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Evaluating Deterrent Effects on Pollinator Contact Exposure to Fluorescent Powder in White Clover-Infested Turfgrass

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Short title: Pollinator contact exposure

Abstract

Pollinators are susceptible to insecticide residues when foraging on weedy flowers in turfgrass systems. Deterrent practices may mitigate this risk by reducing pollinator visits; however, their effectiveness in limiting contact exposure of pollinators has not been thoroughly evaluated. Two trials were conducted using a randomized complete block design with three temporal blocks to assess the effectiveness of deterrent practices in preventing contact exposure of actively trapped honey bees (*Apis mellifera*) and passively trapped insects to fluorescent powder-treated white clover (*Trifolium repens* L.) inflorescences in turfgrass. Deterrent treatments included mowing the same morning before fluorescent powder application, spraying with a premix of 2,4-D, MCPP, and dicamba two d before powder treatment, or no deterrent before powder application. Fluorescent powder was extracted from 1,440 honey bee specimens collected by active trapping at 4 and 28 hours after treatment. Mowing and synthetic auxin herbicides pre-treatment reduced the number of fluorescent powder-exposed honey bees by at least 75% and 93%, respectively. Among exposed honey bees, mowing and herbicide treatments reduced powder concentration by at least 75% and 90%, respectively. Honey bee visitation was positively correlated with *T. repens* inflorescence density, explaining 81% of visitation variability. Mowing transiently decreased *T. repens* floral density by 85%, but recovered by 7 d, while herbicides resulted in complete loss of floral resources by 7 d. Blue vane traps captured 1,117 bees from 23 species, over 96% of which were native, while yellow sticky cards collected 384 insects from the Lepidoptera, Diptera, and Coleoptera orders. Despite differences in honey bee exposure, deterrent treatments did not affect the exposure of passively trapped pollinators to fluorescent powder, likely due to strong visual attraction of traps. Results suggest that mowing and synthetic auxin herbicides effectively deter honey bees from *T. repens* inflorescences, reducing their exposure risk.

Keywords: Best practices; integrated pest and pollinator management; native bees; native pollinators; pollinator conservation; urban landscapes; UV-absorbing flowers.

Introduction

Weedy flowers provide pollen and nectar resources to pollinators in urban landscapes (Bretagnolle and Gaba 2015; Hicks et al. 2016). It is expected that dandelion (*Taraxacum officinale* F.H. Wigg.) and white clover (*Trifolium repens* L.) could be visited by up to 50 different species of pollinators, including 37 species of bees for floral rewards in an urban turfgrass system (Larson et al. 2014). Lowenstein et al. (2019) found that *T. repens* inflorescences attracted the most pollinator visits among 106 plant taxa, including ornamental and weedy flowers, in an urban landscape study documenting 1,815 plant-pollinator interactions. Due to the common occurrence of weeds in turfgrass systems and pollinator interaction for floral rewards (Jaiswal and Joseph 2024), the threat associated with pollinator exposure to insecticide-treated *T. repens* flowers also increases (Gels et al. 2002; Larson et al. 2013). Insecticide applications are commonly utilized in managed turfgrass systems as part of controlling insect pest outbreaks (Held and Potter 2012; Larson et al. 2017). Besides direct contact, the same systemic insecticides also pose a risk to beneficial insects due to the potential for residue transfer to the visiting pollinator through the nectar of insecticide-treated weedy flowers (Larson et al. 2013; Larson et al. 2014). Furthermore, honey bees (*Apis mellifera*, Hymenoptera: Apidae) and bumble bees (*Bombus impatiens*, Hymenoptera: Apidae) were not able to discriminate between neonicotinoid-treated and nontreated inflorescences of *T. repens* (Larson et al. 2013).

