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Effect of herbicides on pollinator foraging behavior and flower morphology in white clover (*Trifolium repens* L.)–infested turfgrass

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Abstract

The recent decline in pollinator abundance is a cause of concern for sustaining global food production. Several common weeds of managed turfgrass systems attract honeybees and other wild pollinators. As turfgrass often requires treatment with insecticides that harm bees, best practices are needed to prevent bees from visiting weed-infested turf areas that will be treated for insect pests. Weed control tactics can protect pollinator exposure to insecticides by reducing the floral resources afforded to bees from turfgrass weeds. Three field studies were conducted in 2021 and 2022 to evaluate the effect of various herbicides and herbicide formulation constituents on pollinator foraging and white clover floral morphology in managed tall fescue turfgrass. Treatments included a nontreated control; MCPP; 2,4-D; dicamba; Trimec Classic™ (2,4-D, MCPP, dicamba); Speedzone™ (carfentrazone, 2,4-D, MCPP, dicamba); and an herbicide-formulation constituent (inert ingredients of Speedzone™). All response variables were evaluated for 8 d, starting from one day before treatment and ending 6 d after treatment (DAT). The herbicide formulation constituent did not alter white clover flower density, floral discoloration, floral quality, or insect visitation compared to nontreated plots. Herbicides reduced flower density and floral quality to the same extent, but MCPP discolored white clover floral tissue 16% per day and less than all other herbicides except dicamba. Floral quality completely declined in approximately 5 d following any herbicide treatment. Bee visitation to white clover–infested turf increased by 3 bees min^{-1} for every 100 white clover blooms m^{-2}. Honeybees and other insects vacated herbicide-treated areas in less than 2 d, despite minimal effects on floral quality and density at that time. The data suggest that practitioners could apply insecticides 2 d after auxin herbicide treatment and avoid harm to pollinators, but additional work is needed to directly measure pollinator exposure following such treatments.

Introduction

Pollination is an essential ecosystem service necessary for the reproduction of over 80% of total plant species and 35% of global crop production (Schowalter 2022). Honeybees and wild pollinators are estimated to contribute over $18 billion annually through improved crop production via rendering pollination services to more than 100 crops in the United States (USDA 2022). However, recent declines in pollinator abundance are a cause of concern for sustaining global food production (van der Sluijs and Vaage 2016). A recent survey representing 7% of the total managed honey-producing colonies in the United States reported a loss of 45.5% of the total managed honeybee colonies in 2021 (Steinhauer et al. 2021). Whereas current data indicate that pollinator decline is driven by biotic stressors, habitat loss, and competitive displacement of honeybees and wild pollinators by introduced parasites and pathogen populations, pesticides have been cited as a likely abiotic contributor that negatively interacts with other factors (Goulson et al. 2015). Pesticides also appear to gain more attention in relation to pollinator decline compared with other factors (Leska et al. 2021).

Several common weeds of managed turfgrass systems, including dandelion (*Taraxacum officinale* F.H. Wigg.) and white clover, attract honeybees, bumble bees (*Bombus* spp.), hoverflies (Syrphidae), and other pollinators during flowering (Larson et al. 2014). A recent survey conducted by the Weed Science Society of America reported that white clover is one of the most common weeds in turfgrass (Van Wychen 2020) and is one of the most common attractants to pollinators via providing floral rewards in urban landscapes (Larson et al. 2014). Highly managed turfgrasses primarily depend on insecticide applications to prevent damage that can result in stand loss from foliar or root-feeding insects (Held and Potter 2012). Neonicotinoids are the most widely used insecticides in turfgrass management and pose a severe threat to pollinator health in weed-infested areas (Larson et al. 2017). When bumble bees (*Bombus impatiens*) foraged on clothianidin-treated white clover blooms in managed turfgrass, mortality increased and colony growth decreased (Larson et al. 2013). Systemic insecticides, especially imidacloprid, thiamethoxam, and chlorpyrifos, leave residues that are harmful to pollinators when ingested, whereas pyrethroids and neonicotinoids can harm pollinators by both contact exposure and ingestion (Sanchez-Bayo and Goka 2014).

