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EFFECTS OF THE HERBICIDE DICAMBA ON NONTARGET PLANTS AND POLLINATOR VISITATION

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Abstract: Nearly 80% of all pesticides applied to row crops are herbicides, and these applications pose potentially significant ecotoxicological risks to nontarget plants and associated pollinators. In response to the widespread occurrence of weed species resistant to glyphosate, biotechnology companies have developed crops resistant to the synthetic-auxin herbicides dicamba and 2,4-dichlorophenoxyacetic acid (2,4-D); and once commercialized, adoption of these crops is likely to change herbicide-use patterns. Despite current limited use, dicamba and 2,4-D are often responsible for injury to nontarget plants; but effects of these herbicides on insect communities are poorly understood. To understand the influence of dicamba on pollinators, the authors applied several sublethal, drift-level rates of dicamba to alfalfa (*Medicago sativa* L.) and *Eupatorium perfoliatum* L. and evaluated plant flowering and floral visitation by pollinators. The authors found that dicamba doses simulating particle drift (≈1% of the field application rate) delayed onset of flowering and reduced the number of flowers of each plant species; however, plants that did flower produced similar-quality pollen in terms of protein concentrations. Further, plants affected by particle drift rates were visited less often by pollinators. Because plants exposed to sublethal levels of dicamba may produce fewer floral resources and be less frequently visited by pollinators, use of dicamba or other synthetic-auxin herbicides with widespread planting of herbicide-resistant crops will need to be carefully stewarded to prevent potential disturbances of plant and beneficial insect communities in agricultural landscapes. *Environ Toxicol Chem* 2016;35:144–151. © 2015 SETAC

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INTRODUCTION

Herbicides are the dominant pesticide used on most farms, accounting for nearly 40% of total pesticide use worldwide [1]. In the United States, herbicide use accounts for almost 50% of pesticide use, including nearly 80% of pesticide applications in row crops [1,2]. Over the past 2 decades, widespread use of herbicides has mostly been driven by high adoption rates of genetically modified, herbicide-resistant crops, which allow specific herbicides, like glyphosate, to be applied over the growing crop without risking injury. Glyphosate-resistant crops account for a large percentage of the acreage of several common agronomic crops (e.g., at least 93% of soybeans, 68% of maize, and 71% of cotton in the United States are glyphosate-resistant) [3–5]; however, the popularity of glyphosate-resistant crops has fostered overreliance on this 1 active ingredient, resulting in 29 weed species worldwide that are resistant to glyphosate [6,7].

The expanding problem of glyphosate-resistant weeds has prompted agricultural companies to engineer varieties of crop species that are resistant to herbicides in addition to glyphosate. Recently, agricultural companies have developed crops that are resistant to dicamba or 2,4-dichlorophenoxyacetic acid (2,4-D) [8,9], 2 synthetic-auxin herbicides that are highly effective at controlling broadleaf weeds, a feature likely to drive high adoption rates following commercialization [10]. Currently, to prepare for crop planting, farmers apply these 2 herbicides in tolerant cereal crop fields early in the growing season; nevertheless, the active ingredients are frequently responsible for sublethal, off-target damage to nearby sensitive crops and noncrop plants [11–15]. Nontarget plants are commonly exposed to dicamba or 2,4-D through particle drift, which can occur when droplets of herbicide solutions are carried away aerially from application equipment [16]. Certain formulations of dicamba and 2,4-D can also evaporate off of a crop field after an application and move as vapor drift [12,17], although newer formulations of both compounds can greatly reduce this risk [14]. Importantly, nontarget injury in agricultural landscapes is likely to increase as farmers adopt these new transgenic traits and use these herbicides later in the year when more foliage is fully emerged [10]. In addition, exposure of plants in agroecosystems to sublethal doses of dicamba and 2,4-D may impose stress that alters host-plant quality with potential repercussions for insect communities, particularly herbivorous species [18–20] and pollinators, which are sensitive to floral quantity and quality [21,22].

Pollinators are extremely important for global agricultural production. Many crops, especially fruits and vegetables, require pollination; and emerging evidence suggests that insect-mediated pollination can improve yield and quality over self-pollination [23]. Of course, insect pollination is less critical for some grain-crop and forage-crop systems; however, in many regions high-value, insect-pollinated vegetable crops are grown near row crops. Wind pollinates cereal crop species such as corn and wheat, whereas others like soybean do not require pollinators to achieve acceptable yields; however, production of crops like canola or alfalfa grown for seed relies on insect pollination [24,25]. In fact, pollination of alfalfa for

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seed production in the United States is worth more than $5 billion per year, most of which is attributable to the alfalfa leafcutter bee (*Megachile rotundata* Fabricius [Hymenoptera: Megachilidae]). Moreover, pollination services for all crops requiring direct pollination annually exceed $15 billion, and wild bee communities provide nearly $3.5 billion worth of these pollination services [26,27]. Further, because of its large colony sizes and history of management practices, the honey bee (*Apis mellifera* L. [Hymenoptera: Apidae]) is the most common managed pollinator species globally and provides pollination worth $11.5 billion annually [26]. Yet, the influences of herbicides, which often affect plant flowering, on honey bees and other flower-visiting insect species are not well understood.

Some herbicides may have lethal or sublethal, direct and/or indirect effects on pollinators, whereas others do not appear to cause any effects. For instance, herbicides such as paraquat are highly toxic to honeybees when applied topically [28]. In contrast, when fed to honey bee colonies, the herbicides 2,4-D and 2,4-trichlorophenoxyacetic acid do not appear toxic to adult bees but can negatively influence brood development [29,30]. Others, like dicamba and picloram, do not appear to be directly toxic to adult honey bees or brood [29,30]. Indirect effects of herbicides on pollinators may be mediated by floral resources. For instance, extremely low doses of 2,4-D can up-regulate nectar production of cotton, although this increase did not alter honey bee visitation rates [31]. Sublethal doses of other herbicides, on the other hand, can reduce nectar sources and floral density up to 85%, a reduction likely to influence pollinator visitation and resource availability [18,20,32]. Because pollen is the sole source of protein for bees, reduction in pollen quantity and quality (i.e., protein concentration) from herbicide exposure could result in negative consequences for development and survival of wild and managed bee larvae [33,34].