Best practices for protecting pollinators from insecticide exposure in turfgrass systems include managing weedy flowers through mowing or herbicide applications (Godara et al. 2023; Larson et al. 2015). Godara et al. (2023) noted that honey bees and other pollinators evacuate a treated plot within 2 days of synthetic auxin herbicide application, although *T. repens* flower quality persisted for up to 5 days in weedy turfgrass. McDougall et al. (2021) found reduced pollinator abundance, diversity, richness, and evenness in a peach orchard [*Prunus persica* (L.) Batsch var. *nectarine* ‘Fantasia’] where synthetic auxin herbicide was used to remove weedy flowers from the groundcover. Mowing significantly reduced imidacloprid and clothianidin residues in *T. repens* nectar by 99% compared to non-mowed flowers, yet it decreased the survival rate of an anthrocorid bug *Orius insidiosus* by 26% when they fed on guttation from imidacloprid-treated creeping bentgrass (*Agrostis stolonifera* L.), even post-mowing (Larson et al. 2015). These studies suggest the need to employ deterrent strategies to limit the foraging of

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pollinators on weedy flowers in turfgrass, thereby reducing insecticide exposure. However, insect visitation data from previous studies were typically collected from short observations, 1 to 2 minutes between approximately 10:00 AM and 4:00 PM, potentially underestimating pollinator activity beyond these times (Bohnenblust et al. 2016; Godara et al. 2023). A more comprehensive assessment of honey bee contact exposure in weed-infested turfgrass following mowing or herbicide application could enhance our understanding of how effective these practices are in deterring the insects.

Previous researchers utilized fluorescent tracers for evaluating deposition rates of pesticides on plants and soil (McWhorter and Wooten 1961; Menger et al. 2020; Staniland 1960, 1961) and for spray drift studies (Fritz et al. 2011; Szarka et al. 2021). Fluorescent powders (DayGlo Corp., Cleveland, OH) are also commonly used as a marker for arthropod species in mark-release-recapture studies due to economic viability, environmental safety, and ease of handling (Hagler and Jackson 2001; Hagler et al. 2011; Perry et al. 2017; Rodriguez-Saona et al. 2020). Previous research utilized a Saturn yellow, fluorescent powder (DayGlo Corp. Cleveland, OH), which yields a vivid fluorescent green color at 494 nm when exposed to excitement wavelength in the ultraviolet (UV) spectrum (Sobeck et al. 2021). Research methods involving beneficial insects, such as pollinators, should utilize a nonlethal marking approach to evaluate their movement patterns (Lövei and Ferrante 2024). Fluorescent powder is nonlethal and persists for up to three weeks on insect bodies, which can be evaluated qualitatively for traces of fluorophore under UV light (Diouf et al. 2022). Koch and WeiBer (1997) used fluorescent powder as a proxy for insecticides to test honey bee exposure in flowering apple orchards (*Malus* spp.) and Phacelia (*Phacelia tanacetifolia* Benth.) fields. Byrne et al. (1996) applied fluorescent powder at 123 kg ha^{-1} to a melon (*Cucumis melo* L.) field to track the migration behavior of whitefly (*Bemisia tabaci*, Hemiptera: Aleyrodidae). The fluorescent powder was also used to mimic pollen on flowers in field experiments to estimate the pollen dispersal by pollinators (Campbell 1985), making fluorescent powder a suitable choice for application to insects and weedy flowers.

However, research investigating the effectiveness of deterrent practices aimed at preventing pollinator foraging on weedy flowers before insecticide application in managed turfgrass is lacking. The objective of this study was to assess the contact exposure of actively

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trapped honey bees and passively captured insects to fluorescent powder-treated *T. repens* inflorescences in tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.; syn. *Lolium arundinaceum* (Schreb.) S.J. Darbyshire] turfgrass after implementing our proposed deterrent practices. We hypothesized that honey bee exposure to fluorescent powder, utilized as a proxy for insecticide application, would be reduced after implementing deterrent treatments to *T. repens* inflorescences in managed turfgrass systems.

