were tree or shrub species. In addition, no net loss of indigenous seed producing plants was detected. Missed strips and patches, which accounted for up to 25% of the sampled area, buffered treatment effects. **(Canada)**

5. Bell, F.W., and D.G. Pitt. 2007. Seasonal susceptibility of boreal plants: red raspberry phenology as a bioindicator of optimum within-season timing of glyphosate applications. *The Forestry Chronicle* 83: 733-741.
   See Crop Tree Productivity Section.

6. Bigler, F. and R. Albajes. 2011. Indirect effects of genetically modified herbicide tolerant crops on biodiversity and ecosystem services: the biological control example. *Journal of Consumer Protection and Food Safety* 6(Suppl 1): S79-S84.
   Weeds can directly affect arthropods by providing favorable microclimates, food, shelter, mating sites and oviposition substrates. Indirect effects on herbivores and higher trophic level arthropods can occur if weed species interact with each other and with crop plants modifying plant physiology, chemical and visual cues and competing for resources. Hence, weed management can interfere in many different ways with arthropods. Evidence from literature is that our understanding of the mechanisms underlying the observed effects is still very limited. Higher plant diversity does not necessarily mean increased biological control. Genetically Modified Herbicide-Tolerant (GMHT) crops offer new possibilities for weed management with potential consequences for conservation biological control. For example, farmers have more flexibility in timing herbicide applications (early vs. late overall sprays), and band spraying in row crops have been shown to modify weed abundance and composition in favor of a number of predators and parasitoids. There is some evidence that maximum weed control with overall glyphosate spray of GMHT crops results in reduced weed biomass and lower arthropod abundance, including beneficial species. Conversely, many articles show that maintaining some weed enhances arthropod densities and natural biological control. GMHT crops may increase adoption of minimum and no tillage systems with possible effects on weeds and arthropods. Weed management systems with GMHT plants have a great potential to alter plant diversity in crops in favor of conservation biological control. However, careful consideration of other management options such as crop and herbicide rotation should be evaluated to make sustainable use of the benefits of GMHT crops. **(Switzerland)**

7. Binimelis, R., W. Pengue, and I. Monterroso. 2009. “Transgenic treadmill”: Responses to the emergence and spread of glyphosate-resistant johnsongrass in Argentina. *Geoforum* 40(4): 623-633.
   The broad-spectrum herbicide glyphosate has become the largest-selling crop-protection product worldwide. The increased use of glyphosate is associated with the appearance of a growing number of tolerant or resistant weeds, with socio-environmental consequences apart from the loss of productivity. In 2002, a glyphosate-resistant biotype of johnsongrass (*Sorghum halepense* (L.)) appeared in Argentina and now covers at least 10,000 ha. This paper analyzes the driving forces behind the emergence and spread of this weed and also examines management responses and their implications. Preventive strategies against glyphosate-resistant johnsongrass fail because of the institutional setting. Reactive measures, however, transfer the risks to the society and the environment through the introduction of novel genetically modified crops that allow the use of yet more herbicide. This in turn

reinforces the emergence of herbicide-resistant weeds, constituting a new phenomenon of intensification, the "transgenic treadmill". **(Argentina)**

8. Brodman, R., W.D. Newman, K. Laurie, S. Osterfeld, and N. Lenzo. 2010. Interaction of an aquatic herbicide and predatory salamander density on wetland communities. *Journal of Herpetology* 44(1): 69-82.

See Amphibians, Aquatic Invertebrates and Plants, and Algae Section.

9. Carrera, M.F. and H.A. Carreras. 2011. Effects of the application of glyphosate on chemical-physiological parameters of *Usnea amblyoclada* (Müll. Arg.) Zahlbr. *Ecologia Austral* 21: 353-361.

The expansion in the use of glyphosate as herbicide in order to control weeds, mainly in transgenic soybean cultivation, causes biological and ecological deterioration in the ecosystem. The species *U. amblyoclada* has been used as biomonitor “in situ”, in order to estimate this herbicide´s effect in the lichenic flora. Thus, the samples were transplanted during a 3-month period in a soybean field and in a control area. After that, physiological parameters indicating lichenic damage were established. Furthermore, the effect of different herbicide doses on the lichen’s physiology was tested in the laboratory. The results showed a meaningful decrease in the photosynthetic pigments after the fumigation with glyphosate. A significant decrease of the contents was observed of feofitina ‘a’ and ‘b’ and, in addition, an increase in the concentrations of the products of oxidations of cellular membranes. These results were observed also in the experiments in laboratory. In this way, the damaging effects of glyphosate on the lichen *U. amblyoclada* and the usefulness of this species as biomonitor in agricultural areas were demonstrated. **(Argentina)**

10. Cerdeira, A.L. and S.O. Duke. 2006. The current status and environmental impacts of glyphosate-resistant crops: a review. *Journal of Environmental Quality* 35(5): 1633-1658.

Glyphosate [N-(phosphonomethyl) glycine]-resistant crops (GRCs), canola (*Brassica napus* L.), cotton (*Gossypium hirsutum* L.), maize (*Zea mays* L.), and soybean [*Glycine max* (L.) Merr.] have been commercialized and grown extensively in the Western Hemisphere and, to a lesser extent, elsewhere. Glyphosate-resistant cotton and soybean have become dominant in those countries where their planting is permitted. Effects of glyphosate on contamination of soil, water, and air are minimal, compared to some of the herbicides that they replace. No risks have been found with food or feed safety or nutritional value in products from currently available GRCs. Glyphosate-resistant crops have promoted the adoption of reduced- or no-tillage agriculture in the USA and Argentina, providing a substantial environmental benefit. Weed species in GRC fields have shifted to those that can more successfully withstand glyphosate and to those that avoid the time of its application. Three weed species have evolved resistance to glyphosate in GRCs. Glyphosate-resistant crops have greater potential to become problems as volunteer crops than do conventional crops. Glyphosate resistance transgenes have been found in fields of canola that are supposed to be non-transgenic. Under some circumstances, the largest risk of GRCs may be transgene flow (introgression) from GRCs to related

species that might become problems in natural ecosystems. Glyphosate resistance transgenes themselves are highly unlikely to be a risk in wild plant populations, but when linked to transgenes that may impart fitness benefits outside of agriculture (e.g., insect resistance), natural ecosystems could be affected. The development and use of failsafe introgression barriers in crops with such linked genes is needed. **(Brazil/U.S.A.)**

11. Cerdeira, A.L., D.L.P. Gazziero, S.O. Duke, and M.B. Matallo. 2011. Agricultural impacts of glyphosate-resistant soybean cultivation in South America. *Journal of Agricultural and Food Chemistry* 59(11):5799-5807.

    In the 2009/2010 growing season, Brazil was the second largest world soybean producer, followed by Argentina. Glyphosate-resistant soybeans (GRS) are being cultivated in most of the soybean area in South America. Overall, the GRS system is beneficial to the environment when compared to conventional soybean. GRS resulted in a significant shift toward no-tillage practices in Brazil and Argentina, but weed resistance may reduce this trend. Probably the highest agricultural risk in adopting GRS in Brazil and South America is related to weed resistance due to use of glyphosate. Weed species in GRS fields have shifted in Brazil to those that can more successfully withstand glyphosate or to those that avoid the time of its application. Five weed species, in order of importance, *Conyza bonariensis* (L.) Cronquist*, Conyza canadensis* (L.) Cronquist*, Lolium multiflorum* Lam., *Digitaria insularis* (L.) Mez ex Ekman, and *Euphorbia heterophylla* L., have evolved resistance to glyphosate in GRS in Brazil. *Conyza* spp. are the most difficult to control. A glyphosate-resistant biotype of *Sorghum halepense* L. has evolved in GRS in Argentina and one of *D. insularis* in Paraguay. The following actions are proposed to minimize weed resistance problem: (a) rotation of GRS with conventional soybeans in order to rotate herbicide modes of action; (b) avoidance of lower than recommended glyphosate rates; (c) keeping soil covered with a crop or legume at intercrop intervals; (d) keeping machinery free of weed seeds; and (d) use of a preplant nonselective herbicide plus residuals to eliminate early weed interference with the crop and to minimize escapes from later applications of glyphosate due to natural resistance of older weeds and/or incomplete glyphosate coverage. **(Brazil)**

12. Cerdeira, A.L., D.L.P. Gazziero, S.O. Duke, M.B. Matallo, and C.A. Spadotto. 2007. Review of potential environmental impacts of transgenic glyphosate-resistant soybean in Brazil. *Journal of Environmental Science and Health* Part B 42(5): 539-549.

    Transgenic glyphosate-resistant soybeans (GRS) have been commercialized and grown extensively in the Western Hemisphere, including Brazil. Worldwide, several studies have shown that previous and potential effects of glyphosate on contamination of soil, water, and air are minimal, compared to those caused by the herbicides that they replace when GRS are adopted. In the USA and Argentina, the advent of glyphosate-resistant soybeans resulted in a significant shift to reduced- and no-tillage practices, thereby significantly reducing environmental degradation by agriculture. Similar shifts in tillage practiced with GRS might be expected in Brazil.

Transgenes encoding glyphosate resistance in soybeans are highly unlikely to be a risk to wild plant species in Brazil. Soybean is almost completely self-pollinated and is a non-native species in Brazil, without wild relatives, making introgression of transgenes from GRS virtually impossible. Probably the highest agricultural risk in adopting GRS in Brazil is related to weed resistance. Weed species in GRS fields have shifted in Brazil to those that can more successfully withstand glyphosate or to those that avoid the time of its application. These include *Chamaesyce hirta* (erva-de-Santa-Luzia), *Commelina benghalensis* (trapoeraba), *Spermacoce latifolia* (erva-quente), *Richardia brasiliensis* (poaia-branca), and I*pomoea* spp. (corda-de-viola). Four weed species, *Conyza bonariensis*, *Conyza canadensis* (buva), *Lolium multiflorum* (azevem), and *Euphorbia heterophylla* (amendoim bravo), have evolved resistance to glyphosate in GRS in Brazil and have great potential to become problems. **(Brazil)**

13. Cobb, T.P., D.W. Langor, and J.R. Spence. 2007. Biodiversity and multiple disturbances: boreal forest ground beetle (Coleoptera: Carabidae) responses to wildfire, harvesting, and herbicide. *Canadian Journal of Forest Research* 37(8): 1310-1323.

    See Terrestrial Invertebrates Section.

14. Damgaard, C., B. Strandberg, S.K. Mathiassen, and P. Kudsk. 2011. The combined effect of nitrogen and glyphosate on the competitive growth, survival and establishment of *Festuca ovina* and *Agrostis capillaris*. *Agriculture, Ecosystems and Environment* 142(3): 374-381.

    In order to study the combined effect of nitrogen and glyphosate on biodiversity in agricultural areas, a replicated long-time field experiment with glyphosate and nitrogen treated plots was set-up. The experiment allowed a quantitative estimation of the effect of glyphosate and nitrogen on competitive growth, survival and establishment of the dominating species during and between growing seasons. It was found that the observed ecological success of *Festuca ovina* relative to *Agrostis capillaris* in glyphosate treated plots was primarily due to altered competitive plant growth during the growing season rather than an immediate die back following spraying. Overall, interaction of herbicide and fertilizer on plant competitive growth, survival and establishment were demonstrated, and it was suggested that positive interactions between glyphosate and nitrogen may be important for the ecological success of *A. capillaris* in field margins. The used method of combining pin-point data and Bayesian state-space competition models may be applied in other ecological studies. For example, the method may be used in predicting the effects of altered weed control strategies on the botanical composition of the agro-ecosystem. **(Denmark)**

15. Dampier, J.E., E; N. Luckai, F.W. Bell, and W.D. Towill. 2007. Do tree-level monocultures develop following Canadian boreal silviculture? Tree-level diversity tested using a new method. *Biodiversity and Conservation* 16(10): 2933-2948.

    Concern about forestry practices creating tree-level monoculture plantations exists. Our study investigates tree diversity responses for six early seral boreal

forest plantations in Ontario, Canada, representing three conifer species; black spruce (*Picea mariana*), white spruce (*P. glauca*), and jack pine (*Pinus banksiana*), 14 release treatments, and 94 experimental units. Dominance-diversity curves and Simpson's indices of diversity and evenness indicate tree alpha diversity. We propose a new method for assessing diversity, using percentage of theoretical species maximum (%TSM) which is determined by comparing post-disturbance richness (S) with a theoretical species maximum (TSM). Our results support the hypothesis that alternative vegetation release treatments generally do not reduce tree species diversity levels (%TSM) relative to untreated plots. The only %TSM ($P \leq 0.05$) comparison that produced less diversity than in control plots was repeated annual treatments of Vision herbicide at one of the black spruce study sites. Our results generally support the hypothesis that tree monocultures do not develop after vegetation release. Only one out of 94 experimental units developed into a tree layer monoculture (Simpson's reciprocal diversity index=1). Again this was one of the repeated annual treatments of Vision herbicide at one of the black spruce study sites—a treatment which is atypical of Canadian forest management. **(Canada)**

16. Duke, S.O. and S.B. Powles. 2008. Glyphosate: a once-in-a-century herbicide. *Pest Management Science* 64(4): 319-325.

    Since its commercial introduction in 1974, glyphosate [N-(phosphonomethyl) glycine] has become the dominant herbicide worldwide. There are several reasons for its success. Glyphosate is a highly effective broad-spectrum herbicide, yet it is very toxicologically and environmentally safe. Glyphosate translocates well, and its action is slow enough to take advantage of this. Glyphosate is the only herbicide that targets 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), so there are no competing herbicide analogs or classes. Since glyphosate became a generic compound, its cost has dropped dramatically. Perhaps the most important aspect of the success of glyphosate has been the introduction of transgenic, glyphosate-resistant crops in 1996. Almost 90% of all transgenic crops grown worldwide are glyphosate resistant, and the adoption of these crops is increasing at a steady pace. Glyphosate/glyphosate-resistant crop weed management offers significant environmental and other benefits over the technologies that it replaces. The use of this virtually ideal herbicide is now being threatened by the evolution of glyphosate-resistant weeds. Adoption of resistance management practices will be required to maintain the benefits of glyphosate technologies for future generations. **(U.S.A.)**

17. Fairchild, J.F., A.L. Allert, J.S. Riddle, D.R., and Gladwin. 2002. Efficacy of glyphosate and five surfactants for controlling giant salvinia. *Journal of Aquatic Management* 40: 53-58.

    See Fish Section.

18. Ferreira, E.A., J.B. Santos, A.A. Silva, L. Vargas, and M.R. Reis. 2006. Glyphosate application for Italian ryegrass biotype control and impact on soil microbiota. *Planta Daninha, Viçosa-MG* 24(3): 573-578.

    See Microbiota and Fungi Section.

19. Firbank, L.G. and F. Forcella. 2000. Genetically modified crops and farmland biodiversity. *Science* 289(5484): 1481-1482.
See Birds Section.

20. Flueck, W.T. and J.M. Smith-Flueck. 2006. Herbicides and forest biodiversity: an alternative perspective. *Wildlife Society Bulletin* 34(5): 1472-1478.
Effects of herbicide use on forest biodiversity was the topic of a special section in the winter 2004 issue of *The Wildlife Society Bulletin*. In acknowledging public concerns regarding the toxic effects of herbicides, several of the contributing authors argued that these effects are negligible and that intensifying wood production would be beneficial for forest biodiversity and conservation by reducing habitat conversions. We contend there are other important environmental consequences; hence, responding to increased opportunities for selling wood products by augmenting supply through intensifying production should not be the only option. We argue that it is also important to develop mechanisms to reduce the demand for forest products. We believe the focus of the special section was too narrow, particularly with respect to benefiting biodiversity, because herbicide use also intensifies the export of wood products and, thus, nutrients. Other factors that must be considered include soil acidification caused by biomass export and fertilizer application, as well as additional acidification resulting from aerial emissions. In addition, because of mineral cycle dynamics constraints, intensively managed forests may not be sustainable for wood production, and less so for forest-dependent animals. Extensively exploited forests may deplete mineral reserves, and any intensification likely would speed up the declines. We believe the indirect impact from herbicides through accelerating mineral export and loss needs to be addressed, in particular how it may affect mammals' ability to accumulate essential trace elements. We contend using fertilizer applications as a corrective measure at the landscape level would be cost-prohibitive. Thus, heralding that herbicides, a tool to intensify wood production, benefit forest biodiversity appears premature, given the time scale of forest growth and soil development. **(Argentina)**

21. Fortier, J. and C. Messier. 2006. Are chemical or mechanical treatments more sustainable for forest vegetation management in the context of the TRIAD? *The Forestry Chronicle* 82(6): 806-818.
Chemical and mechanical forest vegetation management (FVM) treatments are analyzed and compared to assess which is the most sustainable in intensively managed plantations in the context of the TRIAD. At the biological and ecological level, herbicides have been found to have more impacts on flora and fauna compared to mechanical treatments, but the differences are of short duration. The effects of noise generated by manual or mechanical brushing on wildlife have not been investigated, however. Local application of herbicide at the base of the tree should further lower these impacts. At the social level, the general public has a negative perception of chemical treatments, while mechanical treatments are well perceived. However, in terms of worker safety chemical treatments are less risky than manual brushing (brushsaw or chainsaw). At the economic level, herbicides globally cost less and are more effective at increasing fibre production than

mechanical operations. We conclude that it is difficult to assess globally what is the most sustainable option to control competing vegetation. However, the careful use of herbicide may be the most sustainable option if the added productivity thus obtained is used to increase protected areas and ecosystem-based management, as is intended with the TRIAD concept. **(Canada)**

22. Ge, X., D.A. d'Avignon, J.J.H. Ackerman, B. Duncan, M.B. Spaur, and R.D. Sammons. 2011. Glyphosate-resistant horseweed made sensitive to glyphosate: low-temperature suppression of glyphosate vacuolar sequestration revealed by ^{31}P NMR. *Pest Management Science* 67(10):1215-1221.

    Horseweed has been the most invasive glyphosate-resistant (GR) weed, spreading to 16 states in the United States and found on five continents. The authors have previously reported that GR horseweed employs rapid vacuolar sequestration of glyphosate, presumably via a tonoplast transporter, substantively to reduce cytosolic glyphosate concentrations[1]. It was hypothesized that glyphosate sequestration was the herbicide resistance mechanism. If resistance is indeed endowed by glyphosate sequestration, suppression of sequestration offers the potential for controlling GR horseweed at normal herbicide field-use rates. Low-temperature ^{31}P NMR experiments performed *in vivo* with GR cold-acclimated horseweed showed markedly suppressed vacuolar accumulation of glyphosate even 3 days after glyphosate treatment. [In stark contrast, 85% of the visible glyphosate was sequestered 24 h after spraying warm-acclimated GR horseweed.] Cold-acclimated GR horseweed treated at normal use rates and maintained at low temperature succumbed to the lethal effects of glyphosate over a 40 day period. Treatment of GR horseweed in the field when temperatures were cooler showed the predicted positive herbicidal response. Low temperature markedly diminishes vacuolar sequestration of glyphosate in the GR horseweed biotype, yielding a herbicide response equivalent to that of the sensitive biotype. This supports the recent hypothesis[1] that glyphosate sequestration is the resistance mechanism employed by GR horseweed. **(U.S.A.)**

23. Grebner, D.L., A.W. Ezell, J.D. Prevost, and D.A. Gaddis. 2011. Kudzu control and impact on monetary returns to non-industrial private forest landowners in Mississippi. *Journal of Sustainable Forestry* 30(3): 204-223.

    Kudzu—*Pueraria montana* var. *lobata* (Willd.)—was initially planted in the southern United States and subsequently spread throughout the countryside following changes in land use. Kudzu covers more than 2.8 million ha which prevents uses such as timber production and establishment of carbon plantations. Using data collected on sites in Mississippi, this study examines the after-tax monetary trade-offs of controlling kudzu using different herbicide regimes. The results suggest that the most cost-effective way to control kudzu patches is to apply Escort XP using an aerial applicator. This application is appropriate for both young and old kudzu patches and whether the landowner intends to afforest the site with pine or oak. **(U.S.A.)**

24. Guynn, D.C Jr., S.T. Guynn, T.B. Wigley, and D.A. Miller. 2004. Herbicides and forest biodiversity—what do we know and where do we go from here? *Wildlife Society Bulletin* 32(4): 1085-1092.

    Use of herbicides to control competing vegetation in young forests can increase wood volume yields by 50–150%. However, increasing use of herbicides in forest management has caused widespread concerns among the public and biologists about direct toxicity to wildlife and indirect effects through habitat alteration. Abundant research has indicated that forest herbicide treatments target biochemical pathways unique to plants, do not persist in the environment, and have few toxic effects when operationally applied. Herbicides affect forest biodiversity by creating short-term declines in plant species diversity, altering vegetative structure, and potentially changing plant successional trajectories. For wildlife species, effects vary but generally are short-term. Despite these findings, public opinion against forest herbicides often has limited or restricted their use, likely due to people's values associated with forests and a lack of technical knowledge. Future research efforts on relationships between forest herbicides and biodiversity should address landscape and site-specific issues, be based on rigorous experimental design, be relevant to public concerns, include comparisons of herbicide treatments with alternative treatments excluding herbicides, examine use of chemical mixtures, and determine the social, economic, and possible long-term ecological consequences of treatments. **(U.S.A.)**

25. Haeussler, S., L. Bedford, A. Leduc, Y. Bergeron, and J.M. Kranabetter. 2002. Silvicultural disturbance severity and plant communities of the southern Canadian boreal forest. *Silva Fennica* 36(1): 307-327.

    Boreal forest ecosystems are adapted to periodic disturbance, but there is widespread concern that conventional forest practises degrade plant communities. We examined vegetation diversity and composition after clearcut logging, mechanical and chemical site preparation in eight 5- to 12-yr old studies located in southern boreal forests of British Columbia and Quebec, Canada to find useful indicators for monitoring ecosystem integrity and to provide recommendations for the development and testing of new silvicultural approaches. Community-wide and species-specific responses were measured across gradients of disturbance severity and the results were explained in terms of the intermediate disturbance hypothesis and a simple regeneration model based on plant life history strategies. Species richness was 30 to 35% higher 5 to 8 years after clearcut logging than in old forest. Total and vascular species diversity generally peaked on moderately severe site treatments, while non-vascular diversity declined with increasing disturbance severity. On more-or-less mesic sites, there was little evidence of diversity loss within the range of conventional silvicultural disturbances; however, there were important changes in plant community composition. Removing soil organic layers caused a shift from residual and resprouting understory species to ruderal species regenerating from seeds and spores. Severe treatments dramatically increased non-native species invasion. Two important challenges for the proposed natural dynamics-based silviculture will be 1) to find ways of maintaining populations of sensitive non-vascular species and forest mycoheterotrophs, and 2) to create

regeneration niches for disturbance-dependent indigenous plants without accelerating non-native species invasion. **(Canada)**

26. Harmoney, K.R., P.W. Stahlman, P.W. Geier, and K.R. Hickman. 2010. Rate and timing of glyphosate application to control Caucasian Old World bluestem (*Bothriochloa bladhii*). *Invasive Plant Science and Management* 3(3): 310-314.

Caucasian Old World bluestem (OWB) has escaped into native rangelands and could have unknown effects on distribution, utilization, growth, and reproduction of native plant and animal species. This trial was established to determine the rate and timing of glyphosate application that will provide the greatest OWB suppression. Treatments included glyphosate applied early at the five-leaf stage of growth with 1.12, 2.24, or 3.36 kg glyphosate ai ha^{-1} (1, 2, or 3 lb ai ac^{-1}), sequential application with an early glyphosate application of 1.12, 2.24, or 3.36 kg ha^{-1} followed by a second application 8 wk later of either 1.12 or 2.24 kg ha^{-1}, and a late-only application of 1.12, 2.24, or 3.36 kg ha^{-1}. Sequential glyphosate applications with at least one of the early or late applications being 2.24 kg ha^{-1} or more reduced OWB frequencies from over 87% to below 30% each year. During a moist year, all sequential application treatments reduced OWB frequency to 12% or less. Frequency of OWB the year after application was directly related (r^2 = 0.91) to the total amount of glyphosate applied during dry conditions. Seed heads were absent or nearly absent in all sequential application treatments, with the exception of glyphosate at 1.12 kg ha^{-1} applied early and late during the dry season. Sequential application of glyphosate that includes one treatment either early or late of 2.24 or 3.36 kg ha^{-1} appears to be the most effective treatment to reduce established OWB during dry years. During years of adequate moisture, a single late application of 2.24 or 3.36 kg ha^{-1} or sequential applications with 1.12 kg ha^{-1} at each application is as adequate as sequential applications with greater rates for reducing OWB frequency and achieving OWB control. **(U.S.A.)**

27. Harrington, T.B. and J.H. Miller. 2005. Effects of application rate, timing, and formulation of glyphosate and triclopyr on control of Chinese privet (*Ligustrum sinense*). *Weed Technology* 19(1): 47-54.

Chinese privet is a nonnative shrub that has invaded mesic forests throughout the southeastern United States during the past century. Foliar sprays of glyphosate and triclopyr were tested in three factorial experiments that included wide ranges of application rate, timing, and formulation to refine methods for controlling Chinese privet. For spring (April) and fall (October and December) applications, percentage control of privet cover averaged 93 to 100% and 49 to 70% for glyphosate and triclopyr treatments, respectively, whereas for summer (June and August) applications, control averaged 67 to 69% and 14 to 26%, respectively (study 1). However, privet control was not influenced by variation in herbicide rates of 1.7, 3.4, 5.0, or 6.7 kg ae/ha compared with each of the five application timings. No differences were found in August comparisons of liquid vs. dry glyphosate products or water-soluble vs. oil-soluble triclopyr products for each of the four rates (study 2). In a comparison of low rates of glyphosate applied in August with or without trenching of plot perimeters to isolate privet clumps (study 3), control increased from

12 to 65% as rate increased from 0 to 0.8 kg ae/ha, suggesting that rate responses may occur at lower values than those tested in studies 1 and 2. Isolation of privet clumps by trenching did not have a statistically detectable effect on privet susceptibility to glyphosate. Low rates of glyphosate (1.7 kg ae/ha or possibly lower) will provide effective control of privet when applied in the spring or fall. **(U.S.A.)**

28. Homyack, J.A., D.J. Harrison, and W.B. Krohn. 2004. Structural differences between precommercially thinned and unthinned conifer stands. *Forest Ecology and Management* 194: 131-143.

Effects of precommercial thinning (PCT) in young, high-density forest stands on the growth and yield of crop trees has been well-studied, but information about the response of habitat characteristics and structural attributes that are related to abundance and diversity of wildlife populations is deficient. We examined changes in habitat characteristics and forest structure that occurred with PCT and stand development in commercial spruce-fir stands within the Acadian forest of northern Maine. We selected 30 regenerating, herbicide-treated conifer stands (17 treated with PCT) of three development classes (1, 6, or 11 years post-PCT) and measured 27 variables that described the characteristics and structure of the understory, overstory, woody debris, or ground cover within these stands. The application of PCT accelerated some characteristics of stand development, resulting in a reduction of understory structure and complexity, which conflicts with previous studies of non-herbicide-treated forest that reported increases in understory complexity after thinning. Near-ground cover, overstory cover, and understory structure described >80% of the variation in vegetation structure between thinned and unthinned stands. Horizontal cover, an overstory to understory contrast, and a gradient of herbaceous vegetation described >75% of the variation that occurred as regenerating stands (thinned and unthinned) developed through time. The forest structure in regenerating stands treated with PCT may have negative effects on wildlife that are dependent on the structure of early successional forest, but may positively affect species that use more mature forest. **(U.S.A.)**

29. Homyack, J.A., D.J. Harrison, and W.B. Krohn. 2005. Long-term effects of precommercial thinning on small mammals in northern Maine. *Forest Ecology and Management* 205(1-3): 43-57.

See Mammals Section.

30. Homyack, J.A., D.J. Harrison, and W.B. Krohn. 2007. Effects of precommercial thinning on snowshoe hares in Maine. *Journal of Wildlife Management* 71(1): 4-13.

See Mammals Section.

31. Irvine, I.C., M.S. Witter, C.A. Brigham, and J.B.H. Martiny. 2011. Relationships between methylobacteria and glyphosate with native and invasive plant species: implications for restoration. *Restoration Ecology* (in press) Article first published online: 13 DEC 2011. DOI:10.1111/j.1526-100X.2011.00850.x

After removing invasive plants, whether by herbicides or other means, typical restoration design focuses on rebuilding native plant communities while disregarding

soil microbial communities. However, microbial–plant interactions are known to influence the relative success of native versus invasive plants. Therefore, the abundance and composition of soil microorganisms may affect restoration efforts. We assessed the effect of herbicide treatment on phytosymbiotic pink-pigmented facultative methylotrophic (PPFM) bacteria and the potential consequences of native and invasive species establishment post-herbicide treatment in the lab and in a coastal sage scrub (CSS)/grassland restoration site. Lab tests showed that 4% glyphosate reduced PPFM abundance. PPFM addition to seeds increased seedling length of a native plant (*Artemisia californica*) but not an invasive plant (*Hirschfeldia incana*). At the restoration site, methanol addition (a PPFM substrate) improved native bunchgrass (*Nassella pulchra*) germination and size by 35% over controls. In a separate multispecies field experiment, PPFM addition stimulated the germination of *N. pulchra*, but not that of three invasive species. Neither PPFM nor methanol addition strongly affected the growth of any plant species. Overall, these results are consistent with the hypothesis that PPFMs have a greater benefit to native than invasive species. Together, these experiments suggest that methanol or PPFM addition could be useful in improving CSS/grassland restorations. Future work should test PPFM effects on additional species and determine how these results vary under different environmental conditions. **(U.S.A.)**

32. Jadhav, A., M. Hill, and M. Byrne. 2008. Identification of a retardant dose of glyphosate with potential for integrated control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach. *Biological Control* 47(2): 154-158.

    Water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae) has a negative impact on aquatic ecosystems in South Africa. Attempts to use biological control against the weed have been hampered by low temperatures, with slow development of biocontrol agents, and excessively eutrophic waters which enable the plants to proliferate and grow at greater rates than the agents. The failure of biological control has encouraged the use of herbicides which also hinder the biocontrol agents, predominantly by destroying their food source. This study tested a broad spectrum herbicide, glyphosate, to identify whether there is a dose which will retard vegetative growth (production of ramets and leaves) but not kill the water hyacinth plants. A concentration of 0.8% achieved this goal while having no direct detrimental effects on two water hyacinth biocontrol agents, *Neochetina eichhorniae* and *N. bruchi*. The results show that application of retardant doses of herbicide might be used for integrated control of *E. crassipes* and this needs to be tried under natural field conditions. **(South Africa)**

33. Krueger-Mangold, J., R.L. Sheley, and B.D. Roos. 2002. Maintaining plant community diversity in a waterfowl production area by controlling Canada thistle (*Cirsium arvense*) using glyphosate. *Weed Technology* 16(2): 457-463.

    Our objective was to maximize Canada thistle control and plant community diversity in a waterfowl production area administered by the U.S. Fish and Wildlife Service. We tested three rates (1.5, 3.0, and 4.5 ai/ha) of glyphosate applied during spring, summer, or fall using two application methods. The lowest rate of glyphosate decreased the Canada thistle density by about 30% relative to the control.

Glyphosate applied in the fall decreased Canada thistle density below that of the control more consistently than when applied in spring or summer. Wick application generally resulted in less Canada thistle biomass than did broadcast application. Species richness was generally higher when glyphosate was wick applied, and all rates of this application method increased species richness when compared with the control. We recommend fall wick application of glyphosate at 1.5 kg ai/ha to control Canada thistle near the riparian areas. This application provided optimum Canada thistle control, while maintaining species richness important for waterfowl. **(U.S.A.)**

34. Lautenschlager, R.A. and T.P. Sullivan. 2002. Effects of herbicide treatments on biotic components in regenerating northern forests. *The Forestry Chronicle* 78(5): 695-731.

We reviewed literature, primarily since 1990, that documents effects of herbicide treatments on major biotic components in northern forested ecosystems. Vegetation changes are responsible for changes in all other biotic components. Non-conifer vegetation is commonly reduced for two to five years following broadcast herbicide treatments. Fungal components, however, seem relatively unaffected. Short-term vegetation reductions in cover, density, and related biomass, if they occur, are species and/or vegetation group specific; longer-term changes are linked to conifer stocking, site quality, and the ability of conifers to dominate treated sites. Herbicide treatments do not reduce, and may increase, stand- and landscape-level plant species richness. Those treatments seldom produce mono-cultures when used by foresters for boreal or boreal mixedwood management. The active ingredients in the herbicide products used in forestry in northern ecosystems have no direct effect on the general health (survival, growth, reproduction) of animals in treated areas. Specific, stand-level forest management practices, particularly effects of site preparation and conifer release, must be examined in relation to the landscape mosaic and the desired future forest conditions. At broad scales, across boreal and boreal mixedwood ecosystems, conifers have been consistently replaced by hardwoods since Europeans began harvesting timber from those ecosystems. Herbicides provide a safe, effective tool for restoring conifers in previously conifer-dominated ecosystems. Forest scientists presently have a reasonable understanding of effects of a variety of herbicide treatments on conifer growth and a variety of environmental components. However, they need to continually update that understanding relative to treatments (replicates, chemicals, combinations, or timing) that may be used in the future. **(Canada)**

35. Lautenschlager, R.A. and T.P. Sullivan. 2004. Improving research into effects of forest herbicide use on biota in northern ecosystems. *Wildlife Society Bulletin* 32(4): 1061-1070.

Studies of indirect effects of forest herbicide use on biota have improved dramatically during the last two decades, though further improvements are still needed. Based on our experience, we provide recommendations designed to ensure continued improvements to general field research, including forest herbicide-wildlife research. Specifically, we suggest that researchers should: 1) use a combination of public concerns and existing scientific information to focus research efforts (i.e., the

appropriate foundation for this type of research is social and ecological); 2) predict and test social and ecological consequences of herbicide and alternative treatments on components of concern in forested landscapes, using scales (time and space) that are operationally, ecologically, and socially meaningful; 3) understand the ecology of biotic components of interest and their interaction with other components in shared ecosystems; 4) determine the appropriate integrative currency so that a synthesis of effects on ecosystem or economic parameters can be developed; 5) document treatment delivery and consequences for plants targeted for suppression and for improved growth; and 6) appreciate that herbicides could hold the key to a variety of wildlife management and vegetation (habitat) restoration efforts. **(Canada)**

36. Lautenschlager, R.A., J.H. Pedlar, and C.M. Nielsen. 2003. Ice storm damage: Effects of competition and fertilization on near-ground vegetation. *The Forestry Chronicle* 79(1): 54-62.

    Increasing ice damage to tree canopies led to increased cover of near-ground deciduous tree species, herbaceous species, and total vegetative cover but reduced fern cover in managed sugar maple stands in southeastern Ontario. Near-ground vegetation did not respond to the addition of fertilizers [2000 kg/ha of dolomitic lime, 200 kg/ha of both phosphorus (P) and potassium (K), or both lime and P and K]. Vegetation management with glyphosate in these stands reduced near-ground deciduous tree cover 86%, while grass and sedge cover were reduced 69%, and shrub cover was reduced 98% two years after treatment. Although species richness was initially reduced by vegetation management, species richness levels on treated plots were comparable to, or higher than, those on untreated plots by two years after treatment. **(Canada)**

37. Lindgren, C.J., T.S. Gabor, and H.R. Murkin. 1999. Compatibility of glyphosate with *Galerucella calmariensis*; a biological control agent for purple loosestrife (*Lythrum salicaria*). *Journal of Aquatic Plant Management* 37: 44-48.

    By integrating *Galerucella calmariensis* with glyphosate there is potential to achieve both immediate and sustained control of purple loosestrife (*Lythrum salicaria*). The objective of this study was to determine the compatibility of glyphosate on the oviposition and survival of adult *G. calmariensis* and on the ability of *G. calmariensis* third instar larvae to pupate to teneral adults. Our results revealed glyphosate (formulated as Roundup®) at a concentration of 2% (2.43 L/acre) and 4% solution (4.86 L/acre) had no impact on the ability of *G. calmariensis* third instar larvae to pupate to new generation adults. To examine the effect of a 2% solution of glyphosate on adult *G. calmariensis* oviposition and survival, adults were randomly divided between a direct contact group (adults sprayed directly), an indirect contact group (host plants with adults were sprayed), and a control group. Our results revealed that glyphosate does not impact *G. calmariensis* oviposition or adult survival. The results of this study indicate that *G. calmariensis* is compatible with glyphosate indicating that further field studies examining integrated control strategies for purple loosestrife are warranted. **(Canada)**

38. Lindgren, P.M.F. and T.P. Sullivan. 2001. Influence of alternative vegetation management treatments on conifer plantation attributes: abundance, species diversity, and structural diversity. *Forest Ecology and Management* 142: 163-182.
This study was designed to test the hypothesis that alternative vegetation management treatments (manual cutting and cut-stump applications of glyphosate herbicide) would decrease plant community abundance, species diversity, and structural diversity of young mixed conifer plantations in southern British Columbia, Canada. The experimental design consisted of nine operational-sized plantations, stratified into three blocks (1 control, 1 manual, and 1 cut-stump plantation per block), with five permanent strip-transects to sample vegetation within each plantation. Vegetation management treatments did not significantly (p>0.10) affect the crown volume index of herb, shrub, or coniferous tree layers. However, both manual and cut-stump treatments significantly reduced crown volume index of deciduous trees in the first post-treatment year (p=0.05 and p<0.01, respectively). Due to prolific regrowth of stump sprouts, the manual treatment effect did not last beyond the first post-treatment year. In contrast, the cut-stump treatment impeded sprouting and, relative to control and manual treatments, continued to significantly suppress deciduous growth for at least 4 years (p<0.05). Species richness, diversity, and turnover of the herb, shrub, and tree layers were not significantly (p>0.10) different between treatments and control. Similarly, the structural diversity of herb, shrub, and tree layers were also not significantly (p>0.10) different between treatments and control. By opening the canopy and decreasing the dominance of the deciduous tree layer, both manual and cut-stump treatments showed greater total structural diversity (herb, shrub, and tree layers combined) relative to the control. However, differences in total structural diversity between treatments and control were, for the most part, not significant (p>0.10). Therefore, these vegetation management treatments affected only the volume of the targeted deciduous tree layer and did not adversely affect the species richness, diversity, turnover, or structural diversity of the plant community. These results may be applicable to other temperate forest ecosystems where conifer release is practiced in young plantations. **(Canada)**

39. Linz, G.M. and H.J. Homan. 2011. Use of glyphosate for managing invasive cattail (*Typha* spp.) to disperse blackbird (Icteridae) roosts. *Crop Protection* 30(2): 98-104.
Hybrid cattail (*Typha* x *glauca* Godr.) has become the dominant emergent vegetation in many wetlands of central North America's Prairie Pothole Region (PPR). Hybrid cattail, an invasive species, can outcompete native emergents and form a dense canopy that alters the original physiognomy and ecological processes of the wetland. Blackbirds (Icteridae), which number in the millions in late summer in the PPR, use cattails for reproduction, loafing and roosting. Ripening crops, especially sunflower, planted near wetland roost sites can sustain considerable economic damage from blackbirds. Producers of sunflower in North Dakota and South Dakota can obtain assistance from the U.S. Department of Agriculture's Wildlife Services unit to prevent blackbird damage. Beginning in 1991, Wildlife Services began aerially spraying cattails with glyphosate herbicide to reduce roosting substrate and lessen the severity of localized sunflower damage. As the

program enters its 20th year, we review published research aimed at assessing the ecological effects and efficacy of glyphosate use in wetlands. Additionally, we incorporate unpublished data gathered to enhance the program's environmental safety and efficacy. **(U.S.A.)**

40. Londo, J.P., M.A. Bollman, C.L. Sagers, E.H. Lee, and L.S. Watrud. 2011. Changes in fitness-associated traits due to the stacking of transgenic glyphosate resistance and insect resistance in *Brassica napus* L. *Heredity* 107(4): 328-337.

    Increasingly, genetically modified crops are being developed to express multiple 'stacked' traits for different types of transgenes, for example, herbicide resistance, insect resistance, crop quality and tolerance to environmental stresses. The release of crops that express multiple traits could result in ecological changes in weedy environments if feral crop plants or hybrids formed with compatible weeds results in more competitive plants outside of agriculture. To examine the effects of combining transgenes, we developed a stacked line of canola (*Brassica napus* L.) from a segregating F_2 population that expresses both transgenic glyphosate resistance (CP4 EPSPS) and lepidopteran insect resistance (Cry1Ac). Fitness-associated traits were evaluated between this stacked genotype and five other *Brassica* genotypes in constructed mesocosm plant communities exposed to insect herbivores (*Plutella xylostella* L.) or glyphosate-drift. Vegetative biomass, seed production and relative fecundity were all reduced in stacked trait plants when compared with non-transgenic plants in control treatments, indicating potential costs of expressing multiple transgenes without selection pressure. Although costs of the transgenes were offset by selective treatment, the stacked genotype continued to produce fewer seeds than either single transgenic line. However, the increase in fitness of the stacked genotype under selective pressure contributed to an increased number of seeds within the mesocosm community carrying unselected, hitchhiking transgenes. These results demonstrate that the stacking of these transgenes in canola results in fitness costs and benefits that are dependent on the type and strength of selection pressure, and could also contribute to changes in plant communities through hitchhiking of unselected traits. **(U.S.A.)**

41. Madison, L.A., T.G. Barnes, and J.D. Sole. 2001. Effectiveness of fire, disking, and herbicide to renovate tall fescue fields to northern bobwhite habitat. *Wildlife Society Bulletin* 29(2): 706-712.

    See Birds Section.

42. Man, R., J.A. Rice, and G.B. MacDonald. 2010. Five-year light, vegetation, and regeneration dynamics of boreal mixedwoods following silvicultural treatments to establish productive aspen–spruce mixtures in northeastern Ontario. *Canadian Journal of Forest Research* 40(8): 1529-1541.

    See Crop-Tree Productivity Section.

43. Matarczyk, J.A. A.J. Willis, J.A. Vranjic, and J.E. Ash. 2002. Herbicides, weeds and endangered species: management of bitou bush (*Chrysanthemoides monilifera* ssp.

rotundata) with glyphosate and impacts on the endangered shrub, *Pimelea spicata*. *Biological Conservation* 108(2): 133-141.

Environmental weed invasion threatens the biodiversity of native species. Unfortunately, managing these weeds may also affect biodiversity adversely. A recent example occurred when glyphosate, a herbicide used to control the highly invasive weed, bitou bush (*Chrysanthemoides monilifera* ssp. *rotundata*), accidentally drifted over a small population of an endangered shrub, *Pimelea spicata*. Following concerns that the affected population would not recover and, thereby, cause the local extinction of *P. spicata*, we conducted a series of glasshouse and field experiments to explore the impacts of glyphosate on this endangered species. Seedlings and young plants of *P. spicata*, in which the tap root was undeveloped, were killed by a single application of glyphosate. Older plants with a well developed tap root also died back initially, but about 50% of individuals re-sprouted. This re-growth was associated with a significant decrease in tap root diameter, implying that further disturbance, including repeated treatment with glyphosate, would kill plants by impairing their potential for recovery. Unlike some sclerophyllous native shrubs, the tolerance of *P. spicata* to glyphosate was limited, even when its growth was slowed artificially by limiting water availability. Winter applications of glyphosate to manage infestations of bitou bush will impact adversely on populations of *P. spicata* and may also affect the other rare and endangered species whose survival is threatened by this species, even though some natives are unaffected by the herbicide. Protecting native biodiversity from bitou bush will involve sustainable weed management that minimises impacts on non-target native species. **(Australia)**

44. McLaren, B., K. Emslie, T. Honsberger, T. McCready, F.W. Bell, and R. Foster. 2011. Monitoring and understanding mammal assemblages: experiences from Bending Lake, Fallingsnow, and Tom Hill. *The Forestry Chronicle* 87(2): 225-234.

We monitored mammal assemblages in treatment areas in three studies, two involving competition control (with live capture) in Ontario and one involving commercial thinning (with snow tracking) in Alberta. Abundant and opportunistic species were relatively unaffected by treatments, while species preferring open habitats or a dense shrub layer thrived in herbicide-treated and thinned areas, respectively. A few populations declined, but returned to levels in reference areas within two years of treatment. Most populations fluctuated both seasonally and annually, making other trends difficult to detect. We discuss several issues related to using a broadcast approach to mammal monitoring, including design improvements, with a view towards better future decisions in an adaptive management framework. **(Canada)**

45. Melander, B., N. Holst, A.C. Grundy, C. Kempenaar, M.M. Riemens, A. Verschwele, and D. Hansson. 2009. Weed occurrence on pavements in five North European towns. *Weed Research* 49(5): 516-525.

Weeds on pavements in urban areas are unwanted mainly because they cause an untidy appearance or sometimes structural damage. Glyphosate has been the principal weed control method for years, but policies in several European towns

have changed to lower dependence on herbicides. Instead, less effective and more species-dependent non-chemical methods are used, but little is known about the pavement flora. Consequently, we surveyed the flora on pavements in five North European towns [Braunschweig (DE), Malmö (SE), Næstved (DK), Royal Leamington Spa (UK) and Wageningen (NL)] by recording weed species and their coverage in 56 recording points randomly placed in each town. Weeds were recorded at several dates in 2005 and 2006 and no weed control was applied apart from sweeping. Weed coverage increased during the survey (averaging 1.4% in late 2006) and was highest in the towns having the strictest policies limiting herbicide use. Most coverage (averaging 2%) was found along the pavement edge away from the road. Poa annua was the most frequently recorded species, followed by bryophytes (mainly mosses), Sagina procumbens and perennial grasses. Grasses and some other species frequently found, notably Taraxacum officinale, should receive particular attention when planning a non-chemical weed control campaign on pavements. **(Northern Europe**)

46. Mihajlovich, M. and P. Blake. 2004. An evaluation of the potential of glyphosate herbicide for woodland caribou habitat management*. Alces* 40: 7-11.

Two studies evaluating the effects of glyphosate used for habitat management on caribou lichens and dwarf shrubs were undertaken. Glyphosate substantially reduced blueberry cover at all rates tested in both cutover and uncut areas. Glyphosate did not affect caribou lichen cover. **(Canada)**

47. Miller, K.V. and J.H. Miller. 2004. Forestry herbicide influences on biodiversity and wildlife habitat in southern forests. *Wildlife Society Bulletin* 32(4): 1049-1060.

In the southern United States, herbicide use continues to increase for timber management in commercial pine (*Pinus* spp.) plantations, for modifying wildlife habitats, and for invasive plant control. Several studies have reported that single applications of forestry herbicides at stand initiation have minor and temporary impacts on plant communities and wildlife habitat conditions, with some reports of enhanced habitat conditions for both game and nongame species. Due to the high resiliency of floral communities, plant species richness and diversity rebound rapidly after single herbicide treatments, with short- and long-term compositional shifts according to the selectivity and efficacy of the herbicide used. Recently, however, a shift to the Southeast in North American timber supplies has resulted in increased forest management intensity. Current site-preparation techniques rely on herbicide combinations, often coupled with mechanical treatments and ≥1 years of post-planting applications to enhance the spectrum and duration of vegetation control. This near-total control of associated vegetation at establishment and more rapid pine canopy closure, coupled with shortened and repeated rotations, likely will affect plant diversity and wildlife habitat quality. Development of mitigation methods at the stand and landscape levels will be required to minimize vegetative and wildlife impacts while allowing continued improvement in pine productivity. More uncertain are long-term impacts of increasing invasive plant occupation and the projected increase in herbicide use that will be needed to reverse this worsening situation. In addition, the potential of herbicides to meet wildlife management objectives in areas where

traditional techniques have high social costs (e.g., prescribed fire) should be fully explored. **(U.S.A.)**

48. Mozdzer, T.J., C.J. Hutto, P.A. Clarke, and D.P. Field. 2008. Efficacy of imazapyr and glyphosate in the control of non-native *Phragmites australis*. *Restoration Ecology* 16(2): 221-224.

The cosmopolitan common reed (*Phragmites australis*) has been expanding into previously unoccupied wetland habitats throughout North America. This invasion by a non-native haplotype of *Phragmites* has become a major concern due to a reduction in plant diversity, reduction of faunal biodiversity, and changes in ecosystem structure. A randomized complete block design was used to compare the efficacy of two herbicides, glyphosate (Rodeo, Dow AgroSciences, IN, U.S.A.) and imazapyr (Habitat, BASF Corporation, NC, U.S.A.), on 1-ha *Phragmites* monoculture in a shallow borrow pit. Six foliar experimental treatments were applied consisting of (1) 2% glyphosate formulation, June application; (2) 2% glyphosate formulation, September application; (3) 2% imazapyr formulation, June application; (4) 2% imazapyr formulation, September application; (5) 5% imazapyr formulation, June application; and (6) 5% imazapyr, September application. Experimental plots were monitored yearly for two years after treatment. Relative importance values (RIV) were determined to assess the efficacy of herbicide treatments. We report that imazapyr foliar application is statistically superior to glyphosate in reducing *Phragmites* RIV, with no significant differences between the 2 and 5% formulations. Both herbicides are more effective in reducing *Phragmites* RIV if applied early in the growing season (June). No significant differences in non-*Phragmites* plant recolonization were observed between herbicide treatments over the two-year time course. These results suggest that imazapyr is superior in reducing *Phragmites* RIV, and that earlier applications of herbicides may be more effective on *Phragmites*. However, managers must note that adjacent nontarget plant species may be negatively affected by earlier treatments. **(U.S.A.)**

49. Newmaster, S.G. and F.W. Bell. 2002. The effects of silvicultural disturbances on cryptogam diversity in the boreal-mixedwood forest. *Canadian Journal of Forest Research* 32(1): 38-51.

In northern forests, cryptogams (spore producing plants) occupy a key position in forest ecosystem diversity and function. Forest harvesting and silvicultural practices have the potential to reduce cryptogam diversity. This project uses four blocks that were mechanically site prepared, planted with a single conifer species, and subsequently subjected to five conifer release treatments: (1) motor-manual cleaning, (2) mechanical brush cutting, (3) aerial application of triclopyr, (4) aerial application of glyphosate, and (5) control (untreated clearcut). Five 10 x 10 m subplots were installed in each of the five treatment plots and the uncut forest on the four blocks. Botanical surveys were conducted before and 1-5 years after treatments. Species richness and abundance, Shannon's and Heip's indices, and rank abundance diagrams clearly show that richness and abundance were affected by silvicultural treatments. Vegetation management treatments resulted in significant reductions in cryptogam diversity, to the point that only a few colonists and drought-

tolerant species remained. Cryptogam diversity was ranked in the following order: forest > clearcut > mechanical clearing > herbicide treatment. Herbicide treatments had the greatest initial effect on species richness, species abundance, and diversity indices. Cryptogam diversity showed signs of recovery 5 years after treatments. Missed strips (untreated areas) within a clearcut provided a refuge for remnant communities of forest cryptogams that could play a key role in the rehabilitation forest diversity. **(Canada)**

50. Owen, M.D.K. 2008. Weed species shifts in glyphosate-resistant crops. *Pest Management Science* 64(4): 377-387.

The adoption of glyphosate-based crop production systems has been one of the most important revolutions in the history of agriculture. Changes in weed communities owing to species that do not respond to current glyphosate-based management tactics are rapidly increasing. Clearly, glyphosate-resistant crops (GRCs) do not influence weeds any more than non-transgenic crops. For most crops, the trait itself is essentially benign in the environment. Rather, the weed control tactics imposed by growers create the ecological selection pressure that ultimately changes the weed communities. This is seen in the adoption of conservation tillage and weed management programs that focus on one herbicide mode of action and have hastened several important weed population shifts. Tillage (disturbance) is one of the primary factors that affect changes in weed communities. The intense selection pressure from herbicide use will result in the evolution of herbicide-resistant weed biotypes or shifts in the relative prominence of one weed species in the weed community. Changes in weed communities are inevitable and an intrinsic consequence of growing crops over time. The glyphosate-based weed management tactics used in GRCs impose the selection pressure that supports weed population shifts. Examples of weed population shifts in GRCs include common waterhemp [*Amaranthus tuberculatus* (Moq ex DC) JD Sauer], horseweed (*Conyza canadensis* L), giant ragweed (*Ambrosia trifida* L) and other relatively new weed problems. Growers have handled these weed population shifts with varying success depending on the crop. **(U.S.A.)**

51. Pitt D.G., A.E. Morneault, P. Bunce, and F.W. Bell. 2000. Five years of vegetation succession following vegetation management treatments in a jack pine ecosystem. *Northern Journal of Applied Forestry* 17(3): 100–109.

Five years of data on vegetation dynamics and succession are provided for six operational release treatments applied to three 2- to 4-yr-old jack pine (*Pinus banksiana Lamb.*) plantations in central Ontario. Treatments included 3 yr of annual noncrop vegetation removal, conventional aerial spray with glyphosate (1.42 kg ae/ha), ground application of glyphosate with a mist blower, basal-bark application of triclopyr, motor-manual cutting (brush saw), and no treatment. Conventional aerial spraying and annual removal resulted in the greatest jack pine crop growth, with trees exceeding 90% crown closure, 7 cm in groundline diameter, and 3 m in height (stem volume index = 5.1 dm^3) after 5 growing seasons. The cover of herbaceous plants was highest (30–50%) in the aerial spray plots during the observation period. Deciduous tree, shrub, and fern species remained well represented on these plots,

although total cover and height were low (≤35% and 1 m, respectively). Mist-blower and brush-saw plots contained mid-sized pine (3.5 dm^3) with 69% crown closure. In contrast, untreated and basal-bark plots contained the smallest pine (2.3 dm^3 and 31% crown closure), likely caused by heavy competition and herbicide damage, respectively. On mist-blower and basal-bark plots, good height growth was observed on untreated deciduous trees; low-shrub and fern cover remained high (46 and 30%, respectively); and herbaceous cover increased gradually to 22%. On brush-saw plots, recovery of woody cover was rapid, but height growth was relatively slow. Deciduous trees and tall shrubs dominated untreated sites (> 70% cover) by the end of the fifth growing season. Successional trends suggest that aerial spray and annual removal treatments will produce pure jack pine stands at maturity; mist blower, basal bark, and brush-saw treatments may produce mixedwood stands; and untreated plots will likely be dominated by hardwoods. **(Canada)**

52. Ristau, T.E., S.H. Stoleson, S.B. Horsley, and D.S. deCalesta. 2011. Ten-year response of the herbaceous layer to an operational herbicide-shelterwood treatment in a northern hardwood forest. *Forest Ecology and Management* 262: 970-979.

Shelterwood seed cutting in conjunction with herbicide site preparation has proven effective at regenerating Allegheny hardwood forests, but the long-term impact of this silvicultural system on herbaceous vegetation has not been determined. From 1994 to 2004, we studied the impacts of operational herbicide site preparation using glyphosate plus sulfometuron methyl herbicides in the context of a shelterwood seed cut. Our study took place on 10 partially cut sites on the Allegheny National Forest in northwestern Pennsylvania. Half of each site received herbicide and half did not in a split-plot design with repeated measures. Fences were erected after year six because deer impact had increased. Resilience of individual species and the community were determined using measures of percent cover by species or species groups and indices of diversity and similarity comparing post-treatment to pre-treatment conditions and controls. In the short term, abundance of all species was reduced and there were four fewer species on average in treated areas. No species was eliminated by herbicide across all sites in the long term. Graminoids were more abundant on treated plots after year six. Targeted ferns remained less abundant on treated than control plots after 10 years. Species richness recovered within 4 years following treatment. Shannon Diversity and Shannon Evenness were greater in treated than in control plots over the full study period, but the differences were not significant in any single year. The richness-based Jaccard index of similarity did not differ between control and treatment plots after year two, while relative abundance influenced indices showed significant differences through year eight. Results suggest that herbaceous layer vegetation is resilient to the disturbance created by herbicide-shelterwood treatments. **(U.S.A.)**

53. Sciegienka, J.K., E.N. Keren, and F.D. Menalled. 2011. Interactions between two biological control agents and an herbicide for Canada thistle (*Cirsium arvense*) suppression. *Invasive Plant Science and Management* 4(1): 151-158.

See Microbiota and Fungi Section.

54. Scursoni, J., F. Forcella, J. Gunsolus, M. Owen, R. Oliver, R. Smeda, and R. Vidrine. 2006. Weed diversity and soybean yield with glyphosate management along a north-south transect in the United States. *Weed Science* 54(4): 713-719.

There are many concerns about the effects of repeated use of glyphosate in glyphosate-resistant (GR) crops, including two that are seemingly contradictory. These are (1) weed escapes and (2) loss of weed diversity. Weeds that escape glyphosate treatment represent species that likely will become troublesome and difficult to control in the future, and identifying these future problems may allow more effective management. In contrast, complete weed control directly reduces the weed component of agroecosystem biodiversity and may lower other components indirectly (e.g., weed-dependent granivores). During 2001 and 2002 effects of glyphosate and conventional weed control treatments on weed community composition and GR soybean yields were studied. Field studies were conducted along a north-south transect of sites spanning a distance of 1600 km from Minnesota to Louisiana. Low-intensity use (single application yr^{-1}) of glyphosate allowed more escapes and maintained higher weed diversity than high-intensity use (two applications yr^{-1}) of glyphosate, and it was equivalent to or even higher than diversity in non-GR systems. Although the same weeds escaped from low- and high-intensity glyphosate treatments, frequency of escapes was higher with less intensive use. These results suggest that limited use of glyphosate would not have profound effects on weed diversity. In addition, crop yield did not differ between GR and non-GR treatments at high latitudes, but below 40^{o} N latitude, with a longer cropping season, yields with low-intensity glyphosate use decreased by about 2% per degree latitude because of competition from escaped weeds. **(U.S.A.)**

55. Shepard, J.P., J. Creighton, and H. Duzan. 2004. Forestry herbicides in the United States: an overview. *Wildlife Society Bulletin* 32(4): 1020-1027.

Herbicides are used in forestry to manage tree-species composition, reduce competition from shrubs and herbaceous vegetation, manipulate wildlife habitat, and control invasive exotics. There are no national statistics on extent of forestry herbicide use. A survey of 13 forest products companies found that 51 distinct applications of 1–3 herbicides were used and that 11 applications (with 6 active ingredients) accounted for 90% of the area reported treated. Reported rates were always lower than maximum labeled rate, and average rates ranged from 10–42% of the labeled maxima. Herbicides were used on 74,464 hectares in the National Forest System (including rangeland) in 2001. Another survey of forestry herbicide use by all ownerships in the southern United States in 2002 reported 985,237 hectares treated. Public concerns over use of herbicides in forests include toxicity to humans, pets, livestock, and wildlife and effects of herbicides on wildlife habitat. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) provides a comprehensive system of toxicity testing and regulates sale and use of herbicides. However, there are concerns about FIFRA testing: use of a small number of sentinel species, testing only active herbicide ingredients, and not testing the mixtures of ≥2 herbicides commonly used in forestry. Recent litigation suggests that aerial application of pesticides (including herbicides) is a point-source discharge of pollutants into waters of the United States and thus requires a Clean Water Act (CWA) permit. These

lawsuits have created confusion about how 2 federal statutes (FIFRA and CWA) relate with regard to pesticides, and new policy clarification is being developed. Other litigation has initiated a process to improve consultation among federal agencies regarding potential for pesticides to affect threatened and endangered species. In summary, herbicides are vital for commercial timber management and have applications for managing wildlife habitat. Federal regulations and water-quality monitoring indicate that use of herbicides in forestry constitutes low risk to humans and wildlife. **(U.S.A.)**

56. Smith, H., L.G. Firbank, and D.W. Macdonald. 1999. Uncropped edges of arable fields managed for biodiversity do not increase weed occurrence in adjacent crops. *Biological Conservation* 89(1): 107-111.

Ten experimental management regimes, designed to quantify benefits to weed control and wildlife conservation on uncropped field edges of expanded width, were examined for effects on the weed flora within adjacent arable crops. The treatments involved cutting, sowing and herbicide regimes, with differing effects on plant and invertebrate populations. The relative abundance of all plant species within the adjacent crop edge was monitored using permanent quadrats between 1987 and 1991. The field edge management regimes affected neither the total relative plant abundance, nor the relative abundance of most common species, within the adjacent crop edge. While frequencies of *Avena* spp were initially greatest adjacent to margins cut in spring and autumn, this effect was lost through time, and the greater abundance of *Phleum pratense* and *Leucanthemum vulgare* adjacent to the field edges in which they had been sown presented no threat to good husbandry. The management of uncropped arable field edges to enhance biodiversity is very unlikely to affect weed levels within the crop, especially where they contain, or are sown with, non-invasive perennial species. **(United Kingdom)**

57. Strandberg, B., M.B. Pedersen, and N. Elmegaard. 2005. Weed and arthropod populations in conventional and genetically modified herbicide tolerant fodder beet fields. *Agriculture, Ecosystems and Environment* 105(1): 243-253.

The introduction of genetically modified herbicide tolerant (GMHT) crops has raised concerns from both scientists and non-governmental organisations about possible effects on arable flora and fauna due to the changes in herbicide application and management that such crops involve. Three consecutive studies were performed, covering flora and fauna in fields of GMHT and conventional fodder beets over the season, at different locations and under different spraying regimes. At all locations and in the 3 years, a denser and more diverse weed flora and arthropod fauna were found in GMHT beets in early and mid-summer than in conventional beets when glyphosate-treatment occurred at or after label recommendation. Following the herbicide applications the GMHT fields had fewer weed species and seeds and lower weed densities and biomass than conventional fields. However, application of glyphosate earlier than recommended resulted in an extremely low weed diversity, density and biomass during the entire season. Timing of the first glyphosate applications, i.e. the duration of the herbicide free period, was essential in terms of biodiversity improvements. In the long term reduced production of weed

seeds in GMHT fields may deplete the weed flora if the GMHT strategy becomes widely adopted. **(Denmark)**

58. Sullivan, T.P., D.S. Sullivan, P.M.F. Lindgren, and J.O. Boateng. 2002. Influence of conventional and chemical thinning on stand structure and diversity of plant and mammal communities in young lodgepole pine forest. *Forest Ecology and Management* 170(1):173-187.

Silvicultural practices that provide a wide variety of vegetative composition and structure (habitats) in young stands should help manage for biological diversity across forested landscapes. This study was designed to test the hypotheses that: (i) abundance and diversity of stand structure attributes (species diversity and structural diversity of herb, shrub and tree layers) and forest floor small mammal communities, and (ii) relative habitat use by large herbivores, will increase from unthinned to conventionally thinned to chemically thinned stands of young lodgepole pine (Pinus contorta) forest. Replicate study areas were located near Summerland, Kelowna and Williams Lake in south-central British Columbia, Canada. Each study area had three treatments: a conventionally thinned, a chemically thinned and an unthinned stand. Pre-commercial thinning was conducted in 1993. Coniferous stand structure and understory vegetation were measured prior to thinning in 1993 and 5 years later in 1998. Small mammal populations were sampled intensively from 1993 to 1998. Relative habitat use by large herbivores was sampled in 1998. Our results indicate that chemical thinning of young lodgepole pine stands produced an aggregated pattern of crop trees compared with stands subjected to conventional thinning. Diameter growth of crop trees in the chemically thinned stands was similar to that in the conventionally thinned, but also to that in unthinned stands. Although horizontal stratification (aggregates of trees) was enhanced, vertical stratification (structural diversity of vegetation) was less in the chemically than conventionally thinned stands. Abundance and diversity of understory vegetation and small mammal communities were generally unaffected by stand thinning in these particular installations. Relative habitat use by mule deer (*Odocoileus hemionus*) occurred in a gradient from highest in the conventionally thinned stand to lowest in the unthinned stand. Habitat use by snowshoe hares (*Lepus americanus*) tended to have the opposite trend. Moose (*Alces alces*) exhibited no difference in habitat use among stands. Thus, although there were few differences among treatment stands, chemical thinning could be used to develop an aggregated pattern of crop trees in pre-commercially thinned stands to maintain habitat for herbivores such as snowshoe hares and mule deer. Understory plant and forest floor small mammal communities would be maintained in these stands as well. **(Canada)**

59. Sullivan, T.P. and D.S. Sullivan. 2003. Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems. *Environmental Reviews* 11: 37-59.

The conservation of biological diversity (biodiversity) is becoming an integral part of the sustainable management of forest- and agro-ecosystems. A vital part of these systems is weed control, or vegetation management, to enhance crop production. Because of its widespread use and environmental compatibility, this review was

designed to evaluate glyphosate herbicide in terms of: (i) its role as a disturbance agent and (ii) a measure of its impact on species diversity of terrestrial plants and animals. Our analyses were based on 60 published studies of terrestrial plants and animals in temperate zone forest- and agro-ecosystems. Species richness and diversity of vascular plants was either unaffected or increased, particularly herbaceous species, in response to glyphosate. Responses of plant species in forest ecosystems differ from those in agro-ecosystems where glyphosate is used to repeatedly reduce non-crop vegetation in most situations. Richness and diversity of songbirds appeared little affected by glyphosate-induced habitat alteration. In studies on small mammal communities and glyphosate use, none found significant reductions in species richness or diversity. As for avian responses, some small mammal species declined temporarily whereas others increased in abundance. The impact of glyphosate on large mammalian herbivores was measured by abundance of animals and food plants and by habitat use. Hares (*Lepus* spp. L.) and deer (*Odocoileus* spp. Rafinesque and *Capreolus capreolus* L.) were little affected, whereas reductions in plant biomass and related moose (*Alces alces* L.) forage and habitat use generally occur for 1-5 years after treatment. Studies on terrestrial invertebrates covered a wide range of taxa with variable responses in abundance to glyphosate treatments. The magnitude of observed changes in mean species richness and diversity of vascular plants, birds, and small mammals, from the effects of herbicide treatment, were within the mean values of natural fluctuations of these variables. The biological significance of this impact is limited to shifts in species composition based on changes in floral composition and structure of habitats. Management for a mosaic of habitats within forest and agricultural landscapes, which provide a range of conditions for plant and animal species, should help ameliorate the short-term changes in species composition accompanying vegetation management with glyphosate. **(Canada)**

60. Swift, K. and F.W. Bell. 2011. What are the environmental consequences of using silviculturally effective forest vegetation management treatments? *The Forestry Chronicle* 87(2): 201-216.

In this paper, we present examples of stand-level consequences of using forest vegetation management treatments in boreal and temperate forest ecosystems in Canada. Specifically, we address several selected indicators: air and water quality, soils and nutrients, plant diversity, and wildlife habitat. For each of these, we discuss direct and indirect effects of five broad categories of treatments: (1) silviculture and harvesting systems and (2) physical, (3) thermal, (4) cultural, and (5) chemical/biological treatments. Our emphasis is on forest vegetation management treatments that are currently used in Canada to manage conifers. By applying regulations and best management practices, conducting landscape-level analyses and developing longer-term monitoring programs resource managers can minimize the effects of FVM treatments on the environmental indicators presented in this paper. **(Canada)**

61. Wagner, R.G., M. Newton, E.C. Cole, J.H. Miller, and B.D. Shiver. 2004. The role of herbicides for enhancing forest productivity and conserving land for biodiversity in North America. *Wildlife Society Bulletin* 32(4): 1028–1041.
    See Crop Tree Productivity Section.

62. Wardle, D.A., K.I. Bonner, G.M. Barker, G.W. Yeates, K.S. Nicholson, (*et al.*). 1999. Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. *Ecological Monographs* 69(4): 535-568.
    The consequences of permanent loss of species or species groups from plant communities are poorly understood, although there is increasing evidence that individual species effects are important in modifying ecosystem properties. We conducted a field experiment in a New Zealand perennial grassland ecosystem, creating artificial vegetation gaps and imposing manipulation treatments on the reestablishing vegetation. Treatments consisted of continual removal of different subsets or "functional groups" of the flora. We monitored vegetation and soil biotic and chemical properties over a 3-yr period. Plant competitive effects were clear: removal of the C_3 grass *Lolium perenne* L. enhanced vegetative cover, biomass, and species richness of both the C_4 grass and dicotyledonous weed functional groups and had either positive or negative effects on the legume *Trifolium repens* L., depending on season. Treatments significantly affected total plant cover and biomass; in particular, C_4 grass removal reduced total plant biomass in summer, because no other species had appropriate phenology. Removal of C_3 grasses reduced total root biomass and drastically enhanced overall shoot-to-root biomass ratios. Aboveground net primary productivity (NPP) was not strongly affected by any treatment, indicating strong compensatory effects between different functional components of the flora. Removing all plants often negatively affected three further trophic levels of the decomposer functional food web: microflora, microbe-feeding nematodes, and predaceous nematodes. However, as long as plants were present, we did not find strong effects of removal treatments, NPP, or plant biomass on these trophic groupings, which instead were most closely related to spatial variation in soil chemical properties across all trophic levels, soil N in particular. Larger decomposer organisms, i.e., Collembola and earthworms, were unresponsive to any factor other than removal of all plants, which reduced their populations. We also considered five functional components of the soil biota at finer taxonomic levels: three decomposer components (microflora, microbe-feeding nematodes, predaceous nematodes) and two herbivore groups (nematodes and arthropods). Taxa within these five groups responded to removal treatments, indicating that plant community composition has multitrophic effects at higher levels of taxonomic resolution. The principal ordination axes summarizing community-level data for different trophic groups in the soil food web were related to each other in several instances, but the plant ordination axes were only significantly related to those of the soil microfloral community. There were time lag effects, with ordination axes of soil-associated herbivorous arthropods and microbial-feeding nematodes being related to ordination axes representing plant community structure at earlier measurement dates. Taxonomic diversity of some soil organism groups was linked to plant removals or to plant diversity. For herbivorous arthropods, removal of C_4 grasses enhanced diversity; there were negative

correlations between plant and arthropod diversity, presumably because of negative influences of C_4 species in the most diverse treatments. There was evidence of lag relationships between diversity of plants and that of the three decomposer groups, indicating multitrophic effects of altering plant diversity. Relatively small effects of plant removal on the decomposer food web were also apparent in soil processes regulated by this food web. Decomposition rates of substrates added to soils showed no relationship with treatment, and rates of CO_2 evolution from the soil were only adversely affected when all plants were removed. Few plant functional-group effects on soil nutrient dynamics were identified. Although some treatments affected temporal variability (and thus stability) of soil biotic properties (particularly CO_2 release) throughout the experiment, there was no evidence of destabilizing effects of plant removals. Our data provide evidence that permanent exclusion of plant species from the species pool can have important consequences for overall vegetation composition in addition to the direct effects of vegetation removal, and various potential effects on both the above- and belowground subsystems. The nature of many of these effects is driven by which plant species are lost from the system, which depends on the various attributes or traits of these species. **(New Zealand)**

63. Warren, R.S., P.E. Fell, J.L. Grimsby, E.L. Buck, G.C. Rilling, and R.A. Fertik. 2001. Rates, patterns, and impacts of *Phragmites australis* expansion and effects of experimental *Phragmites* control on vegetation, macroinvertebrates, and fish within tidelands of the lower Connecticut River. *Estuaries* 24(1): 90-107.

    See Fish Section.

64. Washburn, B.E. and T.G. Barnes. 2000. Postemergence tall fescue (*Festuca arundinacea*) control at different growth stages with glyphosate and AC 263,222. *Weed Technology* 14(1): 223-230.

    Effective methods are needed to eradicate tall fescue (*Festuca arundinacea*) and convert tall fescue fields into habitats more suitable to wildlife species. The objectives of this study were to determine the efficacy of (1) a single glyphosate application during the spring or fall and (2) AC 263,222, alone and in combination with glyphosate, applied during four different tall fescue growth stages for control of established tall fescue. Studies were conducted during 1996 to 1998 in fields dominated by tall fescue located in central Kentucky. Pre- and posttreatment plant communities were described to quantify differences in vegetative characteristics due to herbicide applications. Glyphosate at 2.2 kg ai/ha applied during the spring or fall was effective in reducing tall fescue to less than 12% cover. AC 263,222 at 0.2 kg ai/ha, AC 263,222 at 0.2 kg/ha plus glyphosate at 0.6 kg/ha, or AC 263,222 at 0.2 kg/ha plus glyphosate at 1.1 kg/ha applied during the spring growth, boot, summer dormancy, and fall growth stages were equally effective, reducing tall fescue cover to less than 3% at 2 to 7 mo after treatment. Glyphosate and AC 263,222 are effective tools for the initial removal of tall fescue. **(U.S.A.)**

65. Watrud, L.S., G. King, J.P. Londo, R. Colasanti, B.M. Smith, R.S. Waschmann, and E.H. Lee. 2011. Changes in constructed *Brassica* communities treated with glyphosate drift. *Ecological Applications* 21(2): 525-538.

We constructed a mixed-species community designed to simulate roadside and field edge plant communities and exposed it to glyphosate drift in order to test three hypotheses: (1) higher fitness in transgenic *Brassica* carrying the *CP4 EPSPS* transgene that confers resistance to glyphosate will result in significant changes in the plant community relative to control communities; (2) given repeated years of glyphosate drift selective pressure, the increased fitness of the transgenic *Brassica* with *CP4 EPSPS* will contribute to an increase in the proportion of transgenic progeny produced in plant communities; and (3) the increased fitness of *Brassica* carrying the *CP4 EPSPS* transgene will contribute to decreased levels of mycorrhizal infection and biomass in a host species (*Trifolium incarnatum*). Due to regulatory constraints that prevented the use of outdoor plots for our studies, in 2005 we established multispecies communities in five large cylindrical outdoor sunlit mesocosms (plastic greenhouses) designed for pollen confinement. Three of the community members were sexually compatible *Brassica* spp.: transgenic glyphosate-resistant canola (*B. napus*) cultivar (cv.) RaideRR, glyphosate-sensitive non-transgenic *B. napus* cv. Sponsor, and a weedy *B. rapa* (GRIN Accession 21735). Additional plant community members were the broadly distributed annual weeds *Digitaria sanguinalis*, *Panicum capillare*, and *Lapsana communis*. Once annually in 2006 and 2007, two mesocosms were sprayed with glyphosate at 10% of the field application rate to simulate glyphosate drift as a selective pressure. After two years, changes were observed in community composition, plant density, and biomass in both control and treatment mesocosms. In control mesocosms, the weed *D. sanguinalis* (crabgrass) began to dominate. In glyphosate drift-treated mesocosms, *Brassica* remained the dominant genus and the incidence of the *CP4 EPSPS* transgene increased in the community. Shoot biomass and mycorrhizal infection in *Trifolium incarnatum* planted in 2008 were significantly lower in mesocosms that had received glyphosate drift treatments. Our results suggest that, over time, glyphosate drift can contribute to persistence of *Brassica* that express the *CP4 EPSPS* transgene and that increased representation of *Brassica* (a non-mycorrhizal host) within plant communities may indirectly negatively impact beneficial ecosystem services associated with arbuscular mycorrhiza. **(U.S.A.)**

66. Wilson, S.D. and M. Pärtel. 2003. Extirpation or coexistence? Management of a persistent introduced grass in a prairie restoration. *Restoration Ecology* 11(4): 410-416.

Introduced perennial grasses are one of the greatest constraints to prairie restoration. Herbicides suppress but do not eliminate introduced grasses, so we explored the interaction of herbicide with two additional controls: heavy clipping (to simulate grazing) and competition from native species. A 50-year-old stand of the introduced perennial grass *Agropyron cristatum* (crested wheatgrass) in the northern Great Plains was seeded with native grasses and treated with herbicide annually for 7 years in a factorial experiment. Clipping was applied as a subplot treatment in the final 3 years. Both herbicide and clipping significantly reduced the cover of *A. cristatum*, but clipping produced an immediate and consistent decrease, whereas herbicide control varied among years. The cover of *A. cristatum* decreased significantly with increasing cover of a seeded native grass, *Bouteloua gracilis* (blue

grama), suggesting that both top-down (i.e., grazing) and bottom-up (i.e., resource competition) strategies can contribute to *A. cristatum* control. No treatment had any effect on the seed bank of *A. cristatum*. Even in the most effective control treatments, *A. cristatum* persisted at low amounts (approximately 5% cover) throughout the experiment. The cover of *B. gracilis* increased significantly with seed addition and herbicide, and, after 7 years, was similar to that in undisturbed prairie. The total cover of native species increased significantly with clipping and herbicide, and species richness was significantly higher in plots receiving herbicide. Clipping season had no effect on any variable. In summary, no method extirpated *A. cristatum*, but clipping reduced its cover by 90% and doubled the cover of native species. Extirpation might not be a realistic goal, but relatively simple management allowed coexistence of native species. **(Canada)**

Birds

1. Buffington, J.M., J.C. Kilgo, R.A. Sargent, K.V. Miller, and B.R. Chapman. 2000. Effects of restoration techniques on breeding birds in a thermally-impacted bottomland hardwood forest. *Ecological Engineering* 15: S115-S120.