Because of the emergence of colony collapse disorder [35], there is a renewed interest in the effects of pesticides on honey bee health and their potential role in colony collapse disorder. Recent evidence has found 121 different active ingredients of pesticides associated with honey bee hives [36]. Seventeen of these compounds were herbicides, but this did not include dicamba and 2,4-D; however, the technical analyses used were inappropriate for detecting these synthetic-auxin herbicides, so the possibility remains that they could be found in hives (C. Mullin, The Pennsylvania State University, University Park, PA, USA, personal communication). Nevertheless, given pending commercialization of the new transgenic, herbicide-resistant crops and the associated likelihood of more extensive use of dicamba and 2,4-D over large areas later in the season [10], the possibility will greatly increase for bees to encounter these herbicides or plants affected by them. Furthermore, because synthetic-auxin herbicides are selectively toxic to broadleaf plants and not grasses, low-dose exposures that do not result in plant mortality may have significant impacts on the timing, amount, and quality of flower production by the plant species that sustain pollinator populations. In the present study, we assessed how sublethal doses of dicamba to plants influence floral production, resource quality, and rates of visitation by honey bees and other flower-visiting taxa. To evaluate the influence of dicamba in agricultural and natural contexts, we conducted field experiments with 2 insect-pollinated plant species commonly found near herbicide-resistant crops: alfalfa (*Medicago sativa* L.), and common boneset (*Eupatorium perfoliatum* L.).

MATERIALS AND METHODS

Pollinator visitation to M. sativa

To assess the effects of drift-level doses of dicamba on *M. sativa* flowering and pollinator visitation, we conducted a dose–response field experiment during the summer of 2012 at the Russell E. Larson Agricultural Research Farm of the Pennsylvania State University (Rock Springs, PA, USA). Six dicamba treatments (0 g acid equivalent [a.e.]/ha, 0.0056 g a.e./ha, 0.056 g a.e./ha, 0.56 g a.e./ha, 5.6 g a.e./ha, and 56.1 g a.e./ha) were arranged in a randomized complete block design (6.1×2.4-m plots) and replicated 4 times. Based on a 561 g a.e./ha field application, a current label rate for corn [37], these values correspond to the lowest observed effect rate for dicamba exposures on sensitive soybean plants (0.0056 g a.e./ha [14]), vapor drift exposures (< 0.56 g a.e./ha [14,17]), a particle drift exposure (5.6 g a.e./ha [38–40]), or a serious misapplication event (56.1 g a.e./ha). The farm manager established the alfalfa field in April 2004 with Genoa® NK seed (Helena Chemical) at a rate of 20 kg/ha. Although alfalfa is not typically grown for seed in Pennsylvania, it was used because it is a common perennial crop species that flowers and provides an important resource to many types of pollinators. Five meters separated plots to minimize drift from nearby treatments. Three weeks prior to applying the herbicide, we harvested the entire alfalfa field and then, when alfalfa was approximately 15 cm tall (≈V4 growth stage) on 2 July between 6:30 AM and 7:00 AM, sprayed each plot with 1 of 5 rates (see above) of the low-volatility diglycolamine formulation of dicamba (Clarity® herbicide; BASF) or water as a control (total carrier volume of 187 L/ha). We applied dicamba using a backpack CO_2 sprayer with a 3.05-m handheld boom equipped with AIXR11002 nozzles (Teejet Technologies) calibrated at 24 kPa, walking at a speed of 1.2 m/s. To prevent severe damage from the potato leafhopper (*Empoasca fabae* [Harris]) and ensure flowering, on 9 July we applied the insecticide lambda-cyhalothrin to the field at 23.3 g active ingredient/ha. On 23 July, when *M. sativa* plants were consistently flowering across the site, we assessed the abundance and diversity of floral visitors to a distinct group of flowering stems per plot (2–3 stems) averaging 16 flower clusters per group (range 7–36 at rates of 0–5.6 g/ha, often 0 at a rate of 56.1 g/ha) for 3 min. Visitors were visually identified to the lowest taxon possible. Because we observed very few visitors (6 total floral visits by all insect taxa combined), on 26 July a single standard honey bee hive was placed approximately 50 m from the field edge. Then on 30 July and 9 August, we evaluated floral visitation, counted flowers per stem as described above, and used these data to calculate the number of visits per minute. In addition to counts on groups of flowering stems, we counted for 1 min the number of honey bees we saw in each whole plot. All observations were made between 12:00 PM and 3:00 PM on sunny days.

We analyzed alfalfa flowering and pollinator visitation responses to dicamba exposure using either the log-logistic or the $\log_{10}$-linear regression model. We analyzed the number of alfalfa flower clusters per stem and honey bees per plot per minute as responses to dicamba dose by fitting log-logistic dose–response curves using the nls package in R [41,42], excluding pollinator visits from the first sample date because we did not observe enough visits to draw meaningful conclusions. Because high doses of dicamba are fatal to alfalfa and we did not have doses high enough to populate the lower limit of the curve [43], we fit a 3-parameter log-logistic function with the lower asymptote set to 0 (Equation 1) for

flower clusters per stem and the number of bees per plot per minute on each date.

$$Y_i = D/(1 + \exp\{b^*[\log_{10}(Dose_i) - \log_{10}(e_{50})]\}) \quad (1)$$

The log-logistic function is commonly used to describe herbicide dose–response data because its parameters are biologically interpretable, where D is the upper limit, $Dose_i$ represents the doses tested, e is the dose that causes a 50% reduction in the response variable (50% effective dose [ED50]), and b is the slope of the curve at the ED50 dose [44].

Synthetic-auxin herbicides can cause hormesis, a stimulatory effect to crops at low doses. To test for hormesis, we compared our logistic models to the Cedergreen hormesis model [45] using Akaike's information criterion [46]. For all variables, the log-logistic models showed a better fit, indicating no evidence for hormesis.

Data for total pollinator visits per plot and honey bee visits per alfalfa plant showed a very poor fit to log-logistic models and instead indicated a log-linear pattern. In these models, we regressed total visits against a logarithm of dicamba dose, such that the control dose was set to 0 and treatment doses were evenly spaced on an integer scale with 0.0056 g ha^{-1} equal to 1 [47].