Materials and Methods

A field study was conducted in 2024 using a randomized complete block design with three treatments, each replicated three times across two trials (Table 1). Treatments were randomly assigned to one of three sites and blocked by time (Table 1). Three tall fescue turfgrass sites were chosen, each with a minimum *T. repens* floral density of 25 inflorescences m^{-2} over an area of at least 446 m² (Figure 1). Within each site, a 44.6 m² plot served as the experimental unit for applying fluorescent powder and deterrent treatments (Figure 1). At each site, a 0.5-m wide border was mowed to a height of 3 cm, which was about one-third of the original grass height, around each experimental unit to act as a visual barrier for preventing the inadvertent transfer of fluorescent powder into the sampling area due to human traffic during data collection (Figure 1). To ensure the independence of the experimental units, no two plots were placed closer than 4.5 km apart (Table 1), as this distance is beyond the typical foraging range of honey bees, which is up to 2 km (Steffan-Dewenter and Kuhn 2003).

Deterrent treatments included: 1) mowing (HRS216, American Honda Motor Co., Inc., Alpharetta, GA) the plot to a height of 5 cm to remove approximately 40% of the turfgrass canopy including most of the *T. repens* inflorescences at 0 d before applying fluorescent powder; 2) spraying the plot with a premix of 2,4-D, MCPP, and dicamba (Trimec Classic®, PBI Gordon Corp., Shawnee, KS) at 1.52 kg ai ha^{-1}, 2 d before applying fluorescent powder; 3) nontreated control with no deterrent treatment but fluorescent powder application at 0 d, referred to as a positive control (Table 1). Turfgrass is typically mowed to remove no more than one-third of the turf canopy (Patton 2025). Our treatments of 40% removal were based on preliminary experiments to maximize white clover bloom removal without significant harm to turfgrass (data not shown). Likewise, synthetic auxin herbicide premix was applied 2 d before fluorescent

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powder as honey bees and other pollinators vacated the herbicide-treated area within 2 d after treatment based on previous visual observations (Godara et al. 2023). Data collection was conducted for an 8-d period, initiated 1 d before applying deterrent treatments to collect baseline data for floral density and pollinator visitation, and concluded 6 d after application. Mowing, fertilization, or any other turf protection product applications were suspended at all sites to maintain consistent flower density and pollinator activity throughout the experiment duration. Temporal blocks were spaced at least 22 d apart and timed to avoid periods of rainfall.

Herbicide treatment was applied using a CO_2-pressurized backpack sprayer calibrated to deliver 374 L ha^{-1} using four extended-range flat fan XR11006 spray nozzles (TeeJet® Technologies, Wheaton, IL) at 4.8 km hr^{-1}. A fluorescent powder (ECO 17 Saturn Yellow®, DayGlo® Color Corp., Cleveland, OH) with 4.5 µm particle size was used to assess pollinator exposure to treated areas after deployment of deterrent treatments. The fluorescent powder was mixed with sand particles, ranging in size from 0.25 to 1 mm, at a ratio of 1:4 using an electric motor-driven mixer (Proforce® Equipment, Inc., Miami, FL) to achieve a uniform blend. The sand was found in preliminary experiments (data not shown) to improve the flow of the powder blend through a custom 1.8 m × 1.2 m, five-screen applicator with 0.6 cm^2 openings and 25 cm between screen layers. This custom device was positioned 20 times in each plot where a premeasured quantity of fluorescent powder-sand blend was dispensed at a rate of 73 kg fluorescent powder ha^{-1}.

Active honey bee sampling. Before applying deterrent treatments, 20 live honey bees were collected using vacuum-based catching devices (Bugzooka™, Trimax™ Locks/Wyers Products Group, Inc., Centennial, CO) from each experimental unit to establish a baseline for fluorescence in the laboratory before any exposure to applied fluorescent powder. An additional 80 honey bees were captured in equal measure at 4 and 28 hours after treatment (HAT) of fluorescent powder from areas outside the treated plots but within the 446 m^2 *T. repens* inflorescences-infested zone with similar catching devices mentioned above (Figure 1). Each honey bee was captured individually in a catch tube to avoid cross-contamination of residues. The honey bee specimens in the catch tube were then euthanized in a chloroform-based kill jar for 60 seconds. A chloroform-based kill jar accommodated over 20 catch tubes and concurrently reduced the handling time at each site. Samples were immediately placed on ice and transported to the

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Virginia Tech Glade Road Research Facility (37.23°N, 80.44°W) in Blacksburg, VA, for analysis of fluorescent powder exposure. Upon arrival, the samples were stored in a freezer at -20°C until the extraction process began.