   We evaluated the effects of revegetation techniques on breeding bird communities in a bottomland hardwood forest impacted by thermal effluent. In 1993, sections of the Pen Branch bottomland on the Savannah River Site, South Carolina, were herbicide-treated (glyphosate), burned, and planted; other sections were planted only while others were unaltered and served as controls. Few differences in the avian community occurred at 1 and 2 years post-treatment among treatments. Plots that were herbicide-treated, burned, and planted had greater species richness in 1994 and abundance in 1995 than sections that were planted only ($P < 0.05$). Bird species composition differed slightly among treatments and White-eyed Vireos (*Vireo griseus*), Common Yellowthroats (*Geothlypis trichas*), Indigo Buntings (*Passerina cyanea*), and Red-winged Blackbirds (*Agelaius phoeniceus*) were the most abundant species in the corridor. Revegetation techniques used to restore this thermally-impacted bottomland had little effect on the avian communities 1 and 2 years post-treatment. **(U.S.A.)**

2. Chamberlain, D.E, S.N. Freeman, and J.A. Vickery. 2007. The effects of GMHT crops on bird abundance in arable fields in the UK. *Agriculture, Ecosystems and Environment* 118(1): 350-356.

   Bird surveys were carried out in summer 2000 and winter 2000/2001 on 24 sugar beet fields, 11 maize fields, 10 spring rape fields and (for winter-only) 23 winter rape fields divided into conventional and genetically modified herbicide tolerant (GMHT) treatments. These fields were a sub-sample of those used in the farm scale evaluation (FSE [Firbank, L.G, Heard, M.S., Woiwod, I.P., Hawes, C., Haughton, A.J., Champion, G.T., Scott, R.J., Hill, M.O., Dewar, A.M., Squire, G.R., May, M.J., Brooks, D.R., Bohan, D.A., Daniels, R.E., Osborne, J.L., Roy, D.B., Black, H.I.J., Rothery, P., Perry, J.N., 2003. An introduction to the farm-scale evaluations of genetically modified herbicide tolerant crops. J. Appl. Ecol. 40, 2-16.]). The study aimed to compare bird abundance between GMHT and conventional crop treatments. In the summer, the abundance of yellowhammers *Emberiza citrinella* and of granivores collectively was significantly greater on conventional than GMHT sugar beet. Abundance of granivores and species richness was significantly greater on conventional than GMHT maize, but only after the application of herbicides to the GMHT treatment. No significant differences were detected in spring oilseed rape. No significant differences were detected prior to herbicide application in any crop. In winter, granivores were more abundant on bare plough following conventional sugar beet treatment than following GMHT treatment. Woodpigeon *Columba palumbus*, blackbird *Turdus merula* and corvids were more abundant on maize stubbles following GMHT treatment. These differences were in accord with likely differences in food availability ascertained from previous research carried out under the FSE. **(United Kingdom)**

3. Easton, W.E. and K. Martin. 2002. Effects of thinning and herbicide treatments on nest-site selection by songbirds in young managed forests. *Auk* 119(3): 685–694.

Routine silvicultural practices continue to alter the structure and composition of forests after logging by removing deciduous vegetation from regenerating coniferous forests. We identified nest trees and surveyed vegetation in a 5 m radius surrounding songbird nests (nest patch) and compared the nest patches to available habitat in nine 11-22 year old conifer plantations (22-47 ha) where 90-96% of deciduous stems were removed by two treatments: manual thinning, and manual thinning plus application of glyphosate (herbicide). The control and two treatments were replicated three times. We characterized the nest patches of five species: Warbling Vireo (*Vireo gilvus*), Dusky Flycatcher (*Empidonax oberholseri*), Swainson's Thrush (*Catharus ustulatus*), American Robin (*Turdus migratorius*), and Chipping Sparrow (*Spizella passerina*). During three post-treatment years, areas treated with thinning plus herbicide remained depauperate of deciduous vegetation whereas thinned sites experienced deciduous regrowth. Despite variation in the density of deciduous trees and in the type of tree used for nesting in the control and treatments, nest patches were positively correlated with the amount of remaining deciduous vegetation, representing habitat that either escaped or recovered from silvicultural treatments. That relationship was stronger in areas with the fewest deciduous trees. Nests were more likely to be successful in areas with more willow. Within a mosaic of managed forest stands, birds appear to use the same proximate habitat cues for selecting a nest patch despite varying fitness consequences across different silviculture regimes. Although birds appeared to compensate for changes in stand habitat by finding patches of untreated vegetation or altering the type of tree they nested in, there was a reproductive cost for some species. **(Canada)**

4. Firbank, L.G. and F. Forcella. 2000. Genetically modified crops and farmland biodiversity. *Science* 289(5484): 1481-1482.

Scientists in Great Britain have proposed a mathematical model that seeks to measure the impact of genetically modified herbicide-tolerant sugar beet crops on weed populations in British fields and the consequences for farmland birds that are dependent on the weed seeds for food. **(United Kingdom)**

5. Homan, H.J., G.M. Linz, R.C. Carlson, and W.J. Bleier. 2003. Spring distribution of ring-necked pheasants (*Phasianus colchicus*) following cattail reduction with glyphosate herbicide. *Wildlife Research* 30(2): 159-166.

To reduce blackbird (Icteridae) damage to field crops in the north-central United States, dense stands of cattail (*Typha* spp.) are thinned with glyphosate herbicide. The stands become unusable as roosting and loafing sites, which helps to protect susceptible crops nearby, particularly sunflower (*Helianthus annus*). Landscape-level impacts of cattail management on non-target avian species have not been studied. We measured use of upland breeding territories by male ring-necked pheasants (*Phasianus colchicus*) following cattail reduction in wetlands used by pheasants for overwintering. In August 1992, glyphosate was applied to all wetlands with ≥ 70% cattail coverage in four 23-km^2 study blocks in south-eastern North Dakota. Four other blocks were used for controls. Habitat use was inferred from

territorial crowing counts. No treatment effect or treatment*year interaction (all $P \geq 0.05$) was evident during 2 years of post-treatment observations. Although the herbicide eradicated large contiguous stands of cattail that pheasants had used for winter cover, surface water levels rose in 1993, which created additional cattail growth in untreated wetlands within the blocks. The additional cattail may have lessened the effect of the herbicide treatments. During drier periods, when cattail growth slows, cattail reduction could affect use of upland breeding sites. We recommend more research to assess the effects of glyphosate during drier periods. **(U.S.A.)**

6. Jones, J.D.J. and M.J. Chamberlain. 2004. Efficacy of herbicides and fire to improve vegetative conditions for northern bobwhites in mature pine forests. *Wildlife Society Bulletin* 32(4): 1077-1084.

Declining northern bobwhite (*Colinus virginianus*) populations during the past 30 years have prompted managers to seek ways to improve habitat quality for this species. Reductions in frequency of prescribed fire throughout considerable expanses of mature pine (*Pinus* spp.) forests have resulted in closed-canopy conditions, predominantly woody understory conditions, and a loss of early-successional habitats needed by bobwhites. Herbicides, particularly in conjunction with prescribed fire, may be useful for managing these pine forests to benefit the bobwhite and other early-successional species, but effects of herbicides in combination with fire are not well understood. Therefore, we used 3 similar-aged, mature pine stands to evaluate vegetative response to selective herbicides with prescribed fire with respect to bobwhite nesting and broodrearing habitats. Our treatments were imazapyr with fire, imazapyr combined with glyphosate with fire, and dormant-season prescribed fire only. Plant diversity tended to decline on herbicide treatments during the first year but increased substantially on imazapyr plots during the second growing season following a burn. Bobwhite food plants increased following application of imazapyr during the first growing season and were greater for both herbicide treatments than burning alone during the second growing season. Abundance of hardwoods declined on both herbicide treatments. However, no treatments produced bare-ground percentages known to be selected by bobwhites, and only prescribed fire alone created and maintained suitable escape cover. Overall, imazapyr with fire provided the greatest net improvement in vegetative conditions for bobwhites and retained floristic diversity. We recommend that managers target areas in which vegetative conditions have progressed to where burning alone is incapable of restoring early-successional plant communities needed by the bobwhite and other species and apply imazapyr with fire to create diverse, patchy habitat for bobwhites. **(U.S.A.)**

7. King, D.I. and B.E. Byers. 2002. An Evaluation of powerline rights-of-way as habitat for early-successional shrubland birds. *Wildlife Society Bulletin* 30(3): 868-874.

Recent population declines among bird species that breed in early-successional shrubland habitats in the eastern United States have been associated with declines in habitat availability. Forest succession has eliminated shrublands in many locations, but powerline rights-of-way constitute a potential reservoir of shrubland

habitat for birds. We studied 2 populations of an early-successional shrubland bird, the chestnut-sided warbler (*Dendroica pensylvanica*), in powerline rights-of-way in western Massachusetts over five breeding seasons to evaluate the potential conservation value of these habitats. Our goals were to 1) measure reproductive success and adult survival of birds nesting in powerline rights-of-way, 2) test for edge-related increases in nest predation that might compromise the health of bird populations in powerline rights-of-way, and 3) evaluate whether reproductive success and adult survival rates of birds nesting in powerline rights-of-way were sufficient to maintain these populations. Our results indicated that nesting and fledging success in these populations were high, but nest success was marginally lower (P=0.09) near edges in 1 of 2 years for which distances from nests to edge were measured. Also, reproductive success and adult survival were sufficient at both sites to balance losses from mortality, suggesting that powerline rights-of-way can support populations of early-successional shrubland birds. **(U.S.A.)**

8. Lautenschlager, R.A. and T.P. Sullivan. 2002. Effects of herbicide treatments on biotic components in regenerating northern forests. *Forestry Chronicle* 78(5): 695-731.

See Biodiversity and Restoration Section.

9. Madison, L.A., T.G. Barnes, and J.D. Sole. 2001. Effectiveness of fire, disking, and herbicide to renovate tall fescue fields to northern bobwhite habitat. *Wildlife Society Bulletin* 29(2): 706-712

Fields dominated by tall fescue (*Festuca arundinacea*) are common throughout the southeastern United States and are poor habitat for northern bobwhites (*Colinus virginianus)*. Our study examined effectiveness of controlled burning, disking, and Round-Up™ herbicide applications to improve bobwhite habitat in fescue-dominated fields. We conducted the study on 4 Kentucky Department of Fish and Wildlife Resources Wildlife Management Areas (WMA). On each WMA we divided a field into 16 0.1-ha plots, and at each field we randomly assigned 2 plots to the following treatments: control, fall burning, fall disking, spring burning, spring disking, spring herbicide application, summer burning, or summer disking. We measured the vegetation structure, seed production, and floristic composition within each treatment plot from fall 1990 to summer 1994. The spring herbicide application most effectively reduced tall fescue coverage. Fescue coverage was reduced for one year following disturbance by fall, spring, and summer disking, but had become similar to control plots and pre-treatment conditions by the second year post-treatment. Fall, spring, and summer burning did not reduce tall fescue coverage. Fall-disked plots improved habitat for bobwhite winter feeding during winter 1993, whereas herbicide-treated plots provided the best winter feeding habitat during winter 1994. Herbicide-treated plots provided the best habitat quality for bobwhite nesting in summer 1993, but no treatment satisfied nesting habitat requirements in summer 1994. **(U.S.A.)**

10. Marc, J., R. Bellé, J. Morales, P. Cormier, O. Mulner-Lorillon. 2004. Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the

prevention of G2/M transition. *Toxicological sciences: an official journal of the Society of Toxicology* 82(2): 436-442.

See Amphibians, Aquatic Invertebrates and Plants, and Algae Section.

11. May, M.J., G.T. Champion, A.M. Dewar, A. Qi, and J.D. Pidgeon. 2005. Management of genetically modified herbicide-tolerant sugar beet for spring and autumn environmental benefit. *Proceedings of the Royal Society B* 272: 111-119.

When used in genetically modified herbicide-tolerant (GMHT) crops, glyphosate provides great flexibility to manipulate weed populations with consequences for invertebrates and higher trophic levels, for example birds. A range of timings of band and overall spray treatments of glyphosate to GMHT sugar beet were compared with a conventional weed control programme in four field trials over 2 years. Single overall sprays applied between 200 and 250 accumulated day degrees (above a base air temperature of 3°C; °Cd) and band applied treatments applied at 10% or 20% ground cover within the crop rows generally gave significantly greater weed biomass and seed rain than conventional treatments, while later band sprays (more than 650 °Cd) reduced seed return. Two overall sprays of glyphosate produced low weed biomass and generally lowest seed return of all treatments but tended to give some of the highest yields. However, the early overall sprays (200-250°Cd) and band sprays gave as good or better yields than the conventional and were generally equivalent to the two overall-spray programme. Viable seeds in the soil after the experiment were generally higher following the early overall (200-250°Cd) and the band spray treatments than following the conventional. The results show that altered management of GMHT sugar beet can provide alternative scenarios to those of the recent Farm Scale Evaluation trials. Without yield loss they can enhance weed seed banks and autumn bird food availability compared with conventional management, or provide early season benefits to invertebrates and nesting birds, depending on the system chosen. Conventional weed control does not have the flexibility to enable these scenarios that benefit both agriculture and environment, although there may be some options for increasing weed seed return in autumn. **(United Kingdom)**

12. Peterson, R.K., and A.G. Hulting. 2004. A comparative ecological risk assessment for herbicides used on spring wheat: the effect of glyphosate when used within a glyphosate-tolerant wheat system. *Weed Science* 52: 834-844.

Glyphosate-tolerant spring wheat currently is being developed and most likely will be the first major genetically engineered crop to be marketed and grown in several areas of the northern Great Plains of the United States. The public has expressed concerns about environmental risks from glyphosate-tolerant wheat. Replacement of traditional herbicide active ingredients with glyphosate in a glyphosate-tolerant spring wheat system may alter ecological risks associated with weed management. The objective of this study was to use a Tier 1 quantitative risk assessment methodology to compare ecological risks for 16 herbicide active ingredients used in spring wheat. The herbicide active ingredients included 2,4-D, bromoxynil, clodinafop, clopyralid, dicamba, fenoxaprop, flucarbazone, glyphosate, MCPA, metsulfuron, thifensulfuron, tralkoxydim, triallate, triasulfuron, tribenuron, and trifluralin. We compared the relative risks of these herbicides to glyphosate to

provide an indication of the effect of glyphosate when it is used in a glyphosate-tolerant spring wheat system. Ecological receptors and effects evaluated were avian (acute dietary risk), wild mammal (acute dietary risk), aquatic vertebrates (acute risk), aquatic invertebrates (acute risk), aquatic plants (acute risk), nontarget terrestrial plants (seedling emergence and vegetative vigor), and groundwater exposure. Ecological risks were assessed by integrating toxicity and exposure, primarily using the risk quotient method. Ecological risks for the 15 herbicides relative to glyphosate were highly variable. For risks to duckweed, green algae, groundwater, and nontarget plant seedling emergence, glyphosate had less relative risk than most other active ingredients. The differences in relative risks were most pronounced when glyphosate was compared with herbicides currently widely used on spring wheat. **(U.S.A.)**

13. Rodgers, R.D. 2002. Effects of wheat-stubble height and weed control on winter pheasant abundance. *Wildlife Society Bulletin* 30(4): 1099-1112.

    Recent changes in agriculture on the semi-arid central High Plains have serious implications for pheasants (*Phasianus colchicus*) and other farmland wildlife. Of greatest concern are increased herbicide use accompanying intensification of crop rotations and the shorter wheat (*Triticum aestivum*)-stubble heights produced by a shift to semi-dwarf wheat varieties and increasingly powerful combines. From 1990-1995, I investigated the effect of stubble height and post-harvest weed control on subsequent winter abundance of pheasants in wheat stubble. Increasing wheat cutting height from a mean of 22 cm to 46 cm produced a nearly 9-fold average increase in indices of winter pheasant abundance in wheat-stubble test blocks where no post-harvest weed control was performed. Post-harvest weed growth was positively affected by wheat-stubble height, probably due to taller stubble's ability to better conserve limited moisture by reducing ground-level air movement. Herbicide application to stubble reduced indices of winter pheasant abundance by >80%, and tillage reduced those indices by >90%, compared to untreated fields. Herbicide application to wheat stubble and reduced stubble height are considered major causes of the long-term decline of pheasants on the central High Plains. This research and a companion agronomic study have shown that increased stubble height and post-harvest weed growth in wheat stubble are integral components of a modified wheat-fallow rotation that provides superior habitat quality, soil conservation benefits, and greater profitability than other wheat-fallow systems currently in use. The benefits of greater wheat-stubble height can also be applied in more intensive wheat-row-crop-fallow rotations. **(U.S.A.)**

14. Stoleson, S.H., T.E. Ristau, D.S. deCalesta, and S.B Horsley. 2011. Ten-year response of bird communities to an operational herbicide–shelterwood treatment in a northern hardwood forest. *Forest Ecology and Management* 262(7): 1205-1214.

    Use of herbicides in forestry to direct successional trajectories has raised concerns over possible direct or indirect effects on non-target organisms. We studied the response of forest birds to an operational application of glyphosate and sulfometuron methyl herbicides, using a randomized block design in which half of each 8 ha block received herbicide and the other acted as a control, on shelterwood

seed-cut Allegheny hardwood stands in northwestern Pennsylvania. We monitored birds using 50 m radius point counts in two pretreatment years and for 10 years post-treatment (1992-2004). Fences were erected six years after herbicide treatment in response to increased deer browsing at a subset of sites. Avian responses to herbicide treatment varied by nesting guild: we detected no response by cavity-nesters, but documented declines in the shrub-, ground- and canopy-nesting guilds. Responses were short-lived (2-4 years post-treatment), but shrub-nesters did not recover until fencing provided regenerating vegetation respite from browsing. Thus, apparent responses of birds to herbicide were confounded with deer browsing in this study. High species turnover, even within control plots, suggests that avian communities may be assessed more appropriately assessed at larger spatial scales than those used in this study (6.5-8 ha). **(U.S.A.)**

15. Sullivan, T.P. and D.S. Sullivan. 2003. Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems. *Environmental Reviews* 11: 37-59.

See Biodiversity and Restoration Section.

16. Zimmerman, G., F.W. Bell, J. Woodcock, A. Palmer, and J. Paloniemi. 2011. Response of breeding songbirds to vegetation management in conifer plantations established in boreal mixedwoods. *The Forestry Chronicle* 87(2): 217-224.

We examined the response of breeding songbird communities 11 years after four vegetation management treatments were applied. Post-treatment vegetation was characterized by manually interpreting large-scale aerial photography and estimating proportional cover of eight vegetation classes. Songbird abundance was assessed by territory mapping. Using GIS layers, number of registrations and average vegetation proportions were compared among treatments. Ordination of the relative abundance of the 11 most frequent bird species suggested differences between Vision® and untreated areas. These results show that effects of vegetation management on songbirds may be more persistent than previous studies suggest and that monitoring should continue. **(Canada)**

Crop Tree Productivity

1. Balneaves, J.M. 1982. Grass control for radiata pine establishment on droughty sites. *New Zealand Journal of Forestry* 27(2): 259-276.

   Survival and growth of radiata pine (*Pinus radiata* D. Don) seedlings in the warm dry-summer climate of Canterbury are markedly influenced by competition from grasses and other herbaceous weeds. Weed control not only produced an immediate increase in growth but also had a positive effect on tree growth for the following eight years. Site improvement by either ripping or weed control had a marked effect on tree growth. A combination of the two further increased survival, height growth, and total basal area. With the option of either ripping or weed control, the results suggested that post-planting weed control would give better value for money. Further work on the effects of fertiliser application in the presence or absence of weed competition indicated that failure to achieve good weed control will result in an increase in weed competition and may reduce the survival and growth of radiate pine seedlings. Broadcast vegetation control resulted in better survival and growth of tree seedlings. If, however, spot application is desired because of costs, then the “Spot Gun" applicator may be used with hexazinone. Best results can be achieved with the flat fan nozzle. Costs for such an operation on hill country are around $45/ha. **(New Zealand)**

2. Bell, F.W. and S.G. Newmaster. 2002. The effects of silvicultural disturbances on the diversity of seed-producing plants in the boreal mixedwood forest. *Canadian Journal of Forest Research* 32(7): 1180-1191.

   See Biodiversity and Restoration Section.

3. Bell, F.W. and D.G. Pitt. 2007. Seasonal susceptibility of boreal plants: red raspberry phenology as a bioindicator of optimum within-season timing of glyphosate applications. *The Forestry Chronicle* 83: 733-741.

   In Canada, forest managers operating under public licenses are under pressure from the public to cease using herbicides or at minimum reduce the quantity of active ingredient applied in the environment. Lacking in their decision-making toolbox is information about biological cues that could help optimize herbicide performance. In 1990, two rates of the herbicide glyphosate, 1.1 and 1.7 kg acid equivalent (a.e.) ha^{-1}, were applied bi-weekly between July 21 and September 25 using a backpack sprayer to release jack pine (*Pinus banksiana* Lamb.) seedlings from red raspberry (*Rubus idaeus* L. var. *strigosus* (Michx.) Maxim.) competition. On average, the higher application rate reduced raspberry cover by at least 6% more than the lower rate ($p < 0.01$). Control of raspberry was poor with the earliest application, peaked with mid- to late-summer applications, and decreased with late-season applications. Peak jack pine performance, as measured by stem volume index, followed a mid-August application at the low rate. Earlier applications resulted in substantial herbicide injury and later applications were not as effective at reducing raspberry competition. The optimum timing for jack pine performance corresponded with the period between the beginning of raspberry’s floricane senescence (i.e., end of full flowering) and the initiation of primocane senescence (i.e., fruit maturation).

Seedlings released in mid-August maintained a growth advantage over other seedlings from the fifth through to the tenth year of this study. Discerning forest managers may choose to use phenological cues from the target species, such as red raspberry, as a bioindicator of glyphosate efficacy. **(Canada)**

4. Bell, F.W., M. Kershaw, I. Aubin, N. Thiffault, J. Dacosta, and A. Wiensczyk. 2011. Ecology and traits of plant species that compete with boreal and temperate forest conifers: An overview of available information and its use in forest management in Canada. *The Forestry Chronicle* 87(2): 161-174.

   In boreal and temperate forests in Canada, at least 71 plant species, including trees, shrubs, herbs, grasses, and ferns, have the potential to significantly reduce the growth of conifer regeneration. A thorough understanding of the autecology of these plants—their response to their environment - can help resource managers to improve their approaches to vegetation management, thereby maximizing crop tree growth and survival. In this paper, we highlight key sources of information about the autecology of the major species that compete with forest conifers, including books and field guides, journal series, Web sites, and plant trait databases. We suggest ways that this information can be applied in resource management, recommend approaches for maintaining and updating this information, and underline the needs for developing a single, consolidated, comprehensive source of such information for use by resource managers and researchers. Information gaps are also briefly discussed. **(Canada)**

5. Bell, F.W., R.A. Lautenschlager, R.G. Wagner, D.G. Pitt, J.W. Hawkins, and K.R. Ride. 1997. Motor–manual, mechanical, and herbicide release affect early successional vegetation in northwestern Ontario. *The Forestry Chronicle* 73(1): 61-68.

   Cover and height of vegetation before and one growing season after: 1) motor-manual cutting, 2) mechanical brush cutting (Silvana Selective/Ford Versatile), 3) aerial application of Release® (a.i. triclopyr) herbicide, 4) aerial application of Vision® (a.i. glyphosate) herbicide, and 5) control (no treatment) were quantified. Multivariate analysis permitted the study of vegetation response as a whole, while accounting for correlations that exist among the individual vegetation groups. Univariate analysis were used to study the responses of individual vegetation groups. Although no pre-treatment differences in percent cover were observed (P = 0.128), deciduous tree, shrub, forb, grass, and sedge groups responded differently to the treatments after one growing season (P < 0.018). Post-treatment cover of deciduous tree and shrub groups was lower in herbicide treated plots than in cut plots. Forb, grass and sedge covers varied greatly among treatments. Brush saw and Silvana Selective treatments decreased cover of deciduous trees. Release® decreased cover of deciduous trees and shrubs. Vision® decreased cover of deciduous trees, shrubs and ferns. Cover of all vegetation groups increased on the untreated control. Among the conifer release treatments examined, Vision® reduced woody and herbaceous vegetation most. **(Canada)**

6. Bell, F.W., J. Dacosta, M. Penner, A. Morneault, A. Stinson, B. Towill, N. Luckai, and J. Winters. 2011. Longer-term volume trade-offs in spruce and jack pine plantations following various conifer release treatments. *The Forestry Chronicle* 87(2): 235-250.

   We assessed growth responses 10 years post treatment for 31 combinations of site, species, and treatments from six studies in Ontario, Canada, to determine if conifer release treatments increase gross total conifer volumes but decrease gross total stand volumes in boreal forests. Treatments included single and multiple herbicide application or motor-manual and mechanical conifer release. Treatment effects on 10^{th}-year gross total preferred conifer and total stand volumes ranged from −49% to +556% and −71% to +116%, respectively, compared to the untreated controls. We projected net merchantable stand volumes (NMV) from 10 years post treatment to 60 years of age. These projections indicate that NMV of preferred conifers at age 60 could range from 7.4 m^3 ha^{-1} to 232.4 m^3 ha^{-1}. The variation in observed and predicted volumes can be attributed to site characteristics, tree species, ecology, and treatment efficiency. **(Canada)**

7. Bell, F.W., J. Parton, N. Stocker, D. Joyce, D. Reid, M. Wester, A. Stinson, G. Kayahara and B. Towill. 2008. Developing a silvicultural framework and definitions for use in forest management planning and practice. *The Forestry Chronicle* 84(5): 678-693.

   Lack of a management framework on which to base silviculture options has plagued the forest management planning process in Ontario. The Forest Management Planning Manual for Ontario's Crown Forests directs that strategic silvicultural options be developed and identified in terms of the (i) applicable forest unit, (ii) associated assumptions, and (iii) extent to which they can be used on a forest management unit. In this paper, we describe a framework for classifying management (or silviculture) intensity and propose definitions for extensive, basic, intensive, and elite intensities of silviculture to support the framework's use in planning and application. We outline how the Canadian Ecology Centre – Forestry Research Partnership, a research partnership between Tembec Inc., the Ontario Ministry of Natural Resources, and Natural Resources Canada, is considering applying these in the forest management planning process. The framework and definitions can be used to develop strategic silviculture options within an active adaptive management approach. This framework should help to reduce uncertainties associated with forest development, treatment costs, response to treatments, and success rates provided appropriate monitoring. The framework and definitions described were specifically developed for silviculture related to reforestation of even-aged boreal forests. **(Canada)**

8. Biring, B.S., P.G. Comeau, and P. Fielder. 2003. Long-term effects of vegetation control treatments for release of Engelmann spruce from a mixed-shrub community in Southern British Columbia. *Annals of Forest Science* 60: 681-690.

   In British Columbia, vegetation management treatments are widely used to ensure successful establishment of young stands and achievement of free-growing requirements. A study was established in 1991 to examine the effectiveness of vegetation control treatments for release of Engelmann spruce (*Picea engelmannii*

Parry) seedlings from a mixed-shrub community. The study consisted of eight treatments replicated three times in a completely randomized design. The treatments comprised six combinations of spring, summer and annual repeated manual cutting, a single application of glyphosate, and an untreated control. Controlling the mixed-shrub community one-year after planting using glyphosate and manual cutting treatments significantly improved spruce survival. Repeated manual cutting significantly improved survival over that achieved with only a single treatment. Consequently, the density of well-spaced trees was significantly increased in the repeated manual cutting and glyphosate treatments. In 2001, the untreated control only has 27% of well-spaced spruce trees that are free growing compared to more than 50%, 75% and 83% in single cutting, repeated manual cutting and glyphosate treatment, respectively. Treatments significantly increased height and groundline diameter from the third through the seventh year but not in year ten. Continued mortality of suppressed seedlings after year seven is a probable cause of lack of treatment differences in the tenth year. However, height-to-diameter ratio was significantly reduced in year ten for all treatments over the control and for repeated versus single cutting treatments. Ten-years after treatment, significant differences in vegetation community percent cover, richness, and diversity were not detected among treatments. **(Canada)**

9. Boateng, J.O., J.L. Heineman, J. McClarnon, and L. Bedford. 2006. Twenty year responses of white spruce to mechanical site preparation and early chemical release in the boreal region of northeastern British Columbia. *Canadian Journal of Forest Research* 36(10): 2386-2399.

   The effects of six mechanical site preparation treatments, two stock-type treatments, and early chemical release on survival and growth of planted white spruce (*Picea glauca* (Moench) Voss) were studied in the BWBSmw1 biogeoclimatic zone of northeastern British Columbia. After 20 years, spruce height and diameter were larger in all mounding treatments than in the control. Early results suggested better spruce performance on large than small mounds, but after 20 years, growth was equally good on small mounds as on mounds with 20 cm mineral capping. Spruce planted on hinge positions in the Bräcke patch and blade scarification treatments did not survive or grow well. Early chemical release improved spruce growth equally as well as the mounding treatments. Twenty year spruce survival averaged 71% in the 14 and 20 cm mound treatments, 60% in the early chemical release treatment, and ≤35% in the Bräcke patch and blade scarification treatments. A large stock type was also planted in untreated ground and, after 20 years, had similar survival and growth as the standard stock type. Differences in survival had a large effect on basal area at age 20 years. Trend analysis showed that treatments diverged into two distinct groups with regard to spruce size during the 20 year span of the study. **(Canada)**

10. Boateng, J.O., J.L. Heineman, L. Bedford, G.J. Harper, and A.F. Nemec. 2009. Long-term effects of site preparation and postplanting vegetation control on *Picea glauca* survival, growth and predicted yield in boreal British Columbia. *Scandinavian Journal of Forest Research* 24(2): 111-129.

The 19-20-year effects of mechanical site preparation, windrow burning, chemical site preparation, and postplanting vegetation control on survival and growth of planted white spruce are reported from two boreal sites in British Columbia, Canada. Survival differed between treatments at both sites, but was relatively good (≥77%) even in untreated plots. Current data regarding the proportion of spruce that were physically overtopped by vegetation and previous results from related soils and vegetation studies suggest that lasting reductions in tall shrub and aspen abundance were more important to spruce growth than early microenvironmental effects associated with manipulating the rooting environment. At Inga Lake, postplanting vegetation control produced a 13-fold increase in spruce volume over the control after 19 years, which was statistically equivalent to increases resulting from fine mixing, plow-inverting and windrow burning site preparation treatments. At Iron Creek, chemical site preparation and plow-inverting quadrupled spruce volume, whereas mounding, patch scarification and disc trenching were ineffective. Growth and yield simulations using treatment-specific site index curves for Inga Lake suggested that rotation length could be shortened by 12-16 years through the use of site preparation or postplanting vegetation control. However, untreated areas, due to the relatively good survival of white spruce at age 19, were predicted to produce equivalent volume if left to grow to mean annual increment culmination age. **(Canada)**

11. Borders, B.E. and R.L. Bailey. 2001. Loblolly pine—pushing the limits of growth. *Southern Journal of Applied Forestry* 25(2): 69-74.

With mean annual increments up to 5.4 cords/ac/yr, six loblolly pine (*Pinus taeda* L.) research sites in Georgia produced yields on par with other results from intensively managed loblolly plantations around the world. Cultural treatments in the Georgia study include complete control of vegetation other than the planted pines with multiple applications of herbicide, annual fertilization, the combination of complete vegetation control and annual fertilization, and an intensive mechanical site preparation treatment. Complete vegetation control resulted in higher yield production at ages 10 to 12 yr than the intensive mechanical treatment at all locations. This regime also resulted in higher yield production than the annual fertilization treatment at four of six locations. Volume mean annual increment for 10- to 12-yr-old plantations with the combination treatment of complete vegetation control and annual fertilization ranged from 325 to 490 ft^3/ac, growth rates comparable to those obtained at other high biomass production areas throughout the world. Our economic evaluation based on these results shows that 8 to 12% real rates of return are feasible from investments in intensive loblolly pine plantations in the southeastern United States. **(U.S.A.)**

12. Boreham, G.R. and R.N. Pallett. 2009. The influence of tree improvement and cultural practices on the productivity of *Eucalyptus* plantations in temperate South Africa. *Southern Forests* 71(2): 85-93.

The South African forestry industry is dependent on a resource of fast-growing, low-cost wood to remain globally competitive. Land availability and the legislative requirements for afforestation in South Africa are limiting factors for future growth

and therefore increasing the productivity per unit area of existing land offers the largest potential to improving forest productivity and reducing the unit cost of wood production. Operational gain integrates all the components of the deployment process that have an influence on final stand yield, and these include tree breeding efficiency, propagation efficiency, plant use efficiency, matching species to sites, stand density and early growth through cultural practices. The aim of this study was to gain increased understanding of the interactions between intensive levels of silvicultural management and the deployment of improved genotypes of a number of commercial *Eucalyptus* species, across a range of sites, with varying levels of productivity. To demonstrate the influence of factors associated with productivity gains, a series of five trials were established, on five different sites, within the warm and cool temperate regions of KwaZulu-Natal. At each site and for each factor, a treatment that would exhibit superior productivity was deliberately chosen over a second treatment representing inferior but not uncommon operational practice. The four main factors tested at each site were species (recommended relative to an alternative choice), genetic level (unimproved relative to some level of improvement), planting density (recommended 1 667 stems ha^{-1} relative to a suboptimal 1 111 stems ha^{-1}), and silvicultural intensity (combination of fertiliser, water and optimum weeding termed 'high', relative to a suboptimal level 'low'). Significant productivity improvements at approximately five years (mid-rotation) were demonstrated for the main factors across site productivities and species. Silvicultural treatments contribute slightly more to productivity improvements than tree improvement and species choice. In this trial series, achieving high stocking and fertilising with adequate weed control influenced *Eucalyptus* productivity to a greater degree than deploying improved genetic material and matching the correct species to the site. The influence of genetics and species has increased over the past three years. In general, interactions between the main factors remain absent, highlighting the additive nature of productivity improvements for each of the main factors. **(South Africa)**

13. Burgess, D., G. Adams, T. Needham, C. Robinson, and R. Gagnon. 2010. Early development of planted spruce and pine after scarification, fertilization and herbicide treatments in New Brunswick. *The Forestry Chronicle* 86(4): 444-454.

Scarification, fertilization and herbicide responses were investigated within nine years of planting black, Norway and white spruce, and jack pine seedlings in a clearcut in a mixedwood forest in New Brunswick, Canada. The study was designed as a complete, randomized block split-plot design with three replicates. Tree survival overall was 84%. Only jack pine survival improved significantly, with silvicultural treatment increasing from 51% to 78% with operational and to 82% with intensive herbicide applications, and to 84% after scarification alone. As expected, jack pine had greater early growth than spruce. Herbicide reduced competition and dramatically increased early growth of all four tree species, and scarification generally interacted with herbicide to further increase growth. Mean height at age nine increased with silvicultural treatments from 1.6 m to 4.0 m for jack pine, 1.2 m to 2.8 m for black spruce and 0.8 m to 2.2 m for Norway and white spruce. Foliar NPK nutrient concentrations generally increased after fertilization and herbicide

applications, as did soil NPK availability when examined using ion exchange resins. Major plant competitors were trembling and largetooth aspen, red maple, beaked hazel and white birch. Higher competition after treatment at the New Brunswick site under more operational conditions was a key factor in lowering its tree productivity compared with a similar research study with much more aggressive competition control in northern Ontario involving both jack pine and black spruce. **(Canada)**

14. Carter, M.C. and C.D. Foster. 2004. Prescribed burning and productivity in southern pine forests: a review. *Forest Ecology and Management* 191(1): 93-109.

Fire is an ancient tool but still widely used in the management of southern pine forests. Fire is a relatively inexpensive tool and has a number of beneficial uses such as removing logging debris, reducing wildfire risk, increasing the abundance of certain understory species, and maintaining or restoring certain ecological conditions. However, recent studies demonstrate that fire can play a significant role in regulating the productivity of certain ecosystems. Burning releases large quantities of carbon and essential nutrients to the atmosphere as gases and particulates. The adverse impact of these releases on air quality is widely recognized, but the potential impact of nutrient losses and changes in soil productivity have received less attention from southern pine managers. The effect of fire on nitrogen (N) pools is especially significant since N availability is one of the most common limiting factors in forest productivity. The amount of N lost during the burning of forest fuels is directly related to fuel consumption and ranges from 3 to 6 kg N Mg^{-1} of fuel consumed. The combined losses of N and other elements through harvesting and burning appear to exceed considerably the rate of replacement by natural processes, and may necessitate the regular application of fertilizer to maintain the nutrient capital of the site. Burning results in a short-term increase in soil available N and other nutrients immediately after burning, which stimulates the growth of understory vegetation; a desirable effect in some settings but also a source of competition for newly planted pine seedlings. A more thorough understanding of the biogeochemical effects of prescribed fire of varying frequencies and severities is needed to make optimum use of this tool in sustainable forest management. **(U.S.A.)**

15. Comeau, P.G., B.S. Biring, and G.J. Harper. 2000. Effectiveness of repeated manual cutting and glyphosate for release of Engelmann spruce from mixed-shrub herb vegetation. *Western Journal of Applied Forestry* 15(3): 154-162.

This study, initiated in 1992, examined the effectiveness of repeated cutting for controlling vegetation in a mixed-shrub-herb community to release planted Engelmann spruce (*Picea engelmannii*) seedlings. Eight treatments were compared: (a) cut once in spring of 1992, (b) cut once in summer of 1992, (c) 3 yr of cutting once annually in the spring (mid to late June) starting in 1992, (d) 3 yr of cutting once annually in the summer (late July) starting in 1992, (e) 3 yr of cutting twice annually, in the spring and in the summer starting in 1992, (f) 2 yr of cutting twice annually, in the spring and in the summer starting in 1993, (g) application of glyphosate herbicide at 2.1 kg ai/ha during late August, 1992, and (h) an untreated control. Treatments were replicated 3 times in 30 X 30 m plots. Glyphosate

treatment (g) and spring cutting treatments (c, e, and f) significantly reduced vegetation (vascular plant) cover and height compared to the control in 1993 and 1994. There were no significant differences in vegetation cover among treatments in year 5 (1996), but vegetation height was significantly lower in the glyphosate treatment (g) and repeated cutting treatments (c, d, e, and f) compared to the control. After 5 yr, spruce survival was high for both control (86%) and treated (> 91%) plots, with no significant (P< 0.05) differences detected among treatments. Repeated cutting treatments (c, d, e, and f) and glyphosate treatment (g) significantly increased root collar diameter (rcd) over that of untreated (h) seedlings, and repeated summer cutting (d) significantly increased seedling height. Three years of repeated cutting (c, d, and e) increased rcd by 54%, and glyphosate (g) increased rcd by 46% compared to the control (h). At the end of year 5, seedlings receiving 3 yr of cutting (e) were not significantly larger than seedlings receiving only 2 yr of cutting (f). **(Canada)**

16. Comeau, P.G. and G.J. Harper. 2009. Effects of vegetation control treatments for release of Engelmann spruce from a mixed-shrub community in southern British Columbia – Year 15 results. *The Forestry Chronicle* 85(4): 583-592.

    Fifteen-year results from a study initiated in 1992 to compare the effectiveness of repeated cutting treatments with treatments involving single cutting or a single application of glyphosate herbicide are presented. While treatments resulted in significant improvements in diameter and height of planted spruce at ages 3 to 7, these differences were no longer significant in year 15. All vegetation control treatments significantly increased survival in year 15 but did not significantly affect average spruce size. Despite apparent initial treatment effects on vegetation cover, the treatments applied in this study have had little lasting effect on non-crop vegetation. **(Canada)**

17. Cyr, G. and N. Thiffault. 2009. Long-term black spruce plantation growth and structure after release and juvenile cleaning: A 24-year study. *The Forestry* Chronicle 85(3): 417-426.

    Vegetation management is crucial to meet growth and yield objectives in conifer plantations. But, the combined and long-term effects of mechanical release and juvenile cleaning on growth and stand structure have yet to be documented in black spruce plantations. A long-term study was carried out in Quebec (Canada) to evaluate the interactions between initial mechanical release at age 2 years and juvenile cleaning at age 14 years (i.e., a second release treatment) on planted black spruce survival and dimensions at age 24 years. Population structure and stand species composition were also assessed. Results showed that release and juvenile cleaning had an additive, positive effect on survival, diameter at breast height (DBH), height, crown width, crown length, and the last 5-year DBH and height increments. Juvenile cleaning effects were of higher magnitude than release effects, especially on 5-year DBH increment. Combination of both treatments reduced DBH and height variability of saplings, whereas juvenile cleaning alone resulted in a higher proportion of saplings occupying higher height classes. Total merchantable basal area was constant among treatments. But, without juvenile cleaning, hardwoods

occupied a higher proportion of the basal area and were taller than spruces. In a context of sustainable forest management, in which conifer plantations are expected to offer high wood yield, our results demonstrate the importance of juvenile cleaning following initial mechanical release to promote crop tree growth and yield. **(Canada)**

18. Dacosta, J., K. Szuba, F.W. Bell, T. Moore, K. Lennon, J. Leach, D. Bazeley, and N. Luckai. 2011. Modelling landscape-level effects of reduced herbicide use in two forests in northern Ontario. *The Forestry Chronicle* 87(2): 290-309.

    In Ontario, forest management planning requires that legal obligations to sustain forest composition and pattern, wildlife habitat, and other values are met, while at the same time addressing, for example, wood supply needs, costs, forest access, and stakeholder concerns. One of the latter is pressure to reduce herbicide use. Stand-level effects of vegetation management alternatives have been documented, but how these effects scale up to the landscape-level and affect the achievement of social, ecological, and economic objectives embedded in forest management plans (FMPs) remains uncertain. We applied nine modelling scenarios in the context of approved FMPs to explore the potential landscape-level effects of replacing herbicide use with an alternative (brush saw) for two large forests in northeastern Ontario. Results of non-spatial and spatial modelling over 60 years suggested that although herbicides are applied to only 25% to 34% of the harvested area in these forests, reductions in use would affect: (i) overall wood supply, with 14% to 44% less conifer and 6% to 17% less hardwood available; (ii) habitat supply, with less habitat for species preferring recent disturbances and more habitat for species preferring mature and older forest; (iii) costs, with wood transportation cost increasing by 16% to 20% and increased spending on silviculture; (iv) size and distribution of cutblocks and disturbance patches, with more small patches; and (v) the extent of the active road network, which would increase. **(Canada)**

19. Dampier, J.E.E., F.W. Bell, M. St-Amour, D.G. Pitt, and N.J. Luckai. 2006. Cutting versus herbicides: Tenth-year volume and release cost-effectiveness of sub-boreal conifer plantations. *The Forestry Chronicle* 82(4): 521-528.

    Few cost-effectiveness studies of vegetation management in conifer plantations are reported in the literature. This study provides follow-up cost-effectiveness analysis from research conducted at the Fallingsnow Ecosystem Project in northwestern Ontario, Canada with the objective of determining the relationship between release treatment costs and planted white spruce (Picea glauca [Moench] Voss) stem volume ($\$ m^{-3}$) ten years after alternative release treatments. Treatment cost estimates for 2003 were calculated by applying 1993 time-study data to estimated 2003 market costs for each treatment component. Untreated control plots had no treatment costs and were not included in the analysis. Including them will always suggest that doing nothing will be the most cost-effective, regardless how limited spruce volume is. The most cost-effective treatment was the aerial application of herbicide Vision ($\$12.16\ m^{-3}$), followed by the aerial application of herbicide Release ($\$12.18\ m^{-3}$), cutting with brushsaw ($\$38.38\ m^{-3}$) and mechanical tending by Silvana Selective ($\$42.65\ m^{-3}$). No cost differences were found between the herbicide treatments ($p = 0.998$) or between the cutting treatments ($p = 0.559$).

The herbicide treatments were three-fold more cost-effective than the cutting treatments (p = 0.001). This analysis only considered the planted conifer component of these young stands. **(Canada)**

20. Fortier, J. and C. Messier. 2006. Are chemical or mechanical treatments more sustainable for forest vegetation management in the context of the TRIAD? *The Forestry Chronicle* 82(6): 806-818.

See Biodiversity and Restoration Section.

21. Fournier, A., A. Bouchard, and A. Cogliastro. 2007. Artificial regeneration of hardwoods in early successional shrub communities using two clearing intensities and herbicide application. *Northern Journal of Applied Forestry* 24(3): 184-191.

In southern Quebec, returning abandoned farmland to forest production presents a management opportunity. The shrub communities, which naturally colonize abandoned agricultural land, could be enriched by planting hardwood species that occurred in the precolonial forests. This study examines the growth of four hardwood species (*Acer saccharum*, *Fraxinus americana*, *Prunus serotina*, and *Juglans nigra*) planted on former pastures now covered by shrubby vegetation. Retaining part of this shrubby vegetation may produce improved growth in the planted trees. The experimental plantations were established in 1998 in two sites with different soil conditions and consisted of various treatments to control competition. Analyses seek to determine the effect of these treatments on (i) the light conditions, (ii) the cover of competing vegetation, and (iii) the growth and vigor of planted trees. Results show that increasing shrub clearing intensity has reduced the cover of tall competing vegetation after 5 years. However, light conditions and the cover of low competing vegetation around planted trees no longer vary significantly among treatments after 5 years. Strip clearing (SC) improved the growth of white ash and total clearing improved the growth of black walnut, with respect to site considered. Herbicide use was beneficial for the majority of species. SC presents a useful alternative for hardwood plantations. However, low competing vegetation control remains an important factor for increasing planted tree productivity in these managed shrub communities. **(Canada)**

22. Fu, S., F.W. Bell, and H.Y.H. Chen. 2007. Long-term effects of intensive silvicultural practices on productivity, composition, and structure of northern temperate and boreal plantations in Ontario, Canada. *Forest Ecology and Management* 241(1): 115-126.

Long-term interactive effects of site preparation, brush control and fertilization treatments on productivity, composition and structure were examined on jack pine (*Pinus banksiana* Lamb.), black spruce (*Picea mariana* [Mill.] B.S.P.), white pine (*Pinus strobus* L.) and white spruce (*Picea glauca* [Moench] Voss) plantations, 15 years after planting in Ontario, Canada. For all crop species, growth performance increased significantly with site preparation intensity on sites without brush control, but did not change on sites with brush control. The effect of brush control and site preparation on stand volume of crop trees varied among plantations while fertilization had no effect. For the jack pine plantation, stand volume of crop trees

increased with brush control and site preparation intensity, while volume of non-crop trees did not differ among treatments. For the black spruce plantation, brush control increased and site preparation marginally affected stand volume of crop trees. However, brush control decreased non-crop tree hardwood volumes, resulting in a significant increase in total stand volume. In the white pine and white spruce plantations, stand volume of crop trees increased while that of non-crop trees declined, resulting in no difference in total stand volume. However, there was a significant shift of stand composition from hardwood dominated, mixed, to crop trees dominated with silvicultural intensity. Height structure changed with increasing silvicultural intensity for all plantations, showing a shift from a hardwood dominated multi-layer canopy (a canopy of two or more layers mixed of crop trees and non-crop trees) to a crop tree dominated canopy with an understory of naturally established shade-tolerant seedlings. We conclude that intensive silviculture is a useful tool for managing stand composition and structure of boreal and northern temperate plantations, but increases in total stand volume may not occur as expected. **(Canada)**

23. Fu, S., H.Y.H. Chen, F.W. Bell, M. Sharma, J.R. Delaney, and G, Peterson. 2008. Effects of timing of glyphosate application on jack pine, black spruce, and white spruce plantations in northern Manitoba. *The Forestry Chronicle* 84(1): 37-45.

    Deferring conifer release treatments is sometimes necessary, but its effects on crop tree performance are not well understood. We investigated the effects of deferring glyphosate applications on jack pine (*Pinus banksiana* Lamb.), black spruce (*Picea mariana* [Mill.] BSP), and white spruce (*Picea glauca* [Moench.] Voss.) plantations established on a dry site with moderate competition and a moist site with heavier competition in The Pas, northern Manitoba. At each site, experimental treatments included no herbicide and one glyphosate (formulated as Vision®) at 1.42 kg acid equivalent glyphosate ha^{-1} application between one and five years after planting with three replications. Survival, root collar diameter, and height of the three conifers were repeatedly measured for ten consecutive years following planting. Survival of the three conifers was not affected by glyphosate application. For all the three species, root collar diameter and stand volume of crop trees increased significantly in glyphosate-treated plots, compared to control plots. Varying the timing of glyphosate application between year 1 and 5 resulted in a similar conifer dimensions. Glyphosate application significantly improved height growth of black spruce and marginally white spruce, but not jack pine. On these sites, glyphosate reduced the cover of woody species, but grass, forbs and shrubs rapidly captured the newly available growing space. A two variable regression analysis revealed that grass cover negatively affected stand volume of all three conifer species. Cover of tall shrub, forbs, and interaction of cover and height of tall shrubs were the next most important variables to explain jack pine, black spruce, and white spruce volume growth, respectively. **(Canada)**

24. Greifenhagen, S., D.G. Pitt, M.C. Wester, and F.W. Bell. 2005. Juvenile response to conifer release alternatives on aspen-white spruce boreal mixedwood sites. Part II: Quality of aspen regeneration. *The Forestry Chronicle* 81(4): 548-558.

This study, a component of the Fallingsnow Ecosystem Project, was designed to investigate the effects of conifer release alternatives on the quality of regenerating trembling aspen (*Populus tremuloides* Michx.). A randomized block design was used to compare untreated aspen with aspen growing in areas treated with two broadcast cutting treatments (brushsaw and Silvana Selective) and two broadcast herbicide treatments (glyphosate and triclopyr). The glyphosate treatment virtually eliminated aspen, whereas triclopyr tended to top-kill aspen, resulting in lateral dominance. Both fall cutting treatments generated prolific aspen root suckering and stump sprouting. Stain was common in aspen across the study site in damaged, untreated, and post-treatment stems, indicating that stain develops rapidly in young aspen suckers. Stems damaged by the treatments had higher incidences of decay (33% of cut stems and 10% of herbicide-damaged stems) than untreated aspen (8% of stems); however, decay volume was low for all treatments (1-4% of total stem volume affected). The location of decay (e.g., near ground level in cut stems) and presence of stem crooks in herbicide-treated aspen are important effects of the treatments on aspen quality Armillaria root disease, which was found throughout the study site, was more prevalent in roots of treatment-damaged and untreated aspen than in suckers that originated post-treatment. These differences can be attributed to proximity to parent stumps, prevalence of root wounds, and older age of damaged and untreated stems. **(Canada)**

25. Groot, A. 1999. Effects of shelter and competition on the early growth of planted white spruce (*Picea glauca*). *Canadian Journal of Forest Research* 29(7): 1002-1014.

Overstory manipulation and vegetation control treatments were applied at three experimental locations in northern Ontario, Canada, to examine shelter and competition effects on planted white spruce *(Picea glauca* (Moench) Voss). Overstories were nearly pure trembling aspen (*Populus tremuloides* Michx.) or aspen–conifer mixedwoods. Overstory treatments included clear-cutting, uniform shelterwood (40% canopy removal), strip shelterwood (widths from 0.5 to 1.0*H*, where *H* is the height of dominants), patch shelterwood (diameter about 1.0*H*), narrow strips (width 0.25*H*), and intact overstory. Vegetation-control treatments included herbicide and no-herbicide treatments. Second-year seedling growth was poorest under intact overstories and in 0.25*H* strips, and vegetation control had little effect on growth in this situation. Vegetation control in clearcuts increased seedling diameter but not height growth. In shelterwood treatments, however, vegetation control often increased both diameter and height growth. Greatest diameter tended to occur in clearcuts with vegetation control, whereas greatest height growth tended to occur in shelterwoods with vegetation control. These differing responses likely occur because diameter growth is influenced primarily by light availability, but height growth is additionally affected by other environmental factors. Combining early vegetation control along with shelterwood treatments appears to provide the optimum environment for establishing white spruce. **(Canada)**

26. Harper, G.J., P.G. Comeau, and B.S. Biring. 2005. A comparison of herbicide and mulch mat treatments for reducing grass, herb, and shrub competition in the BC

interior Douglas-fir zone—Ten-year results. *Western Journal of Applied Forestry* 20(3): 167-176.

We present results from a study established in 1993 in the Interior Douglas-fir biogeoclimatic zone of southwestern British Columbia to test the effectiveness of several herbicide and mulch mat treatments for reducing grass, herbaceous, and shrub competition. The effectiveness of the preplant herbicide applications of hexazinone (Velpar L) at 2.1 kg ai/ha, glyphosate (Vision) at 2.1 and 1.0 kg ai/ha, and the installation of plastic mulch mats (Tredegar 90 × 90-cm mats) for controlling competing vegetation, improving seedling growth and survival of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings and improving soil moisture availability was evaluated. Ten-year results indicate that application of hexazinone and glyphosate enhanced the survival, diameter, and height growth of planted Douglas-fir seedlings. These herbicide treatments provided effective control of grass and herbaceous vegetation for three to four growing seasons. Plastic mulch mats did result in improvements in growth and survival, but these were not statistically different from untreated seedlings. The results of soil moisture monitoring from within the various treatments indicated delay in the onset of soil drought as a key determinant of the seedling growth improvement noted. **(Canada)**

27. Harper, G.J., K. Polsson, and J. Goudie. 2008. Modeling vegetation management treatments with the Tree and Stand Simulator. *The Forestry Chronicle* 84(1): 53-59.

The Tree and Stand Simulator (TASS) has been used for over 20 years in British Columbia to generate yield tables for managed stands. In order to explore the impacts of weed control on site productivity we chose two vegetation management research trials where 10- to 15-year post-treatment data were available (Boston Bar and Mica research sites). Tree survival and height growth results were used to adjust the TASS input parameters to simulate the various brushing treatments. At the Boston Bar site, all vegetation reduction treatments shortened the Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) physical rotation age by up to 10 years and culmination mean annual increment (cMAI) was increased 8% to 11% relative to the untreated control. At the Mica site, the glyphosate and all repeated manual cutting treatments resulted in a shortening of the Engelmann spruce (*Picea engelmannii* Parry) rotation age by seven years and increased cMAI by approximately 11% to 12%. **(Canada)**

28. Harrington, T.B., R.G. Wagner, S.R. Radosevich, and J.D. Walstad. 1995. Interspecific competition and herbicide injury influence 10-year responses of coastal Douglas-fir and associated vegetation to release treatments. *Forest Ecology and Management* 76(1): 55-67.

Responses of competing vegetation and planted Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) were studied for 10 years after six herbicide and manual release treatments in the Washington and Oregon Coast Ranges. Research objectives were to quantify regional, long-term responses of vegetation to various levels of competition, light and soil water availability, and intensity versus importance of factors influencing Douglas-fir growth. Three treatments reduced shrub cover relative to the untreated check: triclopyr in year 1, glyphosate in years

1–5, and repeated control (via several herbicide applications) in years 1–10. Reductions in woody cover from glyphosate stimulated increases in herb cover in years 3 and 5, while repeated control reduced herb cover in years 1, 2 and 5. Through year 10, Douglas-fir survival (86–99%) varied little among treatments. Visual symptoms of herbicide injury to Douglas-fir from triclopyr (45% of trees) and glyphosate (17% of trees) were associated with 0.1 – 0.2 m reductions in first-year height. After adjusting for tree size, Douglas-fir growth in stem basal area 2 years after triclopyr was less than that of the untreated check, suggesting prolonged effects of herbicide injury. Because it sustained low levels of interspecific competition, caused minimal tree injury, and prevented overtopping cover from red alder (*Alnus rubra* Bong.), repeated control was the only treatment in which Douglas-fir size (9.8 m height and 21 cm basal diameter in year 10) significantly exceeded ($P \leq 0.02$) that of the untreated check (7.8 m height and 12 cm diameter). **(U.S.A.)**

29. Haywood, J.D. 1994. Tenth-year results of herbaceous weed control in a loblolly pine plantation. *Southern Journal of Applied Forestry* 18(3): 105-109.

    Herbaceous weed control influenced the growth of planted loblolly pine (*Pinus taeda* L.) over a 10-yr-period. Five treatments were examined: (1) Untreated control: seedlings were planted in the established herbaceous vegetation; (2) Glyphosate: glyphosate was broadcast in September 1981 before Planting; (3) Disked: plots were cross-disked in September 1981 before planting; (4) Glyphosate-PPWC: glyphosate was broadcast before planting as in Treatment 2, and postplant weed control (PPWC) herbicides were broadcast yearly for 4 yr (1982, atrazine plus simazine: 1983, atrazine plus oxyfluorfen; 1984 and 1985, hexazinone); and (5) Disked-PPWC: plots were disked before planting as in Treatment 3, and the PPWC herbicides were broadcast as in Treatment 4. Four years of PPWC did not affect survival and resulted in greater height, dbh, and volume per loblolly pine through 10 growing seasons. The disked-PPWC plots were the most productive through 8 growing seasons, but higher than average mortality after 8 yr on the disked-PPWC treatment resulted in the glyphosate-PPWC plots producing more volume per acre after 10 growing seasons. Total volume production was 253 inside bark ft^3/ac greater on the two PPWC treatments than on the untreated controls. **(U.S.A.)**

30. Haywood, J.D. and A.E. Tiarks. 1990. Eleventh-year results of fertilization, herbaceous, and woody plant control in a loblolly pine plantation. *Southern Journal of Applied Forestry* 14(4): 173-177.

    Through 11 years, fertilization at planting significantly increased the stemwood volume (outside bark) per loblolly pine (*Pinus taeda* L.) on an intensively prepared moderately well-drained fine sandy loam site in northern Louisiana. Four years of herbaceous plant control significantly increased pine survival, and because herbaceous plant control increased survival, it resulted in a significant increase in total stand volume. Woody plant control no longer produced significant results by age 11. **(U.S.A.)**

31. Haywood, J.D., J.C. Goelz, M.A.S. Sayer, and A.E. Tiarks. 2003. Influence of fertilization, weed control, and pine litter on loblolly pine growth and productivity and

understory plant development through 12 growing seasons. *Canadian Journal of Forest Research* 33(10): 1974-1982.

On a silt loam soil in central Louisiana, three cultural treatments were applied to a seedling loblolly pine (*Pinus taeda* L.) plantation. The treatments were in a 2 × 2 × 2 factorial design: (1) no fertilization or a broadcast application of 177 kg N/ha and 151 kg P/ha; (2) no herbicides applied or broadcast or spot applications of hexazinone, sulfometuron methyl, or glyphosate herbicides and felling as required to control competing vegetation during the first three growing seasons; and (3) no litter applied or broadcast application of pine litter to form a 10 to 15 cm layer in the first growing season. Through 12 growing seasons, the fertilization or herbicide treatment significantly increased stand growth ($\alpha = 0.05$), and these two treatments had an additive effect (no treatments, 209 m^3/ha; fertilization, 328 m^3/ha; herbicide, 280 m^3/ha; fertilization and herbicide, 362 m^3/ha). However, because litter application probably had a minor fertilization effect, the fertilizer and litter combination produced the greatest yield (370 m^3/ha). The herbicide and litter combination adversely affected pine survival, and so applying all three treatments was no more effective than fertilization alone. The loblolly pine overstory was the dominant factor influencing the long-term development of the understory. **(U.S.A.)**

32. Heineman, J.L., S.W. Simard, D.L. Sachs, and W.J. Mather. 2005. Chemical, grazing, and manual cutting treatments in mixed herb–shrub communities have no effect on interior spruce survival or growth in southern interior British Columbia. *Forest Ecology and Management* 205(1-3): 359-374.

Interior spruce (*Picea engelmannii* Parry × *glauca* [Moench] Voss) plantations are commonly weeded of herbs, ferns, and shrubs using foliar glyphosate, livestock grazing, or manual brushing treatments, but it is unknown whether they result in improved conifer survival or growth. We found that despite reducing vegetation for 1–5 years, these treatments applied to mixed herb–shrub communities in southern interior British Columbia had no effect on 5-year survival or growth of interior spruce. Biogeoclimatic zone was the factor that most influenced spruce performance, with greater survival and height growth in the Interior Cedar Hemlock (ICH) zone than in the colder, higher elevation Engelmann Spruce–Subalpine Fir (ESSF) zone. Glyphosate caused larger reductions in vegetation cover and height than did grazing or manual cutting. Glyphosate also reduced plant species richness after 5 years, but none of the treatment methods affected species or structural diversity of the plant community. Structural richness was slightly greater in the ICH than ESSF zone. Our results indicate that the vegetation management treatments operationally applied to herb–shrub communities in southern interior British Columbia have been ineffective at releasing interior spruce. **(Canada)**

33. Hoepting, M.K., R.G. Wagner, J. McLaughlin, and D.G. Pitt. 2011. Timing and duration of herbaceous vegetation control in northern conifer plantations: 15th-year tree growth and soil nutrient effects. *The Forestry Chronicle* 87(3): 398-413.

A 15-year re-measurement of a study designed to identify the optimum timing and duration of herbaceous vegetation control in plantations of four commercial conifer species was completed in northern Ontario. Few differences were revealed in

conifer growth when contrasting early and delayed timing of vegetation control. Conversely, each conifer species responded positively to increased duration of vegetation control, with stand volume gains of up to 209% achieved with four to five years of vegetation control following planting. Compared to earlier assessments, the timing of vegetation control appeared less important than duration. Diminishing returns in the fastest-growing species (jack pine [*Pinus banksiana* Lamb.] and red pine [*Pinus resinosa* Ait.]) are consistent with intraspecific competition related to the onset of crown closure in these stands. Quantification of a suite of soil nutrient pools along the gradient of increased duration of vegetation control indicated that the more intensive levels of vegetation control did not adversely affect the assessed soil nutrient pools in red pine or jack pine, but a cautionary approach should be considered for white pine (*Pinus strobus* L.) and black spruce (*Picea mariana* [Mill.] BSP) where some declines were evident. Vegetation control for two to three years following planting should maximize early conifer growth potential without adverse longer-term effects on soil nutrient pools. **(Canada)**

34. Homagain, K., C.K. Shahi, M. Leitch, N. Luckai, and F.W. Bell. 2011. Differences in extrinsic tree quality and value of fibre production following alternative vegetation management treatments in northwestern Ontario. *The Forestry Chronicle* 87(2): 251-259.

    We examined differences in stem quality, and volume and value of fibre produced by planted white spruce 16 years after vegetation management treatments in northwestern Ontario. Forest Vegetation Simulator ($FVS^{Ontario}$) was used to project the total and merchantable volume to age 70 and BUCK-2 was used to optimize the resulting product mix. Projected value was based on 2009 prices for hog fuel, pulpwood and SPF (spruce–pine–fir) eastern green lumber prices. At 16 years posttreatment, gross total volumes in herbicide-treated and mechanically cut plots were significantly higher (120%–165% and 94%–98%, respectively) than that in control plots (14.73 m^3 ha^{-1}). Based on height, diameter, and taper criteria, observed tree quality did not differ among treatments. The projected value of the fibre produced was 36% to 53% higher in herbicide- treated plots and 24% to 37% higher in mechanically cut plots than in control plots ($18 486.76 per ha). **(Canada)**

35. Homagain, K., C.K. Shahi, N. Luckai, M. Leitch, and F. W. Bell. 2011. Benefit–cost analysis of vegetation management alternatives: An Ontario case study. *The Forestry Chronicle* 87(2): 260-273.

    Vegetation management practices are an integral component of forest management. In this paper, we report results of stand-level benefit–cost analyses of 12 vegetation management treatments applied at six study sites in northern Ontario. Forest Vegetation Simulator ($FVS^{Ontario}$) was used to project gross total and merchantable volumes to 70 years of age, and BUCK-2 was used to optimize potential products. Net present value (NPV), benefit–cost ratio (BCR), and internal rate of return (IRR) were calculated using 2009 constant dollars and variable real discount rates. Aerial herbicide treatments produced the highest NPV, BCR, and IRR. Internal rates of return of 4.32%, 2.90%, 2.82% and 2.50% for aerial herbicide, manual brush cutting, ground-applied herbicide, and brush cutting plus herbicide

treatments, respectively, indicated that all of the vegetation management alternatives evaluated are economically viable. **(Canada)**

36. Imo, M. and V.R. Timmer. 1999. Vector competition analysis of black spruce seedling responses to nutrient loading and vegetation control. *Canadian Journal of Forest Research* 29(4): 474-486.

Impacts of methods to overcome competing vegetation (fertilization, herbicide application, and shading) on conventional and nutrient-loaded black spruce (Picea mariana (Mill.) BSP) seedlings were examined on six forest site types in northeastern Ontario using vector competition analysis. Four sites (*Alnus*; hardwood; mixedwood, herb rich; and mixedwood, herb poor) were selected to represent high competition, and two sites (feathermoss and *Vaccinium*) to represent low-competition forest sites. Although similar in biomass before planting, loaded seedlings had greater N, P, and K content than nonloaded seedlings. After planting, loaded seedlings outperformed growth of nonloaded seedlings under all vegetation-control treatments on all sites. Herbicide application eliminated noncrop vegetation but resulted in significantly higher seedling biomass only on the high-competition *Alnus* site. Field fertilization stimulated weed growth resulting in seedling suppression on the high-competition sites, while nutrient loading countered weed competition on most sites. Interpretation of these results by vector competition analysis revealed that, without fertilization, competition for nutrients was the primary factor limiting seedling growth, while competition for light and (or) moisture was greater after fertilizer addition on weed-prone sites. Similar interpretation revealed no seedling-weed interactions in the less competitive sites. **(Canada)**

37. Jobidon, R. 1998. Comparative efficacy of biological and chemical control of the vegetative reproduction in *Betula papyrifera* and *Prunus pensylvanica*. *Biological Control* 11(1): 22-28.

The effectiveness of various treatments to control stump sprouting of *Betula papyrifera* and *Prunus pensylvanica* was examined under field conditions in Quebec. The experiment involved six stump sprouting-control treatments organized in a randomized complete block design with 15 repetitions: two isolates of the indigenous fungus *Chondrostereum purpureum* (biological control), a heat-killed blank of the inoculum of *C. purpureum*, bialaphos (biorational control), glyphosate (chemical control), and the untreated control. Control of stump sprouting was evaluated 1 and 2 years after treatment by the proportion of stumps with living sprouts and by the mean number of living and dead sprouts per stump. One and 2 years after treatment, biological control was comparable to chemical control in terms of frequency of sprouting and mean number of sprouts per stump, for *B. papyrifera*. While the two fungal isolates affected stump sprouting in *B. papyrifera* similarly, *P. pensylvanica* showed two significantly different responses, suggesting that virulence could vary among isolates of *C. purpureum*, particularly for less susceptible species. One year after treatment with *C. purpureum*, an infection analysis was performed to assess the extent of colonization within the stumps of both species. Results suggest that the extent of colonization was not related to the level of control of stump sprouting. **(Canada)**

38. Jobidon, R. and L. Charette. 1997. Effets, après 10 ans, du dégagement manuel simple ou répété et de la période de coupe de la végétation de compétition sur la croissance de l'épinette noire en plantation. *Canadian Journal of Forest Research* 27(12): 1979-1991.

    The effectiveness of manual release to favor growth and survival of black spruce seedlings was determined in eastern Quebec plantations. Up to three releases over as many consecutive years, and two weed cutting methods, were tested for two types of competing vegetation composed of either raspberry and fireweed (Squatec site) or shade intolerant deciduous trees (Lake Anna site). Five weed cutting periods, from June through October, were also tested at this last site. Seedling growth and survival were not affected by the number of releases. At the Squatec site, seedlings released with a brush cutter were 24% larger in diameter at the collar, and on average 12% higher, than untreated seedlings after 10 years. Weed cutting during the growth season (June through September) enhanced seedling growth. At the Lake Anna site, the best growth enhancement was produced by cutting out all competing vegetation. Seedlings in these plots were 53% larger in diameter at the collar and on average 79% higher than control seedlings. Effects of the release treatments on height and diameter growth increased with time relative to untreated seedlings, showing that treatments had a long-lasting effect. Furthermore, a variance analysis of skewness and kurtosis coefficients was used to determine the effect of competition on the structure of seedling dimensions. After 10 years at the Squatec site, diameter and height distributions were skewed to the left and to the right, respectively. After the same lapse of time at Lake Anna, height and diameter distributions in released plots were skewed to the right opposite to the left in untreated plots. At both experimental sites, seedling dimensions were arranged in a hierarchical manner nearly always similar between released and control plots as a result of competition effects that were lasting since the time of release. Results suggest that release effects should be examined not only on the average growth of seedlings but also on stand structure. **(Canada)**

39. Jobidon, R., L. Charette, and P.Y. Bernier. 1998. Initial size and competing vegetation effects on water stress and growth of *Picea mariana* (Mill.) BSP seedlings planted in three different environments. *Forest Ecology and Management* 103(2): 293-305.

    Three experimental sites in Québec were planted with four different sizes of containerized black spruce seedlings (*Picea mariana* (Mill.) BSP). We examined the water stress experienced by each stock size of black spruce seedlings in relation to different competing vegetation covers and also the effect of the water stress on spruce growth during the first three growing seasons. The sites consisted of one abandoned agricultural field and two forest locations. Containers of sizes 45–110, 45–340, 15–700, and 12–1000 were employed to produce the four different sizes of spruce seedlings. At each site, the experimental protocol used a split-plot in a randomized complete block design, in which the presence of a competing vegetation cover (weedy and bare plots) was assigned to the whole plot, while a specific seedling size was assigned to each subplot. At each experimental site both the

predawn xylem water potential Ψ_{xp} and the midday value Ψ_{xm} were measured three times during each of the first three growing seasons. Data were analysed as a completely randomized split-split-plot design, where selection of seedlings in time was considered as the whole plot. The competing vegetation tended to protect the spruce seedlings from excessive water loss, without depressing the soil-water potential (SWP) to the point of reducing the moisture available to the seedlings. Both Ψ_{xp} and Ψ_{xm} were found to decrease significantly with increasing initial seedling size. The increased water stress experienced by the large stock of spruce seedlings had an effect on the absolute growth rate (AGR) in height on only one experimental site. The AGR was impaired by the presence of a competing vegetation cover, but more severely for the smaller stock-size than the larger. The short-term effect of a competition should be based on radial growth; height growth and mortality are not early indicators of such effect. These results emphasize the need to produce a large stock of spruce seedlings with well-developed root systems and root growth capacity, even though only moderate water stress was observed during the first three years of plantation growth. **(Canada)**

40. Jobidon, R., V. Roy, and G. Cyr. 2003. Net effect of competing vegetation on selected environmental conditions and performance of four spruce seedling stock sizes after eight years in Québec (Canada). *Annals of Forest Science* 60(7): 691-699.

A study was established in 1993 to determine the response of four black spruce (*Picea mariana*) and white spruce (*P. glauca*) stock sizes on two sites located in Québec (Canada), each representing a different type of competing vegetation. At each site, a split-split-plot design with 15 to 17 replicates was used, in which the presence of competition (weedy and bare plots), seedling initial size, and spruce species were assigned respectively to the whole plot, the subplot, and the sub-subplot. Larger initial seedling size provided a greater competitive ability for light and had higher growth rates than the standard stock size for both species. Growth gains from combining plantation of large stock with vegetation control were multiplicative. Non-crop vegetation significantly lowered the seasonal profile of 10-cm depth soil temperature on both sites. This study shows that early release treatment is required on sites dominated by raspberry-hardwood competition complex and planting large spruce stock on such harsh competition sites will help reduce the need for repeated vegetation control. **(Canada)**

41. Jobidon, R., F. Trottier, and L. Charette. 1999. Chemical or manual weed control in black spruce plantations? Case study in the balsam fir-white birch (ecosystem) in Quebec. *The Forestry Chronicle* 75(6): 973-979.

This study aimed at comparing manual and chemical release treatments in black spruce plantations within the balsam fir-white birch ecosystems located in the Lower-Saint-Lawrence and inland Gaspe region of Quebec. Three experimental sites characterized by competition dominated by red raspberry (*Rubus idaeus*) were selected for the study. On each site, a completely randomized block design with seven repetitions was used. Each block was composed of four plots, each representing a vegetation treatment: a manual release, a manual release to be

repeated two years later, a chemical release using Vision (Monsanto Canada), and no treatment on a control plot. The effects of treatment on spruce growth were assessed 1, 2, and 5 years after treatment. The spruce seedlings benefited from the release treatments. After five years, spruce diameter was 25.2 to 42.9% superior, height was 12.5 to 24.8% superior and the mean volume index was 89.3 to 168.6% superior to seedlings on control plots. Moreover, growth profiles over time for the released and control seedlings were not parallel; the seedlings in the released plots showed a growth rate which was superior to that of the control plots, and differences in growth trajectories increased over time. For the three experimental sites, chemical release showed no significant advantage over manual release when assessed after five years of spruce growth variables. This result is attributed to a relatively poor vegetation regrowth after treatment. A manual release treatment was applied a second time on only one of the three sites. In this case, spruce demonstrated a significantly higher growth than on plots which were only manually released once. The survival rate was not significantly affected by either of the treatments except when seedlings suffered mechanical damage. This study supports the hypothesis whereby sites located within the balsam fir-white birch ecosystems for which vigor of competing vegetation is moderate, a manual release treatment produces similar results to a chemical release treatment in terms of black spruce growth in the ensuing years. **(Canada)**

42. Jones, P.D., A.W. Ezell, and S. Demarais. 2010. Growth response of loblolly pine (*Pinus taeda* L.) 3–5 years after plantation establishment using different management intensities. *Journal of Sustainable Forestry* 29(5): 486–502.

The upward trend of intensive management in Southern pine forests is expected to continue, both in area and intensity level. Much of the Mississippi Lower Coastal Plain (LCP) is managed intensively using some combination of mechanical site preparation, chemical site preparation, and herbaceous weed control (HWC). We studied pine growth response and competition control on loblolly pine (*Pinus taeda* L.) plantations 3–5 years following establishment using five combinations of chemical site preparation, mechanical site preparation, and HWC. Treatments were designated a priori as 1 (least intensive) through 5 (most intensive) largely on the basis of anticipated impact on the vegetation community. We measured pine height and diameter at breast height (dbh); woody stem density; hardwood basal area (BA); coverage of herbaceous plants, understory woody plants, and pine trees; and estimated differences in pine response using age-shift calculations at age 5. Pine height and dbh were correlated with treatment intensity; treatment 5 maintained an average advantage of 1.4-m height and 2.5-cm dbh over treatment 2, the least responsive treatment. Woody stem density varied widely and was not affected by treatment; understory woody coverage was also unaffected by treatment. Coverage of herbaceous plants was reduced in treatments receiving broadcast HWC, and in treatment 2, where slower establishment of pines may have acted as a release for competing woody plants. Age-shift gains relative to treatment 2 ranged from 0.4–1.0 years. Based on year 5 measurements of hardwood BA, it is likely that treatments 1 and 2 will fall further behind treatments 3–5 as the stands mature. Greatest control

of competing vegetation and maximum growth of pines was achieved with the most intensive treatment. **(U.S.A.)**

43. Kogan, M. and C. Alister. 2010. Glyphosate use in forest plantations. *Chilean Journal of Agricultural Research* 70(4): 652-666.