Pollinator visitation to E. perfoliatum

Because limited information is available about the effects of herbicides on native noncrop, nonweedy plants [48], we assessed the effects of drift-level doses of dicamba on flowering of, and pollinator visitation to, a native perennial flowering-plant species. We chose *E. perfoliatum* because it can occur naturally in field edges throughout the eastern United States and is highly attractive to honey bees and other insect species [49–51] (N. DeBarros, 2010, Master's thesis, The Pennsylvania State University, University Park, PA, USA). In spring 2013 we purchased *E. perfoliatum* plants (1-L pots; Missouri Wildflowers Nursery) and on 15 May placed the newly arrived plants outdoors in a large mesh cage (1.8 × 1.8 × 3.7 m), to prevent herbivory, and watered as needed. On 10 June, to facilitate growth, we repotted the plants into 7.6-L pots (Griffin Greenhouse). When plants were approximately 1 m tall (11 July) and before flowering began, we applied, using a backpack CO_2 sprayer (conditions described above in *Pollinator visitation to* M. sativa), 1 of 5 dicamba treatments (0 g a.e./ha, 0.56 g a.e./ha, 5.6 g a.e./ha, 56.1 g a.e./ha, and 280.5 g a.e./ha; Clarity herbicide) to 10 plants except for the 280.5 g a.e./ha dose, which we applied to 7 plants. Because of the limited number of plants, we omitted the 2 doses lower than 0.56 g a.e./ha because we did not see any effects at these rates in the present study's alfalfa experiment and added the 280.5 g a.e./ha rate to ensure that we saw complete inhibition of flowering. Herbicide was applied between 8:20 AM and 8:40 AM, and plants of different treatments were separated by 20 m for 48 h to reduce risk of herbicide drift.

To assess floral visitation by pollinators to dicamba-treated *E. perfoliatum*, we placed all of our potted plants and a single honey bee hive for approximately 1 mo (29 July–23 August 2013) at a commercial farm near Port Matilda, Pennsylvania, USA. We placed the standard bee hive on the edge of a large, partially mowed hay field and randomly positioned our plants in 2 separate rows along a fencerow approximately 100 m from the hive. Individual plants were separated by 1 m and rotated once per week to account for location effects. When plants were placed in the field, we measured plant height to the apical tip. From 31 July to 22 August, for 3 d per week and twice per day, we counted for 1 min the number of pollinators visiting each individual plant. We sight-identified visitors to the lowest taxonomic level possible but did not collect individuals because we did not want to disrupt the pollinator community, with the exception of a 1-time collection of 3 individual scatopsid flies to confirm their identity. Because new *E. perfoliatum* inflorescences emerge during late morning and pollinator activity is highest after mid-morning, all observations were performed between 11:30 AM and 3:30 PM on sunny days. On each sample date, we also recorded the number of flower heads per plant and on 18 August measured, using a caliper, the diameter of 3 flower heads per plant to estimate floral area. After 22 August, we removed plants from the field site to a grass area in proximity to our research laboratory, where we continued for 4 wk to record weekly the number of flower heads per plant to track the influence of dicamba on flower production and particularly to determine if plants dosed with higher rates of dicamba would eventually flower. Ten weeks after dicamba exposure, plants were cut at soil level, dried (50 °C for 72 h), and weighed for biomass.

As above with our alfalfa experiment, detailed in *Pollinator visitation to* M. sativa, we analyzed the response of *E. perfoliatum* and its pollinators to dicamba using either the log-logistic or the log-linear model. We used log-logistic models for the responses of flower heads per plant on 22 August; time to initial and maximum flowering; mean visits per square centimeter of floral area by halictids, *Bombus* spp., and scatopsids; and mean visits per plant per minute (averaged over the entire observation period) by halictids, honey bees, *Bombus* spp., scatopsids, syrphids, and "other" flies. Because the number and size of flowers produced per plant could vary substantially among plants with the same treatment dose, we examined *E. perfoliatum* pollinator visitation both on a per plant basis and standardized on a per square centimeter floral area basis. We recorded the date of initial flowering for all plants that flowered after 30 July; however, in a few instances plants flowered prior to this date, in which case we used 30 July as the initial date. We removed from the analyses 1 plant sprayed with 5.6 g a.e./ha because this plant was 20-cm larger than all of the other plants at the time of spraying and it bloomed similarly to plants dosed with 0 g a.e./ha and 0.56 g a.e./ha. Because we had rates high enough to populate the lower asymptote of the curve (C = lower limit), we fit a 4-parameter log-logistic function (Equation 2) for flowers per plant.

$$Y_i = C + (D - C)/(1 + \exp\{b^*[\log_{10}(Dose_i) - \log_{10}(e_{50})]\}) \quad (2)$$

We analyzed *E. perfoliatum* height, biomass/ramet, and mean visits per square centimeter of floral area by honey bees, syrphids, and "other" flies using $\log_{10}$-linear regression with dose on a logarithmic scale because the nonlinear regression analyses suggested that the relationships between these responses and dicamba dose were log-linear. Visitation by the other insect groups that we observed was not high enough to allow for meaningful analysis. We standardized biomass by the number of ramets to satisfy homogeneity of variance assumptions for regression.

Pollen protein analysis

To assess effects of dicamba on the nutritional content of *E. perfoliatum* pollen, we analyzed the protein concentration of *E. perfoliatum* pollen using the Bradford assay, which uses Coomassie Brilliant Blue G-250 to dye proteins in solution that can absorb light at 595 nm [52]. We collected pollen samples

Table 1. Summary of curve parameters (± standard error) and fit (r^2) for the 3-parameter log-logistic dose–response curves for alfalfa flower clusters per stem and the number of honey bees per plot per minute in response to dicamba dose[a]

Response variable	Date	b (slope)	d (upper limit)	e (ED50)	r^2
Number of flower clusters per stem	21 d postapplication	3.45 (10.35)	7.81 (0.64)	7.71 (7.72)	0.538
	28 d postapplication	1.07 (1.48)	7.58 (0.92)	13.17 (10.53)	0.401
	38 d postapplication	0.27 (0.14)	7.12 (0.70)	105.35 (144.10)	0.338
Number of honey bees per plot per minute	28 d postapplication	1.91 (1.41)	10.45 (0.90)	21.30 (16.46)	0.491
	38 d postapplication	2.29 (10.77)	8.34 (0.84)	58.90 (33.84)	0.161

[a]We used the squared Pearson correlation of predicted and observed values for each curve to approximate the r^2 statistic.
ED50 = 50% effective dose; b = slope of the curve at the ED50 dose; d = upper limit; e = dose that causes a 50% reduction in the response variable, the lower limit was set to 0 for all variables.

from 4 individual flowering plants from the plant group sprayed with water only and 3 individual plants from each of the 0.56 g a.e./ha and 5.6 g a.e./ha dicamba-treated groups. Plants injured by the higher rates of dicamba did not flower adequately for pollen collection. We collected fresh pollen using a small paintbrush by gently brushing the pollen off of flowers into a glass container daily for 2 wk from 27 August through 9 September. We stored pollen at –23 °C until analysis. To prepare the samples for analysis, we divided the pollen into 3 1-mg biological replications in 1.5-mL Eppendorf microcentrifuge tubes for each individual plant. We added 3 drops of 0.1 M NaOH to each sample and then ground samples with a microcentrifuge pestle to facilitate breaking of pollen walls. After grinding, each tube was filled with 1.5 mL of 0.1 M NaOH and vortexed. We refrigerated all samples at 4 °C for 24 h.

