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Predicting sublethal effects of herbicides on terrestrial non-crop plant species in the field from greenhouse data

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The response of greenhouse-grown wild plant species to herbicide exposure could be related to the response of the same species when grown in the field.

Abstract

Guidelines provided by OECD and EPPO allow the use of data obtained in greenhouse experiments in the risk assessment for pesticides to non-target terrestrial plants in the field. The present study was undertaken to investigate the predictability of effects on field-grown plants using greenhouse data. In addition, the influence of plant development stage on plant sensitivity and herbicide efficacy, the influence of the surrounding vegetation on individual plant sensitivity and of sublethal herbicide doses on the biomass, recovery and reproduction of non-crop plants was studied. Results show that in the future, it might well be possible to translate results from greenhouse experiments to field situations, given sufficient experimental data. The results also suggest consequences at the population level. Even when only marginal effects on the biomass of non-target plants are expected, their seed production and thereby survival at the population level may be negatively affected.

Keywords: Non-crop terrestrial plants; Glufosinate ammonium; Biomass; Seed production; Life cycle

1. Introduction

During the past two decades, the interest for vegetation on edges immediately surrounding arable fields has increased significantly. As a consequence, a great deal of concern has arisen regarding effects of pesticides on these margins, in The Netherlands (De Snoo, 1995), other parts of Europe (Marrs et al., 1993), Canada and the United States (Boutin et al., 2004).

Herbicides in particular may have a large effect on such networks. These chemicals may alter biodiversity as they can affect plant species composition, diversity, development, growth, or morphology. Plants are an important part of the habitat in relation to other organisms such as birds and insects, providing them with food, shelter, and an environment to reproduce (Freemark and Boutin, 1995; Moreby and Southway, 1999). Changes in the species composition of field margins due to herbicide applications to adjacent fields have been observed in previous studies (De Snoo, 1999; Jobin et al., 1997; Marrs et al., 1989).

Issues such as herbicide doses and allowable distances to field margins for spraying are currently being discussed (De Jong et al., 2007; De Snoo, 1995). However, insufficient knowledge is available on the effects of sublethal doses of herbicides on non-target, non-crop terrestrial plants required to estimate these distances and doses.

The European and Mediterranean Plant Protection Organization (EPPO) Council has provided a standard for the environmental risk assessment of plant protection products such as pesticides to non-target terrestrial higher plants (European and Mediterranean Plant Protection Organization, 2003) with a tiered approach. They present a definition of a non-target

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plant that we will use in this paper: a non-crop plant located outside the treatment area.

However, a number of factors that need to be dealt with during the collection of data required for the risk assessment are not described in this approach. Risk assessments of herbicide phytotoxicity are often performed with data obtained from greenhouse experiments with single plant species, which may over- or underestimate effects. The advantage of greenhouse experiments is that they can be easily standardized. To date, very few studies have investigated the possibilities of directly predicting effects in the field from greenhouse data (Breeze et al., 1992; Fletcher and Johnson, 1990; Wright and Thompson, 2001). Those few used greenhouse and field data that originated from different studies in which either the plant species or origin of the plants differed. A direct comparison was not made in any of these studies. Such knowledge may be very useful during the development of risk assessment protocols.

The present study was undertaken to investigate the predictability of effects on field-grown plants using greenhouse data and to investigate the effects on vegetation assemblages, so-called mesocosms, in a greenhouse. In addition, the sensitivity of plants during different developmental stages, their recovery and effects on the next generation (seed production and germination) were addressed.

2. Description of the experimental set-up

2.1. Experiment 1: Sublethal effects of glufosinate ammonium on four non-crop species

To test the effects of glufosinate ammonium on the aboveground biomass, seed production, seed germination and recovery of different species grown in the greenhouse and in the field, seeds of *Chenopodium album*, *Stellaria media*, *Poa annua*, and *Echinochloa crus-galli* were obtained from a commercial seed supplier (Medigran, Hoorn, The Netherlands, http://www.medigran.nl). Seven hundred and sixty-eight 0.5-L pots were filled with a peat/sand mixture (2:1). Seeds of the four species were scattered over the soil surface (one species per pot) and covered with a thin layer of sifted soil. The species were sown in such a manner that emergence of all species would coincide. Half of the pots were randomly arranged in four blocks in a greenhouse (day/night temperature 18/12 °C and a 16:8 h light/dark period) and watered in trays. The other half was randomly placed in four blocks in a field adjacent to the greenhouse located in Wageningen, The Netherlands. The experiment started in the first week of May 2005. After emergence the number of plants was thinned to four per pot. Half of the pots from both the greenhouse and the field were sprayed 2 weeks after emergence (WAE) with glufosinate ammonium and the other half at 4 WAE. Four pots per treatment remained unsprayed as control. After treatment, the plants were placed back in the position they were in prior to treatment. Glufosinate ammonium is a broad-spectrum contact herbicide and is used to control a wide range of weeds after crop emergence, or for total vegetation control on non-cultivated land. It inhibits the activity of the enzyme glutamine synthetase which causes the accumulation of ammonia, leading to cell destruction and inhibition of photosynthesis.

