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Author manuscript

Environ Toxicol Chem. Author manuscript; available in PMC 2018 September 10.

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Published in final edited form as:

Environ Toxicol Chem. 2017 October ; 36(10): 2799–2813. doi:10.1002/etc.3839.

PLANT REPRODUCTION IS ALTERED BY SIMULATED HERBICIDE DRIFT TO CONSTRUCTED PLANT COMMUNITIES

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Abstract

Herbicide drift may have unintended impacts on native vegetation, adversely affecting individual species and plant communities. To determine the potential ecological effects of herbicide drift, small plant community plots were constructed using 9 perennial species found in different Willamette Valley (OR, USA) grassland habitats. Studies were conducted at 2 Oregon State University (Corvallis, OR, USA) farms in 2 separate years, with single and combined treatments of 0.01 to 0.2 × field application rates (f.a.r.) of 1119 g ha⁻¹ for glyphosate (active ingredient [a.i.] of 830 g ha⁻¹ acid glyphosate) and 560 g ha⁻¹ a.i. for dicamba. Plant responses were percentage of cover, number of reproductive structures, mature and immature seed production, and vegetative biomass. Herbicide effects differed with species, year, and, to a lesser extent, farm. Generally, 0.1 to 0.2 × f.a.r. of the herbicides were required to affect reproduction in *Camassia leichtlinii*, *Elymus glaucus*, *Eriophyllum lanatum*, *Festuca idahoensis*, *Iris tenax*, and *Prunella vulgaris*. *Eriophyllum lanatum* also had a significant increase in percentage of immature seed dry weight with 0.01 × f.a.r. of dicamba or the combination of glyphosate plus dicamba. Other species showed similar trends, but fewer significant responses. These studies indicated potential effects of low levels of herbicides on reproduction of native plants, and demonstrated a protocol whereby species growing in a constructed plant community can be evaluated for ecological responses. *Environ Toxicol Chem* 2017;36:2799–2813. Published 2017 Wiley Periodicals Inc. on behalf of SETAC. This article is a US government work and, as such, is in the public domain in the United States of America.

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Supplemental Data – The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3839.

Conflict of Interest – The authors declare they have no conflicts of interest.

Data availability – USEPA-generated data from the present study are available on Agency's Environmental Dataset Gateway (<https://edg.epa.gov/metadata/catalog/main/home.page>) or from the corresponding author.

This article includes online-only Supplemental data.

Keywords

Ecotoxicology; Herbicide; Plant toxicology; Native plants; Glyphosate; Dicamba

INTRODUCTION

Fragments of noncrop habitats in agricultural lands are important reservoirs of biodiversity for native plants [1]. These seminatural to natural communities provide needed services for agriculture such as pollination and pest control [2]. Pesticide spray drift from crops to nearby (nontarget) native plants could have important impacts on plant community structure and function. For example, studies have shown that exposure to herbicide drift “can alter the structure of nontarget plant communities in favor of introduced and invasive species, particularly grass species” [3]. Herbicide use in agriculture also can have secondary impacts on farmland wildlife, primarily mediated through a variety of toxic effects on plants and changes in habitat composition, heterogeneity, and interspersed [4].

While the information required for pesticide registration is substantial, key information gaps and scientific uncertainties currently hinder our ability to assess the ecological risk of herbicide drift to native plant communities [5]. There have been suggestions of changes needed in pesticide registration protocols to protect these nontarget plants and communities [5–7]. Especially important is selection of species, because, as stated by Carpenter and Boutin [8], “if conservation of wild species is the primary intention, then ecologically relevant test species should be favored in phytotoxicity testing.” Furthermore, most assessments of the impacts of herbicides on nontarget plants are based on short-term studies, which “may seriously underestimate the impacts of low doses of herbicides particularly on slower growing species” [9]. There also has been limited information on the effects herbicides have on plant reproduction, although impacts on flowering and seed production have been demonstrated in both greenhouse and field studies [8,10–13].

Although plants grown in pots have provided useful information in terms of effects on nontarget, especially native, plants, there is concern that these tests using single species cannot be adequately extrapolated to field situations for ecological risk assessment. Kleijn and Snoeijs [14] found that because the results of greenhouse and field experiments did not coincide, the extrapolation of the results of pot experiments to field conditions was “difficult and inappropriate.” Damgaard et al.’s [15] dose–response experiments on a single species and competition experiments with 2 species in containers, both in a greenhouse, indicated differences in response even though the experiments were performed under similar conditions. Dalton and Boutin [3] stated, “To date, there exists no sound scientific rationale for assuming that single-species greenhouse tests provide adequate measures of protection to nontarget plants.” Thus, studies are needed with different plant species growing in combination under field conditions.

Previously, we described how nontarget plant species can be used successfully in greenhouse simulated herbicide drift studies, including species that may be at risk from herbicide drift in grasslands of the Willamette Valley of Oregon (USA) [16]. Other greenhouse studies indicated reproductive responses in *Pisum sativum* L. [17] and in *Brassica rapa* L. [18]

exposed to simulated drift levels of herbicides. In a recent study [19], native plants were exposed to combinations of glyphosate and dicamba to address important issues concerning weed management systems being developed for crops genetically engineered to be resistant to glyphosate and other herbicides [20]. We have also conducted field studies with small constructed plant communities indicating that effects of herbicides on individual species in a community may affect whole-community response to herbicide exposure [21].

The present study expands on our previous research with native plants to consider more complex constructed communities of 9 perennial species growing in the field. We determined effects on ecological fitness of the species as indicated by changes in reproduction, which can have important consequences for community structure and function. In light of the development of multiple herbicide-resistant technologies, we investigated the effects of combinations of 2 herbicides, glyphosate and dicamba, as well as the individual herbicides.

METHODS

Plot establishment

Small permanent plots were established at 2 Oregon State University experimental farms (Botany and Hyslop) in the Willamette Valley (OR, USA). At each farm, strips of land for establishing the plots were flamed with a propane torch in early April 2008 to control weeds. Within these strips, at each farm 200 small plots (0.56 m², 0.75 m on a side) were laid out in 2 blocks, each with 2 rows of 50 plots. Later, unwanted vegetation in and around the plots was removed either by mowing or by hand to minimize soil disturbance.

Nine perennial native plant species found in Oregon's Willamette Valley were planted in each plot. Details concerning these species are found in Table 1. Species were selected to represent multiple reproductive strategies and phenologies; thus, they may not necessarily occur in the same habitat. Each species was planted in identical locations in each plot so that they would always experience the same interspecific competition (Figure 1), with 25 individual plant locations at a 0.15-m spacing. No 2 individuals of the same species were planted next to each other. Only the 9 central plants in the middle 0.45 m × 0.45 (0.2 m²) square portion of each plot were measured, with the 16 outer plants serving as a border.

Plants of *Elymus glaucus* Buckley (ELGL), *Eriophyllum lanatum* (Pursh) Forbes (ERLA), *Festuca idahoensis* Elmer subsp. *roemerii* (Pavlick) S. Aiken (FEID), *Prunella vulgaris* L. subsp. *lanceolata* (W. Bartram) Hultén (PRVU), *Lupinus rivularis* Douglas ex Lindl. (LURI), and *Ranunculus occidentalis* Nutt. (RAOC) were started in a greenhouse in late winter 2007 with seeds planted in a native Willamette Valley soil in cone cells (approximately 130 mL capacity). Seeds of ERLA, PRVU, and RAOC were stratified (for 6, 4, and 2 wk, respectively, at 5 °C) prior to planting. *Fragaria virginiana* Duchesne (FRVI) plants were obtained as bare root seedlings, which were then transplanted into cone cells as for the other species. Seedlings of these 7 species were kept in a lathhouse, and then transplanted into the experimental field plots in late April and early May 2008. Plants were watered in mid May 2008 at both sites. In November 2008, *Camassia leichtlinii* (Baker) S. Watson (CALE) and *Iris tenax* Douglas ex Lindl. (IRTE) bulbs were planted, and dead plants of the other species

were replaced in the field plots. Plots at the Hyslop farm were watered prior to planting CALE and IRTE. (It had rained before planting at the Botany farm.) No additional water was used at either farm after this time. Plots were weeded regularly, and perimeters of the blocks of plots were mowed as needed.