To perform the Bradford assay we used the Bio-Rad Protein Assay Kit microassay 300-μL microplate protocol with bovine γ-globulin as the protein standard [53]. Because of the high protein concentration of the pollen, we diluted 50 μL of each replicate into 100 μL 0.1 M NaOH in each well of a sterile untreated BD Falcon 96-well plate. We measured the absorbance of standards and samples at 595 nm using a SpectraMax 190 spectrophotometer (Molecular Devices). Using SoftMax Pro Ver 4.0 software [54], we used linear regression to determine the protein standard curve from which we calculated the protein concentrations (micrograms of protein per milligram of pollen) of the samples. To analyze protein content of pollen in response to dicamba rate, we used linear regression with dose on a logarithmic scale [47].

RESULTS

Pollinator visitation to M. sativa

Depending on time after application, alfalfa flowering had a varied response to sublethal doses of dicamba. On 23 July, 21 d postapplication, we found that a dose of 56.1 g a.e./ha completely inhibited flowering; and our log-logistic function estimated that a dose of 7.7 g a.e./ha reduced alfalfa flowering by 50% compared with the control (Table 1). At 28 d after application, an estimated dose of 13.2 g a.e./ha reduced flowering by 50% and flowers were difficult to find in plots treated with a dose of 56.1 g a.e./ha of dicamba (Table 1), whereas the particle drift-level dose of 5.6 g a.e./ha reduced flowering by 33%. At 38 d postapplication (9 August), none of the treatments we tested reduced flowering by even 50%, a point which our model estimates would have occurred at nearly 105 g a.e./ha (Table 1).

At 28 d post–herbicide application, our log-logistic regression model revealed that the number of honey bees present per plot was reduced by 50% at an estimated dicamba dose of 21.3 g a.e./ha (Table 1). On 9 August (38 d postapplication) alfalfa plants treated with high doses began to flower well enough to attract bees; however, the presence of honey bees in our plots was nearly 50% less in plots treated with a dose of 56.1 g a.e./ha than our control plants (Table 1). Although the number of honey bees was lower in plots dosed with high rates of dicamba, we did not detect a significant relationship between the total number of floral visits by all pollinators and dicamba dose at either 28 d or 38 d post–herbicide application (Table 2); we did not record any visits in plots treated with 56.1 g a.e./ha at 28 d post–herbicide application because there were so few flowers in these plots. Similarly, we did not detect any relationships between the number of honey bee visits per plant per minute and dicamba dose at 28 d or 38 d post–herbicide application (Table 2). Although honey bees were the most common visitors recorded, other insects that we observed visiting flowers sporadically included several lepidopteran species (particularly hesperiid species), syrphids and other flies, halictid bees, *Bombus* spp., cantharid beetles, and Japanese beetle (*Popillia japonica* [Newman]).

Table 2. Best-fit log-linear regression equations relating dicamba dose to floral visitation for alfalfa and dose to plant height and floral visitation for *Eupatorium* perfoliatum

Experiment	*Y*	Model	*F*	r^2	*p*
Alfalfa	Total number of visits at 28 d postapplication	$Y = -0.188X + 1.31$	2.63	0.107	0.119
	Total number of visits at 38 d postapplication	$Y = -0.0095X + 0.47$	0.07	0.003	0.790
	Number of honey bee visits per plant at 28 d postapplication	$Y = -0.079X + 0.66$	1.19	0.051	0.287
	Number of honey bee visits per plant at 38 d postapplication	$Y = -0.095X + 0.51$	0.65	0.029	0.430
E. perfoliatum	Plant height (cm)[a]	$Y = -5.13X + 128.50$[a]	19.74[a]	0.310[a]	<0.001[a]
	Plant biomass per ramet (g)[a]	$Y = -2.00X + 16.70$[a]	11.91[a]	0.213[a]	0.001[a]
	Number of honey bee visits per cm^2 of floral area per minute[a]	$Y = -0.0049X + 0.017$[a]	10.35[a]	0.187[a]	0.002[a]
	Number of syrphid fly visits per cm^2 of floral area per minute[a]	$Y = -0.0076X + 0.024$[a]	9.87[a]	0.180[a]	0.003[a]
	Number of "other" fly visits per cm^2 of floral area per minute[a]	$Y = -0.0085X + 0.027$[a]	10.78[a]	0.197[a]	0.002[a]

[a]Significant relationships.

Table 3. Summary of curve parameters (± standard error) and fit (r^2) for dose–response curves for the number of *Eupatorium perfoliatum* flower heads per plant and visitation by various pollinators per plant or per square centimeter of floral area in response to dicamba dose[a]

Response variable	b (slope)	c (lower limit)	d (upper limit)	e (ED50)	r^2
Flower heads per plant at 42 d postapplication	1.81 (1.34)	−1.51 (58.01)	557.66 (74.32)	2.43 (1.82)	0.564
Initial time to flowering in days postapplication	−2.83 (11.14)	19.94 (1.64)	57.22 (7.17)	9.20 (18.64)	0.701
Time to maximum flowering in days postapplication	−4.17 (14.25)	47.94 (1.19)	69.00 (3.08)	5.10 (1.73)	0.689
Mean total visits per plant per minute	3.55 (27.95)	–0.003 (0.13)	2.1 (0.11)	6.67 (9.26)	0.849
Mean scatopsid fly visits per plant per minute	1.84 (1.65)	–0.03 (4.97)	37.20 (6.38)	1.33 (1.26)	0.428
Mean honey bee visits per plant per minute	1.12 (1.23)	0.01 (0.63)	4.76 (0.74)	0.65 (0.41)	0.410
Mean halictid bee visits per plant per minute	7.41 (68.25)	0.12 (1.09)	9.00 (1.01)	4.08 (11.92)	0.496
Mean *Bombus* spp. visits per plant per minute	1.32 (0.90)	0.004 (0.49)	4.39 (0.60)	1.10 (0.75)	0.511
Mean syrphid fly visits per plant per minute	11.10 (53.95)	0.11 (0.23)	1.62 (0.33)	0.77 (1.19)	0.309
Mean "other" fly visits per plant per minute	0.45 (0.89)	0.00004 (0.001)	0.03 (0.007)	5.33 (0.60)	0.164
Mean total visits per cm^2 of floral area per min.	2.24 (10.44)	−0.0005 (0.05)	0.30 (0.03)	6.45 (4.66)	0.553
Mean scatopsid fly visits per cm^2 of floral area per min.	1.15 (0.58)	−0.005 (0.03)	0.29 (0.04)	2.43 (1.49)	0.558
Mean *Bombus* spp. visits per cm^2 of floral area per min.	1.49 (1.75)	0.001 (0.003)	0.03 (0.004)	0.84 (0.39)	0.407
Mean halictid bee visits per cm^2 of floral area per minute	4.44 (15.62)	0.00001 (0.02)	0.08 (0.01)	4.95 (2.30)	0.439

[a]We used the squared Pearson correlation of predicted and observed values for each curve to approximate the r^2 statistic.
SED50 = 50% effective dose; b = the slope of the curve at the ED50 dose; c = lower limit; d = upper limit; e = dose that causes a 50% reduction in the response variable (ED50).