Field-grown plants of all species were of a similar size as greenhouse-grown plants at 2 WAE.

Dosages were 0, 0.04, 0.2, 0.4, 2, and 4 L Finale® per ha (which corresponded with doses of 0, 6, 30, 60, 300 and 600 g glufosinate ammonium per ha). The recommended field dose for this herbicide ranges from 3 to 5 L per ha, depending on the crop. The expected deposition of glufosinate ammonium when Finale is applied at 3 L per ha, varies from 111.0 g active ingredient (a.i.) per ha at 1 m, 36.2 g a.i. per ha at 4 m to 3.86 g a.i. per ha at 10 m from the field edge (calculated with the IDEFICS model, Holterman et al., 1997). The herbicide was applied in a 4 m wide by 2 m deep spray chamber. The sprayer consisted of a 1 m wide movable spray boom with three Teejet XR11004 (class Medium spray quality) flat fan nozzles (Spraying Systems Co., Wheaton, IL, USA, http://www.teejet.com) that delivered 400 L per ha. The height between nozzle and the soil surface of the pots was 50 cm.

Fresh weight was used as effect parameter. A preliminary comparison between the effect of glufosinate ammonium on the fresh and dry weight of plants did not show significant differences (Riemens, et al., 2004). Furthermore, the effects of sublethal doses on fresh and dry shoot weight of *Brassica napus* in a previous study carried out in both greenhouse as well as field were highly correlated and the coefficients of variance were similar for dry and fresh weight, (De Jong and Udo de Haes, 2001). The aboveground fresh weight of all species was determined at 4 weeks after treatment (WAT) and at seed setting (SS). For *C. album* and *S. media* plants seed setting already occurred at 4 WAT, so all plants were harvested at that moment, both in the field as well as in the greenhouse.

Seeds were collected and counted per pot. After storage at 10 °C in a dark room in which they were shielded from light and moisture, four lots of 20 seeds were randomly chosen per pot for a germination experiment. Germination tests were conducted in a greenhouse at day/night temperatures of 24/12 °C and a 16:8 h light/dark period. Each seed lot was allowed to germinate in a plastic pot (6 × 5 × 5 cm) filled with sterilized soil. Germinated and emerged seeds were regularly counted and removed from each pot for 21 days. Experiments were conducted from May 2005 until February 2006.

2.2. Experiment 2: Effects of glufosinate ammonium on mesocosms

Mesocosms were composed of eight species in 5 L pots filled with a peat/sand mixture (2:1). Each mesocosm consisted of four monocotyledons, *P. annua*, *E. crus-galli*, *Elymus repens*, *Panicum milliaceum*, and four dicotyledons, *Solanum nigrum*, *S. media*, *C. album*, and *Centaurea cyanus*. All seed reproducing species were seeded in such a manner that emergence of the species would coincide. Since *E. repens* reproduces vegetatively, cuttings of the root system were placed

into the soil in such a manner that its emergence would coincide with the emergence of the other species.

Monocotyledons and dicotyledons were placed alternately in the pots and thinned to eight plants per species per pot after emergence. The experiment started in May 2004. The pots were randomly arranged in a greenhouse with a day/night temperature of 18/12 °C and a 16:8 h light:dark period, and were watered in the trays. At 4 weeks after emergence the pots were sprayed with the same herbicide and doses and in the same manner as in Experiment 1. The first visual symptoms of herbicide injury were recorded at 2 days after treatment based on four categories: no visible injury (1), yellow spots or leaftips (2), yellow spots or leaftips and wilting of the plant (3), and necrosis of plant tissue (brown coloration) and wilting of the plant (4). After 4 weeks, the total fresh weight of the eight plants from each species per pot was determined.

3. Statistical analysis

3.1. Experiment 1: Sublethal effects of glufosinate ammonium on four non-crop species

3.1.1. Fresh weight

The aboveground fresh weight reduction compared to control per dose was calculated for each plant species and analyzed using nonlinear regression analysis with a logistic growth curve: $y = c + (d - c)/1 + e^{-b(\log(\text{dose}) - \log(e))}$ (Seefeldt et al., 1995) with four parameters b, c, d, and e. The lower limit, c, was set at 0. The upper limit (d), the slope (b) and the ED50 (dose at which an effect of 50% can be observed) (e) were estimated. Because the fresh weight reduction compared to control was plotted on the y-axis the values of the upper limit, d, were always estimated around 1 and not significantly different between treatments. Regressions were performed using the statistical program R (Team RDC, 2003) (http://www.R-project.org), as described by Nielsen et al. (2004), and Ritz and Streibig (2005). Parameter estimates were compared using a two way analysis of variance using Genstat 8th edition (Payne et al., 2006). Fisher's Least Significant Difference test was used to compare means.