Each honey bee specimen was processed to extract fluorescent powder, creating individual samples for body rinse analysis. Preliminary tests established that an *N*, *N*-dimethylformamide (Spectranalyzed®, Fisher Scientific, Fair Lawn, NJ) and water (1:9) extraction method achieved a 91 ± 3.6% efficiency in removing fluorescent powder from honey bees after two rinses (data not shown). Each rinse consisted of 2 mL of the rinsing solution. Fluorescent powder intensity was measured using a QEPRO-XR spectrometer (Ocean Insight, Inc., Orlando, FL) equipped with a PX-2 pulsed xenon lamp (Ocean Insight). Excitation energy in the UV spectrum was redirected after blocking visible light by a bandpass filter (Ocean Insight) into the spectrometer using a 1-cm cuvette-shaped piece of Teflon (CVD-Diffuse, Ocean Insight) placed at a 45° angle in the fluorescence cuvette holder (Square One Cuvette Holder, Ocean Insight) for calibration. The amount of fluorescent powder per bee was quantified using standard curves that correlated fluorescence intensity with powder concentration (Fritz et al. 2011), allowing comparison across different treatments (Figure 2). For preliminary experiments and baseline fluorescence detection, the standard curve was developed using fluorescent powder that had not been exposed to solar radiation (Figure 2). For honey bees collected at 4 and 28 hours after treatment (HAT) of fluorescent powder, the standard curve was adjusted to account for solar radiation exposure by placing Petri dishes (100 mm diameter, Kimble® Kimax®, Dwk Life Sciences, Millville, NJ) in the field accordingly (Figure 2). The average fluorescence intensity from 20 honey bees, collected for baseline detection in each plot, was subtracted from all other samples as background noise before data analysis.

Passive pollinator sampling. Double-sided yellow sticky cards (20 cm × 14 cm, Alpha Scents, Inc., West Linn, OR) were deployed at a height of 25 cm above turfgrass canopy for 28 HAT of fluorescent powder (Figure 1). Two sticky cards were installed in the center of the treated area, while the other two were randomly positioned in one of the cardinal directions outside the treated area (Figure 1). Each card was evaluated under a stereoscope (SMZ1270, Nikon Corporation, Tokyo, Japan) equipped with a UV light source (Everbeam®, Surrey, Canada) emitting 315 to 405 nm with a peak at 365 nm for the presence or absence of fluorescent powder on

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lepidopterans, hover flies, and beetles captured on both sides. These taxa were selected based on their higher probability to interact with inflorescences. Two blue vane traps (BanfieldBio™ Inc., Seattle, WA) were installed, inside and outside of the treated area for 28 HAT of fluorescent powder (Figure 1). These traps were composed of two 3 mm thick, 24 ×13 cm polypropylene cross vanes placed perpendicularly at the top of a blue funnel placed over 2-liter capacity clear plastic jars (12 cm diameter and 18 cm height). Plastic jars were installed 5 cm below the turf canopy, and blue-colored funnels and vanes were at least 12 cm above the turf canopy. Joshi et al. (2015) reported blue vane traps as the most effective trap type for collecting bees after a 3-year study, which recorded over 14,500 bees from 118 species. Previous researchers used an ethylene glycol-based medium to trap insects (Joshi et al. 2015; Turley et al. 2022), but we utilized double-sided yellow sticky cards in the clear jar to trap the insects and to avoid dilution of fluorescent powder concentration over the study period. Bee species from vane traps were identified using guides and published dichotomous keys (Ascher and Pickering 2020; Levenson and Youngstead 2019; Williamson et al. 2018).