Pollinator visitation to E. perfoliatum

Flowering of *E. perfoliatum* was very sensitive to dicamba dose. Forty-two days after application (22 August), a dose of 2.43 g a.e./ha, less than the particle drift level of 5.6 g a.e./ha we applied, reduced the number of flower heads by half compared with the water control (Table 3 and Figure 1). We also found a reduction in the number of flowers per plant and observed delays in both the initial date of flowering (slope [b] = –2.83, ED50 = 9.20 g a.e./ha) and the amount of time to maximum flowering (slope [b] = –4.17, ED50 = 5.10 g a.e./ha), both of which were noticeable at doses of 5.6 g a.e./ha or higher (Table 3). All plants flowered in the 0 g a.e./ha, 0.56 g a.e./ha, and 5.6 g a.e./ha treatments; but only 40% of plants flowered when dosed with 56.1 g a.e./ha, though they flowered several weeks after we recorded visitation data. Those dosed with 280.5 g a.e./ha never flowered. Moreover, the 2 highest doses almost completely inhibited flowering for 42 d following application (Figure 1); only 1 plant dosed with a rate of 56.1 g a.e./ha bloomed during this period, and it did so minimally. The plants dosed with 56.1 g a.e./ha that did flower began flowering 57 d postapplication. Expectedly, dicamba dose negatively influenced height ($P < 0.001$) and biomass ($P = 0.001$) of *E. perfoliatum* (Table 2). Importantly, we did not detect any relationship between protein concentrations in pollen (micrograms of protein per microgram of pollen) of *E. perfoliatum* and dicamba rate on a $\log_{10}$ scale ($Y = –0.56X + 140$, $F = 0.01$, $r^2 = 0.001$, $P = 0.939$; Figure 2).

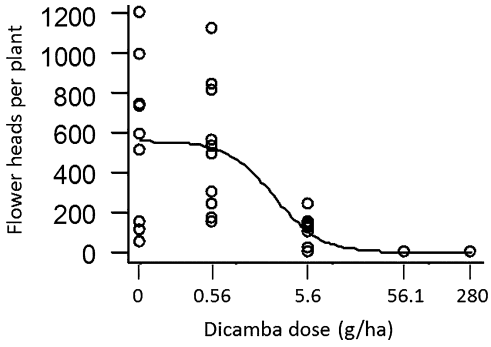

Figure 1. Flower heads per *Eupatorium perfoliatum* plant at 42 d postapplication for 5 doses of dicamba.

The most common insect visitors to *E. perfoliatum* flowers were halictid bees, *Bombus* spp., honey bees, crabronid wasps, scatopsid flies, syrphid flies, and "other" flies. All pollinator species visited plants treated with water only or the 0.56 g a.e./ha rate of dicamba more frequently than plants treated with higher doses (Table 4). Consistent with the decreases in flowering by *E. perfoliatum*, the dicamba dose of 6.67 g a.e./ha reduced total insect floral visits by 50% (Table 4 and Figure 3), and doses between 0.65 g a.e./ha and 4.08 g a.e./ha reduced taxon-specific floral visits for halicitids, *Bombus* spp., honey bees, scatopsids, and syrphids by 50% compared with the control (Tables 2 and 3). Notably, a 6.45 g a.e./ha rate of dicamba also reduced total floral visits per square centimeter of floral area by 50% compared with control plants (Tables 3 and 4). Visitation patterns by individual taxa per square centimeter of floral area showed similar patterns and declined by 50% at rates between 0.84 g a.e./ha and 4.95 g a.e./ha (Table 3) for most taxa, but visitation by honey bees, syrphids, and "other" flies decreased log-linearly as dicamba dose increased (Table 2). In general, pollinator visitation patterns were similar when expressed on either a per plant or a per square centimeter floral area basis (Table 3). At the 2 highest doses, we observed minimal visitation by any of the groups. For the entire (present) study, when visits from all taxa were combined, we recorded

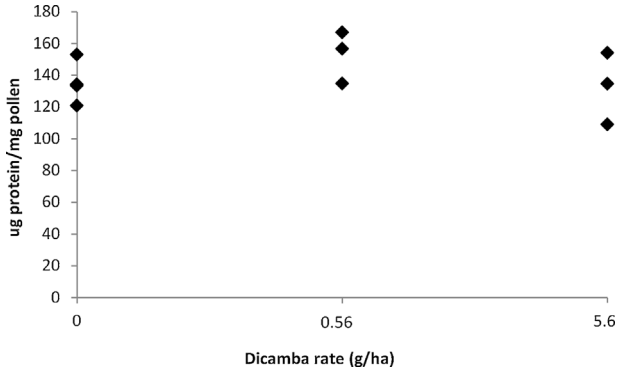

Figure 2. Protein concentration (micrograms of protein per milligram of pollen) of *Eupatorium perfoliatum* pollen collected from plants sprayed with 3 rates of dicamba ($Y = –0.56X + 140$, $F = 0.01$, $r^2 = 0.001$, $p = 0.939$).

Table 4. Total number of visits by the 10 most common insect groupings to flowers on *Eupatorium perfoliatum* plants treated with 5 doses of dicamba

Dicamba dose (g acid equivalent/ha)	Syrphidae	Scatopsidae	Halictidae	Crabronidae	*Apis mellifera*	*Bombus* spp.	Sphecidae	"Other" flies	Micro-Hymenoptera	Coleoptera
0	16	372	79	13	47	44	3	19	5	7
0.56	16	309	101	9	27	46	0	23	7	9
5.61	3	57	14	7	6	5	1	14	3	3
56.1	0	0	2	2	0	1	0	2	0	0
280.50	0	0	0	0	0	0	0	0	0	0

70 more visits to control plants than to plants dosed with the 0.56 g a.e./ha rate of dicamba. Similarly, we recorded 20 more honey bee visits and 63 more scatopsid fly visits to control plants than plants dosed with the 0.56 g a.e./ha rate of dicamba (Table 4). In contrast, we recorded 22 more visits by halictid bees to plants treated with the 0.56 g a.e./ha rate of dicamba than control plants (Table 4). Other visitors that we observed uncommonly included coleopteran species (particularly cerambycids, cantharids, and Japanese beetle), vespid, and chrysidid wasps, and colletid bees.