3.1.2. Comparison of effects on field and greenhouse-grown plants

The estimated ED10, 20,…,90-values of the greenhouse-grown plants were log-transformed and plotted against the log-transformed ED10,20,…,90-values of the field-grown plants. The relationship was analyzed with linear regression analysis using Genstat 8th edition (Payne et al., 2006) for all species together and for each species separately.

3.1.3. Seed production and emergence

The percentage seed production and the percentage seedling emergence, both relative to the control, were calculated per dose for each plant species, location and age if seed production was sufficient for analysis. The percentages were arcsine transformed (Sokal and Rohlf, 1981). After the appropriate checks for normality, a two-way analysis of variance with a randomized block design was used. Fisher's Least Significant Difference test was used to compare means.

Box plots were made with the number of seeds per plant fresh weight (g) on the y-axis and the glufosinate ammonium dose (g a.i./ha) on the x-axis to compare the reduction in seed production with the reduction in plant fresh weight per dose.

3.2. Experiment 2: Effects of glufosinate ammonium on mesocosms

The aboveground fresh weight reduction per dose was calculated for each plant species, the total vegetation, the dicotyledons and the monocotyledons in the mesocosms and analyzed using nonlinear regression analysis as described above for fresh weight in Experiment 1.

The effect of surrounding vegetation was studied for plants sprayed with glufosinate ammonium at 4 weeks after emergence and harvested at 4 weeks after treatment. The doses at which plants show a certain effect level (the ED10, 20,…,90-values) were compared between individually greenhouse-grown plants and plants of the same species grown in the vegetation assemblages. In order to make this comparison, selectivity indices (Ritz and Streibig, 2005), defined as the ratio between the effective dose for a species in a vegetation and the effective dose for the same species grown individually, were calculated for each species and plotted against the corresponding effect level. If this ratio is larger than 1, the species will benefit from the surrounding vegetation, if the ratio is smaller, the effects of the surrounding vegetation on the sensitivity of the species will be negative.

4. Results

4.1. Experiment 1: Sublethal effects of glufosinate ammonium on four non-crop species

4.1.1. Effect of plant development stage on sensitivity

The ED50-values of the dose–response curves of field-grown plants sprayed in an earlier developmental stage (2 WAE) were significantly ($p < 0.05$) smaller than the ED50-values of field-grown plants sprayed in a later stage (4 WAE) for *S. media*, *E. crus-galli* and *C. album* (Table 1). This indicates that field-grown plants are more sensitive to glufosinate ammonium when treated in an earlier developmental stage than when treated in a later developmental stage. However, there was no difference between the ED50-values of *P. annua* field-grown plants sprayed in different developmental stages (Table 1). Greenhouse-grown plants showed no effect of plant development stage on their sensitivity; the ED50-values between plants sprayed at 2 WAE and 4 WAE did not significantly differ (Table 1).

4.1.2. Recovery of plants

Recovery was studied by comparing the fresh weight of the plants harvested at 4 WAT with the fresh weight of plants of the same species at SS. Only the recovery of the monocotyledons, *E. crus-galli* and *P. annua*, was studied since seed setting of the

Table 1
Experiment 1: Fresh weight reduction compared to control

Species	Treatment	Parameter estimate ± SE	
		Slope (*b*)	ED50 (*e*) (g active ingredient/ha)
Chenopodium album	Field 2 WAE 4 WAT	1.93 ± 1.05** ^{b}	72.0 ± 7.36* ^{b}
	Field 4 WAE 4 WAT	0.73 ± 0.16* ^{b}	430.1 ± 52.49* ^{a}
	Greenhouse 2 WAE 4 WAT	6.60 ± 2.09** ^{a}	38.1 ± 0.85** ^{b}
	Greenhouse 4 WAE 4 WAT	0.87 ± 0.10** ^{b}	51.4 ± 3.87** ^{b}
Poa annua	Field 2 WAE 4 WAT	0.94 ± 0.54* ^{cd}	294.1 ± 76.53** ^{bc}
	Field 2 WAE SS	1.00 ± 0.83* ^{cd}	276.11 ± 182.41** ^{bcd}
	Field 4 WAE 4 WAT	0.68 ± 0.21* ^{d}	696.1 ± 19.97* ^{a}
	Field 4 WAE SS	1.59 ± 0.61* ^{bc}	405.9 ± 63.13* ^{b}
	Greenhouse 2 WAE 4 WAT	2.52 ± 0.69** ^{a}	65.9 ± 2.47*** ^{e}
	Greenhouse 2 WAE SS	1.75 ± 0.20** ^{b}	108.8 ± 9.99*** ^{de}
	Greenhouse 4 WAE 4 WAT	1.06 ± 0.14* ^{bcd}	211.8 ± 27.28*** ^{cde}
	Greenhouse 4 WAE SS	1.35 ± 0.38* ^{bc}	276.4 ± 19.59*** ^{bcd}
Echinochloa crus-galli	Field 2 WAE 4 WAT	1.34 ± 0.78* ^{a}	248.3 ± 39.19* ^{b}
	Field 2 WAE SS	1.25 ± 0.49* ^{a}	336.8 ± 70.87* ^{b}
	Field 4 WAE 4 WAT	1.40 ± 1.05* ^{a}	689.0 ± 86.89* ^{a}
	Field 4 WAE SS	1.15 ± 0.68* ^{a}	590.7 ± 10.78* ^{a}
	Greenhouse 2 WAE 4 WAT	1.68 ± 0.45* ^{a}	28. 1 ± 2.53** ^{c}
	Greenhouse 2 WAE SS	1.58 ± 0.22** ^{a}	62.1 ± 14.83** ^{c}
	Greenhouse 4 WAE 4 WAT	2.45 ± 0.77* ^{a}	58.36 ± 5.87*** ^{c}
	Greenhouse 4 WAE SS	2.10 ± 0.88* ^{a}	56.8 ± 13.23** ^{c}
Stellaria media	Field 2 WAE 4 WAT	2.66 ± 1.18* ^{a}	180.9 ± 38.53* ^{b}
	Field 4 WAE 4 WAT	0.66 ± 0.25* ^{a}	356.1 ± 56.36* ^{a}
	Greenhouse 2 WAE 4 WAT	1.08 ± 0.52** ^{b}	23.5 ± 1.81** ^{c}
	Greenhouse 4 WAE 4 WAT	0.49 ± 0.07*** ^{b}	19.8 ± 2.80** ^{c}