Floral density and honey bee visitation. Four quadrants, each measuring 3.34 m^2 were established in each direction (north, south, east, west) within and outside the treated area at each site to collect data on floral density and visible honey bee visitation (Figure 1). Flower density was assessed by manually counting all *T. repens* inflorescences within each quadrant at 8:00 AM on evaluation timings. Honey bee visitation was measured in each quadrant by visually recording the number of unique foragers physically interacting with inflorescences for one-minute period per plot, conducted at 12:00 PM and 2:00 PM, as bees were expected to be actively foraging. Data were collected at each site before initiating a deterrent treatment application and at 0 d, 1 d, and 7 d after treatment (DAT) of fluorescent powder. Floral density data were converted to the number of inflorescences m^{-2} and honey bee visitation data were reported as insect visits min^{-1} m^{-2}. Floral density was further converted to the percent reduction of initial flower density before applying deterrent treatments at each trial site.

Data analysis. Treatment was considered as a fixed effect, while trial and replication were considered random effects in the model statement. The number of fluorescent powder-exposed honey bees, average concentration on exposed honey bees, and flower density response variables were analyzed using ANOVA via PROC MIXED in SAS v 9.4 (SAS Institute, Cary, NC). Data

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for response variables were tested for normality using PROC UNIVARIATE and Shapiro-Wilk statistic, and homogeneity of variance was confirmed by visually inspecting plotted residuals and other metrics using the DIAGNOSTIC option of the PLOT procedure. Both bee and other insect counts from the pane and yellow sticky traps, respectively, were analyzed with the same procedure mentioned above. Data for the total number of bees captured per vane trap was $\log_{10}$-transformed, and the proportion of individuals marked were square-root-transformed to comply with normality. The mean square of the treatment effect was evaluated using the mean square error associated with random variable interaction (McIntosh 1983). Means were separated using Fisher's protected LSD test with a significance level of 0.05. Two linear regressions, using PROC REG, were calculated to assess the relationship between fluorescence intensity and the concentration of fluorescent powder and for floral density and honey bee visitations.

Results and Discussion

The treatment effect was significant for the number of fluorescent powder-exposed honey bees at 4 (P = 0.0114) and 28 HAT (P = 0.0021), and not dependent on the trial (Table 2). A mowing event before fluorescent powder application reduced the number of exposed honey bees by at least 75% compared to the nontreated control at 4 and 28 HAT (Table 2). A single mowing event reduced pollinator visitation on weedy flowers including *T. repens* by at least 50% in a grassland habitat (Phillips et al. 2019). However, a premix of synthetic auxin herbicides reduced the number of fluorescent powder-exposed honey bees by ≥ 94% at 4 and 28 HAT (Table 2). Baucom et al. (2025) also reported a 93% reduction in pollinator visitation based on visual observations after weedy flowers were treated with dicamba, altering plant-pollinator interactions. Synthetic auxin herbicides performed better in deterring honey bees at 28 HAT, as the number of fluorescent powder-exposed honey bees was higher in mowed plots compared to herbicide-treated plots (Table 2).

The treatment effect was significant on the average concentration of fluorescent powder on exposed honey bees at both 4 HAT (P = 0.0256) and 28 HAT (P = 0.0391) and not affected by trial (Table 2). At 4 HAT, honey bees from sites without deterrent treatments were exposed to 61.28 µg of fluorescent powder per honey bee, which was reduced to 13.27 µg in mowed plots and to 0 µg in herbicide-treated plots (Table 2). Larson et al. (2015) observed that even after