During the 2008 growing season, the LURI plants grew faster and overshadowed the other species. Thus, in fall 2008, the LURI plants were cut off at the base, the stumps were painted with glyphosate (Roundup Original®) to kill the root systems, and over the winter and spring 2009 the stumps were pulled out. To replace LURI, seedlings of *Potentilla gracilis* Douglas ex Hook. (POGR) were started in cones in a greenhouse during the winter of 2008 to 2009, and transplanted into the locations previously used by LURI in March 2009.

Plot selection

Prior to the experiments in 2010 and 2011, all plots at both farms were surveyed, and live plants were inventoried to determine whether any plants had died over the previous winter. Dead material was removed from all plots at both farms, and the plots were weeded. Dead plants were replaced with transplanted new seedlings from the same species. Based on this survey and plant replacements, in 2010, 81 plots were available for use (all 9 species present) at the Botany farm and 70 plots were available at the Hyslop farm; in 2011, 77 plots were available for use at each farm. In each year, 64 plots at each farm were selected from available plots for use in the present study, based on species completeness and similar plant sizes. Data for species with replaced plants were not used for statistical analysis for that year.

Baseline measurements of the 9 interior plants in each of the selected plots at each farm were conducted prior to herbicide treatments. Measurements included plant height (natural canopy, no extension of leaves) and 2 widths, the maximum plant width, and a second width perpendicular to the maximum width. Plots then were randomly assigned to 8 treatments with 8 plots per treatment using the initial baseline measurements for the largest species, ERLA, to prestratify the 64 plots into 8 complete blocks at each farm in each year.

The selection process resulted in reuse of some plots from a previous year's study. In 2010, 6 of the Botany farm plots and 5 of the Hyslop farm plots were reused from a preliminary 2009 study. Because these plots had been previously designated as no spray, they were assumed to be unaffected by any herbicide treatments. In addition, these 11 plots were used for a variety of different treatments in 2010, lessening the chance of any bias as a result of previous use. In 2011, because of a large loss of plants over the winter, 63 plots that had been used in 2010 were reused for treatments: 36 plots at the Botany farm and 27 at the Hyslop farm. Of these plots, 11 Botany farm plots and 4 Hyslop farm plots were no-spray or carrier control treatments in 2010, and 5 plots from the Botany farm and 2 from the Hyslop farm had also been no-spray plots in 2009. These plots were assumed to be unaffected by any herbicide treatments. The other 48 reused plots had been treated with a variety of different herbicide treatments in 2010. After the stratification and blocking based on size used to assign plots to treatments, between 2 and 6 reused plots from 2010 were assigned to each 2011 treatment. Because of the stratification and blocking procedures used to consider size in assigning treatments, and use of a covariate (pretreatment percent cover) in the

analysis of variance, any previous year treatment effects were factored into the results for 2011.

ForFEID it was determined in 2011 that what had been assumed to be *F. idahoensis* in some plots was actually another grass species. Thus, data from these plots were not used in 2010 or 2011. In 2011, 4 of these plots were from the control treatment at the Botany farm, resulting in only 4 replicate plots. Even though this was only one-half the number of replicate plots for most other treatments, the data were still used for comparison with the other treatments.

Herbicide treatments

Herbicides were applied to the experimental plots during late April in each year at a time when the chance for rain would be less in the Willamette Valley, when the native plants would be actively growing, and when herbicides may be applied to Willamette Valley crops. Herbicide treatments were designed to represent possible spray-drift scenarios, using concentrations that could occur to native plants near the edge of agricultural fields [22]. Glyphosate was applied as Roundup Original (41% active ingredient [a.i.] N-[phosphonomethyl] glycine; Monsanto). Dicamba was applied as Banvel (6-dichloro-2-methoxybenzoic acid, Micro Flo). All herbicide treatments included the carrier Preference® (nonionic surfactant blend, WinField Solutions) at 0.5% v/v. Glyphosate treatments were based on a label field application rates (f.a.r.) of 1119 g ha⁻¹ a.i. (830 g ha⁻¹ acid glyphosate). Dicamba treatments were based on a label f.a.r. of 560 g ha⁻¹ a.i. In 2010, there were 8 treatments: nospray (untreated), carrier control (solution with just surfactant), 0.01 and 0.1 × f.a.r. of glyphosate or dicamba alone, and mixtures of 0.01 or 0.1 × f.a.r. each of glyphosate and dicamba. In 2011, the lowest herbicide levels were dropped (0.01 × f.a.r.) and replaced with higher levels of 0.2 × f.a.r. of glyphosate, 0.2 × f.a.r. of dicamba, and the mixture of 0.2 × f.a.r. of dicamba and glyphosate to enhance the potential to detect herbicide effects on the plants. Herbicides were applied using a unicycle plot sprayer (Mater Engineering) equipped with 2 spray nozzles (TeeJet TP80015; Spraying Systems) at 60-cm spacing on a hand-held wand. The sprayer was checked before treatments to ensure uniform spray rates, and barriers were placed between plots during spray events to prevent drift onto neighboring plots. Treatment dates in each year are shown in Table 2. A light rain fell at the Hyslop farm following application in both years.

One sample of each herbicide solution was collected following application and analyzed by an independent laboratory (glyphosate by high-pressure liquid chromatography–mass spectrometry (HPLC–MS) or HPLC–fluorescence detector, and dicamba by HPLC–MS, to confirm nominal concentrations. Average laboratory concentrations were close to the target nominal concentrations (range –10 to +14%, average –1%) for dicamba at both farms and in both years, and for glyphosate at both farms in 2011. In 2010, laboratory concentrations of glyphosate were an average of 19 and 52% higher than nominal concentrations at the Botany and Hyslop farms, respectively. There was no evidence that the nominal concentrations nor that the lab results were in error for these low-concentration application rates; thus, the nominal concentrations were assumed out of convention and used (as a fraction of f.a.r.) for the statistical analysis.

Plant measurements

To select plots for use in 2010 or 2011, the volume for each species in each plot was calculated based on measurements taken the month prior to treatment (data not included). Each plant's volume was assumed to be a cone, using a modification of the formula: volume = $\pi/3 \times \text{height} \times (\text{geometric mean width}/2)^2$, where the geometric mean width = $(w1 \times w2)^{0.5}$. Height was measured from the soil to the top of the plant, and width was measured at 2 locations: w1 was the broadest diameter of the plant, and w2 was the width perpendicular to the broadest diameter.

Percent cover was the amount of the 0.2-m² plot surface area covered by a species based on visual overhead observation. Before the initial measurements, the square around the perimeter of the central 9 plants was marked and pegged so that all measurements would be taken in the same location. At the beginning of each set of measurements, a plot delineator made of 1-cm diameter polyvinylchloride pipe was placed around the perimeter of the plot using the pegs as guides. Percent cover for each species was determined by comparing the area covered with measuring frames (circles, squares, and rectangles made from 0.46-cm diameter wood and plastic tubing) representing 1, 2, 3, 5, 10, 15, 20, 25, and 40% of the plot area. The exact area within each frame was determined by taking digital pictures of the area covered and verifying the area using computer software (ArcGis, Environmental Systems Research Institute). If percent cover for a species was less than the most appropriate size frame, a smaller frame was used to determine how much cover to subtract out from the total. If the percent cover was more than a frame, additional frames were used and the total cover was based on the sum of the cover from all frames. Cover values recorded as <0.10% were replaced with 0.05% in data files.

Prior to herbicide treatment, percent cover measurements (cover 0, Table 2) were made for use as a prestratification variable for treatment assignment, as well as a covariate to adjust for initial plant size in the statistical analysis. Percent cover was then measured approximately every 2 wk after the herbicide treatments during the growing season (cover 1 through cover 5). For the Hyslop farm in 2011, there was a sixth sampling period after treatment (cover 6) because of extended growth. Data from 10 wk after treatment (cover 5) were used to determine overall effects of the herbicides on plant size, except for CALE and RAOC where the data from 6 wk after treatment (cover 3) were used. These 2 species had many plants with 0 cover 10 wk after treatment because of senescence and loss of shoots. The percent cover is also reported for ERLA cover 3 for comparison with the cover 5 data in 2010. Data for other cover sampling periods are not reported in the present study.