Parameter estimates ± SE (standard error) of dose–response curves of the reduction of the aboveground fresh weight of greenhouse-grown and field-grown plants sprayed at 2 and 4 weeks after emergence (2 and 4 WAE) and harvested at 4 weeks after treatment (4 WAT) and at seed setting (SS) relative to the control treatment, versus glufosinate ammonium dose. Regression equation: $Y = d/1 + e^{-b(\log(\text{dose}) - \log(e))}$.
***$p < 0.001$, ** $p < 0.01$, *$p < 0.05$. Different letters within a column within a species indicate significant differences at the 5% level.

dicotyledons coincided with the first measurement time at 4 weeks after treatment and this required harvesting of those plants.

None of these plants were able to recover significantly (Table 1). The ED50-values at 4 weeks after treatment did not differ from the ED50-values at seed setting ($p > 0.05$), except for the ED50-values of field-grown *P. annua* plants treated in a later developmental stage for which the effect was larger at SS than at 4 WAT.

4.1.3. Comparison of effects on field and greenhouse-grown plants

The dose–response curves for all species differed significantly ($p < 0.05$) for the plants grown in the greenhouse and the plants grown in the field (Table 1). The greenhouse-grown plants had a smaller ED50-value than the field-grown plants and were more affected at high doses than were the field-grown plants.

The relation between the log-transformed ED10, 20,…,90-values from the greenhouse data and the log-transformed ED10, 20,…,90-values of the field data is shown in Fig. 1. The parameter estimates per species are shown in Table 2. These data show that a linear relationship exists between the ED-values of greenhouse and field-grown plants treated with glufosinate ammonium on a logarithmic scale for each species. The results were compared with a previous field study with glufosinate ammonium on established vegetations containing both dicotyledons and monocotyledons. In that study the no observed effect concentration (NOEC) was 256 g.a.i./ha (De Snoo et al., 2003). We predicted the greenhouse dose corresponding with this NOEC to be 52 g a.i./ha with a 95% confidence interval of 31–89 g a.i./ha. This is consistent with the actual NOEC value of 68 g a.i./ha for the total aboveground weight. This value lies well within the calculated greenhouse range for the total aboveground biomass from the field data of De Snoo et al. (2003).

4.1.4. Effects on seed production and seedling emergence

Stellaria media was the only species that produced enough seeds in the greenhouse for analysis. Seed production of young

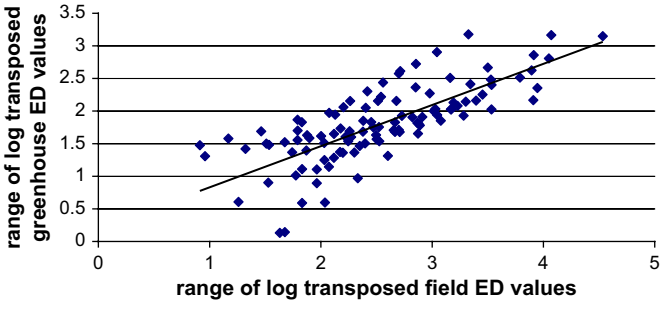

Fig. 1. The linear relationship between the ED10, 20,…, 90-values of greenhouse-grown plants and field-grown plants for all species on a logarithmic scale: log(greenhouse ED-value) = 0.1989 + 0.6314 * (log(field ED-value)), with $R^2 = 0.566$.

