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Sublethal effects of the herbicide glufosinate ammonium on crops and wild plants: short-term effects compared to vegetative recovery and plant reproduction

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Abstract Current guidelines for phytotoxicity testing rely heavily on short-term testing of primarily crop species to predict the sensitivity of non-target, wild plants to herbicides. However, little is known on how plants recover following initial growth inhibitions in standard 14–28 day greenhouse tests conducted for pesticide assessment and registration. The objectives of this study were to assess the ability of plant species to recover (biomass and reproduction) when tested at the juvenile stage (routine regulatory testing), comparing crop and wild species and using the herbicide glufosinate ammonium. Ten crops and 10 wild species were tested with a one-time exposure to glufosinate ammonium in a greenhouse. Half the plants of each species (9 doses × 6 replicates) were harvested 3 weeks after being sprayed (short-term). The remaining plants were harvested several weeks later, coinciding with seed set or natural senescence (long-term). Total aboveground biomass and several endpoints related to crop production and plant reproduction were measured. Calculated IC50 values (dosage that results in a 50% decrease in the biomass of a plant as compared to the untreated controls) based solely on aboveground biomass, for species harvested in the long-term were generally higher than those obtained in the short-term (with two exceptions), indicating recovery over time. Crop species did not differ from wild species in terms of sensitivity. However, in seven out of 12 cases where reproduction was measurable, reproductive endpoints were more sensitive than either short or long-term biomass endpoints, indicating the importance of examining these parameters in phytotoxicity testing. Glufosinate ammonium was found to be phytotoxic at low doses (2.64–7.74% g ai/ha of the label rate).

Keywords Glufosinate ammonium · Herbicide · IC50 · Non-target plant species · Plant recovery · Plant reproduction · Regulatory testing

Introduction

Current regulatory guidelines for phytotoxicity testing rely heavily on the use of crops to predict herbicidal effects on wild plant species (USEPA 1996). Although some efforts were recently made to include wild plant species within these guidelines (OECD 2006), testing with non-crop species is not a frequent, current practice**.** The reluctance to use wild species as indicators has often been attributed to difficulties in germination and cultivation. Crops are often chosen because they are considered fast growing and predictable; however, recent work has found that many wild species can be easily utilized in phytotoxicity evaluations (Olszyk et al. 2008; White et al. 2009; Boutin et al. 2010). Comparisons of crop and wild species in terms of sensitivities to herbicides have produced mixed results. McKelvey et al. (2002) observed that, in general, wild species were generally less sensitive than the most sensitive crop species for numerous herbicides; however, this finding was based primarily on visual estimates of effects. Olszyk et al. (2008), found that wild species were often more variable in sensitivity than standard crop species, but often fell within the range of sensitivities of the crop species, whereas Boutin and Rogers (2000) noted that risk assessments based on crop data alone were not necessarily protective of sensitive wild species. At present there is no

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consensus as to which type of species should be used in phytotoxicity testing for protection of native/wild plants.

Another area of concern lies in the duration of testing. Guidelines recommend a growing period of approximately 14–28 days after herbicide exposure (at the 2–4 leaf seedling stage) for phytotoxicity evaluations. This short-term evaluation primarily addresses the phytotoxic effects at the juvenile stage in plant development. Previous studies have identified that young plants (seedlings) are often more sensitive to herbicides than adults in terms of decreases in biomass after spray (Breeze et al. 1992; Fletcher et al. 1996; Tharp et al. 1999; Boutin et al. 2000). The long-term ability of young plants sprayed at sub-lethal doses to recover has rarely been assessed; however, Riemens et al. (2009) found that plants harvested at later dates were often able to compensate for initial losses of biomass, rebounding to levels comparable to controls. In addition, Marrs et al. (1991a) observed damage symptoms on plants up to four meters away from the origin of spray of three herbicides (MCPA, mecoprop and glyphosate) 4 weeks after spray; however, a trend towards recovery was observed at the end of the growing season (20 weeks) for some species. Understanding recovery rates would therefore provide important information on the abilities of wild species to persist in a natural environment that is exposed to herbicide drift.

Even if plants are able to compensate for biomass loss in the long-term, measurements of biomass alone may not be enough to truly understand the effects on a plant's future ability to reproduce. Though recovery may occur in certain species, the potential energetic expenses paid to overcome this barrier may have negative affects on the reproductive success of the individual through reductions in seed or fruit outputs, and subsequent long-term effects through diminished seedbanks (Khan and Donald 1992). This could be exacerbated in natural environments, where both intra- and inter-specific competition for resources (including light, nutrients, etc.), especially with non-sensitive species, as well as other factors including pathogens, predators and parasites, could further dampen the ability to recover. A plant that does not receive a lethal dosage may experience delays in flowering or fruit production, which could potentially inhibit seed output that year if it does not occur within the growing season (i.e. before frost). Such reductions would subsequently negatively affect both farmers, through reduced yields (and hence profits), and native wildlife, through loss of food resources.

Evidence of decreases in reproductive success without similar effects on biomass has been widely cited in the literature for field studies. Fawcett and Slife (1978) observed decreases in seed production and subsequent viability of seeds in several species of weeds sprayed with 2,4-D or dalapon, even after plants had begun to recover (new growth). Riemens et al. (2008) observed larger effects of low doses of glufosinate on seed production in *Stellaria media* than on above-ground biomass. Kjær et al. (2006) observed that spray drift rates of approximately 5% (at budding stage) and 15% (at flowering stage) of metsulfuron resulted in losses of 100% of berries from branches of hawthorn (*Crataegus monogyna*); however, numbers and dry weights of leaves and flowers were unaffected. Marrs et al. (1989) observed that decreases in seed yields of various wild species occurred at greater distances from the origin of spray than decreases in biomass. Other studies have observed similar decreases in seed production (Fletcher et al. 1996; Taylor and Oliver 1997; Schmenk and Kells 1998; Riemens et al. 2009) and crop yield (Bhatti et al. 1995; Al-Khatib et al. 2003; Pfleeger et al. 2008) to low doses of herbicides simulating drift that are lower than decreases in biomass/vegetative growth as compared to the controls.

Knowledge of recovery potential and long-term effects on populations can provide great insight into the effects of herbicides at the community level (Barnthouse 2004), where even slight decreases in growth rates, or reproductive rates, can put wild plant species at a greater disadvantage than those species which are highly tolerant (Marrs et al. 1991b). Such disturbance events (spraying) may lead to a simplification of the community, with changes to dominant species and species structure due to resistant species (also "invading" species; late growers, etc.) gaining competitive edges over susceptible ones (Malone 1972; Marrs 1985; Boutin and Jobin 1998; Riemens et al. 2004). It has been shown that over time, plants (and communities) can recover (Marrs et al. 1991a); however, exposure to herbicides may cause longer-term changes in community structure (Malone 1972) and plant relative abundance (Freedman et al. 1993). Recovery of plants can also be very slow, taking years to get back to pre-treatment population/health levels (Marrs 1985; Crone et al. 2009), thus providing an opportunity for new species to colonise (Marrs 1985).