DISCUSSION

Our results indicate that sublethal doses of dicamba approximating particle drift events can delay, reduce, or prevent flowering of plant species found in agricultural landscapes and lead to reduced visitation by pollinators. For alfalfa, rates of dicamba higher than 5.6 g a.e./ha were disruptive, mainly causing delays in flowering. For alfalfa grown for seed, similar delays caused by herbicide drift could cause yield losses, but the degree of loss would depend on the acreage damaged and the extent of the dicamba injury. Based on the present study's results, reductions in flowers caused by low drift-level doses of dicamba ($\leq$0.56 g a.e./ha, 0.1% of a field application rate) onto alfalfa would appear to be minimal, whereas higher drift doses (0.56–5.6 g a.e./ha, 0.1–1% of a field application rate) could reduce seed production. In contrast, sulfonylurea herbicides caused reductions in flowering and seed size of several plant species at higher drift level rates (10% of the field rate) but not at lower drift rates (<10%) where few effects on flowering or reproductive tissues were observed [18]. Importantly, drift levels of dicamba in our higher range (0.56–5.6 g a.e./ha) have been reported from empirical fieldwork based on expected use rates for dicamba-resistant crops; in fact, vapor drift doses as high as 0.56 g a.e./ha and particle drift doses up to 5.6 g a.e./ha traveled up to 21 m from the treated area [14,40]. It should be noted, however, that because alfalfa and many other species bloom for several weeks, the length of the flowering period could mitigate some of the negative effects of a single herbicide-drift event on yield. In many locations, multiple drift events in a season may be possible, and the effects of multiple drift exposures within a season are not well understood.

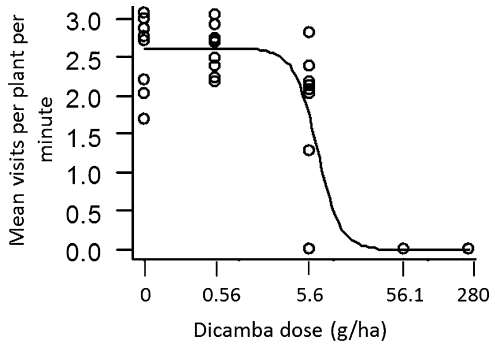

Figure 3. Mean visits by all taxa combined per *Eupatorium perfoliatum* plant per minute from 30 July through 22 August 2013 to plants sprayed with 5 doses of dicamba.

Similar to the results with alfalfa, we also detected reductions and delays in flowering by *E. perfoliatum* following dicamba exposure. It should be noted, however, that the present analysis likely underestimates the potential delay in initial flowering because we neglected to record the date of initial flowering for several plants dosed with 0 g a.e./ha and 0.56 g a.e./ha. Nevertheless, dicamba significantly delayed flowering, even at the lower doses we tested. Importantly, the ED50 values we observed for flowering of *E. perfoliatum* were very low (<10 g a.e./ha) and often substantially lower than levels required to cause a 50% reduction in biomass (10–80 g a.e./ha) of several other native plant species [55]. Although reduced and delayed flowering by a noncrop plant species such as *E. perfoliatum* may not have direct economic implications, it could negatively influence pollinators and other flower-visiting insect species inhabiting agricultural landscapes, like natural enemies, that rely on flowering noncrop plant species for food [50,51,56]. Moreover, although many pollinator species are generalists, reductions and delays in plant flowering caused by herbicide injury could perturb pollinator communities. For instance, if delayed flowering prevents floral resources from being available when early-season pollinators are active, these early-emerging individuals might not be able to find sufficient food resources to support their activities or fuel growth of their offspring [57,58]. Reduced flowering in addition to a delay could have compounding effects on pollinators in some locations. In the case of more specialized pollinator species, herbicide-induced delays could decouple local plant-pollinator phenology, possibly reducing populations of specialized pollinator species if herbicide damage is widespread and the specialized floral visitors cannot find necessary food resources [59]. We should note, however, that some broadleaf plant species have a higher tolerance for dicamba and 2,4-D, and these species may better survive drift events and provide some floral resources [60,61]. Importantly, our data show that nutritional composition of pollen is similar for plants that flower after being injured by dicamba, which suggests that negative effects on pollinators would be more likely to result from an overall reduction in the quantity of pollen resources of sensitive plant species than differences in nutrition associated with visiting damaged versus undamaged plants.

In addition to delays in flowering, as dicamba dose increased we also observed reductions in plant size and flowering by *E. perfoliatum* (Table 2). This reduction in plant size may negatively influence *E. perfoliatum* as it competes with other plant species. The reduction in flowers is likely to result in decreased seed production, which may cause potential changes in

plant community composition, particularly if *E. perfoliatum* and other susceptible broadleaf plants in field margins receive repeated exposures of herbicides over time. Indeed, plant communities have been shown to change in response to herbicide exposure over multiple seasons, including reductions in broadleaf plant species and the overall broadleaf community, changes that can then influence associated arthropod communities [20,61].

CONCLUSION

Sublethal doses of dicamba (0.1–1% of the field application rate) caused delays and reductions in flowering of 2 susceptible and agroecologically significant plant species. These sublethal effects of dicamba on plant flowering indicate potential negative consequences for plant and flower abundance, and possibly crop yield (e.g., seed production), in areas at risk for exposure to drift-level doses of synthetic-auxin herbicides. Decreases in amounts of flowering led to reduced pollinator visitation to plants injured by dicamba, suggesting that widespread nontarget damage from dicamba and similar herbicides may adversely affect pollinator communities. The protein composition of pollen, however, does not appear to change if plants are able to flower, so effects on pollinator communities may be more likely to result from fewer resources because herbicide-damaged plants produce reduced floral displays. If alfalfa and *E. perfoliatum* can serve as proxies for some of the flowering plant species that occur in agricultural landscapes, it seems likely that drift-level doses of dicamba could widely influence availability of floral resources from sensitive plant species in proximity to crop fields for pollinators, particularly with repeated drift exposures, potentially reducing the quantity of floral resources that support pollinator communities or even other guilds of beneficial insects, such as predators or parasitoids, that are also frequent visitors to flowers [50,51,56]. It is also notable that dicamba can alter growth and development of insect herbivores when they feed on plants stressed by sublethal doses [19], so dicamba and other herbicides may more widely influence arthropod communities in agroecosystems than is currently recognized. Previous research has determined that the majority of floristic diversity in agroecosystems resides in small fragments of seminatural habitat around arable fields [62,63]. Thus, sustaining pollinators and other beneficial insects that rely on plant diversity will require protecting remaining noncrop habitats from herbicide drift. Commercialization of synthetic-auxin resistant crops is likely to lead to a 2-fold to 70-fold increase in total applications of dicamba and 2,4-D and a corresponding increase in risk of nontarget damage to plants [10]. Taken together, the present study's results strongly support the need for enhanced stewardship of synthetic-auxin herbicides, like dicamba and 2,4-D, to minimize their influence on nontarget plant species as growers begin to use more of these active ingredients over larger areas and later in the season in association with new herbicide-resistant crop varieties.