Under Chilean conditions the lack of weed control at forest tree establishment results in an average of at least 60% less biomass accumulation during the first year of growth of radiate pine or eucaliptus, and glyphosate offers a series of advantages in forestry weed management because its activity in both herbaceous weed groups, monocots and dicots, as well as annuals, biennials and perennials. Also, its efficacy in woody undesirable vegetation makes glyphosate a very important herbicide that can be applied to control herbaceous and woody weeds as pre-planting and during the second or third years of trees growth as strip applications. The aim of this review is to discuss the main uses of glyphosate in reforestation worldwide, during the first 2 yr after tree establishment, as broadcast application over the top of the forest trees and the most important factors that could affect glyphosate efficacy as a forest herbicide, like weed growth stage, application technique, volume and water quality, rainfastness, dew effect and the use of extra adjuvant with formulated glyphosate. **(Chile)**

44. Lamhamedi, M.S., P.Y. Bernier, C. Hébert, and R. Jobidon. 1998. Physiological and growth responses of three sizes of containerized *Picea mariana* seedlings outplanted with and without vegetation control. *Forest Ecology and Management* 110(1): 13-23.

Three different stock sizes of containerized black spruce (*Picea mariana* [Mill.] B.S.P.) seedlings were planted in an abandoned agricultural field. The small planting stock was of a conventional type produced in 110 cm^3 containers. The experimental medium and large stock types were produced in 340 and 700 cm^3 containers, respectively. Gas exchange, xylem water potential and dry masses were measured six times during each of the first two growing seasons in field plots with and without vegetation control. During the first growing season, the effect of planting shock masked most physiological and growth differences among seedling types. During the second growing season, in plots with vegetation control, small and medium seedlings had similar values of physiological variables and of growth as measured by relative growth rates (RGR), but the large seedlings showed lower values of both net photosynthesis and of RGR, a difference attributed to low initial quality of the root system in the larger seedlings. In plots without vegetation control, the trend was identical, but differences were not significant; the greater height of the larger seedlings, and the resulting greater access to light, compensated for their lower initial quality. The similarity in response between the medium and the small seedlings shows that a fourfold increase in shoot size (1.68–6.82 g) in the initial size and a doubling of the shoot : root ratio (2.17–4.54) of the planting stock did not result in increased planting shock or reduced growth in these containerized conifer stock types. The results also show the importance of the interaction between stock height and the vertical light profile created by the competing vegetation in the final assessment of stock performance. **(Canada)**

45. LaSala, A.V., J.K. Dawson, and A.N. Goodwin. 2004. Productivity and economic implications of various silvicultural thinning regimes in Tasmanian regrowth eucalypt forests. *Tasforests* 15: 19-28.

    Thinning of native eucalypt forests is employed in the densest even-aged regrowth stands. A series of simulated regimes involving different combinations of thinnings at ages of approximately 17, 32 and 47 years were applied to assessment data from high quality, young stands using growth and economic models. The objective was to evaluate the regimes on the basis of their ability to maximise sawlog volume and to determine their economic implications. Net Present Value (NPV) was calculated for each regime discounted back to the time of pre-commercial thinning (PCT) intervention using real discount rates of 5% and 10%. Regimes which ranked highest in terms of NPV did not rank highest in terms of sawlog volume, and vice versa. Sawlog yields in all thinned regimes exceeded those in the unthinned stands over a 65-year rotation, and consisted of larger sawlogs. A regime involving PCT plus one commercial thinning produced more sawlog volume at age 65 years than an unthinned regime at age 80 years. All regimes involving one or more commercial thinnings resulted in higher NPVs than allowing these stands to grow on without intervention until rotation ages of up to 80 years. The preferred thinning regime will vary according to the primary management objective. **(Australia)**

46. Lautenschlager, R.A., F.W. Bell, R.G. Wagner, and J.A. Winters.1997. The Fallingsnow Ecosystem Project: Comparing conifer release alternatives in northwestern Ontario. *The Forestry Chronicle* 73(1): 35-38.

    The Fallingsnow Ecosystem Project documents the ecological consequences of alternative conifer release treatments (motor-manual [clearing/brush saw], mechanical [Silvana Selective/Ford Versatile tractor], helicopter-applied herbicides Vision® [a.i. glyphosate], Release® [a.i. triclopyr], and control [no treatment] in young spruce (*Picea* spp.) plantations. Here a series of papers that quantify the effects of these alternative treatments on major environmental components, as well as their production rates and costs is introduced. In general, one growing season after treatment, untreated plots tended to have consistently larger, more diverse populations of the biotic components examined. Biotic and abiotic changes caused by the cutting treatments were more similar to each other than to those resulting from the herbicide treatments, but statistical differences among treatments were few. Herbicide treatments were the most productive and least costly, and about 60% of the active ingredient was deposited on the target (aspen [*Populus tremuloides* Michx.]) foliage. **(Canada)**

47. Lautenschlager, R.A., J.H. Pedlar, J.A. Winters, and C.M. Nielsen. 2003. Ice storm damage: Effects of competition and fertilization on the growth of sugar maple trees. *The Forestry Chronicle* 79(1): 63-69.

    Treatment plots in blocks established in productive sugar maple (*Acer saccharum*) bushes throughout southeastern Ontario were used to quantify effects of fertilizers, vegetation control and interactions on maple growth following the 1998

ice storm. Treatments were applied during the spring of 1999. Increment cores from six mature sugar maple trees in each plot in each block were extracted and examined during October 2001. Maple growth was highly variable before the storm, but fell to a 30-year low during both the first and second year after the storm. Growth reductions increased with increasing crown damage. Treatment-related statistical differences were marginal; however, the data suggest that phosphorus and potassium additions and competition control improved the growth of ice-damaged mature sugar maple trees. The combination of competition control and fertilization increased growth of ice-damaged maple the most. **(Canada)**

48. Lautenschlager, R.A. and T.P. Sullivan. 2002. Effects of herbicide treatments on biotic components in regenerating northern forests. *The Forestry Chronicle* 78(5): 695-731.

See Biodiversity and Restoration Section.

49. Little, K.M. and C.A. Rolando. 2001. The impact of vegetation control on the establishment of pine at four sites in the summer rainfall region of South Africa: scientific paper. *Southern African Forestry Journal* 192(1): 31-40.

Four trials designed to study the impact of intensive, selective and commercial vegetation control (operational) practices on pine establishment were implemented across an altitudinal and climatic range of sites in the summer rainfall region of South Africa. The trial sites incorporated one high altitude (1650 m a.s.l.) cool temperate site, two mid-altitude (1000 m a.s.l.) warm temperate sites and a low altitude (60 m a.s.l.) sub-tropical site. Treatments implemented at each trial included a weedy and a weedfree control, operational weed control, selective control of herbaceous or woody vegetation types as well as a ringweeding treatment at three sites. The results indicate that the abundance and type of vegetation at a site varies as a function of the local physiographic and environmental conditions as well as historical land-use. At the high altitude site the competitive vegetation was less abundant than at the mid-low altitude sites where vigorous woody vegetation dominated. Due to the differential growth of vegetation across the sites, tree growth responses to intensive and selective vegetation control were site dependent. There were no significant tree growth responses to vegetation management at the high altitude site. Relative to the weedfree control, tree growth suppression was highest on the weedy and woody treatment plots at the warmer, mid-low altitude sites. No significant suppression of tree growth occurred where the vegetation was kept away from the trees, on the ring-weeded and operational treatment plots. Herbaceous vegetation caused significant tree growth suppression only where climatic conditions were conducive to extended seasonal growth. **(South Africa)**

50. Little,K.M. and C.*A*. Rolando. 2002. Post-establishment vegetation control in a *Eucalyptus grandis x E. camaldulensis* stand: research note. *Southern African Forestry Journal* 193(1): 77-80.

In eucalypt plantations, the effect of shading following canopy closure reduces the growth of competing vegetation. In some eucalypt stands full canopy closure is not achieved and the important characteristic of complete shading is lost. This

results in stands with the potential to support competitive levels of postestablishment vegetation. A trial was established on a two year old *Eucalyptus grandis* x *E. camaldulensis* stand to determine the competitive effect of post-establishment vegetation on tree growth. A weedfree treatment was implemented annually over a period of five years and compared to a weedy control to determine the time period for the post-establishment vegetation to reach competitive levels. There was no significant tree growth or survival response to competition from post-establishment vegetation for the duration of the trial. **(South Africa)**

51. Little, K.M. and J. van Staden. 2003. Interspecific competition affects early growth of a *Eucalyptus grandis* x *E. camaldulensis* hybrid clone in Zululand, South Africa. *South African Journal of Botany* 69(4): 505-513.

    To determine the effects of the onset and development of vegetation competition on tree performance, a Eucalyptus hybrid clone (GC304) was planted in a field trial in Zululand, South Africa. Nine vegetation management treatments, imposed from planting, included a weedy control treatment, a manually weeded treatment, a chemically weeded treatment (glyphosate), a 1.2m row and a 1.2m inter-row weeding, a 0.5m radius ring weeding, a complete weeding except for a 0.5m radius ring around the tree (no ring weeding), and the use of two legume cover-crops, *Mucuna puriens* (L.) DC. (velvet bean) and *Vigna unguiculata* (L.) Walp. (cowpea). The different treatments applied during establishment resulted in the differential growth of the trees as determined by measurements of tree height and crown diameter. This occurred from as early as 60 days after planting. The degree of competition could be directly related to the type of vegetation (cover-crops or naturally occurring weeds) and its proximity to the tree. The predominant vegetation on this site, yellow nutsedge (*Cyperus esculentus* L.), was able to colonise the site rapidly, causing severe and early competition. There were strong indications that this initial competition was mainly for moisture and possibly also for nutrients, rather than competition for light. Initially, trees in those treatments that had vegetation within their immediate vicinity were most affected (weedy control, inter-row weeding and no ring weeding). With time, tree performance was more closely related to an increase in the percentage of the area kept free of vegetation. At 180 days after planting the ranking of the top five treatments in relation to the area kept free of vegetation was: manually weeded treatment (100% of area free of vegetation) > chemically weeded treatment (100% of area free of vegetation) > no ring weeding (90% of area free of vegetation) > row weeding (40% of area free of vegetation) > ring weeding (10% of area free of vegetation). The planting of cover-crops, although beneficial in terms of the suppression of competing vegetation, also caused significant tree suppression. This occurred despite the fact that their initial biomass accumulation was slower than that of the natural weed population. Of the two cover-crops, the use of a velvet bean cover-crop was not considered suitable due to its vigorous vining habit which adversely affected growth form. **(South Africa)**

52. Little, K.M., C.A. Rolando, and C.D. Morris. 2007. An integrated analysis of 33 *Eucalyptus* trials linking the onset of competition-induced tree growth suppression

with management, physiographic and climatic factors. *Annals of Forest Science* 64(6): 585-591.

One of the greatest difficulties associated with controlling competitive vegetation during the establishment of eucalypts relates to the timing and planning of 'weeding' operations. This may be due to site related variability in vegetation species distribution and abundance, climatic conditions and methods of site preparation. Using data from 33 eucalypt vegetation management trials, multivariate statistical techniques were used to determine whether any climatic, physiographic or management related variables could be related to the time taken for competition-induced tree growth suppression to occur. Altitude, the method of site preparation (burning versus not burning) and the interaction between these two factors were significantly related to the timing of tree growth suppression. Regardless of the method of site preparation, the onset of competition-induced tree growth suppression occurred earlier at lower altitudes, where the vegetation was more diverse and vigorous. At higher altitudes, burning appears to stimulate the earlier growth of vegetation, reducing the time for competition-induced tree growth suppression to occur. **(South Africa)**

53. Maguire, D.A., D.B. Mainwaring, R. Rose, S.M. Garber, and E.J. Dinger. 2009. Response of coastal Douglas-fir and competing vegetation to repeated and delayed weed control treatments during early plantation development. *Canadian Journal of Forest Research* 39(6): 1208-1219.

A key silvicultural decision in managing young conifer plantations is determining the number and timing of release treatments to control competing vegetation. Three coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) plantations were treated under eight alternative herbicide regimes during the first 5 years after planting to test treatment effects on vegetation dynamics and seedling growth. After termination of herbicide treatments, competing vegetation developed at a rate similar to that of check plots, reaching 40%-60% cover in the first growing season and approaching 100% by the third. Recovery of competing vegetation was slightly more rapid with greater number of previous releases. Annual volume growth of seedlings was negatively correlated with current cover of competing vegetation, but competitive effects from previous years were fully accounted for by initial tree size. Under 4 years of release, delaying treatment by 1 year reduced volume attained at the end of 5 years by about 15%. Plots receiving 5 consecutive years of weed control reached the 5year volume of check plots in only 3.9 years, implying an age shift of 1.1years. Increasing the number of operational release treatments significantly improved seedling growth in the short term, but long-term growth effects must be monitored to determine the economically optimal regime. **(U.S.A.)**

54. Mallik, A.U., F.W. Bell and Y. Gong. 2002. Effectiveness of delayed brush cutting and herbicide treatments for vegetation control in a seven-year-old jack pine plantation in northwestern Ontario, Canada. *Silva Fennica* 36(2): 505-519.

Efficacy of three conifer release treatments, i) single application of glyphosate (Vision™) herbicide, ii) multiple application of glyphosate herbicide, and iii) motor-manual brush cutting for controlling competing plants, particularly trembling aspen

(*Populus tremuloides*), pin cherry (*Prunus pensylvanica*), green alder (*Alnus viridis* spp. *crispa*), and beaked hazel (*Corylus cornuta* spp. *cornuta*), was studied in a seven-year-old jack pine (*Pinus banksiana*) plantation in northwestern Ontario, Canada. The single and multiple glyphosate applications were equally effective in controlling trembling aspen and pin cherry, causing over 90% stem mortality. The brushsaw treatment caused an initial decrease followed by an increase in stem density of these two species. A high degree of stem thinning by natural mortality in the untreated control plots was observed in trembling aspen (23–46%) and pin cherry (41–69%) over four years. As with trembling aspen and pin cherry, stem density of green alder and beaked hazel initially decreased and then increased following the brushsaw treatment, mainly due to resprouting. Stem mortality in green alder and beaked hazel was 45% and 97%, respectively, two years after the operational glyphosate treatment. Competition index (CI) was low (mean CI = 52, ranging from 18 to 115) in all the plots including the untreated control. There was a significant increase in basal diameter of jack pine in the brushsaw and herbicide-treated plots compared to the control three years after the treatments. Jack pine seedlings in the brushsaw and glyphosate treated plots were taller compared to that of control but differences were not significant. Lower species richness and diversity were recorded in the herbicide-treated plots compared to the brushsaw and control plots in the third growing season following treatment. **(Canada)**

55. Man, R., J.A. Rice, and G.B. MacDonald. 2010. Five-year light, vegetation, and regeneration dynamics of boreal mixedwoods following silvicultural treatments to establish productive aspen–spruce mixtures in northeastern Ontario. *Canadian Journal of Forest Research* 40(8): 1529-1541.

Given the extent of boreal mixedwoods in Canada and the challenges of maintaining their conifer component following harvest, we investigated the effects of intimate mixtures versus a mosaic spatial arrangement on mixedwood establishment and growth in northeastern Ontario. The silvicultural treatments were preharvest aerial herbicide spray, postharvest ground herbicide in conifer corridors, partial cutting, conventional conifer plantation with postharvest aerial herbicide, and an untreated reference stand. Fifth-year results suggest that preharvest herbicide application followed by clearcutting controlled trembling aspen (*Populus tremuloides* Michx.) regeneration density and height growth nearly as effectively as postharvest herbicide in conifer corridors or the conventional conifer plantation treatments. Partial cutting reduced aspen regeneration in both harvested and leave corridors but did not affect shrub cover. Survival of spruce regeneration did not differ among silvicultural treatments; however, more spruce seedlings progressed from small to intermediate height classes in the preharvest spray and partial cut than in the postharvest herbicide treatment plots. Based on short-term responses in light availability, vegetation cover, and regeneration as well as cost comparisons among options, the treatment objectives were generally met: the stand has the desired species density and composition of a healthy and productive mixedwood. **(Canada)**

56. Messina, M.G. 1990. Herbicides increase growth responses to fertiliser in a 5-year-old *Eucalyptus regnans* plantation. *New Zealand Journal of Forestry Science* 20(2): 168-175.

A 5-year-old *Eucalyptus regnans* plantation in the Napier district of the North Island of New Zealand was treated with 200 kg diammonium phosphate (DAP) + 250 kg urea/ha, or 200 kg DAP + 500 kg urea/ha, and with a 2% solution of glyphosate applied at a rate of approximately 3 litres a.i./ha, in an incomplete factorial design. Glyphosate was first applied as a weed wiping with a tractor-mounted wick in November 1984. A second application was broadcast with a tractor-mounted directed-spray system in February 1985. DAP was applied in October 1984 and urea in December 1984. Reduction of herbaceous/shrubby competition significantly (P=0.05) increased 20-month diameter at breast height and volume increments, but did not affect height increment. Fertilizer treatment significantly increased height increment (with no significant difference between treatment rates), but did not affect diameter or volume increment over the whole 20-month study period. Neither herbicide nor fertilizer treatment significantly influenced foliar nutrient concentrations. The results indicated that weed control can be as important as fertilizer in enhancing eucalypt growth. **(New Zealand)**

57. Miller, J.H., B.R. Zutter, R.A. Newbold, M.B. Edwards, and S.M. Zedaker. 2003. Stand dynamics and plant associates of loblolly pine plantations to midrotation after early intensive vegetation management - A southeastern United States regional study. *Southern Journal of Applied Forestry* 27(4): 1-16.

Increasingly, pine plantations worldwide are grown using early control of woody and/or herbaceous vegetation. Assured sustainable practices require long-term data on pine plantation development detailing patterns and processes to understand both crop-competition dynamics and the role of stand participants in providing multiple attributes such as biodiversity conservation and wildlife habitat. This study examined loblolly pine (*Pinus taeda* L.) plantations across 13 southeastern sites grown for 15 yr with near-complete control of woody, herbaceous, and woody plus herbaceous components during the first 3-5 yr compared to no plant control. This multiple objective experiment (the COMProject) documents stand dynamics at the extreme corners of a response surface that encompasses most conditions of woody and herbaceous competition common to pine plantations in the region. This is the first of two companion reports. After 15yr, patterns of stand development remained significantly altered by early control treatments and were influenced most by the amounts of hardwoods and shrubs present or controlled. Herbaceous components were more similar across the region. Associated plants in these plantations included 68 species of trees, 33 species/genera of shrubs, and 140 genera of herbaceous and semiwoody plants, woody vines, clubmoss, and ground lichen--241 total taxa or an estimated 490 total species-more richness than previously reported or assumed. Hardwood rootstock numbers were on average maintained at fairly constant levels from yr 1-15 when not controlled, with no initial lag phase evident for reestablishment, indicating prior stand origin. Dynamics of associated vegetation were significantly altered with woody control initially increasing herbaceous cover, while herbaceous control increased hardwood cover and decreased shrub cover.

After early herbaceous control, hardwood basal area (BA) was increased by an average of 28%. After rapid early colonization, herbaceous plants began to decline on all treatments about yr 8 as pine and/or hardwood canopy cover reached a total of 50-60%, while woody vines continued to increase. By age 15, plant component richness remained significantly changed by early treatments at all locations, most notably fewer tree species after early woody control. **(U.S.A.)**

58. Miller, J.H., B.R. Zutter, S.M. Zedaker, M.B. Edwards, and R.A. Newbold. 1995. Early plant succession in loblolly pine plantations as affected by vegetation management. *Southern Journal of Applied Forestry* 19(3): 109-126.

A common study design has been used at 13 locations across the South to examine loblolly pine (*Pinus taeda* L.) plantations established using four vegetation control treatments after mechanical site preparation: (a) No Control, (b) Woody Control, (c) Herbaceous Control for 4 yr, and (d) Total Control. This research, the Competition Omission Monitoring Project (COMP), is monitoring both pine growth and plant succession. During the first 8 yr, the cover of herbaceous components and prevalent genera, along with pine, woody (nonpine), and total herbaceous cover were estimated annually in September. Stem numbers and heights of arborescent and nonarborescent woody species were measured during the first 5 yr and yr 8. There were 101 prevalent genera of herbaceous plants and 76 species/genera of woody plants present on the study sites, with a core group common to most. Herbaceous cover was rapidly reestablished on No Control and Woody Control treatments, with greater than 80% cover in the first year. After the first year, herbaceous cover steadily declined on No Control plots and was sustained when woody plants were eliminated. In general, grasses dominated the herbaceous layer (mainly *Andropogon* and *Panicum* spp.) with cover peaking in yr 4. Woody control increased the actual cover of both grasses and forbs, but only the relative proportion of forbs, which peaked in yr 1-2. Woody control also increased the actual cover of vines and semiwoodies (mainly nontargeted *Rubus* spp.) by yr 6-8, but only the relative cover of semiwoodies. Development of the pine canopy cover was similar with woody and herbaceous control, but pine heights were greater with herbaceous control. Interestingly, herbaceous control did not increase total woody cover until year 8, but the proportion of arborescent tree to nonarborescent shrub cover was increased. Most arborescent species and rootstocks became established in the first year. **(U.S.A.)**

59. Miller, J.H., B.R. Zutter, S.M. Zedaker, M.B. Edwards, and R.A. Newbold. 2003. Growth and yield relative to competition for loblolly pine plantations to midrotation - A southeastern United States regional study. *Southern Journal of Applied Forestry* 27(4): 237-252.

Loblolly pine (*Pinus taeda* L.) plantations were studied across 13 southeastern sites grown for 1.5 yr with near-complete control of woody, herbaceous, and woody plus herbaceous components during the first 3-5 yr. This multiple objective experiment (the COMProject) documents stand dynamics at the extreme corners of the response surface that encompasses most competition conditions common to pine plantations. This is the second of two companion reports. Merchantable pine

volume after 15 yr with early, near complete competition control reached 2,350-4,415ft^3/ac by site compared to 1,132-2,965ft^3/ac on the no controls. With control of both woody and herbaceous competition, 15 yr volumes were increased by 23-121% and gains increased as hardwoods and shrubs increased on the no controls. Early woody control increased merchantable pine volume on 11 sites by 14-118%, while herbaceous control yielded somewhat less on average, a 17-50% increase on ten sites. No gains and some volume losses occurred when control of one component released severe competition from an enhanced remaining component, otherwise gains were generally additive for control of both components. Pine volume was decreased by about 1% for each 1 ft^2/ac of hardwood basal area (BA) present at age 15. Annual measurements determined that culmination of current annual increment (CAI) with control of both competition components occurred in yr 8-11 at 250-470ft^3/ac/yr. CAIs for pine height, BA, and volume were decreased by about 5-27% when growing season rainfall (March-October) was less than 36 in. Mean annual increment had not culminated for any treatment at any location by yr 15 and ranged from 195-250 ft^3/ac/yr with both woody and herbaceous control. Fusiform rust mainstem galls [*Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Hedge. & Hunt) Burdsall & Snow] in high severity areas increased additively with control of both components, more so with herb control. Contrary to the widespread assumption that hardwood out-compete pine, the hardwood proportion of stand BA decreased from yr 5-15 on sites where hardwood BA in yr 5 exceeded 10 ft^2. **(U.S.A.)**

60. Miller, J.H., B.R. Zutter, S.M. Zedaker, M.B. Edwards, J.D. Haywood, and R.A. Newbold. 1991. A regional study on the influence of woody and herbaceous competition on early loblolly pine growth. *Southern Journal of Applied Forestry* 15(4): 169-179.

    A common study design has been installed at 14 locations Southside in track the growth of loblolly pine established with four different competition control treatments: (a) no control, (b) woody control, (c) herbaceous control, and (d) total control after site preparation. This region-wide investigation is the Competition Omission Monitoring Project (COMP). During the first 5 years, the general pine response by treatment has been greater growth in height, diameter, and volume index as follows: total control > herbaceous control > woody control > no control. Generally, diameter growth was more responsive than height to vegetation control treatments. On13 plantation study sites, pine volume after 5 years with total control averaged about fourfold more than with no control. Volume was increased by an average of 67% with woody control, while herbaceous control increased volume by 171%, showing the greater average influence of herbaceous competition during the initial 5 years. The magnitude of growth reductions by competitors varied by site and intensity. **(U.S.A.)**

61. Mitchell, R.J., B.R. Zutter, and D.B. South. 1988. Interaction between weed control and loblolly pine, *Pinus taeda*, seedling quality. *Weed Technology* 2(2): 191-195.

    Third-year heights, third-year root-collar diameters, and 3 yr volume growth of loblolly pine seedlings were examined in relation to a) root-collar diameter class at

time of planting and b) herbaceous weed control. Treatments were a) no weed control and b) complete control for 2 yr. For both weed control treatments, means for third-year heights, groundline diameters, and volume growth were related positively to initial seedling diameter. The influence of initial seedling diameter on third-year diameters and heights did not differ among weed control treatments. However, the influence of initial diameter on volume growth did differ among weed control treatments. Thus, when using herbaceous weed control, additional gains in early volume growth can be realized by planting seedlings with large root-collar diameters. **(U.S.A.)**

62. Monleon, V.J., M. Newton, C. Hooper, and J.C. Tappeiner II. 1999. Ten-year growth response of young Douglas-fir to variable density varnishleaf ceanothus and herb competition. *Western Journal of Applied Forestry* 14(4): 208-213.

The effect of different densities of varnishleaf ceanothus (*Ceanothus velutinus* var. *laevigatus*) and herbaceous vegetation control on stem diameter, height, and volume of plantation Douglas-fir (*Pseudotsuga menziesii* vat. *menziesii*) seedlings was examined during the 10 yr following planting. Initial densities of ceanothus ranged between 0 and 15,000 seedlings/ha and were obtained by interplanting ceanothus germinants or chemical thinning after clearcutting and broadcast-burning. Herbaceous vegetation control was achieved by a single application of glyphosate following planting, with shrub seedlings covered. Ceanothus density in the range of 0 to 6,750 plants/ha did not have an effect on Douglas-fir diameter, height, or volume at age 10; however, Douglas-fir growth was significantly decreased when ceanothus densities reached 15,000 plants/ha. Ten years after planting, Douglas-fir volume in the treatments with 6,750 ceanothus/ha or less was 1.7 times greater than that in the 15,000 ceanothus/ha treatment. On the other hand, removal of herbaceous vegetation after planting significantly increased tree diameter, height, and volume, regardless of ceanothus density. Even 10 yr after the application of the treatment, trees without early herb competition grew faster and had mean dbh, height, and volume that were 1.02 cm, 0.55 m, and 12.98 dm^3/tree greater respectively than those with herbs. Thus, a treatment at plantation establishment to control herbaceous vegetation and to reduce ceanothus density to less than 7,000 plants/ha will ensure an increase in growth and stocking for at least 10 yr. **(U.S.A.)**

63. Perie, C. and A.D. Munson. 2000. Ten-year responses of soil quality and conifer growth to silvicultural treatments. *Soil Science Society of America Journal* 64(5): 1815-1826.

The development of sustainable forestry practices and credible certification systems relies on continuous monitoring of indicators. In the present study, carried out at the Petawawa Research Forest (Ontario, Canada), we evaluated the impacts of three intensive silvicultural treatments: scarification, fertilization, and herbicide treatment, applied alone or in combination—on indicators of organic layer quality, foliar nutrition, and tree growth—10 yr after establishment of eastern white pine (*Pinus strobus* L.) and white spruce [*Picea glauca* (Moench) Voss] plantations. We compared these 10-yr results with measurements made 3 to 4 yr after plantation establishment. In both 1989 and 1996, the herbicide treatment had the greatest

effect on organic layer quality. In 1996, compared with the no-treatment control, herbicide application reduced organic C mass by 46%, total N mass by 15%, and acid phosphatase activity by 64%. These negative effects were offset when herbicide was applied in combination with fertilizer. The negative impact of herbicide on microbial biomass C noted in 1990 was no longer evident in 1996. In herbicide-treated plots, the nitrate-dominated cycle observed 1989–1990 was replaced by an ammonium-dominated cycle in 1996. Although herbicide application negatively affected soil quality, it increased tree growth and generally improved foliar nutrition; thus organic layer and tree responses were not correlated. The indicators used were sensitive to changes in the ecosystem over time and signaled soil impacts that could have consequences for long-term productivity. **(Canada)**

64. Pitt, D.G. and F.W. Bell. 2005. Juvenile response to conifer release alternatives on aspen-white spruce boreal mixedwood sites. Part I: Stand structure and composition. *The Forestry Chronicle* 81: 538-547.

    Stand structure and composition for planted white spruce (*Picea glauca* (Moench) Voss) and other naturally regenerating commercial species were compared seven years after the testing of five conifer release alternatives on three boreal mixedwood sites. No release resulted in aspen (*Populus tremuloides* Michx.) -dominated stands with 89% stocking and the highest basal areas (BAs, 5.1 m^2/ha) and stem volume indices (SVIs, 10.7 m^3/ha) observed. Release by manual or machine cutting increased planted spruce BA and SVI by 67 and 38%, respectively. However, this treatment also caused significant root and stump suckering of aspen, more than doubling stem densities and increasing stocking by 12% over untreated areas. Although cutting reduced the height of aspen from 6 m (untreated) to 2–3 m, equal to or just taller than planted spruce, it is likely that future growth will result in deciduous-dominated mixedwoods. Broadcast foliar application with Release® herbicide temporarily reduced the size of aspen, without causing the increased regeneration observed following cutting. This produced a more varied stand structure that promoted the stature of planted spruce, doubling dominant spruce stocking, BAs, and SVIs, and leading to a more balanced mixedwood. Broadcast release with Vision® herbicide produced conifer-dominated stands with few deciduous stems; these areas contained the lowest observed BAs (1.7 m2/ha) and SVIs (1.9 m3/ha). Relatively low planting densities (1350 sph), coupled with near complete deciduous removal in these plots, created very open-grown conditions that threaten overall productivity and stem quality of the spruce. The five approaches tested are capable of producing a range of stand conditions found in a healthy boreal mixedwood landscape. **(Canada)**

65. Pitt D.G., A.E. Morneault, P. Bunce, and F.W. Bell. 2000. Five years of vegetation succession following vegetation management treatments in a jack pine ecosystem. *Northern Journal of Applied Forestry* 17(3): 100–109.

    See Biodiversity and Restoration Section.

66. Pitt, D.G., R.G. Wagner, and W.D. Towill. 2004. Ten years of vegetation succession following ground-applied release treatments in young black spruce plantations. *Northern Journal of Applied Forestry* 21(3): 123-134.

Responses of planted black spruce [*Picea mariana* (Mill.) BSP] and associated vegetation were studied for 10 years after conifer release options on two northeastern Ontario sites. Six treatments were compared to untreated check plots, including directed foliar application of glyphosate herbicide, basal bark treatment with triclopyr herbicide, glyphosate capsule injection with the EZ-Ject system, spot-treatment with hexazinone herbicide, manual cutting with brushsaw, and five growing seasons of annual vegetation removal with repeat applications of glyphosate. Ten years after treatment, black spruce survival averaged 86% and varied little among treatments ($P > 0.5$). Annual vegetation removal treatments resulted in nearly complete domination by spruce, with treated trees exhibiting 16–55% gains in height and 112–476% gains in stem volume growth over untreated trees. Despite rigorous vegetation control on these plots, each of the vegetation groups studied were well represented at the end of the observation period, including deciduous trees, tall shrubs, low shrubs, forbs, ferns, and grasses/sedges. Directed foliar treatment provided good control of herbaceous and woody vegetation around individual crop trees, providing an 8–46% gain in height and a 43–246% gain in stem volume growth. Both spruce and hardwoods shared dominance on these plots. Spot treatments with hexazinone provided similar short-term reductions in herbaceous vegetation, but tended to release shrub species that had a negative net effect on spruce growth. The other release treatments provided only short-term reductions in woody vegetation, which ultimately led to young stands dominated by deciduous tree species. **(Canada)**

67. Richardson, B., M.O. Kimberley, J.W. Ray, and G.W. Coker. 1999. Indices of interspecific plant competition for *Pinus radiata* in the central north island of New Zealand. *Canadian Journal of Forest Research* 29(7): 898-905.

Pinus radiata D. Don was grown on its own and with a range of densities of either buddleia (*Buddleja davidii* Franchet) or broom (*Cytisus scoparius* L.), two important forest weed species, in a field trial at Rotorua, New Zealand. Tree growth from the time of planting to age 3 was modelled as a function of tree size and a competition modifier. The competition modifier is, in effect, a multiplier that reduces tree growth according to the degree of competition defined by a competition index (CI). A range of CIs, with some sensitivity to both weed and tree growth and development over time, were individually incorporated into the modifier and evaluated. The "best" CI combined measures of weed height relative to tree height, proximity of the weed to the tree, and weed abundance, and was negatively correlated with an index of light availability. For a given value of CI, the effect on tree growth was independent of weed species. For diameter growth, the effect of CI was independent of tree age. However, for height growth the negative effect of a given CI value was much higher in year 3 than in years 1 and 2. This suggest that competition has an immediate effect on diameter but a delayed effect on height growth. **(New Zealand)**

68. Riginos, C. 2009. Grass competition suppresses savanna tree growth across multiple demographic stages. *Ecology* 90(2): 335-340.

Savanna ecosystems, defined by the codominance of trees and grasses, cover one-fifth of the world's land surface and are of great socioeconomic and biological importance. Yet, the fundamental question of how trees and grasses coexist to maintain the savanna state remains poorly understood. Many models have been put forward to explain tree-grass coexistence, but nearly all have assumed that grasses do not limit tree growth and demography beyond the sapling stage. This assumption, however, has rarely been tested. Here I show that grass can strongly suppress the growth of trees. I removed grass around trees of three size classes in an *Acacia drepanolobium* savanna in Laikipia, Kenya. For even the largest trees, grass removal led to a doubling in growth and a doubling in the probability of transitioning to the next size class over two years. These results suggest that grass competition in productive (nutrient-rich) savannas may limit tree growth as much as herbivory and fire (the main factors thought to determine tree demography within a rainfall region) and should be incorporated into savanna models if tree-grass coexistence and savanna dynamics are to be understood. **(Kenya)**

69. Schönau, A.P.G. 1984. Silvicultural considerations for high productivity of *Eucalyptus grandis. Forest Ecology and Management* 9(4): 295-314.

Eucalyptus grandis Hill ex Maiden is undoubtedly one of the most widely used tree species for new afforestation in the warmer climates of the world. This paper indicates its importance for afforestation to supply timber for end uses ranging from industrial to household. The site requirements for this species in respect of climate, soil and physiography are indicated. *Eucalyptus grandis* requires a warm temperate climate, since it is sensitive to frost, and an annual rainfall of not less than 900 mm. Soils should be deep and friable and, if these conditions are met and periods of soil moisture deficit are not too long, highly leached soils with a low nutrient status show excellent growth of this species. Due to the specific climatic requirements the species is grown at a wide range of altitudes in the tropics and sub-tropics. The interrelationship between these site requirements and stocking, silvicultural techniques and management are stressed. The silvicultural operations of site preparation, establishment, fertilizing, weeding, pruning, thinning and regeneration are discussed in relation to optimum productivity for this species. The management requirements and working standards for these operations are given. It is concluded that intensive establishment methods will give optimum productivity irrespective of the scale of the enterprise. Proper site selection is of prime importance for this species which is very site demanding but extremely responsive to ameliorating treatments. **(South Africa)**

70. Scott, D.A. and T.J. Dean. 2006. Energy trade-offs between intensive biomass utilization, site productivity loss, and ameliorative treatments in loblolly pine plantations. *Biomass and Bioenergy* 30(12): 1001-1010.

Loblolly pine plantations are the most important source of forest products in the US and the slash remaining after conventional harvest represents a significant potential source of bioenergy. However, slash removal in intensive harvests might,

under some circumstances, reduce site productivity by reducing soil organic matter and associated nutrients. Two complimentary studies in the Gulf Coastal Plain of the southeastern US were designed to test whether harvest intensity (level of biomass removal) could have a negative long-term impact on site productivity. Harvesting tree crowns in addition to the merchantable bole had a negative impact (18%) on pine biomass accumulation by age 7–10 years on 15 of 19 research blocks. Sites at risk of harvest-induced reductions in productivity were relatively unproductive prior to harvest and had low soil phosphorus (P) concentrations. Intensive harvesting, fertilization, and chemical control of non-crop vegetation were all energy efficient; the additional biomass energy gained through these practices was two-orders of magnitude greater than the energy needed to conduct the activities. Harvest of slash for bioenergy in the Gulf Coastal Plain of the southeastern US has the potential to reduce productivity on infertile soils, but fertilization has the potential to restore and even improve productivity on those sites in an energy-efficient way. **(U.S.A.)**

71. Sharma, M., F.W. Bell, R.G. White, A. Morneault, and W.D. Towill. 2010. Seedling size and woody competition most important predictors of growth following free-to-grow assessments in four boreal forest plantations. *The Forestry Chronicle* 86(2): 213-224.

Improvements to forest management decisions require accurate and quantifiable information. We examined the effects of various classes of competitors on crop tree growth in the context of free-to-grow standards using regression analysis. We found that seedling size accounted for most of the variation in height and volume growth of jack pine (*Pinus banksiana* Lamb.) and black spruce (*Picea mariana* [Mill.] BSP) plantations. Including herbaceous and woody competition as explanatory variables explained the additional variation on crop tree growth significantly. In the plantation initiation phase (years 2 to 6), the presence of herbaceous competitors generally reduced conifer growth but in the first part of the stem-exclusion phase (years 7 to 12) increased their growth. In all four boreal plantations in this study, woody competi- tors reduced conifer growth in both the initiation and stem-exclusion phases. These results have relevance to forest managers who develop and/or use free-to-grow surveys. **(Canada)**

72. Shiver, B.D. and D.B. Warnell. 2002. Twelve-year results of a loblolly pine site preparation study in the piedmont and upper coastal plain of South Carolina, Georgia, and Alabama. *Southern Journal of Applied Forestry* 26(1): 32-36.

A designed experimental study was installed at 25 separate locations in the Piedmont and Upper Coastal Plain regions of South Carolina, Georgia, and Alabama with the objective of evaluating the effects of different site preparation treatments, both chemical and mechanical, on growth and yield of cutover site prepared loblolly pine (*Pinus taeda* L.) plantations. The following six site preparation treatments were included: (1) burn, (2) chop and burn, (3) shear, pile and disk, (4) chop, herbicide and burn, (5) herbicide and burn, and (6) herbicide, burn and complete vegetation control. The herbicide, burn and complete vegetation control treatment significantly increased mean dbh, mean height, stand basal area, and total and merchantable volume compared to all other treatments. The burn-only treatment consistently

ranked worst compared to all other treatments. There were significant differences in mean dbh, mean height, stand basal area, and total and merchantable volume between: (1) the herbicide and burn, which is an operational chemical treatment, and (2) the average of the chop and burn treatment and the shear, pile and disk treatment, which are operational mechanical treatments. In all cases the operational chemical treatment performed significantly better than the average of the two operational mechanical treatments. Average 12-yr-old merchantable volumes (ft^3/ac) across all locations by treatment were: burn (846), chop and burn (1,445), shear, pile and disk (1,740), chop, herbicide and burn (1,669), herbicide and burn (1,919), and herbicide, burn and complete vegetation control (2,546). There were no apparent trends in percent fusiform infection levels across site preparation treatment intensity levels. **(U.S.A.)**

73. Simard, S.W., S.M. Hagerman, D.L. Sachs, J.L. Heineman, and W.J. Mather. 2005. Conifer growth, *Armillaria ostoyae* root disease, and plant diversity responses to broadleaf competition reduction in mixed forests of southern interior British Columbia. *Canadian Journal of Forest Research* 35(4): 843-859.

    Broadleaf trees are routinely removed from conifer plantations during vegetation management treatments, but whether the removal increases tree productivity or affects root disease and plant diversity is unknown. The effects of manual and chemical reduction of paper birch (*Betula papyrifera* Marsh.) and trembling aspen (*Populus tremuloides* Michx.) on conifer survival, growth, root disease incidence, and plant community diversity were investigated for 5 years in Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) and lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) plantations in southern interior British Columbia. Broadleaves were reduced by manual, girdling, and cut-stump glyphosate treatments for 5 years but most severely following cut-stump glyphosate and with a delay due to slow death following girdling. Conifer survival was reduced for 3-5 years following manual cutting or girdling of birch because of a 1.5- to 4-fold increase in mortality due to *Armillaria ostoyae* (Romagn.) Herink, but this did not occur following cut-stump glyphosate treatment of birch or manual cutting of aspen. Conifer diameter increased with treatment intensity and productivity of the vegetation complex. Competition thresholds were identified for diameter but not survival, although *Armillaria*-caused mortality tended to increase near the minimum growth threshold. Structural diversity increased following manual cutting and cut-stump glyphosate because birch dominants were removed and understory layers increased, but species richness and diversity were unaffected. Forest managers can expect increased conifer growth with birch removal but also small increases in mortality due to *Armillaria ostoyae* root disease following manual treatments and loss of large birch trees in all treatments. **(Canada)**

74. Simard, S.W., M.D. Jones, D.M. Durall, G.D. Hope, R.J. Stathers, N.S. Sorensen, and B.J. Zimonick. 2003. Chemical and mechanical site preparation: effects on *Pinus contorta* growth, physiology, and microsite quality on grassy, steep forest sites in British Columbia. *Canadian Journal of Forest Research* 33(8): 1495-1515.

Site preparation alleviates the effects of pinegrass (*Calamagrostis rubescens* Buckl.) interference on conifers in British Columbia, but little is known about interference mechanisms and appropriate site preparation methods for steep slopes. In this study, lodgepole pine (*Pinus contorta* Dougl. ex Loud.) was planted in pinegrass controls and small (90 × 90 cm) and large (180 × 90 cm) patches where (*i*) only pinegrass was removed using glyphosate or (*ii*) both pinegrass and the forest floor were removed using an excavator. Treatments were replicated three times in east- and west-facing clearcuts and effects were followed for 9 years. Two-year pine survival was 78% in the control and >97% in large patches. All patch treatments improved pine growth, but it was greatest in large chemical patches during the initial 6 years and in both large patch treatments thereafter. Removal of the forest floor reduced foliar and soil nutrients, increased bulk density and soil water availability, decreased porosity and aggregate stability, and reduced ectomycorrhizal diversity and richness. These changes were not observed in chemical patches. All patch treatments increased soil temperatures and reduced frost relative to controls, but more so in large patches. Pinegrass can suppress early pine performance, and large chemical patches that retain the forest floor are best for relieving the multiple environmental stressors. **(Canada)**

75. Smethurst, P.J. and E.K.S. Nambiar. 1989. Role of weeds in the management of nitrogen in a young *Pinus radiata* plantation. *New Forests* 3(3): 203-224.

Pinus radiata trees were grown on a podzolized sandy soil at a second rotation site under the following treatments: total weed control, total weed control plus ammonium nitrate, strip weed control and no weed control. During the first two summers after planting the differences in needle water potential between trees under no, strip or total weed control were very small. Despite similar rates of net N-mineralization in strip and total weed control treatments, which averaged 64 kg ha^{-1} yr^{-1} in the 0-15 cm soil depth, weeds in the strip weed control treatment reduced soil mineral-N concentrations by 50-80%, leaching of N by the end of the first growing season by 45%, foliar-N concentrations by 4-14% and stem biomass at 20 months after planting by 46%. Although N-uptake by above-ground vegetation (trees plus weeds) was 49% higher in the strip weed control treatment, the amount of N apportioned to trees during the first 20 months after planting was reduced from 15.5 to 9.0 kg ha^{-1}. These effects of weeds were even more pronounced in the no weed control treatment. Since weeds had little effect on the needle water potential of trees and the annual rates of N-mineralization, but adversely affected N-uptake by trees, results indicate that weeds directly competed with trees for N, and thereby aggravated N-deficiency in trees. Application of ammonium nitrate after complete weed control increased foliar-N concentrations, and N-uptake and growth of trees, but also induced severe stem deformation. **(Australia)**

76. South, D.B., J.B. Zwolinski, and D.G. M. Donald. 1993. Interactions among seedling diameter grade, weed control, and soil cultivation for *Pinus radiata* in South Africa. *Canadian Journal of Forest Research* 23(10): 2078-2082.

A study was established in the southern Cape Province, South Africa, to examine survival and growth in response to seedling grade and various methods of site

preparation. Second-year performance was examined in relation to (*i*) initial groundline diameter, (*ii*) intensity of weed control, and (*iii*) method of soil cultivation. Weeds were controlled with (*i*) manual release 1 year after planting (the standard method used by the Department of Water Affairs and Forestry) or (*ii*) total weed control for 1 year involving the use of herbicides and additional hoeing. Soil cultivation treatments included (*i*) pitting by hand, (*ii*) pitting with a mechanical auger, (*iii*) ripping, and (*iv*) ripping plus disking. The influence of initial seedling diameter on survival was significant. Seedlings with a 2-mm groundline diameter averaged 62% survival, whereas 5-mm seedlings averaged 85% survival. Use of large-diameter stock improved survival regardless of soil cultivation or weed control treatments. For survival, there was an interaction between soil cultivation treatments and the use of herbicides. Total weed control with herbicides and hoeing improved survival for plots that received ripping or disking treatments. However, seedling survival was decreased where herbicides were used on plots where pits were used. On average, seedlings with larger diameters grew better than those with smaller diameters. However, small-diameter seedlings with total weed control grew better than large-diameter seedlings with standard weed control. Average seedling volume after 2 years was 33% greater for high-grade seedlings (2.4 dm^3) than for low-grade seedlings (1.8 dm^3). However, there were interactions between soil cultivation and weed control treatments for height and diameter growth. With the standard method of weed control, ripping improved height and diameter growth. Total weed control with herbicides and hoeing greatly improved growth for all treatments, and as a result, little or no differences in average seedling volume were observed between pitting and ripping treatments. **(South Africa)**

77. South, D.B., J.B. Zwolinski, and H. Kotze. 2001. Early growth responses from weed control and planting larger stock of *Pinus radiata* are greater than that obtained from mechanical soil cultivation. *New Forests* 22(3): 199-211.

Early growth of two grades of bare-root *Pinus radiata* D.Don seedlings were studied in response to four soil cultivation treatments and two weed control treatments. Soil cultivation treatments included (i) pits made by hand, (ii) pits made with an auger, (iii) ripping alone, and (iv) ripping plus disking. Weed treatments included (i) manual release 1 year after planting or (ii) total weed control for 1 year involving the use of herbicides and additional hoeing. Bare-root seedlings were separated into either medium (4.1-mm average root-collar diameter; height 25–31 cm) or small (2.8-mm average root-collar diameter; height 14–20 cm) size classes. Growth on this site was excellent and 7 years after treatment, trees in the least expensive treatment averaged 12.8 m in height. Use of medium seedlings and extra weed control increased merchantable volume by 20 m^3 ha^{-1} and 19 m^3 ha^{-1}, respectively. When combined, the increase was 39 m^3 ha^{-1}. However, none of the soil cultivation treatments caused a significant increase in merchantable volume. Disking on this site proved to be of no benefit. A boundary-line analysis was used to examine the marginal returns from investing in intensive silviculture. **(South Africa)**

78. South, D.B., J.H. Miller, M.O. Kimberley, and C.L. Vanderschaaf. 2006. Determining productivity gains from herbaceous vegetation management with 'age-shift' calculations. *Forestry* 79(1): 43-56.

Gains in stand volume that result from competition control and fertilization are sometimes reported as 'percentage gains'. Because percentage gains arithmetically decline over time as stand volume increases, plantation managers have difficulty in using percentage gains to project growth and revenues. The 'age-shift' method quantifies the year advancements in stand growth due to silvicultural treatments and, for herbaceous vegetation management, it has been proposed that this metric is less likely to change after the juvenile growth phase. To test the sensitivity of the 'age-shift' method to time and hardwood competition, we used 20-year volume data from 11 loblolly pine (*Pinus taeda* L.) studies that had early complete herbaceous and woody competition control. Volume growth gains were expressed in terms of percentages and 'age-shifts'. On all sites with no woody competition, percentage gains declined from age 8 years to age 20 years. In contrast, age-shift estimates on these plots either remained constant or increased over time. However, in four cases where woody basal areas were greater than 4 m^2 ha^{-1} at age 15 years, age-shift gains due to herbaceous control decreased and eventually resulted in volume losses. When evaluating the response to early herbaceous competition control, age-shift calculations have promise as a useful predictive tool on sites with low levels of hardwood competition. Five methods for calculating age-shift are presented. **(U.S.A.)**

79. Strong, W.L. and S.S. Sidhu. 2005. Prolonged herbicide-induced vegetation changes in a regenerating boreal aspen clearcut. *Journal of Environmental Management* 77(3): 194-204.

A soil-active herbicide (hexazinone) was applied (0, 2, and 4 kg/ha of active ingredient) in a 3-year-old regenerating boreal *Populus tremuloides* Michx. (aspen) clearcut to determine its effect on the compositional and structural development of the vegetation. Woody stem densities and plant foliar cover were evaluated prior to and 2, 6, and 17 years after treatment. Herbicide treatment at the 2 and 4 kg/ha rates reduced tree and total woody stem densities relative to the 0 kg/ha level. The 4 kg/ha level reduced stem densities by 27% 17 years after treatment. The primary reductions occurred in *Amelanchier alnifolia* (Nutt.) Nutt. *ex* M. Roemer (saskatoon) and *Rosa acicularis* Lindl. (wild rose); whereas *Corylus cornuta* Marsh. (beaked hazelnut) and *Viburnum edule* (Michx.) Raf. (low-bush cranberry) stem densities increased. Notable herbicide-caused foliar cover reductions at the 4 kg/ha level occurred in *Eurybia conspicua* (Lindl.) Nesom. (showy aster), *Mertensia paniculata* (Ait.) G. Don. (tall mertensia), *Rubus pubescens* Raf. (dewberry), and *Spiraea betulifolia* Pallas (spiraea), but *Aralia nudicaulis* L. (sarsaparilla), *Cornus canadensis* L. (bunchberry), and *Symphyotrichum ciliolatum* (Lindl.) A.&D. Lve (Lindley's aster) increased. Less distinctive but similar changes occurred in the 2 kg/ha treatment. Total plant cover, species richness, and species dominance concentration were similar among treatments. Eight distinctive forest understory-types were recognized among treatments in Year 17. Between the 0 and 4 kg/ha treatments, five understory-types differed in their frequency of occurrence. Hexazinone did not

improve the survival of silviculturally planted *Picea glauca* (Moench) Voss (white spruce) seedlings relative to untreated sites, but the 4 kg/ha treatment level did increase *Pinus contorta* Dougl. *ex* Loud. (lodgepole pine) survival from 12 to 34%. Surviving seedlings had significantly greater height and basal diameter growth than those at the 0 kg/ha sites, particularly the 4 kg/ha treatment. **(Canada)**

80. Sutherland, B. and F.F. Foreman. 2000. Black spruce and vegetation response to chemical and mechanical site preparation on a boreal mixedwood site. *Canadian Journal of Forest Research* 30(10): 1561-1570.

The growth and development of outplanted black spruce (*Picea mariana* (Mill.) BSP) and competing vegetation five growing seasons after mechanical and chemical site preparation treatments are presented. The largest stem volume increase for black spruce coupled with the lowest vegetation indices for competing trees and shrubs were recorded on the treatment consisting of chemical site preparation with liquid hexazinone applied at 3.1 kg active ingredient (a.i.)-ha^{-1} followed by chemical tending in the second and fourth growing season with glyphosate applied at 1.78 kg a.i.-ha^{-1}. Black spruce stem volume growth was second highest and the vegetation indices for competing trees and shrubs the highest, on plots treated with hexazinone site preparation. Among mechanical treatments, black spruce stem volume was highest on plots treated with mixed-mound site preparation. No other mechanical site-preparation treatment improved the growth of black spruce over boot-screef site preparation alone. The vegetation index of trembling aspen (Populus tremuloides Michx.) was reduced on mixed-mound and area-mixed site preparation treatments. The vegetation index of red raspberry (*Rubus idaeus* L.) was reduced on area-mix and area- and strip-screef treatments. By the fifth growing season, site-preparation treatment had little effect on the comparative growth of grasses and forbs. High-speed strip-mixing with 80 cm wide strips spaced at 2-m centres, on deep, fertile, silty foams of Site Region 3W-Lake Nipigon, does not appear feasible as an alternative to chemical site preparation or conventional manual and mechanical site preparation. **(Canada)**

81. Sutton, R.F. 1995. White spruce establishment: initial fertilization, weed control, and irrigation evaluated after three decades. *New Forests* 9(2): 123-133.

Outplantings of white spruce (*Picea glauca* [Moench] Voss) in a factorial experiment in 1962 (P62) and 1963 (P63), near Ottawa, Ontario, addressed the problem of prolonged unsatisfactory growth of outplanted white spruce, a condition commonly termed check. Applied in the first 2 growing seasons, the treatments (3 levels of nitrogenous fertilization ×± weed control ×± irrigation) subjected the trees to differential fertility and soil moisture on each of three soils (“clay”, “loam”, and “sand”). There were 5 (P62) and 6 (P63) replications of 9-tree plots. The P62 Loam planting was destroyed by fire in 1966. Initial survival in the P63 Clay was too low to warrant long-term monitoring. Monitoring of the P62 Clay planting was discontinued after 20 years when it was clear that site factors unrelated to the experimental treatments were controlling stand development. After 30 growing seasons in the P62 Sand and 29 growing seasons in the P63 Sand and P63 Loam plantings, neither initial nitrogenous fertilization nor initial irrigation was influencing performance, but

on all three sites the positive effect of initial weed control remained significant (*P*<0.001). **(Canada)**

82. Sutton, R.F., L. Bedford, L. Stordeur, and M. Grismer. 2001. Site preparation for establishing interior spruce in British Columbia: Trials at Upper Coalmine and Mackenzie. *Western Journal of Applied Forestry* 16(1): 9-17.

Two trials ("Upper Coalmine" and "Mackenzie") were begun in the 1980s in the Prince George Forest Region, B.C., to evaluate a total of 13 site preparation treatments for establishing interior spruce (*Picea glauca* × *engelmannii*) in the Engelmann Spruce-Subalpine Fir and Sub-Boreal Spruce biogeoclimatic zones. Treatments included mounding (with various thicknesses of mineral soil "capping"), patch scarification, blading, disk trenching, and herbiciding. In each trial, five blocks each contained one 48 or 80 tree plot/ treatment. Trees were monitored for 10 yr at Mackenzie and 15 yr at Upper Coalmine. Large mounds have had consistent biological success. Tree seedling response to blading was site specific; blading at Mackenzie was not significantly inferior to the best (Ministry mound) treatment, but at Upper Coalmine was no better than the untreated control. **(Canada)**

83. Swift, K. and F.W. Bell. 2011. What are the environmental consequences of using silviculturally effective forest vegetation management treatments? *The Forestry Chronicle* 87(2): 201-216.

See Biodiversity and Restoration Section.

84. Thiffault, N. and V. Roy. 2011. Living without herbicides in Québec (Canada): historical context, current strategy, research and challenges in forest vegetation management. *European Journal of Forest Research* 130(1): 117-133.

Vegetation management is crucial to meeting the objectives of forest plantations. Following public hearing processes, chemical herbicides were banned on Crown forest lands in Québec (Canada) in 2001. Release now mainly relies on mechanical treatments. Our objectives are to review the historical context and the research conducted over the past 15 years that has led to the province's current vegetation management strategy and to identify the major challenges of vegetation management being faced in Québec in the context of intensive silviculture and ecosystem-based management. Research has led to an integrated management model without herbicides, adapted to the ecological characteristics of reforestation sites. The Québec experience illustrates how, on most sites, vegetation management that is based on early reforestation, the use of tall planting stock and intensive mechanical release brings crop trees to the free-to-grow stage without the use of herbicides and without resulting in major effects on vegetation diversity. This vegetation management strategy is an asset in the implementation of ecosystem-based management. However, research demonstrates that mechanical release alone does not promote optimal crop-tree growth, due to rapid resprouting or suckering of competitors and competition from herbaceous species. Therefore, the current strategy poses important challenges in the management of plantations where the objective is to maximise wood production. **(Canada)**

85. Thiffault, N., R. Jobidon, and A.D. Munson. 2003. Performance and physiology of large containerized and bare-root spruce seedlings in relation to scarification and competition in Québec (Canada). *Annals of Forest Science* 60: 645-655.

In Québec (Canada), the use of large planting stock is being applied in combination with scarification, since herbicide use is forbidden in public forest. Large containerized and bare-root stock of black spruce were planted on two sites located within the sub-boreal mixedwood region of eastern Québec. We analyzed data to detect main effects and interactions among scarification, competing vegetation and stock type on seedling growth and physiology during the first three growing seasons. Scarification did not improve seedling water relations, third-year height and ground-level diameter, and foliar nutrient concentration. After three years, the two stock types showed similar water relations and nutritional status but the large containerized seedlings performed slightly better than the large bare-root stock in terms of diameter and height growth. Competing vegetation greatly reduced seedling diameter, foliar-N concentration, compared to competition-free seedlings. We discuss results in relation with treatment effects on above- and belowground resource availability to newly planted conifers. **(Canada)**

86. Thiffault, N., B.D. Titus, and M.T. Moroni. 2010. Silviculture and planted species interact to influence reforestation success on a Kalmia-dominated site - a 15-year study. *The Forestry Chronicle* 86(2): 234-242.

Successful regeneration following harvesting or natural disturbance is a fundamental prerequisite for sustainable forest management. However, some regenerating stands have poor juvenile growth rates, which compromise sustainable management objectives. In particular, the presence of some ericaceous species that proliferate after forest disturbance, such as *Kalmia angustifolia*, can slow succession of boreal stands to the point that ecosystem retrogression is induced. We used data from a silvicultural field trial established in central Newfoundland to evaluate how various combinations of silvicultural treatments (trench scarification, herbicide application, fertilization at planting) influenced growth of three conifer species planted on a *Kalmia*-dominated cutover. Ground-level diameter (GLD), height, diameter at breast height (DBH), and percent *Kalmia* cover were assessed at the end of 15 growing seasons after planting. We detected several interactions between silvicultural treatments and planted conifer species. Globally, height and estimates of foliar biomass of all conifer species responded positively to scarification. Fifteen-year height in both scarified and unscarified treatments was in the order *Picea mariana* < *Pinus banksiana* < *Larix laricina*. Black spruce and jack pine height increased when *Kalmia* was controlled with herbicide, but height of tamarack was not. The use of herbicide significantly increased 15-year GLD and volume index of all three conifer species, but only black spruce responded positively to fertilization at planting. Our results confirm that species-specific responses to silvicultural treatments are to be expected when managing *Kalmia*-dominated sites. Although chemical vegetation management has great silvicultural potential, our results suggest that mechanical site preparation can also be effective in promoting early conifer seedling growth that leads to rapid canopy closure. It is anticipated that canopy closure will lead to

exclusion of *Kalmia* later in the rotation through natural successional pathways. **(Canada)**

87. Thomas, K.D., W.J. Reid, and P.G. Comeau. 2001. Vegetation management using polyethylene mulch mats and glyphosate herbicide in a coastal British Columbia hybrid poplar plantation: Four-year growth response. *Western Journal of Applied Forestry* 16(1): 26-30.

This study was initiated in 1995 to gather information on the effectiveness of opaque polyethylene mats and glyphosate herbicide treatment on growth and yield of hybrid poplar plantations on south coastal British Columbia. Immediately after planting 40 cm tall hybrid poplar cuttings, 60 × 60 cm opaque polyethylene mats were installed. All treatment plots were mechanically cultivated in one direction twice yearly from 1995 to 1997. This practice left an uncultivated strip between trees running in the same direction as the cultivation. The uncultivated strip was approximately 1 m wide, and represented approximately one-third (0.04 ha) of the total plot area (0.13 ha). Glyphosate was applied at a rate of 2.1 kg active ingredient (ai)/ha in water to the uncultivated strips in the herbicide treatment plots in midsummer of the second and third growing seasons. The opaque polyethylene mats significantly improved poplar height growth only for the first growing season. After four growing seasons, hybrid poplars treated with glyphosate were significantly taller (983 cm) than those in either the mat (915 cm) or control treated plots (902 cm). Diameter at breast height (dbh) was also significantly greater in the herbicide treated plots (88 mm) than in either the mat (78 mm) or control (77 mm) treated plots. Total volume was also significantly greater in herbicide plots (23 m^3/ha) than in either the mat (17 m^3/ha) or control (17 m^3/ha) plots. Basal area and total volume were 14% and 37%, respectively, greater in the herbicide plots than in the mat and control plots following the fourth growing season. **(Canada)**

88. Thompson, D.G. and D.G. Pitt. 2003. A review of Canadian forest vegetation management research and practice. *Annals of Forest Science* 60: 559-572.

Research and practice in Canadian forest vegetation management was reviewed for the period 1990 to present. Results indicate continued evolution toward a more integrated and ecologically sound program with appropriate focus on key competitors and crop species. Increasing collaboration between academia, government and industry has resulted in > 666 new scientific publications, substantially augmenting the existing knowledge base. The development of (*Chondrostereum purpureum*) as the first biocontrol agent in Canadian forest vegetation management and the use of nutrient-loaded seedlings to enhance establishment success are considered key research highlights. Recent trends in operational practice include a move toward more intensive management on higher quality sites and adoption of innovative approaches (e.g. nutrient loaded seedlings, larger planting stock) and advanced technologies (e.g. electronic guidance in aerial herbicide applications). The lack of long-term growth response data and economic analyses demonstrating positive cost/benefits remain as shortcomings, however continued development of the program will undoubtedly enhance sustainable wood supply and minimize impact on the forest environment. **(Canada)**

89. Wagner, R.G. 2000. Competition and critical-period thresholds for vegetation management decisions in young conifer stands. *The Forestry Chronicle* 76(6): 961-968.

    Thresholds define the time when management action is required to prevent a loss in yield, but have remained relatively elusive in forest vegetation management. Hundreds of studies quantifying the effects of competing vegetation in young forest stands, however, have produced reasonably consistent patterns and magnitudes of tree responses. These consistencies reveal a set of general guidelines that can be used to assist forest managers in deciding when vegetation management treatments are needed. Among the variety of vegetation management thresholds that have been defined, competition and critical-period thresholds can be interpreted from existing forest vegetation research. Competition thresholds define the vegetation density at which yield loss begins to occur and varies depending on whether the manager's objective is to maximize survival, height increment, basal area growth, or biomass. These interactions also appear to vary depending on whether woody or herbaceous plants are the principal competitors. The critical-period threshold defines the time period when vegetation control must occur to prevent yield loss. Results from one critical-period study indicate that capturing the potential for conifer growth requires control of vegetation for the first several years after planting. **(U.S.A.)**

90. Wagner, R.G., G.H. Mohammed, and T.L. Noland. 1999. Critical period of interspecific competition for northern conifers associated with herbaceous vegetation. *Canadian Journal of Forest Research* 29(7): 890-897.

    Using critical-period analysis, we examined the temporal effects of interspecific competition from herbaceous vegetation on seedlings of jack pine (*Pinus banksiana* Lamb.), red pine (*Pinus resinosa* Ait.), eastern white pine (*Pinus strobus* L.), and black spruce (*Picea mariana* (Mill.) BSP) during the first 5 years after planting. The critical period is the time period during stand development when interspecific competition reduces tree growth. We found both similarities and differences in responses among tree species. Gains in stem volume index associated with increasing duration of vegetation control (expressed by weed-free curves) differed among species. In contrast, declines in stem volume index with increasing duration of competition after planting (expressed by weed-infested curves) were equal among species. Critical periods for stem volume index were shorter for shade-intolerant jack and red pine (1 and 2 years after planting) than for more shade-tolerant white pine and black spruce (1-3 years for spruce and 1-4 years for white pine). Intolerant species had greater absolute stem volume growth, but smaller relative declines from continuous association with herbaceous vegetation (85, 81, 78, and 67% for white pine, black spruce, red pine, and jack pine, respectively). Herbaceous vegetation did not affect survival and had a variable influence on height growth of all species. **(Canada)**

91. Wagner, R.G., K.M. Little, B. Richardson, and K. McNabb. 2006. The role of vegetation management for enhancing productivity of the world's forests. *Forestry* 79(1): 57-79.

The management of competing vegetation has evolved with forest management over the past half century and is now an integral part of modern forestry practice in many parts of the world. Vegetation management, primarily using herbicides, has proven especially important in the establishment of high-yield forest plantations. There has been a substantial amount of research quantifying the wood yield gains from the management of competing vegetation over the past few decades. We reviewed results from 60 of the longest-term studies in North America (Canada and US), South Africa, South America (Brazil) and New Zealand/Australia. About three-quarters of the studies reported 30–500 per cent increases in wood volume from the most effective vegetation treatments. In North America, where the longest-term studies for a variety of tree species were between 10 and 35 years old (or from 20–100 per cent of rotation age), gains in wood volume ranged from 4–11 800 per cent in Pacific north-western forests, 14–5840 per cent in the south-eastern forests, and 49–5478 per cent in northern forests. In South Africa and South America (Brazil), several full-rotation (6–8 years) studies with eucalyptus indicate 29–122 per cent and 10–179 per cent increases in wood volume yield, respectively, from effective vegetation management. In New Zealand, time gains of 1 to 4 years from early vegetation control in radiata pine plantations translated into 7–27 per cent increases in wood volume yield over a 25- to 30-year rotation. **(U.S.A.)**

92. Wagner, R.G., J. Flynn, R. Gregory, C.K. Mertz, and P. Slovic. 1998. Acceptable practices in Ontario's forests: Differences between the public and forestry professionals. *New Forests* 16(2): 139-154.

    We compare and contrast survey responses to statements about 1) environmental values, 2) agreement with forest management goals and approaches, 3) perceptions of risk, 4) trust in science and government, and 5) acceptability of forestry practices between the public and three groups of forestry professionals (government biologists, government foresters, and industry foresters) in Ontario. The survey emphasized issues surrounding forest vegetation management due to the contentious nature of herbicide use. Responses were gathered from a 140-question telephone survey administered from September and November 1994 to 1,500 members of the general public and 201 forestry professionals across the province. Forestry professionals tended to be less supportive of some environmental values and forest management goals, perceive everyday and forestry activities to be less risky, be more trusting of science and government, and be more accepting of forestry activities than the general public. Among the three groups of forestry professionals, industry foresters tended to be most different from the public, followed by government foresters, and government biologists. These differences reveal potential sources of conflict and miscommunication between the public and forest managers. Recognizing these differences can help improve communications with the public about forest management plans. **(Canada)**

93. Wagner, R.G., M. Newton, E.C. Cole, J.H. Miller, and B.D. Shiver. 2004. The role of herbicides for enhancing forest productivity and conserving land for biodiversity in North America. *Wildlife Society Bulletin* 32(4): 1028–1041.

Herbicide technology has evolved with forest management in North America over the past 60 years and has become an integral part of modern forestry practice. Forest managers have prescribed herbicides to increase reforestation success and long-term timber yields. Wildlife managers and others interested in conserving biodiversity, however, have often viewed herbicide use as conflicting with their objectives. Do herbicides increase forest productivity, and are they compatible with the objectives of wildlife management and biodiversity conservation? Results from the longest-term studies (10–30 years) in North America suggest that the range of wood volume yield gains from effectively managing forest vegetation (primarily using herbicides) is 30–450% in Pacific Northwest forests, 10–150% in the southeastern forests, and 50–450% in northern forests. Most of the 23 studies examined indicated 30–300% increases in wood volume yield for major commercial tree species and that gains were relatively consistent for a wide range of site conditions. Meeting future demands for wildlife habitat and biodiversity conservation will require that society's growing demand for wood be satisfied on a shrinking forestland base. Increased fiber yields from intensively managed plantations, which include the use of herbicides, will be a crucial part of the solution. If herbicides are properly used, current research indicates that the negative effects on wildlife usually are short-term and that herbicides can be used to meet wildlife habitat objectives. **(U.S.A.)**

94. Wiensczyk, A., K. Swift, A. Morneault, N. Thiffault, K. Szuba, and F.W. Bell. 2011. An overview of the efficacy of vegetation management alternatives for conifer regeneration in boreal forests. *The Forestry Chronicle* 87(2): 175-200.

In this paper, we discuss the broad array of treatments that could be used to control competitive vegetation in conifer plantations in the boreal forests of Canada. We present vegetation management alternatives screened based on their treatment efficacy, which we defined as their ability to (a) control competitive vegetation and (b) not cause undue damage to conifer seedlings. The treatments reviewed range from pre-harvest (preventative) to post-plant release (reactive) treatments, and are organized into five categories: (i) silvicultural and harvest systems, (ii) physical treatments such as mechanical site preparation, cutting, girdling and mulching; (iii) thermal treatments such as prescribed fire and steaming; (iv) cultural treatments such as seedling culture, cover cropping, and grazing; and (v) chemical and biological spray treatments. We based our assessment of treatment efficacy on previous reviews, expert opinion, and published literature. We conclude on the need to further assess the effectiveness of forest vegetation management strategies in the context of multi-purpose plantations that consider ecological, social and silvicultural objectives. **(Canada)**

95. Zhao, D., M. Kane, B.E. Borders, M. Harrison, and J.W. Rheney. 2009. Site preparation and competing vegetation control affect loblolly pine long-term productivity in the southern Piedmont/Upper Coastal Plain of the United States. *Annals of Forest Science* 66(7): 705-713.

A site preparation study was established in 1986 to evaluate the effect of different site preparation treatments on growth and yield of loblolly pine (*Pinus taeda* L.) plantations on the Piedmont and Upper Coastal Plain regions of the southern

United States. Site preparation treatments included: (1) burn only, (2) chop-burn, (3) shear-pile-disk, (4) chop-herbicide-burn, (5) herbicide-burn, and (6) herbicide-burn-herbicide. The data from the available 19 installations at age 21 were analyzed with separate analysis of variance and a multilevel nonlinear mixed-effects modeling approach. The herbicide-burn-herbicide treatment significantly increased average Dbh, height, basal area and volume per hectare compared to all other treatments. The burn only treatment consistently ranked worst and was followed by the chop-burn treatment. The shear-pile-disk and chop-herbicide-burn treatments had similar overall growth pattern, and will approach the same level of pine volume as the herbicide-burn treatment. Loblolly pine mean annual increment in volume (m^3 ha^{-1} y^{-1}) at age 21 by treatment were: herbicide-burn-herbicide (17.9), shear-pile-disk (16.1), herbicide-burn (15.9), chop-herbicide-burn (15.4), chop-burn (14.3), and burn (11.2). An additional chop or herbicide treatment to the burn treatment significantly increased loblolly pine yield. Complete control of both herbaceous and woody completion enhanced long-term pine productivity. **(U.S.A.)**

96. Zutter, B.R. and J.H. Miller. 1998. Eleventh-year response of loblolly pine and competing vegetation to woody and herbaceous plant control on a Georgia flatwoods site. *Southern Journal of Applied Forestry* 22(2): 88-95.

    Through 11 growing seasons, growth of loblolly pine (*Pinus taeda* L.) increased after control of herbaceous, woody, or both herbaceous and woody vegetation (total control) for the first 3 years after planting on a bedded site in the Georgia coastal flatwoods. Gains in stand volume index from controlling either herbaceous or woody vegetation alone were approximately two-thirds that from controlling both types of vegetation. Pine response through age 11 was approximately equal for herbaceous control alone and woody control alone, whereas response through age 5 was greater with control of only herbaceous vegetation. The impact of woody vegetation should continue to have a strong effect on pine growth through midrotation because of its continued development. This is in contrast to herbaceous weeds that have greatly decreased in abundance since age 6. **(U.S.A.)**

97. Zutter, B.R., J.H. Miller, H.L. Allen, S.M. Zedaker, M.B. Edwards, and R.A. Newbold. 1999. Fascicle nutrient and biomass responses of young loblolly pine to control of woody and herbaceous competitors. *Canadian Journal of Forest Research* 29(7): 917-925.

    Individual fascicle mass and foliar nutrient content and concentration of young loblolly pine (*Pinus taeda* L.) were evaluated on 13 locations of a regionwide competition study in the southeastern United States. The study included a factorial combination of two levels of herbaceous weed control treatment (none, treated) and two levels of woody treatment (none, treated) following site preparation. At pine age 2 years, herbaceous treatment (HT) and woody treatment (WT) had a positive effect on individual fascicle biomass and content of N, P, and K at nearly all and at least half of the locations, respectively. In general, these effects mirrored responses noted for seedling diameter and height. N concentration increased and P concentration decreased at about half of the locations, while Ca and Mg concentrations decreased on nearly all locations with HT. By age 6 years, effects of HT and WT on fascicle

mass and nutrient concentrations and contents became neutral or more neutral across the locations. This is attributed in part to the greater nutrient demand of larger crop pines and associated competition components. A notable exception from neutral effects at age 6, typically occurring on sites with high levels of woody vegetation, was the positive response in K concentration or content and negative response in Ca and Mg concentrations with WT. **(U.S.A.)**

Fish

1. Ayoola, S.O. 2008. Toxicity of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*) juvenile. *African Journal of Agricultural Research* 13(12):825-834.

Fish are particularly sensitive to a wide variety of agrochemicals including glyphosate herbicide that may arise from not only deliberate discharge of these chemicals into waterways but also from approved agricultural practices. In the present study, the toxicity of glyphosate an herbicide was investigated with emphasis on histopathological effects. Nile tilapias (*Oreochromis niloticus*) juvenile 15 ± 1.0 g were exposed to 0, 2, 9, 30, 97, 310 mg/l of glyphosate. The lethal concentration (LC_{50}) value of glyphosate was 1.05 mg/l for 96 h of exposure. Glyphosate concentration corresponding to the 96 h LC_{50} values for juvenile tilapia was used to study the effects of glyphosate exposure for 4 days in inducing histopathological changes of gills, livers, kidneys and brains. In the gills, filament cell proliferation, lamellar fusion, lamellar cell hyperplasia, and epithelial lifting were observed. In the liver, there was vacuolation of hepatocytes and necrosis. Kidney lesion consisted of hyaline droplets in the tubular epithelial cells and pkynosis. The brain lesion also consisted of generalized neuronal degeneration and spongiosis. The changes in these tissues occur predominantly in the 96 h exposure. Respiratory stress, erratic swimming and instant death of fish were observed in exposed fish, which varied with the concentration of the toxicant and this showed that mortality increased with increase in concentration. Glyphosate is highly toxic to Nile tilapias, which are more susceptible to this herbicide; therefore their use near fish farm or in area close to aquatic environment should be discouraged. **(Nigeria)**

2. Ayoola, S.O. 2008. Histopathological effects of glyphosate on juvenile African catfish (*Clarias gariepinus*). *American-Eurasian Journal of Agricultural & Environmental Science* 4(3): 362-367.

Most aquatic herbicides have undergone some toxicity testing for effects on non-target aquatic organisms, little of this testing has been conducted on early life stages of *Clarias gariepinus.* African catfish *C. gariepinus* were exposed to acute concentrations of glyphosate for 96hrs. The lethal concentration (LC_{50}) value of glyphosate was 0.295 mg/l for 96h of exposure. The mean mortality percentages were 0, 43, 73, 93 and 96% in the order of concentration of 0, 19, 42, 94, 207 and 455 mg/l, respectively. Glyphosate concentration corresponding to the 96h LC_{50} value for juvenile *C. gariepinus* was used to study the effects of glyphosate exposures in inducing histopathological changes of gills, liver, kidney and brain. In the gills, cellular infiltrations were observed. In the liver there was fatty degeneration, severe fat vacuolation, diffuse hepatic necrosis and darkly stained specks of necrotic nuclei and infiltration of leukocytes. In kidney there was haematopoietic necrosis and severe pyknotic nuclei. The brain showed mononuclear infiltration, neuronal degeneration and spongiosis. These changes occurred predominantly in the 96h exposure. Respiratory stress, erratic swimming and death of fish were observed in exposed fish which varies with the concentration of the toxicant and its showed that mortality increased with increasing in concentration. Glyphosate is toxic to juvenile

fish. *C. gariepinus* are more susceptible to herbicide, therefore their use on/near fish farm or in area close to aquatic environment should be discouraged. **(Nigeria)**

3. Cattaneo, R., B. Clasen, V.L. Vania, C.C. de Menezes, A. Pretto, B. Baldisserotto, A. Santi, and L.A. de Avila. 2011. Toxicological responses of Cyprinus carpio exposed to a commercial formulation containing glyphosate. *Bulletin of Environmental Contamination and Toxicology* 87(6): 597 – 602.