In this paper, we seek to evaluate the current regulatory guidelines for phytotoxicity testing. Our objectives were 4-fold: (1) to further evaluate the usefulness of crops as test species in herbicide phytotoxicity testing as compared to wild/naturalized plant species; (2) to evaluate the validity of short-term (3 week) testing at the juvenile (2–4 leaf) stage as an adequate indicator for long-term phytotoxicity effects on aboveground biomass; (3) to determine if biomass endpoints (both short- and long-term) are equivalent to reproductive (i.e. seed, fruit, etc.) endpoints in long-term studies and (4) to determine the hazardous dosage of an herbicide (glufosinate ammonium) based on short- and long-term tests with biomass and reproductive endpoints. Our study was conducted under greenhouse conditions

following current regulatory guidelines with half of the plants harvested 21 days (3 weeks) following glufosinate exposure, and the remaining plants harvested several weeks later and used to assess recovery.

Materials and methods

Herbicide background information

Glufosinate ammonium is a broad spectrum herbicide used for the control of many weed species, as well as a desiccant for certain crops. Its primary mode of action is through the inhibition of glutamine synthetases (Lea and Ridley 1989), which leads to a build-up of ammonium in the cell (Wendler et al. 1990) and disruptions in photorespiratory pathways, inhibiting photosynthesis (Lacuesta et al. 1992; Merkel et al. 2004). Translocation of glufosinate ammonium after absorption is highly variable (and species dependent) and is often limited by its fast action at the sites of contact resulting in rapid phytotoxicity (necrosis), though small rates of transportation, through phloem, to roots and floral parts have been reported (Steckel et al. 1997; Beriault et al. 1999; Pline et al. 1999; Skora Neto et al. 2000). The half-life of glufosinate in bare topsoil is fairly short (approximately 1 week); however, small amounts can still be detected in spring water run-off following snow melt (Siimes et al. 2006). Estimates based on sales in Canadian provinces from 1994 to 2003 predict that approximately 211,500 kg ai (active ingredient) of glufosinate ammonium may be used annually in Canada (>50% in Manitoba), ranking it as the 15th mostly widely applied herbicide in the country (Brimble et al. 2005).

Experimental design

Ten crop species (4 monocots from 2 families; 6 dicots from 5 families) and 10 wild species (4 monocots from 2 families; 6 dicots from 6 families) were used in all experiments (Table 1). Species selection was performed following guidelines established by the USEPA and OECD (USEPA 1996; OECD 2006). All seeds were obtained through commercial seed suppliers. Experiments were carried out at the National Wildlife Research Centre (Environment Canada) greenhouses in Ottawa, ON, from June to December 2009. Average temperature ranged from 17 ± 3 to 39 ± 5°C and photosynthetic active radiation (PAR) ranged from 285 (cloudy day) to 1951 μmol m^{-1} s^{-2} (sunny day).

Seeds of all species were sown separately in trays of soil consisting of a mixture of 3:1 Promix-BX (with Mycorrhize®) to horticultural sand. All trays were placed in the greenhouses after sowing with the exception of *Juncus dudleyi,* which were first stratified for 1 month in a 2–4°C dark refrigerator prior to being placed in the greenhouses. Seedlings were transplanted into 10 cm diameter by 9 cm high pots approximately 1–2 weeks after emergence, depending on the size and vigour of the seedlings. For each species, 120 seedlings in total were transplanted to ensure that enough healthy plants of each species were available for both the short-term (juvenile stage) and long-term (adult/reproductive stage) experiments.

Glufosinate ammonium (Ignite® SN—Bayer CropScience Inc.) containing 150 g/L ai was used in all herbicide trials. A 100% dosage of 750 g ai/ha was chosen based on recommendations provided on the label by BayerCrop Science (http://pr-rp.pmra-arla.gc.ca/PR_SOL/pr_web.ve1?p_ukid=11766) as the maximum dosage that should be applied to fields for weed control. Eight doses of glufosinate ammonium, following a geometric progression of 1.9 were prepared (1, 1.9, 3.4, 6.9, 13, 24.8, 47 and 89%; 7.5–667.5 g ai/ha) based on the abovementioned recommended dosage. No surfactants were added. All herbicide stocks were stored in a 2–4°C refrigerator prior to application, and were used within a week of initial preparation.

All glufosinate ammonium applications were performed using a track spray booth (de Vries Manufacturing, Hollandale, MN, USA) equipped with a TeeJet 8002E flat-fan nozzle (Spraying Systems, Wheaton, IL, USA) that delivered 6.75 mL/m^2 of solution at a pressure of 206.84 kPa. The track-spray booth was calibrated prior to each spraying session. Six replicates, each consisting of one plant per pot, were used for each dose/control. This amounted to 108 plants per species [8 doses + controls × 6 replicates × 2 treatments (short- vs. long-term)] or a total of 2160 sprayed plants throughout the experimental period. Plants were deemed ready for spray once they had reached the 3–6 true leaf stage. Prior to spraying, plants of a given species were sorted and grouped by size across all doses for both the short-term and long-term treatments (i.e. six size groupings, one for each replicate × 18 plants for 9 doses × 2 treatments) in order to ensure size uniformity. Plants were then randomly assigned numerical ID tags in order to prevent potential bias during harvest measurements. All plants were well hydrated prior to spraying in order to maintain humidity for glufosinate efficacy (Anderson et al. 1993). Short and long-term treatment plants were sprayed at the same time, using the same prepared herbicide batches. The control plants for both treatments were not sprayed. Newly sprayed plants were isolated from the main experimental greenhouses for a period of 24 h to avoid potential volatilization and drift of glufosinate, and were not watered until they were returned to the greenhouses in order to optimize glufosinate absorption as specified on the product label (i.e. avoiding rainfall). All plants of a given species (both treatments) were randomized within set blocks in the greenhouses, and

Table 1 Crop and wild plant species tested with glufosinate ammonium

Species	Common name	Lifespan	Family
Crops			
Monocots			
Allium cepa var. "Southport White Globe" L.	Garden onion	Perennial	Liliaceae
Avena sativa L.	Common oat	Annual	Poaceae
Lolium perenne L.	Perennial ryegrass	Annual/Perennial	Poaceae
Zea mays var. "Sunnyvee" L.	Corn	Annual	Poaceae
Dicots			
Brassica oleracea var. *italica* Plenck	Broccoli	Perennial	Brassicaceae
Cucumis sativus var. "Pot Luck Hybrid" L.	Cucumber	Annual	Cucurbitaceae
Fagopyrum esculentum Moench	Buckwheat	Annual	Polygonaceae
Helianthus annuus var. "Teddybear" L.	Common sunflower	Annual	Asteraceae
Lactuca sativa var. "Oakleaf" L.	Garden lettuce	Ann./Bien./Perenn.	Asteraceae
Solanum lycopersicum var. "Tiny Tim" L.	Garden tomato	Annual/Perennial	Solanaceae
Wild			
Monocots			
*Bouteloua gracilis (*Willd. ex Kunth) Lag. ex Griffiths	Blue grama	Perennial	Poaceae
Elymus canadensis L.	Canada wildrye	Perennial	Poaceae
Juncus dudleyi Wiegand	Dudley's rush	Perennial	Juncaceae
Phalaris arundinacea L.	Reed canarygrass	Perennial	Poaceae
Dicots			
Capsella bursa-pastoris (L.) Medik.	Shepherd's purse	Annual	Brassicaceae
Hypericum perforatum L.	Common St. Johnswort	Perennial	Clusiaceae
Melilotus officinalis (L.) Lam.	Melilot	Ann./Bien./Perenn.	Fabaceae
Phytolacca americana L.	American pokeweed	Perennial	Phytolaccaeae
Potentilla recta L.	Sulphur cinquefoil	Perennial	Rosaceae
Solanum dulcamara L.	Climbing nightshade	Perennial	Solanaceae

All information obtained from the USDA Plants Database (plants.usda.gov)

were rotated regularly to ensure uniform exposure to greenhouse conditions.