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mowing, pollinator exposure to insecticide-associated risk was not entirely eliminated, with up to 36 ng of imidacloprid and clothianidin per gram of nectar remaining in newly formed *T. repens* inflorescences. By 28 HAT, the concentration of fluorescent powder on honey bees from no deterrent plots was 23.46 µg per bee, but this decreased to ≤ 5.82 µg in plots treated with mowing or herbicide (Table 2). The average exposure level was reduced by 28 HAT in the positive control group (Table 2). This reduction can be partly explained by the limited lightfastness of the fluorescent powder, where UV irradiation resulted in over 85% decrease in emission intensity within 24 hours (Sobeck et al. 2022). However, this impact was largely corrected through the use of in-field standards for generating standard curves at 4 and 28 HAT (Figure 2). Additional factors possibly contributing to the lower exposure levels over time include dew, which enhances the aggregation of powder particles to one another and to plant surfaces (Ossola and Farmer 2024), therefore decreasing their dislodgeability. Honey bee grooming behavior, including autogrooming and allogrooming, can also contribute to the active removal of powder particles from their bodies (Carroll and Brown 2024).

Honey bee visitation was positively correlated with *T. repens* inflorescence density, with 81% of the variation in honey bee visitation explained by changes in floral density (Figure 3). A similar positive linear correlation between floral density and pollinator visitation has been documented in previous studies on canola (*Brassica napus* L. 'Wichita'), winter pea (*Pisum sativum* L. ssp. *arvense*), and red clover (*Trifolium pratense* L. 'Mammoth') (Ellis and Barbercheck 2015). Further, Godara and Askew (2024) found that honey bee evacuation from herbicide-treated turfgrass areas was influenced by rapid changes in UV reflection from treated weedy flowers but ultimately driven by a reduction in floral density. In no deterrent plots, there was a 14% decline in *T. repens* floral density by 7 DAT (Table 3). Zalenski (1964) observed a similar natural decline, linking it to reduced light penetration at the base of high-density plants, which slows vegetative and floral induction. At 0.25 DAT, *T. repens* inflorescence density was reduced to 85% in mowed plots and 44% in herbicide-treated plots (Table 3). Mowing led to a rapid decline in floral density compared to areas treated with synthetic auxins, where herbicide application resulted in a ~20% reduction in floral density per d post-treatment (Godara et al. 2023). Mowing did not completely eliminate floral resources in weed-infested turfgrass; flowers at lower heights in the canopy can persist even after mowing events (Lerman et al. 2018). *T. repens* shows high phenotypic plasticity in internode length, influenced by management

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intensity, which in turn affects inflorescence height and seed production (Caradus et al. 2023). However, in plots treated with synthetic auxins, there was a 100% decline in *T. repens* floral density, while nontreated and mowed plots showed less than a 15% reduction at 7 DAT (Table 3), due to floral regeneration following mowing. MacRae et al. (2005) also reported over 90% control of *T. repens* inflorescences with synthetic auxin herbicides like 2,4-D and clopyralid.

Blue vane traps captured 23 distinct bee species from the Apidae, Halictidae, and Megachilidae families (Table 4). Among the total of 1117 bees captured, over 96% were native bees and comprised of 1080 insect specimens (Table 4). Previous research by Larson et al. (2014) also documented 21 species of bees visiting *T. repens* inflorescences in turfgrass systems. Deterrent treatment did not affect the number of bee species collected per trap, possibly driven by strong visual cues from the light reflectance properties of the trap in the 400 to 600 nm range (Acharya et al. 2022). Kimoto et al. (2012) also found that the number of sweat bees (*Lasioglossum*) captured via blue vane traps was unaffected by management intensity. No effect of deterrent treatment ($P > 0.05$) was documented on bee exposure to fluorescent powder collected from blue vane traps. Yellow sticky cards captured 284, 21, and 79 insects from Lepidoptera, Diptera, and Coleoptera orders, respectively, when established for 28 HAT (Table 4). Deterrent treatments did not affect ($P > 0.05$) the exposure of Lepidoptera, Diptera, and Coleoptera insects collected from yellow sticky cards to fluorescent powder (data not shown). As with blue vane traps, previous research suggests that the strong visual attraction of Lepidoptera and Diptera insects to yellow traps (Laubertie et al. 2006; Meagher 2001) can attract insects from several m distances. Our deterrent treatments primarily targeted impacts on *T. repens* inflorescences (Table 3, Figure 3), but if insects were more attracted to the passive traps, then their capture rates would remain consistent regardless of habitat modifications with mowing or herbicide application. Venn and Kotze (2014) also observed that the abundance and richness of beetles (Carabidae) were not affected by intensive mowing compared to unmanaged areas for one year, potentially attributed to their functional role.