   The effects of commercial glyphosate herbicide formulation on the activity of acetylcholinesterase (AChE) enzyme and oxidative stress were studied in *Cyprinus carpio* exposed for 96 h to 0.0, 0.5, 2.5, 5.0 and 10.0 mg/L and then allowed to equal recovery period in water without herbicide. The activity of AChE was inhibited in the brain and in the muscle after exposure. However, after recovery period brain and muscle AChE activity increased. Brain thiobarbituric acid reactive species (TBARS) were measured as an indicator of oxidative stress. Increased TBARS levels were observed with all concentrations tested of the glyphosate formulation, and remained increased after the recovery period. The results recorded clearly indicate lipid peroxidation and anti-AChE action induced by Roundup® exposure. **(Brazil)**

4. Cavalcante, D.G.S.M., C.B.R. Martinez, and S.H. Sofia. 2008. Genotoxic effects of Roundup® on the fish *Prochilodus lineatus*. *Mutation Research: Genetic Toxicology and Environmental Mutagenesis* 655: 41-46.

   Glyphosate-based herbicides, such as Roundup®, represent the most extensively used herbicides worldwide, including Brazil. Despite its extensive use, the genotoxic effects of this herbicide are not completely understood and studies with Roundup® show conflicting results with regard to the effects of this product on the genetic material. Thus, the aim of this study was to evaluate the genotoxic effects of acute exposures (6, 24 and 96 h) to 10 mg L^{-1} of Roundup® on the neotropical fish *Prochilodus lineatus*. Accordingly, fish erythrocytes were used in the comet assay, micronucleus test and for the analysis of the occurrence of nuclear abnormalities and the comet assay was adjusted for branchial cells. The results showed that Roundup® produces genotoxic damage in erythrocytes and gill cells of *P. lineatus*. The comet scores obtained for *P lineatus* erythrocytes after 6 and 96 h of exposure to Roundup® were significantly higher than respective negative controls. For branchial cells comet scores were significantly higher than negative controls after 6 and 24 h exposures. The frequencies of micronucleus and other erythrocyte nuclear abnormalities (ENAs) were not significantly different between Roundup® exposed fish and their respective negative controls, for all exposure periods. In conclusion, the results of this work showed that Roundup® produced genotoxic effects on the fish species *P lineatus*. The comet assay with gill cells showed to be an important complementary tool for detecting genotoxicity, given that it revealed DNA damage in periods of exposure that erythrocytes did not. ENAs frequency was not a good indicator of genotoxicity, but further studies are needed to better understand the origin of these abnormalities. **(Brazil)**

5. Cavaş, T., and S. Könen. 2007. Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate

formulation using the micronucleus test and the comet assay. *Mutagenesis* 22(4): 263-268.

Glyphosate is a widely used broad-spectrum weed control agent. In the present study, an *in vivo* study on the genotoxic effects of a technical herbicide (Roundup) containing isopropylamine salt of glyphosate was carried out on freshwater goldfish *Carassius auratus*. The fish were exposed to three doses of glyphosate formulation (5, 10 and 15 ppm). Cyclophosphamide at a single dose of 5 mg/l was used as positive control. Analysis of micronuclei, nuclear abnormalities and DNA damage were performed on peripheral erythrocytes sampled at intervals of 48, 96 and 144 h posttreatment. Our results revealed significant dose-dependent increases in the frequencies of micronuclei, nuclear abnormalities as well as DNA strand breaks. Our findings also confirmed that the alkaline comet assay and nuclear deformations in addition to micronucleus test on fish erythrocytes *in vivo* are useful tools in determining the potential genotoxicity of commercial herbicides. **(Turkey)**

6. Chatterjee, A., S. Adhikari, S.P. Adhikari, and S. Ayyappan. 2004. Fish as bioindicators for waiting period of pesticides. *Indian Journal of Fisheries* 51(3): 271-276.

Experiments were conducted in semi-pond as well as aqueous media with glyphosate, butachlor and cypermethrin for their waiting period and degradation studies using the survivabilities of *Labeo rohita*, *Channa punctatus*, *Anabas testudineus* and *Heteropneustes fossilis* as bio-indicators. The results showed that the waiting period of glyphosate, butachlor and cypermethrin existed in between 13 and 18 days; 91 and 120 days and 7 and 10 days, respectively, in semi-pond conditions. The degradation of pesticides as measured by fish survivability percentage indicated that though in case of glyphosate, a period of 18 days showed 100 percent fish survivability both in semi-pond and aqueous media, in case of cypermethrin and butachlor, there was a difference of about seven days and 20 days respectively, in between semi-pond system and only aqueous system to achieve 100 percent fish survivability which demonstrated that soil in the semi-pond system could play a vital role in pesticide degradation. Moreover, in glyphosate-treated water, 0.721 mg/l more dissolved phosphorus was recorded after 12 days, while in butachlor-treated water, 0.596 mg/l more dissolved nitrogen was observed after 90 days and in cypermethrin-treated water, 0.340 mg/l more dissolved nitrogen was recorded after seven days as compared to control. **(India)**

7. Chattopadhyay, A., S. Adhikari, S.P. Adhikary, and S. Ayyappan. 2007. Influences of environmental factors and antidote addition on glyphosate toxicity to freshwater fish, *Labeo rohita* (Hamilton). *Chemistry and Ecology* 23(4): 279-287.

The effect of glyphosate (a potential herbicide: trade name glycel™, isopropyl amine salt of glyphosate, 41% w/w and inert materials 59% w/w) has been investigated on the survival rate of a freshwater fish *Labeo rohita*. In the present study, we also analysed the influence of some environmental factors (e.g. pH, calcium, and salinity levels of water) and of antidote application (fresh cow dung) on the glyphosate toxicity. A concentration of glyphosate at 12.31 a.i. ha^{-1} m^{-1} was very effective in inhibiting the growth of some aquatic submerged macrophytes, and so

this dose was chosen as a toxicant to the fish. Survival rate of the fish was considered as an index of glyphosate toxicity. The results showed that the toxicity of glyphosate persisted for c. 18 d after which the herbicide degraded, and the survival rate of *Labeo rohita* (mean weight 24.5±0.3 g and mean length 14.5±0.25 cm) was 100%. We observed the total mortality of the fish up to the 12 d of the experiment. The toxic effect of the glyphosate was significantly reduced (P<0.05) at pH 6.8-7.6, at salinity 1.0 ppt, and at water calcium level c. 200 mg l^{-1}. Our results showed that a dose of 30 ml l^{-1} (9990 kg $ha^{-1}m^{-1}$) of fresh cow dung (3.33% w/v in water) reduced the toxicity of glyphosate and the survival rate of the fish reached 100% up to 96 h from the application. **(India)**

8. de Menezes, C.C., M.B. da Fonseca, V.L. Loro, A. Santi, R. Cattaneo, B. Clasen, A. Pretto, and V.M. Morsch. 2011. Roundup effects on oxidative stress parameters and recovery pattern of *Rhamdia quelen*. *Archives of Environmental Contamination and Toxicology* 60(4): 665-671.

Antioxidant enzymes and oxidative stress indicators were evaluated in fish exposed to different concentrations of the herbicide Roundup 48% (Monsanto, St. Louis, MO): control (none), 0.45, or 0.95 mg/l. After exposure for 8 days to herbicide, fish were transferred to clean water for a recovery response period (also 8 days). Herbicide increased thiobarbituric acid reactive species in liver and muscle at the higher concentration and in the brain at both concentrations. Protein carbonyl in liver increased after exposure. Catalase (CAT) and superoxide dismutase (SOD) activities and ascorbic acid levels in liver did not change in fish exposed to both concentrations. Glutathione S-transferase (GST) levels decreased at both concentrations. The nonprotein thiol levels decreased at the 0.95 mg/l concentration. During the recovery period, some of the parameters that had altered, such as protein carbonyl content, later recovered. However, some enzymes reacted during this period, e.g., GST increased its activity, possibly indicating a compensatory response against the toxic conditions. In contrast, CAT and SOD activities decreased during the recovery period, indicating herbicide toxicity. Oxidative stress that occurred during the exposure period was likely due to the increased lipid peroxidation and protein carbonyl content. The results concerning oxidative and antioxidant profiles indicate that short-term exposure to herbicide is capable of causing oxidative stress in fish tissues. **(Brazil)**

9. El-Shebly, A.A. and M.A.H. El-kady. 2008. Effects of glyphosate herbicide on serum growth hormone (GH) levels and muscle protein content in Nile tilapia (*Oreochromis niloticus* L.*). Research Journal of Fisheries and Hydrobiology* 3(2): 84-88.

Glyphosate herbicide is one the most toxic pollutants for aquatic ecosystem. Changes in serum concentrations of growth hormone (GH) and muscle protein content in *O. niloticus* treated with different concentrations of glyphosate herbicide under laboratory conditions were evaluated. Nile tilapia *Oreochromis niloticus* (mean initial weight of 57.61 ± 4.8g and initial length 14.87±1.7cm) were exposed to different concentrations of Roundup, a glyphosate (acid equivalent) herbicide: 0 (control), 1, 3, and 5 mg/L for 96 h (short-term) of exposure. Unexposed fish (control) exhibited significantly higher serum GH levels (p<0.05) compared to treated

fish. The GH levels and muscle protein content decreased significantly with increasing glyphosate concentrations. GH levels and muscle protein percentage may be the good early indicators of herbicide contamination in tilapia species. **(Egypt)**

10. Everett, K.D.E. and H.W. Dickerson. 2003. *Ichthyophthirius multifiliis* and *Tetrahymena thermophila* tolerate glyphosate but not a commercial herbicidal formulation. *Bulletin of Environmental Contamination and Toxicology* 70(4): 731-738.

***** We concluded that *Tetrahymena thermophila* would likely survive herbicidal applications of technical grade glyphosate in aquatic systems and that *Ichthyophthirius multifiliis* would not be controlled by glyphosate. In contrast, at herbicidal glyphosate concentrations (6 oz/gal or 4.7% x 1.11*M* = 52 m*M*), Roundup would kill both *T. thermophila* and *I. multifiliis*. Roundup was at least 100-times more lethal than technical grade glyphosate to *T. thermophila* and *I. multifiliis*, based on equivalent glyphosate concentrations. These findings were consistent with observed differences in the lethality of glyphosate and Roundup in salmon, trout, and carp. Our findings that relatively high technical-grade glyphosate concentrations had no effect on *T. thermophila* and *I. multifiliis* suggests that these protozoa do not use the shikimate pathway or that glyphosate is not taken up by these ciliates. Use of glyphosate is likely to increase as genetically engineered Roundup Ready® crops become popular. These findings increase our understanding of glyphosate sensitivity in ciliated protozoa and permit the strategic application of glyphosate-containing herbicides and near aquaculture systems. **(U.S.A.)**

11. Ezeri, G.N.O. 2002. Effect of herbicidal control of water hyacinth on fish health at the Ere Channel, Ogun State, Nigeria. *Journal of Applied Sciences and Environmental Management* 6(1): 49-52.

A herbicidal control of water hyacinth,WH (*Eichhornia crassipes,* Martius Solms - Laubach) was carried out by applying glyphosate (N-phosphyonomethyl glycerine) containing 360g/l glyphosate in the form of 480g/l isopropylamine salt at the rate of 2.16kg active ingredient (a.i./ha) by a fixed wing, AG-CAT Schweizer plane at the Ere fishing channel. Pathological studies revealed that of the total number of fishes examined prior to the chemical application, 334 (5%) had fin-rot, 2541 (38%) abrasion, 802 (12%) lesions, 334 (5%) ulcerations, 1805 (27%) sloughing of their body slime. None had tumours or nodules. The post application examination of fishes revealed that 5806 (7%) had fin-rot, 8294 (10%) abrasion, 4147 (5%) lesions, 1244 (1.5%) ulcerations and 4145 (5%) sloughing of body slime. None had tumours or nodules. The total number of fish that showed signs of infection prior to herbicidal application was 516 (86.9%) while it was 23,636 (28.49%) for post application of herbicide. The total number of fish caught prior to herbicidal treatment was 6,686 (7.46%) while a total number of 82,943 fish (92.54%) were caught after treatment. No fish mortality was observed throughout the post treatment monitoring. In this multi-disciplinary work, it was established that glyphosate at 2.16 a.i./ ha controlled WH and associated weeds within four weeks of application without any intrinsic deleterious effect on fish and aquatic fauna. **(Nigeria)**

Fish

12. Fairchild, J.F., A.L. Allert, J.S. Riddle, and D.R. Gladwin. 2002. Efficacy of glyphosate and five surfactants for controlling giant salvinia. *Journal of Aquatic Plant Management* 40: 53-58.

Giant salvinia (*Salvinia molesta* Mitchell) is a non-native, invasive aquatic fern that was recently introduced to the southern United States. The aggressive nature of the species has led to concerns over its potential adverse impacts to native plants, fish, and invertebrates. We conducted a study to determine the efficacy of glyphosate [isopropylamine salt of N-(phosphono-methyl)glycine] and several surfactants for control of giant salvinia. Studies were conducted over a 42-day period using static renewals (twice weekly) with 4% Hoagland's medium (10 mg/L N equivalent) in replicated 2-L containers. Five concentrations of glyphosate (0, 0.45, 0.91, 1.82, and 3.60% v:v) and five surfactants (0.25% concentration, v:v; Optima™, Kinetic™, Mon 0818™, Cygnet Plus™, and LI-700™) were applied with a pressurized sprayer as a single surface application in a fully nested experimental design. Untreated giant salvinia grew rapidly and exhibited an increase of 800% wet weight biomass over the 42-day test duration. Glyphosate, with and without surfactants, exhibited efficacy at concentrations as low as 0.45% of the commercial formulation. Glyphosate with Optima was the only mixture that resulted in complete mortality of plants with no regrowth. **(U.S.A.)**

13. Filizadeh, Y. and I.H. Rajabi. 2011. Toxicity determination of three sturgeon species exposed to glyphosate. *Iranian Journal of Fisheries Sciences* 10(3): 383-392.

Glyphosate, N-(phosphonomethyl) glycine, has been widely used to control agricultural weeds in the north of Iran. However, it is also supposed to have adverse effects on natural sturgeon population. The present study was undertaken to evaluate the acute toxicity of glyphosate to three different sturgeon species (*Huso huso, Acipenser stellatus*, and *A. persicus*) under laboratory conditions. Fish were exposed to one of ten glyphosate concentrations (10 to 100 mg l^{-1} with 10 mg l^{-1} intervals), along with a control group. The values of the median lethal concentration (LC50) for each experimental species were estimated using a standard probit regression analyses after each 6, 12, 24, 48, 96, and 168 hours as exposure times. Results showed that increase in glyphosate exposure times up to 168 hours was simultaneous to decrease of the lethal concentration (LC50). 96-h LC50 of glyphosate for *H. huso, A. stellatus* and *A. persicus* were 26.4, 23.2 and 27.5 mg l^{-1}, respectively. Glyphosate exhibited a slight to moderate toxicity in sturgeon species. However, it may negatively affect the natural population of sturgeons through decreasing of fry mass, smaller size of yolk sac and the initiation of unsafe behaviors. **(Iran)**

14. Glusczak, L., D. dos Santos Miron, M. Crestani, M.Braga da Fonseca, F. Araujo Pedron, M.F. Duarte, and V.L.P. Vieira. 2006. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens*). *Ecotoxicology and Environmental Safety* 65(2): 237- 241.

In this study, teleostean fish *Leporinus obtusidens* (piava) were exposed to different concentrations of Roundup, a glyphosate (acid equivalent) herbicide: 0 (control), 3, 6, 10, and 20 mg/L for 96 h (short-term). Acetylcholinesterase (AChE)

activity was verified in brain and muscle tissues. Metabolic parameters in the liver and muscle (lactate, glycogen, glucose, protein, and ammonia), as well as some hematological parameters, were determined. Unexposed fish exhibited significantly higher brain AChE activity when compared to that of the muscle (*P*<0.05) (13.8+/-0.76 and 6.1+/-1.31 micromol/min/g protein, respectively). Results indicated that AChE activity significantly decreased in the brain of fish exposed to all glyphosate concentrations tested, but in the muscle this parameter was not altered. In addition, fish exposed to all glyphosate concentrations showed a significant increase in hepatic glycogen and glucose, but a significant reduction in muscle glycogen and glucose. Lactate and protein of fish exposed to all glyphosate concentrations presented a significant decrease in the liver, but did not change significantly in the muscle. Levels of ammonia in both tissues increase in fish at all glyphosate concentrations. Exposure to this herbicide produced a decrease in all hematological parameters tested. These results indicate that AChE activity as well as metabolic and hematological parameters may be good early indicators of herbicide contamination in *L. obtusidens*. **(Brazil)**

15. Glusczak, L., D. dos Santos Miron, B.S. Moraes, R.R. Simões, M.R.C. Schetinger, V.M. Morsch, and V.L. Loro. 2007. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). *Comparative Biochemistry and Physiology, Part C* 146(4): 519- 524.

    Silver catfish (*Rhamdia quelen*; Teleostei) were exposed to commercial formulation Roundup®, a glyphosate herbicide: 0 (control), 0.2 or 0.4 mg/L for 96 h. Fish exposed to glyphosate showed an increase in hepatic glycogen, but a reduction in muscle glycogen at both concentrations tested. Glucose decreased in liver and increased in muscle of fish at both herbicide concentrations. Glyphosate exposure increased lactate levels in liver and white muscle at both concentrations. Protein levels increased in liver and decreased in white muscle while levels of ammonia in both tissues increased in fish at both glyphosate concentrations. Specific AChE activity was reduced in brain after treatments, no changes were observed in muscle tissue. Catalase activity in liver did not change during of exposure. Fish exposed to glyphosate demonstrated increased TBARS production in muscle tissue at both concentrations tested. For both glyphosate concentrations tested brain showed a reduction of TBARS after 96 h of exposure. The present results showed that in 96 h, glyphosate changed AChE activity, metabolic parameters and TBARS production. The parameters measured can be used as herbicide toxicity indicators considering environmentally relevant concentration. **(Brazil)**

16. Glusczak, L., V.L. Loro, A. Pretto, B.S. Moraes, A. Raabe, M.F. Duarte, M.B. da Fonseca, C.C. de Menezes, and D.M. Valladão. 2011. Acute exposure to glyphosate herbicide affects oxidative parameters in piava (*Leporinus obtusidens*). *Archives of Environmental Contamination and Toxicology* 61(4): 624- 630.

    In recent years, commercial glyphosate herbicide formulations have been widely used in agriculture to control aquatic weeds. These pesticides may result in disruption of ecological balance, causing damage to nontarget organisms including fish. Teleostean fish (*Leporinus obtusidens*) were exposed to commercial glyphosate

herbicide formulation at 0 (control), 3, 6, 10 or 20 mg L^{-1} for 96 h. The effects of herbicide on plasmatic metabolic parameters, thiobarbituric acid reactive substances (TBARS), catalase activity, protein carbonyl, and mucus layer parameters were studied. Plasmatic glucose and lactate levels increased but protein levels showed reduction after herbicide exposure. TBARS levels in brain showed a reduction at all tested concentrations. However, liver demonstrated increased TBARS levels at all tested concentrations, whereas in white muscle TBARS production did not change after exposure to herbicide. Fish exposed to all concentrations of glyphosate showed increase in liver catalase activity and protein carbonyl. Herbicide exposure increased protein and carbohydrate levels of the mucus layer at all tested concentrations. The present results showed that, in 96 h, glyphosate changed toxicological parameters analyzed in piava. Parameters measured in this study may be useful in environmental biomonitoring. **(Brazil)**

17. Grisolia, C.K. 2002. A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis* 518(2):145-150.

A comparative analysis between mouse and fish erythrocyte micronuclei (MN) assays was carried out using cyclophosphamide, mitomycin C and various pesticides such as alliete, brestanid, decis 25 CE (deltamethrin), kelthane 480 CE (dicofol), roundup (glyphosate), imazapyr and thiram. The aim of this study was to evaluate the fish species *Tilapia rendalli* as a suitable organism for the detection of genotoxicants in water. The clastogens cyclophosphamide and mitomycin C induced MN in both test-systems. Insecticides: decis 25 CE increased *T. rendalli* MN frequencies at doses of 1.0 and 5.0 mg/kg, but not at the highest dose, and in mice there was no MN induction. Kelthane 480 CE also induced a significant MN frequency in *T. rendalli*, but not in mice. Fungicides: alliete and brestanid induced MN only in *T. rendalli*, while thiram was negative in both assays. Herbicides: imazapyr induced MN in *T.rendalli* at the maximum tolerated dose only, while roundup induced MN at three dosed levels. In mice both herbicides presented negative results. This study revealed that fish MN assay can be used as a genotoxicological test-system since some methodological particularities were observed. **(Brazil)**

18. Guilherme, S, I. Gaivão, M.A. Santos, and M. Pacheco. 2010 European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup®-a glyphosate-based herbicide. *Mutagenesis* 25(5): 523-530.

The glyphosate-based herbicide, Roundup®, is among the most used pesticides worldwide. Due to its extensive use, it has been widely detected in aquatic ecosystems representing a potential threat to non-target organisms, including fish. Despite the negative impact of this commercial formulation in fish, as described in literature, the scarcity of studies assessing its genotoxicity and underlying mechanisms is evident. Therefore, as a novel approach, this study evaluated the genotoxic potential of Roundup® to blood cells of the European eel (*Anguilla anguilla*) following short-term (1 and 3 days) exposure to environmentally realistic concentrations (58 and 116 microg/l), addressing also the possible association with

oxidative stress. Thus, comet and erythrocytic nuclear abnormalities (ENAs) assays were adopted, as genotoxic end points, reflecting different types of genetic damage. The pro-oxidant state was assessed through enzymatic (catalase, glutathione-S-transferase, glutathione peroxidase and glutathione reductase) and non-enzymatic (total glutathione content) antioxidants, as well as by lipid peroxidation (LPO) measurements. The Roundup® potential to induce DNA strand breaks for both concentrations was demonstrated by the comet assay. The induction of chromosome breakage and/or segregational abnormalities was also demonstrated through the ENA assay, though only after 3-day exposure to both tested concentrations. In addition, the two genotoxic indicators were positively correlated. Antioxidant defences were unresponsive to Roundup®. LPO levels increased only for the high concentration after the first day of exposure, indicating that oxidative stress caused by this agrochemical in blood was not severe. Overall results suggested that both DNA damaging effects induced by Roundup® are not directly related with an increased pro-oxidant state. Moreover, it was demonstrated that environmentally relevant concentrations of Roundup® can pose a health risk for fish populations. **(Portugal)**

19. Guilherme, S., I. Gaivão, M.A. Santos, and M. Pacheco. 2012. DNA damage in fish (*Anguilla anguilla*) exposed to a glyphosate-based herbicide – Elucidation of organ-specificity and the role of oxidative stress. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis* 743(1-2):1-9.

Organophosphate herbicides are among the most dangerous agrochemicals for the aquatic environment. In this context, Roundup®, a glyphosate-based herbicide, has been widely detected in natural water bodies, representing a potential threat to non-target organisms, namely fish. Thus, the main goal of the present study was to evaluate the genotoxic potential of Roundup® in the teleost fish *Anguilla anguilla*, addressing the possible causative involvement of oxidative stress. Fish were exposed to environmentally realistic concentrations of this herbicide (58 and 116μgL^{-1}) during one or three days. The standard procedure of the comet assay was applied to gill and liver cells in order to determine organ-specific genetic damage. Since liver is a central organ in xenobiotic metabolism, nucleoids of hepatic cells were also incubated with a lesion-specific repair enzyme (formamidopyrimidine DNA glycosylase - FPG), in order to recognise oxidised purines. Antioxidants were determined in both organs as indicators of pro-oxidant state. In general, both organs displayed an increase in DNA damage for the two Roundup® concentrations and exposure times, although liver showed to be less susceptible to the lower concentration. The enzyme-modified comet assay showed the occurrence of FPG-sensitive sites in liver only after a 3-day exposure to the higher Roundup® concentration. The antioxidant defences were in general unresponsive, despite a single increment of catalase activity in gills (116μgL^{-1}, 3-day) and a decrease of superoxide dismutase activity in liver (58μgL^{-1}, 3-day). Overall, the mechanisms involved in Roundup®-induced DNA strand-breaks showed to be similar in both organs. Nevertheless, it was demonstrated that the type of DNA damage varies with the concentration and exposure duration. Hence, after 1-day exposure, an increase on pro-oxidant state is not a necessary condition for the induction of DNA-damaging

effects of Roundup®. By increasing the duration of exposure to three days, ROS-dependent processes gained preponderance as a mechanism of DNA-damage induction in the higher concentration. **(Portugal)**

20. Hued, A.C., S. Oberhofer, and M. de los Ángeles Bistoni. 2012. Exposure to a commercial glyphosate formulation (Roundup®) alters normal gill and liver histology and affects male sexual activity of *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes). *Archives of Environmental Contamination and Toxicology* 62(1): 107-117.

    Roundup is the most popular commercial glyphosate formulation applied in the cultivation of genetically modified glyphosate-resistant crops. The aim of this study was to evaluate the histological lesions of the neotropical native fish, *Jenynsia multidentata*, in response to acute and subchronic exposure to Roundup and to determine if subchronic exposure to the herbicide causes changes in male sexual activity of individuals exposed to a sublethal concentration (0.5 mg/l) for 7 and 28 days. The estimated 96-h LC_{50} was 19.02 mg/l for both male and female fish. Gill and liver histological lesions were evaluated through histopathological indices allowing quantification of the histological damages in fish exposed to different concentrations of the herbicide. Roundup induced different histological alterations in a concentration-dependent manner. In subchronic-exposure tests, Roundup also altered normal histology of the studied organs and caused a significant decrease in the number of copulations and mating success in male fish exposed to the herbicide. It is expected that in natural environments contaminated with Roundup, both general health condition and reproductive success of *J. multidentata* could be seriously affected. **(Argentina)**

21. Jiraungkoorskul, W., E.S. Upatham, M. Kruatrachue, S. Sahaphong, S. Vichasri-Grams, and P. Pokethitiyook. 2003. Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*). *Environmental Toxicology* 18(4): 260-267.

    In *Oreochromis niloticus* that had been exposed for 3 months to sublethal concentrations (5 and 15 ppm) of the commercial glyphosate herbicide ($C_3H_8NO_5P$) Roundup, the organs exhibited varying degrees of histopathological change. In the gills filament cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial lifting, and aneurysm were observed. In the liver there were vacuolation of hepatocytes and nuclear pyknosis. Kidney lesions consisted of dilation of Bowman's space and accumulation of hyaline droplets in the tubular epithelial cells. The structural damages could be correlated to the significant increase ($p \leq 0.05$) in aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase activities in the second and third months of exposure. The results indicated that long-term exposure to glyphosate at sublethal concentrations had adverse effects on the histopathological and biochemical alterations of the fish. **(Thailand)**

22. Kannan C. and R.M. Kathiresan. 2002. Herbicidal control of water hyacinth and its impact on fish growth and water quality. *Indian Journal of Weed Science* 34(1&2): 92-95.

Fish

Imazapyr at 0.5 kg ha^{-1} resulted in maximum reduction in fresh weight of water hyacinth with 100% control. Paraquat, 2,4-D and glyphosate reduced the growth of water hyacinth. Water quality in relation to dissolved oxygen and pH was affected by all treatments. Paraquat at 0.9 kg ha^{-1}, 2,4-D at 1.0 kg ha^{-1} and glyphosate 2.2 kg ha^{-1} resulted in reduced dissolved oxygen content compared to the untreated control where common carps were cultured without any water hyacinth or herbicide treatments. However, the dissolved oxygen content in imazapyr treated water was reduced to 0.35 ppm. Presence of water hyacinth alone also reduced dissolved oxygen content to 2.59 ppm. All herbicidal treatments lowered the water pH and resulted in higher mortality of common carp. Imazapyr treatment had the highest common carp mortality (71.4%). In contrast, glyphosate treated water hyacinth resulted in only 14.3% mortality of common carp. **(India)**

23. Kelly, D.W., R. Poulin, D.M. Tompkins, and C.R. Townsend. 2010. Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival. *Journal of Applied Ecology* 47(2): 498-504.

Anthropogenic pollution and disease can cause both lethal and sub-lethal effects in aquatic species but our understanding of how these stressors interact is often not known. Contaminants can reduce host resistance to disease, but whether hosts are impacted at environmentally relevant concentrations is poorly understood. We investigated the independent and combined effects of exposure to the common herbicide glyphosate and the trematode parasite *Telogaster opisthorchis* on survival and the development of spinal malformations in juvenile *Galaxias anomalus*, a New Zealand freshwater fish. We then investigated how exposure to a glyphosate concentration gradient (0·36, 3·6, 36 mg active ingredient (a.i.) L^{-1}) affected the production and release of the infective cercarial stage of the parasite by its snail intermediate host *Potamopyrgus antipodarum*. Survival of juvenile fish was unaffected by exposure to glyphosate alone (at an environmentally relevant concentration; 0·36 mg a.i. L^{-1}) or by *T. opisthorchis* infection alone. However, simultaneous exposure to infection and glyphosate significantly reduced fish survival. Juvenile fish developed spinal malformations when exposed either to infections alone or to infections and glyphosate, with a trend towards greater severity of spinal malformation after exposure to both stressors. All snails exposed to the highest glyphosate concentration (36 mg a.i. L^{-1}) died within 24 h. Snails exposed to a moderate concentration (3·6 mg a.i. L^{-1}) produced significantly more *T. opisthorchis* cercariae than snails in the control group or the low concentration group (0·36 mg a.i. L^{-1}; the same concentration as in the fish experiment). *Synthesis and applications.* This is the first study to show that parasites and glyphosate can act synergistically on aquatic vertebrates at environmentally relevant concentrations, and that glyphosate might increase the risk of disease in fish. Our results have important implications when identifying risks to aquatic communities and suggest that threshold levels of glyphosate currently set by regulatory authorities do not adequately protect freshwater systems. **(New Zealand)**

24. Kreutz, L.C., L.J.G. Barcellos, A. Marteninghe, E.D. dos Santos, and R. Zanatta. 2010. Exposure to sublethal concentration of glyphosate or atrazine-based

herbicides alters the phagocytic function and increases the susceptibility of silver catfish fingerlings (*Rhamdia quelen*) to *Aeromonas hydrophila* challenge. *Fish & Shellfish Immunology* 29(4): 694-697.

The resistance of fish to microorganisms challenge depends mainly on the efficacy of the immune response. Most studies on the natural immune response of fish have focused on the effect of diets and immunostimulants. Few studies correlated the presence of commonly used agrichemical and susceptibility to infection by aquatic microorganism. Thus, this study aimed to investigate the effect of glyphosate and atrazine-based herbicides on immune cell phagocytosis and susceptibility of silver catfish to *Aeromonas hydrophila* infection. Following exposure to sublethal concentrations of glyphosate or atrazine (10% of the $LC_{50\text{-}96\ h}$), a significant decrease in the number of intracelomatic cells and phagocytic index could be observed. In addition, silver catfish fingerlings exposed to glyphosate or atrazine were more susceptible to intracelomatic challenge with pathogenic *A. hydrophila*. Thus, the presence of these herbicides on the water alters the natural immune response to bacterial and possibly to other aquatic microorganism. **(Brazil)**

25. Kreutz,L.C., L.J.G. Barcellos, D. Anziliero, T.O. Silva, D. Martins, M. Lorenson, A. Marteninghe, and L.B. da Silva. 2008. Acute toxicity test of agricultural pesticides on silver catfish (*Rhamdia quelen*) fingerlings. *Ciencia Rural* 38(4): 1050-1055.

Toxicity risks of agricultural pesticides to fishes are pivotal. Currently, many questions remain unsolved regarding to the toxicity of commonly used pesticides to silver catfish (*Rhamdia quelen*), a South American catfish. The present studies have been designed to investigate the acute toxicity and the lethal concentration (LC50) of four herbicides, two fungicides and two insecticides to silver catfish fingerlings. All experiments were carried out in triplicates, in a static bioassay system, using commercially available pesticides. The data was analyzed through the Trimmed Spearman-Karber method available from the Environmental Protection Agency. The 96hLC50 and 95% lower and upper confidence limits, respectively, for the following pesticides were determined: glyphosate (7.3mg L-1; 6.5-8.3), atrazine (10.2mg L-1; 9.1-11.5), atrazine+simazine (10.5mg L-1; 8.9-12.4), mesotrione (532.0mg L-1; 476.5-594), tebuconazole (5.3mg L-1; 4.9-5.7), methylparathion (4.8mg L-1; 4.3-5.3), strobulurin and triazol (9.9mg L-1; 8.7-11.2). Diflubenzuron was also tested and caused no fish mortality up to 1g L-1. The toxic concentration of these pesticides to silver catfish fingerlings fell above the concentration used for application in the field and, except following accidental application or misplacing of empty recipients, it should not cause fish mortality. Nonetheless, the data obtained will be useful to study the long-term effect of these products on the hematological, biochemical, hormonal and immunological parameters of silver catfish and related fish species in South Brazil. **(Brazil)**

26. Kreutz, L.C., L.J.G. Barcellos, S. de Faria Valle, T. de Oliveira Silva, D. Anziliero, E.D. dos Santos, M. Pivato, and R. Zanatta. 2011. Altered hematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate. *Fish and Shellfish Immunology* 30(1): 51-57.

Fish

Using agrichemicals to control unwanted species has become a necessary and common worldwide practice to improve crop production. Although most currently used agrichemicals are considered relatively safe, continuous usage contributes for soil and water contamination and collateral toxic effects on aquatic species. Few studies correlated the presence of agrichemicals on fish blood cells and natural immune system. Thus, in this study, silver catfish (*Rhamdia quelen*) were exposed to sublethal concentrations (10% of the $LC_{50\text{-}96\ h}$) of a glyphosate based herbicide and hematological and natural immune system parameters were evaluated. Silver catfish fingerlings exposed to glyphosate for 96 h had a significant reduction on blood erythrocytes, thrombocytes, lymphocytes and total leukocytes in contrast to a significant increase in the number of immature circulating cells. The effect of glyphosate on natural immune system was evaluated after 24h or 10 days exposure by measuring the phagocytic index of coelomic cells, and lysozyme, total peroxidase, bacteria agglutination, bactericidal activity and natural complement hemolytic activity in the serum of fingerlings. A significant reduction on phagocytic index, serum bacteria agglutination and total peroxidase was observed only after 24h exposure to glyphosate. In contrast, fingerlings exposed to glyphosate for 10 days had a significant lower serum bacteria agglutination and lysozyme activity. Glyphosate had no effect on serum bactericidal and complement natural hemolytic activity after 24h or 10 days exposure. Nonetheless, the information obtained in this study indicates that glyphosate contaminated water contributes to alter blood cells parameters and to reduce the activity of natural immune components important to mediate fish resistance to infecting microorganisms. **(Brazil)**

27. Langiano, V.C. and C.B.R. Martinez. 2008. Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. *Comparative Biochemistry and Physiology, Part C* 147(2): 222-231.

The toxicity of Roundup, a glyphosate-based herbicide widely used in agriculture, was determined for the Neotropical fish *Prochilodus lineatus*. The 96 h-LC_{50} of Roundup was 13.69 mg L^{-1}, indicating that this fish is more sensitive to Roundup than rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). These differences should be considered when establishing criteria for water quality and animal well-being in the Neotropical region. Short-term (6, 24 and 96 h) toxicity tests were then performed to evaluate the effects of sub-lethal concentrations of the herbicide (7.5 and 10 mg L^{-1}) to *P. lineatus*. Roundup did not interfere with the maintenance of the ionic balance and there was no significant alteration in plasma cortisol levels in Roundup-exposed fish. However an increase in plasma glucose was noted in fish exposed to 10 mg L^{-1} of the herbicide, indicating a typical stress response. Catalase liver activity also showed an increase in fish exposed to 10 mg L^{-1} of the herbicide, suggesting the activation of antioxidant defenses after Roundup exposure. In addition, Roundup induced several liver histological alterations that might impair normal organ functioning. Therefore, short-term exposure to Roundup at sublethal concentrations induced biochemical, physiological and histological alterations in *P. lineatus*. **(Brazil)**

Fish

28. Li, S.N. and R. K. Kole. 2004. Response of gill ATPase and liver esterase of *Pseudorasobora parva* to a two month exposure to glyphosate and metsulfuron methyl. *Toxicological and Environmental Chemistry* 86(4): 239-245.

    Responses of gill ATPase and liver esterase of topmouth gudgeon, *Pseudorasobora parva* under sublethal exposure to glyphosate (WSC 41% as isopropyl amine salt) at 1.0, 5.0 and 25 mg/L and metsulfuron methyl (technical 98.2%) at 0.0095, 0.085 and 0.85 mg/L of water were measured at 8th, 16th, 24th and 65th days of exposure. Two way analysis of variance (ANOVA) followed by least significant difference (LSD) test and parameter estimates indicated a significant inhibitory effect on gill ATPase activity (max. 57%) by metsulfuron methyl and liver esterase activity by glyphosate (max. 43%) as compared to the control, but the differences in the residual activities among the concentration levels of the herbicides were not significant. The activities changed significantly with the sampling times except gill ATPase activity under metsulfuron methyl exposure. In most of the cases, the maximum inhibitory effect on the enzymes was recorded on the 8th day and over-recovering appeared with time. **(China)**

29. Lushchak, O.V., O.I. Kubrak, J.M. Storey, K.B. Storey, and V.I. Lushchak. 2009. Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. *Chemosphere* 76(7): 932-937.

    The formulation of Roundup consists of the herbicide glyphosate as the active ingredient with polyethoxylene amine added as a surfactant. The acute toxicity of Roundup (particularly of glyphosate) to animals is considered to be low according to the World Health Organization, but the extensive use of Roundup may still cause environmental problems with negative impact on wildlife, particularly in an aquatic environment where chemicals may persist for a long time. Therefore, we studied the effects of Roundup on markers of oxidative stress and antioxidant defense in goldfish, *Carassius auratus*. The fish were given 96 h exposure to Roundup at concentrations of 2.5-20 mg L^{-1}. Exposure to Roundup did not affect levels of lipid peroxides (LOOH) in goldfish brain or liver, and in kidney only the 10 mg L^{-1} treatment elevated LOOH by 3.2-fold. Herbicide exposure also had no effect on the concentrations of protein thiols or low molecular mass thiols in kidney, but selective suppression of low molecular mass thiols by 26-29% occurred at some treatment levels in brain and liver. Roundup exposure generally suppressed the activities of superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione reductase and glucose-6-phosphate dehydrogenase in fish tissues. For example, SOD activities were reduced by 51-68% in brain, 58-67% in liver and 33-53% in kidney of Roundup treated fish. GST activity decreased by 29-34% in liver. However, catalase activity increased in both liver and kidney of herbicide-exposed fish. To our knowledge this is the first study to demonstrate a systematic response by the antioxidant systems of fish to Roundup exposure. **(Ukraine)**

30. Menéndez-Helman, R.J., G.V.Ferreyroa, M. dos Santos Afonso, and A. Salibián. 2012. Glyphosate as an acetylcholinesterase inhibitor in *Cnesterodon decemmaculatus*. *Bulletin of Environmental Contamination and Toxicology* 88(1): 6-9.

Fish

The toxic effect of sublethal concentrations (1, 17.5 and 35 mg L^{-1} of pure glyphosate was evaluated on acetylcholinesterase (AChE) activity in the fish species, *Cnesterodon decemmaculatus*. Acute bioassays (96 h) under laboratory conditions were conducted and homogenates for each specimen corresponding to the anterior, middle and posterior body sections were performed. Fish survival was 100%, even at the highest concentration tested (35 mg L^{-1}), in accordance with the low lethal toxicity reported for glyphosate. However, a significant inhibitory effect on AChE activity was recorded even for the lowest herbicide concentration tested (1 mg L^{-1}), in the homogenates corresponding to the anterior body section. The inhibition ranged from 23 to 36%. The analytical determination of glyphosate in assay media by ion chromatography, was used to verify its stability. These results indicate that AChE-a neurotoxicity biomarker-in *C. decemmaculatus* may be affected by exposure to environmentally relevant concentrations of glyphosate. **(Argentina)**

31. Modesto, K.A., and C.B.R. Martinez. 2010. Roundup® causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. *Chemosphere* 78(3): 294-299.

This work aimed to evaluate Roundup effects on biochemical biomarkers of the neotropical fish *Prochilodus lineatus*. Fish were acutely exposed (6, 24 and 96 h) to 10 mg L^{-1} of Roundup® (RD) or only water (control) and samples of liver, for antioxidants analysis, and brain and muscle, for acetylcholinesterase (AChE) determination, were collected. Fish exposed to RD for 24h showed reduction on superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities, and increased glutathione (GSH) content. After 24 and 96h, fish of RD group showed increased glutathione-S-transferase (GST) activity and lipid peroxidation. AChE activity was inhibited in brain after 96h and in muscle after 24 and 96h of exposure. Thus, acute exposure to RD stimulated the biotransformation pathway, with increased GST, but interfered on the antioxidant defenses, with reduction of SOD and GPx activity, leading to the occurrence of lipid peroxidation. Inhibition of AChE showed that RD acts as a contaminant with anti-AChE action. **(Brazil)**

32. Modesto, K.A., and C.B.R. Martinez. 2010. Effects of Roundup Transorb on fish: hematology, antioxidant defenses and acetylcholinesterase activity. *Chemosphere* 81(6): 781-787.

Roundup Transorb® (RDT) is a glyphosate-based herbicide containing a mixture of surfactants. The objective of this work was to evaluate the effects of this herbicide on the Neotropical fish *Prochilodus lineatus*. Juvenile fish were acutely exposed (6, 24 and 96 h) to 1 mg L^{-1} of RDT (RDT 1), 5 mg L^{-1} of RDT (RDT 5) or only water (control) and blood samples for hematological analysis, liver for antioxidants analysis, and brain and muscle for acetylcholinesterase (AChE) determination, were collected. RDT effects were more evident in fish exposed to the higher concentration of the herbicide. Hematologic alterations appeared only after 96 h exposure, when fish showed an increase in the hematocrit and in the number of both red and white blood cells. After 6h exposure fish showed a transient reduction in superoxide dismutase and catalase activity. RDT also inhibited glutathione-S-transferase, after 6 and 24h of exposure. The reduction in these enzymes is probably related to the

occurrence of lipid peroxidation (LPO) in fish exposed to the herbicide for 6h. LPO returned to control levels after 24 and 96 h exposure to RDT, when fish showed an increased activity of glutathione peroxidase. The content of reduced glutathione also increased after 96 h exposure. Thus, after 24 and 96 h the antioxidant defenses were apparently enough to combat ROS, preventing the occurrence of oxidative damage. The exposure to RDT for 96 h led to an inhibition of AChE in brain and muscle at rates which may not be considered a life-threatening situation. **(Brazil)**

33. Okonkwo, F.O. and C.E.C.C. Ejike. 2011. Simulation of heavy metal contamination of fresh water bodies: toxic effects in the catfish and its amelioration with co-contamination with glyphosate. *Journal* of *Applied Sciences and Environmental Management* 15(2): 341-345.

The toxic implications of fresh water contamination with zinc in the catfish, *Clarias albopunctatus* (Lamonte and Nicole, 1927), and the effect of a co-contamination with a sub-lethal dose of glyphosate (Roundup) was studied using the static bioassay model. Thirty six fish were divided into 3 equal groups. Fish in Group 1 were placed in normal tap water, and served as the control group, while fish in Groups 2 and 3 were placed in water contaminated with $ZnSO_4$ and $ZnSO_4$ + glyphosate, respectively. The study lasted for 96 hours (though sampling was done at the 48th hour). Biochemical markers of toxicity were measured and the fish liver and gill histology were studied using standard protocols. The results show that ZnSO4 was significantly toxic to the fish only after 96 hours. Co-contamination of the water with both toxicants was found to ameliorate the toxic effects of ZnSO4 significantly. The metal chelating property of glyphosate may be responsible for the observed attenuation of toxicity in the fish in Group 3. **(Nigeria)**

34. Ortiz-Ordoñez, E., E. Uría-Galicia, R.A. Ruiz-Picos, A.G. Sánchez Duran, Y. H. Trejo, J.E. Sedeño-Díaz, and E. López- López. 2011. Effect of Yerbimat herbicide on lipid peroxidation, catalase activity, and histological damage in gills and liver of the freshwater fish *Goodea atripinnis*. *Archives of Environmental Contamination and Toxicology* 61(3): 443-452.

The use of herbicides for agricultural and aquatic weed control has increased worldwide. These substances are potentially toxic pollutants because they induce the production of reactive oxygen species for biological systems and exert oxidative stress in nontarget organisms living in the treated aquatic systems. Recent evidence suggests differences in the toxicity of glyphosate in the form of an active ingredient compared to the toxicity of glyphosate in combination with surfactants, such as those found in commercial formulations. In Mexico, one of the most widely used glyphosate-based herbicides is Yerbimat, which has agricultural as well as aquatic weed control applications. However, there are no aquatic toxicity data, particularly regarding native fish. Therefore, we determined the acute toxicity of commercial-formulation Yerbimat in a static bioassay at 96 h (LC_{50}). We also determined its toxicity at 96 h in sublethal concentrations to assess the lipid peroxidation levels (LPX), catalase activity, hepatic glycogen content, and histological damage in the liver and gills of the fish *Goodea atripinnis* associated with chronic exposure (75 days). The LC_{50} was 38.95 ± 0.33 mg/L. The results of the short-term exposure

study indicate that Yerbimat can potentially induce oxidative stress in *G. atripinnis*, because LPX was increased in the gills and liver. Catalase activity was reduced in the gills but increased in the liver, whereas hepatic glycogen was depleted. Chronic exposure was associated with histopathological damage in the gills and liver, some of which was irreversible. Yerbimat represents a potential risk for aquatic biota; therefore, we recommend that its application be carefully considered. **(Mexico)**

35. Osten, J.R., A. Ortíz-Arana, L. Guilhermino, and A.M.V.M. Soares. 2005. In vivo evaluation of three biomarkers in the mosquitofish (*Gambusia yucatana*) exposed to pesticides. *Chemosphere* 58(5): 627-636.

In this study, the acute toxicity and the in vivo effects of commercial chlorpyrifos, carbofuran and glyphosate formulations on cholinesterase (ChE), glutathione S-transferase (GST) and lactate dehydrogenase (LDH) activities of the mosquitofish (*Gambusia yucatana*) were investigated. In a first phase of the study, head and muscle ChE were characterized with different substrates (acetylthiocholine iodide, s-butyrylthiocholine iodide and propionylthiocholine iodide) and the selective inhibitors eserine hemisulfate, 1,5-bis(4-allyldimethylammoniumphenyl)-pentan-3-one dibromide (BW284C51), and N,N'-diisopropylphosphorodiamic acid (iso-OMPA). The results obtained suggest that the enzyme present in both head and muscle of *G. yucatana* is mainly acetylcholinesterase (AChE). Acute toxicity was evaluated by exposing fish to several concentrations of single pesticides and of a mixture of chlorpyrifos/glyphosate. LC_{50} values were determined after 96 h of exposure, except in the case of carbofuran for which LC_{50} was calculated after 24 h since almost all the fish died within this period. LC_{50} values were 0.085 mg/l for chlorpyrifos, 17.79 mg/l for glyphosate, 0.636 mg/l for carbofuran and 0.011 mg/l for the chlorpyrifos/glyphosate mixture. A Toxic Unit approach was used to compare the toxicity of chlorpyrifos and glyphosate when occurring in a mixture with their toxicities as single compounds. Synergistic effects of chlorpyrifos and glyphosate when present in a mixture were found. At the end of each bioassay (24 h for carbofuran, 96 for the other substances/mixture), effects on biomarkers were analyzed. Muscle LDH activity was not altered by any of the three pesticides tested. Gill GST activity was significantly inhibited (40%) by carbofuran after 24 h of exposure to concentrations equal or higher than 0.06 mg/l. ChE muscle and head activity were significantly inhibited (50% and 30%, respectively) by carbofuran at concentrations equal or higher than 0.25 mg/l. Chlorpyrifos induced a significant inhibition of both muscle and head ChE (80% and 50%, respectively) after 96 h of exposure to concentrations equal or higher than 0.05 mg/l. Carbofuran did not induce significant alterations of fish ChE. The ChE EC_{50} determined for chlorpyrifos/glyphosate mixture (0.070 mg/l) was higher than the correspondent value calculated for chlorpyrifos alone (0.011 mg/l) suggesting an antagonistic effect of glyphosate on ChE inhibition by chlorpyrifos. ChE activity of *G. yucatana* seems to be a good biomarker to diagnose the exposure of wild populations of this species exposed to anticholinesterase pesticides. **(Mexico)**

Fish

36. Peterson, R.K., and A.G. Hulting. 2004. A comparative ecological risk assessment for herbicides used on spring wheat: the effect of glyphosate when used within a glyphosate-tolerant wheat system. *Weed Science* 52: 834-844.

See Birds Section.

37. Rossi, S.C., M.D. da Silva, L.D.S. Piancini, C.A.O. Ribeiro, M.M. Cestari, and H.C.S. de Assis. 2011. Sublethal effects of waterborne berbicides in tropical freshwater fish. *Bulletin of Environmental Contamination and Toxicology* 87(6): 603-607.

The study evaluated the sublethal effects of the herbicides glyphosate (Roundup) and diuron (Hexaron) and the mixture of them, used extremely in agriculture, through biomarkers in fish. The glutathione S-transferase activity increased (74%) and catalase activity decreased (37%) at the higher exposure concentration of Hexaron in comparison to the control group, suggesting an activation of this metabolism route. Membrane damage was observed at the higher exposure of Roundup and in the mixture group compared to the control group, which can be related to the nuclear alterations observed in these exposed groups. The cholinesterase activity was also inhibited (37%) in mixture group compared to the control group and no gill morphology damage was found. The results suggested a potential synergic effect in some analysed parameters. **(Brazil)**

38. Rouleau, C., and J. Kohli. 2008. Distribution of ^{14}C-labelled atrazine, methoxychlor, glyphosate, and bisphenol-A in goldfish studied by whole-body autoradiography (WBARG). *Water Quality Research Journal of Canada* 43(4): 265-274.

Nonpersistent contaminants represent thousands of chemicals used as pesticides, pharmaceuticals, personal care products, additives, etc. Because of this diversity, the assessment of the environmental risks they may pose for the environment represents a formidable task. Identification of target organs is key information needed to orient further research on newly-investigated organic xenobiotics. We used whole-body autoradiography to visualize the distribution of ^{14}C-labelled atrazine, methoxychlor, glyphosate, and bisphenol-A in goldfish (*Carassius auratus*) and identify target organs. Fish were exposed for 2 days (glyphosate and bisphenol-A) and 7 days (atrazine and methoxychlor) to the radiolabelled compounds at a concentration of 15 nM. They were then frozen, embedded in carboxymethylcellulose gel, 20-µm-thick cryosections were collected, freeze-dried, and exposed to phosphor screens to visualize the tissue distribution of radioactivity. Goldfish did not accumulate glyphosate. The three other compounds were accumulated, mostly in the gall bladder. Nevertheless, unforeseen accumulation sites were observed; atrazine accumulated in the uveal tract of the eye, high levels of radioactivity were found in the cerebrospinal fluid of goldfish exposed to methoxychlor, and an important accumulation of bisphenol-A was seen in urine, oral mucosa, esophagus, and intestinal lumen. The potential toxicological consequences of the accumulation of these chemicals at very specific locations within the fish body are discussed and further research suggested. **(Canada)**

39. Salbego, J. A. Pretto, C.R. Gioda, C.C. de Menezes, R. Lazzari, J.R. Neto, B. Baldisseerotto, and V.L. Loro. 2010. Herbicide formulation with glyphosate affects

growth, acetylcholinesterase activity, and metabolic and hematological parameters in piava (*Leporinus obtusidens*). *Archives of Environmental Contamination and Toxicology* 58(3): 740-745.

The teleost fish *Leporinus obtusidens* (piava) was exposed to different concentrations of Roundup, a commercial herbicide formulation containing glyphosate (0, 1, or 5 mg L^{-1}), for 90 days. Acetylcholinesterase (AChE) activity was verified in brain and muscle. Hepatic and muscular metabolic parameters as well as some hematological parameters were determined. The results showed that brain AChE activity was significantly decreased in fish exposed to 5 mg L^{-1} Roundup, whereas muscular AChE activity was not altered. Both Roundup concentrations significantly decreased liver glycogen without altering the muscle glycogen content. Hepatic glucose levels were reduced only in fish exposed to 5 mg L^{-1} Roundup. Lactate levels in the liver and muscle significantly increased in fish exposed to both Roundup concentrations. Hepatic protein content remained constant at 1 mg L^{-1} but increased at 5 mg L^{-1} Roundup. In the muscle however, protein content decreased with increasing exposure concentration. The herbicide exposure produced a decrease in hematological parameters at both concentrations tested. The majority of observed effects occur at environmental relevant concentrations, and in summary, the results show that Roundup affects brain AChE activity as well as metabolic and hematologic parameters of piavas. Thus, we can suggest that long-term exposure to Roundup causes metabolic disruption in *Leporinus obtusidens*. **(Brazil)**

40. Senapati, T., A.K. Mukerjee, and A.R. Ghosh. 2009. Observations on the effect of glyphosate based herbicide on ultra structure (SEM) and enzymatic activity in different regions of alimentary canal and gill of *Channa punctatus* (Bloch). *Journal of Crop and Weed* 5(1): 236-245.

Glyphosate is the isopropyl amine salt of N-(Phosphonomethyl)-glycine, a broad-spectrum nonselective herbicide, which has been extensively used to control annual and perennial weeds in agricultural, forest and aquatic systems. The ultrastructural changes in different regions of alimentary canal and gill were observed by Scanning Electron Microscopic study on a non-target aquatic teleostea fish, *Channa punctatus*. Fishes were exposed to herbicide at a dose of 4 mgl^{-1} generally used by farmers to control weeds in water bodies for a period of 45 days in laboratory condition with a control. Severe damage, shrinkage and degeneration of pentagonal cellular contour of stratified epithelial cells (SEC) were observed in gill. Shrinkage of SEC resulting in degeneration of microridges was observed in buccopharynx. Slight necrosed and distorted SEC was observed in oesophagus. Severe mucus secretion was observed in stomach. Erosion on the apical surface of mucosal folds and columnar epithelial cells (CEC) and necrosis of CEC was also noticed in stomach. Obliteration of CEC along its entire length from basement membrane was observed in the intestinal portion. After 45 days treatment by glyphosate protease activity was slightly reduced in stomach and intestine in comparison to control fish. Amylase activity reduced in oesophagus and intestine in treated condition. Lipase activity was also reduced slightly in stomach and intestine of glyphosate treated fish. **(India)**

Fish

41. Slaninova, A., M. Smutna, H. Modra, and Z. Svobodova. 2009. A review: oxidative stress in fish induced by pesticides. *Neuro Endocrinology Letters* 30 Supplement 1: 2-12.

The knowledge in oxidative stress in fish has a great importance for environmental and aquatic toxicology. Because oxidative stress is evoked by many chemicals including some pesticides, pro-oxidant factors' action in fish organism can be used to assess specific area pollution or world sea pollution. Hepatotoxic effect of DDT may be related with lipid peroxidation. Releasing of reactive oxygen species (ROS) after HCB exposure can be realized via two ways: via the uncoupling of the electron transport chain from monooxygenase activity and via metabolism of HCB major metabolite pentachlorophenol. Chlorothalonil disrupts mitochondrial metabolism due to the impairment of NADPH oxidase function. Activation of spleen macrophages and a decrease of catalase (CAT) activity have been observed after endosulfan exposure. Excessive release of superoxide radicals after etoxazole exposure can cause a decrease of CAT activity and increase phagocytic activity of splenocytes. Anticholinergic activity of organophosphates leads to the accumulation of ROS and resulting lipid peroxidation. Carbaryl induces changes in the content of glutathione and antioxidant enzymes activities. The antioxidant enzymes changes have been observed after actuation of pesticides deltamethrin and cypermethrin. Bipyridyl herbicides are able to form redox cycles and thereby cause oxidative stress. Low concentrations of simazine do not cause oxidative stress in carps during sub-chronic tests while sublethal concentrations of atrazin can induce oxidative stress in bluegill sunfish. Butachlor causes increased activity of superoxide dismutase -catalase system in the kidney. Rotenon can inhibit the electron transport in mitochondria and thereby increase ROS production. Dichloroaniline, the metabolite of diuron, has oxidative effects. Oxidative damage from fenpyroximate actuation is related to the disruption of mitochondrial redox respiratory chain. Low concentration of glyphosate can cause mild oxidative stress. **(Czech Republic)**

42. Solomon, K.R., A. Anadón, G. Carrasquilla, A.L. Cerdeira, and J. Marshall, and L.-H. Sanin. 2007. Coca and poppy eradication in Colombia: environmental and human health assessment of aerially applied glyphosate. *Reviews of Environmental Contamination and Toxicology* 190: 43-125.

See Human Health Section.

43. Sopin´ska, A., A. Grochoła, and J. Niezgoda. 2000. Influence of water pollution with Roundup herbicide on fish health. *Journal Medycyna Weterynaryjna* 56(9): 593-597.

The effects of sublethal concentrations of Roundup [glyphosate] (4, 6, 8 mg/litre) in water on non-specific immunity and the liver and kidneys was investigated in 120 healthy carp weighing 60-80 g. Non-specific immunity, phagocytic activity of blood and kidney neutrophils and macrophages, and lysozyme level all decreased during the experiment. Histopathology revealed degenerative changes of the liver and kidneys. Changes in the liver were characterized by oedema, vacuolar degeneration and focal fibrosis. In kidneys, degenerative changes related to tubular cells were noted, as well as necrosis of haemopoietic tissue and focal fibrosis. It is concluded that Roundup is toxic to carp. **(Poland)**

Fish

44. Soso, A.B., L.J.G. Barcellos, M.J. Ranzani-Paiva, L.C. Kreutz, R.M. Quevedo, D. Anziliero, *et al.* 2007. Chronic exposure to sub-lethal concentration of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundiá (*Rhamdia quelen*). *Environmental Toxicology and Pharmacology* 23(3): 308-313.

This work was carried out to verify the effect of a glyphosate-based herbicide on Jundiá hormones (cortisol, 17β-estradiol and testosterone), oocyte and swim-up fry production. Earthen ponds containing Jundiá females were contaminated with glyphosate (3.6mg/L); blood samples were collected from eight females from each treatment immediately before, or at 1, 10, 20, 30 and 40 days following contamination. A typical post-stress rise in cortisol levels was observed at the 20th and 40th days following exposure to glyphosate. At the 40th day, 17β-estradiol was decreased in the exposed females. A similar number of oocytes were stripped out from females from both groups; however, a lower number of viable swim-up fry were obtained from the herbicide exposed females, which also had a higher liver-somatic index (LSI). The results indicate that the presence of glyphosate in water was deleterious to *Rhamdia quelen* reproduction, altering steroid profiles and egg viability. **(Brazil)**

45. Stehr, C.M., T.L. Linbo, D.H. Baldwin, N.L. Scholz, and J.P. Incardona. 2009. Evaluating the effects of forestry herbicides on fish development using rapid phenotypic screens. *North American Journal of Fisheries Management* 29(4): 975-984.

Herbicides are used to control invasive or noxious plants on public lands throughout the western United States. These chemicals are often applied in the upper reaches of watersheds that provide spawning and rearing habitat for anadromous species of Pacific salmon *Oncorhynchus* spp., steelhead *O. mykiss*, and bull trout *Salvelinus confluentus*. As a consequence, natural resource managers must often weigh the ecological benefits of using herbicides to control nonnative plants against the potential for unintended toxicological impacts on salmonids, particularly during early life stages. However, the effects of chemical control agents on fish development have not been widely investigated. Their use in the vicinity of aquatic habitats, including areas that support threatened and endangered species, is therefore a concern. To address this uncertainty, we used the zebrafish *Dania rerio* as a model experimental system for investigating developmental toxicity, which involved conducting rapid and sensitive phenotypic screens for potential developmental defects resulting from exposure to six herbicides (picloram, clopyralid, imazapic, glyphosate, imazapyr, and triclopyr) and several technical formulations (Tordon K, Transline, Habitat, Plateau, Garlon 3A, and Renovate). Zebrafish embryos were exposed continuously through 5 d of development at nominal concentrations ranging from 3 ug/L to 10 mg/L. Detailed screens were used to examine aspects of ontogeny from early development (gastrulation and segmentation) through organogenesis, hatching, and morphology as free-swimming larvae. Growth was measured at the end of each exposure interval. To detect defects in neural development (sensorimotor integration), the escape reflex of larvae was monitored at 3, 4, and 5 d after fertilization. No developmental toxicity was

observed in response to the six individual herbicides or the different technical formulations. The absence of toxicity at relatively high exposure concentrations suggests that noxious weed control activities are not likely to pose a direct threat to the health of salmonids at early life stages. **(U.S.A.)**

46. Szarek, J, A. Siwicki, A. Andrzejewska, E. Terech-Majewska, T. Banaszkiewicz. Effects of the herbicide Roundup™ on the ultrastructural pattern of hepatocytes in carp (*Cyprinus carpio*). *Marine Environmental Research* 50(1-5): 263-266.

    Experimental studies were performed on healthy, 80-100 g carp (*Cyprinus carpio*). Fish were exposed by emersion in Roundup™ (205 mg of glyphosate/l or 410 mg of glyphosate/l) in concentrations of 40- to 20-fold lower than those used in practice. Electron microscopy revealed that the herbicide caused appearance of myelin-like structures in carp hepatocytes, swelling of mitochondria and disappearance of internal membrane of mitochondria in carp at both exposure concentrations. It means that Roundup™ was harmful to carp when used in applied concentrations. Results of these studies enhance our knowledge of ultrastructural pathomorphology of fish organs following exposure to Roundup. **(Poland)**

47. Tierney, K.B., P.S. Ross, H.E. Jarrard, K.R. Delaney, and C.J. Kennedy. 2006. Changes in juvenile coho salmon electro-olfactogram during and after short-term exposure to current-use pesticides. *Environmental Toxicology and Chemistry* 25(10): 2809-2817.

    For anadromous salmonids, olfaction is a critical sense, enabling return migration. In recent years, several pesticides have been identified that interfere with salmonid olfaction at concentrations in the µg/L range; thus, they may pose a risk to species longe]ity. In the present study, we investigated the acute effects of five agricultural pesticides on juvenile coho salmon *(Oncorhynchus kisutch)* olfaction using the electro-olfactogram (EOG), a measure of odorant-evoked field potentials. Electro-olfactogram responses to the odorant L-serine were measured during and following a 30-min exposure of the left olfactory rosette to chlorothalonil, endosulfan, glyphosate acid, iodocarb (IPBC), trifluralin, and 2,4-dichlorophenoxyacetic acid. With the relatively insoluble pesticides endosulfan and trifluralin, decreases in EOG amplitude were only apparent at relatively high concentrations (100 and 300 µg/L, respectively) following 20 min of exposure and were absent for chlorothalonil (1 mg/L). With the water-soluble herbicide glyphosate, significant EOG reductions occurred within 10 min of exposure to 1 mg/L and more rapidly with higher concentrations. Recovery of EOG post-glyphosate exposure was concentration-dependent, and complete recovery was not observed with some concentrations at 60 min postexposure. Dichlorophenoxyacetic acid only affected EOG at high concentration (100 mg/L), where it eliminated EOG within 2 min of exposure. With IPBC, EOG was decreased at 25 min of exposure to 1 µg/L; higher concentrations caused decreases to occur more rapidly. Excluding IPBC and glyphosate, all EOG reductions occurred at concentrations greater than the current Canadian water-quality guidelines and reported 96-h lethality values. Our results show that olfactory neurons can be impaired rapidly by some current-use pesticides, even at exposures in the low-µg/L range. **(Canada)**

Fish

48. Tierney, K.B, M.A. Sekela, C.E. Cobbler, B. Xhabija, M. Gledhill, S. Ananvoranich, and B.S. Zielinski. 2011. Evidence for behavioral preference toward environmental concentrations of urban-use herbicides in a model adult fish. *Environmental Toxicology and Chemistry* 30(9): 2046-2054.

Fish live in waters of contaminant flux. In three urban, fish-bearing waterways of British Columbia, Canada, we found the active ingredients of WeedEx®, KillEx®, and Roundup® herbicide formulations (2,4-D, dicamba, glyphosate, and mecoprop) at low to high ng/L concentrations (0.26 to 309 ng/L) in routine conditions, i.e., no rain for at least one week. Following rain, these concentrations increased by an average of eightfold, suggesting runoff as a major route of herbicide introduction in these waterways. To determine whether fish might be able to limit point-source exposures through sensory-driven behaviors, we introduced pulses of representative herbicide mixtures to individual adult zebrafish (a model species) in flow-through tanks. Fish did the opposite of limit exposure; they chose to spend more time in pulses of herbicide mixtures representative of those that may occur with rain events. This attraction response was not altered by a previous 4-d exposure to lower concentrations of the mixtures, suggesting fish will not learn from previous exposures. However, previous exposures did alter an attraction response to an amino acid prevalent in food (L-alanine). The present study demonstrates that fish living within urban waterways may elect to place themselves in herbicide-contaminated environments and that these exposures may alter their behavioral responses to cues necessary for survival. **(Canada)**

49. Tsui, M.T.K. and L.M. Chu. 2008. Environmental fate and non-target impact of glyphosate-based herbicide (Roundup) in a subtropical wetland. *Chemosphere* 71(3): 439-446.

See Water Quality Section.

50. Ugaddan, G.R. and P.P. Ocampo. 2009. Brain acetylcholinesterase (AChE) activity and liver melanomacrophage centers (MMCs) formation in Nile tilapia (*Oreochromis niloticus* L.) following exposure to glyphosate herbicide. *Asia Life Sciences* 18(1): 73-85.

This study aimed to determine the pattern of brain acetylcholinesterase (ACK) activity and melanomacrophage centers (MMCs) proliferation in *Oreochromis niloticus* L. upon exposure to sublethal doses of glyphosate herbicide. In the dose-response experiment, significant differences in main brain AChE values was found among treatment groups (KW = 27.67, $P<0.05$). No linear relationship, however, was detected between treatment doses and rate of AChE inhibition. The time-course pattern experiment showed that the length of exposure period influences the rate of ACK inhibition (KW = 30.66, $P<0.05$), and that moderate negative linear relationship exist between length of exposure and rate of enzyme inhibition. In a recovery experiment, it was found that brain AChE activity recovered 7 days after the removal of test fish from the-treated water; and at I V, day period, AChE activity appeared fully recovered. Liver MMCs were found to develop in fish upon exposure to sublethal concentration of glyphosate. Increasing doses of the test compound

caused an increase in the number of liver MMCs; however, their sizes were independent from treatment doses. Increase in the length of exposure period also caused an increase in the number of MMCs but again the size of aggregates was found to be independent from the length of exposure period. **(Philippines)**

51. Warren, R.S., P.E. Fell, J.L. Grimsby, E.L. Buck, G.C. Rilling, and R.A. Fertik. 2001. Rates, patterns, and impacts of Phragmites australis expansion and effects of experimental Phragmites control on vegetation, macroinvertebrates, and fish within tidelands of the lower Connecticut River. *Estuaries* 24(1): 90-107.

Phragmites expansion rates (linear at 1-3% yr^{-1}) and impacts of this expansion on high marsh macroinvertebrates, aboveground production, and litter decomposition from *Phragmites* and other marsh graminoids were studied along a polyhaline to oligohaline gradient. These parameters, and fish use of creeks and high marsh, were also studied in *Phragmites* control sites (herbicide, mowing, and combined herbicide/mow treatments). *Phragmites* clones established without obvious site preferences on oligohaline marshes, expanding radially. At higher salinities, *Phragmites* preferentially colonized creekbank levees and disturbed upland borders, then expanded into the central marsh. Hydroperiods, but not salinities or water table, distinguished *Phragmites*-dominated transects. Pooled samples of *Phragmites* leaves, stems, and flowers decompose more slowly than other marsh angiosperms; *Phragmites* leaves alone decompose as or more rapidly than those of cattail. Aboveground *Phragmites* production was 1,300 to 2,400 g m^{-2} (about 23% of this as leaves), versus 600-800 g m^{-2} for polyhaline to mesohaline meadow and 1,300 g m^{-2} for oligohaline cattail-sedge marsh. Macroinvertebrates appear largely unaffected by *Phragmites* expansion or control efforts; distribution and densities are unrelated to elevation or hydroperiod, but densities are positively related to litter cover. Dominant fish captured leaving flooded marsh were *Fundulus heteroclitus* and *Anguilla rostrata*; both preyed heavily on marsh macroinvertebrates. *A. rostrata* and *Morone americana* tended to be more common in *Phragmites*, but otherwise there were no major differences in use patterns between *Phragmites* and brackish meadow vegetation. SAV and macroalgal cover were markedly lower within a *Phragmites*-dominated creek versus one with *Spartina*-dominated banks. The same fish species assemblage was trapped in both plus a third within the herbicide/mow treatment. Fish biomass was greatest from the *Spartina* creek and lowest from the *Phragmites* creek, reflecting abundances of *F. heteroclitus*. Mowing depressed *Phragmites* aboveground production and increased stem density, but was ineffective for control. *Phragmites*, *Spartina patens*, and *Juncus gerardii* frequencies after herbicide-only treatment were 0.53-0.21; total live cover was <8% with a heavy litter and dense standing dead stems. After two growing seasons *Agrostis stolonifera*/*S. patens*/*J. gerardii* brackish meadow characterized most of the herbicide/mow treatment area; *Phragmites* frequency here was 0.53, contributing 3% cover. Both values more than doubled after four years; a single treatment is ineffective for long-term *Phragmites* control. **(U.S.A.)**

Fish

52. Zhu, G.N., Z.Y. Lou, and J.H. Sun. 2000. Study on toxicity and environmental safety of glyphosate to aquatic organisms. *Journal of Zhejiang University (Agriculture and Life Sciences)* 26(3): 309-312.

The toxicity of glyphosate to aquatic organisms and dosage responses were investigated. Glyphosate showed low toxicity to fish (*Pseudorasbora parva*, $LC_{50,24\text{-}96h}$ > 403.8 mg/litre), daphnia (*Daphnia pulex*, $LC_{50,24h}$ = 20.04 mg/litre) and algae (*Scenedesmus obliquus*, $LC_{50,24\text{-}120h}$ > 308.6 mg/litre). Daphnia was the most sensitive of the organisms to glyphosate. The results also showed that the disappearance of glyphosate in water was very fast, being reduced to less than 0.01 mg/litre on the 6th day after application. Glyphosate could be absorbed on sediments, however, and thus disappeared more slowly. **(China)**

Human Health

1. Acquavella, J.F., B.H. Alexander, J.S. Mandel, C. Gustin, B. Baker, P. Chapman and M. Bleeke. 2004. Glyphosate biomonitoring for farmers and their families: results from the Farm Family Exposure Study. *Environmental Health Perspectives* 112(3): 321-326.

   Glyphosate is the active ingredient in Roundup agricultural herbicides and other herbicide formulations that are widely used for agricultural, forestry, and residential weed control. As part of the Farm Family Exposure Study, we evaluated urinary glyphosate concentrations for 48 farmers, their spouses, and their 79 children (4-18 years of age). We evaluated 24-hr composite urine samples for each family member the day before, the day of, and for 3 days after a glyphosate application. Sixty percent of farmers had detectable levels of glyphosate in their urine on the day of application. The geometric mean (GM) concentration was 3 ppb, the maximum value was 233 ppb, and the highest estimated systemic dose was 0.004 mg/kg. Farmers who did not use rubber gloves had higher GM urinary concentrations than did other farmers (10 ppb vs. 2.0 ppb). For spouses, 4% had detectable levels in their urine on the day of application. Their maximum value was 3 ppb. For children, 12% had detectable glyphosate in their urine on the day of application, with a maximum concentration of 29 ppb. All but one of the children with detectable concentrations had helped with the application or were present during herbicide mixing, loading, or application. None of the systemic doses estimated in this study approached the U.S. Environmental Protection Agency reference dose for glyphosate of 2 mg/kg/day. Nonetheless, it is advisable to minimize exposure to pesticides, and this study did identify specific practices that could be modified to reduce the potential for exposure. **(U.S.A.)**

2. Anadón, A., J Del Pino, M.A. Martínez, V. Caballero, I. Ares, I. Nieto, M. R. Martínez-Larrañaga. 2008. Neurotoxicological effects of the herbicide glyphosate. *Toxicology Letters* 1805: S164.

   Glyphosate is a nonselective herbicide that inhibits plant growth through interference with the production of essential aromatic amino acids. Glyphosate have been extensively investigated for the potential to produce adverse health effects in humans. Studies point out that glyphosate may be a factor in the birth defects (autism, attention-deficit, and hyperactivity disorder) observed among children of herbicide applicators. Government regulatory agencies are reviewing the available scientific data to reevaluate the safety of glyphosate. The purpose of this work is to describe neurotoxicological effects following the administration of glyphosate (75, 150 and 800 mg/kg/day, orally for 5 days) in male Wistar rats (n = 6/group). Animals were sacrificed 24 h following the last dose of glyphosate and the brains were removed. The frontal cortex, midbrain and striatum were dissected and analyzed for the content of the neurotransmitters 5-hydroxytryptamine (5-HT) and itsmetabolite 5-hydroxy-3-indole acetic acid (5-HIAA), and dopamine (DA) and itsmetabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), using a HPLC method with electrochemical detection. A serotonin and dopamine depleting, dose-dependent, effects were produced by glyphosate. Glyphosate, at the highest dose,

decreased 5-HT levels in frontal cortex (32%, P < 0.001), midbrain (22%, P < 0.001) and striatum (49%, P < 0.001). Similarly, glyphosate, at the highest dose, decreased DA levels in frontal cortex (53%, P < 0.01), midbrain (15%, P < 0.001) and striatum (22%, P < 0.001) respect to controls. Also, glyphosate caused a statistically significant increase in the metabolites of serotonin and dopamine. **(Spain)**

3. Benachour, N. and G.-E. Séralini. 2009. Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chemical Research in Toxicology* 22(1): 97-105.

We have evaluated the toxicity of four glyphosate (G)-based herbicides in Roundup (R) formulations, from 10^5 times dilutions, on three different human cell types. This dilution level is far below agricultural recommendations and corresponds to low levels of residues in food or feed. The formulations have been compared to G alone and with its main metabolite AMPA or with one known adjuvant of R formulations, POEA. HUVEC primary neonate umbilical cord vein cells have been tested with 293 embryonic kidney and JEG3 placental cell lines. All R formulations cause total cell death within 24 h, through an inhibition of the mitochondrial succinate dehydrogenase activity, and necrosis, by release of cytosolic adenylate kinase measuring membrane damage. They also induce apoptosis via activation of enzymatic caspases 3/7 activity. This is confirmed by characteristic DNA fragmentation, nuclear shrinkage (pyknosis), and nuclear fragmentation (karyorrhexis), which is demonstrated by DAPI in apoptotic round cells. G provokes only apoptosis, and HUVEC are 100 times more sensitive overall at this level. The deleterious effects are not proportional to G concentrations but rather depend on the nature of the adjuvants. AMPA and POEA separately and synergistically damage cell membranes like R but at different concentrations. Their mixtures are generally even more harmful with G. In conclusion, the R adjuvants like POEA change human cell permeability and amplify toxicity induced already by G, through apoptosis and necrosis. The real threshold of G toxicity must take into account the presence of adjuvants but also G metabolism and time-amplified effects or bioaccumulation. This should be discussed when analyzing the in vivo toxic actions of R. This work clearly confirms that the adjuvants in Roundup formulations are not inert. Moreover, the proprietary mixtures available on the market could cause cell damage and even death around residual levels to be expected, especially in food and feed derived from R formulation-treated crops. **(France)**

4. Benachour, N., H. Sipahutar, S. Moslemi, C. Gasnier, C. Travert, and G.E. Séralini. 2007. Time- and dose-dependent effects of Roundup on human embryonic and placental cells. *Archives of Environmental Contamination and Toxicology* 53(1): 126-133.