Table 2
Experiment 1: Comparison of effects on field and on greenhouse-grown plants

Species	Parameter estimates ± SE		R^2
	a (constant)	*b* (slope)	
Poa annua	0.97 ± 0.12***	0.47 ± 0.05***	0.74
Echinochloa crus-galli	0.26 ± 0.18*	0.55 ± 0.07***	0.89
Chenopodium album	0.39 ± 0.18*	0.56 ± 0.07***	0.80
Stellaria media	1.19 ± 0.20***	1.10 ± 0.08***	0.83

Parameter estimates ± SE (standard error) of the relationship between the greenhouse and field ED10, 20,…90-values for the individual species: log (greenhouse ED-value) = $a + b$ * (log(field ED-value)).
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

plants (treated at 2 WAE) was similar to that of older plants (treated at 4 WAE) ($p = 0.972$). Therefore, seed production of both groups was analyzed as one. Seed production was strongly affected by glufosinate ammonium dose ($p < 0.001$). Seed production was already reduced at the lowest dose and no seeds were produced at all at the highest dose (Table 3). The relative effect (%) on seed production was greater than that on fresh weight (%) (Fig. 2).

Seedling emergence was unaffected for seeds from young treated plants. Seedling emergence was reduced with increasing dose (Table 3) ($p < 0.001$) for seeds produced by older treated plants.

4.2. Experiment 2: Effects of glufosinate ammonium on mesocosms

4.2.1. Visual symptoms and effects on biomass

The first visual symptoms on the species in the mesocosms were observed at 2 days after treatment. No visual symptoms were observed at the two lowest doses. At 30 g active ingredient/ha (a.i./ha), *P. annua* showed no visual effects, while *E. repens*, *C. album*, *S. nigrum*, and *S. media* had yellow spots, *P. milliaceum* and *E. crus-galli* had yellow leaftips, and *C. cyanus* had yellow spots and was wilting. At doses of 60 and 300 g a.i./ha all species showed yellow spots and were wilting. At the highest dose applied, necrotic spots appeared on the leaves of all species.

Table 3
Experiment 1: Seed production and emergence

Dose (g active ingredient/ha)	% Seed production relative to control	% Seedling emergence	
		Young plants (2 WAE)	Older plants (4 WAE)
0	100[a]	95.42×	94.28×
6	66.98[b]	98.22×	95.92×
30	41.96[c]	98.59×	79.62◆
60	18.89[d]	95.69×	81.22◆
300	1.40[e]	95.63×	52.44†
600	0[e]	96.87×	83.44◆
Fisher's LSD	17.60	13.33	

Back transformed percentages of seed production relative to the control treatment per glufosinate ammonium dose for greenhouse-grown *Stellaria media* plants sprayed at 2 and 4 weeks together, and back transformed percentages of seedling emergence per glufosinate ammonium dose for seeds from greenhouse-grown *Stellaria media* plants sprayed at 2 and 4 weeks after emergence (WAE).
Different letters indicate significant differences at $p < 0.05$ for seed production. Different symbols indicate significant differences at $p < 0.05$ for seedling emergence.

The ED50-value of the monocotyledon-curve was significantly ($p < 0.001$) higher than the ED50-value of the dicotyledon-curve (parameters of the dose–response curves are shown in Table 4). No significant differences were found for the slopes of the curves ($p > 0.05$), indicating that the monocotyledons in the mesocosms were less affected by glufosinate ammonium treatments compared to the dicotyledons in the same mesocosms.

The estimated parameters of the dose–response curves for individual species (Table 4) confirm the trend that monocotyledons in a vegetation are less affected by sublethal glufosinate ammonium doses than the dicotyledons in the same vegetation. Except for *S. nigrum* which had a higher ED50 than those of *P. annua* and *E. crus-galli*, the ED50-values of all individual monocotyledons were higher than the ED50-values of the individual dicotyledons.

4.2.2. Effect of surrounding vegetation

The selectivity indices remained constant and below one for *C. album*: the dose before a certain effect can be observed is always five times lower when the species is grown in a mixture, compared to the single species situation (Fig. 3). Glufosinate ammonium reduces the biomass of dicotyledons more than the biomass of monocotyledons at similar doses. Therefore, it is disadvantageous for *C. album* plants to grow in mixtures with monocotyledons. However, for *S. media* plants it is advantageous to grow in a mixture at low effect levels (that is at low doses), even when monocotyledons are present (Fig. 3). *S. media* is a small plant that probably receives less of the applied dose due to the shelter provided by the other species when grown in a mixture. At a certain point, the applied dose reaches a threshold above which the provided shelter becomes insufficient and the competitive ability of *S. media* will be reduced compared to that of the monocotyledons. At high doses it will be disadvantageous for *S. media* to grow in mixtures containing monocotyledons. The monocotyledons in the mixture, *P. annua* and *E. crus-galli*, respond in an opposite way; at high doses it will be advantageous to grow in a mixture with dicotyledons, whereas it will be disadvantageous at low dosages.