Our study shows that pre-treating with mowing or synthetic auxin herbicides significantly reduces the number of honey bees exposed to fluorescent powder by at least 75% and 93%, respectively. Out of the exposed honey bees, the mowing and herbicide treatments reduced the fluorescent powder concentration by at least 75% and 90%, respectively. Mowing temporarily

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decreases the floral density of *T. repens* by 85%, but the impact diminishes by 7 DAT, allowing pollinator food resources to recover. In contrast, synthetic auxin herbicides cause a complete loss of floral resources by 7 DAT, leading to a long-term elimination of *T. repens* inflorescences. These deterrent practices vary from the permanent removal of pollinator food sources using synthetic auxin herbicides in areas requiring frequent insecticide applications to the short-term suppression of inflorescences via mowing, which permits floral regeneration in areas with less pest pressure. While this study provides valuable insight into how deterrent practices can mitigate pollinator exposure risk, several constraints of the experimental design should be considered. These experiments were conducted twice during 2024, which limits the ability to account for interannual variation in environmental conditions, weed phenology, and pollinator activity. Factors such as differences in temperature, precipitation, clover growth dynamics, and pollinator abundance across years could influence the magnitude of deterrent effects observed. Blue vane traps captured 1117 insect specimens representing 23 bee species, while 384 insects from Lepidoptera, Diptera, and Coleoptera orders were captured on yellow sticky cards. Despite differences observed in contact exposure of actively sampled honey bees, deterrent treatments did not affect the exposure of passively trapped pollinators to fluorescent powder or bee abundance in each blue vane trap. Our results highlight the intricate relationships between pollinators, weedy floral resources, and turfgrass management, emphasizing the need for developing integrated strategies that reconcile pest management with pollinator conservation. Future research will further explore the effects of deterrent treatments across various insect functional groups.

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Competing Interests

The authors declare none.

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Table 1. Research experiments were conducted in Virginia to evaluate the effectiveness of deterrent treatments in preventing contact exposure of pollinators to fluorescent powder-treated *Trifolium repens* inflorescences in tall fescue turfgrass.

Trial	Deterrent treatment	GPS coordinates	Location[a]	Fluorescent powder application[b]
1	No deterrent	37.21489°N, 80.36061°W	BCC	May 22, 2024
1	Mowing	37.20526°N, 80.56497°W	HF	
1	2,4-D, MCPP, dicamba	37.21686°N, 80.41154°W	TRC	
1	No deterrent	37.20542°N, 80.56460°W	HF	June 21, 2024
1	Mowing	37.21676°N, 80.41125°W	TRC	
1	2,4-D, MCPP, dicamba	37.21526°N, 80.36081°W	BCC	
1	No deterrent	37.23313°N, 80.43644°W	GRRF	July 15, 2024
1	Mowing	37.21540°N, 80.36090°W	BCC	
1	2,4-D, MCPP, dicamba	37.20562°N, 80.56423°W	HF	
2	No deterrent	37.21266°N, 80.41281°W	TRC	August 12, 2024
2	Mowing	37.20487°N, 80.56509°W	HF	
2	2,4-D, MCPP, dicamba	37.21635°N, 80.36133°W	BCC	
2	No deterrent	37.20461°N, 80.56558°W	HF	September 3, 2024
2	Mowing	37.21659°N, 80.36147°W	BCC	
2	2,4-D, MCPP, dicamba	37.23337°N, 80.43662°W	GRRF	
2	No deterrent	37.21676°N, 80.36206°W	BCC	September 30, 2024
2	Mowing	37.21318°N, 80.41267°W	TRC	
2	2,4-D, MCPP, dicamba	37.20258°N, 80.56443°W	HF	

[a]Abbreviations: BCC, Blacksburg Country Club; GRRF, Glade Road Research Facility; HF, Homefield Farm; TRC, Turfgrass Research Center. All sites were in Montgomery County, Virginia, and were separated by at least a 4.5 km radius from each other. The three treatments were randomly assigned to these sites.