Thus, commonly used pesticides have come under increased scrutiny from government regulators regarding potential risks to pollinators (EPA 2022). Current insecticide regulations to reduce pollinator exposure include confusing terminology on insecticide labels, such as “Do not apply this product or allow it to drift to blooming crops or weeds if bees are visiting the area.” The US Environmental Protection Agency recommended revisions on the label language to improve pollinator health, with more specificity in the environmental hazards section about pollinating insect hazard statements (EPA 2017). Current mitigation practices include not spraying areas where bees may visit, spraying areas at times when bees are not expected to visit, and mowing to remove weedy blooms before insecticide treatment to turf (NCIPMC 2016). However, the risk associated with insecticides is not eliminated by mowing because of subcanopy blooms that remain following the mowing event. In addition, white clover blooms that emerge 1 to 2 wk following imidacloprid or clothianidin treatment to mowed turf contained between 6.2 and 26 ng of insecticide active ingredient per gram of nectar that had presumably moved systemically from treated foliage to newly developed blooms (Larson et al. 2015).

Another practice to mitigate potential harm to pollinators from insecticide residues is to treat turf with an herbicide prior to insecticide application. The National Pesticide Information Center classified 2,4-D and dicamba toxicity to honeybees as partially nontoxic, meaning that acute toxicity to herbicide exposure (LD_{50}) is ≥11 μg bee^{-1} (Bunch et al. 2012; Gervais et al. 2008), which is 2,973 times less toxic than imidacloprid (Gervais et al. 2010). Additionally, Morton et al. (1972) classified 2,4-D and dicamba as relatively nontoxic to honeybees based on feeding studies containing 0 to 1,000 ppm herbicide mixed with 60% sucrose syrup and fed to honeybees. Similarly, MCPP is considered nontoxic to bees (NCBI et al. 2022). These three active ingredients are found in a wide variety of products marketed for broadleaf weed control in turfgrass (McCarty et al. 2010; Shaner 2014). Despite high levels of detected herbicide and fungicide residues in the pollen of 32 Maine apiaries, herbicides comprised none of the honeybee risk quotient, fungicides contributed less than 5% risk quotient via dermal exposure, and insecticides comprised all the risk quotient associated with oral exposure and most of that associated with dermal exposure (Drummond et al. 2018).

Despite herbicides being reasonably nontoxic to honeybees, few studies have evaluated their use to prevent honeybee exposure to insecticide-treated areas. Only one study assessed the effects of herbicide treatments on floral quality and pollinator floral visitation 3 wk after treatment (Bohnenblust et al. 2016), but few studies evaluated the herbicidal effect on floral intensity (MacRae et al. 2005; Schmitz et al. 2013). Herbicide application to weedy flowers can affect pollinator foraging by reducing flower density (Schmitz et al. 2013) and affecting nectar availability (Kearns et al. 1998). The speed at which pollinators vacate herbicide-treated areas is unknown, and practitioners need this information to schedule insecticide application intervals. We hypothesized that insect foragers would vacate herbicide-treated areas in step with white clover floral decline. We further hypothesized that formulation constituents used in herbicide products would decrease, but not eliminate, pollinator foraging to white clover–infested turf. Our objectives were to assess the temporal influence, assessed daily, of several herbicides and a formulation constituent on white clover floral quality, digitally assessed floral discoloration, bloom density, and pollinator foraging visits to weedy turf.

Table 1. List of treatments with formulation and rates evaluated in the field experiments at the Virginia Tech Glade Road Research Facility and Virginia Tech Turfgrass Research Center, Blacksburg, VA, in 2021 and 2022.[a]

Treatment[a]	Formulation	Rate
	kg ai L^{-1}	kg ai ha^{-1}
Nontreated control	–	–
MCPP	0.18	0.84
2,4-D	0.36	1.68
Dicamba	0.24	1.12
Trimec Classic™ (2,4-D, MCPP, dicamba)	0.33	1.52
Speedzone™ (carfentrazone, 2,4-D, MCPP, dicamba)	0.26	1.23
Speedzone™ inert formulation constituents	–	1.23

[a]All the evaluated herbicides and herbicide-formulation constituent were manufactured by PBI Gordon Corp., Shawnee, KS.