Short-term (juvenile stage) experiment

All healthy (non-necrotic) above-ground biomass of all plants within the short-term treatment was harvested 21 days (3 weeks) following glufosinate exposure. For *Allium cepa*, total biomass also included the below-ground onion bulb. All plants were bagged separately and dried within a drying oven for at least 72 h at approx. 70°C prior to weighing. Dry biomass for all plants was then recorded (dead plants were scored as having zero biomass).

Long-term (adult/reproduction stage) experiment

Due to the length of time of the study, and to minimize the risk of plants becoming pot bound, the long-term treatment plants of *Zea mays*, *Brassica oleracea*, *Cucumis sativus*, *Helianthus annuus*, *Elymus canadensis*, *Phytolacca americana* and *Potentilla recta* were carefully transplanted into larger pots (18 cm height × 15 cm diameter) in order to prevent stress. With the exception of *Zea mays* (which was transplanted prior to the short-term plants being harvested), all of the above-mentioned species were transplanted after the harvest of the short-term treatments. In addition, all plant species were supplemented with 50 mL of a prepared solution consisting of 2.5 mL/L of 20–20–20 "All Purpose Plant Food" fertilizer (Optimum Hydroponix) at approximately 40 and 64 days after exposure, with the exception of *Capsella bursa-pastoris* which was harvested before the 40 day mark due to fruit maturation and natural leaf senescence of the controls. *Cucumis sativus, Helianthus annuus*, *Solanum lycopersicum*, *Hypericum perforatum, Potentilla recta*, *and Solanum dulcamara* were fertilized one additional time, while *Brassica oleracea* received two extra fertilizations when the controls showed signs of early stress (yellowing leaves).

Long-term plants were allowed to grow until fruit/seed production occurred or until the controls began to show signs of natural senescence or stress, at which point all plants of the given species were harvested (Table 2).

Depending on the species, different parameters of reproductive success or crop yield were measured (Table 2) in addition to the standard measurement of dry biomass as described for the short-term treatment. In the case of the long-term plants, all above-ground tissues, including seeds, fruits, etc., were summed to obtain total above-ground biomass. Seed counts for *Fagopyrum esculentum* and pod counts for *Capsella bursa-pastoris* (when ripe) were taken on multiple occasions in order to avoid losses due to shedding; whereas seed counts for *Avena sativa* and *Elymus canadensis* and fruit counts for *Juncus dudleyi* and *Phytolacca americana* were performed only on the day of harvest. Seed counts for *Melilotus officinalis* were performed after drying. Measurements of tomato fruit (*Solanum lycopersicum*) fresh weight were performed 1 h after watering to ensure adequate hydration. Fresh fruit weights of picked, ripe tomatoes were taken on multiple occasions and the fruits were subsequently dried for biomass calculation. Total fruit weight was summed for each tomato plant prior to analysis. *Helianthus annuus* seedhead mass was determined after drying. All dried floral parts were physically removed from each seedhead prior to weighing in order to obtain a more accurate representation of seed production. Tiller counts for *Bouteloua gracilis* were made at the time of harvest. For both *Hypericum perforatum* and *Solanum dulcamara*, flowering status (yes or no) and primary apical meristem health (alive or dead) was recorded. In addition, the height of the remaining primary apical meristem (from base of the soil to site of apical meristem necrosis) was measured for *Hypericum perforatum* plants, while maximum stem height (cm) from the base of the soil to the apex was recorded for *Solanum dulcamara*. Fresh crop yields for *Allium cepa* (bulb and leaves included) and *Lactuca sativa* (leaves only), 1 h after watering, were also recorded. Only dry biomass measurements were recorded for all remaining species due to lack of, or inconsistencies in, flowering/fruiting.

Statistical analysis

Inhibition concentration values (IC50), defined as the dosage that results in a 50% decrease in the biomass of a plant as compared to the untreated controls, were calculated through non-linear regression models (Environment Canada 2005) for all species and treatments using Systat version 13. If either the assumption of normality of residuals (Shapiro–Wilk Test) or homogeneity of variance (Levene's Test) could not be met, even with data transformations, then the linear interpolation method for sublethal toxicity, also known as the inhibition concentration approach (ICPIN), was used (Norberg-King 1993). In some cases, doses that had 100% mortality rates were removed from the non-linear model if the preceding lower dose also had high mortality, in order to preserve the assumptions of normality and/or homogeneity. Their removal was found not to affect the curvature of the fitted line or the estimated IC50 values. This process was repeated for all other reproductive success parameters measured in the long-term treatment.

To assess differences between crop and wild species, comparisons of IC50 values were made using *t*-tests or non-parametric Mann–Whitney *U* tests (when the assumptions of normality of residuals or homogeneity of variance were violated) for the short- and long-term treatments. In the cases where an IC50 could not be determined (no model could be fitted), or when predicted IC50 values were greater than the maximum applied dosage of 667.5 g ai/ha, the maximum value of 667.5 was applied, and the non-parametric Mann–Whitney *U* test was performed.

Additional analyses of the relationship between the death of the primary apical meristem (Yes/No) and flowering (Yes/No) in the long-term treatments was carried out for *Hypericum perforatum* and *Solanum dulcamara* separately using Pearson χ^2 tests. This was performed to assess the usages of apical meristem length and maximum height, respectively, as surrogates for reproductive success measurements since models could not be fitted for fruit/seed data for both species (only two *Hypericum* controls flowered/fruited and *Solanum* controls did not produce fruit), and a potential link between the loss of primary meristems and delays in flowering had been observed. Pearson correlation analyses were also performed in order to verify if dry weight measurements were representative of the fresh crop yields of both *Allium cepa* and *Lactuca sativa*.

Hazardous dosage (HD_5) calculations were performed in E_TX (Aldenberg and Slob 1993) using the calculated IC50 values, based on total plant biomass, of the crops and wild species separately for the short- and long-term treatments. HD_5 determination is related to the species sensitivity distribution of IC50s, and provides estimates of the maximum HD_5 that would provide protection of 95% of all plant species with confidence intervals of 50% [$HD_5(50)$] or 95% [$HD_5(95)$]. HD_5 values based on crop yields ($n = 6$) and reproductive success ($n = 8$) were calculated separately for crop and wild species respectively. Crop yield was analyzed separately since it provides an indication of potential agronomic impacts due to pesticide drift. In addition, further HD_5 calculations were made using only the species ($n = 12$) for which reproductive fitness data was available as a group (Table 2). This was performed in order to assess differences in sensitivity of measurements between the use of biomass at the short- and long-term stages and the other fitness parameters (seeds, fruit, height, etc.) measured at the long-term stage. In all cases, log-logistic distributions (assumptions met) were used for HD_5

Table 2 Time after spray (days) and parameters measured, other than dry biomass (with rationale), for all species in the long-term (adult/reproduction) treatment