The percent cover represented the percentage of the 0.2-m² square surface area of plot covered by a plant. Plants inside plots that were not covered by other plants were measured first, as moving foliage around will change the area of a plant. If a plant had foliage that went outside the border, only the foliage inside the border was measured. If part or the entire plant inside the plot was covered by another plant, the plant that was covering it was moved aside to determine the plant's cover. This overlap between species was noted in both years, and in 2011 the percent overlap per plot was directly measured using frames. Total bare ground in the plot was measured directly in 2010 using the frames; but in 2011, it was calculated using the formula $(100 - [\text{sum of percent covers for all 9 species}]) - \text{overlap}$

percent cover. Because overlap was included either indirectly as in 2010 or measured directly as in 2011, the total coverage of the plot accounted for by plants and bare ground was greater than 100% of the surface area of the plot. The overlap and percent ground coverage were for informational purposes only. For FRVI, the original mother plant was included in the measurements, as were all offspring as long as they remained within the sampling frame. For all species, if there was no cover noted at measurement time, a zero was used.

At each percent cover measuring period, notes were made regarding whether plants had buds, flowers, fruits, or seeds (data not included). For species with reproductive structures, the structures were collected over multiple periods as they became dry, beginning in mid-June for CALE and continuing until the end of September for ERLA and PRVU. For ERLA, seed structures were also included from 2 plants collected in September 2010 that resembled buds and had few mature seeds. Seed weight data from 1 plant were not used because of uncertainty regarding the value. Reproductive structure collection was on the same day for a species across plots at a farm, but continued on a second day if there were large numbers of structures. Some CALE and IRTE, and to a lesser extent other species seed, were lost from the seed structures prior to collection; however, data were used from the seed that were present. Seed production was measured as seed dry weight, with seed heads dried at 60°C after collection in the field. After drying, seeds were removed from reproductive structures over time, and then cleaned, if necessary, using Clipper Office Tester Seed Cleaning Equipment (A.T. Ferrell). This equipment used a shaker and blower to separate the heavier seeds from the lighter chaff. For all species except PRVU, the seeds were classified as immature or mature, with mature seeds having solid bodies and the immature seeds softer bodies as determined by squeezing with a forceps. The heavier (mature) and lighter (immature) seeds were separated using the seed cleaner and checked for filling using an AO Spencer dissecting microscope under 15 × magnification. Plants of FRVI never produced seeds, and RAOC plants produced only a few seeds, which could not be collected. The RAOC seeds were exposed and unprotected from the elements. The seeds were fragile, and as they reached maturity, or close to it, they were dislodged from the plant by a breeze or by being brushed by the adjacent plants. For PRVU in 2011, atypical inflorescences that did not produce seeds were collected separately and counted by plot. These seed heads had extruded bracts versus the tightly adhering bracts occurring in normal seed heads.

Because of the high number of seed heads for ERLA, it was not possible to remove and weigh all the seeds. Thus, for many plots, seed heads were counted, and approximately 25 heads (or 5% of the total number) were randomly picked for processing to remove seeds and determine seed maturation. The average mature and immature seed weights per seed head were then determined for this sample and multiplied by the number of seed heads per plot to obtain total mature and immature seed weight per plot. For all species where data were present, the percent immature seed weight was calculated as (immature seed weight/ total seed weight) × 100. While the seeds were in storage, mice ate some of the ERLA (8 plots) and PRVU seeds (19 plots) from 2011. These plots were omitted prior to statistical analysis. For PRVU, because the number of omitted plots was large for total seed weight, the data were not analyzed statistically and were used only for information purposes.

The experimental work was terminated in each year when the summer dry period had reached a point when few, if any, new reproductive structures were produced and plants were dying back or had died. In 2011 shoot biomass was collected after seed collection was completed. Because shoots of CALE and RAOC died back first, their status was evaluated on 18 July 2011. Because nearly all shoots had died back, the CALE plants were harvested on 20 July 2011. However, because RAOC shoots were gone from nearly all plots, there were no RAOC biomass data. Above-ground biomass for all other species was harvested at the Botany farm from 20 to 22 September 2011 and at the Hyslop farm from 27 to 30 September 2011.

Experimental design and statistical analysis

The present field study used a restricted randomization design to examine the effects of low levels of herbicides on reproduction and growth of 9 species of perennial plants. The species were grown together in small plots so that below-ground and above-ground competition could occur. However, statistical analysis of plant competition and herbicide responses was beyond the scope of the present study. Prior to each experimental year, the 64 plots were stratified into 8 blocks with 8 plots per block according to plant size; the first block consisted of the 8 smallest plant sizes, the second block consisted of the next 8 smallest plant sizes, and so on. The herbicide treatments were randomly allocated to the prestratified plots so that the average plant size was the same for each treatment. The treatments were designed to test for interactions between 2 herbicides used alone or in combination at 2 concentrations, with all herbicide treatments also including a carrier. There were carrier control plants (0 herbicide) and nospray plants to test for effects of herbicides and carrier alone, respectively. There were 8 replicate plots for each of the 8 herbicide, carrier control, and no-spray treatments, with the replicates stratified and blocked at a farm as indicated in the section Plot selection. The present study was repeated at 2 farms and in 2 yr, but with different concentrations and different plots as feasible in the 2 yr.

The statistical analysis considered covariance based on percent cover measurements prior to herbicide treatments. Data were not used for statistical analysis for a parameter if >15% of the plot values were missing because of environmental conditions or other factors. Data for percent immature seed dry weight were used as long as there were adequate numbers of plots to carry out statistical analysis and plots without seeds were excluded from analysis. However, because the number of plots may be considerably less than the 128 possible, the results should be approached with caution. Data for parameters not analyzed statistically were still presented in the Supplemental Data figures to illustrate general trends.

Initially, data from both years and farms were combined and analyzed using analysis of covariance (ANCOVA) to test for year, farm, and herbicide treatment main effects and their interactions, with pretreatment percent cover as the covariate to adjust for variation in plant size for each year. The treatments were carrier controls; 2 levels (0.01 and $0.1 \times \text{f.a.r.}$ in 2010, or 0.1 and $0.2 \times \text{f.a.r.}$ in 2011) for each of the glyphosate, dicamba, and glyphosate plus dicamba treatments; and no-spray controls. When data for the 2 yr were combined, the ANCOVA tests for 2- and 3-factor interactions involving treatment considered only common treatments in both years and both farms: carrier (0 herbicide) controls, no-spray plants, and

0.1 × f.a.r. of glyphosate, dicamba, and glyphosate plus dicamba. The across-year analysis was carried out for the 2 species that produced the most seeds (ERLA and ELGL) and 2 other species that produced a substantial amount of seeds in each year (CALA and IRTE). The analysis was for key reproduction parameters that were of primary interest in the present study. Because the across-year analysis indicated year × treatment effects, and did not consider all treatments each year, we focused on individual year responses. Subsequently, an ANCOVA was run for each year separately for each parameter so that the treatment and farm main effects and their interaction could be tested using all of the treatment levels each year.

Prior to analysis, the seed dry weight and shoot dry weight data were log- transformed, as treatment effects were assumed to be additive on a log scale. If necessary because of zero values, a small value (1 order of magnitude smaller than smallest value) was added prior to log transformation. The percent cover and percent immature seed dry weight data were arcsine transformed ($= \arcsin[\sqrt{\text{percentage}/100}]$), and the number of reproductive structures were square root- transformed to stabilize the variance. Because of heterogeneity of variance for most species and response parameters, a weighted ANCOVA was used, with weights proportional to the inverse of the variances for the transformed data for each treatment and farm. Variance plots were checked, and no extra weighing of zero values was necessary because of discontinuities of data.