Roundup® is the major herbicide used worldwide, in particular on genetically modified plants that have been designed to tolerate it. We have tested the toxicity and endocrine disruption potential of Roundup (Bioforce®) on human embryonic 293 and placental-derived JEG3 cells, but also on normal human placenta and equine testis. The cell lines have proven to be suitable to estimate hormonal activity and toxicity of pollutants. The median lethal dose (LD_{50}) of Roundup with embryonic cells

is 0.3% within 1 h in serum-free medium, and it decreases to reach 0.06% (containing among other compounds 1.27 mM glyphosate) after 72 h in the presence of serum. In these conditions, the embryonic cells appear to be 2–4 times more sensitive than the placental ones. In all instances, Roundup (generally used in agriculture at 1–2%, i.e., with 21–42 mM glyphosate) is more efficient than its active ingredient, glyphosate, suggesting a synergistic effect provoked by the adjuvants present in Roundup. We demonstrated that serum-free cultures, even on a short-term basis (1 h), reveal the xenobiotic impacts that are visible 1–2 days later in serum. We also document at lower non-overtly toxic doses, from 0.01% (with 210 μM glyphosate) in 24 h, that Roundup is an aromatase disruptor. The direct inhibition is temperature-dependent and is confirmed in different tissues and species (cell lines from placenta or embryonic kidney, equine testicular, or human fresh placental extracts). Furthermore, glyphosate acts directly as a partial inactivator on microsomal aromatase, independently of its acidity, and in a dose-dependent manner. The cytotoxic, and potentially endocrine-disrupting effects of Roundup are thus amplified with time. Taken together, these data suggest that Roundup exposure may affect human reproduction and fetal development in case of contamination. Chemical mixtures in formulations appear to be underestimated regarding their toxic or hormonal impact. **(France)**

5. Bolognesi, C., G. Carrasquilla, S. Volpi, K.R. Solomon, and E.J.P. Marshall. 2009. Biomonitoring of genotoxic risk in agricultural workers from five Colombian regions: association to occupational exposure to glyphosate. *Journal of Toxicology and Environmental Health* Part A 72(15-16): 986-997.

In order to assess possible human effects associated with glyphosate formulations used in the Colombian aerial spray program for control of illicit crops, a cytogenetic biomonitoring study was carried out in subjects from five Colombian regions, characterized by different exposure to glyphosate and other pesticides. Women of reproductive age (137 persons 15-49 yr old) and their spouses (137 persons) were interviewed to obtain data on current health status, history, lifestyle, including past and current occupational exposure to pesticides, and factors including those known to be associated with increased frequency of micronuclei (MN). In regions where glyphosate was being sprayed, blood samples were taken prior to spraying (indicative of baseline exposure), 5 d after spraying, and 4 mo after spraying. Lymphocytes were cultured and a cytokinesis-block micronucleus cytome assay was applied to evaluate chromosomal damage and cytotoxicity. Compared with Santa Marta, where organic coffee is grown without pesticides, the baseline frequency of binucleated cells with micronuclei (BNMN) was significantly greater in subjects from the other four regions. The highest frequency of BNMN was in Boyaca, where no aerial eradication spraying of glyphosate was conducted, and in Valle del Cauca, where glyphosate was used for maturation of sugar cane. Region, gender, and older age (≥35 yr) were the only variables associated with the frequency of BNMN measured before spraying. A significant increase in frequency of BNMN between first and second sampling was observed in Narino, Putumayo, and Valle immediately (<5 d) after spraying. In the post-spray sample, those who reported direct contact with the eradication spray showed a higher quantitative frequency of

BNMN compared to those without glyphosate exposure. The increase in frequency of BNMN observed immediately after the glyphosate spraying was not consistent with the rates of application used in the regions and there was no association between self-reported direct contact with eradication sprays and frequency of BNMN. Four months after spraying, a statistically significant decrease in the mean frequency of BNMN compared with the second sampling was observed in Narino, but not in Putumayo and Valle del Cauca. Overall, data suggest that genotoxic damage associated with glyphosate spraying for control of illicit crops as evidenced by MN test is small and appears to be transient. Evidence indicates that the genotoxic risk potentially associated with exposure to glyphosate in the areas where the herbicide is applied for coca and poppy eradication is low. **(Colombia)**

6. Bradberry, S.M., A.T. Proudfoot, and J.A. Vale. 2004. Glyphosate poisoning. *Toxicological Reviews* 23(3): 159-167.

Glyphosate is used extensively as a non-selective herbicide by both professional applicators and consumers and its use is likely to increase further as it is one of the first herbicides against which crops have been genetically modified to increase their tolerance. Commercial glyphosate-based formulations most commonly range from concentrates containing 41% or more glyphosate to 1% glyphosate formulations marketed for domestic use. They generally consist of an aqueous mixture of the isopropylamine (IPA) salt of glyphosate, a surfactant, and various minor components including anti-foaming and colour agents, biocides and inorganic ions to produce pH adjustment. The mechanisms of toxicity of glyphosate formulations are complicated. Not only is glyphosate used as five different salts but commercial formulations of it contain surfactants, which vary in nature and concentration. As a result, human poisoning with this herbicide is not with the active ingredient alone but with complex and variable mixtures. Therefore, it is difficult to separate the toxicity of glyphosate from that of the formulation as a whole or to determine the contribution of surfactants to overall toxicity. Experimental studies suggest that the toxicity of the surfactant, polyoxyethyleneamine (POEA), is greater than the toxicity of glyphosate alone and commercial formulations alone. There is insufficient evidence to conclude that glyphosate preparations containing POEA are more toxic than those containing alternative surfactants. Although surfactants probably contribute to the acute toxicity of glyphosate formulations, the weight of evidence is against surfactants potentiating the toxicity of glyphosate. Accidental ingestion of glyphosate formulations is generally associated with only mild, transient, gastrointestinal features. Most reported cases have followed the deliberate ingestion of the concentrated formulation of Roundup® (41% glyphosate as the IPA salt and 15% POEA). There is a reasonable correlation between the amount ingested and the likelihood of serious systemic sequelae or death. Advancing age is also associated with a less favourable prognosis. Ingestion of >85mL of the concentrated formulation is likely to cause significant toxicity in adults. Gastrointestinal corrosive effects, with mouth, throat and epigastric pain and dysphagia are common. Renal and hepatic impairment are also frequent and usually reflect reduced organ perfusion. Respiratory distress, impaired consciousness, pulmonary oedema, infiltration on chest x-ray, shock, arrythmias, renal failure requiring haemodialysis, metabolic acidosis and hyperkalaemia may

supervene in severe cases. Bradycardia and ventricular arrhythmias are often present pre-terminally. Dermal exposure to ready-to-use glyphosate formulations can cause irritation and photo-contact dermatitis has been reported occasionally; these effects are probably due to the preservative Proxel® (benzisothiazolin-3-one). Severe skin burns are very rare. Inhalation is a minor route of exposure but spray mist may cause oral or nasal discomfort, an unpleasant taste in the mouth, tingling and throat irritation. Eye exposure may lead to mild conjunctivitis, and superficial corneal injury is possible if irrigation is delayed or inadequate. Management is symptomatic and supportive, and skin decontamination with soap and water after removal of contaminated clothing should be undertaken in cases of dermal exposure. **(United Kingdom)**

7. De Roos, A.J., A. Blair, J.A. Rusiecki, J.A. Hoppin, M. Svec, M. Dosemeci, D.P. Sandler, and M.C. Alavanja. 2005. Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study. *Environmental Health Perspectives* 113(1): 49-54.
   Glyphosate is a broad-spectrum herbicide that is one of the most frequently applied pesticides in the world. Although there has been little consistent evidence of genotoxicity or carcinogenicity from *in vitro* and animal studies, a few epidemiologic reports have indicated potential health effects of glyphosate. We evaluated associations between glyphosate exposure and cancer incidence in the Agricultural Health Study (AHS), a prospective cohort study of 57,311 licensed pesticide applicators in Iowa and North Carolina. Detailed information on pesticide use and other factors was obtained from a self-administered questionnaire completed at time of enrollment (1993-1997). Among private and commercial applicators, 75.5% reported having ever used glyphosate, of which > 97% were men. In this analysis, glyphosate exposure was defined as a) ever personally mixed or applied products containing glyphosate; b) cumulative lifetime days of use, or "cumulative exposure days" (years of use × days/year); and c) intensity-weighted cumulative exposure days (years of use × days/year × estimated intensity level). Poisson regression was used to estimate exposure-response relations between glyphosate and incidence of all cancers combined and 12 relatively common cancer subtypes. Glyphosate exposure was not associated with cancer incidence overall or with most of the cancer subtypes we studied. There was a suggested association with multiple myeloma incidence that should be followed up as more cases occur in the AHS. Given the widespread use of glyphosate, future analyses of the AHS will allow further examination of long-term health effects, including less common cancers. (**U.S.A.)**

8. El-Demerdash, F.M., M.I. Yousef, and E.I. Elagamy. 2001. Influence of paraquat, glyphosate, and cadmium on the activity of some serum enzymes and protein electrophoretic behavior (in vitro). *Journal of Environmental Science and Health* B36(1): 29-42.
   In vitro study for the determination of the toxicity of some pesticides (glyphosate and paraquat) and cadmium chloride ($CdCl_2$) on the activities of serum acetylcholinesterase (AChE), lactate dehydrogenase (LDH), aspartate

aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AlP), and acid phosphatase (AcP) is described. Changes in electrophoretic patterns of serum proteins were also tested. Results revealed that glyphosate was effective on all enzymes except AcP. Its IC_{50} values (the concentration of compound that inhibits 50% of the enzyme activity in 1 h at 37ºC) were 714.3, 750, 54.2, 270.8, and 71.4 m*M* for AChE, LDH, AST, ALT, and AlP, respectively. The inhibitory effect of paraquat varied markedly among all enzymes. The IC_{50} values of paraquat were 321.4 and 750 m*M* for AST and ALT, respectively. It had mild effect on AChE and LDH; and no effect on the activities of AlP and AcP. The effect of $CdCl_2$ was pronounced with AChE, ALT, AlP, and AcP, and no effect on LDH and AST was found. The corresponding IC_{50} values were 77.7, 22.2, 33.3, and 83.3 m*M* for AChE, ALT, AlP, and AcP, respectively. Polyacrylamide gel electrophoretic patterns of serum proteins showed marked differences with glyphosate and $CdCl_2$ but not with paraquat. The results suggest that the in vitro enzyme-activity test seems to have a potential for the assessment of pesticide and heavy metal toxicity. **(Egypt)**

9. Elie-Caille, C., C. Heu, C. Guyon, and L. Nicod. 2010. Morphological damages of a glyphosate-treated human keratinocyte cell line revealed by a micro- to nanoscale microscopic investigation. *Cell Biology and Toxicology* 26(4): 331-339.

    Among the molecules to which the human skin is exposed, glyphosate is used as an herbicide. Glyphosate has been shown to induce in vitro cutaneous cytotoxic effects, concomitant with oxidative disorders. In this following study, we focused on dynamic events of the loss of HaCaT cell integrity appearing after a glyphosate treatment. In these conditions, we showed that glyphosate is able to disrupt HaCaT cells and to induce intracellular oxidative cascade. In this aim, we optimized the conditions of cell treatment playing on exposure time (from 24 h to 30 min), which directly modify the cell viability profile (glyphosate 50% inhibition concentration from 28 to 53 mM) and allow to track cells along the treatment as an "induction and visualization" process. The combination of atomic force and fluorescence microscopic approaches offered opportunities to lead in parallel an investigation of the membrane surface and of the intracellular disorders, through cytoskeleton, nuclear, and oxidative stress marker targeting. The originality of our approach relies on monitoring all events derived from oxidative stress in process and performed by simultaneous cytotoxic induction and nanoscale cell visualization. We revealed a transition from spread and globular to elongated cell morphology, with a drastic cell size reduction, after a dose- and time-dependent glyphosate treatment; a redistribution of cell surface protrusions was also pointed out. All these membrane damages, added to observations of disorganized cytoskeleton, condensed chromatin, and overproduction of oxidative reactive species, lead us to conclude that glyphosate acts in induction of apoptotic process. **(France)**

10. Gasnier C, C. Dumont, N. Benachour, E. Clair, M.-C. Chagnon, and G.-E. Séralini. 2009. Glyphosate based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology* 262: 184-191.

    Glyphosate-based herbicides are the most widely used across the world; they are commercialized in different formulations. Their residues are frequent pollutants

in the environment. In addition, these herbicides are spread on most eaten transgenic plants, modified to tolerate high levels of these compounds in their cells. Up to 400 ppm of their residues are accepted in some feed. We exposed human liver HepG2 cells, a well-known model to study xenobiotic toxicity, to four different formulations and to glyphosate, which is usually tested alone in chronic *in vivo* regulatory studies. We measured cytotoxicity with three assays (Alamar Blue®, MTT, ToxiLight®), plus genotoxicity (comet assay), anti-estrogenic (on ERalpha, ERbeta) and anti-androgenic effects (on AR) using gene reporter tests. We also checked androgen to estrogen conversion by aromatase activity and mRNA. All parameters were disrupted at sub-agricultural doses with all formulations within 24h. These effects were more dependent on the formulation than on the glyphosate concentration. First, we observed a human cell endocrine disruption from 0.5 ppm on the androgen receptor in MDA-MB453-kb2 cells for the most active formulation (R400), then from 2 ppm the transcriptional activities on both estrogen receptors were also inhibited on HepG2. Aromatase transcription and activity were disrupted from 10 ppm. Cytotoxic effects started at 10 ppm with Alamar Blue assay (the most sensitive), and DNA damages at 5 ppm. A real cell impact of glyphosate-based herbicides residues in food, feed or in the environment has thus to be considered, and their classifications as carcinogens/mutagens/reprotoxics is discussed. **(France)**

11. Gasnier, C., N. Benachour, E. Clair, C. Travert, F. Langlois, C. Laurant, C. Decroix-Laport, and G.-E. Seralini. 2010. Dig1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines. *Journal of Occupational Medicine and Toxicology* 5(1): 29-41.

    Worldwide used pesticides containing different adjuvants like Roundup formulations, which are glyphosate-based herbicides, can provoke some in vivo toxicity and in human cells. These pesticides are commonly found in the environment, surface waters and as food residues of Roundup tolerant genetically modified plants. In order to know their effects on cells from liver, a major detoxification organ, we have studied their mechanism of action and possible protection by precise medicinal plant extracts called Dig1. The cytotoxicity pathways of four formulations of glyphosate-based herbicides were studied using human hepatic cell lines HepG2 and Hep3B, known models to study xenobiotic effects. We monitored mitochondrial succinate dehydrogenase activity and caspases 3/7 for cell mortality and protection by Dig1, as well as cytochromes P450 1A1, 1A2, 3A4 and 2C9 and glutathione-S-transferase to approach the mechanism of actions. All the four Roundup formulations provoke liver cell death, with adjuvants having stronger effects than glyphosate alone. Hep3B are 3-5 times more sensitive over 48 h. Caspases 3/7 are greatly activated in HepG2 by Roundup at non-cytotoxic levels, and some apoptosis induction by Roundup is possible together with necrosis. CYP3A4 is specifically enhanced by Roundup at doses 400 times less than used in agriculture (2%). CYP1A2 is increased to a lesser extent together with glutathione-S-transferase (GST) down-regulation. Dig 1, non cytotoxic and not inducing caspases by itself, is able to prevent Roundup-induced cell death in a time-dependant manner with an important efficiency of up to 89%, within 48 h. In addition, we evidenced that it prevents Caspases 3/7 activation and CYP3A4 enhancement, and not GST

reduction, but in turn it slightly inhibited CYP2C9 when added before Roundup. Roundup is able to provoke intracellular disruption in hepatic cell lines at different levels, but a mixture of medicinal plant extracts Dig1 can protect to some extent human cell lines against this pollutant. All this system constitutes a tool for studying liver intoxication and detoxification. **(France)**

12. Gehin, A., Y.C. Guillaume, J. Millet, C. Guyon, and L. Nicod. 2005. Vitamins C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach. *International Journal of Pharmaceutics* 288(2): 219-226.

    The purpose of this study was to investigate and compare the cytotoxicity of glyphosate alone or included in Roundup 3 plus modulated by the cytoprotective effects of additional antioxidants such as Vitamin C and Vitamin E on the human keratinocytes cell line HaCaT. An experimental design which allows to minimize the number of experiments was carried out to determine the optimal conditions for cytoprotection against herbicide-induced toxicity. It was shown that HaCaT cell line provides a useful model to study components with toxicity or antioxidant activity. Our results indicated that (i) glyphosate-based formulations can be responsible for oxidative damage to human epidermal cells, (ii) antioxidant compounds should be associated to herbicide formulations to decrease their deleterious effects on human skin. The use of an experimental design connected with the simplex method can be consider to be a fast technique to classify, with a limited number of experiments, the respective role of five parameters in the in vitro cytoprotection by antioxidants of herbicide-induced toxicity. **(France)**

13. Goldstein, D.A., J.F. Acquavella, R.M. Mannion, and D.R. Farmer. 2002. An analysis of glyphosate data from the California Environmental Protection Agency pesticide illness surveillance program. *Journal of Toxicology. Clinical Toxicology* 40(7): 885-892

    Glyphosate is among the pesticides most frequently reported to the California EPA Pesticide Illness Surveillance Program. We analyzed glyphosate-related calls to the Pesticide Illness Surveillance Program in order to assess the number of reports involving systemic symptoms and to better understand the nature and severity of reported cases. Data on glyphosate and other pesticides are available for the years 1982-1997 including: type of exposure (agricultural/other); target organ(s) affected (skin/eye/respiratory/systemic); exposure(s); an assessment of causal relationship (possible, probable, or definite); and limited medical text. Of 815 total glyphosate calls, most involved topical irritation of the eye (*n* = 399), skin (*n* = 250), upper airway (*n* = 7), or combinations of these sites (*n* = 32) without systemic symptoms. Of the 187 systemic cases, only 22 had symptoms recorded as probably or definitely related to glyphosate exposure alone. The reported symptoms were not severe, expected to be limited in duration, and frequently inconsistent with the route of exposure and/or previous experience with glyphosate. We conclude that call volume is not a reliable indicator of the actual incidence or severity of glyphosate-related incidents in California. **(U.S.A.)**

Human Health

14. Hewitt, A.J., K.R. Solomon and E.J.P. Marshall. 2009. Spray droplet size, drift potential, and risks to nontarget organisms from aerially applied glyphosate for coca control in Colombia. *Journal of Toxicology and Environmental Health*. Part A 72(15): 921-929.

    A wind tunnel atomization study was conducted to measure the emission droplet size spectra for water and Glyphos (a glyphosate formulation sold in Colombia)+Cosmo-flux sprays for aerial application to control coca and poppy crops in Colombia. The droplet size spectra were measured in a wind tunnel for an Accu-Flo nozzle (with 16 size 0.085 [2.16 mm] orifices), under appropriate simulated aircraft speeds (up to 333 km/h), using a laser diffraction instrument covering a dynamic size range for droplets of 0.5 to 3,500 µm. The spray drift potential of the glyphosate was modeled using the AGDISP spray application and drift model, using input parameters representative of those occurring in Colombia for typical aerial application operations. The droplet size spectra for tank mixes containing glyphosate and Cosmo-Flux were considerably finer than water and became finer with higher aircraft speeds. The tank mix with 44% glyphosate had a $D_{v0.5}$ of 128 µm, while the value at the 4.9% glyphosate rate was 140 µm. These are classified as very fine to fine sprays. Despite being relatively fine, modeling showed that the droplets would not evaporate as rapidly as most similarly sized agricultural sprays because the nonvolatile proportion of the tank mix (active and inert adjuvant ingredients) was large. Thus, longer range drift is small and most drift that does occur will deposit relatively close to the application area. Drift will only occur downwind and, with winds of velocity less than the modeled maximum of 9 km/h, the drift distance would be substantially reduced. Spray drift potential might be additionally reduced through various practices such as the selection of nozzles, tank mix adjuvants, aircraft speeds, and spray pressures that would produce coarser sprays. Species sensitivity distributions to glyphosate were constructed for plants and amphibians. Based on modeled drift and 5th centile concentrations, appropriate no-spray buffer zones (distance from the end of the spray boom as recorded electronically ±5%) for protection of sensitive plants were 50-120 m for coca spray scenarios and considerably lower for poppy spray scenarios. The equivalent buffer zone for amphibia was 5 m. The low toxicity of glyphosate to humans suggests that these aerial applications are not a concern for human health. **(Colombia)**

15. Hokanson, R., R. Fudge, R. Chowdhary, and D. Busbee. 2007. Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. *Human & Experimental Toxicology* 26(9): 747-752.

    Gene expression is altered in mammalian cells (MCF-7 cells), by exposure to a variety of chemicals that mimic steroid hormones or interact with endocrine receptors or their co-factors. Among those populations chronically exposed to these endocrine disruptive chemicals are persons, and their families, who are employed in agriculture or horticulture, or who use agricultural/horticultural chemicals. Among the chemicals most commonly used, both commercially and in the home, is the herbicide glyphosate. Although glyphosate is commonly considered to be relatively non-toxic, we utilized *in vitro* DNA microarray analysis of this chemical to evaluate its

capacity to alter the expression of a variety of genes in human cells. We selected a group of genes, determined by DNA microarray analysis to be dysregulated, and used quantitative real-time PCR to corroborate their altered states of expression. We discussed the reported function of those genes, with emphasis on altered physiological states that are capable of initiating adverse health effects that might be anticipated if gene expression were significantly altered in either adults or embryos exposed *in utero*. **(U.S.A.)**

16. Hultberg, M. 2007. Cysteine turnover in human cell lines is influenced by glyphosate. *Environmental Toxicology and Pharmacology* 24(1): 19-22.

Pesticides are widely spread in the environment and there is a lack of knowledge concerning the impact of these substances on the human cell. In the present study the effect of low doses of the pesticides bentazon, metalaxyl and glyphosate on the cellular metabolism of glutathione and cysteine was examined in HeLa and hepatoma cell cultures. No effect was observed when the cells were exposed to bentazon or metalaxyl. However, significant changes in the intra- and extracellular concentration of cysteine, a precursor for glutathione synthesis, were detected when glyphosate was added to the medium. This finding was observed in the presence of micromolar concentration range of glyphosate, and is relevant when compared to concentrations observed in monitoring programmes. **(Sweden)**

17. Koller, V.J., M. Fürhacker, A. Nersesyan, M. Mišík, M. Eisenbauer, and S. Knasmueller. 2012. Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. *Archives of Toxicology* DOI: 10.1007/s00204-012-0804-8.

Glyphosate (G) is the largest selling herbicide worldwide; the most common formulations (Roundup, R) contain polyoxyethyleneamine as main surfactant. Recent findings indicate that G exposure may cause DNA damage and cancer in humans. Aim of this investigation was to study the cytotoxic and genotoxic properties of G and R (UltraMax) in a buccal epithelial cell line (TR146), as workers are exposed via inhalation to the herbicide. R induced acute cytotoxic effects at concentrations >40 mg/l after 20 min, which were due to membrane damage and impairment of mitochondrial functions. With G, increased release of extracellular lactate dehydrogenase indicative for membrane damage was observed at doses >80 mg/l. Both G and R induced DNA migration in single-cell gel electrophoresis assays at doses >20 mg/l. Furthermore, an increase of nuclear aberrations that reflect DNA damage was observed. The frequencies of micronuclei and nuclear buds were elevated after 20-min exposure to 10–20 mg/l, while nucleoplasmatic bridges were only enhanced by R at the highest dose (20 mg/l). R was under all conditions more active than its active principle (G). Comparisons with results of earlier studies with lymphocytes and cells from internal organs indicate that epithelial cells are more susceptible to the cytotoxic and DNA-damaging properties of the herbicide and its formulation. Since we found genotoxic effects after short exposure to concentrations that correspond to a 450-fold dilution of spraying used in agriculture, our findings indicate that inhalation may cause DNA damage in exposed individuals. **(Austria)**

Human Health

18. Mañas, F., L. Peralta, J. Raviolo, H.G. Ovando, A. Weyers, L. Ugnia, M.G. Cid, I. Larripa, and N. Gorla. 2009. Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests. *Environmental Toxicology and Pharmacology* 28(1): 37-41.

    It was evaluated the genotoxicity of glyphosate which up to now has heterogeneous results. The comet assay was performed in Hep-2 cells. The level of DNA damage in the control group (5.42±1.83 arbitrary units) for tail moment (TM) measurements has shown a significant increase (p<0.01) with glyphosate at a range concentration from 3.00 to 7.50mM. In the chromosome aberrations (CA) test in human lymphocytes the herbicide (0.20-6.00mM) showed no significant effects in comparison with the control group. In vivo, the micronucleus test (MNT) was evaluated in mice at three doses rendering statistical significant increases at 400mg/kg (13.0±3.08 micronucleated erythrocytes/1000 cells, p<0.01). In the present study glyphosate was genotoxic in the comet assay in Hep-2 cells and in the MNT test at 400mg/kg in mice. Thiobarbituric acid reactive substances (TBARs) levels, superoxide dismutase (SOD) and catalase (CAT) activities were quantified in their organs. The results showed an increase in these enzyme activities. **(Argentina)**

19. Mañas, F., L. Peralta, J. Raviolo, H.G. Ovando, A. Weyers, L. Ugnia, M.G. Cid, I. Larripa, and N. Gorla. 2009. Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. *Ecotoxicology and Environmental Safety* 72(3): 834-837.

    Formulations containing glyphosate are the most widely used herbicides in the world. AMPA is the major environmental breakdown product of glyphosate. The purpose of this study is to evaluate the in vitro genotoxicity of AMPA using the Comet assay in Hep-2 cells after 4h of incubation and the chromosome aberration (CA) test in human lymphocytes after 48h of exposition. Potential in vivo genotoxicity was evaluated through the micronucleus test in mice. In the Comet assay, the level of DNA damage in exposed cells at 2.5-7.5mM showed a significant increase compared with the control group. In human lymphocytes we found statistically significant clastogenic effect AMPA at 1.8mM compared with the control group. In vivo, the micronucleus test rendered significant statistical increases at 200-400mg/kg. AMPA was genotoxic in the three performed tests. Very scarce data are available about AMPA potential genotoxicity. **(Argentina)**

20. Marc, J., O. Mulner-Lorillon, and R. Bellé. 2004. Glyphosate-based pesticides affect cell cycle regulation. *Biology of the Cell* 96: 245-249.

    Cell-cycle dysregulation is a hallmark of tumor cells and human cancers. Failure in the cell-cycle checkpoints leads to genomic instability and subsequent development of cancers from the initial affected cell. A worldwide used product Roundup 3plus, based on glyphosate as the active herbicide, was suggested to be of human health concern since it induced cell cycle dysfunction as judged from analysis of the first cell division of sea urchin embryos, a recognized model for cell cycle studies. Several glyphosate-based pesticides from different manufacturers were assayed in comparison with Roundup 3plus for their ability to interfere with the

cell cycle regulation. All the tested products, Amega, Cargly, Cosmic, and Roundup Biovert induced cell cycle dysfunction. The threshold concentration for induction of cell cycle dysfunction was evaluated for each product and suggests high risk by inhalation for people in the vicinity of the pesticide handling sprayed at 500 to 4000 times higher dose than the cell-cycle adverse concentration. **(France)**

21. Marc, J., R. Bellé, J. Morales, P. Cormier, and O. Mulner-Lorillon. 2004. Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the prevention of G2/M transition. *Toxicological Sciences* 82(2): 436-442.

    A glyphosate containing pesticide impedes at 10 mM glyphosate the G2/M transition as judged from analysis of the first cell cycle of sea urchin development. We show that formulated glyphosate prevented dephosphorylation of Tyr 15 of the cell cycle regulator CDK1/cyclin B *in vivo*, the end point target of the G2/M cell cycle checkpoint. Formulated glyphosate had no direct effect on the dual specific cdc25 phosphatase activity responsible for Tyr 15 dephosphorylation. At a concentration that efficiently impeded the cell cycle, formulated glyphosate inhibited the synthesis of DNA occurring in S phase of the cell cycle. The extent of the inhibition of DNA synthesis by formulated glyphosate was correlated with the effect on the cell cycle. We conclude that formulated glyphosate's effect on the cell cycle is exerted at the level of the DNA-response checkpoint of S phase. The resulting inhibition of CDK1/cyclin B Tyr 15 dephosphorylation leads to prevention of the G2/M transition and cell cycle progression. **(France)**

22. Marc, J., O. Mulner-Lorillon, S. Boulben, D. Hureau, G. Durand, and R. Belle. 2002. Pesticide Roundup provokes cell division dysfunction at the level of CDK1/cyclin B activation. *Chemical Research in Toxicology* 15: 326-331.

    To assess human health risk from environmental chemicals, we have studied the effect on cell cycle regulation of the widely used glyphosate-containing pesticide Roundup. As a model system we have used sea urchin embryonic first divisions following fertilization, which are appropriate for the study of universal cell cycle regulation without interference with transcription. We show that 0.8% Roundup (containing 8 mM glyphosate) induces a delay in the kinetic of the first cell cleavage of sea urchin embryos. The delay is dependent on the concentration of Roundup. The delay in the cell cycle could be induced using increasing glyphosate concentrations (1-10 mM) in the presence of a subthreshold concentration of Roundup 0.2%, while glyphosate alone was ineffective, thus indicating synergy between glyphosate and Roundup formulation products. The effect of Roundup was not lethal and involved a delay in entry into M-phase of the cell cycle, as judged cytologically. Since CDK1/cyclin B regulates universally the M-phase of the cell cycle, we analyzed CDK1/cyclin B activation during the first division of early development. Roundup delayed the activation of CDK1/cyclin B in vivo. Roundup inhibited also the global protein synthetic rate without preventing the accumulation of cyclin B. In summary, Roundup affects cell cycle regulation by delaying activation of the CDK1/cyclin B complex, by synergic effect of glyphosate and formulation products. Considering the universality among species of the CDK1/cyclin B

regulator, our results question the safety of glyphosate and Roundup on human health. **(France)**

23. Marc, J., M. Le Breton, P. Cormier, J. Morales, R. Bellé, and O. Mulner-Lorillon. A glyphosate-based pesticide impinges on transcription. 2005. *Toxicology and Applied Pharmacology* 203(1): 1-8.

Widely spread chemicals used for human benefits may exert adverse effects on health or the environment, the identification of which are a major challenge. The early development of the sea urchin constitutes an appropriate model for the identification of undesirable cellular and molecular targets of pollutants. The widespread glyphosate-based pesticide affected sea urchin development by impeding the hatching process at millimolar range concentration of glyphosate. Glyphosate, the active herbicide ingredient of Roundup, by itself delayed hatching as judged from the comparable effect of different commercial glyphosate-based pesticides and from the effect of pure glyphosate addition to a threshold concentration of Roundup. The surfactant polyoxyethylene amine (POEA), the major component of commercial Roundup, was found to be highly toxic to the embryos when tested alone and therefore could contribute to the inhibition of hatching. Hatching, a landmark of early development, is a transcription-dependent process. Correlatively, the herbicide inhibited the global transcription, which follows fertilization at the 16-cell stage. Transcription inhibition was dose-dependent in the millimolar glyphosate range concentration. A 1257-bp fragment of the hatching enzyme transcript from *Sphaerechinus granularis* was cloned and sequenced; its transcription was delayed by 2 h in the pesticide-treated embryos. Because transcription is a fundamental basic biological process, the pesticide may be of health concern by inhalation near herbicide spraying at a concentration 25 times the adverse transcription concentration in the sprayed microdroplets. **(France)**

24. Martínez, A., I. Reyes, and N. Reyes. 2007. Cytotoxicity of the herbicide glyphosate in human peripheral blood mononuclear cells. *Biomédica* 27(4):594-604.

Glyphosate is a broad-spectrum, non-selective herbicide and commonly used to eliminate weeds in agricultural and forest settings. Studies evaluating glyphosate toxicity in animals and environment show that commercial formulations of glyphosate are more toxic than the active component itself. Technical grade glyphosate was compared with the commercial formulation Roundup® in their respective toxicities on human peripheral blood mononuclear cells. Human peripheral blood mononuclear cells were exposed to different concentrations of glyphosate, either technical grade or in the form of Roundup® for 24 h, 48 h, 72 h, and 96 h. Cytotoxicity was assayed by trypan blue dye exclusion method and reduction of (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2Htetrazolium-5-carboxyanilide inner salt)XTT reagent. Both technical grade glyphosate and Roundup® formulation were toxic to human peripheral blood mononuclear cells. Cytotoxicity of Roundup® was higher than cytotoxicity of glyphosate, since the LC50 (50% lethal concentration) determined by the trypan blue exclusion method at 24 h was the equivalent of 56.4 microg/ml of glyphosate in the form of Roundup® and 1,640 microg/ml (1.64 mg/ml) for technical grade glyphosate. This in vitro study confirmed the toxic effects on human cells by glyphosate and its

commercial preparations. Commercial formulations were more cytotoxic than the active component alone, supporting the concept that additives in commercial formulations play a role in the toxicity attributed to glyphosate-based herbicides. **(Colombia)**

25. Mesnage, R., E. Clair, S. Gress, C. Then, A. Székács, and G.-E. Séralini. 2012. Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. *Journal of Applied Toxicology* DOI: 10.1002/jat.2712.

The study of combined effects of pesticides represents a challenge for toxicology. In the case of the new growing generation of genetically modified (GM) plants with stacked traits, glyphosate-based herbicides (like Roundup) residues are present in the Roundup-tolerant edible plants (especially corns) and mixed with modified *Bt* insecticidal toxins that are produced by the GM plants themselves. The potential side effects of these combined pesticides on human cells are investigated in this work. Here we have tested for the very first time Cry1Ab and Cry1Ac *Bt* toxins (10 ppb to 100 ppm) on the human embryonic kidney cell line 293, as well as their combined actions with Roundup, within 24 h, on three biomarkers of cell death: measurements of mitochondrial succinate dehydrogenase, adenylate kinase release by membrane alterations and caspase 3/7 inductions. Cry1Ab caused cell death from 100 ppm. For Cry1Ac, under such conditions, no effects were detected. The Roundup tested alone from 1 to 20 000 ppm is necrotic and apoptotic from 50 ppm, far below agricultural dilutions (50% lethal concentration 57.5 ppm). The only measured significant combined effect was that Cry1Ab and Cry1Ac reduced caspases 3/7 activations induced by Roundup; this could delay the activation of apoptosis. There was the same tendency for the other markers. In these results, we argue that modified *Bt* toxins are not inert on nontarget human cells, and that they can present combined side-effects with other residues of pesticides specific to GM plants. **(France)**

26. Mladinic, M., P. Perkovic, and D. Zeljezic. 2009. Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay. *Toxicology Letters* 189(2): 130-137.

Possible clastogenic and aneugenic effects of pesticides on human lymphocytes at concentrations likely to be encountered in residential and occupational exposure were evaluated with (and without) the use of metabolic activation (S9). To get a better insight into the content of micronuclei (MN) and other chromatin instabilities, lymphocyte preparations were hybridized using pancentromeric DNA probes. Frequency of the MN, nuclear buds (NB) and nucleoplasmic bridges (NPB) in cultures treated with glyphosate slightly increased from 3.5microg/ml onward. Presence of S9 significantly elevated cytome assay parameters only at 580microg/ml. No concentration-related increase of centromere (C+) and DAPI signals (DAPI+) was observed for glyphosate treatment. Terbuthylazine treatment showed a dose dependent increase in the number of MN without S9 significant at 0.0008microg/ml and higher. At concentration lower than 1/16 LD50 occurrence of C+MN was significantly elevated regardless of S9, but not dose related, and in the

presence of S9 only NBs containing centromere signals were observed. Carbofuran treatment showed concentration-dependent increase in the number of MN. The frequency of C+MN was significant from 0.008microg/ml onward regardless of S9. Results suggest that lower concentrations of glyphosate have no hazardous effects on DNA, while terbuthylazine and carbofuran revealed a predominant aneugenic potential. **(Croatia)**

27. Mladinic, M., S. Berend, A.L. Vrdoljak, N. Kopjar, B. Radic, and D. Zeljezic. 2009. Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes *in vitro*. *Environmental and Molecular Mutagenesis* 50(9): 800-807.

In the present study we evaluated the genotoxic and oxidative potential of glyphosate on human lymphocytes at concentrations likely to be encountered in residential and occupational exposure. Testing was done with and without metabolic activation (S9). Ferric-reducing ability of plasma (FRAP), thiobarbituric acid reactive substances (TBARS) and the hOGG1 modified comet assay were used to measure glyphosate's oxidative potential and its impact on DNA. Genotoxicity was evaluated by alkaline comet and analysis of micronuclei and other nuclear instabilities applying centromere probes. The alkaline comet assay showed significantly increased tail length (20.39 μm) and intensity (2.19%) for 580 μg/ml, and increased tail intensity (1.88%) at 92.8 μg/ml, compared to control values of 18.15 μm for tail length and 1.14% for tail intensity. With S9, tail length was significantly increased for all concentrations tested: 3.5, 92.8, and 580 μg/ml. Using the hOGG1 comet assay, a significant increase in tail intensity was observed at 2.91 μg/ml with S9 and 580 μg/ml without S9. Without S9, the frequency of micronuclei, nuclear buds and nucleoplasmic bridges slightly increased at concentrations 3.5 μg/ml and higher. The presence of S9 significantly elevated the frequency of nuclear instabilities only for 580 μg/ml. FRAP values slightly increased only at 580 μg/ml regardless of metabolic activation, while TBARS values increased significantly. Since for any of the assays applied, no clear dose-dependent effect was observed, it indicates that glyphosate in concentrations relevant to human exposure do not pose significant health risk. **(Croatia)**

28. Monroy, C.M., A.C. Cortés, D.M. Sicard, and H.G. de Restrepo. 2005. Cytotoxicity and genotoxicity of human cells exposed in vitro to glyphosate. *Biomédica* 25(3): 335-345.

Glyphosate is a broad-spectrum non-selective herbicide, used to eliminate unwanted weeds in agricultural and forest settings. Herbicide action is achieved through inhibition of aromatic amino acid biosynthesis in plant cells. Since this is not a conserved mechanism between human and plant cells, glyphosate is considered to be a low health risk substance for humans. However, the occurrence of possible harmful side effects of glyphosate use is not well documented and controversial. Toxicity and genotoxicity studies indicate that glyphosate is not harmful, although several investigations suggest that it can alter various cellular processes in animals. Therfore this has potential as a health and environmental risk factor in areas where glyphosate is widely used. The present study evaluated glyphosate cytotoxic and

genotoxic effects in normal human cells (GM38) and human fibrosarcoma (HT1080) cells. Acute and chronic cytotoxicity were determined through the exposure of cultured cells to graded concentrations of glyphosate, and cell viability analysis was performed with crystal violet and Trypan blue staining. Genotoxicity was determined using the comet assay and data significance was evaluated with Dunnet's test. For chronic cytotoxicity a dose-dependent effect was observed in both GM38 and HT1080 cells after treatment with 5.2-8.5 mM and 0.9-3.0 mM glyphosate, respectively. In the acute cytotoxicity study, GM38 cells exposed to 4.0-7.0 mM glyphosate and HT1080 cells exposed to 4.5-5.8 mM glyphosate, had cell viability counts higher than 80%. Genotoxic effects were evidenced in GM38 cells at glyphosate concentrations of 4.0-6.5 mM and in HT1080 cells at glyphosate concentrations of 4.75-5.75 mM. The levels of cytotoxicity and genotoxicity of glyphosate occurring in mammalian cells suggested that its mechanism of action is not limited to plant cells. **(Colombia)**

29. Mose, T., M.B. Kjaerstad, L. Mathiesen, J.B. Nielsen, S. Edelfors, and L.E. Knudsen. 2008. Placental passage of benzoic acid, caffeine, and glyphosate in an ex vivo human perfusion system. *Journal of Toxicology and Environmental Health* Part A 71(15): 984-991.

    Ex vivo perfusion of the human term placenta is a method to study placental transfer without extrapolation from animal to human and with no ethical concerns for mother and child. However, ex vivo placenta perfusion has a limited potential within chemical screening and testing as the method is time-consuming. This study was an attempt to construct data needed to develop quantitative structure-activity relationship (QSAR) models that are able to predict placental transfer of new compounds. Placental transfer is a biological activity that statistically may be related to the physiochemical properties of a given group of compounds. Benzoic acid, caffeine, and glyphosate were chosen as model compounds because they are small molecules with large differences in physiochemical properties. Caffeine crossed the placenta by passive diffusion. The initial transfer rate of benzoic acid was more limited in the first part of the perfusion compared to caffeine, but reached the same steady-state level by the end of perfusion. The transfer of glyphosate was restricted throughout perfusion, with a lower permeation rate, and only around 15% glyphosate in maternal circulation crossed to the fetal circulation during the study period. **(Denmark)**

30. Nakashima K., T.Yoshimura, H. Mori, M. Kawaguchi, S. Adachi, T. Nakao, and F. Yamazaki. 2002. Effects of pesticides on cytokines production by human peripheral blood mononuclear cells--fenitrothion and glyphosate. *The Japanese Journal of Toxicology* 15(2): 159-165.

    In patients with pesticides poisoning, human immune system seems to be damaged. However, the effects of pesticides on human immune system, especially on cytokines production, have not been understood well. We investigated the effects of fenitrothion (MEP), an organophosphorus insecticide, and glyphosate (GLP), a phosphorus containing amino acid-type herbicide, on cytokines production by human peripheral blood mononuclear cells (PBMC). MEP inhibited the proliferative activity

of PBMC at ranging from 1 to 500 microM in a concentration-dependent manner, whereas GLP had a slight inhibitory effect even at 1000 microM. The production of IFN-gamma and IL-2 was inhibited by MEP in a concentration-dependent manner, but GLP slightly inhibited their production only at 1000 microM. The production of TNF-alpha and IL-1 beta was not affected by MEP and GLP at the concentrations which significantly inhibited the proliferative activity and T cell-derived cytokine production. MEP inhibited the production of T cell-derived cytokine (IFN-gamma and IL-2), which indicates that MEP might have the potential of immunosuppressive action. On the other hand, GLP might be a pesticide with only a little damage to the immune system, according to the results in cytokines production. These results suggest that pesticides inhibit the immune system differently, and the grasp of immune condition might be useful for the prognostic presumption and the infectious danger of degree in patients with pesticides poisoning. **(Japan)**

31. Paz-y-Miño, C., M.E. Sánchez, M. Arévalo, M.J. Muñoz, T. Witte, G.O. De-la-Carrera, and P.E. Leone. 2007. Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. *Genetics and Molecular Biology* 30(2): 456-460.

We analyzed the consequences of aerial spraying with glyphosate added to a surfactant solution in the northern part of Ecuador. A total of 24 exposed and 21 unexposed control individuals were investigated using the comet assay. The results showed a higher degree of DNA damage in the exposed group (comet length = 35.5 µm) compared to the control group (comet length = 25.94 µm). These results suggest that in the formulation used during aerial spraying glyphosate had a genotoxic effect on the exposed individuals. **(Ecuador)**

32. Pieniążek, D., B. Bukowska, and W. Duda. 2004. Comparison of the effect of Roundup Ultra 360 SL pesticide and its active compound glyphosate on human erythrocytes. *Pesticide Biochemistry and Physiology* 79(2): 58-63.

The effects of exposure of human erythrocytes to different concentrations of Roundup Ultra 360 SL and its active compound glyphosate were studied. We studied hemolysis after 1, 5, and 24 h incubation; lipid peroxidation, hemoglobin oxidation, the level of reduced glutathione, and the activity of catalase after 1 h. Human erythrocytes were incubated with 100-1500 ppm (100 mug/ml erythrocytes at 5% hematocrite) Roundup Ultra 360 SL and glyphosate. We have found that after 1 h of incubation only Roundup Ultra 360 SL increased the level of methemoglobin, products of lipid peroxidation at 500 ppm and hemolysis at 1500 ppm [Curr. Top. Biophys. 26 (2002) 245], while its active compound glyphosate increased the level of methemoglobin and the level of lipid peroxidation at much higher dose-1000 ppm. At the same time hemolysis was observed to only at the highest dose of glyphosate (1500 ppm) and the longest time of incubation (24h). Both Roundup Ultra 360 SL and glyphosate did not cause statistically significant changes in the level of GSH, but increased the activity of catalase. Roundup Ultra 360 SL provokes more changes in the function of erythrocytes than its active substance glyphosate, which is probably a result of the properties of additives. Taking into account the limited accumulation of Roundup Ultra 360 SL and glyphosate in the organism as well as the fact that the threshold doses which caused changes in erythrocytes for Roundup Ultra 360 SL

were only 500 and 1000 ppm for glyphosate, one may conclude that this pesticide is safe towards human erythrocytes. **(Poland)**

33. Potrebić, O., J. Jović-Stosić, S. Vucinić, J. Tadić, and M. Radulac. 2009. Acute glyphosate-surfactant poisoning with neurological sequels and fatal outcome. *Military-Medical and Pharmaceutical Review* 66(9): 758-762.

    Clinical picture of severe glyphosate-surfactant poisoning is manifested by gastroenteritis, respiratory disturbances, altered mental status, hypotension refractory to the treatment, renal failure, shock. Single case report indicated possible neurotoxic sequels of glyphosate-surfactant exposure with white matter lesions and development of Parkinsonism. We described a patient with massive white matter damage which led to vigil coma and lethal outcome. A 56-year old woman ingested about 500 mL of herbicide containing glyphosate isopropylamine salt. The most prominent manifestation of poisoning included hypotension, coma, hyperkaliemia, respiratory and renal failure. The patient was treated in intensive care unit by symptomatic and supportive therapy including mechanical ventilation and hemodialysis. The patient survived the acute phase of poisoning, but she developed vigil coma. Nuclear magnetic imagining revealed extensive bilateral lesions of the brain stem white matter and pons. The outcome of reported poisoning may be the consequence of glyphosate-surfactant neurotoxic effect or/and ischemia, especially in the episodes of marked hypotension during hemodialysis. Considering recommendation of early hemodialysis as the treatment of choice, even before renal failure development, we point out the importance of careful planning of dialysis modality in hemodynamically instable patient and recommend continuous dialysis methods. **(Serbia)**

34. Potti, A. and I. Sehgal. 2005. Exposure to pesticides increases levels of uPA and uPAR in pre-malignant human prostate cells. *Environmental Toxicology and Pharmacology* 19(2): 215-219.

    Pesticides are associated with prostate carcinogenesis and mortality; however, their exact mechanisms of action are poorly defined. We have used a transformed but non-tumorigenic human prostate epithelial line to determine the effect of common herbicides and insecticides on expression of urokinase and its receptor, uPAR. The herbicide Roundup and insecticides Lorsban and Warrior induced uPA while Lorsban and Warrior also induced uPAR. Furthermore, a combination of Roundup + Lorsban or Roundup + Warrior produced greater increases in uPA and uPAR than when agents were used alone. Both active and "inactive" chemicals within these pesticides are important for the effects observed as the neat chemicals alone failed to induce uPA and were less potent inducers of uPAR. Thus, specific pesticide formulations, especially when combined, can increase uPA and uPAR expression in vitro in transformed prostate epithelial cells. **(U.S.A.)**

35. Poulsen, M.S., E. Rytting, T. Mose, and L.E. Knudsen. 2009. Modeling placental transport: correlation of *in vitro* BeWo cell permeability and *ex vivo* human placental perfusion. *Toxicology in Vitro* 23(7): 1380-1386.

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The placental passage of three compounds with different physicochemical properties was recently investigated in *ex vivo* human placental perfusion experiments (caffeine, benzoic acid, and glyphosate) [Mose, T., Kjaerstad, M.B., Mathiesen, L., Nielsen, J.B., Edelfors, S., Knudsen, L.E., 2008. Placental passage of benzoic acid, caffeine, and glyphosate in an ex vivo human perfusion system. J. Toxicol. Environ. Health, Part A 71, 984-991]. In this work, the transport of these same three compounds, plus the reference compound antipyrine, was investigated using BeWo (b30) cell monolayers. Transport across the BeWo cells was observed in the rank order of caffeine>antipyrine>benzoic acid>glyphosate in terms of both the apparent permeability coefficient and the initial slope, defined as the linear rate of substance transferred to the fetal compartment as percent per time, a parameter used to compare the two experimental models. The results from the *in vitro* studies were in excellent agreement with the *ex vivo* results (caffeine ~antipyrine>benzoic acid>glyphosate). However the transfer rate was much slower in the BeWo cells compared to the perfusion system. The advantages and limitations of each model are discussed in order to assist in the preparation, prediction, and performance of future studies of maternal-fetal transfer. **(Denmark)**

36. Prasad, S., S. Srivastava, M. Singh, and Y. Shukla. 2009. Clastogenic effects of glyphosate in bone marrow cells of Swiss albino mice. *Journal of Toxicology* 2009(5): 308985-6.

Glyphosate (N-(phosphonomethyl) glycine, $C_3H_8NO_5P$), a herbicide, used to control unwanted annual and perennial plants all over the world. Nevertheless, occupational and environmental exposure to pesticides can pose a threat to nontarget species including human beings. Therefore, in the present study, genotoxic effects of the herbicide glyphosate were analyzed by measuring chromosomal aberrations (CAs) and micronuclei (MN) in bone marrow cells of Swiss albino mice. A single dose of glyphosate was given intraperitoneally (*i.p.*) to the animals at a concentration of 25 and 50 mg/kg b.wt. Animals of positive control group were injected *i.p.* benzo(a)pyrene (100 mg/kg b.wt., once only), whereas, animals of control (vehicle) group were injected *i.p.* dimethyl sulfoxide (0.2 mL). Animals from all the groups were sacrificed at sampling times of 24, 48, and 72 hours and their bone marrow was analyzed for cytogenetic and chromosomal damage. Glyphosate treatment significantly increases CAs and MN induction at both treatments and time compared with the vehicle control ($P < 0.05$). The cytotoxic effects of glyphosate were also evident, as observed by significant decrease in mitotic index (MI). The present results indicate that glyphosate is clastogenic and cytotoxic to mouse bone marrow. **(India)**

37. Richard, S., S. Moslemi, H. Sipahutar, N. Benachour, and G.-E. Seralini. 2005. Differential effects of glyphosate and roundup on human placental cells and aromatase. *Environmental Health Perspectives* 113(6): 716-720.

Roundup is a glyphosate-based herbicide used worldwide, including on most genetically modified plants that have been designed to tolerate it. Its residues may thus enter the food chain, and glyphosate is found as a contaminant in rivers. Some agricultural workers using glyphosate have pregnancy problems, but its mechanism

of action in mammals is questioned. Here we show that glyphosate is toxic to human placental JEG3 cells within 18 hr with concentrations lower than those found with agricultural use, and this effect increases with concentration and time or in the presence of Roundup adjuvants. Surprisingly, Roundup is always more toxic than its active ingredient. We tested the effects of glyphosate and Roundup at lower nontoxic concentrations on aromatase, the enzyme responsible for estrogen synthesis. The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation in microsomes or in cell culture. We conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. We suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation. **(France)**

38. Roberts, D.M., N.A. Buckley, F. Mohamed, M. Eddleston, D.A. Goldstein, A. Mehrsheikh, M.S. Bleeke, and A.H. Dawson. 2010. A prospective observational study of the clinical toxicology of glyphosate-containing herbicides in adults with acute self-poisoning. *Clinical Toxicology* 48(2): 129-136.

The case fatality from acute poisoning with glyphosate-containing herbicides is approximately 7.7% from the available studies but these have major limitations. Large prospective studies of patients with self-poisoning from known formulations who present to primary or secondary hospitals are needed to better describe the outcome from acute poisoning with glyphosate-containing herbicides. Furthermore, the clinical utility of the glyphosate plasma concentration for predicting clinical outcomes and guiding treatment has not been determined. To describe the clinical outcomes, dose-response, and glyphosate kinetics following self-poisoning with glyphosate-containing herbicides. This prospective observational case series was conducted in two hospitals in Sri Lanka between 2002 and 2007. We included patients with a history of acute poisoning. Clinical observations were recorded until discharge or death. During a specified time period, we collected admission (n = 216, including five deaths) and serial (n = 26) blood samples in patients. Severity of poisoning was graded using simple clinical criteria. Six hundred one patients were identified; the majority ingested a concentrated formulation (36%, w/v glyphosate). Twenty-seven percent were asymptomatic, 63.7% had minor poisoning, and 5.5% of patients had moderate to severe poisoning. There were 19 deaths (case fatality 3.2%) with a median time to death of 20 h. Gastrointestinal symptoms, respiratory distress, hypotension, altered level of consciousness, and oliguria were observed in fatal cases. Death was strongly associated with greater age, larger ingestions, and high plasma glyphosate concentrations on admission (>734 µg/mL). The apparent elimination half-life of glyphosate was 3.1 h (95% CI = 2.7-3.6 h). Despite treatment in rural hospitals with limited resources, the mortality was 3.2%, which is lower than that reported in previous case series. More research is required to define the mechanism of toxicity, better predict the small group at risk of death, and find effective treatments. **(Sri Lanka)**

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39. Sato, C., Y. Kamijo, K. Yoshimura, and T. Ide. 2011. Aseptic meningitis in association with glyphosate-surfactant herbicide poisoning. *Clinical Toxicology* 49(2): 118-120.

The mechanisms underlying early central nervous system (CNS) signs and symptoms of glyphosate-surfactant herbicide (GlySH) poisoning are unclear. A 58-year-old woman ingested approximately 150 mL of GlySH containing 41% glyphosate and 15% polyoxyethyleneamine. Two days later, she was admitted in the Emergency Center in a semicomatose state. Acute respiratory distress syndrome, circulatory collapse, acute renal failure, and disseminated intravascular coagulopathy were diagnosed. Meningitis was also suspected as she demonstrated Kernig's sign and significant neck stiffness with rigidity of the extremities as well as consciousness disturbance and fever (38.4°C). Investigations of cerebrospinal fluid (CSF) revealed the presence of glyphosate (122.5 μg/mL), significant elevation of IL-6 (394 μg/mL), and pleocytosis (32 cells/μL) with monocyte dominance. All bacteriological and virological tests were later found to be negative. She recovered completely after responding to aggressive supportive care in the intensive care unit. All signs and symptoms suggesting meningitis resolved as the concentration of glyphosate in CSF decreased. She was discharged on day 39 of hospitalization. These findings suggest that the present case involved aseptic meningitis in association with GlySH poisoning. CNS signs and symptoms induced by aseptic meningitis should be considered in cases of glyphosate-surfactant herbicide poisoning. **(Japan)**

40. Schriks, M., M.B. Heringa, M.M.E. van der Kooi, P. de Voogt, and A.P. van Wezel. 2010. Toxicological relevance of emerging contaminants for drinking water quality. *Water Research* 44(2): 461-476.

See Water Quality Section.

41. Shepard, J.P., J. Creighton, and H. Duzan. 2004. Forestry herbicides in the United States: an overview. *Wildlife Society Bulletin* 32(4): 1020-1027.

See Biodiversity and Restoration Section.

42. Solomon, K.R., A. Anadón, G. Carrasquilla, A.L. Cerdeira, and J. Marshall, and L.-H. Sanin. 2007. Coca and poppy eradication in Colombia: environmental and human health assessment of aerially applied glyphosate. *Reviews of Environmental Contamination and Toxicology* 190: 43-125.

The production of coca and poppy as well as the processing and production of cocaine and heroin involve significant environmental impacts. Both coca and poppy are grown intensively in a process that involves the clearing of land in remote areas, the planting of the crop, and protection against pests such as weeds, insects, and pathogens. The aerial spray program to control coca and poppy production in Colombia with the herbicide glyphosate is conducted with modern state-of-the-art aircraft and spray equipment. As a result of the use of best available spray and navigation technology, the likelihood of accidental off-target spraying is small and is estimated to be less than 1% of the total area sprayed. Estimated exposures in humans resulting from direct overspray, contact with treated foliage after reentry to

fields, inhalation, diet, and drinking water were small and infrequent. Analyses of surface waters in five watersheds showed that, on most occasions, glyphosate was not present at measurable concentrations; only two samples had residues just above the method detection limit of 25 µg/L. Concentrations of glyphosate in air were predicted to be very small because of negligible volatility. Glyphosate in soils that are directly sprayed will be tightly bound and biologically unavailable and have no residual activity. Concentrations of glyphosate plus Cosmo- Flux will be relatively large in shallow surface waters that are directly oversprayed (maximum instantaneous concentration of 1,229 µg AE/L in water 30 cm deep); however, no information was available on the number of fields in close proximity to surface waters, and thus it was not possible to estimate the likelihood of such contamination. The formulation used in Colombia, a mixture of glyphosate and Cosmo- Flux, has low toxicity to mammals by all routes of exposure, although some temporary eye irritation may occur. Published epidemiological studies have not suggested a strong or consistent linkage between glyphosate use and specific human health outcomes. An epidemiology study conducted in Colombia did not show any association between time to pregnancy in humans and the use of glyphosate in eradication spraying. The mixture of glyphosate and Cosmo-Flux was not toxic to honeybees. The mixture was, however, more toxic to the alga *Selenastrum*, the cladoceran *Daphnia magna*, fathead minnow, and rainbow trout than formulated glyphosate (Roundup) alone. Studies on the use of glyphosate in agriculture and forestry have shown that direct effects on nontarget organisms other than plants are unlikely. Indirect effects on terrestrial arthropods and other wildlife may be the result of habitat alteration and environmental change brought about by the removal of plants by glyphosate. Because of the lack of residual activity, recovery of glyphosate-treated areas in Colombia is expected to be rapid because of good plant growth conditions. However, return to the conditions of tropical old-growth forest that existed before clear-cutting and burning may take hundreds of years, not from the use of glyphosate but because of the clear-cutting and burning, which are the primary cause of effects in the environment. The risk assessment concluded that glyphosate and Cosmo-Flux did not present a significant risk to human health. In the entire cycle of coca and poppy production and eradication, human health risks associated with physical injury during clear-cutting and burning and the use of pesticides for protection of the illicit crops were judged to be considerably more important than those from exposure to glyphosate. For the environment, direct risks from the use of glyphosate and Cosmo-Flux to terrestrial mammals and birds were judged to be negligible. Moderate risks could occur in aquatic organisms in shallow surface waters that are oversprayed during the eradication program. However, the frequency of occurrence and extent to which this happens are unknown as data on the proximity of surface waters to coca fields were not available. Considering the effects of the entire cycle of coca and poppy production and eradication, clear-cutting and burning and displacement of the natural flora and fauna were identified as the greatest environmental risks and are considerably more important than those from the use of glyphosate for the control of coca and poppy. **(Colombia)**

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43. Thompson, D.G. and D.G. Pitt. 2009. Frequently asked questions (FAQs) on the use of herbicides in Canadian Forestry. *Technical Note No. 112. Natural Resources Canada CFS, Great Lakes Forestry Centre, Sault Ste. Marie, ON*. 7 p.

The focus of this FAQ document is on the use of herbicides and the potential risks, particularly to wildlife, that may be associated with their use. This information has been presented to citizens' groups across Ontario in recent years and has been refined based on feedback received. The objective of this document is to present the scientific facts about herbicide use in forest management and thus better inform discussions related to forest vegetation management techniques. Comprehensive review of this topic is beyond the scope of this article. **(Canada)**

44. Thompson, D., J. Leach, M. Noel, S. Odsen, and M. Mihajlovich. 2012. Aerial forest herbicide application: Comparative assessment of risk mitigation strategies in Canada. *The Forestry Chronicle* 88(2): 176-184.

Herbicide use in forest management is subject to controversy due to potential risks to human and environmental health. Provinces with substantial forest herbicide use are the focus of this comparative assessment of risk mitigation strategies for aerial application of forestry herbicides. This paper explores risk mitigation procedures surrounding treatment prescriptions, herbicide planning and permitting, and operational treatment, against a background of legislative and regulatory requirements. The three major-use provinces have similarly high levels of risk mitigation, including detailed herbicide application plan requirements, use of electronic guidance systems, buffering of environmental sensitivities, pre-spray reconnaissance flights and post-spray auditing. Notable differences include standardizing use of rotary-wing aircraft, use of low-drift nozzles, the rigor applied to aircraft calibration and use of block monitors for detailed meteorological monitoring. These techniques are generally unique to Alberta and are considered best management practices. The routine use of validated aerial dispersal and expert decision support systems (e.g., AgDisp, SprayAdvisor) is recommended, as it could provide significant added value to generic and spatially explicit risk mitigation with multiple applications. It is the opinion of the authors that aerial herbicide applications as performed in all three major-use jurisdictions are highly protective of human and environmental health. **(Canada)**

45. Vasiluk, L.J. Pinto, and M.M. Moore. 2005. Oral bioavailability of glyphosate: studies using two intestinal cell lines. *Environmental Toxicology and Chemistry* 24(1): 153-160.

Glyphosate is a commonly used nonselective herbicide that inhibits plant growth through interference with the production of essential aromatic amino acids. In vivo studies in mammals with radiolabeled glyphosate have shown that 34% of radioactivity was associated with intestinal tissue 2 h after oral administration. The aim of our research was to investigate the transport, binding, and toxicity of glyphosate to the cultured human intestinal epithelial cell line, Caco-2, and the rat small intestinal crypt-derived cell line, ileum epithelial cells-18 (IEC-18). An in vitro analysis of the transport kinetics of [^{14}C]-glyphosate showed that 4 h after exposure, approximately 8% of radiolabeled glyphosate moved through the Caco-2 monolayer

in a dose-dependent manner. Binding of glyphosate to cells was saturable and approximately 4 x 10(11) binding sites/cell were estimated from bound [14C]. Exposure of Caco-2 cells to ≥10 mg/ml glyphosate reduced transmembrane electrical resistance (TEER) by 82 to 96% and increased permeability to [3H]-mannitol, indicating that paracellular permeability increased in glyphosate-treated cells. At 10-mg/ml glyphosate, both IEC-18 and Caco-2 cells showed disruption in the actin cytoskeleton. In Caco-2 cells, significant lactate dehydrogenase leakage was observed when cells were exposed to 15 mg/ml of glyphosate. These data indicate that at doses >10 mg/ml, glyphosate significantly disrupts the barrier properties of cultured intestinal cells. **(Canada)**

46. Wauchope, R.D., T.L. Estes, R. Allen, J.L. Baker, A.G. Hornsby, R.L. Jones, R.P. Richards, and D.I. Gustafson. 2002. Predicted impact of transgenic, herbicide-tolerant corn on drinking water quality in vulnerable watersheds of the mid-western USA. *Pest Management Science* 58(2): 146-160.

    See Water Quality Section.

47. Williams, G.M., R.Kroes, and I.C. Munro. 2000. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. *Regulatory Toxicology and Pharmacology* 31: 117-165.

    Reviews on the safety of glyphosate and Roundup herbicide that have been conducted by several regulatory agencies and scientific institutions worldwide have concluded that there is no indication of any human health concern. Nevertheless, questions regarding their safety are periodically raised. This review was undertaken to produce a current and comprehensive safety evaluation and risk assessment for humans. It includes assessments of glyphosate, its major breakdown product [aminomethylphosphonic acid (AMPA)], its Roundup formulations, and the predominant surfactant [polyethoxylated tallow amine (POEA)] used in Roundup formulations worldwide. The studies evaluated in this review included those performed for regulatory purposes as well as published research reports. The oral absorption of glyphosate and AMPA is low, and both materials are eliminated essentially unmetabolized. Dermal penetration studies with Roundup showed very low absorption. Experimental evidence has shown that neither glyphosate nor AMPA bioaccumulates in any animal tissue. No significant toxicity occurred in acute, subchronic, and chronic studies. Direct ocular exposure to the concentrated Roundup formulation can result in transient irritation, while normal spray dilutions cause, at most, only minimal effects. The genotoxicity data for glyphosate and Roundup were assessed using a weight-of-evidence approach and standard evaluation criteria. There was no convincing evidence for direct DNA damage in vitro or in vivo, and it was concluded that Roundup and its components do not pose a risk for the production of heritable/somatic mutations in humans. Multiple lifetime feeding studies have failed to demonstrate any tumorigenic potential for glyphosate. Accordingly, it was concluded that glyphosate is noncarcinogenic. Glyphosate, AMPA, and POEA were not teratogenic or developmentally toxic. There were no effects on fertility or reproductive parameters in two multigeneration reproduction studies with glyphosate. Likewise there were no adverse effects in reproductive

tissues from animals treated with glyphosate, AMPA, or POEA in chronic and/or subchronic studies. Results from standard studies with these materials also failed to show any effects indicative of endocrine modulation. Therefore, it is concluded that the use of Roundup herbicide does not result in adverse effects on development, reproduction, or endocrine systems in humans and other mammals. For purposes of risk assessment, no-observed-adverse-effect levels (NOAELs) were identified for all subchronic, chronic, developmental, and reproduction studies with glyphosate, AMPA, and POEA. Margins-of-exposure for chronic risk were calculated for each compound by dividing the lowest applicable NOAEL by worst-case estimates of chronic exposure. Acute risks were assessed by comparison of oral LD50 values to estimated maximum acute human exposure. It was concluded that, under present and expected conditions of use, Roundup herbicide does not pose a health risk to humans. **(U.S.A., Netherlands, Canada)**

48. Williams, A.L., R.E. Watson, and J.M. DeSesso. 2012. Developmental and reproductive outcomes in humans and animals after glyphosate exposure: a critical analysis. *Journal of Toxicology and Environmental Health*. Part B, Critical reviews 15(1): 39-96.

    Glyphosate is the active ingredient of several widely used herbicide formulations. Glyphosate targets the shikimate metabolic pathway, which is found in plants but not in animals. Despite the relative safety of glyphosate, various adverse developmental and reproductive problems have been alleged as a result of exposure in humans and animals. To assess the developmental and reproductive safety of glyphosate, an analysis of the available literature was conducted. Epidemiological and animal reports, as well as studies on mechanisms of action related to possible developmental and reproductive effects of glyphosate, were reviewed. An evaluation of this database found no consistent effects of glyphosate exposure on reproductive health or the developing offspring. Furthermore, no plausible mechanisms of action for such effects were elucidated. Although toxicity was observed in studies that used glyphosate-based formulations, the data strongly suggest that such effects were due to surfactants present in the formulations and not the direct result of glyphosate exposure. To estimate potential human exposure concentrations to glyphosate as a result of working directly with the herbicide, available biomonitoring data were examined. These data demonstrated extremely low human exposures as a result of normal application practices. Furthermore, the estimated exposure concentrations in humans are >500-fold less than the oral reference dose for glyphosate of 2 mg/kg/d set by the U.S. Environmental Protection Agency (U.S. EPA 1993). In conclusion, the available literature shows no solid evidence linking glyphosate exposure to adverse developmental or reproductive effects at environmentally realistic exposure concentrations. **(U.S.A.)**

49. Wyatt, S., M.-H. Rousseau, S. Nadeau, N. Thiffault, and L. Guay. 2011. Social concerns, risk and the acceptability of forest vegetation management alternatives: Insights for managers. *The Forestry Chronicle* 87(2): 274-289.

    Although public opinion and social issues have significant influence on policy-making, research on forest vegetation management (FVM) in Canada has a strong

focus on biological aspects, with less attention being paid to social concerns. This paper reviews the social context in which FVM occurs. Individual views about FVM reflect a combination of values, beliefs, and attitude while also including differing perceptions of risks. Public views and the broader social acceptability of management decisions can be linked to five key factors: context, risk, aesthetics, trust, and knowledge. Judgments about acceptability will usually change over time and across specific situations and various segments of a population could make opposing judgments. We identify a variety of public concerns related to FVM in Canada, synthesizing research that can help resource managers consider the social impacts of their choices. We also note particular concerns related to Aboriginal peoples and the FVM workforce. Information about the benefits and disadvantages of FVM options can help resolve public concerns, but using technical information to convince the public is rarely successful. Forest management agencies and resource managers need access to reliable information about social values and concerns to make management decisions that will be socially acceptable. **(Canada)**

50. Yue, Y., Y. Zhang, L. Zhou, J. Qin, and X. Chen. 2008. In vitro study on the binding of herbicide glyphosate to human serum albumin by optical spectroscopy and molecular modeling. *Journal of Photochemistry and Photobiology* B:Biology 90(1): 26-32.

In this study, the interaction mechanism of herbicide glyphosate and human serum albumin (HSA) has been characterized by fluorescence, UV, Fourier transform infrared (FT-IR), Circular dichroism (CD) spectroscopic and molecular modeling methods. The structural characteristics of glyphosate and HSA were probed, and affinity constants were determined under different temperatures. The enthalpy change (DeltaH degrees) and the entropy changes (DeltaS degrees) were calculated to be -21.78kJmol^{-1} and 6.38Jmol^{-1}K^{-1}according to the Van't Hoff equation. These results indicated that glyphosate binds to HSA mainly by hydrogen bond and hydrophobic interaction can also not be excluded, which is in good agreement with the results from modeling experiment. The average binding distance, r, between the donor (HSA) and the acceptor (glyphosate) was evaluated and found to be 2.89nm according to the Förster's theory of non-radiation energy transfer. The alterations of protein secondary structure in the presence of glyphosate were confirmed by the evidences from UV, FT-IR and CD spectroscopes. **(China)**

51. Zhai, H., H.P. Chan, X. Hui, and H.I. Maibach. 2008. Skin decontamination of glyphosate from human skin in vitro. *Food and Chemical Toxicology* 46(6): 2258-2260.

This study compared three model decontaminant solutions (tap water, isotonic saline, and hypertonic saline) for their ability to remove a model herbicide (glyphosate) from an in vitro human skin model. Human cadaver skin was dosed (approximately 375 µg) of [^{14}C]-glyphosate on 3 cm^2 per skin. After each exposure time (1, 3, and 30min post-dosing, respectively), the surface skin was washed three times (4ml per time) with each solution. After washing, the skin was stripped twice with tape discs. Lastly, the wash solutions, strippings, receptor fluid, and remainder of skin were liquid scintillation analyzer counted to determine the amount of

glyphosate. There were no statistical differences among these groups at any time points. The total mass balance recovery at three time exposure points was between 94.8% and 102.4%. The wash off rates (glyphosate in wash solutions) at three different exposure times is 79-101.2%. Thus the three tested decontaminants possess similar effectiveness in removing glyphosate from skin. This in vitro model is not only economic and rapid, but also provides quantitative data that may aid screening for optimal decontaminants. **(U.S.A.)**

Mammals

1. Dallegrave, E., F.D. Mantese, R.T. Oliveira, A.J.M. Andrade, P.R. Dalsenter, and A. Langeloh. 2007. Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. *Archives of Toxicology* 81(9): 665-673.

   Glyphosate is the active ingredient and polyoxyethyleneamine is the surfactant present in the herbicide Roundup® formulation commercialized in Brazil. The aim of this study was to assess the reproductive effects of glyphosate-Roundup® on male and female offspring of Wistar rats exposed during pregnancy and lactation. Dams were treated orally with water or 50, 150 or 450 mg/kg glyphosate during pregnancy (21-23 days) and lactation (21 days). These doses do not correspond to human exposure levels. The results showed that glyphosate-Roundup® did not induce maternal toxicity but induced adverse reproductive effects on male offspring rats: a decrease in sperm number per epididymis tail and in daily sperm production during adulthood, an increase in the percentage of abnormal sperms and a dose-related decrease in the serum testosterone level at puberty, and signs of individual spermatid degeneration during both periods. There was only a vaginal canal-opening delay in the exposed female offspring. These findings suggest that in utero and lactational exposure to glyphosate-Roundup® may induce significant adverse effects on the reproductive system of male Wistar rats at puberty and during adulthood. **(Brazil)**

2. Daruich, J., F. Zirulnik, and M.S. Gimenez. 2001. Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses. *Environmental Research* Section A 85(3): 226-231.

   To prevent health risk from environmental chemicals, particularly for progeny, we have studied the effects of the herbicide glyphosate on several enzymes of pregnant rats. Glyphosate is an organo-phosphorated nonselective agrochemical widely used in many countries including Argentina and acts after the sprout in a systemic way. We have studied three cytosolic enzymes: isocitrate dehydrogenase-NADP dependent, glucose-6-phosphate dehydrogenase, and malic dehydrogenase in liver, heart, and brain of pregnant Wistar rats. The treatment was administered during the 21 days of pregnancy, with 1 week as an acclimation period. The results suggest that maternal exposure to agrochemicals during pregnancy induces a variety of functional abnormalities in the specific activity of the enzymes in the studied organs of the pregnant rats and their fetuses. **(Argentina)**

3. de Bellefeuille, S., L. Bélanger, J. Huot, and A. Cimon. 2001. Clear-cutting and regeneration practices in Quebec boreal balsam fir forest: effects on snowshoe hare. *Canadian Journal of Forest Research* 31: 41-51.

   We compared utilization by the snowshoe hare (*Lepus americanus* Erxleben) of recent clearcuts subjected to three regeneration scenarios commonly used in boreal forest: natural regeneration, plantation with herbicide release (glyphosate), and plantation with manual release (brushsaw). Refuges for snowshoe hare, on a landscape dominated by clearcuts, were also investigated. Colonization of regenerating sites by the hare comes late in the humid boreal forest because clear-

cut stands take more than 10 years to reach the sapling stage. Our sites were in the seedling stage 7–9 years after cutting, and hares avoided them year round because of an inadequate protective cover. Therefore, regeneration treatments did not affect habitat use by the hare on a short-term basis. During the seedling stage, the snowshoe hare were found in the remaining forest which occupied at least 25% of the area of each home range. The preservation of residual forests is thus essential to maintain local populations on an area dominated by commercial clearcuts. **(Canada)**

4. El-Shenawy, N.S. 2009. Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate. *Environmental Toxicology and Pharmacology* 28(3): 379-385.

Glyphosate is the active ingredient and polyoxyethyleneamine, the major component, is the surfactant present in the herbicide Roundup formulation. The objective of this study was to analyze potential cytotoxicity of the Roundup and its fundamental substance (glyphosate). Albino male rats were intraperitoneally treated with sub-lethal concentration of Roundup (269.9mg/kg) or glyphosate (134.95mg/kg) each 2 days, during 2 weeks. Hepatotoxicity was monitored by quantitative analysis of the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities, total protein, albumin, triglyceride and cholesterol. Creatinine and urea were used as the biochemical markers of kidney damages. The second aim of this study to investigate how glyphosate alone or included in herbicide Roundup affected hepatic reduced glutathione (GSH) and lipid peroxidation (LPO) levels of animals as an index of antioxidant status and oxidative stress, respectively, as well as the serum nitric oxide (NO) and alpha tumour necrosis factor (TNF-α) were measured. Treatment of animals with Roundup induced the leakage of hepatic intracellular enzymes, ALT, AST and ALP suggesting irreversible damage in hepatocytes starting from the first week. It was found that the effects were different on the enzymes in Roundup and glyphosate-treated groups. Significant time-dependent depletion of GSH levels and induction of oxidative stress in liver by the elevated levels of LPO, further confirmed the potential of Roundup to induce oxidative stress in hepatic tissue. However, glyphosate caused significant increases in NO levels more than Roundup after 2 weeks of treatment. Both treatments increased the level of TNF-α by the same manner. The results suggest that excessive antioxidant disruptor and oxidative stress is induced with Roundup than glyphosate. **(Egypt)**

5. George, J., S. Prasad, Z. Mahmood, and Y. Shukla. 2010. Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach. *Journal of Proteomics* 73(5): 951-964.

Glyphosate is a widely used broad spectrum herbicide, reported to induce various toxic effects in non-target species, but its carcinogenic potential is still unknown Here we showed the carcinogenic effects of glyphosate using 2-stage mouse skin carcinogenesis model and proteomic analysis Carcinogenicity study revealed that glyphosate has tumor promoting activity Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were

differentially expressed (>2 fold) on glyphosate, 7, 12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) application over untreated control Among them, 9 proteins (translation elongation factor eEF-1 alpha chain, carbonic anhydrase III, annexin II, calcyclin, fab fragment anti-VEGF antibody, peroxiredoxin-2, superoxide dismutase [Cu-Zn], stefin A3, and calgranulin-B) were common and showed similar expression pattern in glyphosate and TPA-treated mouse skin These proteins are known to be involved in several key processes like apoptosis and growth-inhibition, anti-oxidant responses, etc The up-regulation of calcyclin, calgranulin-B and down-regulation of superoxide dismutase [Cu-Zn] was further confirmed by immunoblotting, indicating that these proteins can be good candidate biomarkers for skin carcinogenesis induced by glyphosate Altogether, these results suggested that glyphosate has tumor promoting potential in skin carcinogenesis and its mechanism seems to be similar to TPA. **(India)**

6. Grisolia, C.K. 2002. A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis* 518(2):145-150.

See Fish Section.