Thus, the ratio for most effect levels was significantly ($p < 0.05$) different from 1, indicating a species-specific response to the habitat, i.e., grown in a vegetation or grown individually (Fig. 3), indicating that results from single species experiments can not be translated to effects on these species in mixtures.

5. Discussion

5.1. Comparison of effects on field-grown plants and on greenhouse-grown plants

The current study shows that the aboveground biomass of greenhouse-grown plants is more affected by glufosinate

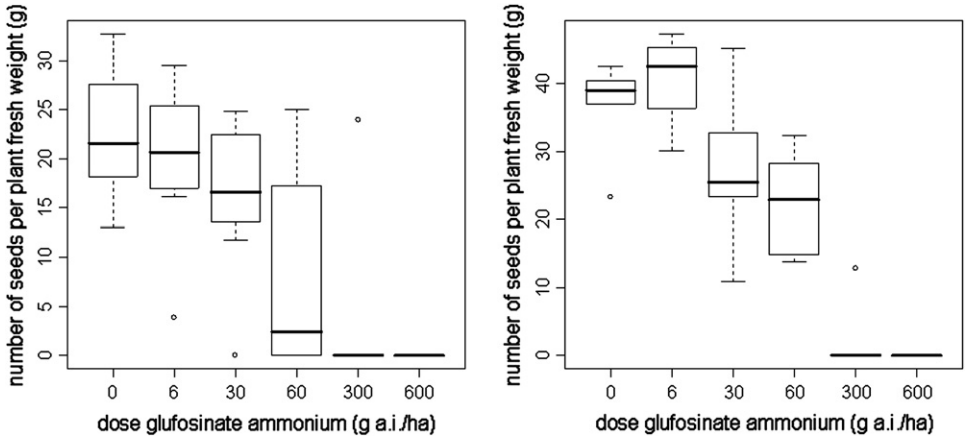

Fig. 2. Box plots of the number of seeds produced per gram plant fresh weight of the plants sprayed at 2 weeks after emergence (left) and the plants sprayed at 4 weeks after emergence (right) versus sublethal doses of glufosinate ammonium.

ammonium than that of field-grown plants. The difference in sensitivity between greenhouse and field-grown plants to glufosinate ammonium may be a result of differences in environmental conditions that promote plant growth rate, such as temperature, relative humidity and light intensity (Petersen and Hurle, 2001; Riethmuller-Haage, 2006).

Previous studies investigated the influence of these climatic conditions on glufosinate ammonium efficacy on *Galium aparine* and *Brassica rapa* in the greenhouse. Both low relative humidity (Anderson et al., 1993) and low light intensity (Petersen and Hurle, 2001) reduced the performance of glufosinate ammonium. The higher efficacy at a high relative humidity may be due to the hydration of the cuticle. Water-soluble compounds such as glufosinate ammonium penetrate the cuticle more easily when it is hydrated (Price, 1982). A low relative humidity, e.g. at field conditions, results in a reduced uptake by the cuticle (Petersen and Hurle, 2001) and hence less efficacy.

The influence of light intensity on the efficacy of glufosinate ammonium can be attributed to the production of toxic ammonia during photorespiration that takes place at high light intensities (Walssgrove et al., 1983). The light intensity under field conditions is usually higher than in the greenhouse (Petersen and Hurle, 2001) making glufosinate ammonium more effective outdoors.

In their greenhouse study with temperatures ranging from 12 to 24 °C, Petersen and Hurle (2001) found no temperature effect on the efficacy of glufosinate ammonium. However, earlier studies showed a reduced efficacy at temperatures below 10 °C (Anderson et al., 1993; Donn, 1982; Langelüddeke et al., 1988; Mathiassen and Kudsk, 1993). In the present study, the temperature in the greenhouse varied from 12 to 18 °C (night/day), whereas the temperature in the field reached temperatures well below 10 °C at night, with a maximum temperature of around 18 °C during daytime.

Table 4
Parameter estimates ± SE (standard error) of the relationship between the aboveground fresh weight of the total vegetation, the monocotyledons, dicotyledons and individual species in the mesocosms sprayed with sublethal doses of Finale versus glufosinate ammonium dose

Species	Parameter estimates ± SE	
	Slope (*b*)	ED50 (*e*) (g active ingredient/ha)
Total vegetation	1.14 ± 0.06***	41.49 ± 4.88***
Monocotyledons	1.30 ± 0.11***	91.45 ± 9.71***
Dicotyledons	1.23 ± 0.07	25.92 ± 3.57***
Poa annua	0.54 ± 0.19**	52.44 ± 33.59
Panicum milliaceum	1.85 ± 0.36***	58.16 ± 12.45***
Echinogloa crus-galli	1.24 ± 0.29***	45.94 ± 16.49**
Elymus repens	1.99 ± 0.57***	203.66 ± 48.99***
Chenopodium album	0.80 ± 0.23***	5.80 ± 4.79*
Centaurea cyanus	0.78 ± 0.19***	6.87 ± 4.61*
Solanum nigrum	1.57 ± 0.33***	53.36 ± 14.92***
Stellaria media	0.99 ± 0.27***	14.35 ± 7.75*