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Data availability—Data, associated metadata, and calculation tools can be requested from E. Bohnenblust (ericbohnenblust@gmail.com).

REFERENCES

1. Grube A, Donaldson D, Kiely T, Wu L. 2011. Pesticides industry sales and usage: 2006 and 2007 market estimates. US Environmental Protection Agency. [cited 2011 March 8]. Available from: http://www.epa.gov/opp00001/pestsales/07pestsales/market_estimates2007.pdf
2. O'Donoghue EJ, Hoppe RA, Banker DE, Ebel R, Fuglie K, Korb P, Livingston M, Nickerson C, Sandretto C. 2011. The changing organization of U.S. farming. USDA Economic Research Service Economic Information Bulletin no. 88. Washington, DC.
3. Gianessi LP. 2008. Economic impacts of glyphosate-resistant crops. *Pest Manag Sci* 64:346–352.
4. Shaw DR, Owen MDK, Dixon PM, Weller SC, Young BG, Wilson RG, Jordan DL. 2011. Benchmark study on glyphosate-resistant cropping systems in the United States. Part 1: Introduction to 2006–2008. *Pest Manag Sci* 67:741–746.
5. US Department of Agriculture Economic Research Service. 2014. Recent trends in GE adoption. [cited 2014 January]. Available from: http://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us/recent-trends-in-ge-adoption.aspx#.UqtekeLAE5Q
6. Powles SB. 2008. Evolved glyphosate-resistant weeds around the world: Lessons to be learnt. *Pest Manag Sci* 64:360–365.
7. Heap I. 2014. International survey of herbicide resistant weeds. [cited 2014 April 8]. Available from: http://www.weedscience.org/
8. Behrens MR, Mutlu N, Chakraborty S, Dumitru R, Jiang WZ, Lavallee BJ, Herman P, Clemente T, Weeks DP. 2007. Dicamba resistance: Enlarging and preserving biotechnology-based weed management strategies. *Science* 316:1185–1188.
9. Wright TR, Shan G, Walsh T, Lira JM, Cui C, Song P, Zhuang M, Arnold N, Lin G, Yau K, Russell SM, Cicchilo RM, Peterson MA, Simpson DM, Zhou N, Ponsamuel J, Zhang Z. 2010. Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. *Proc Natl Acad Sci USA* 107: 20240–20245.
10. Mortensen D, Egan JF, Maxwell B, Ryan M, Smith R. 2012. Navigating a critical juncture for sustainable weed management. *Bioscience* 62:75–84.
11. Auch DE, Arnold WE. 1978. Dicamba use and injury on soybeans (glycine-max) in South Dakota. *Weed Sci* 26:471–475.
12. Behrens R, Lueschen WE. 1979. Dicamba volatility. *Weed Sci* 27:486–493.
13. Association of American Pesticide Control Officials. 2005. Pesticide drift enforcement survey. [cited 2014 February 5]. Available from: http://aapco.org/documents/surveys/DriftEnforce05Rpt.html
14. Egan JF, Mortensen DA. 2012. Quantifying vapor drift of dicamba herbicides applied to soybean. *Environ Toxicol Chem* 31:1023–1031.
15. Egan JF, Barlow KM, Mortensen DA. 2014. A meta-analysis on the effects of 2,4-D and dicamba drift on soybean and cotton. *Weed Sci* 62:193–206.
16. Felsot AS, Unsworth JB, Linders JBHJ, Roberts G, Rautman D, Harris C, Carazo. E. 2011. Agrochemical spray drift: Assessment and mitigation—A review. *J Environ Sci Health B* 46:1–23.
17. Grover R, Yoshida K, Maybank J. 1972. Droplet and vapor drift from butyl ester and dimethylamine salt of 2,4-D. *Weed Sci* 20: 320–324.
18. Boutin C, Lee H, Peart ET, Batchelor PS, Maguire JR. 2000. Effects of the sulfonylurea herbicide metsulfuron methyl on growth and reproduction of five wetland and terrestrial plant species. *Environ Toxicol Chem* 19:2532–2541.
19. Bohnenblust EW, Egan JF, Mortensen DM, Tooker JF. 2013. Direct and indirect effects of the synthetic-auxin herbicide dicamba on two lepidopteran species. *Environ Entomol* 42:586–594.
20. Schmitz J, Schäfer K, Brühl CA. 2013. Agrochemicals in field margins—Assessing the impacts of herbicides, insecticides, and fertilizer on the common buttercup (*Ranunculus acris*). *Environ Toxicol Chem* 32:1124–1131.
21. Williams CF. 2007. Effects of floral display size and biparental interbreeding on outcrossing rates in *Delphinum barberyi* (Ranunculaceae). *Am J Bot* 94:1696–1705.
22. Hanley ME, Franco M, Pichon S, Darvill D, Goulson D. 2008. Breeding system, pollinator choice and variation in pollen quality in British herbaceous plants. *Funct Ecol* 22:592–598.
23. Garrat MPD, Breeze TD, Jenner N, Polce C, Biesmeijer JC, Potts SG. 2014. Avoiding a bad apple: Insect pollination enhances fruit quality and economic value. *Agric Ecosyst Environ* 184:34–40.