The treatment, farm, and year main effects and their interaction were tested using a 0.05 level of significance. However, statistically significant treatment × farm interactions that had *F* values <10% of those for treatment by itself were not considered to be biologically significant if the ordering of the simple treatment means was consistent for both farms. Because large main effects are more likely to have interactions than small main effects, the interactions could be ignored based on the simple means as a guide rather than the interaction sum of squares [23]. If the treatment × farm interaction was statistically and biologically significant, then the ANCOVA was performed separately for each year and farm. Similarly, any covariate × farm interaction that was statistically significant at the 0.05 level was not considered biologically significant if the *F* value was much less than the *F* value for covariate alone.

Post-ANCOVA tests included a set of planned contrasts to test for the overall effects of dicamba and glyphosate individually and combined. Post-ANCOVA partitioned the 7 degrees of freedom for treatment into separate terms for the main effects of dicamba and glyphosate and their interactions, and the treatment contrast comparing the control with the nospray treatment. The test of an additive herbicide response was performed using the planned treatment contrast calculated as the sum of dicamba and glyphosate individually minus 2 × dicamba and glyphosate combined; a significant negative contrast indicated an antagonistic response, and a significant positive contrast indicated a synergistic response as defined by Colby [24]. However, for percent immature seeds, number of atypical inflorescences, and percent atypical inflorescences, a significant negative contrast indicated a synergistic response, and a significant positive contrast indicated an antagonistic response because an increase in the value indicates a detrimental response. Testing for nonadditive effects followed the general procedure of Colby [25] and was by evaluation of overall interaction between dicamba and glyphosate. Results from the data combined and separated

by year and/or farm were used to infer the interactions at $0.1 \times \text{f.a.r.}$ when year was a factor in the analysis, and 0.01 and $0.1 \times \text{f.a.r.}$ for 2010 or 0.1 and $0.2 \times \text{f.a.r.}$ in 2011, alone.

For the individual year analyses, the Bonferroni test was used to compare individual treatments with the carrier control plants. A 1-tailed test was used to compare herbicide treatments to control plants at $p < 0.007$ ($= 0.05/7$). A 2-tailed test was used to compare the no-spray plants with control plants at a 0.05 level of significance with no Bonferroni adjustment. Treatment means and their standard errors were back-transformed from the log, square root, or arcsine transformations in the figures. Weight data were presented on a log scale. For those parameters where there were insufficient data for statistical analysis and the data are just shown to show general trends, the figures were based on the arithmetic means and standard errors.

The PROC GLM analysis of variance procedures in SAS/STAT software were used for these analyses (Ver 9.3 and 9.4 of the SAS System for Windows 7 Enterprise operating systems for PC).

RESULTS

Plant growth and seed production

Most of the native plant species were healthy and well established by the start of each growing season, and only a few plants had to be replaced and were not subsequently used because of loss over the winter. Because of large number of replaced and missing plants for RAOC, the data for this species were not evaluated statistically but were included for comparison purposes only. The species with the greatest percent cover prior to treatments were ERLA, ELGL, and FEID (Supplemental Data, Table S1). The species PRVU and POGR had 2 to 8% cover, whereas CALE, FRVI, IRTE, and RAOC had only 1 to 3% cover or less. For CALE and IRTE, percent cover may have been a poor measure of size because they consisted of only a few tall stems that covered little surface area.

Seed production (total dry weight per plant) varied considerably among species, and between farms and years as indicated by the number of carrier control and total plots with seeds (Supplemental Data, Table S2). The species ERLA, ELGL, FEID, CALE, and IRTE had enough total plots (at least 112 of 128 possible), and plots with seeds (at least 47) were available for successful statistical analysis of herbicide effects on seed production at each farm and in each year. There were enough POGR and PRVU plots and plots with seeds for analysis in 2011 and 2010, respectively. The most prolific species in terms of seed production were ERLA in 2010 and 2011 (Figures 2A and 3A, respectively) and ELGL in 2010 and 2011 (Figure 4A and C, respectively). In 2010, nearly all replicate plants for the carrier control and no-spray treatments had seeds for these species (Supplemental Data, Table S2), even though there was considerable variability in the amount among them. Seed production was more uneven in 2011 for ERLA, when some carrier control plots did not have any seeds and some plots had missing data because of mouse damage.

Four species generally had less seed production, as indicated by total dry weights, in 2010 and 2011 for CALE (Supplemental Data, Figure S1A; Figure 5A), FEID (Supplemental

Data, Figures S2A and S3A, respectively), IRTE (Supplemental Data, Figure S4A; Figure 5C), and PRVU (Supplemental Data, Figures S5A and S6A). Although some POGR plants produced seeds, there were not enough for analysis in 2010 (Supplemental Data, Figure S7A); but there were enough in 2011 (Supplemental Data, Figure S8A). Plants of FRVI never produced seeds, and RAOC plants produced only a few seeds, which could not be collected. Even though the percent immature seed weights could not be calculated if there were no seeds produced, there were adequate numbers of plots with values (at least 65) to conduct statistical analysis for many species at both farms and in both years (Supplemental Data, Tables S3–S8). For IRTE and POGR in 2010 and for CALE, IRTE, and POGR in 2011, there were inadequate numbers of percent immature seed weight values for statistical analysis.

Herbicide effects on reproduction

Table 3 indicates significance across years for key reproductive responses for the most prolific seed-producing species. For 2 of the 8 species $\times$ response combinations, there were significant interactions involving year and treatment (treatment $\times$ year and treatment $\times$ year $\times$ farm). Because inferences of interactions involving treatment and year were limited to the treatment levels common in both years and excluded the $0.01 \times$ f.a.r. and $0.2 \times$ f.a.r. levels, we focused on complete analysis of all treatments by year regardless of whether the interactions were significant. A summary of the statistical analysis results by species, year, farm, and response parameter is shown in Supplemental Data, Table S9, with detailed results from the statistical analysis in Supplemental Data, Tables S3 to S8. Key responses for different species are shown in Figures 2 to 6. Responses for other parameters and species are shown in Supplemental Data, Figures S1 to S14. In general, there were few significant treatment $\times$ farm responses, and thus most data are presented across farms.

There were significant effects on reproduction for all species that produced seeds, except POGR. For ERLA in 2010, total seed weight was reduced by $0.1 \times$ f.a.r. of either glyphosate or dicamba, or their combination across farms (Figure 2A). The number of seed heads was reduced substantially by $0.1 \times$ f.a.r. of glyphosate or glyphosate plus dicamba at the Botany farm, and by $0.1 \times$ f.a.r. of dicamba or glyphosate plus dicamba at the Hyslop farm, compared with the carrier control plants (Figure 2B). In 2011, however, ERLA showed fewer reproductive responses to the herbicides and more variability within treatments compared with 2010. Significant herbicide responses versus carrier controls across farms were found for total seed weight with $0.2 \times$ f.a.r. of glyphosate plus dicamba (Figure 3A), and for seed heads with $0.2 \times$ f.a.r. of dicamba and 0.1 and $0.2 \times$ f.a.r. of the combinations of glyphosate plus dicamba (Figure 3B).

More noticeable than the reduction in total seed production with low levels of herbicides was the dramatic impact on ERLA seed maturity as indicated by the immature seed weight as a percentage of the total immature plus mature seed weight. At both farms in 2010, immature seeds comprised over 50% of the total seed dry weight for many herbicide treatments (Figure 2C). At the Botany farm, herbicide effects were significant not only at the higher drift level of $0.1 \times$ f.a.r. of dicamba and/or glyphosate, but also at a lower drift level of $0.01 \times$ f.a.r. of dicamba or glyphosate plus dicamba (Figure 2C). The only treatment that had no

effect on percent immature seed weight was $0.01 \times \text{f.a.r.}$ of glyphosate alone. A similar pattern occurred at the Hyslop farm, where the percent immature seed dry weight with and 0.01 and $0.1 \times \text{f.a.r.}$ of dicamba, and the combination of glyphosate plus dicamba at $0.01 \times \text{f.a.r.}$ were significantly different from the carrier controls. In 2011, there was again a large increase in percent immature seed dry weight, which was significant for all herbicide treatments across both farms except for $0.1 \times \text{f.a.r.}$ of glyphosate alone (Figure 3C).