Regression equation: $Y = d/1 + e^{-b(\log(\text{dose}) - \log(e))}$.
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

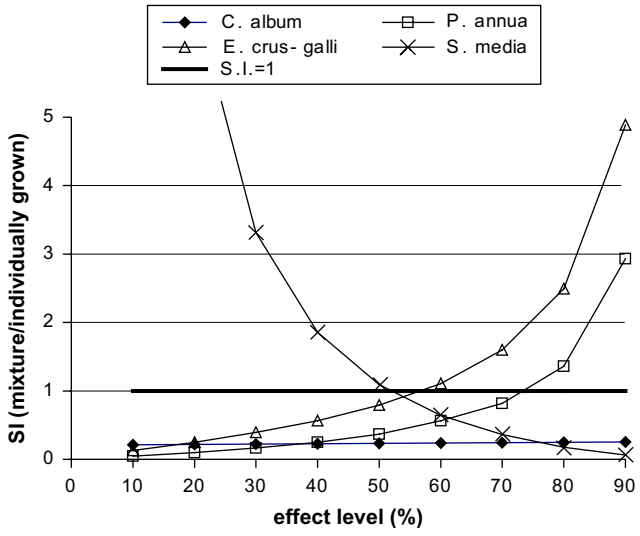

Fig. 3. Selectivity indices (ratio of effective dose of species grown in artificial vegetations/effective dose of the same species grown individually) versus effect level of plants treated with sublethal doses of glufosinate ammonium.

Although light intensity was higher under field conditions than under greenhouse conditions in the present study, the relative humidity and the temperature were lower in the field. We hypothesize that the effect of a low relative humidity and temperature in the field had more influence on the efficacy of the glufosinate ammonium than the higher light intensity. Together with a different structure and/or chemical composition of the cuticle of the field-grown plants this may have resulted in a lower efficacy on the field-grown plants compared to the greenhouse-grown plants.

A relationship was found between the doses resulting in certain effect levels on aboveground fresh weight of greenhouse-grown plants and the doses corresponding to the same effect levels on the aboveground fresh weight of plants grown in the field (Table 2). This relationship was not only found at the individual species level but was also valid for all species tested together (Fig. 1). In wild plant species, the genetic variability within a species can be large between populations from different locations as well as within a population from a specific location. In this study the plants growing in the greenhouse and the field were from the same seed lot, ruling out the genetic variability that may exist between locations, but taking the variability within a location into account. The benefit of this approach is that results are less variable. A main issue for the future is, however, whether it will be possible to use plants from one location in a greenhouse experiment for risk assessment to represent the response of the entire species in the field or that the genetic variability between locations will be too large.

The relationship (Fig. 1) can be used for the total biomass of vegetation composed of several species or for single plants, but not for the prediction of effects on individual species in vegetations. The doses at which certain effects could be observed for species in the vegetations differed from the doses at which the same effects could be observed for those species when grown individually. Depending on the species, these effects increased or decreased with dose (Table 1). In previous studies, effects on individual species grown in a vegetation were either difficult to determine in the field (De Snoo et al., 2003) or depended strongly on the species composition (Marrs and Frost, 1997) and the herbicide used (Marshall, 1988). Differences between the response of individually grown species and the same species grown in a mixture can have several causes.

First of all, the competitive ability of species present in a mixture can be affected by the herbicide treatments and differ per species. As a result, some species will benefit from a higher competitive ability while others will experience an increased competition from the surrounding species for resources. Secondly, some species may benefit from the sheltering effect of other species present during herbicide application, and thus have a reduced exposure. Thirdly, the presence or absence of monocotyledons in the vegetation is known to influence the response of the dicotyledons in a mixture. Marrs and Frost (1997) found that the dicotyledons in their mesocosms responded differently in the presence or absence of grasses in the mixture. In the present study, the mesocosm experiments show that glufosinate ammonium reduced the aboveground weight of the dicotyledons more strongly than that of the monocotyledons, affecting a shift in the species composition. Larger effects on species composition can be expected for herbicides that have a more specific mode of action, targeting specifically on mono- or dicotyledons. However, since it is impossible to separate the effects of herbicides on the inter- and intraspecific interference with neighbors from the effect of shelter (Marrs et al., 1993), it is not possible to determine the contribution of the herbicide to the changes in species composition or changes in the biomass of the individual monocotyledons or dicotyledons. As a result, the prediction of herbicide effects on species in vegetation based on single species experiments is not yet possible.