24. Manning R, Wallis IR. 2005. Seed yield in canola (*Brassica napus* cv. Karoo) depend on the distance of plants from honey bee apiaries. *Aust J Exp Agric* 45:1307–1313.
25. Pitts-Singer TL, Cane JH. 2011. The alfalfa leafcutting bee, *Megachile rotundata*: The world's most intensively managed solitary bee. *Annu Rev Entomol* 56:221–237.
26. Calderone NW. 2012. Insect pollinated crops, insect pollinators and US agriculture: Trend analysis of aggregate data for the period 1992–2009. *PLoS One* 7:e37235.
27. Wratten SD, Gillespie M, Decourtye A, Mader E, Desneux E. 2012. Pollinator habitat enhancement: Benefits to other ecosystem services. *Agric Ecosyst Environ* 159:112–122.
28. Moffett JO, Morton HL, Macdonald RH. 1972. Toxicity of some herbicidal sprays to honey bees. *J Econ Entomol* 65:32–36.
29. Morton HL, Moffett JO. 1972. Ovicidal and larvicidal effects of certain herbicides on honey bees. *Environ Entomol* 1:611–614.
30. Morton HL, Moffett JO, Macdonald RH. 1972. Toxicity of herbicides to newly emerged honey bees. *Environ Entomol* 1:102–104.
31. Moffett JO, Stith LS, Morton HL, Shipman CW. 1980. Effect of 2,4-D (dichlorophenoxyacetic acid) on cotton yield, floral nectar, seed germination, and honeybee visits. *Crop Sci* 20:747–750.
32. Kearns CA, Inouye DW, Waser NM. 1998 Endangered mutualisms: The conservation of plant–pollinator interactions. *Annu Rev Ecol Syst* 29:83–112.
33. Tasei JN, Aupinel P. 2008. Nutritive value of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers (*Bombus terrestris*, Hymenoptera: Apidae). *Apidologie* 39:397–409.
34. Benelli G, Benvenuti S, Desneux N, Canale A. 2014. *Cephalaria transsylvanica*–based flower strips as potential food source for bees during dry periods in European Mediterranean basin countries. *PLoS One* 9:e93153.
35. Stokstad E. 2007. The case of the empty hives. *Science* 316:970–972.
36. Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, vanEngelsdorp D, Pettis JS. 2010. High levels of miticides and agrochemicals in North American apiaries: Implications for honey bee health. *PLoS One* 5:e9754.
37. BASF Corporation. 2008. Clarity herbicide specimen label. Research Triangle Park, NC, USA.
38. Brown RB, Carter MH, Stephenson GR. 2004. Buffer zone and windbreak effects on spray drift deposition in a simulated wetland. *Pest Manag Sci* 60:1085–1090.
39. Carlsen SCK, Spliid NH, Svensmark B. 2006. Drift of 10 herbicides after tractor spray application, 2. Primary drift (droplet drift). *Chemosphere* 64:778–786.
40. Wang M, Rautman D. 2008. A simple probabilistic estimation of spray drift-factors determining spray drift and development of a model. *Environ Toxicol Chem* 27:2617–2626.
41. Ritz C, Streibig JC. 2008. *Nonlinear Regression with R*. Springer Science and Business Media, New York, NY, USA.
42. R Core Team. 2013. *R: A Language* and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
43. Knezevic SZ, Streibig JZ, Ritz C. 2007. Utilizing R software package for dose–response studies: The concept and data analysis. *Weed Technol* 21:840–848.
44. Ritz C, Streibig JC. 2005. Bioassay analysis using R. *J Stat Softw* 12:1–22.
45. Cedergreen N, Ritz C, Streibig JC. 2005. Improved empirical models describing hormesis. *Environ Toxicol Chem* 24:3166–3172.
46. Johnson JB, Omland KS. 2004. Model selection in ecology and evolution. *Trends Ecol Evol* 19:101–108.
47. SPSS. 2012. *IBM SPSS Statistics 21 Core User's Guide*. Somers, NY, USA.
48. Olszyk DM, Burdick CA, Pfleeger TG, Lee EH, Watrud LS. 2004. Assessing the risks to non-target terrestrial plants from herbicides. *J Agric Meteorol* 60:221–242.
49. Robertson C. 1929. *Flowers and Insects: Lists of Visitors of Four Hundred and Fifty Three Flowers*. Science Press, Lancaster, PA, USA.
50. Tooker JF, Hanks LM. 2000. Flowering plant hosts of adult hymenopteran parasitoids of central Illinois. *Ann Entomol Soc Am* 93:580–588.
51. Tooker JF, Hauser M, Hanks LM. 2006. Floral host plants of Syrphidae and Tachinidae (Diptera) of central Illinois. *Ann Entomol Soc Am* 99:96–112.
52. Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254.
53. Bio-Rad Laboratories. 2013. *Quick Start*™ *Bradford Protein Assay Instruction Manual*. Hercules, CA, USA.
54. Molecular Devices. 2001. *SoftMax Pro User Manual*. Sunnyvale, CA, USA.
55. Egan JF, Graham IM, Mortensen DA. 2014. A comparison of the herbicide tolerances of rare and common plants in an agricultural landscape. *Environ Toxicol Chem* 33:696–702.
56. Graham EE, Tooker JF, Hanks LM. 2012. Floral host plants of adult beetles of central Illinois. *Ann Entomol Soc Am* 105:287–297.
57. Awmack CS, Leather SR. 2002. Host plant quality and fecundity in herbivorous insects. *Annu Rev Entomol* 47:817–844.
58. Roulston TH, Cane JH. 2002. The effect of pollen protein concentration on body size in the sweat bee *Lasioglossum zephyrum* (Hymenoptera: Apiformes). *Evol Ecol* 16:49–65.
59. Forrest JRK, Thomson JD. 2011. An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. *Ecol Monogr* 81:469–491.
60. Boutin C, Strandberg G, Carpenter D, Mathiassen SK, Thomas PJ. 2014. Herbicide impact on non-target plant reproduction: What are the toxicological and ecological implications. *Environ Pollut* 185:295–306.
61. Egan JF, Bohnenblust E, Goslee S, Tooker JF, Mortensen DA. 2014. Effects of simulated dicamba herbicide drift on the structure and floral resource provisioning of field edge and old field plant communities. *Agric Ecosyst Environ* 185:77–87.
62. Boutin C, Jobin B. 1998. Intensity of agricultural practices and effects on adjacent habitats. *Ecol Appl* 8:544–557.
63. Liira J, Schmidt T, Aavik T, Arens P, Augenstein I, Bailey D, Billeter R, Bukacek R, Burel F, De Blust G, De Cock R, Dirksen J, Edwards PJ, Hamersky R, Herzog F, Klotz S, Kuhn I, Le Coeur D, Miklova P, Roubalova M, Schweiger O, Smulders MJM, Van Wingerden W, Bugter R, Zobel M. 2008. Plant functional group composition and large-scale species richness in European agricultural landscapes. *J Veg Sci* 19:3–14.