Species	Time (days)	Parameters measured[a] and rationale
Crops		
Allium cepa	91	Harvested before flowering. Measurements of fresh crop weight (incl. bulb) taken as indication of effects on total crop yield
Avena sativa	59	Harvested after seed set when controls began natural senescence. Seed production measured as an indication of crop yield and reproductive success
Lolium perenne	76	Harvested due to lack of new growth and early signs of stress in controls. No reproductive parameters measurable
Zea mays	69	Harvested after cob production when controls began natural senescence. Inconsistencies in cob development (uniformily poor) prevented accurate modelling of dose-reponses, therefore no reproductive parameter was used for analysis. Additional non-experimental corn plants should be grown several weeks after the experimental plants to assure simultaneous presence of anthers and stigmas
Brassica oleracea	141	Harvested before crop production due to stress and senescence of controls. Flower (broccoli) production was rare ($n = 5$), therefore no reproductive parameter could be measured
Cucumis sativus	76	Harvested after fruit formation when controls began natural senescence. No models based on reproductive parameters could be fitted due to inconsistentancies in fruit production and development within all doses. Hand pollination may likely be required to improve fruit production
Fagopyrum esculentum	106	Harvested after seed set when controls began natural senescence. Seed production measured as an indication of crop yield and reproductive success
Helianthus annuus	101	Harvested after seed set when controls began natural senescence. Total seedhead mass was measured as an indication of seed production for crop yield and reproductive output
Lactuca sativa	61	Harvested before flowering. Measurements of fresh crop weight (leaves) taken as indication of effects on total crop yield
Solanum lycopersicum	127	Harvested when controls showed no new signs of further fruit development. Total fresh fruit production (weight) was measured both as an indicator of crop yield and as an indicator of potential seed output (reproduction)
Wild		
Bouteloua gracilis	78	Harvested before flowering since seed production is rare (Coupland, 1950). Asexual reproduction was instead assessed through number of tillers produced
Elymus canadensis	100	Harvested after seed set, before seeds could be shed. Seed production measured as an indication of reproductive success
Juncus dudleyi	72	Harvested after fruit development when controls showed no signs of new growth. Number of fruit produced measured as an indication of potential seed (reproductive) output
Phalaris arundinacea	77	Harvested before flowering due to lack of new growth and early signs of stress in controls. No reproductive parameters measurable
Capsella bursa-pastoris	38	Harvested after pod formation (before shedding) when controls began natural senescence. Due to small seed size, number of pods produced was instead used as an indicator of reproductive output
Hypericum perforatum	139	Harvested late after flowering/fruiting occurred. Due to inconsistent flowering/fruiting in the controls, direct reproductive outputs were not assessable. Instead height of the primary apical meristem (related to death of the meristem) was measured as it appeared to be linked to flowering ability
Melilotus officinalis	66	Harvested after seed set when controls began natural senescence. Seed production measured as an indication of reproductive success
Phytolacca americana	101	Harvested after fruit ripening when controls began natural senescence. Number of fruit produced was measured as an indication of potential seed (reproductive) output
Potentilla recta	147	Harvested before flowering, as no plants showed any signs of bolting. No reproductive parameters measurable
Solanum dulcamara	125	Harvested after initial flowering occurred. Due to frequent loss of flowers and zero fruit production, no direct measurement of reproductive success could be made. Maximum height was instead used as a surrogate as meristem death appeared to be linked to ability to flower

Reproductive or crop yield parameters used in HD_5 calculations are underlined

[a] Total aboveground dry biomass was measured for all plants

determination. If a calculated IC50 value was predicted to be greater than the maximum dosage applied (i.e. greater than 89% or 667.5 g ai/ha), or if no model could be fitted (i.e. full recovery of the species) then the substitute value of 667.5 g ai/ha was used for that species in the HD_5 calculation.

Results

Physical indications of glufosinate ammonium effects were apparent on a majority of the crop and wild plant species within 24 h of application. At the higher doses, early signs of chlorosis and spotted necrosis (drying) were noticeable on the leaves of most dicot broadleaf species (i.e. *Brassica oleracea* leaves were extremely bleached; *Helianthus annuus* leaves became dry and brittle, etc.). Early signs of toxicity in the monocots were less apparent, but often began as chlorosis and/or necrosis at the leaf tips.

Short-term (juvenile) treatment

At the 3 week harvest (Table 3), 50% biomass inhibition concentrations (IC50s) were variable amongst all species. Monocot crops were the least sensitive species group (group mean = 374.98 g ai/ha), with IC50s ranging from 166.88 for *Zea mays* to >667.5 g ai/ha (no effect) for *Lolium perenne*, whereas monocot wild species were found to be slightly more susceptible with IC50s (group mean = 128.66 g ai/ha) ranging from 79.35 for *Phalaris arundinacea* to 165.04 g ai/ha for *Elymus canadensis* (Mann–Whitney *U* Test: $\chi^2 = 5.333$, df = 1, $p = 0.021$). No mortality at any dose was observed in *Allium cepa, Lolium perenne*, *Elymus canadensis* and *Juncus dudleyi*. IC50s for crop (group mean = 71 g ai/ha) and wild (group mean = 66 g ai/ha) dicots did not differ significantly (*t*-test: $t = 0.204$, df = 10, $p = 0.842$). *Cucumis sativus* (crop) and *Capsella bursa-pastoris* (wild) had the lowest IC50s at 3 weeks, 23.59 and 33.37 g ai/ha respectively, while *Helianthus annuus* (crop), *Lactuca sativa* (crop) and *Potentilla recta* (wild) had the highest at 117.3, 116.22 and 109.41 g ai/ha respectively. No individual plant mortality was observed in *Brassica oleracea*, *Hypericum perforatum* or *Potentilla recta*. In terms of overall IC50 values (monocots and dicots combined), no significant difference existed between the crops (mean = 192.39 g ai/ha) and wild species (group mean = 91.30 g ai/ha) (Mann–Whitney *U* Test: $\chi^2 = 1.286$, df = 1, $p = 0.257$) due to high variance.

Long-term (adult/reproductive) treatment

Plants in the long-term treatments were harvested at different times depending on the species (Table 2). In terms of overall plant biomass, species from the long-term treatment, as a whole, had higher IC50 values than those calculated in the short-term treatments, indicating a trend towards recovery. Three crops, *Allium cepa*, *Lolium perenne*, and *Brassica oleracea*, as well as three wild species, *Elymus canadensis, Hypericum perforatum* and *Potentilla recta* exhibited almost full recoveries (IC50s > 635 g ai/ha) in terms of biomass in the long-term, as compared to the short-term treatment. Minor biomass recovery was observed in all remaining species, with the exceptions of two wild species, *Juncus dudleyi* and *Phytolacca americana,* both of which exhibited lower IC50 values in the long-term treatment than in the short-term treatment. In terms of monocots, crops (mean = 484.49 g ai/ha) still tended to have higher IC50s than wild species (mean = 270.43 g ai/ha), but this difference was not significant (Mann–Whitney *U* Test: $\chi^2 = 3.038$, df = 1, $p = 0.081$). Similarly to the short-term data for dicots, no difference was observed between the range of IC50s for crop (mean = 222.72 g ai/ha) and wild dicots (mean = 269.73 g ai/ha) at the end of the long-term treatment (Mann–Whitney *U* Test: $\chi^2 = 0.234$, df = 1, $p = 0.629$). The lowest IC50 values based on biomass in this treatment were once again observed for *Cucumis sativum* (crop) and *Capsella bursa-pastoris* (wild), at 29.17 and 43.36 g ai/ha respectively. Overall, no significant difference between crops (mean = 327.43 g ai/ha) and wild species (mean = 270.41 g ai/ha) was observed in the long-term treatment (Mann–Whitney *U* Test: $\chi^2 = 1.485$, df = 1, $p = 0.223$).