7. Homyack, J.A., D.J. Harrison, and W.B. Krohn. 2005. Long-term effects of precommercial thinning on small mammals in northern Maine. *Forest Ecology and Management* 205(1-3): 43-57.

Precommercial thinning (PCT) is being practiced increasingly throughout the Acadian forest of eastern North America to meet silvicultural objectives; however, effects of this practice on wildlife, both immediately and several years post-treatment are not well understood. Forest dependent small mammals have ecological roles as prey for numerous avian and mammalian predators, dispersers of seeds, fruit, and spores, and contribute to nutrient cycling. Researchers in the northwestern USA have suggested that thinning of young, regenerating clearcuts may increase the abundance and diversity of some forest-dependent small mammals by increasing rates of forest development and enhancing the ecological representation of mid-successional stands across managed landscapes. We examined the effects of PCT within conifer-dominated forest stands 1-, 6-, 11-, and 16-years post-treatment, on abundances of mice, voles, and shrews, and on within-stand structure in the commercially managed, Acadian forests of northern Maine. We live-trapped small mammals on 24 herbicide-treated clearcuts treated with PCT and on 13 similar, unthinned stands during summers of 2000 and 2001. Thinning of mid-successional conifer stands resulted in increased abundances, (red-backed voles, *Clethrionomys gapperi*, $P = 0.008$; masked shrews, *Sorex cinereus*, $P < 0.001$) or had no detectable effect on (deer mice, *Peromyscus maniculatus*, $P = 0.544$; short-tailed shrews, *Blarina brevicauda*, $P = 0.517$) the 4 most common species of Muridae and Soricidae in northern Maine. In general, abundance of deer mice responded more positively to increasing development class and to the number of years since thinning than other species of small mammals. Several within-stand habitat characteristics associated with stand maturity, such as larger stem diameters and a partially open canopy, occurred in thinned stands. Thus, PCT may accelerate the development of

habitat attributes typical of mid-successional conifer stands in intensively managed stands within the Acadian Forest. PCT may increase abundances of small mammal species associated with mid-seral forest conditions at the scale of the forest stand. **(U.S.A.)**

8. Homyack, J.A., D.J. Harrison, and W.B. Krohn. 2007. Effects of precommercial thinning on snowshoe hares in Maine. *Journal of Wildlife Management* 71(1): 4-13.

Snowshoe hares (*Lepus americanus*) are an important prey species and a dominant herbivore across much of their North American range, and researchers have questioned the influences of forestry practices that alter habitat for hares and the potential community-level effects on carnivores. We examined the effects of precommercial thinning (PCT) from 1 to 11 years posttreatment on snowshoe hares. In the commercial forests of northern Maine, USA, we counted and cleared hare pellets twice a year during 2001 and 2002 on >46 km of pellet transects across 30 regenerating conifer stands (17 treated with PCT) previously treated with an aerial application of herbicide. We compared densities of snowshoe hare pellets among 3 development classes with (1 yr after thinning, 6 yr after thinning, and 11 yr after thinning) and without thinning (stands with a similar history of clearcut and herbicide treatment but no thinning). During both years, densities of hares were lower in stands treated with PCT than in similar unthinned stands across the 3 development classes and during both leaf-off and leaf-on seasons ($P < 0.001$). Within both thinned and unthinned stands, hare density was greatest in stands in the 1-year development class when compared to the 6-year and 11-year development classes, but a statistical difference ($P = 0.048$) among classes was evident only during leaf-off seasons. Precommercial thinning was associated with densities of snowshoe hares that were approximately half of those in similar unthinned stands up to at least 11 years posttreatment; however, thinned stands may retain densities of hares greater than stands managed using other forest harvesting regimes. Our results apply to core portions of stands with crop trees spaced at 1.8-2.4-m intervals following complete overstory removal and herbicide treatment. We advocate caution when applying our results to other thinning regimes or across broader spatial scales. **(U.S.A.)**

9. Lautenschlager, R.A. and T.P. Sullivan. 2002. Effects of herbicide treatments on biotic components in regenerating northern forests. *The Forestry Chronicle* 78(5): 695-731.

See Biodiversity and Restoration Section.

10. McComb, B.C., L. Curtis, C.L. Chambers, M. Newton, and K. Bentson. 2008. Acute toxic hazard evaluations of glyphosate herbicide on terrestrial vertebrates of the Oregon coast range. *Environmental Science and Pollution Research* 15(3): 266-272.

See Amphibians, Aquatic Invertebrates and Plants, and Algae Section.

11. McLaren, B., K. Emslie, T. Honsberger, T. McCready, F.W. Bell, and R. Foster. 2011. Monitoring and understanding mammal assemblages: experiences from Bending Lake, Fallingsnow, and Tom Hill. *The Forestry Chronicle* 87(2): 225-234.

See Biodiversity and Restoration Section.

12. Peixoto, F. 2005. Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. *Chemosphere* 61(8): 1115-1122.

The potential toxicity of the herbicide Roundup and its fundamental substance (glyphosate) was tested in bioenergetic functions of isolated rat liver mitochondria. Roundup stimulates succinate-supported respiration twice, with simultaneous collapse of transmembrane electrical potential, while glyphosate used in the same concentrations does not induce any significant effect. Additionally, Roundup depresses state 3 respiration by about 40%, at 15 mM, whereas uncoupled respiration in the presence of FCCP is depressed by about 50%. Depression of uncoupled respiratory activity is mediated through partial inhibition of mitochondrial complexes II and III, but not of complex IV. The phosphorylative system was affected by both a direct and an indirect effect on the F_0F_1 ATPase activity. The addition of uncoupled concentrations of Roundup to Ca^{2+}-loaded mitochondria treated with Ruthenium Red resulted in non-specific membrane permeabilization, as evidenced by mitochondrial swelling in isosmotic sucrose medium. Therefore, the uncoupling of oxidative phosphorylation is also related to the non-specific membrane permeabilization induced by Roundup. Glyphosate alone does not show any relevant effect on the mitochondrial bioenergetics, in opposition to Roundup formulation products. The differences in the toxicity observed could be either attributed to some products of Roundup or to a synergic effect of glyphosate and formulation products. Bearing in mind that mitochondria is provided with a variety of bioenergetic functions mandatory for the regulation of intracellular aerobic energy production and electrolyte homeostasis, these results question the safety of Roundup on animal health. **(Portugal)**

13. Peterson, R.K., and A.G. Hulting. 2004. A comparative ecological risk assessment for herbicides used on spring wheat: the effect of glyphosate when used within a glyphosate-tolerant wheat system. *Weed Science* 52: 834-844.

See Birds Section.

14. Piesova, E. 2005. The effect of glyphosate on the frequency of micronuclei in bovine lymphocytes *in vitro*. *Acta Veterinaria* (Beograd) 55(2-3): 101-109.

Glyphosate is a widely used broad-spectrum herbicide that has expanded its applications on plant varieties that are genetically modified to tolerate glyphosate treatment. The aim of this study was to determine the frequency of micronuclei (W) in bovine peripheral lymphocytes after exposure to glyphosate in vitro. The cytokinesis block micronucleus assay (CBMN) for estimation of genotoxic activity was used. The obtained results indicate that glyphosate weakly induced micronuclei in bovine peripheral lymphocytes. Significant elevations of MNi ($p < 0.05$) were observed at concentrations of glyphosate of 280 µM and 560 µM, respectively. Treatment of bovine lymphocytes did not result in the induction of micronuclei in a dose-dependent manner From cytotoxicity data it is evident that CBPI does not reflect the reduction of cell proliferation. The influence of metabolic activation on the genotoxic activity of glyphosate was investigated, too. When lymphocyte cultures

were treated with glyphosate together with a liver membrane fraction (S9) from Aroclor 1245-induced rat liver, the number of micronuclei in binucleated cells did not increase significantly. **(Slovak Republic)**

15. Romano, R.M., M.A. Romano, M.M. Bernardi, P.V. Furtado, and C.A. Oliveira. 2010. Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. *Archives of Toxicology* 84(4): 309-317.

    Glyphosate is a herbicide widely used to kill weeds both in agricultural and non-agricultural landscapes. Its reproductive toxicity is related to the inhibition of a StAR protein and an aromatase enzyme, which causes an in vitro reduction in testosterone and estradiol synthesis. Studies in vivo about this herbicide effects in prepubertal Wistar rats reproductive development were not performed at this moment. Evaluations included the progression of puberty, body development, the hormonal production of testosterone, estradiol and corticosterone, and the morphology of the testis. Results showed that the herbicide (1) significantly changed the progression of puberty in a dose-dependent manner; (2) reduced the testosterone production, in semineferous tubules' morphology, decreased significantly the epithelium height ($P < 0.001$; control = 85.8 ± 2.8 μm; 5 mg/kg = 71.9 ± 5.3 μm; 50 mg/kg = 69.1 ± 1.7 μm; 250 mg/kg = 65.2 ± 1.3 μm) and increased the luminal diameter ($P < 0.01$; control = 94.0 ± 5.7 μm; 5 mg/kg = 116.6 ± 6.6 μm; 50 mg/kg = 114.3 ± 3.1 μm; 250 mg/kg = 130.3 ± 4.8 μm); (4) no difference in tubular diameter was observed; and (5) relative to the controls, no differences in serum corticosterone or estradiol levels were detected, but the concentrations of testosterone serum were lower in all treated groups ($P < 0.001$; control = 154.5 ± 12.9 ng/dL; 5 mg/kg = 108.6 ± 19.6 ng/dL; 50 mg/dL = 84.5 ± 12.2 ng/dL; 250 mg/kg = 76.9 ± 14.2 ng/dL). These results suggest that commercial formulation of glyphosate is a potent endocrine disruptor in vivo, causing disturbances in the reproductive development of rats when the exposure was performed during the puberty period. **(Brazil)**

16. Shepard, J.P., J. Creighton, and H. Duzan. 2004. Forestry herbicides in the United States: an overview. *Wildlife Society Bulletin* 32(4): 1020-1027.

    See Biodiversity and Restoration Section.

17. Šiviková, K. and J. Dianovskỳ. Cytogenetic effect of technical glyphosate on cultivated bovine peripheral lymphocytes. 2006. *International Journal of Hygiene and Environmental Health* 209(1): 15-20.

    A technical herbicide containing isopropyl amine salt of glyphosate was tested for induction of chromosome aberrations (CA) and sister chromatid exchanges (SCE) in cultured bovine peripheral lymphocytes. Cultures were exposed to a glyphosate formulation at concentrations ranging from 28 to 1120 μmol/l without and with metabolic activation. No clastogenic effect of the herbicide was found. Its genotoxic effect was confirmed in the SCE assay after 24 h of incubation. A statistically significant elevation in SCE induction was observed in each of the donors after application of the product at doses ranging from 56 to 1120 μmol/l. The highest

concentrations (560 and 1120 µmol/l) also caused reduction of mitotic and proliferation indices. In the 2 h-assay with metabolic activation a statistically significant frequency of SCE was observed only in cultures treated with the agent at a concentration of 140 µmol/l. **(Slovak Republic)**

18. Sullivan, T.P. and D.S. Sullivan. 2003. Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems. *Environmental Reviews* 11: 37-59.
    See Biodiversity and Restoration Section.

19. Sullivan, T.P. D.S. Sullivan, P.M.F. Lindgren, and J.O. Boateng. 2002. Influence of conventional and chemical thinning on stand structure and diversity of plant and mammal communities in young lodgepole pine forest. *Forest Ecology and Management* 170(1): 173-187.
    See Biodiversity and Restoration Section.

20. Swift, K. and F.W. Bell. 2011. What are the environmental consequences of using silviculturally effective forest vegetation management treatments? *The Forestry Chronicle* 87(2): 201-216.
    See Biodiversity and Restoration Section.

21. Tatum, V.L. 2004. Toxicity, transport, and fate of forest herbicides. *Wildlife Society Bulletin* 32(4): 1042-1048.
    See Microbiota and Fungi Section.

22. Ward, D., N. Tucker, and J. Wilson. 2003. Cost-effectiveness of revegetating degraded riparian habitats adjacent to macadamia orchards in reducing rodent damage. *Crop Protection* 22(7): 935–940.
    The cost-effectiveness of habitat manipulation as a management strategy for the control of rodent pests in Australian macadamia (*Macadamia integrifolia*) orchards was investigated in a 3-yr study that combines a pest management strategy with a conservation outcome. The manipulation involved the total removal of exotic weeds from an adjacent non-crop riparian habitat, which harboured a high density of the native rodent, *Uromys caudimaculatus*, and the planting of species common to an endangered rainforest ecosystem. Over a 3-yr period, orchard trees adjacent to the restored habitat received 50% less rodent damage than trees adjacent to non-manipulated habitats. A cost–benefit analysis (based on both contractor and farm rates) of the damage reduction obtained after the initial manipulation indicated a break-even point (based on farm rates and a nut in shell price of $2.80/kg) of 3.4 yr. After break-even, this represents an economic benefit to growers that will result in an additional return of approximately $4500 per annum per km of orchard frontage. **(Australia)**

23. Williams, A.L., R.E. Watson, and J.M. DeSesso. 2012. Developmental and reproductive outcomes in humans and animals after glyphosate exposure: a critical

analysis. *Journal of Toxicology and Environmental Health*. Part B, Critical reviews 15(1): 39-96.

See Human Health Section.

Microbiota and Fungi

1. Anderson, J.A. and J.A. Kolmer. 2005. Rust control in glyphosate tolerant wheat following application of the herbicide glyphosate. *Plant Disease* 89(11): 1136-1142.
   In greenhouse and field trials, transgenically modified wheat (*Triticum aestivum*) genotypes with tolerance to glyphosate had extremely low infection types to leaf rust caused by *Puccinia triticina* when treated with a labeled rate of the herbicide glyphosate prior to inoculation with leaf rust. A surfactant solution and a nonglyphosate herbicide had no effect on leaf rust development on the glyphosate tolerant wheat. Glyphosate had a systemic effect in reducing leaf rust development. The leaf rust control by glyphosate decreased with reduced application rates and longer periods of time between glyphosate application and leaf rust infections. The field and greenhouse tests indicated that control of leaf rust in wheat conditioned by glyphosate is transitory and is effective for at least 21, but not more than 35, days after application. Application of glyphosate also reduced infection types on wheat caused by the stem rust fungus, *Puccinia graminis* f. sp. *tritici*. Given these results and evidence from the literature that glyphosate can have adverse effects on other pathogens, including other rust fungi, additional investigation of the fungicidal properties of glyphosate are warranted, with particular attention to the timing of glyphosate application relative to fungal infection. The effects of glyphosate on the soybean rust fungus, *Phakopsora pachyrhizi*, an emerging pathogen in North America, merit immediate investigation. **(U.S.A.)**

2. Araújo, A.S.F., R.T.R. Monteiro, and R.B. Abarkeli. 2003. Effect of glyphosate on the microbial activity of two Brazilian soils. *Chemosphere* 52(5): 799-804.
   Glyphosate [*N*-(phosphonomethyl)-glycine] is a broad-spectrum, non-selective, post-emergence herbicide that is widely used in agricultural. We studied, in vitro, changes in the microbial activity of typical Hapludult and Hapludox Brazilian soils, with and without applied glyphosate. Glyphosate was applied at a rate of 2.16 mg glyphosate kg^{-1} of soil and microbial activity was measured by soil respiration (evolution of CO^2) and fluorescein diacetate (FDA) hydrolysis over a period of 32 days. We found an increase of 10-15% in the CO^2 evolved and a 9-19% increase in FDA hydrolyses in the presence of glyphosate compared with the same type of soil which had never received glyphosate. Soil which had been exposed to glyphosate for several years had the strongest response in microbial activity. Most probable number (MPN) counts showed that after 32 days incubation the number of actinomycetes and fungi had increased while the number of bacteria showed a slight reduction. After the incubation period, high pressure liquid chromatography (HPLC) detected the glyphosate metabolite aminomethyl phosphonic acid (AMPA), indicating glyphosate degradation by soil microorganisms. **(Brazil)**

3. Arfarita, N., T. Imai, A. Kanno, T. Higuchi, K. Yamamoto, and M. Sekine. 2011. Screening of soil-born fungi from forest soil using glyphosate herbicide as the sole source of phosphorus. *Journal of Water and Environment Technology* 9(4): 391-400.
   Glyphosate herbicide was used as screening agent to be the sole source of phosphorus for the isolation of soil-born fungi of forest soil. Three fungal strains were

able to grow consistently in the presence of glyphosate as the sole phosphorus source and were identified as *Fusarium* sp. strain FRP1, *Scopulariopsis* sp. strain FRP2 and *Trichoderma* sp. strain FRP3. On standard medium ten fungal strains were isolated and identified as *Botrytis* sp. strain FR1, *Mucor* sp. strain FR2, *Acremonium* sp. strain FR3, *Trichoderma* sp. strain FR4, Botrytis sp. strain FR5, *Crysosporium* sp. strain FR6, *Scopulariopsis* sp. strain FR7, *Trichoderma* sp. strain FR8, *Botrytis* sp. strain FR9 and *Acremonium* sp. strain FR10. Of the three screened fungal species, *Scopulariopsis* sp. strain FRP2 and *Trichoderma* sp. strain FRP3 were selected for further study because they had the highest ratio of growth diameter. The growth kinetics of *Scopulariopsis* sp. strain FRP2 and *Trichoderma* sp. strain FRP3 were also observed on broth Czapek medium containing glyphosate as the sole source of phosphorus. Synergetic interactions *in vitro* were examined on PDA. The synergetic interactions of *Scopulariopsis* sp. strain FRP2 and *Trichoderma* sp. strain FRP3 as mix culture showed antibiosis inhibition mode. **(Japan)**

4. Babiker, E.M., S.H. Hulbert, K.L. Schroeder, and T.C. Paulitz. 2011. Optimum timing of preplant applications of glyphosate to manage rhizoctonia root rot in barley. *Plant Disease* 95(3): 304-310.

   Rhizoctonia root rot, caused by *Rhizoctonia solani* AG-8 and *R. oryzae*, is considered one of the main deterrents for farmers to adopt reduced-tillage systems in the Pacific Northwest. Because of the wide host range of *Rhizoctonia* spp., herbicide application before planting to control weeds and volunteer plants is the main management strategy for this disease. To determine the effect of timing of glyphosate applications on the severity of Rhizoctonia root rot of barley, field experiments were conducted in 2007, 2008, and 2009 in a field naturally infested with a high level of both *R. solani* and *R. oryzae*. Crop volunteer plants and weeds were allowed to grow over the winter and plots were sprayed with glyphosate at 42, 28, 14, 7, and 2 days prior to planting. As the herbicide application interval increased, there were significant increases in shoot length, length of the first true leaf, and number of healthy seminal roots and a decrease in disease severity. Yield and the number of seminal roots did not show a response to herbicide application interval in most years. The activity of *R. solani*, as measured by toothpick bioassay and real-time polymerase chain reaction, declined over time in all treatments after planting barley. The herbicide application interval required to meet 80 and 90% of the maximum response (asymptote) for all plant and disease measurements ranged from 11 to 27 days and 13 to 37 days, respectively. These times are the minimum herbicide application intervals required to reduce disease severity in the following crop. **(U.S.A.)**

5. Bérubé, M.-È., A. Vanasse, S. Rioux, N. Bourget, Y. Dion, and G. Tremblay. 2012. Effect of glyphosate on Fusarium head blight in wheat and barley under different soil tillages. *Plant Disease* 96(3): 338-344.

   Fusarium head blight (FHB) is a serious disease in the wet conditions of eastern Canada. Tillage practices and herbicide applications have been reported to influence disease intensity. This study aimed to determine the effect of glyphosate on FHB development in wheat and barley and on *Fusarium graminearum* inoculum

production under different soil tillages. The experiment was performed during 2 years (2007 and 2008) at two different sites in Quebec, Canada. Six trials were set in both sites, combining two cereal species (wheat and barley) and three soil tillages: moldboard plow, spring tillage (minimum-till), and direct drilling. For each trial, glyphosate or other herbicides were applied on Roundup Ready soybean the year preceding cereal crops, constituting the main plots. The next year, three wheat and three barley cultivars were sown as subplots. FHB index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON) content, and *F. graminearum* inoculum production were measured. Glyphosate had no significant effect on FHB index, FDK, or DON content, whatever the trial and the site. *F. graminearum* inoculum production was enhanced by glyphosate in only 1 of 12 trials. Cultivar effect was highly significant on DON content. The relationship between *F. graminearum* inoculum from soybean residues and DON content was weak. **(Canada)**

6. Bithell, S.L., R.C. Butler, A. Mckay, and M.G. Cromey. 2009. Effect of glyphosate application to grass weeds on levels of *Gaeumannomyces graminis* var. *tritici* inoculum. *Plant Protection Quarterly* 24(4): 161-167.

   The cereal pathogen *Gaeumannomyces graminis* var. *tritici* (*Ggt*) that causes the disease take-all infects *Elytrigia repens* (couch) and a number of other grass weed species. Soil *Ggt* DNA levels 14 months after couch had been planted into a naturally *Ggt* infected site, in which *Bromus willdenowii* (prairie grass) was also present, indicated that the presence of couch maintained rather than increased *Ggt* levels. Following an early glyphosate application soil *Ggt* levels increased three and eight fold, one and two months respectively after application (86 days before wheat sowing) to plots with couch present. A later glyphosate application (38 days before wheat sowing) also resulted in rapid increases in *Ggt* inoculum levels in plots with couch and to a lesser extent in plots without couch. Results confirm that couch is an important host of *Ggt* with more roots per wheat plant infected, more plants infected and a greater take-all severity in plots with couch present. Early or late glyphosate applications did not affect subsequent levels of take-all in wheat. Prairie grass seedlings were also identified as a significant *Ggt* inoculum source, but this effect was independent of glyphosate and couch treatments. Overall, increases in *Ggt* inoculum levels following glyphosate application are large and relatively rapid. *Ggt* inoculum associated with couch rhizomes is persistent. Finally, very early glyphosate applications will be required to allow time for the break down of couch rhizomes and decline in *Ggt* inoculum levels thus reducing the risk of take-all in subsequent wheat crops. **(New Zealand)**

7. Bogantes-Arias, A., R. Agüero-Alvarado, and R. Mexzón-Vargas. 2006. Weeds control in peach palm (*Bactris gasipaes* K.) for palm hearts: effect on soil and arthropods. *Agronomía Mesoamericana* 17(1): 25-33.

   See Terrestrial Invertebrates Section.

8. Boyette, C.D., R.E. Hoagland, and M.A. Weaver. 2008. Interaction of a bioherbicide and glyphosate for controlling hemp sesbania in glyphosate-resistant soybean. *Weed Biology and Management* 8(1): 18-24.

The bioherbicidal fungus, *Colletotrichum truncatum* (Schwein.) Andrus & Moore, was tested at different inoculum concentrations alone and in combination with, prior to or following treatment with different rates of glyphosate (N -[phosphonomethyl]glycine) (Roundup Ultra) for the control of hemp sesbania (*Sesbania exaltata* [Raf.] Rydb. ex A.W. Hill) in Roundup Ready soybean field plots. *Colletotrichum truncatum* and glyphosate were applied in all pair-wise combinations of 0, 1.25, 2.5, 5.0, and 10.0 × 10 6 spores mL^{-1} (i.e. 3.125, 6.25, 12.5, and 25 × 10 11 spores ha^{-1}), and 0.15, 0.30, 0.60, and 1.2 kg ha^{-1}, respectively. Weed control and disease incidence were enhanced at the two lowest fungal and herbicidal rates when the fungal spores were applied after glyphosate treatment. The application of the fungus in combination with or prior to glyphosate application at 0.30 kg ha^{-1} resulted in reduced disease incidence and weed control regardless of the inoculum's concentration. At the highest glyphosate rates, the weeds were controlled by the herbicide alone. These results suggest that it might be possible to utilize additive or synergistic herbicide and pathogen interactions to enhance hemp sesbania control. **(U.S.A.)**

9. Busse, M.D., A.W. Ratcliff, C.J. Shestak, and R.F. Powers. 2001. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. *Soil Biology and Biochemistry* 33(12): 1777-1789.

We assessed the direct and indirect effect of the herbicide glyphosate on soil microbial communities from ponderosa pine (*Pinus ponderosa*) plantations of varying site quality. Direct, toxic effects were tested using culture media and soil bioassays at glyphosate concentrations up to 100-fold greater than expected following a single field application. Indirect effects on microbial biomass, respiration, and metabolic diversity (Biolog and catabolic response profile) were compared seasonally after 9–13 years of vegetation control using repeated glyphosate applications in a replicated field study. Three pine plantations were selected to provide a range of soil characteristics associated with glyphosate binding (clay, Fe and Al oxide content) and site growing potential from the lowest to the highest in northern California. Glyphosate was toxic to bacteria and fungi from each plantation when grown in soil-free media. Culturable populations were reduced, as was the growth rate and metabolic diversity of surviving bacteria, by increasing concentrations of glyphosate. This toxicity was not expressed when glyphosate was added directly to soil, however. Microbial respiration was unchanged at expected field concentrations (5–50 μg g^{-1}), regardless of soil, and was stimulated by concentrations up to 100-fold greater. Increased microbial activity resulted from utilization of glyphosate as an available carbon substrate. Estimated N and P inputs from glyphosate were inconsequential to microbial activity. Long-term, repeated applications of glyphosate had minimal affect on seasonal microbial characteristics despite substantial changes in vegetation composition and growth. Instead, variation in microbial characteristics was a function of time of year and site quality. Community size, activity, and metabolic diversity generally were greatest in the spring and increased as site quality improved, regardless of herbicide treatment. Our findings suggest that artificial media assays are of limited relevance in predicting glyphosate toxicity to soil organisms and that field rate applications of glyphosate

should have little or no affect on soil microbial communities in ponderosa pine plantations. **(U.S.A.)**

10. Castro, Jr, J.V., M.C.R. Peralba, and M.A.Z. Ayub. 2007. Biodegradation of the herbicide glyphosate by filamentous fungi in platform shaker and batch bioreactor. *Journal of Environmental Science and Health*. Part B 42(8): 883-886.

    The biodegradation conducted by microorganisms on herbicide glyphosate (N-phosphonomethylglycine) was investigated. Five strains of filamentous fungi belonging to the *Fusarium* genre were grown on Czapeck medium without phosphorous and supplemented with the addition of glyphosate. The assays were conducted to determine the ability of use as a phosphorous source, the inhibition caused by presence of herbicide, and the biodegradation in shaker and bioreactor by *Fusarium* strains. It was observed that the herbicide did not show any negative effect on microrganisms by quantity of the biomass. Among the strains tested, no inhibition was noted by the addition of glyphosate even at a high concentration. All strains studied were able to biodegrade it and use the herbicide as a phosphorous source. The formation of consortium was not better than the strains tested in pure culture. The biodegradation in the bioreactor was better than in the shaker. However, there wasn't any influence on biodegradation rate by changing the amount of oxygen in the system. **(Austria)**

11. Clair, E., L. Linn, C. Travert, C. Amiel, G.-E. Séralini, and J.M. Panoff. 2012. Effects of Roundup® and glyphosate on three food microorganisms: *Geotrichum candidum, Lactococcus lactis* subsp. *cremoris* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. *Current Microbiology* 64(5): 486-491.

    Use of many pesticide products poses the problem of their effects on environment and health. Amongst them, the effects of glyphosate with its adjuvants and its by-products are regularly discussed. The aim of the present study was to shed light on the real impact on biodiversity and ecosystems of Roundup®, a major herbicide used worldwide, and the glyphosate it contains, by the study of their effects on growth and viability of microbial models, namely, on three food microorganisms (*Geotrichum candidum, Lactococcus lactis* subsp. *cremoris* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) widely used as starters in traditional and industrial dairy technologies. The presented results evidence that Roundup® has an inhibitory effect on microbial growth and a microbicide effect at lower concentrations than those recommended in agriculture. Interestingly, glyphosate at these levels has no significant effect on the three studied microorganisms. Our work is consistent with previous studies which demonstrated that the toxic effect of glyphosate was amplified by its formulation adjuvants on different human cells and other eukaryotic models. Moreover, these results should be considered in the understanding of the loss of microbiodiversity and microbial concentration observed in raw milk for many years. **(France)**

12. Cook, J.C., R. Charudattan, T.W. Zimmerman, E.N. Rosskopf, W.M. Stall, and G.E. MacDonald. 2009. Effects of *Alternaria destruens*, glyphosate, and ammonium

sulfate individually and integrated for control of dodder (*Cuscuta pentagona*). *Weed Technology* 23(4): 550-555.

Dodder is a serious parasitic weed in the crops in which it is a problem (particularly citrus). *Alternaria destruens* is the active ingredient in a registered bioherbicide for control of dodder species. In greenhouse studies, the treatments applied to citrus parasitized with field dodder were a nontreated control; oil at 7.5% v/v in water; ammonium sulfate at 0.125% w/v in water; glyphosate at 0.02 kg ae/L; *A. destruens* at 1.8 X 10^{10} spores/L; *A. destruens* (1.8 X 10^{10} spores/L) + oil at 7.5% v/v ill water; and a mixture of *A. destruens* (1.8 X 10^{10}, spores/L) + oil at 7.5% v/v in water + glyphosate at 0.02 kg ae/L + ammonium sulfate 0.125% w/v (the mixture treatment). The highest disease or damage severity rating out of all treatments, measured as the area under the disease or damage progress curve (AUDPC), was obtained for the mixture treatment. By 35 d after treatment, all field dodder plants that received the Mixture treatment were dead but the host plant, citrus, was not. These results indicate the feasibility of integrating glyphosate, ammonium sulfate, and *A. destruens* to manage dodder. **(U.S.A.)**

13. Ermakova, I.T., N.I. Kiseleva, T. Shushkova, M. Zharikov, G.A. Zharikov, and A.A. Leontievsky. 2010. Bioremediation of glyphosate-contaminated soils. *Applied Microbiology and Biotechnology* 88(2): 585-594.

Based on the results of laboratory and field experiments, we performed a comprehensive assessment of the bioremediation efficiency of glyphosate-contaminated soddy-podzol soil. The selected bacterial strains *Achromobacter* sp. Kg 16 (VKM B-2534D) and *Ochrobactrum anthropi* GPK 3 (VKM B-2554D) were used for the aerobic degradation of glyphosate. They demonstrated high viability in soil with the tenfold higher content of glyphosate than the recommended dose for the single in situ treatment of weeds. The strains provided a two- to threefold higher rate of glyphosate degradation as compared to indigenous soil microbial community. Within 1–2 weeks after the strain introduction, the glyphosate content of the treated soil decreased and integral toxicity and phytotoxicity diminished to values of non-contaminated soil. The decrease in the glyphosate content restored soil biological activity, as is evident from a more than twofold increase in the dehydrogenase activity of indigenous soil microorganisms and their biomass (1.2-fold and 1.6-fold for saprotrophic bacteria and fungi, respectively). The glyphosate-degrading strains used in this study are not pathogenic for mammals and do not exhibit integral toxicity and phytotoxicity. Therefore, these strains are suitable for the efficient, ecologically safe, and rapid bioremediation of glyphosate-contaminated soils. **(Russia)**

14. Feng, P.C.C., C. Clark, G.C. Andrade, M.C. Balbi, and P. Caldwell. 2008. The control of Asian rust by glyphosate in glyphosate-resistant soybeans. *Pest Management Science* 64(4): 353-359.

Glyphosate is a widely used broad-spectrum herbicide. Recent studies in glyphosate-resistant (GR) crops have shown that, in addition to its herbicidal activity, glyphosate exhibits activity against fungi, thereby providing disease control benefits. In GR wheat, glyphosate has shown both preventive and curative activities against *Puccinia striiformis* f. sp. *tritici* (Erikss) CO Johnston and *Puccinia triticina* Erikss,

which cause stripe and leaf rusts respectively. Laboratory studies confirmed earlier observations that glyphosate has activity against *Phakopsora pachyrhizi* Syd & P Syd which causes Asian soybean rust (ASR) in GR soybeans. The results showed that glyphosate at rates between 0.84 and 1.68 kg ha^{-1} delayed the onset of ASR in GR soybeans. However, field trials conducted in Argentina and Brazil under natural infestations showed variable ASR control from application of glyphosate in GR soybeans. Further field studies are ongoing to define the activity of glyphosate against ASR. These results demonstrate the disease control activities of glyphosate against rust diseases in GR wheat and GR soybeans. **(U.S.A.)**

15. Fernandez, M.R., D. Huber, P. Basnyat, and R.P. Zentner. 2008. Impact of agronomic practices on populations of *Fusarium* and other fungi in cereal and noncereal crop residues on the Canadian Prairies. *Soil & Tillage Research* 100(1): 60-71.

    Fusarium head blight (FHB) is an important disease which has been causing damage to wheat and barley crops in western Canada. Because crop residues are an important source of inoculum, it is important to know the ability of *Fusarium* spp. to colonize and survive in different residue types, and how their populations might be affected by agronomic practices. Sampling of residue types on producers' fields for quantification of *Fusarium* and other fungi was conducted in 2000-2001 in eastern Saskatchewan. *Fusarium* spp. were isolated from most fields, whereas their mean percentage isolation (MPI) was over 50% for cereal and pulse residues, and under 30% for oilseed residues. The most common *Fusarium*, *F. avenaceum*, had a higher MPI in pulse and flax (45-48%) than in cereal or canola (10-22%) residues. This was followed by *F. equiseti*, *F. acuminatum*, *F. graminearum*, *F. culmorum* and *F. poae* which were isolated from all, or most, residue types. Factors affecting *Fusarium* abundance in residues included the current crop, cropping history, and tillage system. In cereal residues, the MPI of *F. avenaceum* was higher when the current crop was another cereal (24%) versus a noncereal (4-8%). When the current crop was another cereal, the lowest MPI of *F. avenaceum* and *F. culmorum* occurred when the field had been in summerfallow (SF) two years previous (*F. avenaceum*: 17% for SF, 28% for a crop; *F. culmorum*: 1% for SF, 4% for a crop); in contrast, *F. equiseti* and *Cochliobolus sativus* were most common in residues of cereal crops preceded by SF (*F. equiseti*: 16% for SF, 10% for a crop; *C. sativus*: 22% for SF, 13% for a crop). The MPI of *F graminearum* was higher when the crop two years previous was an oilseed (7%) versus a cereal (4%). In regards to tillage effects, when the current crop was a cereal, the MPI of *F. avenaceum* was higher under minimum (MT) and zero tillage (ZT) (22-37%) than conventional tillage (CT) (15%), that of *F. graminearum* was lowest under ZT (3% for ZT, 7-11% for CT-MT), whereas that of *C. sativus* was highest under CT (27% for CT, 6-11% for MT-ZT). Under ZT, previous glyphosate applications were correlated positively with *F. avenaceum* and negatively with *F. equiseti* and *C. sativus*. These observations generally agreed with results from previous FHB and root rot studies of wheat and barley in the same region. Percentage isolation of *F avenaceum* from noncereal and of *F. graminearum* from cereal residues were positively correlated with FHB severity

and percentage *Fusarium*-damaged kernels of barley and wheat caused by the same fungi. **(Canada)**

16. Fernandez, M.R., R.P. Zentner, P. Basnyat, D. Gehl, F. Selles, and D. Huber. 2009. Glyphosate associations with cereal diseases caused by *Fusarium* spp. in the Canadian Prairies. *European Journal of Agronomy* 31(3): 133-143.

    Fusarium pathogens cause important diseases, such as root/crown rot and *Fusarium* head blight (FHB), in cereal crops. These diseases can be caused by similar *Fusarium* spp. Common root rot (CRR) is widespread in the western Canadian Prairies, whereas FHB has potential of becoming an important disease in this region. There are no commercially available cereal cultivars with good resistance to these diseases. It is therefore important to identify agronomic practices that could affect levels of *Fusarium* pathogens in cereals. This review deals primarily with the effects of tillage systems and glyphosate use on the development of FHB and CRR in wheat and barley in eastern Saskatchewan. Although the FHB study in 1999-2002 indicated that environment was the most important factor determining FHB development, previous glyphosate use and tillage practice were among the production factors with the greatest association with FHB. Overall, disease was highest in crops under minimum-till management. Previous glyphosate use was consistently associated with higher FHB levels caused by the most important FHB pathogens, *Fusarium avenaceum* and *Fusarium graminearum. Cochliobolus sativus*, the most common CRR pathogen, was negatively associated with previous glyphosate use, while *F. avenaceum*, *F. graminearum*, and other fungi were positively associated, suggesting that glyphosate might cause changes in fungal communities. The Occurrence and isolation of *F. avenaceum* from cereal residues were greater under reduced-till than conventional-till while *C. sativus* was Most Common under conventional-till, and *F. graminearum* was lowest under zero-till. Previous glyphosate applications were again correlated positively with *F avenaceum* and negatively with *C. sativus*. These observations agreed with results from the FHB and CRR studies. These are the first studies that established a relationship between previous glyphosate use and increased *Fusarium* infection of spikes and subcrown internodes of wheat and barley, or *Fusarium* colonization of crop residues. However, because of the close association between noncereal crops, reduced tillage and glyphosate use, it was not possible to completely separate the effects of these factors on *Fusarium* infections. Determining the relative contribution of these popular production trends to the development of diseases caused by *Fusarium* spp. are essential for devising appropriate agronomic recommendations to prevent their further spread in western Canada, and to reduce the impact that these diseases are having in areas where they are already established. The consistent association between previous glyphosate use and *Fusarium* infections also warrants further research to elucidate the nature of this association and the underlying mechanisms determining these effects. **(Canada)**

17. Ferreira, E.A., J.B. Santos, A.A. Silva, L. Vargas, and M.R. Reis. 2006. Glyphosate application for Italian ryegrass biotype control and impact on soil microbiota. *Planta Daninha* 24(3): 573-578.

This study aimed to evaluate the resistance Italian ryegrass (*L. multiflorum*) against glyphosate and the impact of the control of these biotypes on soil respiration and microbial biomass. Two essays were conducted: the first evaluated intoxication and dry mass of the plants of biotypes of three Italian ryegrass population (population 1 - resistant; population 2 - intermediary resistance; and population 3 - sensitive to glyphosate) submitted to different glyphosate rates. The second assay evaluated dry matter of the aereal part, plant height, number of leaves and respiration and microbial mass of the soil cultivated with the resistant and sensitive biotypes, with and without glyphosate application (480 g ha^{-1}). At 14 DAA, death of the susceptible biotype was observed, when treated with rates starting from 200 g ha^{-1} of glyphosate. The resistant ad intermediate resistant biotypes showed glyphosate phytotoxicity of 85% at the highest rate evaluated. The resistant biotype presented the highest dry matter production of the aerial part at 42 DAA and during sprouting, at 72 DAA, compared to the intermediary biotype. The sensitive biotype presented higher plant height, number of leaves and dry mass of the aerial part, to the resistant biotype, without glyphosate. No difference was observed in the microbial activity in the soil among the treatments. **(Brazil)**

18. Gomez, E., L. Ferreras, L. Lovotti, and E. Fernandez. 2009. Impact of glyphosate application on microbial biomass and metabolic activity in a Vertic Argiudoll from Argentina. *European Journal of Soil Biology* 45(2): 163-167.

    The widespread use of genetically modified crops tolerant to glyphosate, has strongly promoted the use of this herbicide. This work evaluates the effect of increasing doses of glyphosate on biomass and metabolic activity and metabolic quotient of soil microbiota under controlled conditions. Commercial formulation of glyphosate (48%) was sprayed over a Vertic Argiudoll placed in trays, at doses of 0.48, 0.96, 1.92 and 3.84 L a.i ha^{-1}. Dosesusually applied in the field are between 0.96 and 1.92 L a.i ha^{-1}. Treatments and control were incubated at 25 degrees C and 75% of water holding capacity. After 4 and 45 days from glyphosate application, carbon from microbial biomass (C-MB), microbial respiration rate (MR), metabolic quotient (qCO_2), and dehydrogenase activity (DA) were determined. The C-MB at 4 days of incubation did not differ ($P < 0.05$) between the control and the doses of 0.48 and 0.96 L, while it was significantly lower in the highest doses. After 45 days, C-BM in the dose of 3.84 L was significantly lower than the control. The MR showed significant differences over the time but not between doses. Significant differences were found in qCO_2 between doses in both periods of incubation. Dehydrogenase activity was significantly higher in the treatments with glyphosate than in the control at the beginning of incubation. Treatment with 0.48 L presented the highest value after 45 days, while the dose of 3.84 L had lowest DA. The variables analyzed showed an initial inhibitory effect that affected the microbial cells. However, this effect was temporary at doses equivalent or higher than those usually applied in the field. **(Argentina)**

19. Harikrishnan, R. and X.B. Yang. 2002. Effects of herbicides on root rot and damping-off caused by *Rhizoctonia solani* in glyphosate-tolerant soybean. *Plant Disease* 86(12): 1369-1373.

Diseases caused by *Rhizoctonia solani* are one of many production constraints in soybean-growing regions. Little information is available about the diseases in soybeans tolerant to different herbicides. In 1998 and 1999, studies were conducted to evaluate the plausible interaction between glyphosate-tolerant soybean and herbicides (glyphosate, imazethapyr, lactofen, and pendimethalin) on damping-off and root rot caused by *R. solani* under greenhouse and field conditions. The herbicides were applied at the product recommended field rate on glyphosate-tolerant (Pioneer 93BOI and Pioneer 9344) and glyphosate-sensitive (BSR 101) soybean grown in soils infested with *R. solani* (isolate AG-4). Root rot and plant stand was significantly affected by soybean cultivars and herbicide treatments in the greenhouse study. A significant cultivar-treatment interaction was detected in the greenhouse study. The interaction implies that the cultivars responded differently to the various herbicides and especially to the herbicide pendimethalin. Plant stands of both cultivars were reduced by *R. solani* alone or in combination with different herbicides compared with the noninoculated control, and this was presumed to be due to damping-off. In a 2-year field study, cultivar and treatment main effects differed with respect to plant stand between years. In 1998, analysis of variance revealed a significant treatment effect on root rot severity but not plant stand. In 1999, analysis of variance revealed a significant effect of treatment on root rot severity and plant stand whereas cultivar showed a significant effect on plant stand only. In 1999, plant stands of both cultivars were similarly affected by most treatments. However, in BSR 101, the *R. solani* + pendimethalin + imazethapyr treatment significantly reduced plant stand compared with the *R. solani* + pendimethalin treatment. Root rot severity was generally low in both years of the field study. Some differential disease responses were detected between glyphosate-tolerant and glyphosate-sensitive cultivars following the application of certain herbicides in greenhouse and field studies. However, glyphosate-tolerant and glyphosate-sensitive cultivars reacted similarly to most herbicide treatments with respect to root rot and damping off. **(U.S.A.)**

20. Hart, M.M., J.R. Powell, R.H. Gulden, K.E. Dunfield, K.P. Pauls, C.J. Swanton, *et al.* 2009. Separating the effect of crop from herbicide on soil microbial communities in glyphosate-resistant corn. *Pedobiologia* 52(4): 253-262.

Glyphosate-resistant (GR) cropping systems change the soil environment by introducing novel compounds and glyphosate into the soil environment. Over one growing season, we examined the effect of both the transgenic corn and the use of glyphosate on two groups of rhizosphere microbes, denitrifying bacteria and fungi. Using quantitative PCR to measure microbe abundance, and terminal restriction fragment length polymorphism (T-RFLP) to measure community structure, we found neither crop type (transgenic or conventional) nor herbicide (glyphosate or conventional) affected rhizosphere denitrifying or fungal communities. Instead, our results showed that seasonality was a significant determinant of denitrifier and fungal abundance as well as their diversity in this study, suggesting in the short term, some microbial communities are robust to changes in their environment by GR crops. **(Canada)**

21. Huber, D., M. Cheng, and B. Winsor. 2005. Association of severe *Corynespora* root rot of soybean with glyphosate-killed ragweed. *Phytopathology* 95(6): S45.

The soilborne pathogen *Corynespora cassiicola* was the predominant fungus isolated from severely stunted soybeans adjacent to glyphosate-killed giant ragweed plants (*Ambrosia trifida*) in Indiana fields. Soybeans adjacent to glyphosate-killed ragweed exhibited dark-brown to black lesions on 90-95% of their roots and hypocotyls. In contrast, soybeans that were not adjacent to dead *Ambrosia trifida*, or that were adjacent to living ragweed plants, exhibited only 5-10% root rot; and a number of different soilborne fungi in addition to *Corynespora* were isolated from these roots. Dead ragweed roots generally yielded pure cultures of *Rhizoctonia* and were not colonized by *Corynespora*. Koch's postulates were completed in the greenhouse where typical hypocotyl lesions developed in 3-5 days and lateral, "fine feeder roots" were extensively rotted by *Corynespora*. Soybean yield reduction was related to the density of glyphosate-killed ragweed plants and ranged from 1.5 kg per dead ragweed to 6 kg per dead ragweed in replicated field plots with and without killed ragweed plants. These field observations indicate that glyphosate or metabolites in dying ragweed root exudates modify the soil environment to predispose adjacent glyphosate-resistant soybean roots to severe *Corynespora* root rot even at temperatures above 20 ^{o}C. **(U.S.A.)**

22. Irvine, I.C., M.S. Witter, C.A. Brigham, and J.B.H. Martiny. 2011. Relationships between methylobacteria and glyphosate with native and invasive plant species: implications for restoration. *Restoration Ecology* (in press) Article first published online: 13 DEC 2011. DOI:10.1111/j.1526-100X.2011.00850.x

See Biodiversity and Restoration Section.

23. Islam, M.S., A.K. Saha, H.Q.M. Mosaddeque, M.R. Amin, and M.M. Islam. 2008. *In vitro* studies on the reaction of fungi *Trichoderma* to different herbicides used in tea plantation. *International Journal of Sustainable Crop Production* 3(5): 27-30.

An experiment was conducted at the Plant Pathology laboratory of Bangladesh Tea Research Institute on September, 2006 paper investigated selected genus of *Trichoderma* and herbicides aimed at defining the effect of different concentrations on the development of mycelium of the isolates studied and spore germination. Five commonly used herbicides like Glyphosate 41SL (2022.66 PPM), Paraquat 20 W/W (746.66 PPM), Bimaster 240/120AS (1680.00 PPM), Kem- Amin 58SL (2165.33 PPM) and T5= Butachlor 5G (281.25 PPM) were considered as treatments. Concentrations (PPM) were calculated on the basis of their doses of applications recommended by BTRI. Herbicides were added with melted media separately maintaining the said concentrations of each. *Trichoderma* was isolated from tea soil by soil dilution method and maintained pure culture on PDA media. After preparation of spore suspension, it was plated with herbicides added PDA media. Every 24 hours observations revealed that Bimaster 240/120AS completely inhibited the growth of *Trichoderma*. Though the fungus structure was seen in the form of spot like sporulation on the PDA treated with Paraquat 20 W/W, Kem- Amin 58SL and Butachlor 5G. But further these could not produce mycelium. Only 21-30% mycelial growth was found in cultured treated with Glyphosate 41SL. **(Bangladesh)**

24. Johal, G.S. and D.M. Huber. 2009. Glyphosate effects on diseases of plants. *European Journal of Agronomy* 31(3): 144-152.

Glyphosate, N-(phosphonomethyl)glycine, is the most extensively used herbicide in the history of agriculture. Weed management programs in glyphosate resistant (GR) field crops have provided highly effective weed control, simplified management decisions, and given cleaner harvested products. However, this relatively simple, broad-spectrum, systemic herbicide can have extensive unintended effects on nutrient efficiency and disease severity, thereby threatening its agricultural sustainability. A significant increase in disease severity associated with the wide spread application of the glyphosate herbicide can be the result of direct glyphosate-induced weakening of plant defenses and increased pathogen population and virulence. Indirect effects of glyphosate on disease predisposition result from immobilization of specific micronutrients involved in disease resistance, reduced growth and vigor of the plant from accumulation of glyphosate in meristematic root, shoot, and reproductive tissues, altered physiological efficiency, or modification of the soil microflora affecting the availability of nutrients involved in physiological disease resistance. Strategies to ameliorate the predisposing effects of glyphosate on disease include judicious selection of herbicide application rates, micronutrient amendment, glyphosate detoxification in meristematic tissues and soil. changes in cultural practices to enhance micronutrient availability for plant uptake, and biological amendment with glyphosate-resistant microbes for nitrogen fixation and nutrient availability. Given that recommended doses of glyphosate are often many times higher than needed to control weeds, we believe the most prudent method to reduce the detrimental effects of glyphosate on GR crops will be to use this herbicide in as small a dose as practically needed. Such a frugal approach will not only curtail disease predisposition of GR crops, but will also benefit the grower and the environment. **(U.S.A.)**

25. Kremer, R.J. and N.E. Means. 2009. Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. *European Journal of Agronomy* 31(3): 153-161.

Current crop production relies heavily on transgenic, glyphosate-resistant (GR) cultivars. Widespread Cultivation of transgenic crops has received considerable attention. Impacts of glyphosate on rhizosphere microorganisms and activities are reviewed based on published and new data from long-term field projects documenting effects of glyphosate applied to GR soybean and maize. Field studies conducted in Missouri, U.S.A. during 1997-2007 assessed effects of glyphosate applied to GR soybean and maize on root colonization and soil populations of *Fusarium* and selected rhizosphere bacteria. Frequency of root-colonizing *Fusarium* increased significantly after glyphosate application during growing seasons in each year at all sites. Roots of GR soybean and maize treated with glyphosate were heavily colonized by *Fusarium* compared to non-GR or GR cultivars not treated with glyphosate. Microbial groups and functions affected by glyphosate included Mn transformation and plant availability; phytopathogen-antagonistic bacterial interactions; and reduction in nodulation. Root-exuded glyphosate may serve as a

nutrient Source for fungi and stimulate propagule germination. The specific microbial indicator groups and processes were sensitive to impacts of GR crops and are part of all evolving framework in developing polyphasic microbial analyses for complete assessment of GR technology that is more reliable than single techniques or general microbial assays. **(U.S.A.)**

26. Kremer, R.J., N.E. Means, and S. Kim. 2005. Glyphosate affects soybean root exudation and rhizosphere micro-organisms. *International Journal of Environmental Analytical Chemistry* 85(15): 1165-1174.

Glyphosate is a non- selective, broad- spectrum herbicide that kills plants by inhibiting the enzyme 5- enolpyruvylshikimic acid- 3- phosphate synthase (EPSPS), which is necessary for synthesis of aromatic amino acids. A secondary mode of action involves infection of roots of glyphosate- susceptible plants by soil- borne micro- organisms due to decreased production of plant protection compounds known as phytoalexins. Varieties of several crops, including glyphosate- resistant (GR) or Roundup Ready soybean, are genetically modified to resist the herbicidal effects of glyphosate and provide farmers with an effective weed- management tool. After glyphosate is applied to GR soybean, glyphosate that is not bound to glyphosate-resistant EPSPS is translocated throughout the plant and accumulates primarily in meristematic tissues. We previously reported that fungal colonization of GR soybean roots increased significantly after application of glyphosate but not after conventional postemergence herbicides. Because glyphosate may be released into soil from GR roots, we characterized the response of rhizosphere fungi and bacteria to root exudates from GR and non- GR (Williams 82; W82) cultivars treated with and without glyphosate at field application rates. Using an immunoassay technique, glyphosate at concentrations > 1000 ng plant^{-1} were detected in exudates of hydroponically grown GR soybean at 16 days post- glyphosate application. Glyphosate also increased carbohydrate and amino acid contents in root exudates in both soybean cultivars. However, GR soybean released higher carbohydrate and amino acid contents in root exudates than W82 soybean without glyphosate treatment. *In vitro* bioassays showed that glyphosate in the exudates stimulated growth of selected rhizosphere fungi, possibly by providing a selective C and N source combined with the high levels of soluble carbohydrates and amino acids associated with glyphosate treatment of the soybean plants. Increased fungal populations that develop under glyphosate treatment of GR soybean may adversely affect plant growth and biological processes in the soil and rhizosphere. **(U.S.A.)**

27. Krzysko-Lupicka, T. and T. Sudol. 2008. Interactions between glyphosate and autochthonous soil fungi surviving in aqueous solution of glyphosate. *Chemosphere* 71(7): 1386-1391.

The survival of autochthonous fungi in soil treated with 1mM aqueous solution of glyphosate was investigated. Significant differences in the total number of fungi in the studied objects were observed, and additionally significant qualitative changes were encountered. The dominating group of fungi belonged to genus Fusarium: *Fusarium solani* H30, *Fusarium solani* H50 and *Fusarium oxysporum* H80. Interactions between the isolated strains of fungi and varying concentrations of

glyphosate were determined. The studied strains possessed high tolerance against the applied doses of glyphosate (0.5-2.0 mM). In the presence of glyphosate (as a sole source of phosphorus) applied in concentrations of 1.0-1.5 mM the increase in dry mass of the tested fungi was highly significant. In the presence of glyphosate the phenotypic changes of studied strains were observed as was shown as the presence of colorants being indicators of such changes. Thus, their color and intensity depended on the age, pH and species present in the culture. The degradation of glyphosate by studied fungi was determined by means of TLC. Two types of compounds were formed. One of them (R_f=0.21-0.35) contained free amino group but was not either glycine nor AMPA. Survival of Fusarium in soil environment is potentially dangerous. **(Poland)**

28. Laatikainen, T. and H. Heinonen-Tanski. 2002. Mycorrhizal growth in pure cultures in the presence of pesticides. *Microbiological Research* 157(2): 127-137.

The effects of pesticides on 64 ectomycorrhizal fungi of boreal forest trees were studied *in vitro*. The pesticides (fungicides: benomyl, chlorothalonil, copper oxychloride, maneb and propiconazole; herbicides: chlorthiamid, glyphosate, hexazinone, linuron and terbuthylazine; insecticide: cypermethrin) were selected as those commonly used in Nordic forest nurseries and afforestation sites. In general, the fungicides proved to be more toxic to ectomycorrhizal fungi than the herbicides and cypermethrin. The fungicides, chlorothalonil and propiconazole, had the clearest inhibitory effect on growth of mycorrhizal fungi. Conversely, maneb, glyphosate and terbuthylazine stimulated the growth of some mycorrhizal fungi. *Leccinum versipelle* and *L. scabrum*, *Paxillus involutus* and *Cenococcum geophilum* were the most sensitive ectomycorrhizal fungi to the various pesticides. **(Finland)**

29. Lane, M., N. Lorenz, J. Saxena, C. Ramsier, and R.P. Dick. 2012. Microbial activity, community structure and potassium dynamics in rhizosphere soil of soybean plants treated with glyphosate. *Pedobiologia - International Journal of Soil Biology* doi:101016/pedobi.2011.12.005.

With the advent of glyphosate [N-(phosphonomethyl)glycine] tolerant crops, soils have now been receiving repeated applications of the herbicide for over 10 years in the Midwestern USA. There is evidence that long-term use of glyphosate can cause micronutrient deficiency but little is known about plant potassium (K) uptake interactions with glyphosate. The repeated use of glyphosate may create a selection pressure in soil microbial communities that could affect soil K dynamics and ultimately K availability for crops. Therefore, the objectives of this study were to characterize the effect of foliar glyphosate applied to GR (glyphosate resistant) soybeans on: (1) rhizosphere microbial community profiles using ester linked fatty acid methyl ester (EL-FAME) biomarkers, (2) exchangeable, non-exchangeable, and microbial K in the rhizosphere soil, and (3) concentrations of soybean leaf K. A greenhouse study was conducted in a 2 × 2 × 3 factorial design with two soil treatments (with or without long-term field applications of glyphosate), two plant treatments (presence and absence of soybean plants), and three rates of glyphosate treatments (0×, 1× at 0.87, and 2× at 1.74 kg ae ha^{-1}, the recommended field rate). After each glyphosate application, rhizosphere soils were sampled and analyzed for

microbial community structure using ester linked fatty acid methyl ester biomarkers (EL-FAME), and exchangeable, plant tissue and microbial biomass K. Glyphosate application caused a significant decrease in the total microbial biomass in soybean rhizosphere soil that had no previous exposure to glyphosate, at 7 days after glyphosate application. However, no significant changes were observed in the overall microbial community structure. In conclusion, the glyphosate application lowered the total microbial biomass in the GR soybean rhizosphere soil that had no previous exposure to glyphosate, at 7 days after glyphosate application; caused no changes in the microbial community structure; and did not reduce the plant available K (soil exchangeable or plant tissue K). **(U.S.A.)**

30. Larson, R.L., A.L. Hill, A. Fenwick, A.R. Kniss, L.E. Hanson, and S.D. Miller. 2006. Influence of glyphosate on Rhizoctonia and Fusarium root rot in sugar beet. *Pest Management Science* 62(12): 1182-1192.

    This study tests the effect of glyphosate application on disease severity in glyphosate-resistant sugar beet, and examines whether the increase in disease is fungal or plant mediated. In greenhouse studies of glyphosate-resistant sugar beet, increased disease severity was observed following glyphosate application and inoculation with certain isolates of *Rhizoctonia solani* Kuhn and *Fusarium oxysporum* Schlecht. f. sp. *betae* Snyd. & Hans. Significant increases in disease severity were noted for *R. solani* AG-2-2 isolate R-9 and moderately virulent *F. oxysporum* isolate FOB13 on both cultivars tested, regardless of the duration between glyphosate application and pathogen challenge, but not with highly virulent *F. oxysporum* isolate F-19 or an isolate of *R. solani* AG-4. The increase in disease does not appear to be fungal mediated, since *in vitro* studies showed no positive impact of glyphosate on fungal growth or overwintering structure production or germination for either pathogen. Studies of glyphosate impact on sugar beet physiology showed that shikimic acid accumulation is tissue specific and the rate of accumulation is greatly reduced in resistant cultivars when compared with a susceptible cultivar. The results indicate that precautions need to be taken when certain soil-borne diseases are present if weed management for sugar beet is to include post-emergence glyphosate treatments. **(U.S.A.)**

31. Lee, C.D., D. Penner, and R. Hammerschmidt. 2000. Influence of formulated glyphosate and activator adjuvants on *Sclerotinia sclerotiorum* in glyphosate-resistant and -susceptible *Glycine max*. *Weed Science* 48(6): 710-715.

    Two *Glycine max* near-isolines, GL2415 (glyphosate sensitive) and GL2600RR (glyphosate resistant), were compared for susceptibility to *Sclerotinia sclerotiorum*, the fungus that causes white mold disease (also known as Sclerotinia stem rot). A formulated isopropylamine salt of glyphosate (RU) at 840, 1,680, and 2,520 g ae ha^{-1}; the RU formulation blank containing only adjuvants (RUFB) at 0.4% (v/v); organosilicone at 0.3% (v/v); a crop oil concentrate at 1.0% (v/v); and a nonionic surfactant at 0.5% (v/v) were evaluated for their influence on V5 *G. max* susceptibility to *S. sclerotiorum* in the field. The same treatments plus a formulated isopropylamine salt of glyphosate lacking adjuvants at 2,520 g ae ha^{-1} and a formulated trimethylsulfonium salt of glyphosate at 2,520 g ae ha^{-1} were applied to

GL2600RR *G. max* in the greenhouse. Results from these studies indicated no differences in fungal growth, disease development, or *G. max* yield for any of these treatments. To determine if any interaction occurred between the chemicals, plants, and *S. sclerotiorum*, leaflets from both cultivars were dipped into and immediately removed from solutions of the RU at 4, 7, and 10% (v/v) and the RUFB and adjuvants at the rates described. The resulting lesion developments were similar across both cultivars but not across chemical treatments. Lesion developments were smaller on leaflets treated with RUFB and RU, but larger on leaflets treated with the other three adjuvants. *Sclerotinia sclerotiorum* mycelia growing on potato dextrose agar were inhibited by high concentrations of RU and of RUFB at 100 mM ae glyphosate and by the three adjuvants at 1% (v/v). Formulated glyphosate lacking adjuvants did not inhibit mycelial growth on potato dextrose agar. The glyphosate resistance trait did not appear to be associated with the susceptibility of *G. max* to *S. sclerotiorum*. Neither the glyphosate resistance trait nor the applications of glyphosate and adjuvants influenced *G. max* susceptibility to *S. sclerotiorum*. **(U.S.A.)**

32. Liphadzi, K.B., K. Al-Khatib, C.N. Bensch, P.W. Stahlman, J. A. Dille, T. Todd, C.W. Rice, M.J. Horak, and G. Head. 2005. Soil microbial and nematode communities as affected by glyphosate and tillage practices in a glyphosate-resistant cropping system. *Weed Science* 53(4): 536-545.

Field experiments were conducted at Ashland Bottoms in northeastern Kansas and at Hays in western Kansas in 2001, 2002, and 2003 to determine the response of soil microbial and nematode communities to different herbicides and tillage practices under a glyphosate-resistant cropping system. Conventional herbicide treatments were a tank mixture of cloransulam plus S-metolachlor plus sulfentrazone for soybean and a commercially available mixture of acetochlor and atrazine for corn. Glyphosate was applied at 1.12 kg ai ha^{-1} when weeds were 10 or 20 cm tall in both corn and soybean. Soil samples were collected monthly at Ashland Bottoms during the growing period for soil microbial biomass (SMB) carbon determination. In addition, substrate-induced respiration (SIR) and BIOLOG substrate utilization were determined at the end of the growing season each year at Ashland Bottoms, and nematode populations were determined at the beginning and the end of the growing season at both sites. Direct effects of glyphosate rates on soil microbial and nematode communities were also studied in a controlled environmnet. Values for SMB carbon, SIR, and BIOLOG substrate utilization were not altered by glyphosate. Nematode community response to the glyphosate treatment was similar under both conventional tillage and no-till environments. Total nematode densities were similar with the glyphosate and conventional herbicide treatments. SMB carbon and BIOLOG substrate utilization did not differ between tillage treatments. Nematode densities were greater under conventional tillage than in the no-till system. This study showed that soil health when glyphosate was applied in a glyphosate-resistant cropping system was similar to that of cropping systems that used conventional herbicides. **(U.S.A.)**

33. Lipok, J., D. Wieczorek, M. Jewgiński, and P. Kafarski. 2009. Prospects of *in vivo* 31P NMR method in glyphosate degradation studies in whole cell system. *Enzyme and Microbial Technology* 44(1): 11-16.

    The degradation of the phosphonate herbicide glyphosate (*N*-phosphonomethylglycine) by four taxonomically distinct microorganisms was studied *in vivo* in whole cell system using phosphorus nuclear magnetic spectroscopy (^{31}P NMR). The time-course of glyphosate metabolization in dense cell cultures was followed by means of ^{31}P NMR up to 21 days after the addition. The results obtained by this non-invasive way confirmed that the cells of *Spirulina platensis* and *Streptomyces lusitanus* biodegrade herbicide. Moreover, phosphorus starvation influenced the rate of glyphosate degradation by *S. platensis*. On the other hand, the results of similar measurements in the cultures of green algae *Chlorella vulgaris* showed that this aquatic plant, however growing in the medium containing 1 mM of *N*-phosphonomethylglycine, did not seem to posses the ability of its biodegradation. Additionally, the use of this method allowed us to find the new fungal strain *Fusarium dimerum*, which is able to biodegrade and utilize the glyphosate as the sole source of phosphorus. The results of our studies on usefulness of *in vivo* ^{31}P NMR for tracing glyphosate degradation in whole cell systems revealed that this non-invasive, one-step method, might be considered as a valuable tool in environmental biotechnology of organophosphonate xenobiotics. **(Poland)**

34. Locke, M.A., R.M. Zablotowicz, and K.N. Reddy. 2008. Integrating soil conservation practices and glyphosate-resistant crops: impacts on soil. *Pest Management Science* 64(4): 457-469.

    Conservation practices often associated with glyphosate-resistant crops, e.g. limited tillage and crop cover, improve soil conditions, but only limited research has evaluated their effects on soil in combination with glyphosate-resistant crops. It is assumed that conservation practices have similar benefits to soil whether or not glyphosate-resistant crops are used. This paper reviews the impact on soil of conservation practices and glyphosate-resistant crops, and presents data from a Mississippi field trial comparing glyphosate-resistant and non-glyphosate-resistant maize (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) under limited tillage management. Results from the reduced-tillage study indicate differences in soil biological and chemical properties owing to glyphosate-resistant crops. Under continuous glyphosate-resistant maize, soils maintained greater soil organic carbon and nitrogen as compared with continuous non-glyphosate-resistant maize, but no differences were measured in continuous cotton or in cotton rotated with maize. Soil microbial community structure based on total fatty acid methyl ester analysis indicated a significant effect of glyphosate-resistant crop following 5 years of continuous glyphosate-resistant crop as compared with the non-glyphosate-resistant crop system. Results from this study, as well as the literature review, indicate differences attributable to the interaction of conservation practices and glyphosate-resistant crop, but many are transient and benign for the soil ecosystem. Glyphosate use may result in minor effects on soil biological/chemical properties. However, enhanced organic carbon and plant residues in surface soils under conservation practices may buffer potential effects of glyphosate. Long-term field research

established under various cropping systems and ecological regions is needed for critical assessment of glyphosate-resistant crop and conservation practice interactions. **(U.S.A.)**

35. Malty, J.D., J.O. Siqueira, and F.M. de Souza Moreira. 2006. Effects of glyphosate on soybean symbiotic microorganisms, in culture media and in greenhouse. *Pesquisa Agropecuaria Brasileira* 41(2): 285-291.

    The effects of the Roundup herbicide on three strains of *Bradyrhizobium elkanii* (BR 29, INPA 80A and INPA 553A), one of *B japonicum* (BR 86), and on three species of arbuscular mycorrhizal fungi (AMF) (*Gigaspora margarita*, *Glomus etunicatum* and *Scutellospora heterogama*), were evaluated in culture media containing increasing concentrations of the herbicide (0-454 µM); evaluations were also made on the effects on nodulation and mycorrhiza colonization of soybean grown in a soil treated with Roundup doses equivalent to 1.25 to 10 L ha^{-1} before sowing. The herbicide inhibited growth of *Bradyrhizobium* spp. and AMF in culture medium. These effects were directly related to increasing concentrations, and varied depending on the strain and species evaluated. However, in vitro inhibition occurred only when concentrations were greater than that recommended for use in the field. Strains BR 29, INPA 553A and INPA 80A showed to be more resistant to glyphosate, when compared to BR 86. Herbicide inhibition on germination and growth of AMF spore germ tubes decreased from *G. etunicatum* to *S. heterogama* and *G. margarita*. Soil application of Roundup before sowing up to a dose equivalent to 10 L ha^{-1} had no effect on noduladon and mycorrhiza colonization of soybean. **(Brazil)**

36. Massenssini, A.M., M.D. Costa, M.R. Reis, and A.A. Silva. 2008. Activity of phosphate solubilizing bacterial isolates in the presence of commercial glyphosate formulations. *Planta Daninha* 26(4): 815-823.

    The objective of this work was to evaluate thee ct of applying different commercial formulations of glyphosate (Roundup Transorb®, Zapp QI®, Roundup NA® e Scout®) on the ability of two bacterial isolates (To 11 and To 66) to solubilize different inorganic phosphates. The ability to solubilize bacterial isolate phosphate was evaluated in relation to three inorganic phosphate sources (calcium, aluminum, and iron phosphates) in the presence of different commercial,formulations of glyphosate. at the concentration of 60 mg L^{-1} acid equivalent, and control treatment without the addition of the herbicides. The effects of the commercial formulations of glyphosate on each bacterial isolate were distinct. Roundup Transorb and Zapp QI led to a significant decrease in the phosphate-solubilizing activity of To 66, while the reverse was observed for Scout. To 11 did not show significant differences in phosphate solubilization in the presence of the commercial formulations tested. Roundup NA and Scout did not change the ability to solubilize phosphate of bacterial isolates, while Roundup Transorb and Zapp QI led to a decrease in the solubilizing activity. **(Brazil)**

37. McMullin, R.T., F.W. Bell, and S.G. Newmaster. 2012. The effects of triclopyr and glyphosate on lichens. *Forest Ecology and Management* 264(15): 90-97.

Two commonly used silvicultural herbicides (triclopyr and glyphosate) were examined for their effects on lichens in northeastern Ontario. One hundred 1 m^2 plots were randomly established throughout a single, open canopy forest stand with tree cover dominated by *Picea mariana* and *Pinus banksiana* and ground cover dominated by lichens. Herbicides were applied to the plots along a gradient of concentrations ranging from 0.71 to 6.72 kg acid equivalent ha^{-1}. The pre- and 1 year post-application abundance (percent cover) of 25 lichen species in 1 m^2 plots were compared. Triclopyr and glyphosate reduced the abundance of 40% and 56% of the lichen species studied, respectively. Tolerance to these herbicides varied among lichen species and a cluster analysis was used to define four tolerance classes. Analysis of variance was used to compare lichen abundance among the tolerance classes and multivariate analysis (canonical correspondence) was used to explore variation in lichen abundance as constrained by species traits post-herbicide application. Except for *Trapeliopsis granulosa*, the most herbicide sensitive species were richly branched. The species with the highest mortality were *Bryoria furcellata*, *Cladonia uncialis*, and *T. granulosa*. In general, lichen response to herbicide treatments cannot be assessed using ecological units; species must be considered individually or, as proposed here, by tolerance class. Study results provide forest managers with improved understanding of the effects of herbicide applications on non-target organisms. **(Canada)**

38. Means, N.E., R.J. Kremer, and C. Ramsier. 2007. Effects of glyphosate and foliar amendments on activity of microorganisms in the soybean rhizosphere. *Journal of Environmental Science and Health*. Part B 42(2): 125-132.

A field study was conducted to determine the effects of glyphosate on microbial activity in the rhizosphere of glyphosate-resistant (GR) soybean and to evaluate interactions with foliar amendments. Glyphosate at 0.84 kg ae ha^{-1} was applied GR soybean at the V4-V5 development stages. Check treatments included a conventional herbicide tank mix (2003 study only) and no herbicides (hand-weeded). Ten days after herbicide application, a commercially available biostimulant and a urea solution (21.0% N) were applied to soybean foliage at 33.5 mL ha^{-1} and 9.2 kg ha^{-1}, respectively. Soil and plant samples were taken 0, 5, 10, 15, 20 and 25 days after herbicide application then assayed for enzyme and respiration activities. Soil respiration and enzyme activity increased with glyphosate and foliar amendment applications during the 2002 growing season; however, similar increases were not observed in 2003. Contrasting cumulative rainfall between 2002 and 2003 likely accounted for differences in soil microbial activities. Increases in soil microbial activity in 2002 suggest that adequate soil water and glyphosate application acted together to increase microbial activity. Our study suggests that general soil microbial properties including those involving C and N transformations are not sensitive enough to detect effects of glyphosate on rhizosphere microbial activity. Measurements of soil-plant-microbe relationships including specific microbial groups (i.e., root-associated *Fusarium* spp.) are likely better indicators of impacts of glyphosate on soil microbial ecology. **(U.S.A.)**

39. Meriles, J.M., S.V. Gil, R.J. Haro, G.J. March, and C.A. Guzmán. 2006. Glyphosate and previous crop residue effect on deleterious and beneficial soil-borne fungi from a peanut-corn-soybean rotations. *Journal of Phytopathology* 154(5): 309-316.

    Bean seedlings (*Phaseolus vulgaris* L.) were transplanted to soil with corn previous crop residue, peanut previous crop residue and no agricultural soil, and treated with a range of glyphosate concentrations. *Trichoderma*, *Gliocladium*, *Fusarium* and *Pythium* soil-borne fungi populations were monitored during 24 days after glyphosate treatment to study the glyphosate and previous crop residue effects on these populations. In addition, those genera of soil-borne fungi were tested to study in vitro toxicity to glyphosate. Independently of glyphosate concentration, the highest population of *Trichoderma* spp. and *Gliocladium* spp. were registered on soil with previous corn residue. *Fusarium* and *Pythium* populations increased proportionally to the increment of glyphosate concentration. No effect of glyphosate was founded on *Trichoderma* and *Gliocladium* populations. The in vitro study results indicated an inhibitory effect of glyphosate on mycelial grown of the most studied soil-borne fungi. **(Argentina)**

40. Mijangos, I., J.M. Becerril, I. Albizu, L. Epelde, and C. Garbisu. 2009. Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and -independent methodologies. *Soil Biology and Biochemistry* 41(3): 505-513.

    We studied, under two different plant compositions, the short-term effects of glyphosate on rhizosphere soil microbial communities through the utilization of cultivation-dependent and -independent techniques. A short-term pot study was carried out using factorial treatments that included two different compositions of forage plant species (triticale *versus* a mixture of triticale and pea) and two concentrations of glyphosate (50 and 500 mg active ingredient kg^{-1} soil, as a commercial formulation, Roundup Plus) arranged in a completely randomized design experiment with four replicates. Control plants (no glyphosate added) were clipped in an attempt to compare two methods of weed control (manual = clipping; chemical = herbicide treatment). Rhizosphere soil was sampled 15 and 30 days after glyphosate treatment and the following soil components were determined: potentially mineralizable nitrogen, ammonium content, community-level physiological profiles using Biolog Ecoplates (TM), DNA microbial biomass and genotype diversity by means of PCR-DGGE. Fifteen days after herbicide treatment, a glyphosate-induced stimulation of the activity and functional diversity of the cultivable portion of the heterotrophic soil microbial community was observed, most likely due to glyphosate acting as an available source of C, N and P. On the other hand, 30 days after herbicide treatment, both the activity and diversity of the rhizosphere soil microbial communities showed an inconsistent response to glyphosate addition. Apart from its intended effect on plants, glyphosate had non-target effects on the rhizosphere soil microbial community which were, interestingly, more enhanced in triticale than in "triticale + pea" pots. Biolog (TM) was more sensitive than PCR-DGGE to detect changes in soil microbial communities induced by glyphosate and plant composition. **(Spain)**

41. Mitchell, J.K., C.N. Yerkes, S.R. Racine, and E.H. Lewis. 2008. The interaction of two potential fungal bioherbicides and a sub-lethal rate of glyphosate for the control of shattercane. *Biological Control* 46(3): 391-399.

    Greenhouse and laboratory experiments were conducted with the potential bioherbicides *Colletotrichum graminicola* (Cg) and *Gloeocercospora sorghi* (Gs) for control of shattercane weed. Single-spray tank mixture applications containing different ratios of the two fungi resulted in additive percent weed biomass losses. Intraspecific (Cg + Cg or Gs + Gs) and interspecific (Cg + Gs or Gs + Cg) sequential applications 1- or 7-days apart indicated antagonistic interactions in percent biomass loss. Application of either fungus with, or 1-3 days prior to, a sub-lethal concentration of glyphosate resulted in an antagonistic percent biomass loss; while application of glyphosate prior to either potential bioherbicide resulted in a synergistic weed disease response. Conidia germination studies conducted both in vitro on agar plates and with leaf impression peels suggest that antagonistic interactions observed in weed disease severity are probably due to the host-pathogen response following infection. **(U.S.A.)**

42. Morjan, W.E., L.P. Pedigo, and L.C. Lewis. 2002. Fungicidal effects of glyphosate and glyphosate formulations on four species of entomopathogenic fungi. *Environmental Entomology* 31(6): 1206-1212.