The prolific seed-producing grass ELGL was not as sensitive to the herbicide treatments as the forb ERLA. In 2010, across farms there were no herbicide effects on either total seed production (Figure 4A) or number of panicles (Supplemental Data, Figure S9A). There was an increase in percent immature seed dry weight with $0.1 \times \text{f.a.r.}$ of glyphosate alone or glyphosate plus dicamba (Figure 4B). In 2011, across farms, there was a significant decrease in number of panicles at $0.2 \times \text{f.a.r.}$ with glyphosate plus dicamba (Figure 4D) but no herbicide effects on seed production (Figure 4C) or percent immature seed dry weight (Figure 4E).

For the other species that produced seeds, responses to the herbicides followed similar trends, but were not as pronounced as for ERLA and ELGL. This may have been, at least in part, because of the low amount of seeds produced and tendency for no seeds to be produced even for a number of carrier control plants.

In 2010, there were no significant effects of the herbicides on total seed dry weight or number of reproductive structures across farms for CALE (Supplemental Data, Figure S1A and B), FEID (Supplemental Data, Figure S2A and B), IRTE (Supplemental Data, Figure S4A and B), or PRVU (Supplemental Data, Figure S5A and B). The percent immature seed dry weight significantly increased with $0.1 \times \text{f.a.r.}$ of glyphosate alone or glyphosate plus dicamba across farms for FEID (Supplemental Data, Figure S2C), and with $0.01 \times \text{f.a.r.}$ of glyphosate plus dicamba for the Hyslop farm for CALE (Supplemental Data, Figure S1C). The increase in percent immature seed dry weight for the Hyslop farm for CALE with $0.1 \times \text{f.a.r.}$ of glyphosate was based on only 1 replicate plant, and thus this response could not be analyzed statistically. Similarly, for IRTE there were increases in percent immature seed dry weight with glyphosate at $0.1 \times \text{f.a.r.}$ at the Hyslop farm and with glyphosate plus dicamba at $0.1 \times \text{f.a.r.}$ at each farm, but the results could not be analyzed statistically because of insufficient data (Supplemental Data, Figure S4C). For POGR, there were not enough seeds or pods for statistical analysis (Supplemental Data, Figure S7A–C).

The addition of the higher $0.2 \times \text{f.a.r.}$ treatments in 2011 resulted in more significant reproductive responses for other species. For CALE, 0.1 or $0.2 \times \text{f.a.r.}$ glyphosate alone or in combination with dicamba caused significant reductions in seed production (Figure 5A) and number of pods (Figure 5B) across farms. For IRTE, 0.1 or $0.2 \times \text{f.a.r.}$ glyphosate alone and $0.2 \times \text{f.a.r.}$ glyphosate plus dicamba caused significant reductions in seed production across farms (Figure 5C). There was a reduction in pods for IRTE with $0.2 \times \text{f.a.r.}$ of glyphosate alone or glyphosate plus dicamba at the Botany farm, and with $0.1 \times \text{f.a.r.}$ of glyphosate alone at the Hyslop farm (Figure 5D). These reductions in seed production and pods for IRTE likely were related to few pods being produced for any plots for these treatments. For PRVU there was a significant reduction in total inflorescences at $0.2 \times \text{f.a.r.}$ of glyphosate

plus dicamba (Figure 6A), but the effect of the treatments on total seed dry weight could not be determined statistically (Supplemental Data, Figure S6A).

For FEID in 2011, there were no significant herbicide effects on total seed dry weight or panicle number (Supplemental Data, Figures S3A,B). The percent immature seed dry weight increased for FEID at the Botany farm with $0.2 \times$ f.a.r. of dicamba or glyphosate alone, and 0.1 or $0.2 \times$ f.a.r. of glyphosate plus dicamba; and at the Hyslop farm for $0.2 \times$ f.a.r. of glyphosate plus dicamba (Supplemental Data, Figure S3C). For POGR, there were no significant treatment effects on total seed dry weight (Supplemental Data, Figure S8A) or pod number (Supplemental Data, Figure S8B). Even though statistical analysis was not feasible because of inadequate numbers of plots with values, in 2011 the percent immature seed weight tended to be greater with the glyphosate and glyphosate plus dicamba treatments than for the carrier controls for CALE (Supplemental Data, Figure S10A), IRTE (Supplemental Data, Figure S11A), and POGR (Supplemental Data, Figure S8C).

For PRVU in 2011, atypical inflorescences occurred for dicamba, and for glyphosate plus dicamba treatment plants, and there were very few, if any, atypical inflorescences for the carrier control, no-spray, or glyphosate-alone plants. The numbers of atypical inflorescences (Figure 6B) and percent atypical inflorescences (Figure 6C) across both farms were significantly higher with both 0.1 and $0.2 \times$ f.a.r. of dicamba alone, and with the combination of glyphosate plus dicamba compared with the controls.

Herbicide effects on vegetative growth

For ERLA, the species exhibiting the greatest seed reduction with herbicide treatments, there were no significant effects of dicamba, glyphosate, or the combination of the 2 herbicides on percent cover 10 wk after treatment versus the controls in 2010 (Figure 2D). In 2011, $0.2 \times$ f.a.r. of glyphosate plus dicamba produced a significant reduction (52%) in percent cover across both farms (Figure 3D), but there was no effect on total vegetative biomass (Figure 3E). The high correlation for ERLA in 2011 between arcsine-transformed week 10% cover and log10 final biomass in 2011 ($r^2 = 0.82$, $n = 121$; data not shown) across all plots for both farms and all treatments indicated that the percent cover measurements may be a good surrogate for overall plant growth for this highly productive species.

There were no significant reductions in percent cover and/ or total vegetative biomass because of herbicide treatment at either farm in either year for most species (Supplemental Data, Figures S1D, S2D, S3D and E, S4D, S5C, S6B and C, S7D, S8D, S9B, S10C, S11B and C, S12A and B, and S13A–C). For ERLA in 2010, there was a significant decrease in percent cover from $0.1 \times$ f.a.r. of glyphosate plus dicamba at week 6 (Supplemental Table S6 and data not shown) but not at week 10 (Figure 2D). This effect on early season growth was not as great as the effects of the herbicides on total seed production. For CALE in 2011, there was a significantly lower percent cover across farms at $0.2 \times$ f.a.r. of dicamba or glyphosate alone, or the combination of glyphosate plus dicamba (Supplemental Data, Figure S10B). The percent cover response for CALE was measured at week 6 after treatment instead of week 10, because the plants essentially died back after week 6. These responses for ERLA and CALE at week 6 may also indicate the possibility for effects on growth earlier in the present study with the higher herbicide concentration, effects that may have no

longer been apparent by the final harvest. For POGR in 2011, there was a reduction in biomass with $0.2 \times$ f.a.r. of glyphosate alone and glyphosate plus dicamba (Supplemental Data, Figure S8E). It was not possible to determine herbicide effects on RAOC in either year (Supplemental Data, Figure S14A and B), because of insufficient data.

There were no significant differences in reproduction or growth between the no-spray and carrier control plants in 2010 and only a few differences in 2011. In 2011, for IRTE and ELGL, percent cover and biomass were higher for plants in the no-spray treatment than in the carrier control across farms (Supplemental Data, Figures S11B and C and S12A and B, respectively). For FEID, percent immature seed dry weight was greater at the Botany farm (Supplemental Data, Figure S3C), and for FRVI, biomass was lower across farms (Supplemental Data, Figure S13C) for the no-spray plants versus the controls. These no-spray effects were rare and small, varied with species, and were detected with a 2-tailed t test versus a 1-tailed test for herbicide effects.

Combined vs. Individual Herbicide Effects

The treatment contrast tests for interaction indicated that nearly all interactions between dicamba and glyphosate were additive across all species, both years, both farms, and the multiple plant responses, with very few antagonistic or synergistic effects (Supplemental Data, Tables S4 and S7). For ERLA there were antagonistic interactions (i.e., less effect with the combinations of glyphosate and dicamba than with the individual herbicides) in 2010 at $0.1 \times$ f.a.r. for total seed dry weight across farms and percent immature seed dry weight at the Botany farm (Supplemental Data, Table S4; Figure 2A and C), and in 2011 for percent immature seed dry weight at $0.2 \times$ f.a.r. across farms (Supplemental Data, Table S7; Figure 3C). There also were antagonistic interactions in 2011 for PRVU for number of atypical and percent atypical inflorescences with $0.2 \times$ f.a.r. across farms (Supplemental Data, Table S7; Figure 6B and C).