Long-term reproductive success

Of the 20 species studied in this experiment, eight failed to generate consistent measurable reproductive success outputs other than biomass (Table 2). *Lolium perenne*, *Phalaris arundinacea* and *Potentilla recta* all failed to flower within the time-span of the experiment, and only biomass could be recorded. *Allium cepa* and *Lactuca sativa* also did not flower; however, measures of fresh crop yield (an indicator of agronomic impact) were, as expected, found to be highly correlated to dry biomass (Pearson correlations = 0.936 and 0.981 respectively). Flowering in *Brassica oleracea* was minimal ($n = 5$) and was not herbicide dose related. Crop/fruit production in *Cucumis sativus* was also inconsistent. Only 10 plants (including only two controls) produced healthy cucumbers, as a large majority of developing cucumber buds were often aborted. However, healthy fruit production did cease at dosages greater than 3.4% (25.5 g ai/ha), mirroring the long-term IC50 (29.17 g ai/ha) for dry biomass in this sensitive species. All *Zea mays* plants, with the obvious exception of those that died, produced male flowers during the course of the experiment (doses 0–89%), and all but one plant (3.4% dose) produced cobs. Cob development, however, was particularly poor (potentially due to a lack of pollen) and highly variable across all doses, and therefore no model could be accurately fitted. Based on average cob dry weight, a 50% reduction would likely have occurred between the 24.8% (avg. 263 mg) and 47% (avg. 274 mg) doses as compared to the controls (avg. 540 mg), which

Table 3 Summary of IC50 values for all plant species tested with glufosinate ammonium calculated for the short-term (ST) and long-term (LT) treatments

Species	Treatment	Model [Transform]	IC50 value (g ai/ha)	95% CI
Crops				
Monocots				
Allium cepa	ST	Gompertz [None]	448.78	292.09; 659.24
	LT[a]	None	>667.5[b]	–
Avena sativa	ST	Gompertz [SQRT]	216.77	171.19; 273.79
	LT	Logistic [None]	250.77	151.76; 413.95
	RP (Seed)	Logistic [None]	149.31	96.05; 231.27
Lolium perenne	ST	Gompertz [SQRT]	1354.19[b]	624.17; 2943.42
	LT	None	>667.5[b]	–
Zea mays	ST	Logistic [None]	166.88	114.08; 244.47
	LT	Gompertz [None]	352.18	307.32; 404.51
Dicots				
Brassica oleracea	ST	ICPIN[c]	45.43	38.95; 58.80
	LT	None	>667.5[b]	–
Cucumis sativus	ST	ICPIN	23.59	22.20; 25.61
	LT	ICPIN	29.17	20.24; 38.13
Fagopyrum esculentum	ST	Logistic [None]	56.02	34.97; 89.36
	LT	Gompertz [None]	141.56	110.69; 180.97
	RP (Seed)	ICPIN	113.6	83.10; 198.39
Helianthus annuus	ST	Logistic [None]	117.3	105.17; 130.52
	LT	ICPIN	149.59	132.32; 208.27
	RP (Seedhead)	ICPIN	145.25	123.62; 207.45
Lactuca sativa	ST	Logistic [None]	116.22	94.06; 143.54
	LT[a]	ICPIN	168.24	126.47; 217.93
Solanum lycopersicum	ST	Gompertz [SQRT]	65.37	57.21; 74.68
	LT	ICPIN	180.26	142.45; 244.24
	RP (Fruit Wt.)	ICPIN	145.89	115.98; 201.67
Wild Species				
Monocots				
Bouteloua gracilis	ST	Gompertz [SQRT]	115.95	85.10; 157.49
	LT	Gompertz [SQRT]	168.04	98.31; 286.73
	RP (Tillers)[d]	Gompertz [None]	101.09	49.58; 205.06
Elymus canadensis	ST	ICPIN	165.04	126.64; 203.64
	LT	Logistic [None]	635.8	392.55; 1031.76
	RP (Seed)	ICPIN	43.08	7.16; 105.49
Juncus dudleyi	ST	ICPIN	154.31	90.16; 284.50
	LT	Logistic [None]	143.88	60.52; 340.19
	RP (Fruit)	ICPIN	49.11	27.40; 114.80
Phalaris arundinacea	ST	Gompertz [None]	79.35	64.61; 97.40
	LT	Gompertz [None]	138.0	106.40; 178.89
Dicots				
Capsella bursa-pastoris	ST	ICPIN	33.37	29.80; 35.97
	LT	Logistic [None]	43.36	37.82; 49.70
	RP (Pods)	ICPIN	41.49	26.20; 58.58
Hypericum perforatum	ST	ICPIN	81.68	30.33; 119.84
	LT	Logistic [None]	1814.52[b]	337.06; 9771.37
	RP (AM Height)[e]	ICPIN	40.99	38.42; 48.35

Table 3 continued

Species	Treatment	Model [Transform]	IC50 value (g ai/ha)	95% CI
Melilotus officinalis	ST	ICPIN	36.08	30.78; 39.49
	LT	Logistic [None]	48.66	36.24; 61.22
	RP (Seed)	ICPIN	31.49	3.56; 36.45
Phytolacca americana	ST	Gompertz [None]	97.17	83.53; 113.02
	LT	ICPIN	77.54	44.97; 107.72
	RP (Fruit)	ICPIN	62.74	7.32; 102.11
Potentilla recta	ST	Logistic [None]	109.41	90.20; 132.35
	LT	Gompertz [None]	1152.45[b]	464.59; 2856.59
Solanum dulcamara	ST	ICPIN	40.68	37.57; 42.45
	LT	ICPIN	113.84	92.76; 147.46
	RP (Height)[e]	ICPIN	94.28	56.98; 144.07

Total above-ground dry plant biomass was used for "ST" and "LT"; reproductive parameters (RP) were measured only for the "LT" treatment and vary with species (chosen parameters within parentheses). Confidence limits (CI) calculated through Wald method (nonlinear regressions) or through bootstraps (ICPIN; Norberg-King 1993)

[a] IC50 for dry biomass was representative of total fresh crop yield (Pearson correlations)

[b] Predicted IC50 is greater than maximum dosage applied (667.5 g ai/ha); the value of 667.5 was therefore applied in analyses

[c] ICPIN = Inhibition Concentration Approach (Norberg-King 1993)

[d] Number of tillers (shoots) produced was representative of asexual reproduction

[e] Height was related to apical meristem death, a parameter that was not independent from flowering (i.e. reproduction)

would represent an IC50 between 186 and 352.5 g ai/ha respectively.

The remaining 12 species all had assessable reproductive parameters. Total seed production, as an indication of reproductive output, was measured in four species: *Avena sativa*, *Fagopyrum esculentum*, *Elymus canadensis*, and *Melilotus officinalis* (Table 2). In all four cases the calculated IC50 based on the seed production parameter was lower than that for total dry biomass in the long-term treatment, indicating that recovery of biomass may not result in equivalent recovery of seed output (Table 3). Most striking is the result for *Elymus canadensis* (Fig. 1, Table 3), where even though plants appeared to fully recover in terms of biomass (Recovery IC50 = 635.8 g ai/ha), total seed production was greatly reduced by 50% at doses as low as 43.08 g ai/ha (approx. 15× lower). This value was approximately one-fourth of the biomass IC50 at the short-term stage; a similar trend also noted in both *Avena sativa* (Table 3; Fig. 1) and *Melilotus officinalis* (Table 3). Due to the amount of time and effort required to harvest and count seeds, the alternate approach of counting/weighing fruit as an indication of seed output was used for *Solanum lycopersicum*, *Capsella bursa-pastoris*, and *Phytolacca americana* (Table 3; Fig. 1). Similarly to the results for seed production, the IC50s for fruit production were also lower than biomass IC50s for the long-term treatment (but only slightly for *Capsella bursa-pastoris*). Of these species, only *Phytolacca americana* had a predicted reproductive IC50 lower than both the short- and long-term biomass IC50s, indicating that fruit production is an important, sensitive variable in this species. In the case of *Helianthus annuus* (Table 3; Fig. 1), the IC50 value calculated from seedhead mass (145.25 g /ha; Table 3; Fig. 1) was equivalent to that obtained for total biomass (149.59 g ai/ha) in the long-term experiment, both of which, in turn, were larger than the calculated short-term biomass IC50 (117.3 g ai/ha).