    Fungicidal effects of glyphosate and glyphosate formulations on the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metchnikoff) Sorokin, *Nomuraea rileyi* (Farlow) Samson, and *Neozygites floridana* Weiser & Muma were evaluated under laboratory conditions. Media previously inoculated with entomopathogenic fungi were exposed to distilled water, glyphosate (active ingredient), seven glyphosate formulations, and five blank formulations (carrier only). The fungicidal activity was determined by measuring inhibition in mycelial growth in solid media (*B. bassiana*, *M. anisopliae*, and *N. rileyi*), and spore concentration in liquid medium (*N. floridana*). Glyphosate did not have fungicidal activity against any of the fungi tested. Fungicidal properties of glyphosate formulations varied among fungal species. *Neozygites floridana* and *M. anisopliae* were susceptible to all glyphosate formulations. RoundUp Ready-To-Use was consistently the glyphosate formulation with one of the strongest fungicidal properties. Fungicidal activity of some formulations had a synergistic effect with glyphosate. RoundUp Original was the only formulation that did not show any interaction on fungicidal activity between glyphosate and the formulation. The results showed that the four fungi tested are susceptible to various glyphosate formulations when exposed to field concentrations. **(U.S.A.)**

43. Njiti, V.N., O. Myers, D. Schroeder, and D.A. Lightfoot. 2003. Roundup ready soybean: Glyphosate effects on *Fusarium solani* root colonization and sudden death syndrome. *Agronomy Journal* 95(5): 1140-1145.

    During 1997, the first year of widespread use of glyphosate (*N*-[phosphonomethyl]glycine) on Roundup Ready (RR) soybean [*Glycine max* (L.) Merr.] a severe sudden death syndrome (SDS) epidemic occurred and several RR cultivars were affected. Effects of glyphosate on colonization of soybean root by

Fusarium solani (Mart.) Sacc f. sp. *glycines* (Fsg) and SDS were evaluated. Five RR cultivar pairs that contrasted for SDS resistance from maturity groups (MG) II to VI were evaluated with and without glyphosate application. The MG Ii and III cultivars were evaluated near Bloomington, Pontiac, and Mahomet in central Illinois and the MG IV, V, and VI cultivars were evaluated near Harrisburg, Ullin, and Valmeyer in southern Illinois. The Fsg root infection severity (IS), colony forming units per gram of root (CFU), SDS leaf scorch disease index (DX), and grain yield were determined. Across environments within each MG, there were no significant effects of glyphosate on IS, CFU, and DX. Significant differences were expected between cultivars but only observed in some MG. There was no significant effect of glyphosate on yield. Significant Glyphosate X Cultivar interactions occurred for yield in MG VI, in favor of the glyphosate sprayed subplots. In this study root colonization by Fsg and SDS leaf symptoms did not significantly increase following the application of glyphosate. Data from this study indicate that the development of SDS on RR soybean is influenced by genotype. Farmers planting RR soybean in Fsg infested fields are encouraged to select cultivars with resistance to SDS. **(U.S.A.)**

44. Pankey, J.H., J.L. Griffin, P.D. Colyer, R.W. Schneider, and D.K. Miller. 2005. Preemergence herbicide and glyphosate effects on seedling diseases in glyphosate-resistant cotton. *Weed Technology* 19(2): 312-318.

    Field experiments were conducted to evaluate the influence of preemergence (PRE) herbicides metolachlor at 1,700 g ai/ha, pyrithiobac at 70 g ai/ha, or pendimethalin at 840 g ai/ha applied alone or with fluometuron at 1,300 g ai/ha and glyphosate postemergence (POST) at 840 g ai/ha on seedling diseases in glyphosate-resistant cotton. Hypocotyl disease severity both years averaged across PRE herbicide treatments was greater after glyphosate application to four-leaf cotton than cotyledon cotton. The PRE herbicide treatments, particularly those including fluometuron, increased root and hypocotyl disease ratings compared with a nontreated control, and a sequential application of glyphosate did not further increase disease severity. Greenhouse experiments using soil infested with *Rhizoctonia solani* confirmed findings from the field study showing that PRE herbicides can predispose cotton to greater seedling disease injury with no increased seedling disease severity associated with application of glyphosate. In the field study, glyphosate applied at cotyledon or four-leaf growth stages decreased disease severity on cotton hypocotyls both years. This inhibitory effect of glyphosate was less evident in the greenhouse study and may have been related to species of fungi present, infestation level, and differences in environmental conditions when compared with the field. **(U.S.A.)**

45. Pasaribu, A., R.B. Mohamad, Y. Awang, R. Othman, and A. Puteh. 2011. Growth and development of symbiotic *Arbuscular mycorrhizal* fungi, *Glomus mossea* (Nicol. and Gerd.), in alachlor and glyphosate treated soils. *African Journal of Biotechnology* 10(55): 11520-11526.

    Herbicides are applied to control weeds in agricultural practices and could also be detrimental to the development of some microorganisms living in the soil ecosystem. This study was conducted to determine the growth and development of

mycorrhizal fungi, *Glomus mossea* (Nicol. and Gerd.), in soils treated with herbicides. Herbicide treatments were alachlor at 1.8, 3.6, 5.4 and 36 µg active ingredient (a.i.) g^{-1} or glyphosate at 1.1, 2.2, 3.3 and 21.6 µg a.i. g^{-1} dry soil, representing 0.5, 1, 1.5 and 10 x their recommended field application rates. Spore germination percentage and hyphal growth length were determined from spores germinated on cellulose membrane filters, sandwiched between the herbicide treated soil layers in Petri-dish after 30 days incubation in darkness at 22 ± 1^{o}C. External and internal hyphae and their active portions were determined from soil samples of the host growing medium and colonized host plant root systems, respectively. The alkaline phosphatase (ALP) and succinate dehydrogenase (SDH) staining techniques were used to determine the respective active portion of the hyphae. Spores from the prepared inoculum source have high germination percentage (93%) in non-herbicide treated soil. Germination of spores and its hyphal growth were not significantly affected in soil treated with alachlor and glyphosate at the recommended field application rates or less. Alachlor reduced the spore germination and hyphal growth significantly at treatments higher than recommended field application rates, but non-significant effect caused by the glyphosate. The development of external hyphae was insignificantly affected in the herbicides treated soils compared with that of the untreated soil. Colonization and development of internal hyphae on host plant roots were not affected by the herbicide treatments to the soil growing medium at recommended field application rates or less. There was a tendency for the higher treatment rates of alachlor (1.5 and 10x) to affect the development of internal mycorrhizal tissues. Application of alachlor or glyphosate herbicide at their recommended field application rates were not harmful to mycorrhizal development and symbiotic colonization of plant roots. Alachlor, at higher treatments than the recommended field application rates affected the presymbiotic stages of the spore germination and the internal mycorrhizal tissues development substantially. **(Indonesia)**

46. Pizzul, L., M del Pilar Castillo, and J. Stenström. 2009. Degradation of glyphosate and other pesticides by ligninolytic enzymes. *Biodegradation* 20(6): 751-759.

The ability of pure manganese peroxidase (MnP), laccase, lignin peroxidase (LiP) and horseradish peroxidase (HRP) to degrade the widely used herbicide glyphosate and other pesticides was studied in separate in vitro assays with addition of different mediators. Complete degradation of glyphosate was obtained with MnP, $MnSO_4$ and Tween 80, with or without H_2O_2. In the presence of $MnSO_4$, with or without H_2O_2, MnP also transformed the herbicide, but to a lower rate. Laccase degraded glyphosate in the presence of (a) 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), (b) $MnSO_4$ and Tween 80 and (c) ABTS, $MnSO_4$ and Tween 80. The metabolite AMPA was detected in all cases where degradation of glyphosate occurred and was not degraded. The LiP was tested alone or with $MnSO_4$, Tween 80, veratryl alcohol or H_2O_2 and in the HRP assay the enzyme was added alone or with H_2O_2 in the reaction mixture. However, these enzymes did not degrade glyphosate. Further experiments using MnP together with $MnSO_4$ and Tween 80 showed that the enzyme was also able to degrade glyphosate in its commercial formulation Roundup® Bio. The same enzyme mixture was tested for

degradation of 22 other pesticides and degradation products present in a mixture and all the compounds were transformed, with degradation percentages ranging between 20 and 100%. Our results highlight the potential of ligninolytic enzymes to degrade pesticides. Moreover, they suggest that the formation of AMPA, the main metabolite of glyphosate degradation found in soils, can be a result of the activity of lignin-degrading enzymes. **(Sweden)**

47. Powell, J.R. and C.J. Swanton. 2008. A critique of studies evaluating glyphosate effects on diseases associated with *Fusarium* spp. *Weed Research* 48(4): 307-318.

With the large-scale adoption of glyphosate-resistant crops in North America, there are concerns that non-target microbial populations might be affected by increased frequency of glyphosate use. Stimulation of fungal species associated with crop diseases, including *Fusarium* spp., has been observed in laboratory and glasshouse experiments. Although field surveys in Saskatchewan detected positive associations between the incidence of Fusarium head blight and application of glyphosate formulations, few field experiments have been successful at demonstrating a stimulatory effect of glyphosate on crop diseases, including diseases associated with *Fusarium* spp. Taken at face value, there is little evidence from experimental field trials to support a causative link between glyphosate and crop diseases associated with *Fusarium* spp. However, we are concerned that the experimental field trials investigating links between glyphosate and *Fusarium* spp. are not representative of interactions that occur under actual farming conditions. In addition, inadequate consideration may have been given to microbial ecology during the design and maintenance of these experimental field trials. At this time, there is insufficient evidence to prove or disprove a link between glyphosate and crop diseases associated with *Fusarium* spp. and this area should receive high research priority, given the rapid and widespread increase in glyphosate use. **(Canada)**

48. Powell, J.R., R.G. Campbell, K.E. Dunfield, R.H. Gulden, M.M. Hart, D. Levy-Booth, *et al.* 2009. Effect of glyphosate on the tripartite symbiosis formed by Glomus intraradices, Bradyrhizobium japonicum, and genetically modified soybean. *Applied Soil Ecology* 41(1): 128-136.

Most soybeans grown in North America are genetically modified (GM) to tolerate applications of the broad-spectrum herbicide glyphosate; as a result, glyphosate is now extensively used in soybean cropping systems. Soybean roots form both arbuscular mycorrhizal (AM) and rhizobial symbioses. In addition to individually improving host plant fitness, these symbioses also interact to influence the functioning of each symbiosis, thereby establishing a tripartite symbiosis. The objectives of this study were to (1) estimate the effects of glyphosate on the establishment and functioning of AM and rhizobial symbioses with GM soybean, and (2) to estimate the interdependence of the symbioses in determining the response of each symbiosis to glyphosate. These objectives were addressed in two experiments; the first investigated the importance of the timing of glyphosate application in determining the responses of the symbionts and the second varied the rate of glyphosate application. Glyphosate applied at recommended field rates had no effect on *Glomus intraradices* or *Bradyrhizobium japonicum* colonization of soybean roots,

or on soybean foliar tissue [P]. N-2-fixation was greater for glyphosate-treated soybean plants than for untreated-plants in both experiments, but only when glyphosate was applied at the first trifoliate soybean growth stage. These data deviate from previous studies estimating the effect of glyphosate on the rhizobial symbiosis, some of which observed negative effects on rhizobial colonization and/or N_2-fixation. We did observe evidence of the response of one symbiont (stimulation of N_2-fixation following glyphosate) being dependent on co-inoculation with the other; however, this interactive response appeared to be contextually dependent as it was not consistent between experiments. Future research needs to consider the role of environmental factors and other biota when evaluating rhizobial responses to herbicide applications. **(Canada)**

49. Prabhu, T., J. Srikanth, and G. Santhalakshmi. 2007. Compatibility of selected pesticides with three entomopathogenic fungi of sugarcane pests. *Journal of Biological Control* 21(1): 73-82.

The compatibility of some insecticides, fungicides and herbicides commonly used for sugarcane with *Beauveria bassiana, B. brongniartii* and *Metarhizium anisopliae* was evaluated in *in vitro* assays. Radial growth, biomass, and spore production did not show a consistent trend for the evaluated insecticides. However, based on percent reduction in spore production, chlorpyrifos (0.04%) was the most toxic to the 3 fungi (100%); lindane (0.04%) was the most toxic to *B. brongniartii* (100%), but the least toxic to *B. bassiana* (26.3%) and *M. anisopliae* (17.1%); monocrotophos (0.036%) was moderately toxic to *B. bassiana* (43.0%) and *M. anisopliae* (35.2%), and the least toxic to *B. brongniartii* (13.4%); malathion (0.10%) was the most toxic to *M. anisopliae* (88.2%) and *B. brongniartii* (69.1%), and moderately toxic to *B. bassiana* (43.0%); and endosulfan (0.035%) was moderately toxic to all 3 species (49.5-58.1%). Carbendazim (0.05%) was completely toxic to all 3 fungi (100%); mancozeb 0.08% was also equally toxic to all 3 fungi (69.5-100.0%). Glyphosate (0.205%) was the most toxic (88.1%) to *B. bassiana*, and moderately toxic to *B. brongniartii* (39.3%) and *M. anisopliae* (58.2%). Atrazine (0.35%) was moderately toxic (40.5-55.7%) to all 3 fungi. 2.4-D (0.20%) was moderately toxic to *B. bassiana* (45.9%) and *B. brongniartii* (63.3%), and least toxic (17.7%) to *M. anisopliae*. The implications of the results in sugarcane pest management involving entomopathogenic fungi are discussed. **(India)**

50. Qiu, Y., R. Zheng, C. Li, and J. Sun. 2006. Effects of glyphosate-isopropylammonium 41AS herbicide on the community structure of soil mesofauna in agroecosystems. *Chinese Journal of Soil Science* DOI: cnki:ISSN:0564-3945.0.2006-05-032

We collected 967 individual soil Mesofauna,which subordinated to 3 Phyla,6 Classes,and 10 orders,in which Collembola and Acarina were the dominant populations.The result showed that compared with the control soil sample,the species and quantities of soil mesofauna treated by Glyphosate-isopropylammonium 41AS herbicide were remarkblely decreased.The species of soil animals was decreased with decreasing population common and rare fauna,while the amount of soil animal was mainly associated with the populations of Collembola and

Acarina.Besides,the dominant population of Collembola and Acarina were decreased with the increasing concentrations of Glyphosate-isopropylammonium 41AS herbicide. **(China)**

51. Ratcliff, A.W., M.D. Busse, and C.J. Shestak. 2006. Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. *Applied Soil Ecology* 34(2): 114-124.

Glyphosate applied at the recommended field rate to a clay loam and a sandy loam forest soil resulted in few changes in microbial community structure. Total and culturable bacteria, fungal hyphal length, bacterial: fungal biomass, carbon utilization profiles (BIOLOG), and bacterial and fungal phospholipid fatty acids (PLFA) were unaffected 1, 3, 7, or 30 days after application of a commercial formulation (Roundup®). In contrast, a high concentration of glyphosate (100 x field rate) simulating an undiluted chemical spill substantially altered the bacterial community in both soils. Increases in total bacteria, culturable bacteria, and bacterial: fungal biomass were rapid following application. Culturable bacteria increased from about 1% of the total population in untreated soil to as much as 25% at the high concentration by day 7, indicating enrichment of generalist bacteria. Community composition in both soils shifted from fungal dominance to an equal ratio of bacteria to fungi. Functional diversity of culturable bacteria, estimated by C substrate utilization, also increased at the high glyphosate concentration, particularly in the clay loam soil. Unlike the other bacterial indices, only minor changes in bacterial PLFA resulted after the third day following the 100x field rate application. Apparently the herbicide resulted in an across-the-board stimulation of bacteria that was not reflected by the finer-scale PLFA community structure. Changes in fungal properties (hyphae, propagules, PLFA biomarkers) were few and transient. We conclude that the commercial formulation of glyphosate has a benign affect on community structure when applied at the recommended field rate, and produces a non-specific, short-term stimulation of bacteria at a high concentration. **(U.S.A.)**

52. Reddy, K.N., H.K. Abbas, R.M. Zablotowicz, C.A. Abel, and C.H. Koger. 2007. Mycotoxin occurrence and *Aspergillus flavus* soil propagules in a corn and cotton glyphosate-resistant cropping systems. *Food Additives and Contaminants* 24(12): 1367-1373.

The effects of cotton-corn rotation and glyphosate use on levels of soil-borne *Aspergillus flavus*, aflatoxin and fumonisin contamination in corn and cotton seed were determined during 2002 - 2005 in Stoneville, Mississippi (USA). There were four rotation systems (continuous cotton, continuous corn, cotton - corn and corn - cotton) for both glyphosate-resistant (GR) and non-GR cultivars-herbicide system arranged in a randomized complete block design with four replications. *Aspergillus flavus* populations in surface (5-cm depth) soil, sampled before planting (March/April), mid-season (June) and after harvest (September), ranged from 1.47 to 2.99 log (10) cfu g^{-1} soil in the four rotation systems. Propagules of *A. flavus* were higher in the continuous corn system compared to the continuous cotton system on three sample dates, and cotton rotated with corn decreased *A. flavus* propagules in three of nine sample dates. Propagules of *A. flavus* were significantly greater in plots

with GR cultivars compared to non-GR cultivars in three samples. In cotton seed, aflatoxin and fumonisin levels were similar (≤ 4 mu g kg^{-1} and non-detectable, respectively) regardless of rotation and glyphosate. In corn grain, aflatoxin was above the regulatory level (≥ 20 µg kg^{-1}) only in GR cultivar in 2004 and 2005. Fumonisin was higher in non-GR cultivar (4 mu g kg^{-1}) regardless of rotation in 2004; however, in 2002, 2003 and 2005, aflatoxin and fumonisin levels were similar regardless of rotation and glyphosate. These results indicate the potential for increased aflatoxin and fumonisin levels (1 of 4 years) in corn; however, climatic conditions encountered during this study did not allow for mycotoxin production. In laboratory incubation studies, fairly high concentrations of glyphosate were required to inhibit *A. flavus* growth; however no short-term effect of soil treatment with glyphosate on *A. flavus* populations were observed. These data suggest that altered populations of *A. flavus* or higher aflatoxin concentrations in corn grain were due to indirect effects of the GR cropping system. **(U.S.A.)**

53. Reis, M.R., A.A. Silva, M.A.M. Freitas, J.L. Pereira, M.D. Costa, M.C. Picanco, *et al.* 2009. Impact of glyphosate associated with insecticide and fungicide application on the microbial activity and potential phosphate solubilization in soil cultivated with Roundup Ready® soybean. *Planta Daninha* 27(4): 729-737.

The objective of this work was to evaluate the microbial activity and phosphate solubilization potential of a soil cultivated with soybean under different phytosanitary management strategies. The experiment was conducted in the field in a Cambic red-yellow Argisol. Ten treatments were arranged in a randomized block design with four replications. The effect of fungicide + herbicide application (endosulphan + tebuconazole) was evaluated in the plots, while weed control management was studied in the subplots (hoed or unhoed control; single-dose or sequential glyphosate application, single-dose fomesafen + fluazifop-p-butil application). Soil samples from the inter-row were collected when plants reached the R_2 stage and were used to measure soil respiratory rate, microbial biomass carbon (MBC), metabolic quotient (qCO_2), phosphate solubilization potential, and pH. The phytosanitary management strategies evaluated did not affect soil respiratory rates. The herbicides tested influenced soil MBC and qCO_2 variables directly related to soil quality. Glyphosate applied in a single dose or sequentially, either in combination with endosulphan + tebuconazole or not, led to lower qCO_2 values (0.075-0.079 mg μg^{-1} d^{-1}) and higher MBC (239.64 - 312.82 µg g^{-1}), indicating less soil disturbance. Higher phosphate solubilizing activity, 425 and 4 72 mg L^{-1}, were observed for the treatments with single-dose or sequential application of glyphosate, respectively, in the absence of endosulfan + tebuconazole. Agrochemical application on soybean shoots affects the activity of soil microorganisms in the plant rhizosphere. **(Brazil)**

54. Sailaja, K.K. and K. Satyaprasad. 2006. Degradation of glyphosate in soil and its effect on fungal population. *Journal of Environmental Science & Engineering* 48(3): 189-90.

Glyphosate application resulted in a decline in soil pH with consequent increase in soil mycoflora suggesting an indirect relationship. Though the composition of mycoflora unchanged, species of aspergilli, fusaria, penicillia and *Trichoderma* were

predominant. HPLC, IR analysis revealed the presence of sarcosine derivative as an intermediary of glyphosate degradation in soil. **(India)**

55. Sanogo, S., X.B. Yang, and P. Lundeen. 2001. Field response of glyphosate-tolerant soybean to herbicides and sudden death syndrome. *Plant Disease* 85(7): 773-779.

Three-year field experiments were conducted to assess the development of sudden death syndrome (caused by *Fusarium solani* f. sp. *glycines*) in three soybean cultivars, tolerant (P9344 and A3071) and nontolerant (BSR101), to glyphosate following foliar application of four herbicides (acifluorfen, glyphosate, imazethapyr, and lactofen) commonly applied to soybeans in the north-central region of the United States. Cultivar A3071 is resistant to sudden death syndrome, whereas cultivars P9344 and BSR101 are susceptible to this disease. There was no statistically significant cultivar-herbicide interaction with respect to the severity of foliar symptoms of the disease and the frequency of isolation of *F. solani* f. sp. *glycines* from roots of soybean plants. Across all herbicide treatments, the level of sudden death syndrome was lower in the disease-resistant cultivar than in the susceptible ones. There was an increase in the disease levels under application of acifluorfen, glyphosate, and imazethapyr compared with nontreated or lactofen-treated plants. The results obtained indicate that the response of glyphosate-tolerant soybeans to sudden death syndrome is not different from the response of conventional soybeans to this disease following application of the selected herbicides, and the resistance of soybean to sudden death syndrome was not changed with application of glyphosate. **(U.S.A.)**

56. Savin, M.C., L.C. Purcell, A. Daigh, and A. Manfredini. 2009. Response of mycorrhizal infection to glyphosate applications and P fertilization in glyphosate-tolerant soybean, maize, and cotton. *Journal of Plant Nutrition* 32(10): 1702-1717.

Glyphosate and phosphorus (P) fertilizer may alter arbuscular mycorrhizal (AM) fungal infection rates of glyphosate-tolerant cotton, maize, and soybean in low-P soil. Microbial biomass, water soluble P, Mehlich-3 P, and acid and alkaline phosphatase activities were not significantly impacted by glyphosate or P in the greenhouse. Phosphorus fertilization decreased mycorrhizal infection rates in cotton and maize and increased shoot biomass and shoot P in soybean in 2005, and decreased mycorrhizal infection in soybean and increased shoot biomass in cotton and maize and shoot P in all three crops in 2006. In pasteurized soil, glyphosate decreased percent mycorrhizal infection in maize, increased infection in cotton, and did not significantly affect infection in soybean. When soil was not pasteurized, glyphosate did not significantly alter mycorrhizal infection in any crop. The potential for glyphosate to alter AM fungal infection in glyphosate-tolerant plants may depend on whether soil microbial communities are compromised by other factors. **(U.S.A.)**

57. Schnurer, Y., P. Persson, M. Nilsson, A. Nordgren, and R. Giesler. 2006. Effects of surface sorption on microbial degradation of glyphosate. *Environmental Science & Technology* 40(13): 4145-4150.

Sorption may affect the bioavailability and biodegradation of pesticides in soils. The aim of this study was to test the effect of surface sorption on microbial utilization of the herbicide glyphosate as a source of phosphorus, nitrogen, or carbon. We added goethite to a humus soil to manipulate the soil's glyphosate sorption capacity. The addition of glyphosate generally either decreased microbial CO_2 production or produced no effect. Additions of glyphosate, in combination with glucose and N, did not change the respiration rate in comparison with the same treatment but without glyphosate. In contrast, glyphosate additions combined with glucose and P decreased microbial growth, whereas the combination with goethite counteracted the negative effect. The different treatments were examined using attenuated total reflectance Fourier transform (ATR-FTIR) spectroscopy; the results suggest that glyphosate was de-carboxylated in the sorbed state. Stimulating microbial growth by the addition of glucose and nitrogen resulted in further oxidation of glyphosate and only phosphate was detectable on the goethite surface after 13 days incubation. Our results show that sorbed glyphosate is microbially degradable, and it retards microbial activity. This study emphasizes the importance of combining quantitative measurements with a molecular-level examination, to better understand biogeochemical processes. **(Sweden)**

58. Sciegienka, J.K., E.N. Keren, and F.D. Menalled. 2011. Interactions between two biological control agents and an herbicide for Canada thistle (*Cirsium arvense*) suppression. *Invasive Plant Science and Management* 4(1): 151-158.

We investigated the single and combined effects of two biological control agents, the stem-mining weevil *Hadroplontus litura* and the pathogen *Pseudomonas syringae* pv. *tagetis*, with a herbicide (reduced or full application of glyphosate: 0.63 kg ae ha^{-1}, or 3.78 kg ae ha^{-1}, respectively) on the growth of Canada thistle, *Cirsium arvense*. We hypothesized that first, although each control method would have a negative effect on Canada thistle shoot biomass, root biomass, and shoot number, the integration of more than one control method would have greater impact than individual control methods. Second, we hypothesized that the order in which control methods are applied affects the outcome of the management program, with a pathogen application following weevil infestation being more effective than one prior to it. Although control methods impacted Canada thistle growth ($P < 0.001$, expect for a nonsignificant impact of glyphosate on shoot number), the combined effect of the three control methods behaved, generally, in an additive manner. A marginal interaction between the pathogen and the herbicide ($P = 0.052$) indicated a slight antagonistic interaction between these control methods. An interaction between the two biological control agents tested ($P < 0.001$) indicated that application of a pathogen prior to the release of weevil larvae could be more deleterious to Canada thistle than a late application. The observed, mostly additive, relationship between biological control agents and herbicides implies that integrating control methods rather than using a single approach could lead to greater Canada thistle control. **(U.S.A.)**

59. Spiridonov, Y.Y., G.E. Larina, L.D. Protasova, V.A. Abubikerov, and M.G. Zharikiov. 2010. Long-term application of the herbicide Roundup in the Central Nonchernozemic zone. *Agricultural Chemistry* 2: 29-36.

    Development of a weed cenosis was studied on a fallow field after the application of organophosphorus herbicides in the Nonchernozem zone. It was found that the biannual (in 2007-2008) application of Roundup at a rate of 3 1/ha reduced the content of impurities by 94-99%. Perennial regular application at a rate of 3-9 l/ha did by 98-100%. A number and abundance of species reduced to 0-15 (153-275 g/square meter) in different years of observations compared to 58 species (796 g/square m on average) in the control treatment (without application of the herbicide). Bird vetch (*Vicia cracca*) and wormwood (*Artemisia vulgaris*) were found to be tolerant (or relatively resistant) to the glyphosate herbicide. Foalfoot (*Tussilago farfara*), common dandelion (*Taraxacum officinale*), couch grass (*Agropyron repens*), maywedd (*Matricaria inodora*), purple deadly (*Lamium purpureum*) were sensitive to Roundup. The residual herbicide increased a population of saprotrophic microflora in soddy-podzolic soil during one month after application. Later the number meaning was close to be normal (1.46 x 10 E7 CFU (colony forming unit per 1 gram of soil). **(Russia)**

60. Tao, B., L. Jiang, X. Shen, F. Luan, and L. Qiu. 2011. Effects of glyphosate on soil microorganisms. *Chinese Journal of Oil Crop Sciences* CNKI:SUN:ZGYW.0.2011-02-014

    Black soil in northeast region of China was used to investigate the effects of glyphosate on soil respiration, soil cultural bacteria, *Rhizobium japonicum* and *Fusarium oxysporum*,to provide a theoretical basis for the evaluation of environmental safety of glyphosate. The results showed that increasing glyphosate concentration increased the inhibition on soil respiration. When glyphosate concentration in soil were $1mg \cdot kg^{-1}$,$10mg \cdot kg^{-1}$ and $100\ mg \cdot kg^{-1}$,the risk index were 1.20,0.322 and 0.076,which were less than the standard index of 20.Glyphosate influenced quantities of soil fungi and actinomycetes. High glyphosate concentration increased the inhibition to population of soil cultural bacteria. With the extended application of glyphosate, soil fungus, bacteria and actinomycetes populations had been restored. Actinomycetes were more sensitive to glyphosate than fungus and bacteria, and soil bacteria had a strong tolerance or degrading ability to glyphosate. Glyphosate impacted on inhibiting *Rhizobium japonicum* and *Fusarium oxysporum*, and the inhibition of *Rhizobium japonicum* was in direct proportion with the concentration of glyphosate. Low glyphosate concentration promoted *Fusarium oxysporum* populations, while high concentration inhibited them. The extended application time of glyphosate decreased the inhibition of glyphosate on *Rhizobium japonicum* and *Fusarium oxysporum*. **(China)**

61. Tatum, V.L. 2004. Toxicity, transport, and fate of forest herbicides. *Wildlife Society Bulletin* 32(4): 1042-1048.

    See Plant and Soil Residues Section.

62. Wardle, D.A., K.I. Bonner, G.M. Barker, G.W. Yeates, K.S. Nicholson, (*et al.*). 1999. Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. *Ecological Monographs* 69(4): 535-568.
See Biodiversity and Restoration Section.

63. Watrud, L.S., G. King, J.P. Londo, R. Colasanti, B.M. Smith, R.S. Waschmann, and E.H. Lee. 2011. Changes in constructed *Brassica* communities treated with glyphosate drift. *Ecological Applications* 21(2): 525-538.
See Biodiversity and Restoration Section.

64. Weaver, M.A., L.J. Krutz, R.M. Zablotowicz, and K.N. Reddy. 2007. Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil. *Pest Management Science* 63(4): 388-393.
Transgenic glyphosate-resistant (GR) soybean [*Glycine max* (L.) Merr.] has enabled highly effective and economical weed control. The concomitant increased application of glyphosate could lead to shifts in the soil microbial community. The objective of these experiments was to evaluate the effects of glyphosate on soil microbial community structure, function and activity. Field assessments on soil microbial communities were conducted on a silt loam soil near Stoneville, MS, USA. Surface soil was collected at time of planting, before initial glyphosate application and 14 days after two post-emergence glyphosate applications. Microbial community fatty acid methyl esters (FAMEs) were analyzed from these soil samples and soybean rhizospheres. Principal component analysis of the total FAME profile revealed no differentiation between field treatments, although the relative abundance of several individual fatty acids differed significantly. There was no significant herbicide effect in bulk soil or rhizosphere soils. Collectively, these findings indicate that glyphosate caused no meaningful whole microbial community shifts in this time period, even when applied at greater than label rates. Laboratory experiments, including up to threefold label rates of glyphosate, resulted in up to a 19% reduction in soil hydrolytic activity and small, brief (<7 days) changes in the soil microbial community. After incubation for 42 days, 32-37% of the applied glyphosate was mineralized when applied at threefold field rates, with about 9% forming bound residues. These results indicate that glyphosate has only small and transient effects on the soil microbial community, even when applied at greater than field rates. **(U.S.A.)**

65. Westerhuis, D., L.L. Vawdrey, and R. Piper. 2007. An *in vitro* study into the effect of glyphosate on *Sclerotium rolfsii*. *Australasian Plant Disease Notes* 2(1): 23-24.
The emergence of banana plants from planted corm pieces is known to be adversely affected by *Sclerotium rolfsii*, a fungal pathogen that is common in soil and plant debris. Glyphosate at 3.6 g/L reduced the radial growth of *S. rolfsii* in pure culture compared with benomyl at 0.5 g/L and an untreated control. This result supports anecdotal observations that glyphosate sprays can inhibit the growth of *S. rolfsii* affecting banana material in the field. **(Australia)**

66. Zabaloy, M.C., J.L. Garland, and M.A. Gomez. 2010. Assessment of the impact of 2,4-dichlorophenoxyacetic acid (2,4-D) on indigenous herbicide-degrading bacteria and microbial community function in an agricultural soil. *Applied Soil Ecology* 46(2): 240-246.

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) may influence soil microbial communities by altering the balance between resident populations. Our objective was to assess the effect of environmentally relevant levels (ERLs) of 2,4-D on microbial community function and on the population dynamics of 2,4-D degrading bacteria using a microcosm approach. The most probable number approach was used to enumerate 2,4-D-degrading soil bacteria. Carbon substrates utilization was tested with a microtiter-based oxygen sensor system to evaluate short-term functional shifts caused by herbicide treatment. Shifts in the community in response to potential toxicity of 2,4-D were assessed in the agricultural soil and a reference forest soil using the pollution-induced community-tolerance (PICT) approach. Results indicated that the agricultural soil had a stable 2,4-D degrading population able to use the herbicide as C and energy source, which increases immediately after an ERL dose of 2,4-D and remains high for about 1 month after exposure has ceased. An enhanced, dose-dependent response to 2,4-D as substrate was observed in the microtiter assay, while heterotrophic bacterial activity appeared mostly unchanged. The PICT assay showed higher tolerance to 2,4-D in the agricultural soil than in the unexposed forest soil. Our results suggest that agricultural use of 2,4-D at recommended level leads to selection for (1) a copiotrophic degrader population and (2) a persistently herbicide-tolerant, but functionally similar, microbial community. **(Argentina)**

Plant and Soil Residues

1. Alexa, E., A. Lazureanu, S. Alda, M. Negrea, and O. Iordanescu. 2008. Researches regarding extractable glyphosate residues from different soils. *Communications in Agricultural and Applied Biological Sciences* 73(4): 861-869.

   Glyphosate (N-phosphonomethyl-glycine) is a systemic, broad spectrum herbicide effective against most plant species, including annual and perennial species and is one of the world's most widely used herbicide. To glyphosate applied treatments, a part of active agent comes in contact with soil surface, adsorbing to soil compounds, while another part remains in soil solution. The adsorbing to soil compounds represents a feat importance conditioning the herbicide presence in soil solution and so, his availability to degradation and dispersion in the environment. In this paper work, the extractable glyphosate residues from soil solution have been determined through HPLC-FLD. Substrates used were Black Chernozem, Typical Gleysoil, Slight Vertisol, with moderate carbonatation. The experimental results indicated that the extractable glyphosate residue fractions from soil diminish (<20%), depending of soil parameters and decrease in this order: Gleysoil, Black Chernozem, Slight Vertisol. **(Romania)**

2. Ando, C., R. Segawa, C. Gana, L. Li, J. Walters, R. Sava, *et al.* 2003. Dissipation and offsite movement of forestry herbicides in plants of importance to native Americans in California national forests. *Bulletin of Environmental Contamination and Toxicology* 71(2): 354-361.

***** Residues of glyphosate, triclopyr, and hexazinone were detected outside the treatment area following application to bracken fern roots, buckbrush shoots, and deerbrush shots. Residues ranged from none-detected to 2.7 µg/g. Of the 240 off-site samples collected, only 19 (7.9%) contained herbicide residues and approximately 33% of the detections were at or close to the detection limits. The glyphosate detected (0.1 µg/g) at two locations on the 6-12 m transect collected 12 wk post-application were most likely due to contamination since they were not detected at this distance at the earlier dates. This is probably the case also for the hexazinone detection (0.01 µg/g) reported at the 7.2-9 m distance. Rain runoff was likely the source of the 0.7-µg/g hexazinone detection at 12-wk post-application of liquid hexazinone. Prominent gullies crossing the road from the treatment area to the transect were observed at week 12. These gullies were not apparent prior to week 12 sampling. It is assumed that herbicide resiudes were transported with rainfall/snowmelt moving the 15-27 m distance and ultimately to be translocated by the sampled plants. Residues were not detected at this monitoring location in samples earlier than week 12. Hexazinone has a high potential to move off-site due to its high water solubility and low absorption to soil, so this detection was not considered unusual. Granular hexazinone was detected at the 1.5-4.5 and 15-21 m distances, with concentrations of 0.1-1 µg/g at 1-3 days follo9wing herbicide application. The detections were probably from herbicide dust deposition on plants. **(U.S.A.)**

3. Baig, M.N., A.L. Darwent, K.N. Harker, and J.T. O'Donovan. 2003. Preharvest applications of glyphosate affect emergence and seedling growth of field pea (*Pisum sativum*). *Weed Technology* 17(4): 655-665.

Field experiments were conducted in 1994 and 1995 at Vegreville, Legal, and Lacombe, AB, to determine the effects of a preharvest application of glyphosate on seedling emergence and growth of field pea. Glyphosate was applied at 0.9 kg ai/ha at each of the three crop development stages, as determined by seed moisture content (SMC), to determinate ('Ascona' and 'Radley') and indeterminate ('Miko' and 'Trapper') cultivars. Applying glyphosate when the SMC was less than 30% had little to no effect on seedling emergence but reduced seedling shoot fresh weight in two of six experiments. Applying glyphosate at SMC above 40% reduced seedling emergence and shoot fresh weight in two and three of the six experiments, respectively. Reductions in seedling emergence and shoot fresh weight were greater from seeds collected from the top than from seeds collected from the bottom one-third of sprayed plants. Differences in response between determinate and indeterminate cultivars occurred, but there was no consistent trend. Given the variable maturity in most fields and on individual pea plants, applications of preharvest glyphosate to peas destined for seed production may decrease seed germination and biomass accumulation. **(Canada)**

4. Bonfleur, E.J., A. Lavorenti, and V.L. Tornisielo. 2011. Mineralization and degradation of glyphosate and atrazine applied in combination in a Brazilian Oxisol. *Journal of Environmental Science and Health* Part B 46(1): 69-75.

The aim of this study was to investigate the behavior of the association between atrazine and glyphosate in the soil through mineralization and degradation tests. Soil treatments consisted of the combination of a field dose of glyphosate (2.88 kg ha^{-1}) with 0, ½, 1 and 2 times a field dose of atrazine (3.00 kg ha^{-1}) and a field dose of atrazine with 0, ½, 1 and 2 times a field dose of glyphosate. The herbicide mineralization rates were measured after 0, 3, 7, 14, 21, 28, 35, 42, 49, 56 and 63 days of soil application, and degradation rates after 0, 7, 28 and 63 days. Although glyphosate mineralization rate was higher in the presence of 1 (one) dose of atrazine when compared with glyphosate alone, no significant differences were found when half or twice the atrazine dose was applied, meaning that differences in glyphosate mineralization rates cannot be attributed to the presence of atrazine. On the other hand, the influence of glyphosate on atrazine mineralization was evident, since increasing doses of glyphosate increased the atrazine mineralization rate and the lowest dose of glyphosate accelerated atrazine degradation. **(Brazil)**

5. Borggaard, O.K. 2011. Does phosphate affect soil sorption and degradation of glyphosate? – A Review. *Trends in Soil Science and Plant Nutrition* 2(1): 16-27.

Glyphosate is worldwide the most used herbicide because it is an efficient weed killer with favorable environmental and toxicological properties such as very low human and animal toxicity. Chemically, glyphosate is a phosphonate, i.e. it resembles phosphate. Consequently, glyphosate is strongly sorbed by the same soil minerals as phosphate, especially aluminium and iron oxides, allophane/imogolite and other variable charge minerals, whereas sorption by 2:1-layer silicates is

modest. However, this similarity between the two compounds indicates that phosphate may affect glyphosate sorption and degradation. Therefore, phosphate may affect the leachability of glyphosate and, in turn, the risk of groundwater contamination with this xenobioticum. Although the effect of phosphate on glyphosate sorption was pointed out shortly after the introduction of the herbicide in 1974, much is still unclear about interactions between glyphosate and phosphate. Therefore, it has been attempted to summarize available knowledge on influence of phosphate on sorption and degradation of glyphosate and its main metabolite, aminomethylphosphonic acid (AMPA) at different scales from the isolated mineral to the field level. The summary has shown that the interaction between glyphosate and phosphate can exhibit great variation from mineral to mineral and from soil to soil according to investigations in several laboratories. Phosphate has been shown to suppress glyphosate sorption by some minerals and soils but for most minerals and soils the effect is limited or even absent. This discrepancy has been interpreted as existence of two kinds of sorption sites including common sites subject to glyphosate-phosphate competition and to sites specific for either glyphosate or phosphate, where the two sorbates are sorbed independently of each other (additive sorption). Phosphate seems to have a positive effect on glyphosate degradation in some soils but no or little effect in other soils. Transport of presumably colloid-bonded glyphosate and AMPA in structured clayey soils with macropores has been demonstrated both in several lysimeter studies and few field trials; the leaching is augmented by heavy rainfall shortly after glyphosate application. However, the role of phosphorus in this transport has not been investigated. In sandy soils without macropores, glyphosate and AMPA do not seem to leach.

These results are in agreement with those of a Danish field trial, where concentrations of glyphosate and AMPA in soil samples and leachates were monitored during the year following glyphosate application to plots on a flat sandy soil that have received different amounts of phosphorus (and lime) since 1944. This trial showed that less than 0.1 g/ha corresponding to 0.013 % of applied glyphosate was translocated during the monitoring period and the leachate concentrations of glyphosate and AMPA were less than the EU drinking water threshold, 0.1 µg/L and often below the detection limit (0.01 µg/L). The contents of glyphosate and AMPA in the soil samples did not change significantly during the monitoring period, except for decreasing glyphosate content in the samples from one of the four plots investigated, but this decrease was ascribed to favorable pH conditions for the microbial degraders because of liming. The very limited translocation of glyphosate and AMPA found in the leachate and subsoil during the monitoring was attributed to transport by the plants as also seen in other investigations. Therefore, a main conclusion of this trial was that the soil phosphorus status did not influence glyphosate and AMPA dissipation at that site. **(Denmark)**

6. Casabé, N., L. Piola, J. Fuchs, M.L. Oneto, L. Pamparato, S. Basack, *et al.* 2007. Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field. *Journal of Soils and Sediments* 7(4): 232-239.

Continuous application of pesticides may pollute soils and affect non-target organisms. Soil is a complex ecosystem; its components can modulate the effects of

pesticides. Therefore, it is recommended to evaluate the potential environmental risk of these compounds in local conditions. We performed an integrated field-laboratory study on an Argentine soya field sprayed with glyphosate and chlorpyrifos under controlled conditions. Our aim was to compare the sensitivity of a series of endpoints for the assessment of adverse effects of the extensive use of these agrochemicals.A RR soya field in a traditional farming area of Argentina was sprayed with glyphosate (GLY) or chlorpyrifos (CPF) formulations at the commercially recommended rates, according to a randomized complete block design with 3 replicates. In laboratory assays, *Eisenia fetida andrei* were exposed to soil samples (0–10 cm depth) collected between the rows of soya. Endpoints linked to behavior and biological activity (reproduction, avoidance behavior and bait-lamina tests) and cellular/subcellular assays (Neutral Red Retention Time — NRRT; DNA damage — Comet assay) were tested. Field assays included litterbag and bait-lamina tests. Physico/chemical analyses were performed on soil samples.GLY reduced cocoon viability, decreasing the number of juveniles. Moreover, earthworms avoided soils treated with GLY. No effects on either reproduction or on avoidance were observed at the very low CPF concentration measured in the soils sampled 10 days after treatment. Both pesticides caused a reduction in the feeding activity under laboratory and field conditions. NRRT was responsive to formulations of CPF and GLY. Comet assay showed significantly increased DNA damage in earthworms exposed to CPF treated soils. No significant differences in DNA migration were observed with GLY treated soils. Litterbag field assay showed no differences between treated and control plots. The ecotoxicological effects of pesticides can be assessed by monitoring the status of communities in real ecosystems or through the use of laboratory toxicity tests. Litterbag field test showed no influence of the treatments on the organic matter breakdown, suggesting a scarce contribution of soil macrofauna. The bait-lamina test, however, seemed to be useful for detecting the effects of GLY and CPF treatments on the activity of the soil fauna. CPF failed to give significant differences with the controls in the reproduction test and the results were not conclusive in the avoidance test. Although the field population density of earthworms could be affected by multiple factors, the effects observed on the reproduction and avoidance tests caused by GLY could contribute to its decrease, with the subsequent loss of their beneficial functions. Biomarkers measuring effects on suborganism level could be useful to predict adverse effects on soil organisms and populations. Among them, NRRT, a lysosomal destabilization biomarker, resulted in demonstrating more sensitivity than the reproduction and avoidance tests. The Comet assay was responsive only to CPF. Since DNA damage can have severe consequences on populations, it could be regarded as an important indicator to be used in the assessment of soil health. Reproduction and avoidance tests were sensitive indicators of GLY exposure, with the former being more labor intensive. Bait-lamina test was sensitive to both CPF and GLY. NRRT and Comet assays revealed alterations at a subcellular level, and could be considered complementary to the biological activity tests. Because of their simplicity, some of these bioassays seemed to be appropriate pre-screening tests, prior to more extensive and invasive testing. This study showed deleterious effects of GLY and CPF formulations when applied at the nominal concentrations recommended for soya crops. Further

validation is needed before these endpoints could be used as field monitoring tools in Argentine soya soils (ecotoxicological risk assessment — ERA tools). **(Argentina)**

7. Ghanem, A., P. Bados, A.R. Estaun, L.F. de Alencastro, S. Taibi, J. Einhorn, *et al.* 2007. Concentrations and specific loads of glyphosate, diuron, atrazine, nonylphenol and metabolites thereof in French urban sewage sludge. *Chemosphere* 69(9): 1368-1373.

   Indirect soil pollution by heavy metals and organics may occur when sewage sludge is used as fertilizer. It is essential to define the nature and amounts of pollutants contained in sewage sludge in order to assess environmental risk. Here, we present results from a one-year monitoring of herbicides (glyphosate, diuron and atrazine) and their major degradates in sewage sludge sampled from three wastewater treatment plants and one composting unit in the vicinity of Versailles, France. The concentrations of these compounds were determined, as well as these of the surfactant nonylphenol. We demonstrated the presence of glyphosate and aminomethylphosphonic acid at the mg kg^{-1} (dry matter) level in all samples. Diuron was detected at the microg kg^{-1} (d.m.) level, whereas its degradate and triazine compounds were below the limits of quantification. Nonylphenol amounts were higher than the future European limit value of 50 mg kg^{-1} (d.m.). **(France)**

8. Goss, R.M., R.E. Gaussoin, and A.R. Martin. 2004. Phytotoxicity of clippings from creeping bentgrass treated with glyphosate. *Weed Technology* 18(3): 575-579.

   Recent advances in genetic engineering have led to the development of glyphosate-resistant (GR) crops for genetic markers and selective weed control. The effects of glyphosate residue on turfgrass clippings could be toxic to non-GR species. The objective of this experiment was to determine whether glyphosate would retain activity within clippings of creeping bentgrass when applied to Kentucky bluegrass and perennial ryegrass. Greenhouse-grown 'Penncross' and GR 'ASR-368' were treated with glyphosate at 2.24 kg/ha. Clippings were collected 1, 3, 7, and 12 d after application and applied to greenhouse-grown Kentucky bluegrass and perennial ryegrass. Kentucky bluegrass and perennial ryegrass dry weight and percent cover were reduced by clippings receiving glyphosate that were harvested 1 and 3 d after glyphosate application from both susceptible and resistant creeping bentgrass. Results indicate that glyphosate remains active in clippings for up to 3 d after treatment within creeping bentgrass clippings. Glyphosate-applied creeping bentgrass clippings will need to be managed to prevent injury to susceptible species. **(U.S.A.)**

9. Kleter, G.A., J.B. Unsworth, and C.A. Harris. 2011. The impact of altered herbicide residues in transgenic herbicide-resistant crops on standard setting for herbicide residues. *Pest Management Science* 67(10): 1193-1210.

   The global area covered with transgenic (genetically modified) crops has rapidly increased since their introduction in the mid-1990s. Most of these crops have been rendered herbicide resistant, for which it can be envisaged that the modification has an impact on the profile and level of herbicide residues within these crops. In this article, the four main categories of herbicide resistance, including resistance to

acetolactate-synthase inhibitors, bromoxynil, glufosinate and glyphosate, are reviewed. The topics considered are the molecular mechanism underlying the herbicide resistance, the nature and levels of the residues formed and their impact on the residue definition and maximum residue limits (MRLs) defined by the Codex Alimentarius Commission and national authorities. No general conclusions can be drawn concerning the nature and level of residues, which has to be done on a case-by-case basis. International residue definitions and MRLs are still lacking for some herbicide–crop combinations, and harmonisation is therefore recommended. **(The Netherlands)**

10. Laitinen, P., S. Rämö, U. Nikunen, L. Jauhiainen, K. Siimes, and E. Turtola. 2009. Glyphosate and phosphorus leaching and residues in boreal sandy soil. *Plant and Soil* 323(1): 267-283.

Glyphosate [(N-(phosphonomethyl)glycine)] is a widely used herbicide and it is known to compete for the same sorption sites in soil as phosphorus. Persistence and losses of glyphosate were monitored in a field with low phosphorus status and possible correlation between glyphosate and phosphorus leaching losses was studied. Glyphosate and its metabolite AMPA (aminomethyl phosphonic acid) residues in soil samples were analysed after a single application in autumn. Twenty months after the application the residues of glyphosate and AMPA in the topsoil (0–25 cm) corresponded to 19% and 48%, respectively, of the applied amount of glyphosate, and traces of glyphosate and AMPA residues were detected in deeper soil layers (below 35 cm). These results indicate rather long persistence for glyphosate in boreal soils. Surface runoff and subsurface drainflow were collected continuously all year round for 20 months and analysed for glyphosate, AMPA, dissolved phosphate, total phosphorus and total suspended solids. The glyphosate concentrations in the surface runoff water were highest, with 99% of the total leaching losses obtained, during the periods of snow melting and soil thawing in the first winter following the autumn application. The total leaching of glyphosate was 5.12 g ha^{-1} and that of AMPA 0.48 g ha^{-1}, corresponding to about 0.51% and 0.07%, respectively, of the applied amount of glyphosate. No residues of glyphosate and AMPA were detected in the subsurface drainflow. The correlations between concentrations of glyphosate and dissolved orthophosphate as well as glyphosate and total phosphorus in surface runoff were significant ($p < 0.01$). **(Finland)**

11. Major, W.W., C.E. Grue, S.C. Gardner, and J.M. Grassley. 2003. Concentrations of glyphosate and AMPA in sediment following operational applications of Rodeo® to control smooth cordgrass in Willapa Bay, Washington, USA. *Bulletin of Environmental Contamination and Toxicology* 71: 912-918.

***** Rodeo® (a.i. glyphosate) is the only herbicide approved to control smooth cordgrass (*Spartina*) in Willapa Bay, Washington. Determining the concentrations of glyphosate and its primary breakdown product, aminomethyl-phosphonic acid (AMPA) in Bay sediments following operational applications is a necessary first step in evaluating bioavailability and the potential for non-target effects. We report concentrations of glyphosate and AMPA in sediment following operational hand and aerial applications of Rodeo® to *Spartina* in the Bay at maximum allowable rates.

Our data suggest degradation of glyphosate is slower in estuarine habitats than freshwater environments, and more similar to that reported for agricultural (including forest). Concentrations of glyphosate we detected in sediments are a fraction of those known to be toxic to aquatic invertebrates. On the basis of these results, we would not expect direct toxic effects on aquatic organisms, even at the highest concentration of glyphosate detected in sediment during our study (16.2 ppm). Our study suggest that a proposed doubling of the aerial application rate to increase efficacy in controlling *Spartina* will pose little hazard to non-targets, and that concerns over non-target effects should be directed at the selection of surfactants rather than the quantity of active ingredient. **(U.S.A.)**

12. Morshed, M.M., D. Omar, R.B. Mohamad, and S.B.A. Wahed. 2011. Determination of glyphosate through passive and active sampling methods in a treated field atmosphere. *African Journal of Agricultural Research* 6(17): 4010-4018.

    The study was carried out to determine the atmospheric residues of glyphosate (N-phosphonomethylglicine) using both passive and active sampling methods in Malaysia's tropical weather conditions. The field was treated with Roundup (Monsanto) @ 2L ha^{-1} using Mistblower (Solo 412). Glyphosate was sampled in 12 h day time pre and post-spray sampling events using three simple and low-cost passive air samplers (cotton gauze, cellulose filter, and PUF) and active sampling using PUF plug and quartz filter cartridges. In pre-spray sampling event, no glyphosate detection was shown in both passive and active sampling. On the other hand, post-spray passive samples data revealed that only cotton gauze among the three passive air samples showed detection in both post-spray events during which the first post-spray (2.49 ng/cm^2) showed significantly higher residue measurement than that of second post-spray period (0.84 ng/cm^2). In active sampling, however, no glyphosate residue was detected in any of the PUF plug samples but detected only in quartz filter samples, revealing that glyphosate is associated with particles rather than vapour in the air. The highest concentration of glyphosate (42.96$\mu g/m^3$) was measured in the air at operator's breathing zone during the 25 min spray application period. In the post-spray active sampling periods, glyphosate residue was significantly far below compared to the spray period concentration. Furthermore, in paired comparison between active and passive sampling methods in terms of residue uptake performance, passive sampling showed significantly better performance than the active sampling method in this study. **(Malaysia)**

13. Perie, C. and A.D. Munson. 2000. Ten-year responses of soil quality and conifer growth to silvicultural treatments. *Soil Science Society of America Journal* 64(5): 1815-1826.

    See Crop Tree Productivity Section.

14. Peruzzo, P.J., A.A. Porta, and A.E. Ronco. 2008. Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina. *Environmental Pollution* 156(1): 61-66.

    Levels of glyphosate were determined in water, soil and sediment samples from a transgenic soybean cultivation area located near to tributaries streams of the

Pergamino-Arrecifes system in the north of the Province of Buenos Aires, Argentina. Field work took into account both the pesticide application and the rains occurring after applications. The pesticide was analysed by HPLC-UV detection, previous derivatization with 9-fluorenylmethylchloroformate (FMOC-Cl). In addition, SoilFug multimedia model was used to analyse the environmental distribution of the pesticides. In the field, levels of glyphosate in waters ranged from 0.10 to 0.70 mg/L, while in sediments and soils values were between 0.5 and 5.0 mg/Kg. Temporal variation of glyphosate levels depended directly on the time of application and the rain events. The results obtained from the application of the model are in accordance with the values found in the field. **(Argentina)**

15. Reynolds, P.E., N.V. Thevathasan, J.A. Simpson, A.M. Gordon, R.A. Lautenschlager, W.F. Bell, D.A. Gresch, and D.A. Buckley. 2000. Alternative conifer release treatments affect microclimate and soil nitrogen mineralization. *Forest Ecology and Management* 133(1): 115-125.

    In 1993, the Fallingsnow Ecosystem Project was initiated in northwestern Ontario to assess the effects of alternativeconiferrelease practices on ecosystem processes, wildlife populations, and spruce production. Coniferreleasetreatments: two herbicide (glyphosate and triclopyr), two cutting treatments (brushsaw and Silvana selective mower), and controls were established on four 30–60 ha clearcut and planted (spruce) blocks. Unharvested controls adjacent to each block constituted a sixth treatment. Objectives of this study were: (1) to quantify soil nitrification rates for the control, glyphosate, brushsaw, and unharvested forest treatments and (2) to relate these rates to soil temperature and moisture. Weather stations and buried fiberglass/resistance soil cells were established in 1994 to monitor soil temperatures and moisture. During the second posttreatment growing season (1995), soil samples were collected at 5, 15, and 30 cm depth and incubated in polyethylene bags at the same depth from which they were collected for 30 days prior to exhumation. The above procedure was repeated for the months of June, July, August, and September. In the third posttreatment growing season (1996), bags were buried (mid-June, mid-July, mid-August) at 5 cm only and exhumed 30 days after burial. Higher levels of nitrate (NO_3^-) were observed for the glyphosate and brushsaw treatments in August 1995 compared with the control and unharvested forest treatments. Rates (μg 100 g^{-1} dry soil per day) of ammonium (NH_4^+) and nitrate production decreased with soil depth and exhibited a distinctive seasonal trend, decreasing as soil temperatures declined. Ammonium production was significantly correlated with soil temperature and moisture, increasing with increasing temperature, and decreasing at higher moisture levels. By the third (1996) posttreatment growing season, no treatment-related differences were observed, and ammonium production was less correlated with soil temperature than during the second (1995) posttreatment growing season. These results affirm that application of glyphosate is the best option evaluated for effective weed control and optimal nutrient release. **(Canada)**

16. Santos, B.M., J.P. Gilreath, C.E. Esmel, and M.N. Siham. 2007. Effects of sublethal glyphosate rates on fresh market tomato. *Crop Protection* 26(2): 89-91.

Two field trials were conducted to determine the effect of low doses of glyphosate on tomato plant growth and marketable yield. The herbicide was applied to the foliage 1 day before transplanting. There was a rapid decline on tomato plant vigour and height as the herbicide dose reached 100 mg/L. Marketable yield was also affected by sublethal herbicide doses, with a 41% yield decrease with 25 mg/L of glyphosate. These results confirm that low glyphosate doses, which could be found in multi-purpose spraying equipment, might severely reduce tomato marketable yield. **(U.S.A.)**

17. Swift, K. and F.W. Bell. 2011. What are the environmental consequences of using silviculturally effective forest vegetation management treatments? *The Forestry Chronicle* 87(2): 201-216.

See Biodiversity and Restoration Section.

18. Tatum, V.L. 2004. Toxicity, transport, and fate of forest herbicides. *Wildlife Society Bulletin* 32(4): 1042-1048.

Public opposition to use of herbicides in forests typically centers around concerns over potential toxicity to wildlife. Characterization of the risk of silvicultural herbicides to wildlife requires an understanding of herbicide toxicity and environmental fate and transport. The fate and chemistry of herbicides and adjuvants within environmental media determine how and which organisms may be exposed and duration of those exposures. The nature of the toxicity of herbicides, adjuvants, and their decomposition products, and levels at which those toxic responses may be observed, determine which organisms or life stages may be most susceptible to any toxic effects and which exposure concentrations and durations can be considered safe. In general, herbicides most commonly used for vegetation management in forestry (glyphosate, triclopyr, imazapyr, sulfometuron, metsulfuron methyl, hexazinone) degrade quickly once they enter the environment and thus are neither persistent nor bioaccumulative. Because modern herbicides have been designed to target biochemical processes unique to plants, they exhibit a low level of direct toxicity to animals. When used according to label instructions, modern silvicultural herbicides pose little risk to wildlife. **(U.S.A.)**

19. Thompson, D., J. Leach, M. Noel, S. Odsen, and M. Mihajlovich. 2012. Aerial forest herbicide application: Comparative assessment of risk mitigation strategies in Canada. *The Forestry Chronicle* 88(2): 176-184.

See Human Health Section.

20. Tsui, M.T.K and L.M. Chu. 2004. Comparative toxicity of glyphosate-based herbicides: aqueous and sediment porewater exposures. *Archives of Environmental Contamination and Toxicology* 46(3): 316-323.

See Amphibians, Aquatic Invertebrates and Plants, and Algae Section.

21. Wardle, D.A., K.I. Bonner, G.M. Barker, G.W. Yeates, K.S. Nicholson, (*et al.*). 1999. Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. *Ecological Monographs* 69(4): 535-568.

See Biodiversity and Restoration Section.

22. Zablotowicz, R.M., C. Accinelli, L.J. Krutz, and K.N. Reddy. 2009. Soil depth and tillage effects on glyphosate degradation. *Journal of Agricultural and Food Chemistry* 57(11): 4867-4871.

The use of glyphosate-resistant crops facilitated the widespread adoption of no-tillage (NT) cropping systems. The experimental objectives were to determine glyphosate sorption, mineralization, and persistence at two depths [0-2 cm (A) and 2-10 cm (B)] in a silt loam managed under long-term conventional tillage (CT) or NT soybean. Relative to the other soils, organic carbon (OC) and fluorescein diacetate (FDA) hydrolytic activity were at least 1.4-fold higher in NT-A. Glyphosate K_d values ranged from 78.2 to 48.1 and were not correlated with OC. Cumulative glyphosate mineralized after 35 days was highest in NT-A soil (70%), intermediate in CT-A and CT-B (63%), and least in NT-B (51%). Mineralization was positively correlated with OC and FDA activity, but negatively correlated with K_d, indicating that sorption decreased bioavailability. Independent of tillage and depth, the half-lives for 0.01 N $CaCl_2$ and 0.1 N NaOH extractable residues (bioavailable residues and residues bound to iron and aluminum oxides, respectively) were ≤1.2 h and ≤14.2 days, respectively. These data indicate that glyphosate sorption and persistence are similar between the surface of NT and CT soils and that the adoption of NT will likely have minimal impact on the risk for nontarget effects of glyphosate on soil microflora or transport in surface runoff. **(U.S.A.)**

Terrestrial Invertebrates

1. Badji, C.A., R.N.C. Guedes, A.A. Silva, A.S. Corrêa, M.E.L.R. Queiroz, and M. Michereff-Filho. 2007. Non-target impact of deltamethrin on soil arthropods of maize fields under conventional and no-tillage cultivation. *Journal of Applied Entomology* 131(1): 50-58.

Deltamethrin is a commonly used insecticide for controlling its key maize pest, the fall armyworm *Spodoptera frugiperda* (Lep., Noctuidae). Its toxicological profile is well known, but its impact on arthropods widely reported as bioindicators, mainly springtails (Collembola) and mites (Oribatida), is yet to be assessed in tropical maize fields. The treatments used to circumvent this shortcoming were conventional cultivation and no-tillage cultivation (with a pre-sowing application of 2,4-D and glyphosate) systems with or without deltamethrin spraying. The deltamethrin residue analysis of soil samples by gas chromatography did not detect the insecticide 24 h after it was sprayed on the maize fields. There was no significant overall effect of deltamethrin based on principal component analysis. However, repeated-measures analyses of variance detected significant impact of deltamethrin in a species of Nitidulidae (Coleoptera). The cultivation system also provided significant impact on Oribatida and Gamasida soil mites and on the same Nitidulidae species referred above, which were more abundant in the conventional cultivation system. Springtails were also significantly affected by the cultivation system showing greater abundance in the conventional system, except Podumorpha. Analyses using only high taxonomic levels did not allow the detection of impact in the ant assemblage assessed. The results suggest that the impact of deltamethrin on soil arthropods from tropical fields varies among species and is lower than expected. The cultivation system imposes more drastic effects on arthropod assemblage. **(Brazil)**

2. Bell, J.R., A.J. Haughton, N.D. Boatman, and A. Wilcox. 2002. Do incremental increases of the herbicide glyphosate have indirect consequences for spider communities? *Journal of Arachnology* 30(2): 288-297.

We examined the indirect effect of the herbicide glyphosate on field margin spider communities. Glyphosate was applied to two replicated (n = 8 per treatment) randomized field experiments over two years in 1997–1998. Spiders were sampled using a modified garden vac monthly from May–October in the following treatments: 1997 comprised 90g, 180g, & 360g active ingredient (a.i.) glyphosate ha^{-1} treatments and an unsprayed control; 1998 comprised 360g, 720g and 1440g a.i. glyphosate ha^{-1} treatments and an unsprayed control. We examined the indirect effect of glyphosate on the spider community using DECORANA (DCA), an indirect form of gradient analysis. We subjected DCA-derived Euclidean distances (one a measure of beta diversity and the other a measure of variability), to the scrutiny of a repeated measures ANOVA design. We found that species turnover and cluster variation did not differ significantly between treatments. We attribute the lack of any effect to a large number of common agricultural species which are never eliminated from a habitat, but are instead significantly reduced. Reduction rather than elimination does not cause the spider communities within these plots to turn over any faster than the control. However, like most other animal communities, the spider community did turn over and change in structure and composition through the

season, regardless of treatment. Using Spearman rank correlations, we found that this within-season species turnover is related to the decline in vegetation height and the increase in percentage dead vegetation cover in the field margin. **(United Kingdom)**

3. Benamú, M.A., M.I. Schneider, and N.E. Sánchez. 2010. Effects of the herbicide glyphosate on biological attributes of *Alpaida veniliae* (Araneae, Araneidae), in laboratory. *Chemosphere* 78(7): 871-876.

In the past decades there has been increasing interest in the study of arthropod predators as effective potential natural enemies to be used in the biological control of agricultural pests. In Argentina, transgenic soybean crops (Round-up Ready, RR) are inhabit by many spider species, some of them in high abundance, being indicative of an import potential for pest predation. This crop is associated with the use of glyphosate, a broad-spectrum herbicide, with low environmental impact, even though since the 80's, several negative effects have been deeply documented on mammals, fishes, amphibians, snails, earthworms, insects, etc. Nowadays, the effects on arthropod physiology, behavior and life history traits as end-points in ecotoxicological evaluations are being recognized. In transgenic soybean crops of Buenos Aires province (Argentina), *Alpaida veniliae* (Araneae, Araneidae) is one of the most abundant orb web weaver spiders. The purpose of this study was to address the effects of glyphosate on some biological attributes of A. veniliae, in laboratory. Results of this study showed no lethal direct effects of Glifoglex on this spider, but it is the first report in literature about sublethal effects of this herbicide on a spider's biological attributes. Negative effects on prey consumption, web building, fecundity, fertility and developmental time of progeny were observed. Although sublethal effects have received less attention than direct lethal effects, they are relevant from an ecological point of view, since the reduction of the arthropod performance may create risks to arthropod biodiversity conservation in agroecosystems. **(Argentina)**

4. Bigler, F. and R. Albajes. 2011. Indirect effects of genetically modified herbicide tolerant crops on biodiversity and ecosystem services: the biological control example. *Journal of Consumer Protection and Food Safety* 6(Suppl 1): S79-S84.

See Biodiversity and Restoration Section,

5. Bogantes-Arias, A., R. Agüero-Alvarado, and R. Mexzón-Vargas. 2006. Weeds control in peach palm (*Bactris gasipaes* K.) for palm hearts: effect on soil and arthropods. *Agronomía Mesoamericana* 17(1): 25-33.

A study was conducted at Los Diamantes Experiment Station, located in Guápiles, Costa Rica during 2000 and 2001 to compare the effects of four sowing densities (20 000, 10 000, 6666 and 5000 plants/ha, with distances of 0.25, 0.50, 0.75 and 1.00 m, respectively) of spineless "palmito" peach palm and two weed management strategies (chemical using glyphosate and physical) on chemical and microbiological characteristics of the soil, insect and weed populations in this crop. No differences were observed among treatments in regard to soil nutriments, except for a larger quantity of K in the plots subjected to physical treatment in the second sampling. Changes among samplings (first and second) were evident for variables

such as the pH, Al and Ca. In the second sampling, the quantities of fungi, bacteria and actinomycetes in the soil were different among treatments. In additional plots with overgrowth, a total of 65 families of insects were gathered. There were important increases in the number of morphospecies and of individuals between the first one and second sampling in families of insects; the largest increment in the number of families, morphospecies and number of individuals was found in plots sown with 0.25 m distance among plants. **(Costa Rica)**

6. Bohan, D.A., C.W.H. Boffey, D.R. Brooks, S.J. Clark, A.M. Dewar, L.G. Firbank, *et al.* 2005. Effects on weed and invertebrate abundance and diversity of herbicide management in genetically modified herbicide-tolerant winter-sown oilseed rape. *Proceedings of the Royal Society B* 272: 463-474.

We evaluated the effects of the herbicide management associated with genetically modified herbicide-tolerant (GMHT) winter oilseed rape (WOSR) on weed and invertebrate abundance and diversity by testing the null hypothesis that there is no difference between the effects of herbicide management of GMHT WOSR and that of comparable conventional varieties. For total weeds there were few treatment differences between GMHT and conventional cropping, but large and opposite treatment effects were observed for dicots and monocots. In the GMHT treatment, there were fewer dicots and more monocots than in conventional crops. At harvest, dicot biomass and seed rain in the GMHT treatment were one-third of that in the conventional, while monocot biomass was threefold greater and monocot seed rain almost fivefold greater in the GMHT treatment than in the conventional. These differential effects persisted into the following two years of the rotation. Bees and butterflies that forage and select for dicot weeds were less abundant in GMHT WOSR management in July. Year totals for Collembola were greater under GMHT management. There were few other treatment effects on invertebrates, despite the marked effects of herbicide management on the weeds. **(United Kingdom)**

7. Boydston, R.A. and M.M. Williams. 2004. Combined effects of *Aceria malherbae* and herbicides on field bindweed (*Convolvulus arvensis*) growth. *Weed Science* 52(2): 297-301.

The effects of a gall mite (*Aceria malherbae*) and sublethal doses of either 2,4-DB or glyphosate on field bindweed growth were evaluated under laboratory conditions. Mite feeding reduced field bindweed shoot biomass 37 to 48% and root biomass 46 to 50%. 2,4-DB at 0.07 to 0.14 kg ae ha^{-1} or glyphosate at 0.14 to 0.28 kg ai ha^{-1} reduced field bindweed root biomass 25 to 52%. Combining *A. malherbae* feeding with either 2,4-DB or glyphosate application reduced root biomass of field bindweed plants more than mites or either herbicide alone. Live *A. malherbae* were present on field bindweed 3 wk after treatment with either herbicide. Combination of *A. malherbae* with sublethal herbicide doses may allow for field bindweed suppression while reducing potential herbicide injury to crops and maintaining *A. malherbae* populations. **(U.S.A.)**

8. Brooks, D.R., D.A. Bohan, G.T. Champion, A.J. Haughton, C. Hawes, M.S. Heard, *et al.* 2003. Invertebrate responses to the management of genetically modified herbicide–tolerant and conventional spring crops. I. Soil-surface-active invertebrates.

Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 358: 1847-1862.

The effects of herbicide management of genetically modified herbicide-tolerant (GMHT) beet, maize and spring oilseed rape on the abundance and diversity of soil-surface-active invertebrates were assessed. Most effects did not differ between years, environmental zones or initial seedbanks or between sugar and fodder beet. This suggests that the results may be treated as generally applicable to agricultural situations throughout the UK for these crops. The direction of the effects was evenly balanced between increases and decreases in counts in the GMHT compared with the conventional treatment. Most effects involving a greater capture in the GMHT treatments occurred in maize, whereas most effects involving a smaller capture were in beet and spring oilseed rape. Differences between GMHT and conventional crop herbicide management had a significant effect on the capture of most surface-active invertebrate species and higher taxa tested in at least one crop, and these differences reflected the phenology and ecology of the invertebrates. Counts of carabids that feed on weed seeds were smaller in GMHT beet and spring oilseed rape but larger in GMHT maize. In contrast, collembolan detritivore counts were significantly larger under GMHT crop management. **(United Kingdom)**

9. Casabé, N., L. Piola, J. Fuchs, M.L. Oneto, L. Pamparato, S. Basack, *et al.* 2007. Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field. *Journal of Soils and Sediments* 7(4): 232-239.

See Plant and Soil Residues Section.

10. Cobb, T.P., D.W. Langor, and J.R. Spence. 2007. Biodiversity and multiple disturbances: boreal forest ground beetle (Coleoptera: Carabidae) responses to wildfire, harvesting, and herbicide. *Canadian Journal of Forest Research* 37(8): 1310-1323.

Rising societal demands for forest resources along with existing natural disturbance regimes suggest that sustainable forest management will increasingly depend on better understanding the cumulative effects of natural and anthropogenic disturbances. In North America, for example, there is increasing economic pressure to salvage log burned forests, although the ecological consequences of combining fire and harvesting on the same sites are unclear. We examined the short-term (2year) responses of boreal forest ground beetles (Coleoptera: Carabidae) to the individual and combined effects of wildfire, harvesting, and herbicide. Ground beetle responses to wildfire and forestry-related disturbances differed strongly and suggested that, although some species may appear to benefit from disturbance combinations (e.g., Sericoda quadripunctata (DeGeer)), these effects are detrimental to others (e.g., Sericoda bembidioides Kirby). Species compositional variability was significantly reduced by disturbance combinations suggesting that multiple disturbances may lead to a simplification of this entire assemblage. In addition, ground beetle responses were correlated with changes in several key habitat parameters such as amount of woody debris, exposed ground, and plant species richness suggesting avenues for future study. Overall, however, our results suggest that efforts to avoid compounding disturbances on any site should be

considered when developing current and future forest management guidelines. **(Canada)**

11. Contardo-Jara, V., E. Klingelmann, and C. Wiegand. 2009. Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes. *Environmental Pollution* 157(1): 57-63.

The bioaccumulation potential of glyphosate and the formulation Roundup Ultra, as well as possible effects on biotransformation and antioxidant enzymes in Lumbriculus variegatus were compared by four days exposure to concentrations between 0.05 and 5 mg L^{-1} pure glyphosate and its formulation. Bioaccumulation was determined using ^{14}C labeled glyphosate. The bioaccumulation factor (BCF) varied between 1.4 and 5.9 for the different concentrations, and was higher than estimated from $logP_{ow}$. Glyphosate and its surfactant POEA caused elevation of biotransformation enzyme soluble glutathione S-transferase at non-toxic concentrations. Membrane bound glutathione S-transferase activity was significantly elevated in Roundup Ultra exposed worms, compared to treatment with equal glyphosate concentrations, but did not significantly differ from the control. Antioxidant enzyme superoxide dismutase was significantly increased by glyphosate but in particular by Roundup Ultra exposure indicating oxidative stress. The results show that the formulation Roundup Ultra is of more ecotoxicological relevance than the glyphosate itself. **(Germany)**

12. Correia, F.V. and J.C. Moreira. 2010. Effects of glyphosate and 2,4-D on earthworms (*Eisenia foetida*) in laboratory tests. *Bulletin of Environmental Contamination and Toxicology* 85: 264-268.

Laboratory tests were conducted to compare the effects of various concentrations of glyphosate and 2,4-D on earthworms (*Eisenia foetida*) cultured in Argissol during 56 days of incubation. The effects on earthworm growth, survival, and reproduction rates were verified for different exposure times. Earthworms kept in glyphosate treated soil were classified as alive in all evaluations, but showed gradual and significant reduction in mean weight (50%) at all test concentrations. For 2,4-D, 100% mortality was observed in soil treated with 500 and 1,000 mg/kg. At 14 days, 30%–40% mortality levels were observed in all other concentrations. No cocoons or juveniles were found in soil treated with either herbicide. Glyphosate and 2,4-D demonstrated severe effects on the development and reproduction of *Eisenia foetida* in laboratory tests in the range of test concentrations. **(Brazil)**

13. da Silva, R.S., G. de Paula Cognato, F.C. Vuaden, M.F.S. Rezende, F.V. Thiesen, M. da Graça Fauth, M.R. Bogo, C.D. Bonan, and R.D. Dias. 2003. Different sensitivity of Ca^{2+}-ATPase and cholinesterase to pure and commercial pesticides in nervous ganglia of *Phyllocaulis soleiformis* (Mollusca). *Comparative Biochemistry and Physiology* Part C 135(2): 215-220.

We measured the effects in vitro of pure and commercial pesticides on Ca^{2+}-activated ATPase and cholinesterase (ChE) activities in the nervous system of the slug *Phyllocaulis soleiformis*. The pesticides used in this study included carbamate and organophosphates, which acts as reversible and irreversible

anticholinesterases, respectively. Both enzymes were insensitive to pure carbofuran (1 mM), glyphosate (1 mM) and malathion (120 μM). However, the carbamate carbofuran, in the commercial formulation Furandan 350S, inhibited ATPase and ChE activities. The organophosphate glyphosate used in the commercial preparation of Gliz 480CS® inhibited ATPase activity and increased cholinesterase activity. These effects are likely due to the action of adjuvant substances of the chemical formulation. The commercial formulation (Malatol 500CE) did not alter enzymes activities. Our results suggest that cholinesterase present in the slug nervous tissue has a different behavior to those identified in vertebrate nervous tissue, since it was insensitive to pure compounds, known as anticholinesterases in vertebrates. Considering the insensitivity of the Ca^{2+}-activated ATPase, we suggested that the purinergic neurotransmission and other roles of ATP might not be affected by the pure pesticides tested. **(Brazil)**

14. Davis, H.N., R.S. Currie, B.W. French, and L.L. Buschman. 2009. Impact of land management practices on Carabids (Coleoptera: Carabidae) and other arthropods on the western high plains of North America. *Southwestern Entomologist* 34(1): 43-59.

This study examined how land management practices can affect the abundance of several arthropods commonly found in agriculture. This work was done in plots that had been subjected to three successive years of an agronomic experiment that evaluated the effects of a wheat, *Triticum aestivum* L., cover crop or no cover crop on weed and water management. After the third growing season, pitfall traps were installed and arthropods were collected and identified. At one location, carabids (Coleoptera: Carabidae) were identified to genus. Four of the genera (*Amara, Anisodactylus, Harpalus,* and *Calathus*) were more common under no-till conditions. Only one genus *(Stenolophus)* was more common in tilled plots. Five genera (*Amara*, *Bradycellus, Scarites, Stenolophus,* and *Calathus*) were more common in plots with a history of more weeds caused by less herbicide use. Carabids were not more abundant in plots with fewer weeds after herbicides had been applied. Past presence of a winter cover crop never reduced carabid numbers, but significantly increased members of two genera *(Harpalus* and *Poecilus).* As a group, carabids at one location were more common in plots without a history of a cover crop. At another location, more carabids were in tilled than nontilled plots. Crickets (Orthoptera: Gryllidae) were more common under no-till conditions. At all locations, wolf spiders (Araneae: Lycosidae) were more common in plots with no tillage and a previous cover crop. Results suggested that surface residues affected carabids, wolf spiders, and crickets. **(U.S.A.)**

15. De La Fuente, E.B., S. Perelman, and C.M. Ghersa. 2010. Weed and arthropod communities in soyabean as related to crop productivity and land use in the Rolling Pampa, Argentina. *Weed Research* 50(6): 561-571.