A synergistic interaction (i.e., greater effect with the combinations of glyphosate and dicamba than with the individual herbicides) occurred for PRVU total inflorescences across farms in 2010 at $0.1 \times$ f.a.r. of glyphosate plus dicamba (Supplemental Table S4; Figure 5B), and in 2011 at $0.2 \times$ f.a.r. of glyphosate plus dicamba (Supplemental Data, Table S7; Figure 6A).

DISCUSSION

Reproductive Responses

The present study clearly indicated that herbicide drift simulated by using a reduced herbicide f.a.r. has the potential to adversely affect seed production of native plant species, likely reducing their ecological fitness. It also indicated that herbicide effects on seed production are not limited primarily to sulfonylurea herbicides as discussed by others [10,11,25,26], but that the widely used herbicides glyphosate and dicamba can also reduce seed production. Previously, adverse effects of glyphosate on seed production were discussed primarily in terms of enhanced weed control and not in terms of implications for native plants. For example, Shuma et al. [27] indicated that glyphosate reduced seed weights

and germination, potentially increasing control for wild oat *Avena fatua* L. [27]. Especially important in the present study were the large increases in percent immature seed weights for many species, which were proportionally greater than the reductions in total seed dry weight. These seed-specific responses were also highlighted by the atypical inflorescences for PRVU, which occurred almost entirely with dicamba or glyphosate plus dicamba treatments (Figure 6B).

Plant reproductive responses to simulated drift of herbicides have been noted before, but not for as many species as in the present study, and infrequently for plants growing under field conditions. In greenhouse studies, Carpenter et al. [28] found that many species had reduced reproduction with chlorimuron. *Avena fatua* L. treated with $0.5 \times$ f.a.r. of glyphosate, especially at 5 d after anthesis, had reduced seed amounts and viability, as well as a reduced dormancy requirement [27]. The authors discussed these effects in terms of reduced *A. fatua* L. ecological fitness (in terms of competition with crops and enhanced weed control [27]), but the same responses could dramatically affect viability if they occurred in native plants. Previously, it was shown that simulated glyphosate drift ($0.1 \times$ f.a.r.) reduced viable *B. rapa* L. flowers available for pollination for plants growing in simulated field plots in large outdoor chambers [29]. The reduction in flowering decreased the likelihood that these plants would be pollinated by transgenic *B. rapa* genotypes, thereby potentially reducing their susceptibility to gene flow, and also reduced the number of *B. rapa* L. seeds available in the soil seed bank. Londo et al. [30] also indicated that sublethal (0.05 – $0.1 \times$ f.a.r.) exposure to glyphosate resulted in deformed and shortened anthers, which appeared unable to release pollen normally.

In greenhouse studies, Rotchés-Ribalto et al. [31] reported reductions in seed production for arable weed species with 2,4- D and tribenuron. Kutman et al. [32] indicated that $0.1 \times$ f.a.r. of glyphosate caused large (up to 85%) reductions in wheat grain yield, especially when plants were treated at the booting stage of development. Olszyk et al. [17] found that approximately $0.01 \times$ f.a.r. of glyphosate caused a 25% reduction, and $0.1 \times$ f.a.r. caused nearly a 100% reduction in seed production in *P. sativum* L. *Pisum sativum* L. plants were less sensitive to dicamba, with a 25% reduction in seed dry weight with $0.07 \times$ f.a.r. Olszyk et al. [18] reported that $0.1 \times$ f.a.r. of glyphosate resulted in a nearly 100% reduction in silique and seed production in *B. rapa* L. (Wisconsin Fast Plants).

Groundbreaking field research by Maars et al. [13] indicated that flowering and seed production of common British plant species found in nature preserves and seminatural habitats could be reduced by common herbicides including glyphosate, using plants in pots exposed in the field. In contrast to the present study, however, Maars et al. [13] reported that reproduction was not necessarily more sensitive to glyphosate than other responses, depending on the species. For example, *P. vulgaris* L. reproduction was equally or less sensitive to herbicides as was growth, because flowering and seed production occurred at the same or lower distances (higher herbicide concentration) from the sprayer than injury or biomass reduction [13].

Gove et al. [9] also evaluated the effects of glyphosate on British plant species treated in a greenhouse and grown in the field and indicated effects at 0.01 to $0.05 \times$ f.a.r. of 2160 g a.i.

ha⁻¹, which were in the same range of rates used in the present study. Gove et al. [9] reported reduced flower production at approximately the same, or at a greater, concentration than that which caused biomass reductions, depending on species.

While past studies with individual species indicated the potential for herbicide effects on plant reproduction, additional field experiments are necessary to assess higher-tier ecological risks to plants [5]. A recent example of such field studies is the research by Schmitz et al. [10,11] on the effects of potential drift of herbicides, insecticides, and fertilizer alone and in combination to field-margin plants. Schmitz et al. [10] demonstrated that $0.3 \times$ f.a.r. of sulfonyleurea herbicides reduced seed production and reduced flower intensity in *Ranunculus acris* L. Schmitz et al. [11] reported that reduced flower and subsequent seed production in *R. acris* L., *Lathyrus pratensis* L., and *Vicia sepium* L. may lead to changes in the composition of plant communities. The herbicide effects on reproduction were associated with reduced occurrence in plots and biomass for some species [11]. Another example of field research was by Bohnenblust et al. [33], who reported that $0.01 \times$ f.a.r. of dicamba reduced the number of flowers in *Medicago sativa* L. grown in field plots and *Eupatorium perfoliatum* L. grown in pots.

The mechanism by which glyphosate affects seed production needs further study, but may involve impacts on the growth regulator indole-3-acetic acid (IAA) during seed formation [34]. Clay and Griffin [34] pointed out that glyphosate inhibits the shikimic acid pathway, which produces the amino acid tryptophan [35], a direct precursor of IAA, which acts as a plant growth regulator [36]. Glyphosate's impact on IAA was suggested as a mechanism for its inhibition of seed germination and emergence [34]. Although this impact on seed physiology may be advantageous as a treatment to reduce weed seed production, as suggested by Clay and Griffin [34], the effects could be adverse for nontarget native plants. The mechanism for dicamba effects on reproduction also needs further research, for example, with genomic studies such as that conducted by Gleason et al. [37], which indicated that dicamba affected auxin metabolism.

Combined vs. individual herbicide effects

Responses to combinations of glyphosate and dicamba tended to resemble the responses to either of the herbicides alone, as seen earlier in our greenhouse studies [19]. For example, in both years the herb ERLA had increases in percent immature seed dry weight with combinations of glyphosate plus dicamba, which most resembled the responses to dicamba alone (Figures 2C and 3C). In 2010, the grass ELGL had a reduction in the percent immature seed dry weight with $0.1 \times$ f.a.r. of glyphosate plus dicamba similar to the reduction with $0.1 \times$ f.a.r. of glyphosate alone (Figure 4B). There were only a few antagonistic or synergistic responses; in most cases, the interactions between dicamba and glyphosate were additive. Previous studies indicated that interactions between herbicides varied widely, with antagonistic responses being more prevalent [38]. Antagonistic responses, especially, may occur if the individual herbicides have large effects on plant responses, as observed previously with several native plants [19]. Although the statistical analysis indicated limited antagonistic effects in the present study, in reality it was not possible to have any additional response to a second herbicide because the effect was so great from the first herbicide. For

example, for ERLA in 2010 (Botany farm) and 2011 (across farms), the increase in percent immature seed dry weight was so large with the individual herbicides that the combination of herbicides could not cause any additional response and would appear to be antagonistic (Figures 2C and 3C, respectively).

In the present study, ERLA appeared to be more sensitive to glyphosate than FEID, POGR or PRVU, species that were previously shown to be more sensitive to glyphosate than ERLA under greenhouse conditions (Table 3 in Olszyk et al. [16]). The reason for this is unknown but may have been related to relative plant size, as discussed by Riemans et al. [39]; that is, smaller species such as POGR, PRVU, and RAOC may have been sheltered and received less of the herbicide than the larger plants.