Reproduction for *Bouteloua gracilis* was measured in terms of the total production of tillers. Flowering was very rare in this species, occurring only in two plants (one each from the 1.9 and 3.4% doses); however, this species has been reported as rarely setting seed, and instead reproduces asexually through tillers (Coupland 1950). The IC50 for number of tillers (101.09 g ai/ha) was found to be less than the IC50 for biomass (168.04 g ai/ha) in the long-term treatment, and only slightly less than the IC50 for biomass in the short-term treatment (115.95 g ai/ha) (Table 3).

To verify if height measurements could be used as indicators of reproductive success for both *Hypericum perforatum* and *Solanum dulcamara*, Pearson χ^2 analyses were performed to determine if there was a relationship between the death of the primary apical meristem and the ability of the plant to flower. Though *Hypericum perforatum* did flower and produce fruit, lack of consistencies within doses (i.e. only two controls flowered) did not allow for an accurate dose–response model for these parameters. However, it was observed that starting at the 6.9% dose, at minimum, 5 out of 6 replicates had lost their primary apical

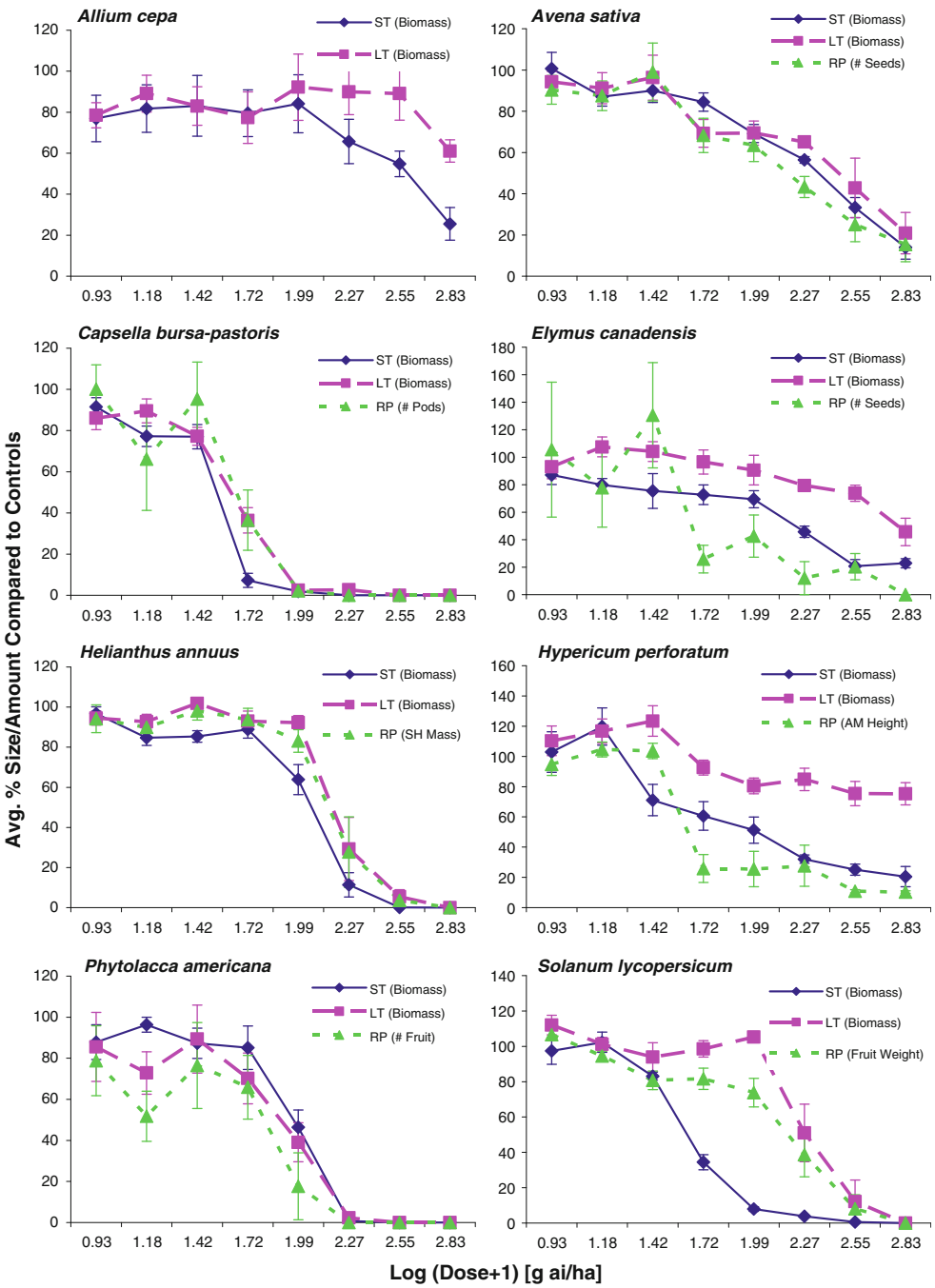

Fig. 1 Dose–response curves for selected species tested with glufosinate ammonium. Curves represent the percent average size (biomass, height) or amounts (# seeds, # fruit) of the sprayed plants as compared to the untreated controls. Total above-ground biomass for plants in the short-term, ST (diamonds), and long-term, LT (squares), treatments are shown for all species; additional reproduction parameters, RP (triangles), used in hazardous dose calculations are also shown. Error bars represent standard error. Note: Scale of the *Y*-axis varies with species. SH = seedhead; AM = apical meristem

meristem. A Pearson χ^2 analysis indicated that the loss of this meristem was not independent from lack of flowering ($\chi^2 = 21.33$, df = 1, $p < 0.001$); therefore, the use of meristem height as an indicator of ability to flower (and thus sexually reproduce) was justified. Based on this parameter, dose–response models predicted a potential reproductive output IC50 of 40.99 g ai/ha (approx. 5.5% dose). This is in contrast to the value predicted by biomass (>667.5 g ai/ha) in the long-term treatment, as it appeared that this species had fully recovered (Table 3; Fig. 1). It is thus likely that more energy was placed into stem regeneration than into flowering and fruiting. Similar findings were made with *Solanum dulcamara*, which flowered but never fruited. Once again, meristem loss and lack of flowering were not independent ($\chi^2 = 7.815$, df = 1, $p = 0.005$). Due to the complete necrosis of the main meristem to the base of the plant, the alternate measure of maximum plant height was used. The IC50 for maximum height (94.28 g ai/ha) was found to be lower than that for biomass in the long-term treatment (113.84 g ai/ha), but

Table 4 Hazardous dose, HD_5s (lowest dose that would protect 95% of all species 50 or 95% of the time), of glufosinate ammonium calculated for crops and for wild species using either short-term (ST) or long-term (LT) biomass IC50s (see Table 2)