In the Rolling Pampa, Argentina, changes in crop management caused changes in weed and arthropod communities and reductions in weed diversity in soyabean. Loss of landscape heterogeneity, caused by an increase in the area planted to soyabean, and herbicide treatment of field margins, may affect weed and arthropod assemblages and reduce species richness. This study focused on the effect of land

use in neighbouring fields, weed management of field margins and crop productivity and history on weed and arthropod communities and their richness inside soyabean fields. Weeds and arthropods were surveyed in a total of 60 soyabean fields in 1999, 2001 and 2002. Neighbouring land use was determined in concentric circles of 500 and 1500 m radius around each field using LANDSAT images, and field margin management (sprayed or non-sprayed) was recorded. Data was analysed using regression and canonical correspondence analysis. Cropping history (number of years of cropping) and percentage of soyabean in concentric circles of 1500 m explained 23% of the variation in weed assemblages, whereas management of field margins and soyabean productivity (mean summer Normalised Difference Vegetation Index) explained 23% of the variation in arthropod assemblages. Perennial, dicotyledon and exotic weed richness and non-herbivore arthropod richness decreased with increasing percentage of soyabean in the surrounding landscape. Results show that weed and arthropod communities respond to different production and landscape variables and that increasing the area planted to soyabean and spraying field margins will put weed and arthropod species and functional groups at risk of extinction. **(Argentina)**

16. Dewar A.M., L.A. Haylock, M.J. May, J. Beane, and R.N. Perry. 2000. Glyphosate applied to genetically modified herbicide-tolerant sugar beet and `volunteer' potatoes reduces populations of potato cyst nematodes and the number and size of daughter tubers. *Annals of Applied Biology* 136(3): 179-187.

    Glyphosate, applied early or later or twice to genetically modified glyphosate-tolerant sugar beet, gave excellent control of planted 'volunteer' potatoes growing within the crop compared to conventional herbicide programmes with or without clopyralid. In three out of four trials, this resulted in significant reductions in the numbers of eggs and cysts of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) where infestations were moderate (23-89 eggs g^{-1} soil). In the fourth trial, which had very high initial populations (130 eggs g^{-1} soil), none of the herbicide treatments had any significant effect on numbers of nematode eggs or cysts. This was probably due to competition for feeding sites, and the early death of the potatoes in all treatments caused by feeding damage by the nematodes and infection by blight, which prevented the nematodes from completing their life cycle. Glyphosate also significantly reduced the number and size of daughter tubers produced, thus helping to prevent a further volunteer problem in the next crop in the rotation. This was achieved by one or two applications of one chemical compared to 2-5 applications of cocktails of conventional herbicides. **(United Kingdom)**

17. Druart, C., M. Millet, R. Scheifler, O. Delhomme, and A. de Vaufleury. 2011. Glyphosate and glufosinate-based herbicides: fate in soil, transfer to, and effects on land snails. *Journal of Soils and Sediments* 11(8): 1373-1384.

    The aim of this work was to assess the transfer and effects of two widely used herbicides on the land snail *Helix aspersa* during long-term exposure under laboratory conditions. Newly hatched snails were exposed for 168 days to soil and/or food contaminated with a formulation of glyphosate (Bypass®) or glufosinate (Basta®) at the recommended field doses and also at 10-fold this dose. Herbicide degradation patterns showed that snails were mainly exposed during the first 28

days. The DT50 of glyphosate and glufosinate was established at 10.6 and 3.7 days, respectively. No significant effects on survival and growth were determined. Concerning genital tract maturation of the snails, exposure to herbicides tended to decrease the development of the albumen gland (inhibition of 43.5 ± 32.8%). The presence of glyphosate (6 mg kg^{-1} dry weight) was demonstrated in snails exposed continuously to this active ingredient at the highest concentration in their food. These results showed a low effect of herbicides at relevant concentrations in soil but the detection of residues in tissues indicated a potential risk of transfer to the food chain. This chronic toxicity bioassay could complete the available tests to assess toxicity of contaminants, and more particularly pesticides, in soil. **(France)**

18. Dutra, B.K., F.A. Fernandes, D.M. Failace, and G.T. Oliveira. 2011. Effect of Roundup® (glyphosate formulation) in the energy metabolism and reproductive traits of *Hyalella castroi* (Crustacea, Amphipoda, Dogielinotidae). *Ecotoxicology* 20(1): 255-263.

    Roundup® (glyphosate formulation) is a nonselective and posts emergent herbicide used for controlling aquatic weeds and different concentrations are used in cultures around the world. The objective of this investigation was to examine the effects of Roundup® (glyphosate formulation) on the biochemical composition, levels of lipoperoxidation, Na^+/K^+ ATPase activity and reproductive traits in the *Hyalella castroi*. Amphipods were collected in summer 2009, in the southern Brazilian highlands. In the laboratory, the animals were kept in aquariums under controlled conditions for 7 days, and after this period they were exposed to 0.36, 0.52, 1.08 and 2.16 mg/l of glyphosate for 7 days. After the period of exposure, the animals were immediately frozen for determination of glycogen, proteins, lipids, triglycerides, cholesterol, levels of lipoperoxidation, and Na^+/K^+ATPase activity. During each day of the cultivation reproductive traits (number of reproductive pairs, ovigerous females and eggs in the marsupium) were observed. All concentrations of Roundup® induced significant decreases in all biochemical parameters and Na^+/K^+ ATPase activity, and significant increase in lipoperoxidation levels. Showing this form a potentially toxic effect at very low concentrations, this pattern of results can lead to significant changes in trophic structure of limnic environments because these amphipods are important links in food chain in these habitats. **(Brazil)**

19. Evans, S.C., E.M. Shaw, and A.L. Rypstra. 2010. Exposure to a glyphosate-based herbicide affects agrobiont predatory arthropod behaviour and long-term survival. *Ecotoxicology* 19(7): 1249-1257.

    Humans commonly apply chemicals to manage agroecosystems. If those chemicals influence the behaviour or survival of non-target arthropods, the food web could be altered in unintended ways. Glyphosate-based herbicides are among the most ubiquitous pesticides used around the world, yet little is known about if and how they might affect the success of terrestrial predatory arthropods in agroecosystems. In this study, we quantified the effects of a commercial formulation of a glyphosate-based herbicide on the activity of three predatory arthropod species that inhabit agricultural fields in the eastern United States. We also measured the survival of the most common species. We tested the reactions of the wolf spider, *Pardosa milvina*, to either direct application (topical) or contact with a treated

substrate (residual). We quantified the reactions of a larger wolf spider, *Hogna helluo*, and a ground beetle, *Scarites quadriceps*, to a compound (topical plus residual) exposure. *Pardosa milvina* reduced locomotion time and distance under topical herbicide exposure, but increased speed and non-locomotory activity time on exposed substrate. Both *H. helluo* and *S. quadriceps* increased non-locomotory activity time under compound herbicide exposure. Over a period of 60 days post-exposure, residually exposed *P. milvina* exhibited lower survivorship compared to topically exposed and control groups. Thus, exposure of terrestrial arthropods to glyphosate-based herbicides affects their behaviour and long-term survival. These results suggest that herbicides can affect arthropod community dynamics separate from their impact on the plant community and may influence biological control in agroecosystems. **(U.S.A.)**

20. Griesinger, L.M., S.C. Evans, and A.L. Rypstra. 2011. Effects of a glyphosate-based herbicide on mate location in a wolf spider that inhabits agroecosystems. *Chemosphere* 84(10): 1461-1466.

Chemical communication is important to many arthropod species but the potential exists for anthropogenic chemicals to disrupt information flow. Although glyphosate-based herbicides are not acutely toxic to arthropods, little is known regarding their effects on natural chemical communication pathways. The wolf spider, *Pardosa milvina*, is abundant in agroecosystems where herbicides are regularly applied and uses air- and substrate-borne chemical signals extensively during mating. The aim of this study was to examine effects of a commercial formulation of a glyphosate-based herbicide on the ability of males to find females. In the field, virgin females, when hidden inside pitfall traps with herbicide, attracted fewer males than females with water. Likewise females in traps with a ring of herbicide surrounding the opening were less likely to attract males than those in traps surrounded by water. We explored the reaction of males to any airborne component of the herbicide in a laboratory two-choice olfactometer experiment. When no female pheromones were present, males were equally likely to select herbicide or water treated corridors and they all moved through the apparatus at similar speeds. When female pheromones were present, the males that selected control corridors moved more slowly than those that selected herbicide and, if we control for the initial decision time, more males selected the control corridors over the herbicide. These data suggest that glyphosate-based herbicides are "info-disruptors" that alter the ability of males to detect and/or react fully to female signals. **(U.S.A.)**

21. Haughton, A.J., J.R. Bell, A. Wilcox, and N.D. Boatman. 2001. The effect of the herbicide glyphosate on non-target spiders: Part I. Direct effects on *Lepthyphantes tenuis* under laboratory conditions. *Pest Management Science* 57(11): 1033-1036.

We examined the toxic effects of glyphosate to adult female *Lepthyphantes tenuis* (Araneae, Linyphiidae), a common spider of agricultural habitats. The overspray technique was used to investigate the effect of the herbicide on forty individuals in each of six glyphosate treatments (2160, 1440, 1080, 720, 360 and 180 g ha^{-1}) and a distilled water control. Spiders collected from the wild were individually placed in exposure chambers and checked every 24 h over a 72-h

experimental period. Mortality of *L. tenuis* remained at less than 10% in all treatments at 24 and 48 h after spray application, and only increased marginally (to 13%) after 72 h. These results support other limited data which suggest that glyphosate is 'harmless' to non-target arthropods. More extended laboratory testing to investigate any side-effects of glyphosate on the life history of *L. tenuis* and other non-beneficial invertebrates is required. **(United Kingdom)**

22. Haughton, A.J., J.R. Bell, N.D. Boatman, and A. Wilcox. 2001. The effect of the herbicide glyphosate on non-target spiders: Part II. Indirect effects on *Lepthyphantes tenuis* in field margins. *Pest Management Science* 57(11): 1037-1042.

We have examined the indirect effect of the herbicide glyphosate on the spider *Lepthyphantes tenuis* in field margins. Glyphosate was applied to a randomised block design field experiment comprising 360, 720 and 1440 g glyphosate AE ha^{-1} treatments and an unsprayed control. Spiders were sampled in each month from June to October 1998. Spider abundance was significantly lower in all the treatments than in the unsprayed control. Abundance was also significantly lower in the 720 and 1440 g treatments than in the 360 g treatment. No significant difference could be detected between the 720 and 1440 g treatments. Poisson regression models showed that patterns of decline in *L. tenuis* were related to increasing dead vegetation and decreasing vegetation height. Glyphosate applications only had a within-season indirect habitat effect on *L. tenuis* as field margins sprayed 16 months after an application of 360 g glyphosate ha^{-1} showed no detrimental effect. **(United Kingdom)**

23. Haughton, A.J., G.T. Champion, C. Hawes, M.S. Heard, D.R. Brooks, D.A. Bohan, *et al.* 2003. Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. II. Within-field epigeal and aerial arthropods. *Philosophical Transactions: Royal Society of London B* 358: 1863-1877.

The effects of the management of genetically modified herbicide-tolerant (GMHT) crops on the abundances of aerial and epigeal arthropods were assessed in 66 beet, 68 maize and 67 spring oilseed rape sites as part of the Farm Scale Evaluations of GMHT crops. Most higher taxa were insensitive to differences between GMHT and conventional weed management, but significant effects were found on the abundance of at least one group within each taxon studied. Numbers of butterflies in beet and spring oilseed rape and of Heteroptera and bees in beet were smaller under the relevant GMHT crop management, whereas the abundance of Collembola was consistently greater in all GMHT crops. Generally, these effects were specific to each crop type, reflected the phenology and ecology of the arthropod taxa, were indirect and related to herbicide management. These results apply generally to agriculture across Britain, and could be used in mathematical models to predict the possible long-term effects of the widespread adoption of GMHT technology. The results for bees and butterflies relate to foraging preferences and might or might not translate into effects on population densities, depending on whether adoption leads to forage reductions over large areas. These species, and the detritivore Collembola, may be useful indicator species for future studies of GMHT management. **(United Kingdom)**

24. Hawes, C., A.J. Haughton, J.L. Osborne, D.B. Roy, S.J. Clark, J.N. Perry, *et al.* 2003. Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide–tolerant crops. *Philosophical Transactions of the Royal Society of London B* 358: 1899-1913.

    Effects of genetically modified herbicide-tolerant (GMHT) and conventional crop management on invertebrate trophic groups (herbivores, detritivores, pollinators, predators and parasitoids) were compared in beet, maize and spring oilseed rape sites throughout the UK. These trophic groups were influenced by season, crop species and GMHT management. Many groups increased twofold to fivefold in abundance between early and late summer, and differed up to 10-fold between crop species. GMHT management superimposed relatively small (less than twofold), but consistent, shifts in plant and insect abundance, the extent and direction of these effects being dependent on the relative efficacies of comparable conventional herbicide regimes. In general, the biomass of weeds was reduced under GMHT management in beet and spring oilseed rape and increased in maize compared with conventional treatments. This change in resource availability had knock-on effects on higher trophic levels except in spring oilseed rape where herbivore resource was greatest. Herbivores, pollinators and natural enemies changed in abundance in the same directions as their resources, and detritivores increased in abundance under GMHT management across all crops. The result of the later herbicide application in GMHT treatments was a shift in resource from the herbivore food web to the detritivore food web. The Farm Scale Evaluations have demonstrated over 3 years and throughout the UK that herbivores, detritivores and many of their predators and parasitoids in arable systems are sensitive to the changes in weed communities that result from the introduction of new herbicide regimes. **(United Kingdom)**

25. Hough-Goldstein, J.A., M.J. Vangessel, and A.P. Wilson. 2004. Manipulation of weed communities to enhance ground-dwelling arthropod populations in herbicide-resistant field corn. *Environmental Entomology* 33(3): 577-586.

    Herbicide treatments were used in glyphosate-resistant field corn, *Zea mays* L., to produce treatments with weeds growing for varying periods of time in a replicated field trial conducted in three different fields over 3 yr. Increased weediness increased the activity-density of *Harpalus pensylvanicus* (DeGeer) (Coleoptera: Carabidae), the most common carabid species collected in pitfall traps. Field crickets, *Gryllus* spp. (Orthoptera: Gryllidae), showed a similar response, with generally higher numbers caught in weedier plots. Other ground-dwelling arthropod species showed variable responses to weediness. Wolf spiders (Lycosidae) showed an apparent response to fresh organic matter and dead weed thatch after herbicide treatments in 2002. Although several arthropods common in the field plots ate second instar western corn rootworms, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), when the rootworms were presented to them on filter paper in the laboratory, rootworms were not consumed when presented to potential predators under a thin layer of soil. Predation on exposed larvae placed in the field for 24 h did not differ by treatment, possibly because the major species that differed by treatment were primarily herbivorous or omnivorous. Corn yields were lower in the weedy check plots all 3 yr, but no significant reduction in yield occurred in treatments with

weeds present for 21, 31, or 41 d after planting, or in plots treated with preemergence herbicide only. **(U.S.A.)**

26. Imura, O., K. Shi, K. Limura, and T. Takamizo. 2010. Assessing the effects of cultivating genetically modified glyphosate-tolerant varieties of soybeans (*Glycine max* (L.) Merr.) on populations of field arthropods. *Environmental Biosafety Research* 9(2): 101-112.

We assessed the effects of cultivating two genetically modified (GM) glyphosate-tolerant soybean varieties (*Glycine max* (L.) Merr.) derived from Event 40-3-2 and a Japanese conventional variety on arthropods under field conditions, with weed control using glyphosate and conventional weed control for two years. Plant height and dry weight of the conventional variety were significantly larger than those of the GM varieties, but the GM varieties bore more pods than the conventional variety. We found arthropods of nine taxonomic orders (Araneae, Acari, Thysanoptera, Homoptera, Heteroptera, Coleoptera, Diptera, Lepidoptera, and Hymenoptera) on the plants. The arthropod incidence (number per plant unit weight pooled for each taxonomic order) on the soybean stems and leaves generally did not differ significantly between the GM and conventional varieties. However, the incidence of Thysanoptera and total incidence (all orders combined) were greater on the GM variety in the second year. The weed control regimes had no significant influence on the arthropod incidence on the soybean stems and leaves. The number of flower-inhabiting Thysanoptera (the dominant arthropod in the flowers) was not significantly different between the GM and conventional varieties. *Asphondylia yushimai* (Diptera, Cecidomyiidae) was more numerous on the pods of the GM variety in both years. Neither the soybean variety nor the weed control regime significantly affected the density of soil macro-organisms. However, the glyphosate weed control affected arthropods between the rows of plants by decreasing the abundances of Homoptera, Heteroptera, Coleoptera and Lepidoptera, and diversity of arthropods. **(Japan)**

27. Jackson, R.E. and H.N. Pitre. 2004. Influence of Roundup Ready® soybean production systems and glyphosate application on pest and beneficial insects in wide-row soybean. *Journal* of *Agricultural and Urban Entomology* 21(2): 61-70.

Roundup Ready® soybean, *Glycine max* (L.) Merrill, in widerow planting systems were investigated in 1997 and 1998 in Mississippi to determine effects of the transgenic crop and glyphosate herbicide on pest and beneficial insects. Populations of adult bean leaf beetle, *Cerotoma trifurcate* (Forster), and threecornered alfalfa hopper, *Spissistilus festinus* (Say), and larvae of green cloverworm, *Hypena scabra* (F.), and velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner), were not affected by genetically altered Roundup Ready soybean or by applications of glyphosate. Numbers of adult big-eyed bug, *Geocoris punctipes* (Say), also were not affected by the transgenic soybean but were influenced by glyphosate applications in 1 of 2 years. *G. punctipes* adults were not directly affected by glyphosate applications, but glyphosate indirectly influenced *G. punctipes* densities in 3 of 11 weeks. These effects were attributed to increased weed densities having a positive effect on *G. punctipes* numbers during this 3-week period. Increased numbers of *G. punctipes* were also found in soybeans receiving delayed applications of glyphosate compared to those receiving glyphosate sprays at recommended times in 1 of 2 years. These

elevated numbers, however, were also related to higher densities of weeds. The results presented herein demonstrated that the Roundup Ready soybean system, including applications of glyphosate, had no detrimental effects on pest and beneficial insects in wide-row soybean plantings. Knowledge of the influence of herbicide-tolerant cropping systems on the occurrence, density, and behavior of pest and beneficial insects in soybean should be beneficial in making future insect pest management decisions. **(U.S.A.)**

28. Jadhav, A., M. Hill, and M. Byrne. 2008. Identification of a retardant dose of glyphosate with potential for integrated control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach. *Biological Control* 47(2): 154-158.

See Biodiversity and Restoration Section.

29. Jasinski, J.R., J.B. Eisley, C.E. Young, J. Kovach, and H. Willson. 2003. Select nontarget arthropod abundance in transgenic and nontransgenic field crops in Ohio. *Environmental Entomology* 32(2): 407-413.

Nontarget arthropod populations were monitored in both transgenic and nontransgenic corn and soybean fields in western and central Ohio. A total of 24 fields, 12 corn and 12 soybean, were inspected weekly from late June through mid August. Half of the cornfields were *Bacillus thuringiensis* (Bt) hybrids and half of the soybean fields were Roundup Ready (RR). Sweep net samples in soybean fields and unbaited Pherocon AM yellow sticky traps in both soybean and cornfields were used to collect nontarget arthropods. Soil samples were also taken from all 24 fields to compare soil mite populations. A select group of 15 readily identifiable arthropods composed mainly of beneficial insects commonly found in field crops, were recorded in weekly sweep net and sticky trap samples for later analysis. Sweep net data from soybean fields revealed no significant differences in nontarget arthropods. Yellow sticky trap data from soybean and corn fields found significantly more green lacewing adults (*Chrysopa* spp.) in six pooled non-RR soybean fields, significantly more rove beetles in one non-Bt cornfield, and significantly more *Orius* spp. in one Bt cornfield. There were also significantly more soil inhabiting mites at one non-RR soybean field. Based on the abundance of these arthropod populations in paired transgenic and nontransgenic fields, few negative effects on the 15 nontarget arthropods selected can be directly associated with transgenic soybean and corn crops in Ohio. **(U.S.A.)**

30. Kaneda, S., S. Okano, Y. Urashima, T. Murakami, and M. Nakajima. 2009. Effects of herbicides, glyphosate, on density and casting activity of earthworm, *Pheretima* (*Amynthas*) *carnosus*. *Japanese Journal of Soil Science and Plant Nutrition* 80(5): 469-476.

Effects of an herbicide, commercial formulation of glyphosate (Roundup), on earthworm cast production on the surface in no-tillage field were investigated by using field and pot experiments. Direct impacts of the herbicide on earthworm mortality, growth and behavior were examined in pot experiment. In field experiments, effects of the herbicide on earthworm density, earthworm casting activity and soil moisture, and effects of litter removing after an application of herbicide on earthworm casting activities. Three treatments, glyphosate application

on fall, application on spring, and no application, were set up in no-till field for the experiment, herbicide impacts on earthworm activity and behavior. Separately three treatments, litter removal treatment after herbicide application, just herbicide application and control, were set up for the experiment, litter removal impact on casting activity. No herbicide impacts on earthworm density were observed, and only one species, *Pheretima* (*Amynthas*) *carnosus* was dominated (95%) in the experiment field. There was no effect of applying herbicide on growth, mortality and behavior of the dominated earthworm in the pot experiment. Herbicide application induced not only increasing earthworm casting activity in spring (p<0.05) and also decreasing the activity in summer (p<0.05). Multiple linear regression analysis revealed that the soil temperature and the soil moisture were important factors for the earthworm cast production rate (p<0.01). Importance of surface litter for the casting activity was observed (p<0.05). This earthworm species produced around 3 kg m^{-2} per year in no-till system. From these results, the herbicide did not have direct harmful impacts on the earthworm, but would effect on earthworm casting activity through variation of environmental factors, such as litter amount, soil temperature, and soil moisture. **(Japan)**

31. Kleter, G.A., C. Harris, G. Stephenson, and J. Unsworth. 2008. Comparison of herbicide regimes and the associated potential environmental effects of glyphosate-resistant crops versus what they replace in Europe. *Pest Management Science* 64(4): 479-488.

While cultivation of transgenic crops takes place in seven of the EU member states, this constitutes a relatively limited part of the total acreage planted to these crops worldwide. The only glyphosate-resistant (GR) crop grown commercially until recently has been soybean in Romania. In addition, large-scale experimental European data exist for GR sugar and fodder beets, and, to a lesser extent, GR oilseed rape. These GR crops are likely to have an impact both on the use of herbicides and on the environmental impact of the latter. From the data on these GR crops, it appears that quantities of herbicides applied to GR beets are decreased while those on GR soybean are slightly increased compared with their conventional counterparts. Depending on the parameters used for prediction or measurement of environmental impacts of GR crops, generally similar or less negative impacts were observed compared with conventional crops. Favourable environmental effects of the glyphosate-containing herbicide regimes on GR crops appear feasible, provided appropriate measures for maintaining biodiversity and prevention of volunteers and gene flow are applied. **(The Netherlands)**

32. Lindgren, C.J., T.S. Gabor, and H.R. Murkin. 1999. Compatibility of glyphosate with *Galerucella calmariensis*; a biological control agent for purple loosestrife (*Lythrum salicaria*). *Journal of Aquatic Plant Management* 37: 44-48.

See Biodiversity and Restoration Section.

33. Lindsay, E.A. and K. French. 2004. The impact of the herbicide glyphosate on leaf litter invertebrates within Bitou bush, *Chrysanthemoides monilifera* ssp *rotundata*, infestations. *Pest Management Science* 60(12): 1205-1212.

Chrysanthemoides monilifera ssp *rotundata* (L) T Norl (Bitou bush) is a serious environmental weed along the southeast coast of Australia. The herbicide glyphosate is commonly used to control *C monilifera* on the New South Wales coastline, but there have been few studies examining the effects of this herbicide on invertebrate communities in the field, especially on sand dunes. Control and impact sites were selected in coastal hind dunes heavily infested with *C monilifera* , and the impact sites were sprayed with a 1:100 v/v dilution of glyphosate- isopropyl 360 g AE litre^{-1} SL (Roundup Biactive). Leaf litter invertebrates were sampled before spraying and after spraying by collecting fixed areas of leaf litter in both the control and impact sites. Samples were sorted for particular invertebrates involved in leaf litter decomposition and some of their predators. This study did not identify any significant direct or indirect effects on leaf litter invertebrate abundance or community composition in the four months following herbicide application. The litter invertebrate assemblages were highly variable on a small spatial scale, with abiotic factors more strongly regulating leaf litter invertebrate numbers than glyphosate application. These results conflict with previous studies, indicating that the detrimental indirect effects herbicide application has on non- target litter invertebrates may depend upon the application rate, the vegetation community and structure and post- spray weather. **(Australia)**

34. Lins, V.S., H.R. Santos, and M.C. Gonçalves. 2007. The effect of the glyphosate, 2,4-D, atrazine e nicosulfuron herbicides upon the Edaphic collembola (Arthropoda: Ellipura) in a no tillage system. *Neotropical Entomology* 36(2): 261-267.

    The use of herbicides is a common and intensive practice in no tillage systems. The herbicides can influence, directly or indirectly, the population of edaphic arthropods. Collembola is a group that functions as a bio-indicator of soil conditions. The degree of abundance and diversity of Collembola provides the level of soil disturbance provoked by agricultural practices. This experiment was designed to compare the influence of herbicides on the population fluctuation of Collembola in a no-till soil preparation system. The work was conducted in a non irrigated no-till area at the Núcleo Experimental de Ciências Agrárias of the Universidade Federal de Mato Grosso do Sul (UFMS), Campus de Dourados, in soil planted with corn as a surface covering, during the period of December, 2002 to December, 2003. The data were analyzed according to a completely randomized model, in a split plot design. The plots received four types of herbicides: glyphosate, atrazine, 2,4-D and nicosulfuron. A fifth plot did not receive any herbicide (control), for a total of five treatment types. The sub plots were represented by their collection times (10, 20, 30 and 40 days after the herbicide applications). Both the type of herbicide and the time of data sampling influenced the Collembola population fluctuation. The treatments with atrazine and 2,4-D caused the most reduction of the population of Collembola, depending on the time of application. **(Portugal)**

35. Londo, J.P., M.A. Bollman, C.L. Sagers, E.H. Lee, and L.S. Watrud. 2011. Changes in fitness-associated traits due to the stacking of transgenic glyphosate resistance and insect resistance in *Brassica napus* L. *Heredity* 107(4): 328-337.

    See Biodiversity and Restoration Section.

36. Lü, L.L., G. Zhang, Y. Li, X. Gao, and W. Fu. 2010. Effects of glyphosate on the growth and development of *Agasicles hygrophila*. *Journal of South China Agricultural University* 31(1): 22-25.

The experiment was designed to assess the effects of different concentrations of glyphosate on the development of *Agasicles hygrophila*. The treatment mass concentrations of glyphosate for eggs, larvae, pupae and adults were 0.41, 0.82, 1.23, 1.64 and 2.05 g/L, respectively. There was no difference in the hatching rate of eggs between the glyphosate treatment and the control group with the concentrations of glyphosate. However, the survival rates of larvae and adults treated by glyphosate were significantly lower than those in the control group. The survival rates of larvae and adults in the control group were 1.59- and 1.31-fold of that in the treated group with 2.05 g/L glyphosate, respectively. **(China)**

37. Marasas, M.E., S.J. Sarandón, and A.C. Cicchino. 2001. Changes in soil arthropod functional group in a wheat crop under conventional and no tillage systems in Argentina. *Applied Soil Ecology* 18(1): 61-68.

Different functional groups of soil arthropodofauna present in the agro-ecosystem can be severely modified by tillage practices. The abundance of different trophic groups subject to conventional tillage (CT) and no tillage (NT) practices were evaluated compared to a natural field boundary (FB) in a wheat crop. Arthropods were captured using pitfall traps and collected every 20 days during 10 months, and grouped according to their habits in predators, phytophagous and detritivorous. Tillage systems affected the abundance of arthropod fauna and the proportion between different functional groups as regards a FB. Predators constituted the most abundant group of all arthropods captured, and their number was higher under NT than under CT. In CT, an increase in predators was observed only in spring, probably associated with a recolonisation from the adjacent plots of NT. Phytophagous was the least representative group in the three evaluated systems, it was higher under cropped plots (NT and CT) than in the FB, and was not affected by tillage practices.

Under NT, the number of predators, remained higher along the crop development. Phytophagous activity in no till plots remains constant. In FB, the number of captured individuals was lower and relatively stable along the whole sampling period than in the cultivated plots.

Ploughing action (CT plots) provoked a decrease in the relative importance of predators and an increase in phytophagous as regards those in NT and FB. Total biomass of captured arthropod fauna showed significant differences between treatments, being higher in NT and lower in CT plots.

It is concluded that tillage systems affect not only the abundance of arthropod fauna but also the proportion between different functional groups. The consequences of these changes for soil quality are discussed later on. **(Argentina)**

38. May, M.J., G.T. Champion, A.M. Dewar, A. Qi, and J.D. Pidgeon. 2005. Management of genetically modified herbicide-tolerant sugar beet for spring and autumn environmental benefit. *Proceedings of the Royal Society B* 272: 111-119.

See Birds Section.

39. Michalková, V. and S. Pekár. 2009. How glyphosate altered the behaviour of agrobiont spiders (Araneae: Lycosidae) and beetles (Coleoptera: Carabidae). *Biological Control* 51(3): 444-449.

Cultivation of herbicide-tolerant strain of GM corn involves applications of a non-selective herbicide. Mortality of arthropod natural enemies resulting from the application of herbicides is negligible. But nothing is known how herbicides affect the behaviour of natural enemies and thus alter their pest control efficacy. Aim of the study was to assess the effect of the Roundup residues on the predatory, defensive, locomotory and reproductive behaviour of epigeic spiders and carabid beetles. Specimens of *Pardosa agricola* (Araneae: Lycosidae) and *Poecilus cupreus* (Coleoptera: Carabidae) were exposed for 2 h to the fresh and 1-day old residues of Roundup Biaktiv (Monsanto, IPA 480 g/l). Predation rate was similar for all treatments in both spiders and beetles. Spiders did not avoid surface treated with herbicide residues more than the control surface, but beetles slightly avoided surface with fresh residues. The speed of locomotion of spiders was not altered by herbicide residues, but beetles exposed to residues crawled at significantly lower speed than the control group. Herbicide residues did not affect the escape efficacy of spiders from a predator. Residues had no detrimental effect on the courtship, mating frequency and duration in spiders. Roundup Bioaktiv thus appears to be harmless to lycosid spiders and only slightly harmful to carabid beetles. The biological control potential of both predators should not be reduced by the application of Roundup Bioaktiv. **(Czech Republic)**

40. Mirande, L., M. Haramboure, G. Smagghe, S. Piñeda, and M.I. Schneider. 2010. Side-effects of glyphosate on the life parameters of *Eriopis connexa* (Coleoptera: Coccinelidae) in Argentina. *Communications in Agriculture and Applied Biological Sciences* 75(3): 367-372.

In Argentina, transgenic soybean crop (Roundup Ready, RR) has undergone a major expansion over the last 15 years, with the consequent increase of glyphosate applications, a broad-spectrum and post emergence herbicide. Soybean crops are inhabited by several arthropods. *Eriopis connexa* Germar (Coleoptera: Coccinelidae) is a predator associated to soybean soft-bodies pest and have a Neotropical distribution. Nowadays, it is being considered a potentially biological control agent in South America. The objectives of this work were to evaluate the side-effects of glyphosate on larvae (third instar) and adults of this predator. Commercial compound and the maximum registered concentrations for field use were employed: GlifoGlex 48 (48% glyphosate, 192 mg a.i./litre, Gleba Argentina S.A.). The exposure was by ingestion through the treated prey (*Rophalosiphum padi*) or by drinking treated water during 48 h for treatment of the adult. The herbicide solutions were prepared using distilled water as solvent. The bioassays were carried out in the laboratory under controlled conditions: 23 ± 0.5 degrees C, 75 ± 5% RH and 16:8 (L:D) of photoperiod. Development time, weight of pupae, adult emergence, pre-oviposition period, fecundity and fertility were evaluated as endpoints. Larvae from glyphosate treatment molted earlier than controls. In addition, the weight of pupae, longevity, fecundity and fertility were drastically reduced in treated organisms. The reductions were more drastic when the treatments were performed at the third larval stage than as adult. The reproduction capacity of the predator was the most affected

parameter and could be related to a hormonal disruption by glyphosate in the treated organisms. This work can confirm the deleterious effects of this herbicide on beneficial organisms. Also, it agrees with prior studies carried out on other predators associated to soybean pest, such as *Chrysoperla externa* (Neuroptera: Chrysopidae) and *Alpaida veniliae* (Araneae: Araneidae). **(Argentina)**

41. Morowati, M. 2000. Histochemical and histopathological study of the intestine of the earthworm (*Pheretima elongata*) exposed to a field dose of the herbicide glyphosate. *The Environmentalist* 20(2): 105-111.

Histopathological and histochemical variations in non-specific esterases of the intestinal epithelial lining of the earthworm (*Pheretima elongata*), exposed to a single field dose of the herbicide glyphosate, were studied on the first, second, third, and fourth week of exposure. A severe cell death was observed in the intestine during the first and second weeks of exposure and mortality exceeded 50 percent. In the third week of exposure, the cells of the intestinal lining of the worms which had survived started regenerating and in the fourth week of exposure the epithelial lining regained its original characteristics and architecture, suggesting the high regenerative power of earthworms. Non-specific esterases showed an intense activity in the first week of the exposure and then decreased in the second week to nearly a minimum, where the entire cell structure was lost. However, the activity of the enzyme remained low until the third week where there was a small rise in the activity, and was confined to certain places in the cytoplasm. In the fourth week, almost a complete regeneration of the epithelial lining as well as the non-specific esterases activity was observed and became quite similar to that of the control worms. These results suggest that glyphosate, even at the recommended field dose, could cause cell death and interfere with non-specific esterases activity of the epithelial lining of the intestine of *P. elongata* causing at least 50 percent mortality in the population of the worms. **(Iran)**

42. Nakamura, A., C.P. Catterall, R.L. Kitching, A.P.N. House, and C.J. Burwell. 2008. Effects of glyphosate herbicide on soil and litter macro-arthropods in rainforest: Implications for forest restoration. *Ecological Management & Restoration* 9(2): 126-133.

Ecological restoration activities, including reforestation, often involve the use of herbicides for the removal of weedy plant cover. Little is known, however, about the effects of herbicides on assemblages of non-target organisms that colonize restored patches. We describe a field experiment to investigate effects of glyphosate herbicide (Roundup® Biactive™) on rainforest-associated soil- and litter-dwelling macro-arthropods. Our experimental protocol differed in two ways from other ecotoxicological studies of herbicides. First, we applied herbicide at a rate considerably greater than the manufacturer's recommended maximum in order to simulate worst-case scenarios that may occur in the practice of forest restoration. Second, our field experiment was carried out under dense canopy cover with sparse understorey vegetation, so that indirect impacts caused by the loss of existing vegetation were eliminated. Paired herbicide-treated and control plots were created within five rainforest remnants on the Maleny plateau of subtropical eastern Australia. Macro-arthropods were collected using litter extraction before,

approximately 3 days after, and 3 months after herbicide application. Responses of arthropods were analysed at two levels of taxonomic resolution: 'coarse' arthropods (arthropods sorted to Order/Class), and ant species. Our results suggest that the use of glyphosate herbicide formulated as Roundup® Biactive™ is suitable for the control of unwanted plants in rainforest restoration sites as it appears to have minimal impact on assemblages of soil and litter macro-arthropods or at least those typical of intact rainforest. **(Australia)**

43. Noel, G.R., Wax, L.M. 2004. *Heterodera glycines* population development on soybean treated with glyphosate. Journal of Nematology 36: 337-338.

A concern when planting genetically modified crops is the potential effect of the technology on nontarget organisms. Glyphosate translocates to the soybean root where the herbicide might affect the biology of *Heterodera glycines*. In 2002 and 2003 Roundup Ready® soybean DSR 320 susceptible to *H. glycines* and DSR 327 resistant to *H. glycines* were grown in field plots in two different fields and either sprayed with glyphosate at the recommended rate and time of application or not sprayed. The experiment was arranged in a randomized complete block design with 12 replications. Nematode reproduction (Pf/Pi) and soybean yield were determined. In both years Pi was below the damage threshold, ranging from 72 to 205 eggs/100 cm^3 soil in 2002 and from 16 to 133 eggs/100 cm^3 soil in 2003. In both years Pf/Pi was greater ($P < .0001$) on DSR 320 when compared with DSR 327. Differences in Pf/Pi between DSR 320 treated or not treated with glyphosate were significant at $P = 0.08$ in both 2002 and 2003. There were no significant differences in Pf/Pi between DSR 327 treated with glyphosate or not treated. Soybean yield was not affected either by *H. glycines* or treatment with glyphosate. Whether or not glyphosate application increases populations of *H. glycines* is not clear. Additional studies in fields with larger numbers of *H. glycines* at planting are needed. **(U.S.A.)**

44. Noel, G.R., Wax, L.M. 2009. *Heterodera glycines* population development on soybean treated with glyphosate. *Nematropica* 39(2): 247-253.

Soybean cyst nematode (*Heterodera glycines*) is a major yield limiting pest in all major soybean producing countries. In the last decade genetically modified soybean tolerant to glyphosate has become widely planted and postemergence application of glyphosate has increased exponentially. Genetically modified crops may affect nontarget microorganisms, either directly or indirectly as in the case of glyphosate application to tolerant crops. Glyphosate translocates to soybean roots where it might affect metabolic sinks such as syncytia produced by *Heterodera glycines*. In 2002-2004 glyphosate tolerant soybean DSR 320 susceptible to *H. glycines* and DSR 327 resistant to *H. glycines* were grown in three different fields and either sprayed with glyphosate at the recommended rate and time of application or not sprayed. Nematode reproduction and soybean yield were determined. In all three years, Pf and Pf/Pi were significantly greater on DSR 320 when compared with DSR 327. In 2004, when Pi exceeded the damage threshold, there was a cultivar × glyphosate interaction for Pf on DSR 320 with greater numbers of eggs recovered from glyphosate treated DSR 320. In 2002 and 2003 Pi was below the damage threshold and no differences in yield occurred between the resistant and susceptible cultivars. However, in 2003 a significant cultivar × glyphosate interaction was

observed for yield of DSR 320. In 2004 a significant difference in yield between DSR 320 and DSR 327 was observed. Glyphosate application affected Pf of *H. glycines* eggs in 2004, the only year Pi exceeded the damage threshold. However, the increase Pf did not translate into crop loss. **(U.S.A.)**

45. Rieux, R., S. Simon, and H. Defrance. 1999. Role of hedgerows and ground cover management on arthropod populations in pear orchards. *Agriculture, Ecosystems and Environment* 73(2): 119-127.

With the failure of conventional chemical control against the pear tree psyllid *Cacopsylla pyri* (L.) (Homoptera: Psyllidae), strategies have been developed to enhance natural arthropod enemies of this pest. Modifying the vegetation adjacent to pear orchards is one integrated pest management (IPM) practice that can increase the agroecosystem plant diversity, thus favoring a natural balance between pest arthropods and their enemies. Different plant species were examined as sources of beneficial arthropods for the pear orchard. Arthropod faunas were sampled in the pear tree (*Pyrus communis* L.) canopy, the ground cover, and in the hedgerow tree species surrounding the orchard. Inter-relationships between pear and hedgerow tree species were studied by Spearman's rank correlation. Ash trees and ivy display a diversified fauna that was correlated with the pear tree community, but not with each other. Their influence on pear tree fauna is different: ash trees host specific psyllids and gall midges (Diptera: Cecidomyiidae), providing food for beneficial pear arthropods and ivy acts as a shelter species for beneficial pear arthropods. Calculation of correlations between the faunas of non-crop and crop plant species appears as a useful tool for estimating the risk (correlation between phytophagous guilds) and benefit (correlation between beneficial guilds) of plants dedicated to environmental crop management.

The following orchard ground covers were tested: (1) bare ground; (2) natural grass cover; and (3) sown ground cover. Multivariate analysis was performed to characterize ground cover arthropod communities. Natural ground cover and sown ground cover sheltered distinct arthropod communities; the natural ground cover beneficial fauna was characterized by spiders and the sown ground cover fauna was characterized by ants. The structure and numbers of the arthropod assemblages collected on pear trees from the three types of ground management were different. The main beneficial arthropods on pear tree were Anthocoridae (Heteroptera) and Miridae (Heteroptera) in the sown area; Empididae (Diptera) and Miridae in the natural ground cover area; and Forficulidae (Dermaptera) and Miridae in the bare ground area. Moreover, the sown ground cover favored a higher beneficial : phytophagous ratio to that in the pear tree canopy. These results imply that interactions are likely to occur between pear trees and hedgerow, and between pear trees and ground cover within the orchard agroecosystem. Manipulating beneficial arthropods through vegetal environment management using selected tree and grass species shows promise in optimizing pear orchard IPM. **(France)**

46. Roy, D.B., D.A. Bohan, A.J. Haughton, M.O. Hill, J.L. Osborne, S.J. Clark, *et al.* 2003. Invertebrates and vegetation of field margins adjacent to crops subject to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified

herbicide-tolerant crops. *Philosophical Transactions of the Royal Society of London* Series B 358: 1879-1898.

The effects of management of genetically modified herbicide-tolerant (GMHT) crops on adjacent field margins were assessed for 59 maize, 66 beet and 67 spring oilseed rape sites. Fields were split into halves, one being sown with a GMHT crop and the other with the equivalent conventional non-GMHT crop. Margin vegetation was recorded in three components of the field margins. Most differences were in the tilled area, with fewer smaller effects mirroring them in the verge and boundary. In spring oilseed rape fields, the cover, flowering and seeding of plants were 25%, 44% and 39% lower, respectively, in the GMHT uncropped tilled margins. Similarly, for beet, flowering and seeding were 34% and 39% lower, respectively, in the GMHT margins. For maize, the effect was reversed, with plant cover and flowering 28% and 67% greater, respectively, in the GMHT half. Effects on butterflies mirrored these vegetation effects, with 24% fewer butterflies in margins of GMHT spring oilseed rape. The likely cause is the lower nectar supply in GMHT tilled margins and crop edges. Few large treatment differences were found for bees, gastropods or other invertebrates. Scorching of vegetation by herbicide-spray drift was on average 1.6% on verges beside conventional crops and 3.7% beside GMHT crops, the difference being significant for all three crops. **(United Kingdom)**

47. Santos, M.J.G., R. Morgado, N.G.C. Ferreira, A.M.V.M. Soares, and S. Loureiro. 2011. Evaluation of the joint effect of glyphosate and dimethoate using a small-scale terrestrial ecosystem. *Ecotoxicology and Environmental Safety* 74(7): 1994-2001.

In the present work a small-scale terrestrial ecosystem (STEM) containing a soil collected from an agricultural field in Central Portugal was used to evaluate the effects of the combination of the herbicide glyphosate and the insecticide dimethoate. Earthworms (*Eisenia andrei*), isopods (*Porcellionides pruinosus*), turnip seeds (*Brassica rapa*), and bait-lamina strips were placed in the STEM. The results showed that the application of the recommended field dose of both pesticides did not cause any effect on the weight variation of earthworms and growth of the plants. The application of the herbicide, even at 5 and 10 times the field dose, increased feeding activity in soil (bait-lamina test), although the application of dimethoate led to a decrease in feeding activity in all concentrations tested. The binary mixtures performed showed that according to the Independent Action model, synergism (higher effect than expected from the single exposures) was observed in both the shoot length and fresh weight of *B. rapa* at 5 times the field dose, but antagonism was observed at 10 times the field dose. Regarding the germination success, synergism was observed at the field dose, but antagonism was detected at 5 times and 10 times the field dose. There was a decrease on the earthworm's weight in all concentrations tested, although no statistical differences were observed in any of the treatments made. Regarding depth distribution of *E. andrei*, worms were found in the upper layer more than it was predicted for all concentrations. In the mixtures with the field and 5 times the field dose there was a decrease in the feeding activity (bait-lamina consumption) by the soil fauna. From the four biomarkers assessed on the isopods (Catalase, Acetylcholinesterase, Glutathione-S-transferase, and Lipid peroxidation), only a significant decrease in the Acetylcholinesterase activity upon dimethoate and the binary mixtures exposures performed with the field dose was

observed and on Lipid peroxidation at the field doses of single and binary exposures. **(Portugal)**

48. Showler, A.T. and S.M. Greenberg. 2003. Effects of weeds on selected arthropod herbivore and natural enemy populations, and on cotton growth and yield. *Environmental Entomology* 32(1): 39-50.

Vegetative diversification with weeds can enhance natural enemy populations and suppress pest-related damage in various crops. Weedy and weed-free cotton (*Gossypium hirsutum* L.) plots were used to study the effects of weediness on selected herbivorous arthropod groups, including the boll weevil (*Anthonomus grandis grandis* Boheman), and natural enemies, boll weevil-induced injury to cotton squares, and cotton plant growth and yield in the Lower Rio Grande Valley of Texas, during 2000 and 2001. The presence of weeds was associated with greater populations of 9 of the 11 prey arthropod groups, and 9 of the 13 natural enemy arthropod groups counted in this study. These trends were mostly evident late in the season when weed biomass was greatest. Weed-free cotton harbored more cotton aphids (*Aphis gossypii* Glover), early in the season and silverleaf whiteflies (*Bemisia argentifolii* Bellows and Perring) later in the season than weedy cotton on some of the sampling dates. Diversity (Shannon's index) within the selected arthropod groups counted in this study was significantly greater in dvac samples from the weed foliage than from weed-free cotton plants during both years, and diversity on weedy cotton plants was greater than on weed-free cotton plants during 2000. Boll weevil oviposition injury to squares was unaffected by weeds, but the higher weed-associated predator populations mainly occurred after most squares had become less vulnerable bolls. Weed competition resulted in lower lint yields of 89% and 32% in the 2 yr. **(U.S.A.)**

49. Sirrine, J.R., D.K. Letourneau, C. Shennan, D. Sirrine, R. Fouch, L. Jackson, *et al.* 2008. Impacts of groundcover management systems on yield, leaf nutrients, weeds, and arthropods of tart cherry in Michigan, USA. *Agriculture, Ecosystems and Environment* 125(1): 239-245.

Three different assemblages of cover crops were planted, fertilizer and herbicide input reduced, and mulch side-delivered in a northern Michigan tart cherry orchard to evaluate effects on tree leaf nutrients, arthropod communities, weed control, and tart cherry yield. The three treatments were compared against the conventional management system, which consisted of sod alleys and herbicide-maintained tree rows. Although fertilizer was reduced by 1/2 and herbicides eliminated in the ground cover management systems (GMSs), there was no evidence of reduced cherry yields. All GMSs provided more plant cover than the unmanipulated sod control. Biomass samples suggested that there was not enough side-delivery mulch to significantly reduce understory weed growth in the tree rows when compared to the non-mulched plots. The GMSs increased species richness, abundance, and diversity (*H'*) of arthropods, despite frequent pesticide applications in all treatments. The results of the economic analysis suggest that cover cropping, half N fertilizer, and herbicide elimination may be a viable alternative to conventional groundcover management in northern Michigan. **(U.S.A.)**

50. Smith, H., L.G. Firbank, and D.W. Macdonald. 1999. Uncropped edges of arable fields managed for biodiversity do not increase weed occurrence in adjacent crops. *Biological Conservation* 89(1): 107-111.
See Biodiversity and Restoration Section.

51. Solomon, K.R., A. Anadón, G. Carrasquilla, A.L. Cerdeira, and J. Marshall, and L.-H. Sanin. 2007. Coca and poppy eradication in Colombia: environmental and human health assessment of aerially applied glyphosate. *Reviews of Environmental Contamination and Toxicology* 190: 43-125.
See Human Health Section.

52. Stamps, W.T., T.W. Woods, M.J. Linit, and H.E. Garrett. 2002. Arthropod diversity in alley cropped black walnut (*Juglans nigra* L.) stands in eastern Missouri, USA. *Agroforestry Systems* 56(2): 167-175.
Knowledge of the complex interactions among trees, crops and their associated fauna is necessary to determine the viability of a particular agroforestry practice. Information is lacking concerning these interactions, particularly in temperate agroforestry practices. We examined the effects of two forages on the growth, nut production, and arthropod communities of alley cropped eastern black walnut, *Juglans nigra* L. Experimental plots of eastern black walnut, intercropped with alfalfa, *Medicago sativa* L., smooth bromegrass, *Bromis inermis* Leyss., or no vegetation were sampled with sweep nets prior to each cutting date for the forages. Comparisons were made between treatments and sampling dates. Tree growth measurements, nut yield and other nut quality measurements were taken at the end of each growing season. There were no differences in tree growth among alleyway treatments. The first season's nut yield was greater from trees with vegetation-free alleyways; otherwise nut production did not differ among the treatments. Arthropods were more numerous and diverse in alley cropped alfalfa than in alley cropped bromegrass or in the vegetation-free controls. Alley cropped bromegrass supported a more diverse population of arthropods than did the vegetation-free control. Arthropod diversity in the tree canopies did not differ among treatments. Alley cropped forages supported a more diverse and even arthropod fauna than did adjacent monocropped forages. We conclude that alley cropped forages had a relatively minor impact on the growth and nut yield of walnut trees. **(U.S.A.)**

53. Strandberg, B., M.B. Pedersen, and N. Elmegaard. 2005. Weed and arthropod populations in conventional and genetically modified herbicide tolerant fodder beet fields. *Agriculture, Ecosystems and Environment* 105(1): 243-253.
See Biodiversity and Restoration Section.

54. Verrell, P. and E. Van Buskirk. 2004. As the worm turns: *Eisenisa fetida* avoids soil contaminated by a glyphosate-based herbicide. *Bulletin of Environmental Contamination and Toxicology* 72: 219-224.
***** Earthworms (Annelida: Oligochaeta) play crucial roles in the formation and maintenance of fertile soils so may be considered biomarker species of soil health and integrity. Here we report the results of three laboratory experiments designed to test for acute effects on *Eisenia fetida* of exposure to a glyphosate-containing

herbicide (Ortho Groundclear Total Vegetation Killer). Our data suggest that exposure to this formulation at the nominal concentration recommended for application (one part Groundclear to four parts water) results in low to negligible acute toxicity in *E. fetida*. However, Groundclear appears to alter locomotor activity in a way that may compromise survival. Worms migrated away from and avoided contaminated soils thus increasing their risk to indirect sources of mortality such as predation, UV radiation and/or desiccation. Whether the responses reported here might impact earthworms and perhaps other components of the soil biotic community in nature is unclear. Our results provide support for the growing appreciation that behavior patterns may be especially sensitive end-points in ecotoxicological studies. **(U.S.A.)**

55. Wardle, D.A., K.I. Bonner, G.M. Barker, G.W. Yeates, K.S. Nicholson, (*et al.*). 1999. Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. *Ecological Monographs* 69(4): 535-568.

See Biodiversity and Restoration Section.

Water Quality

1. Battaglin, W.A., K.C. Rice, M.J. Focazio, S. Salmons, and R.X. Barry. 2009. The occurrence of glyphosate, atrazine, and other pesticides in vernal pools and adjacent streams in Washington, DC, Maryland, Iowa, and Wyoming, 2005-2006. *Environmental Monitoring and Assessment* 155(1-4): 281-307.

   Vernal pools are sensitive environments that provide critical habitat for many species, including amphibians. These small water bodies are not always protected by pesticide label requirements for no-spray buffer zones, and the occurrence of pesticides in them is poorly documented. In this study, we investigated the occurrence of glyphosate, its primary degradation product aminomethylphosphonic acid, and additional pesticides in vernal pools and adjacent flowing waters. Most sampling sites were chosen to be in areas where glyphosate was being used either in production agriculture or for nonindigenous plant control. The four site locations were in otherwise protected areas (e.g., in a National Park). When possible, water samples were collected both before and after glyphosate application in 2005 and 2006. Twenty-eight pesticides or pesticide degradation products were detected in the study, and as many as 11 were identified in individual samples. Atrazine was detected most frequently and concentrations exceeded the freshwater aquatic life standard of 1.8 micrograms per liter (µg/l) in samples from Rands Ditch and Browns Ditch in DeSoto National Wildlife Refuge. Glyphosate was measured at the highest concentration (328 µg/l) in a sample from Riley Spring Pond in Rock Creek National Park. This concentration exceeded the freshwater aquatic life standard for glyphosate of 65 µg/l. Aminomethylphosphonic acid, triclopyr, and nicosulfuron also were detected at concentrations greater than 3.0 µg/l. **(U.S.A.)**

2. Battaglin, W.A., D.W. Kolpin, E.A. Scribner, K.M. Kuivila, and M.W. Sandstrom. 2005. Glyphosate, other herbicides, and transformation products in Midwestern streams, 2002. *Journal of the American Water Resources Association* 41(2): 323-332.

   The use of glyphosate has increased rapidly, and there is limited understanding of its environmental fate. The objective of this study was to document the occurrence of glyphosate and the transformation product aminomethylphosphonic acid (AMPA) in Midwestern streams and to compare their occurrence with that of more commonly measured herbicides such as acetochlor, atrazine, and metolachlor. Water samples were collected at sites on 51 streams in nine Midwestern states in 2002 during three runoff events: after the application of pre-emergence herbicides, after the application of post-emergence herbicides, and during harvest season. All samples were analyzed for glyphosate and 20 other herbicides using gas chromatography/mass spectrometry or high performance liquid chromatography/mass spectrometry. The frequency of glyphosate and AMPA detection, range of concentrations in runoff samples, and ratios of AMPA to glyphosate concentrations did not vary throughout the growing season as substantially as for other herbicides like atrazine, probably because of different seasonal use patterns. Glyphosate was detected at or above 0.1 µg/l in 35 percent of pre-emergence, 40 percent of post-emergence, and 31 percent of harvest season samples, with a maximum concentration of 8.7 µg/l.

AMPA was detected at or above 0.1 µg/l in 53 percent of pre-emergence, 83 percent of post-emergence, and 73 percent of harvest season samples, with a maximum concentration of 3.6 µg/l. Glyphosate was not detected at a concentration at or above the US. Environmental Protection Agency's maximum contamination level (MCL) of 700 µg/l in any sample. Atrazine was detected at or above 0.1 µg/l in 94 percent of pre-emergence, 96 percent of postemergence, and 57 percent of harvest season samples, with a maximum concentration of 55 µg/l. Atrazine was detected at or above its MCL (3 µg/l) in 57 percent of pre-emergence and 33 percent of postemergence samples. **(U.S.A.)**

3. Borggaard, O.K. and A.L. Gimsing. 2008. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. *Pest Management Science* 64(4): 441-456.

   The very wide use of glyphosate to control weeds in agricultural, silvicultural and urban areas throughout the world requires that special attention be paid to its possible transport from terrestrial to aquatic environments. The aim of this review is to present and discuss the state of knowledge on sorption, degradation and leachability of glyphosate in soils. Difficulties of drawing clear and unambiguous conclusions because of strong soil dependency and limited conclusive investigations are pointed out. Nevertheless, the risk of ground and surface water pollution by glyphosate seems limited because of sorption onto variable-charge soil minerals, e.g. aluminium and iron oxides, and because of microbial degradation. Although sorption and degradation are affected by many factors that might be expected to affect glyphosate mobility in soils, glyphosate leaching seems mainly determined by soil structure and rainfall. Limited leaching has been observed in non-structured sandy soils, while subsurface leaching to drainage systems was observed in a structured soil with preferential flow in macropores, but only when high rainfall followed glyphosate application. Glyphosate in drainage water runs into surface waters but not necessarily to groundwater because it may be sorbed and degraded in deeper soil layers before reaching the groundwater. Although the transport of glyphosate from land to water environments seems very limited, knowledge about subsurface leaching and surface runoff of glyphosate as well as the importance of this transport as related to ground and surface water quality is scarce. **(Denmark)**

4. Cooman, K., P. Debels, M. Gajardo, R. Urrutia, and R. Barra. 2005. Use of *Daphnia* spp. for the ecotoxicological assessment of water quality in an agricultural watershed in south-central Chile. *Archives of Environmental Contamination and Toxicology* 48(2): 191-200.

   Because of the importance of surface waters from the Chillán River watershed (Chile) for recreation, agricultural irrigation, and the production of drinking water, local concern about river water quality has increased considerably during the last decade. Agricultural and forestry activities in the watershed, characterized by an intensive use of pesticides, are thought to play an important role in the generation of non-point-source pollution, whereas the discharge of urban wastewater from the city of Chillán constitutes a major point source of pollution. In the present investigation, acute and chronic laboratory bioassays using *Daphnia* spp. were conducted on

surface water samples from 17 river stations located throughout the watershed. Sampling occurred on 6 occasions during a 16-month period (2000 to 2001) and included both high and low flow conditions. Almost all toxic effects observed in summer were directly related to the discharge of urban wastewater, whereas toxicity in rural areas was mainly detected during the winter period when rainfall and river flow are high. Toxicity test results were compared with measured physicochemical water-quality data. Mortality and alterations in reproductive success of *Daphnia* spp. were not consistently reflected in detected chemical pollution. With only one exception (atrazine), detected pesticide concentrations were below known toxicity levels. However, additive and synergistic effects of the presence of a mixture of pesticides could not be excluded as a possible cause of observed toxicity. At several stations, filtering of the water sample led to a strong decrease in toxicity, which suggests the presence of xenobiotics attached to the smaller sediment fraction. Inclusion of sediment chemical analysis and sediment toxicity testing in future work should therefore be encouraged. The presented approach provided information about the adverse effects of human activities on surface water quality in the watershed, not easily obtained from classical monitoring schemes. In specific cases, the approach may represent an economically attractive alternative to physicochemical analyses. Modifications to the proposed methodology should be introduced if the effects of intrastorm and interstorm variability of water quality are to be analyzed. **(Chile)**

5. Edginton, A.N., P.M. Sheridan, H.J. Boermans, D.G. Thompson, J.D. Holt, and G.R. Stephenson. 2004. A comparison of two factorial designs, a complete 3×3 factorial and a central composite rotatable design, for use in binomial response experiments in aquatic toxicology. *Archives of Environmental Contamination and Toxicology* 46(2): 216-223.

   Using an amphibian toxicity testing protocol, comparative studies were conducted to assess the predictive precision, degree of similarity of results and efficiency of a central composite rotatable design (CCRD) in relation to a conventional complete 3×3 factorial design. Data were treated with response surface analysis using generalized linear models (*glm*) and application of profile deviance to generate confidence intervals. Design comparisons were based on studies conducted using the Frog Embryo Teratogenesis Assay—*Xenopus* (FETAX) protocol to examine the interaction of three toxicants at pH levels ranging from 4.5 to 8.5. Test substances included two commercial herbicide formulations based on glyphosate ([N-phosphonomethyl]glycine) isopropylamine salt (Vision, Rodeo) as well as a polyethoxylated tallow amine surfactant blend (MON 0818), which is a key component of the Vision formulation. The generated models from both the CCRD and the factorial designs produced toxicity estimates similar to each other and to previously published results. Trends were also similar to published results in that the surfactant, MON 0818, was comparatively more toxic than Vision, which was more toxic than Rodeo. Further, all toxicants induced higher mortality under alkaline as opposed to acidic conditions. The CCRD was between 66 and 124% more efficient in the Vision and Rodeo experiments in comparison to the complete 3×3 factorial. Thus, the factorial experiment would have required *at least* 66% more observations

to obtain the same precision. There appeared to be no efficiency gain with the use of the CCRD for MON 0818. The CCRD had tighter confidence intervals in 9 of 12 cases across all test substances. Computer simulations using the experimental data for Vision demonstrated that the LCα estimates generated from the 1,000 simulated data sets were very close to the "true" value for both designs. This was based on small bias and mean squared error calculations. Coverage probabilities in both designs were between 91.6 and 95.6%, close to the nominal coverage of 95%. Efficiency comparisons of the simulated Vision data sets demonstrated that the CCRD was more efficient in 93.3% of the comparisons. We suggest that a CCRD using *glm* and profile deviance for statistical analyses be considered an effective and efficient alternative in toxicity studies where interactive effects are of interest. **(Canada)**

6. Kaiser, K. 2011. Preliminary study of pesticide drift into the Maya mountain protected areas of Belize. *Bulletin of Environmental Contamination and Toxicology* 86(1): 56-59.

   In Belize, Central America, many farms surrounding the Protected Areas of the Maya Mountains rely heavily on the application of agrochemicals. The purpose of this study was to test whether orographic drift of glyphosate and organophosphates into the nearby Maya Mountain Protected Areas occurred by collecting phytotelmic water from seven sites over 3 years. Regardless of location within the Maya Mountain Protected Areas, glyphosate was present; organophosphates were more common at ridge sites. Although glyphosate concentrations were low, due to the number of threatened species and the human use of stream water outside the Maya Mountain Protected Areas, better understanding of these effects is warranted. **(Belize)**

7. Kannan C. and R.M. Kathiresan. 2002. Herbicidal control of water hyacinth and its impact on fish growth and water quality. *Indian Journal of Weed Science* 34(1&2): 92-95.

   See Fish Section.

8. Kilbride, K.M. and F.L. Paveglio. 2001. Long-term fate of glyphosate associated with repeated rodeo applications to control smooth cordgrass (*Spartina alterniflora*) in Willapa Bay, Washington. *Archives of Environmental Contamination and Toxicology* 40(2): 179-183.

   Cordgrasses (*Spartina* sp.) are exotic, invasive species that threaten to degrade the intertidal zones of estuaries along the West Coast of North America. Integrated pest management (IPM) strategies primarily focus on the use of aerial and ground applications of Rodeo® in conjunction with mowing, but IPM treatments over multiple years usually are necessary to control *Spartina*. Although information exists regarding the short-term fate and effects to marine biota of a single Rodeo® application to control *Spartina*, little information is available regarding the fate and biotic effects associated with repeated Rodeo® applications necessary for control. Consequently, we conducted a 3-year study to assess the short- and long-term fate and potential effects to marine biota associated with repeated applications of

Rodeo® to control smooth cordgrass in a southwestern Washington estuary. At each of three intertidal locations in Willapa Bay, we established plots on exposed mudflats and along the edge of a *Spartina* meadow that were hand sprayed with Rodeo® (5% solution) and LI-700® (2% solution) during July 1997 and 1998. Glyphosate concentrations in sediment from mudflat plots declined 88% to 96% from 1 day posttreatment in 1997 to 1 year after the second Rodeo® applications in 1999. In contrast, glyphosate concentrations in *Spartina* plots increased 231% to 591% from 1997 to 1999 because *Spartina* rhizomes likely did not readily metabolize or exude it. Comparison of concentrations from mudflat and *Spartina* plots with toxicity test values for marine biota indicates that under worst-case conditions short- and long-term detrimental effects to aquatic biota from repeated application of Rodeo® for *Spartina* control would be highly unlikely. **(U.S.A.)**

9. Kjaer, J., P. Olsen, M. Ullum, and R. Grant. 2005. Leaching of glyphosate and amino-methylphosphonic acid from Danish agricultural field sites. *Journal of Environmental Quality* 34(2): 608-620.

Pesticide leaching is an important process with respect to contamination risk to the aquatic environment. The risk of leaching was thus evaluated for glyphosate (N-phosphonomethyl-glycine) and its degradation product AMPA (amino-methylphosphonic acid) under field conditions at one sandy and two loamy sites. Over a 2-yr period, tile-drainage water, ground water, and soil water were sampled and analyzed for pesticides. At a sandy site, the strong soil sorption capacity and lack of macropores seemed to prevent leaching of both glyphosate and AMPA. At one loamy site, which received low precipitation with little intensity, the residence time within the root zone seemed sufficient to prevent leaching of glyphosate, probably due to degradation and sorption. Minor leaching of AMPA was observed at this site, although the concentration was generally low, being on the order of 0.05 µg L^{-1} or less. At another loamy site, however, glyphosate and AMPA leached from the root zone into the tile drains (1 m below ground surface [BGS]) in average concentrations exceeding 0.1 µg L^{-1}, which is the EU threshold value for drinking water. The leaching of glyphosate was mainly governed by pronounced macropore flow occurring within the first months after application. AMPA was frequently detected more than 1.5 yr after application, thus indicating a minor release and limited degradation capacity within the soil. Leaching has so far been confined to the depth of the tile drains, and the pesticides have rarely been detected in monitoring screens located at lower depths. This study suggests that as both glyphosate and AMPA can leach through structured soils, they thereby pose a potential risk to the aquatic environment. **(Denmark)**

10. Kolpin, D.W., E.M. Thurman, E.A. Lee, M.T. Meyer, E.T. Furlong, and S.T. Glassmeyer. 2006. Urban contributions of glyphosate and its degradate AMPA to streams in the United States. *Science of the Total Environment* 354(2): 191-197.

Glyphosate is the most widely used herbicide in the world, being routinely applied to control weeds in both agricultural and urban settings. Microbial degradation of glyphosate produces aminomethyl phosphonic acid (AMPA). The high polarity and water-solubility of glyphosate and AMPA has, until recently, made their analysis in

water samples problematic. Thus, compared to other herbicides (e.g. atrazine) there are relatively few studies on the environmental occurrence of glyphosate and AMPA. In 2002, treated effluent samples were collected from 10 wastewater treatment plants (WWTPs) to study the occurrence of glyphosate and AMPA. Stream samples were collected upstream and downstream of the 10 WWTPs. Two reference streams were also sampled. The results document the apparent contribution of WWTP effluent to stream concentrations of glyphosate and AMPA, with roughly a two-fold increase in their frequencies of detection between stream samples collected upstream and those collected downstream of the WWTPs. Thus, urban use of glyphosate contributes to glyphosate and AMPA concentrations in streams in the United States. Overall, AMPA was detected much more frequently (67.5%) compared to glyphosate (17.5%). **(U.S.A.)**

11. Kolpin, D.W., D.J. Schnoebelen, and E.M. Thurman. 2004. Degradates provide insight to spatial and temporal trends of herbicides in ground water. *Ground Water* 42(4): 601-608.

    Since 1995, a network of municipal wells in Iowa, representing all major aquifer types (alluvial, bedrock/karst region, glacial drift, bedrock/nonkarst region), has been repeatedly sampled for a broad suite of herbicide compounds yielding one of the most comprehensive statewide databases of such compounds currently available in the United States. This dataset is ideal for documenting the insight that herbicide degradates provide to the spatial and temporal distribution of herbicides in ground water.

    During 2001, 86 municipal wells in Iowa were sampled and analyzed for 21 herbicide parent compounds and 24 herbicide degradates. The frequency of detection increased from 17% when only herbicide parent compounds were considered to 53% when both herbicide parents and degradates were considered. Thus, the transport of herbicide compounds to ground water is substantially underestimated when herbicide degradates are not considered. A significant difference in the results among the major aquifer types was apparent only when both herbicide parent compounds and their degradates were considered. In addition, including herbicide degradates greatly improved the statistical relation to the age of the water being sampled. When herbicide parent compounds are considered, only 40% of the wells lacking a herbicide detection could be explained by the age of the water predating herbicide use. However, when herbicide degradates were also considered, 80% of the ground water samples lacking a detection could be explained by the age of the water predating herbicide use. Finally, a temporal pattern in alachlor concentrations in ground water could only be identified when alachlor degradates were considered. **(U.S.A.)**

12. Peterson, R.K., and A.G. Hulting. 2004. A comparative ecological risk assessment for herbicides used on spring wheat: the effect of glyphosate when used within a glyphosate-tolerant wheat system. *Weed Science* 52: 834-844.

    See Birds Section.

13. Puértolas, L., J. Damásio, C. Barata, A.M.V.M. Soares, and N. Prat. 2010. Evaluation of side-effects of glyphosate mediated control of giant reed (*Arundo donax*) on the structure and function of a nearby Mediterranean river ecosystem. *Environmental Research* 110(6): 556-564.

    See Amphibians, Aquatic Invertebrates and Plants, and Algae Section.

14. Schriks, M., M.B. Heringa, M.M.E. van der Kooi, P. de Voogt, and A.P. van Wezel. 2010. Toxicological relevance of emerging contaminants for drinking water quality. *Water Research* 44(2): 461-476.

    The detection of many new compounds in surface water, groundwater and drinking water raises considerable public concern, especially when human health based guideline values are not available it is questioned if detected concentrations affect human health. In an attempt to address this question, we derived provisional drinking water guideline values for a selection of 50 emerging contaminants relevant for drinking water and the water cycle. For only 10 contaminants, statutory guideline values were available. Provisional drinking water guideline values were based upon toxicological literature data. The maximum concentration levels reported in surface waters, groundwater and/or drinking water were compared to the (provisional) guideline values of the contaminants thus obtained, and expressed as Benchmark Quotient (BQ) values. We focused on occurrence data in the downstream parts of the Rhine and Meuse river basins. The results show that for the majority of compounds a substantial margin of safety exists between the maximum concentration in surface water, groundwater and/or drinking water and the (provisional) guideline value. The present assessment therefore supports the conclusion that the majority of the compounds evaluated pose individually no appreciable concern to human health. **(The Netherlands)**

15. Skark, C., N. Zullei-Seibert, U. Willme, U. Gatzemann, and C. Schlett. 2004. Contribution of non-agricultural pesticides to pesticide load in surface water. *Pest Management Science* 60(6): 525-530.

    Two small creeks, tributaries of the River Ruhr near Schwerte, Federal Republic of Germany, were investigated to reveal the regional agricultural and non- agricultural sources of pesticide inputs and the main pathways to surface water. In addition, the receiving water was monitored for pesticides. The watersheds are situated at the northern margin of the Rhenian Schiefergebirge, a highland landscape in North-Rhine–Westphalia. Solid carboniferous shale is covered by a shallow layer of quaternary unconsolidated rock (porous aquifer thickness <5 m). Occurrence of herbicides such as chlortoluron, isoproturon and terbuthylazine in surface water could be due to their broad agricultural application in regional dominant crops, such as barley, wheat and maize. Occurrence of diuron and glyphosate results from their use in residential settlements and industrial areas as well as from weed control on railway tracks. Atrazine concentrations up to 0.8 µg litre^{-1} indicated recent use of this herbicide, which has been banned since 1991, and was also the result of non-agricultural applications. Pathways for pesticide input to the receiving waters were related to both surface run-off and underground passage. Two-thirds of the observed diuron load in the surface water resulted from an input by

run-off. This was expected as a result of total herbicide application targets to sealed surfaces infringing current regulations and recommendations. Diuron load varied between 0.6 and 1.2% of the estimated amount applied annually in the investigated catchments. Non-agricultural pesticide use contributed more than two-thirds of the whole observed pesticide load in the tributaries and at least one-third in the River Ruhr. **(Germany)**

16. Struger, J., D. Thompson, B. Staznik, P. Martin, T. McDaniel, and C. Marvin. 2008. Occurrence of glyphosate in surface waters of southern Ontario. *Bulletin of Environmental Contamination and Toxicology* 80(4): 378-384.

    Glyphosate in surface waters of southern Ontario (Canada) was studied over a 2-year period. A small percentage of samples exhibited glyphosate concentrations greater than the analytical limit of quantitation (17 µg a.e./L), and the maximum concentration of glyphosate measured was 40.8 µg/L. No samples of roughly 500 analyzed exceeded the Canadian Water Quality Guideline of 65 µg a.e./L considered protective of aquatic life. Typical concentrations of glyphosate in amphibian habitats were well below a range of toxicity thresholds for aquatic organisms, and were thus judged to be unlikely to pose a substantial risk to either sensitive amphibian larvae or other aquatic biota. **(Canada)**

17. Tsui, M.T.K. and L.M. Chu. 2008. Environmental fate and non-target impact of glyphosate-based herbicide (Roundup®) in a subtropical wetland. *Chemosphere* 71(3): 439-446.

    Mai Po Nature Reserve (Hong Kong) is an internationally important wetland for waterbirds. Roundup®, a formulation based on glyphosate, has been used to control the widespread weeds within the reserve for many years but the fate and non-target impact of the herbicide is unknown. To fill this knowledge gap, we applied Roundup® by hand-held sprayer to an estuarine and a freshwater pond in the dry season of year 2002. The surface water and sediment were sampled routinely for glyphosate concentrations following one month of application. *In situ* bioassays using local edible fish species were performed along with the herbicide application. Up to 52% of glyphosate in the surface water was transported to the unapplied regions by wind-driven current in the estuarine pond at 1 DPT (day post treatment). For both ponds, glyphosate concentrations in the water decreased rapidly after 1-3 DPT, but then decreased gradually over time. Both physical adsorption to the bottom sediments and microbial degradation are thought to contribute to these decreases. Interestingly, the persistence of glyphosate in the freshwater pond was longer than in the estuarine system, which is likely due to the considerably higher concentrations of chelating metals (i.e. Cu and Fe) present in the sediment (4.5 and 11-fold higher, respectively) which potentially reduced the bioavailability of glyphosate to the microbial decomposers. Lastly, fishes used in the *in situ* bioassays (both in applied and unapplied areas) showed similar survival rates, indicating that the use of Roundup® at the provided application rate posed no serious hazard. **(China)**

18. Tsui, M.T.K and L.M. Chu. 2004. Comparative toxicity of glyphosate-based herbicides: aqueous and sediment porewater exposures. *Archives of Environmental Contamination and Toxicology* 46(3): 316-323.

    See Amphibians, Aquatic Invertebrates and Plants, and Algae Section.

19. Wauchope, R.D., T.L. Estes, R. Allen, J.L. Baker, A.G. Hornsby, R.L. Jones, R.P. Richards, and D.I. Gustafson. 2002. Predicted impact of transgenic, herbicide-tolerant corn on drinking water quality in vulnerable watersheds of the mid-western USA. *Pest Management Science* 58(2): 146-160.

    In the intensely farmed corn-growing regions of the mid-western USA, surface waters have often been contaminated by herbicides, principally as a result of rainfall runoff occurring shortly after application of these to corn and other crops. In some vulnerable watersheds, water quality criteria for chronic human exposure through drinking water are occasionally exceeded. We selected three settings representative of vulnerable corn-region watersheds, and used the PRZM-EXAMS model with the Index Reservoir scenario to predict corn herbicide concentrations in the reservoirs as a function of herbicide properties and use pattern, site characteristics and weather in the watersheds. We compared herbicide application scenarios, including broadcast surface pre-plant atrazine and alachlor applications with a glyphosate pre-plant application, scenarios in which losses of herbicides were mitigated by incorporation or banding, and scenarios in which only glyphosate or glufosinate post-emergent herbicides were used with corn genetically modified to be resistant to them. In the absence of drift, in almost all years a single runoff event dominates the input into the reservoir. As a result, annual average pesticide concentrations are highly correlated with annual maximum daily values. The modeled concentrations were generally higher than those derived from monitoring data, even for no-drift model scenarios. Because of their lower post-emergent application rates and greater soil sorptivity, glyphosate and glufosinate loads in runoff were generally one-fifth to one-tenth those of atrazine and alachlor. These model results indicate that the replacement of pre-emergent corn herbicides with the post-emergent herbicides allowed by genetic modification of crops would dramatically reduce herbicide concentrations in vulnerable watersheds. Given the significantly lower chronic mammalian toxicity of these compounds, and their vulnerability to breakdown in the drinking water treatment process, risks to human populations through drinking water would also be reduced. **(U.S.A.)**