Significance for ecological risk assessments

As long as the use of chemicals in weed management remains a high priority in agriculture, forestry, and land management, impacts of herbicides on nontarget plants will remain a potential problem. The development of crops with multiple herbicide resistances is increasing concern that subsequent new uses may increase adverse impacts from herbicides to nontarget plants [20]. Despite the importance of potential risks to nontarget plants, current assessments based on individual species phytotoxicity tests may not “reflect relevant ecological processes in terrestrial ecosystems” [5].

A key aspect of ecological risk assessments (the potential effects of herbicides on reproduction) is not addressed with the current plant test protocols, which focus on vegetative growth [40,41]. This is important because measurements of biomass alone may not be enough to truly understand the effects of a plant's future ability to reproduce, and recent studies have demonstrated potential effects of herbicides on flower and seed production [10–12]. The present field study provides a methodology to evaluate herbicide impacts, addressing several of the recommendations of Schmitz et al. [5] for microcosm experiments for terrestrial nontarget plant risk assessments; namely, we used a larger number of species (9), a mix of species considering different plant traits, an older developmental stage, and a whole growing season test duration. Our experimental design along with those used especially by Marrs et al. [13], Schmitz et al. [11,12], Bohnenblust et al. [33], and Brain et al. [42] point to the importance of using field plots or pots placed in the field to determine effects of simulated herbicide drift.

Recently, consideration of reproductive endpoints was recommended as part of the European Union's process to assess risks to nontarget terrestrial plants from pesticides [43]. Recovery from herbicide effects may occur in certain plant species, but the potential costs of recovery may have negative effects on reproduction through reductions in seed or fruit production, with subsequent long-term effects through diminished seed banks of herbicide-sensitive species [8]. When exposed to a sublethal dose of an herbicide, plants may also experience delays in flowering or fruit production, which may negatively affect wildlife via the loss of food [8].

Considerable effort has been made to conserve native plant communities, yet the impacts of pesticide drift on them are still largely unknown [9]. Anthropogenic stressors such as the off-

farm movement of pesticides can induce significant change in plant communities, leading to changes not only in successional trajectories of plant communities [25], but possibly in the diversity and productivity of consumers associated with them. For example, the effects of herbicides on flowering have been shown to alter visitation by pollinating insects [33]. Thus, as stated by Pimental et al. [44], “Maintaining this biological diversity is essential for productive agriculture and forestry.” The present study provides additional evidence that low doses of herbicides can affect individual species reproduction, and hence could impact plant communities and their diversity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Acknowledgment

The authors thank M. Larson from the Senior Environmental Employee program of the National Asian Pacific Center; M. Storm, G. Heine, and F. Senecal of Consolidated Safety Services; US Environmental Protection Agency (USEPA) summer student Oregon State University student intern C. Bidelsbach; and Greater Research Opportunity summer student A. Ramsower for their assistance in carrying out these experiments. The information in this document was funded in part by the USEPA under contracts EP-D-06-013 and EP-D-11-027 to CSS-Dynamac. It has been subjected to review by the National Health and Environmental Effects Research Laboratory’s Western Ecology Division and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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FRVI	PRVU	FRVI	POGR	RAOC
POGR	ELGL	ERLA	PRVU	CALE
IRTE	RAOC	CALE	IRTE	FEID
ERLA	FEID	POGR	FRVI	ERLA
ELGL	IRTE	ROAC	CALE	PRVU

Figure 1.

Arrangement of species in field plots at Botany and Hyslop farms. The plots were 0.75 m on a side with plants on a 0.15-m center-to-center spacing. See Table 1 for definitions of species abbreviations.

Figure 2.

Response of *Eriophyllum lanatum* (Pursh) Forbes to dicamba and/or glyphosate at 2 farms in Oregon's Willamette Valley in 2010. Data are for total seed production (**A**), number of seed heads (**B**), percent immature seed dry weight of total seed dry weight (**C**), and percent cover 10 wk after treatment (**D**). Each bar is average plus unpooled upper average standard error for the Botany and Hyslop farms (**A** and **C**), or across both farms (**B** and **D**). Data are for 8 plots/ treatment for each farm, or 16 plots/treatment across farms (8 from each farm); except for 7 plots with dicamba (DI) or glyphosate (GL) plus DI at $0.01 \times$ the field application rate (f.a.r.) for total seed production and seed heads at the Botany and Hyslop farms, and at the Hyslop farm for DI at $0.1 \times$ f.a.r. and GL at $0.01 \times$ f.a.r. for total seed production, for DI at $0.01 \times$ f.a.r. for seed heads, and for DI or GL at $0.01 \times$ f.a.r. for percent cover. In addition, there were 18 missing plots for percent immature seed across treatments at both farms. An asterisk (*) above a bar indicates a significant difference from the carrier control (CC) for that farm and a plus sign (+) above a bar indicates a significant difference from CC across farms, according to Bonferroni's test. There were no significant herbicide effects for percent

cover in week 10. Numbers following letters in the horizontal axis labels indicate percent of f.a.r. For GL, the f.a.r. was 830 g active ingredient ha⁻¹ acid glyphosate, and for DI the FAR was 560 g ha⁻¹. NS = no spray.

Figure 3.

Response of *Eriophyllum lanatum* (Pursh) Forbes to dicamba and/or glyphosate across 2 farms (Botany and Hyslop) in Oregon's Willamette Valley in 2011. Data are for total seed production (**A**), number of seed heads (**B**), percent immature seed dry weight of total seed dry weight (**C**), percent cover 10 wk after treatment (**D**), and final vegetative biomass (**E**). Each bar is average plus unpooled upper average standard error for 16 plots across farms (8 from each farm) per treatment, except that at the Botany farm there were 7 plots for no-spray (NS) for percent cover; 6 plots with dicamba (DI) or with glyphosate (GL) at $0.2 \times$ the field application rate (f.a.r.) treatment for percent cover and biomass, with carrier control (CC) and GL at $0.2 \times$ f.a.r. for seed dry weight, and with DI or GL at $0.2 \times$ f.a.r. for seed heads.

There were 5 plots at for the Botany farm with DI or GL at $0.1 \times \text{f.a.r.}$, and 4 plots with DI at $0.2 \times \text{f.a.r.}$ for seed dry weight. For the Hyslop farm, there were 7 plots for GL plus DI at 0.1 or $0.2 \times \text{f.a.r.}$ for total seed weight, seed heads, percent cover, and biomass. There were missing percent immature seed dry weight values for 39 plots across treatments and both farms. A plus sign (+) above a bar indicates a significant difference from CC according to Bonferroni's test across both farms. There were no significant herbicide effects for total vegetative biomass. Numbers following letters in the horizontal axis labels indicate the amount $\times \text{f.a.r.}$ For GL, the f.a.r. was $830 \text{ g active ingredient ha}^{-1}$ acid glyphosate, and for DI the f.a.r. was 560 g ha^{-1} .

Figure 4.

Response of *Elymus glaucus* Buckley to dicamba and/or glyphosate across 2 farms (Botany and Hyslop) in Oregon's Willamette Valley. Data for 2010 are for total seed production (**A**) and percent immature seed dry weight of total seed dry weight (**B**). Data for 2011 are for total seed production (**C**), number of panicles (**D**), and percent immature seed dry weight of total seed dry weight (**E**). Each bar is average plus unpooled upper average standard error for 16 plots across farms (8 from each farm) per treatment, except for 2010 seed production at the Botany farm where there were 6 plots for dicamba (DI) at $0.01 \times$ the field application rate (f.a.r.) and 7 plots at the Hyslop farm for glyphosate (GL) and GL plus DI at $0.01 \times$ f.a.r. For percent immature seeds in 2010, at the Botany farm there were 7 carrier control (CC) plots and 5 plots for DI at $0.01 \times$ f.a.r., and at the Hyslop farm there were 7 plots for GL and GL plus DI at $0.01 \times$ f.a.r. In 2011 at the Botany farm there were 7 plots for the NS and GL at 0.1 and $0.2 \times$ f.a.r. for total seed dry weight and panicles; and for percent immature seed there were 15 missing plots across many treatments at both farms. A plus sign (+) above a bar indicates a significant difference from CC across farms according to Bonferroni's test. There were no significant treatment effects in 2010 for total seed dry weight, or in 2011 for total seed dry weight or percent immature seed dry weight. Numbers following letters in the

horizontal axis labels indicate the amount $\times$ f.a.r. For GL, the f.a.r. was 830 g active ingredient ha^{-1} acid glyphosate, and for DI the f.a.r. was 560 g ha^{-1} . NS = no spray.