Grouping	Parameters	*n*	$HD_5(50)$ (g ai/ha)	$HD_5(95)$ (g ai/ha)
Crops only	ST—Biomass	10	19.82	4.99
	LT—Biomass	10	43.84	12.1
	LT—Crop yield[a]	6	58.05	14.92
Wild species only	ST—Biomass	10	28.83	13.2
	LT—Biomass	10	27.68	6.93
	LT—Reproduction	8	25.62	13.06
Reproductive species only (crops and wild)	ST—Biomass	12	28.39	13.86
	LT—Biomass	12	35.8	13.48
	LT—Reproduction	12	27.25	14.05

HD_5s based on crop yield (crops only) or reproductive outputs (wild only) are also shown. Additional HD_5s were calculated using only the species (crop and wild combined) for which additional reproductive parameters, and related IC50s were obtained (see Tables 2 and 3). All models followed a log-logistic species sensitivity distribution

[a] Includes crop yields of *Allium cepa* and *Lactuca sativa* as LT biomass IC50s

higher than that recorded in the short-term treatment (40.68 g ai/ha) (Table 3).

Lastly, *Juncus dudleyi* did produce both flowers and fruit; however, the controls greatly underperformed in terms of the number of fruit capsules produced (avg. 3.67; low = 0, high = 12) as compared to the lower doses (1–3.4%; avg. between 8.33 and 9.67) even though they had equivalent biomass. No non-linear regression models could be fitted with this data; however, an ICPIN model did make an IC50 prediction of 49.11 g ai/ha, based on the large decline in fruit production that occurred between the 3.4% (avg. 9.67) and 6.9% (avg. 3.5) doses.

Hazard dosage (HD) analysis

For crops, the predicted hazard dosages ($HD_5(50)$ and $HD_5(95)$) based on dry biomass in the short-term treatment were approximately half those for the long-term treatment (Table 4). When only crop yield (refer to Table 2) was considered, both the predicted $HD_5(50)$ and $HD_5(95)$ values were greater than those in the short- and long-term treatments. No noticeable difference between $HD_5(50)$ values, based on biomass, for wild species in the short- and long-term treatments was observed; however, the $HD_5(95)$ value in the long-term treatment was approximately half that of the short-term treatment. When only the reproductive (seeds, fruit, etc.) parameters of the wild species were considered, the calculated HD_5 value (25.62 g ai/ha) was similar to the values obtained for both the short (28.83 g ai/ha) and long term (27.68 g ai/ha) treatments when only biomass was considered. Comparing crops to wild species, in terms of the $HD_5(50)$, using short-term treatment data collected from crops may over-predict the sensitivities of wild species to glufosinate ammonium (likely as a result of the extremely sensitive *Cucumis sativus*). However, the opposite would occur with the long-term data as more crops demonstrated full biomass recovery. Based on biomass data the hazard dosage, using the more generally accepted $HD_5(50)$ value, for glufosinate ammonium equates to be between 2.64 and 5.85% of the recommended dosage/label rate (100% = 750 g ai/ha) for crops and 3.69 and 3.84% for wild species. When crop yield is considered, the maximum dosage increases to 7.74%, whereas calculation based on wild species reproductive parameters predict a more consistent 3.42% as compared to the above-mentioned percentages.

When HD_5 values were calculated using only the species for which reproductive success parameters were present ($n = 12$), it was observed that in terms of overall biomass, predictions were lower at the short-term harvest ($HD_5(50) = 28.39$ g ai/ha, $HD_5(95) = 13.86$ g ai/ha) versus the long-term harvest ($HD_5(50) = 35.80$ g ai/ha, $HD_5(95) = 13.48$ g ai/ha). When reproductive IC50s are used instead of the aboveground biomass IC50s in the long-term treatment HD_5 calculation, the predicted $HD_5(50)$ of 27.25 g ai/ha more closely mirrors the value obtained with the short-term biomass data. This indicates that data collected at the 3-week stage, when plants are still becoming established, can be predictive of future reproductive ability. These results indicate a hazard dosage between 3.63 and 4.77% of the label rate for glufosinate ammonium.

Discussion

Phytotoxicity testing: crops versus wild plant species

Assessing the potential impacts of herbicidal spray drift from agricultural fields onto non-target wild vegetation is

becoming a growing concern for pesticide regulatory agencies. Consequently, there is a focus on integrating wild plant species into toxicological exposure studies in conjunction with the standardized list of crops. In this experiment, we found no clear differences between the use of crops and wild plant species in terms of sensitivity (IC50 values) to glufosinate ammonium solely on the basis of biomass at either the short- or long-term treatments. Applications of glufosinate ammonium at the juvenile stage of development caused significant decreases in total aboveground biomass in the majority of species tested, when measured at the 3-week (short-term) stage (Table 3). Similar ranges of IC50 values based on short-term glufosinate ammonium toxicity trials have been reported (63–160 g ai/ha—Tharp et al. 1999). In the case of glufosinate ammonium, crops would appear to be suitable surrogates for wild species when biomass alone is used as an end-point. However, it has been shown in numerous studies that species sensitivity varies considerably with the herbicide tested and that no one plant species is consistently the most or the least sensitive (Fletcher et al. 1985; Marrs et al. 1989; Pestemer and Zwerger 1999; Boutin et al. 2004; Clark et al. 2004). Therefore, if conservation of wild species is the primary intention, then ecologically relevant test species should be favoured in phytotoxicity testing, alongside the agronomically significant species for non-target crop protection. Furthermore, this experiment, and other research (Boutin et al. 2004; White et al. 2009; Boutin et al. 2010) have demonstrated that wild species are easily workable into phytotoxicity testing protocols.

Short-term effects versus recovery of above-ground biomass

Current guidelines for toxicity testing recommend short-term trials (14–28 days) as an adequate indication of the long-term effects on vegetation. This method, however, does not take into account the ability of plants to recover following sub-lethal doses of an herbicide. Previous studies have found that plants can readily recover biomass (e.g., Riemens et al. 2009) and restore metabolic processes (Follak and Hurle 2004) over time following applications of herbicides, thus warranting the evaluation of short-term trials as indicative of herbicidal effects over the long-term. As demonstrated in our results, both crop and wild species, as a whole, recovered biomass over time (Table 3). Of the 20 species tested in this experiment only two species (*Phytolacca americana* and *Juncus dudleyi*) showed further biomass loss with time, whereas the remaining 18 had mild to full biomass recovery (Table 3). Evaluation of biomass endpoints at the 3-week stage (short-term) appears to be slightly more sensitive than biomass endpoints at the adult stage, thus indicating some level of over-protection based on the IC50 data. However, this finding does not appear to be universal for all species as demonstrated in the case of the two above-mentioned species. Riemens et al. (2008) also observed no significant recovery in the vegetative biomass of two grasses (*Poa annua* and *Echinochloa crus-galli*) at the time of seed set as compared to 4 weeks after exposure to glufosinate ammonium.