Materials and Methods

Three field studies were conducted at Blacksburg, VA between 2021 and 2022 in white clover–infested ‘Falcon III’ turf-type tall fescue (*Festuca arundinacea* Shreb) mown at 10 cm. Experiments were initiated at the Virginia Tech Glade Road Research Facility (37.23° N, 80.43° W) on September 28, 2021 and June 22, 2022, and at the Virginia Tech Turfgrass Research Center (37.22° N, 80.41° W) on August 23, 2022. All three experimental sites had a natural infestation of white clover, and plots were selected to contain uniform distribution of >30 flowers m^{-2}. Experiments were implemented as a randomized complete block design with six replications. Treatments included a nontreated control; MCPP; 2,4-D; dicamba; Trimec Classic™ (PBI Gordon Corp., Shawnee, KS); Speedzone™ (PBI Gordon Corp., Shawnee, KS); and a formulation blank (inert ingredients of Speedzone™). A detailed list of treatments with formulation concentration and rates applied is provided in Table 1. In each experiment, blocks were spaced 12 m apart, and 1.83-m by 1.83-m plots within a given block were spaced 3 m apart. All treatments were applied using a CO_2-pressurized backpack sprayer equipped with four Turbo Teejet Induction (TTI) 11006 spray nozzles (TeeJet® Technologies, Wheaton, IL), calibrated to deliver 374 L ha^{-1} of spray solution at 1.6 km h^{-1}. Treatments were applied at approximately 8:00 am on the day following study initiation at each site. No mowing was performed throughout the duration of experiments to prevent any alteration to white clover flower density.

Data were collected each day for 8 d starting the day before treatment and ending 6 DAT. At 7:00 am each morning, white clover flower density was determined by counting all flowers in each plot, and three representative flowers per plot were photographed. Using these photographs, white clover flower quality was visually rated on an index of 1 to 5 for three flowers in each plot and flower discoloration was measured via digital image analysis. The flower quality index of 1, 2, 3, 4, and 5 consisted of a prostrate flaccid peduncle with <10% intact petals, a flaccid peduncle with <25% intact petals, a twisted peduncle with <50% intact petals, slight epinasty with >75% intact petals, and an erect peduncle with >90% intact petals, respectively. Images were analyzed using Turf Analyzer (Green Research Services, LLC, Fayetteville, AR) to quantify white pixels of each flower, and the average pixel count of three subsample flowers in each plot was converted to a percentage reduction compared to the average pixel count of the three nontreated flowers within each replicate. All insect foragers observed for 1 min were recorded for each plot three times each day (~10:00 am, ~1:00 pm, and ~4:00 pm) throughout the experiment as done by other researchers (Larson et al. 2013). Multiple evaluators were employed to assess all insect foragers within 30 min for each assessment time to ensure uniformity in data collection. Insect foragers were separated into honeybees, bumble bees, solitary bees (*Osmia* spp.), hoverflies (*Allograpta obliqua*), wasps (*Vespula* spp.), and butterflies (Hesperiidae, Nymphalidae, Papilionidae, Lycaenidae). Insects were counted only if physical interaction with white clover flowers occurred. Insects that entered the plot area but did not interact with flowers were ignored (Bohnenblust et al. 2016; Boyle et al. 2020).

Data Analysis

All subsamples, including the three temporal pollinator count assessments per day, were averaged prior to ANOVA. For each plot, repeated measures over time were subjected to linear regression to determine the temporal slope over the number of days before an asymptote was reached. For example, a plot that had no clover blooms beyond 4 DAT, would be subject to linear regression to determine temporal trends from 0 to 4 DAT. Functional arguments in Microsoft Excel® (Microsoft Corp., Redmond, WA) were used to test floral quality or insect visitation for each day and 2 d into the future. When the response was stable for 3 d, the slope was returned for the appropriate number of days leading up to stabilization. This method was used because convergence typically could not be reached with nonlinear sigmoidal or hyperbolic equations, and a visual inspection of the data indicated that once insect visitation or white clover floral metrics reached zero values, they remained zeros for the study duration. Slopes were tested for variance homogeneity and analyzed using Proc GLM in SAS (version 9.4, SAS Institute, Cary, NC). Treatment was considered a fixed effect, whereas location and block were considered random. The mean square of treatment effects was tested for all response variables utilizing the mean square associated with random variable "site-year" (MacIntosh 1983). Means were separated using Tukey's HSD ($\alpha = 0.05$).