Figure 5. Response of *Camassia leichtlinii* (Baker) S. Watson (CALE) and *Iris tenax* ex Lindl. (IRFL) and/or dicamba (DI) and/or glyphosate (GL) at 2 farms in Oregon's Willamette Valley in 2011. Data are for CALE total seed production (A) and number (#) of pods (B); and for IRTE, data are total seed production (C) and # of pods (D). Each bar is average plus unpooled upper average standard error across both farms (A–C), or separately for the Botany and Hyslop farms (D). Data are for 16 plots/treatment across farms, or 8 plots/treatment for each farm; except for 7 plots for IRTE at the Hyslop farm with GL at 0.1 $\times$ the field application rate (f.a.r.). A plus sign (+) above a bar indicates a significant difference from carrier controls (CC) across farms, and an asterisk (*) above a bar indicates a significant difference from CC for that farm according to Bonferroni's test. For IRTE pods, all values were zero for GL and GL plus DI at 0.2 $\times$ f.a.r. at the Botany farm, and for GL at 0.1 $\times$ f.a.r. at the Hyslop farm. The least squares standard error for pods from Botany farm GL plus DI at 0.2 $\times$ f.a.r. and Hyslop farm GL at 0.1 $\times$ f.a.r. was negative; thus the standard

error was set at 0. Numbers following letters in the horizontal axis labels indicate the amount $\times$ f.a.r. For GL, the f.a.r. was 830 g active ingredient ha^{-1} acid glyphosate, and for DI the f.a.r. was 560 g ha^{-1} . NS = no spray.

Figure 6.

Response of *Prunella vulgaris* L. subsp. lanceolata (W. Bartram) Hultén to dicamba and/or glyphosate across 2 farms (Botany and Hyslop) in Oregon's Willamette Valley in 2011. Data are for number (#) of total inflorescences (A) number of atypical inflorescences (B), and percentage atypical inflorescences (C). Each bar is average plus unpooled upper average standard error across both farms. Data are 16 plots/treatment across farms (8 from each farm), or 8 plots/treatment for each farm, except for # of inflorescences and atypical inflorescences where there were 7 plots at the Botany farm with carrier control (CC), no-spray (NS), and dicamba (DI) and glyphosate (GL) plus DI at $0.1 \times$ the field application rate (f.a.r.), and 7 plots at the Hyslop farm with DI or GL at $0.2 \times$ f.a.r. For percent atypical inflorescences, there were 21 missing plots across treatments at both farms. A plus sign (+) above a bar indicates a significant difference from CC across farms according to Bonferroni's test. Numbers following letters in the horizontal axis labels indicate the amount

× f.a.r. For GL, the f.a.r. was 830 g active ingredient ha⁻¹ acid glyphosate, and for DI the f.a.r. was 560 g ha⁻¹.

Table 1.

Willamette Valley native perennial plant species used in plant community studies^a

Species	Abb. ^b	Common Name	Growth Habit	Group	Family
<i>Ceanothus leucanthemifolius</i> (Baker) S. Watson	CALE	Large ceano	Forb/herb	Monocot	Liliaceae
<i>Elymus glaucus</i> Buckley	ELGL	Blue wildrye	Graminoid	Monocot	Poaceae
<i>Eriophyllum lanatum</i> (Pursh) Forbes	ERLA	Common woolly sunflower	Forb/herb, subshrub	Dicot	Asteraceae
<i>Festuca idahoensis</i> Elmer subsp. <i>roemerii</i> (Pavlick) S. Aiken	FEID	Roemer's fescue	Graminoid	Monocot	Poaceae
<i>Fragaria virginiana</i> Duchesne	FRVI	Virginia strawberry	Forb/herb	Dicot	Rosaceae
<i>Iris tenax</i> Douglas ex Lindl.	IRTE	Toughleaf iris	Forb/herb	Monocot	Iridaceae
<i>Potentilla gracilis</i> Douglas ex Hook.	POGR	Slender cinquefoil	Forb/herb, subshrub	Dicot	Rosaceae
<i>Prunella vulgaris</i> L. subsp. <i>lanceolata</i> (W. Bartram) Hultén	PRVU	Lance selfheal	Forb/herb	Dicot	Lamiaceae
<i>Ranunculus occidentalis</i> Nutt.	RAOC	Western buttercup	Forb/herb	Dicot	Ranunculaceae

^a Species, growth habit, group, and family based on US Department of Agriculture Plants Database [45]. Subspecies listed only for *Festuca idahoensis* and *Prunella vulgaris*; information on other likely subspecies is available on request.

^b Four-letter abbreviations (Abb.) are based on the first 2 letters of genus and species.

Table 2.

Dates for percent cover measurements and herbicide treatments at each farm in each year^a

<u>Year</u>	<u>Farm</u>	<u>Cover0</u>	<u>Treatment</u>	<u>Cover1</u>	<u>Cover2</u>	<u>Cover3</u>	<u>Cover4</u>	<u>Cover5</u>	<u>Cover6</u>
2010	Botany	28–30 April	11 May	24,25 May	7,8 June	21,22 June	6,7 July	19,20 July	N/A
2010	Hyslop	26–28 April	30 April	17,18 May	1,3 June	14,15 June	29,30 June	12,13 July	N/A
2011	Botany	9,10 May	11 May	23,24 May	6,7 June	20,21 June	5,6 July	18,19 July	N/A
2011	Hyslop	3,4 May	6 May	16,17 May	31May,1 June	14,15 June	27,29 June	11,13 July	25,26 July

^aThe Cover 0 measurements were before herbicide treatment, and Cover 1 through Cover 6 measurements at approximately 14-d intervals after treatment.

Table 3.

Results from ANCOVA considering both 2010 and 2011 for reproductive parameters for key Willamette Valley species treated with low levels of herbicides^a

Species	Parameter	ANCOVA										
		Trt.	Farm	Trt. × Farm	Year	Trt. × Year	Farm × Year	Trt. × Farm × Year	Cov.	Cov. × Farm	Cov. × Year	Cov. × Farm × Year
CALE	Total Seed DW	<0.001 *	0.104	0.445	0.643	0.697	0.965	0.213	0.021 *			
ELGL	Total Seed DW	0.001 *	0.006 *	0.308	<0.001 *	0.967	<0.001 *	0.540	<0.001 *	<0.001 *	<0.001 *	<0.001 *
ELGL	Panicle No.	<0.001 *	0.559	0.334	0.002 *	0.462	0.282	0.136	<0.001 *	0.029 *	<0.001 *	0.019 *
ELGL	% Imm. Seed	<0.001 *	0.016 *	0.363	0.909	0.102	0.191	0.027 *	0.324	0.007 *	0.367	0.077
ERLA	Total Seed DW	<0.001 *	<0.001 *	0.115	0.283	0.186	<0.001 *	0.433	<0.001 *	0.034 *	0.279	<0.001 *
ERLA	Seedhead No.	<0.001 *	0.018 *	0.147	0.054	0.042 *	0.907	0.494	<0.001 *			
ERLA	% Imm. Seed	<0.001 *	0.001 *	0.056	0.074	<0.001 *	0.396	0.068				
IRTE	Total Seed DW	<0.001 *	0.062	0.348	<0.001 *	0.765	0.254	0.534	<0.001 *	0.066	<0.001 *	0.114

^aSpecies abbreviations are as in Table 1. Effects are treatment (Trt; carrier control, no-spray, or herbicides), farm, year, and covariate (Cov.; measured as pretreatment percent cover).

* Significant at $p < 0.05$.

ANCOVA = analysis of covariance; no. = number; Imm. = immature.

Note: Final journal formatting of tables slightly different in terms of lines and spacing.