5.2. Plant development stage and reproduction

Plant development stage played a role in the determination of the plant sensitivity on three out of four species in the present study and elsewhere on several other species treated with metsulfuron methyl (Boutin et al., 2000), chlorsulfuron (Fletcher et al., 1996), glyphosate (Marrs et al., 1991; Ruiter et al., 2000), MCPA and mecoprop (Marrs et al., 1991). Seedlings and young plants were generally more sensitive than older plants. A natural vegetation usually consists of plants in different developmental stages and the balance between young and old plants, the season, and germination period will determine herbicide efficacy.

The difference in sensitivity between younger and older plants was significant for field, but not for greenhouse-grown plants. We hypothesize that this is due to cuticle differences of the plants. The cuticle of young plants differs not only in thickness, but also in chemical composition and fine structure from the cuticle of older plants (Viougeas et al., 1995). These components determine the permeability of the cuticle for herbicides and are influenced by environmental conditions. So the cuticle is determined by plant age itself as well as the environment, thereby causing differences in sensitivity to herbicides. For greenhouse-grown plants, age was the only difference between younger and older plants. The leaves of younger and older plants in the field, however, not only differ in age, but also in environmental conditions experienced during development. So differences in sensitivity are easier to detect between field-grown plants of different developmental stages than between greenhouse-grown plants of different developmental stages.

To determine effects of herbicides in the long term, reproduction is an important factor (Zwerger and Pestemer, 2000). We were unable to compare the seed production between species or between greenhouse and field-grown plants, because only greenhouse-grown *S. media* plants produced enough seeds for analysis. Glufosinate ammonium strongly reduced the seed production of both young and old *S. media* plants. The seed production was reduced more strongly than the aboveground weight at the same doses, in accordance with previous results on chlorsulfuron (Fletcher et al., 1996) and MCPA (Andersson, 1994). These results suggest specific consequences at the population

level. Although only marginal effects are to be expected on the biomass, seed production and thereby survival at the population level can be negatively affected.

Seedling emergence was also reduced, although not for seeds from plants treated at an early developmental stage. Older plants treated with high doses produced seeds that showed reduced germination and emergence.

Results from previous studies support our conclusion that seed production can be affected and that effects on seedling emergence are likely to occur as a result of glufosinate ammonium exposure. These effects were, however, species and herbicide dependent. Andersson (1994) showed that the seed production of *Bilderdykia convolvulus, C. album, Myosotis arvensis*, and *Thlaspi arvense* was reduced by MCPA, while the seed production of *Chamomilla recutita* and *Galium spurium* remained unaffected. He did not find a reduction in seed size. In another study, fluroxypyr reduced the number of large seeds and increased the number of small seeds produced by *Veronica persica* (Champion et al., 1998), possibly due to desiccation. Furthermore, the germination percentage of *V. persica* was found to increase with increasing seed size and therefore decreased with increasing dose (Champion et al., 1998). In the present study, the size of seeds from younger treated plants did not differ between doses ($p = 0.505$), whereas the size of seeds from older treated plants decreased with increasing glufosinate ammonium dose ($p < 0.001$), also possibly due to desiccation. Plants treated in an earlier stage may have been able to recover from desiccation before seed production and thus could produce seeds of a normal size.

5.3. Ways of exposure of plants to herbicides in non-target areas

Plants in non-target areas can be exposed to herbicides via the air, or via run-off. Because the most likely route for most herbicides is exposure through droplet drift (European and Mediterranean Plant Protection Organization, 2003), we chose to simulate drift exposure by spraying the plants and did not consider vapors. However, under certain circumstances, and for some groups of herbicides, volatilization can play an important role (Schweizer and Hurle, 1996; Franzaring et al., 2001; Wittich and Siebers, 2002) and tiered risk assessment protocols for vapor phase toxic compounds have been developed (Dueck, 2003).

The effects of a single application were investigated, ignoring possible cumulative effects of repeated exposures. Repeated exposures can be important since some herbicides are applied to the same field more than once during a growing season. We recommend this aspect be investigated in future research.

6. Conclusion

The risk assessment guideline proposed by the European and Mediterranean Plant Protection Organization (2003) starts with a requirement of exposure studies of six plant species to a single-dose application of a product and then continues with the development of dose–response curves, all in the greenhouse. The relationship between the effects on greenhouse and field-grown plants found in the present study, shows that it might be possible to translate results from greenhouse experiments to field situations in the future. At this moment, however, the relationship was only found for total vegetation and for single species, but not for species grown in a mixture. Furthermore, mainly annual species were used in the experiments because of practical considerations. However, arable field boundary vegetation is known to be composed of both annual and perennial species (Kleijn and Verbeek, 2000). Before we can adopt the use of greenhouse data to predict the effects on vegetations in the field, we will have to investigate which endpoints and exposure time are most suitable for the determination of short- and long-term effects on perennial species.

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