Long-term effects on reproductive outputs

Though plants can and were demonstrated to recover aboveground biomass over time, it is primarily through both sexual and asexual reproduction that plants can maintain their populations within plant communities. Sensitive species exposed to herbicides may experience decreases in fruit production or seed output, as well as delays in flowering, which may result in decreases in their seed banks thus giving more resistant species the opportunity to expand or invade (Hume 1987). Consequently, reproductive outputs should provide a clearer understanding of the true effects of low doses of herbicides in a natural environment. In this experiment, of the 12 species for which reproductive output was assessed, the predicted sensitivity to glufosinate ammonium based on the reproductive endpoints was lower than the biomass endpoints of both the short- and long-term treatments in seven cases, was higher than short-term and lower than long-term in three cases, and was on par with long-term in two cases (Table 3). The fact that some reproductive endpoints were more sensitive than biomass endpoints, even in the short-term treatments, indicates that for some species, analysis of toxicity based solely on aboveground biomass may underestimate the true sensitivity. For instance, if the assumption had been made that both *Elymus canadensis* and *Hypericum perforatum* had fully recovered from glufosinate exposure based on long-term biomass alone (IC50s = 635.8 and >667.5 g ai/ha respectively), then these species would have been severely under-protected in terms of the effects on potential reproductive outputs [IC50 = 43.08 (seeds) and 40.99 (AM height used as surrogate for reproduction) respectively] (Table 3; Fig. 1).

Greater reductions in plant reproductive outputs than in biomass have been demonstrated for several herbicides, particularly sulfonyl ureas. In a greenhouse experiment designed to test the effects of spray timing (based on plant development) on biomass and seed production of several species, Fletcher et al. (1996) observed larger reductions in seed yield (weight) than in plant growth for plants sprayed with less than 1% recommended application rates of chlorsulfuron. Similar results were observed for control-room grown garden pea (*Pisum sativum*), where seed yield was reduced to almost 1% of the controls at chlorsulfuron doses of less than 1% of field application rates, even though

other vegetative parameters appeared unaffected (Fletcher et al. 1995). This result appeared to be herbicide dependent, as application of glyphosate, atrazine, and 2,4-D at similar low-dose rates was observed to have no effect on vegetative growth or reproduction. Al-Khatib et al. (2003) compared visual recovery of field-grown grain sorghum (*Sorghum bicolor*) 2 and 8 weeks after exposure to a variety of herbicides (including glufosinate ammonium). Though plants visually appeared to have recovered at the sub-lethal doses, future crop yield was often still suppressed below levels of estimates of the 8 week response; however, this effect was not consistent across sampled fields.

From a biological standpoint, both early reductions in biomass and overall decreases in reproductive outputs can have substantial impacts on plant community dynamics with cascading effects on wildlife behaviour. Though biomass recovery was noted in this experiment, all plants were grown individually in pots under uniform, controlled conditions, and without direct competition from other plant species. In natural environments subtle decreases in growth of a susceptible species could lead to it being outcompeted for light and nutrients by more resistant, healthier and larger species (Weiner 1990), while decreases in health may make susceptible species more vulnerable to pathogen attacks (Brammall and Higgins 1988; Wang and Freemark 1995). Such events can lead to dominance shifts and simplifications within the community (Hume 1987; Pfleeger and Zobel 1995). Riemens et al. (2004) noted a shift towards increases in monocot biomass relative to dicot biomass for increasing doses of glufosinate ammonium following a 4 week mesocosm study. In a study on forest regeneration following a one-time exposure of hexazinone, it was shown that effects were still present after 17 years (Strong and Sidhu 2005). Though a majority of species had a decrease in canopy cover, some species (possibly due to resistance) did show an increase over control levels. Similarly, a long-term study by Crone et al. (2009) observed that a one-time application of picloram to a natural area for the control of spotted knapweed (*Centaurea maculosa*), had negative long-term effects on the flower production (and hence seed production) in the native perennial, arrowleaf balsamroot (*Balsamorhiza sagittata*) for approximately 4 years, though leaf production (as a non-destructive measure of biomass) was unaffected (Crone et al. 2009).

In addition, seed and fruit loss would directly relate to declines in seedbank numbers, and subsequently affect future seedling recruitment. Delays in flowering can also be problematic from the seasonal perspective. Though both *Hypericum perforatum* and *Solanum dulcamara* did demonstrate biomass recovery, the loss of the primary meristem was found to be related to lack of flowering within the experimental timeframe. Under field conditions, these perennials may not have been able to contribute to their perspective seedbanks before the first frost. In addition, it would be valuable in future studies to assess the ability of the root mass to regenerate by determining plant survival rates following winter.

Full recovery was only recorded for three species (*Allium cepa*, *Brassica oleracea* and *Potentilla recta*) in the experiment (Table 3). Furthermore, *Lolium perenne* was the only species found to be strongly resistant to glufosinate ammonium even at the maximum dosage (89%) applied. However, the effects of glufosinate ammonium exposure on the reproductive outputs of these species could not be assessed due to the lack of, or inconsistencies in, flowering within these species. Based on the results of the other species examined, which showed strong negative effects on reproductive outputs, it may be too early to state that these species had fully recovered on the basis of biomass alone.

Glufosinate ammonium phytotoxicity

The calculation of hazardous dose is based on the log-logistic distribution of IC50 values of given sets of plant species. It differs from our other comparisons of IC50 values (*t*-test and Mann–Whitney *U* test) in that it uses the range of values to extrapolate the maximum dosage of a pesticide that can be applied while still protecting 95% of all plant species (either 50 or 95% of the time). Since it is based on a distribution, the extrapolated value can be outside of the range of IC50s used in its calculation, unlike means or medians. In comparing the calculated $HD_5(50)$ values, evaluating crops at the 3 week (short-term) stage, would appear to be the most sensitive measure of overall glufosinate phytotoxicity. The most sensitive species tested with glufosinate ammonium was the crop *Cucumis sativus* and calculations of the HD_5 values based on crop data was highly influenced by this species. As a comparison, if *Cucumis sativus* had not been selected as a test species, when removed from the HD_5 analysis ($n = 9$), the $HD_5(50)$ for crops would have increased from 19.82 to 28.54 g ai/ha for short-term biomass and from 43.84 to 90.93 g ai/ha for long-term biomass. The inclusion of this species therefore emphasizes the need to continue to assess multiple plant species in phytotoxicological regulatory testing, as finding the "most sensitive" species is not always probable when the goal is to represent the vast number of wild plant species potentially at risk.

For the subset of plants for which reproductive outputs were measured, the hazardous dose calculated based on short-term biomass is more representative of the effect on reproductive outputs than long-term biomass. Nonetheless, the predicted hazardous doses in these cases were all found

to be below 5% of the recommended application rate for glufosinate ammonium. Only long-term crop biomass and crop yield were greater than 5%, but less than 10%. Previous studies into the drift of herbicides from target sites to non-target vegetation have observed spray drifts of approximately 5–10% (Boutin et al. 2004 and references therein). The low hazardous doses determined in this experiment fall within this critical range, thus indicating that glufosinate ammonium spray drift could potentially be highly toxic to non-target plants. Though these results were obtained through controlled greenhouse experiments, it is possible that the same effects could be mimicked, if not exacerbated, in natural areas where environmental conditions, species composition (Kegode and Fronning 2005) and competition could further hamper the survivability of susceptible species.

Conclusion

This study revealed that testing glufosinate ammonium using crops could be overprotective of toxicity for most wild species, contrary to many other studies Nonetheless, most plants were very sensitive to this herbicide, well below the drift level reported in the literature. More importantly, this study demonstrated that reproductive outputs were the most sensitive endpoints for 59% of the species for which some measurements of reproduction could be assessed (seven out of 12 species). In this study it was not possible to measure the rate/time taken to recover following herbicide impact and future research in this area is warranted since it would likely play an important role in plant community dynamics.

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