Results and Discussion

The treatment effect was significant ($P < 0.0001$) for all response variables, including temporal slopes of all bee species foraging, honeybee foraging, white clover flower density, and white clover flower discoloration (Table 2). The bees (honeybees, solitary bees, and bumble bees) represented 79% of the total insects that visited white clover flowers in 3 site-years, with honeybees accounting for 80% of the total bees (data not shown). Furthermore, hoverflies, butterflies, and wasps constituted 18%, 2%, and 1% of the total insect foraging visits (data not shown). These results of insect foraging visits agree with other research where honeybees were the dominant insect visitor on white clover blooms (Goodman and Williams 1994). 2,4-D reduced bee foraging visits by 55% per day and slightly slower than other herbicides, which reduced bee visitation by ≥63% per day. The inert formulation constituents of Speedzone™ did not significantly affect bee foraging visits and resembled the nontreated control (Table 3). 2,4-D controls white clover less effectively than other synthetic auxin herbicide mixtures (Bigham and Schmidt 1965) and is typically recommended to be used in mixture with other products when targeting clovers (Breeden and Brosnan 2011). Reduced herbicidal activity may have played a part in the slightly slower pace of reduced total bee visitation. In contrast, honeybee visitation was reduced by ≥60% per day regardless of the herbicide applied (Table 3). In all cases, insects completely vacated herbicide-treated plots in less than 2 DAT. Although previous studies have not evaluated pollinator visitation in just a few days following treatment, at 4 wk after simulated dicamba drift, pollinator foraging of alfalfa (*Medicago sativa* L.) and common boneset (*Eupatorium perfoliatum* L.) was reduced (Bohnenblust et al. 2016). The authors speculated that dicamba may have reduced nectar production in exposed plants (Bohnenblust et al. 2016). Drift of glyphosate to field-edge plants reduced nectar production 14 DAT (Russo et al. 2022).

It was noted that white clover floral density appeared to drive pollinator foraging frequency, and regression analysis revealed a positive correlation that explained about half of the variance (Figure 1). White clover floral density was reduced by ≥21% per day, irrespective of the herbicide applied; however, only a 3% reduction per day in floral density was observed in nontreated and

Table 2. ANOVA for bees (includes honeybees, bumble bees, and solitary bees), honeybees, white clover flower density, and white clover flower discoloration in the study assessed the effect of herbicide and formulation constituents.[a]

Response variable	F-value	P value
Bee foraging reduction	31.99	<0.0001*
Honeybee foraging reduction	45.26	<0.0001*
White clover flower density	46.57	<0.0001*
White clover flower discoloration	7.61	0.0015*

[a]Asterisks (*) indicate that treatment effects are significant.

Table 3. Treatment effect on the temporal slope of bee foraging, honeybee foraging, and white clover flower density and discoloration, and time to 90% inhibition (I_{90}) of white clover flower quality from three field experiments conducted in Blacksburg, VA in 2021 and 2022.[a]

			White clover		
Treatment	Bees[b]	Honeybee	Flower density	Flower dis-coloration	Flower quality
	Percent reduction per day[c]				I_{90} (d)
Nontreated	2 c	1 b	3 b	– –	– –
MCPP	63 a	67 a	22 a	16 c	5.3 a
2,4-D	55 b	60 a	22 a	24 ab	5.0 a
Dicamba	68 a	67 a	21 a	21 bc	5.1 a
Trimec Classic™	63 a	60 a	21 a	27 ab	5.0 a
Speedzone™	65 a	66 a	23 a	27 a	4.9 a
Formulation[d]	7 c	7 b	3 b	2 d	– –

[a]Means followed by the same letter are not different based on Tukey's HSD at $\alpha = 0.05$.
[b]Bees, included honeybees, bumble bees, and solitary bees.
[c]Percent reduction per day was based on linear slopes of responses for a given number of days where responses were still measured. Zero-loaded data near the end of the assessment period were deleted.
[d]Inert formulation constituents of Speedzone™.

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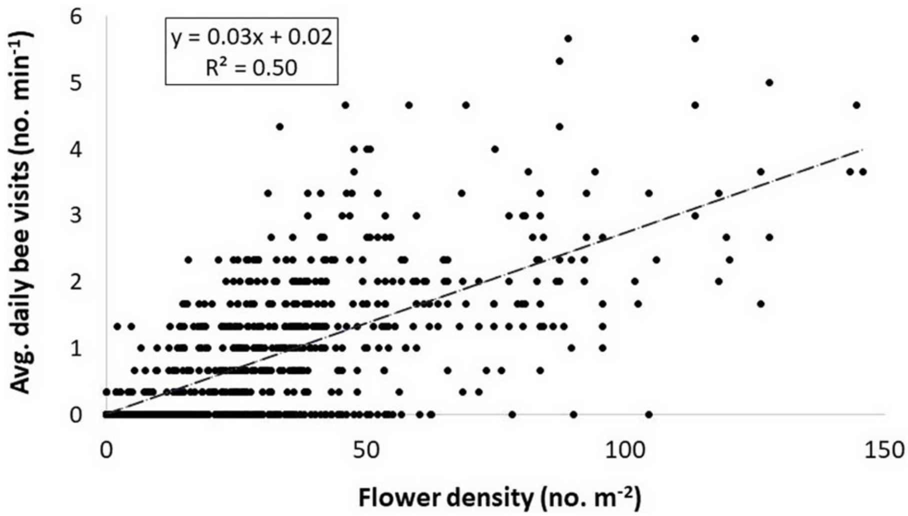

Figure 1. Relationship between flower density and bee (honeybee, bumble bee, solitary bee) foraging visits.

Speedzone™ inert formulation–treated plots (Table 3). MacRae et al. (2005) also observed that clopyralid and 2,4-D reduced white clover floral density. These authors suggested that this floral decline would potentially reduce pollinator exposure to subsequent insecticide applications in apple (*Malus* spp.) orchards. MCPP discolored white clover flowers 16% per day, whereas other herbicides discolored flowers >20% per day (Table 3). Speedzone™ inert formulation–treated flowers were not discolored (Table 3). White clover flower quality was reduced 90% in 4.9 to 5.3 d with no differences between the herbicides evaluated. Flower quality did not change in nontreated and formulation constituent–treated plots, and a time to 90% inhibition (I_{90}) value could not be calculated (Table 3). Although we found no difference in the speed of floral quality loss, Rossouw et al. (2019) reported more visible necrosis on floral buds of grapevines and higher fruit yield losses from simulated 2,4-D drift compared to dicamba and MCPA. All floral quality parameters declined at a pace that was considerably slower than the speed of insect forager vacancy, in contrast to our hypothesis.

Although white clover floral density and quality persisted up to 5 DAT, insect foraging was entirely inhibited at 2 DAT. Nectar depletion is possibly the key factor associated with reduced insect visitation, as King (1964) also observed that nectar secretion in poinsettias (*Euphorbia pulcherrima* Willd. Ex Klotzsch) was inhibited entirely within 2 d after 2,4-D application. This relationship between nectar production and herbicide treatments has not been tested for lawn weeds. More research is needed to reveal the mechanisms associated with reduced insect foraging after herbicide treatment. Researchers aiming to do similar work should pay close attention to floral density between field plots. Despite our efforts to achieve uniformity, plots initially varied from approximately 35 to over 100 white clover blooms m^{-2}. As our herbicide treatments strongly affected pollinator foraging, variable bloom density was not an issue. Treatments that impart more subtle influence on insect foraging may be negatively affected by variable bloom density. Furthermore, plots with <35 blooms m^{-2} may not sufficiently attract pollinators and would lead to experimental error. Future research will evaluate mechanisms, including floral reflectance, nectar production, and herbicide placement, that may explain the rapid evacuation of insects from herbicide-treated white clover.

Practical Implications

Results strongly suggest that honeybees and other insect foragers vacate herbicide-treated areas in fewer than 2 d following treatment, even though the rapid decline in insect visitation does not synchronize with loss of floral density and flower quality metrics. Treating weedy flowers with herbicides 2 d before insecticide application should protect pollinators from exposure to harmful insecticides. This timeline will give practitioners more flexibility compared to previously available research. This research advances our goal to provide stakeholders with additional tools in existing best practices that may help mitigate risks of pollinator exposure to harmful pesticides.

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Competing Interests. The authors declare that PBI Gordon Corp. markets two of the products evaluated in this research but played no role in implementation, data collection, or data interpretation.

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