[b]Fluorescent powder was applied at least 22 d apart for each replication within each trial. Treatments were blocked by time.

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Table 2. Contact exposure of honey bees to fluorescent powder-treated *Trifolium repens* inflorescences in turfgrass at 4 and 28 hours after treatment (HAT) of fluorescent powder.

	Fluorescent powder exposed honey bees[b]				Average concentration on exposed honey bees			
Deterrent treatment[a]	4 HAT		28 HAT		4 HAT		28 HAT	
	----------number----------				----------µg----------			
No deterrent	17	a	16	a	61.28	a	23.46	a
Mowing	4	b	4	b	13.27	b	5.82	b
2,4-D + MCPP + dicamba	0	b	1	c	0.00	b	2.17	b
P-value	0.0114		0.0021		0.0256		0.0391	

[a]Mowing was conducted on same d before applying fluorescent powder; a premix of synthetic auxin herbicides, Trimec Classic at 1.52 kg ai ha^{-1} was applied 2 d before fluorescent powder treatment.

[b]A total of 40 honey bees were actively sampled at each timing per treatment.

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Table 3. *Trifolium repens* inflorescences density 0.25 and 7 d after treatment (DAT) of fluorescent powder.[a]

	Density reduction[b]			
Deterrent treatment[a]	0.25 DAT		7 DAT	
	----------%----------			
No deterrent	2	a	14	a
Mowing	85	c	4	a
2,4-D + MCPP + dicamba	44	b	100	b
P-value	0.0173		0.0202	

[a]Mowing was conducted on the same d before applying fluorescent powder; a premix of synthetic auxin herbicides, Trimec Classic at 1.52 kg ai ha^{-1} was applied 2 d before fluorescent powder treatment.

[b]Reductions are based on the average density of *Trifolium repens* inflorescences before applying deterrent treatments, which were 57, 52, and 47 blooms m^{-2} in nontreated, mowing, and herbicide-treated plots, respectively.

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Table 4. Bee species, butterflies, hoverflies, beetles, and their frequencies captured by passive traps.[a]

Trap type	Order	Family	Common name	Scientific name	Origin	Number captured
Blue vane trap	Hymenoptera	Apidae	Western honey bee	*Apis mellifera*	Nonnative	37
Blue vane trap	Hymenoptera	Apidae	Northern golden bumble bee	*Bombus fervidus*	Native	5
Blue vane trap	Hymenoptera	Apidae	Brown-belted bumble bee	*Bombus griseocollis*	Native	2
Blue vane trap	Hymenoptera	Apidae	Eastern bumble bee	*Bombus impatiens*	Native	98
Blue vane trap	Hymenoptera	Apidae	American bumble bee	*Bombus pensylvanicus*	Native	15
Blue vane trap	Hymenoptera	Apidae	Mikmaq small carpenter bee	*Ceratina mikmaqi*	Native	50
Blue vane trap	Hymenoptera	Apidae	Long-horned bee	*Eucera rosae*	Native	6
Blue vane trap	Hymenoptera	Apidae	Common longhorn bee	*Melissodes communis*	Native	42
Blue vane trap	Hymenoptera	Apidae	Drury's longhorn bee	*Melissodes druriellus*	Native	66
Blue vane trap	Hymenoptera	Apidae	Three-knotted longhorn bee	*Melissodes trinodis*	Native	45
Blue vane trap	Hymenoptera	Apidae	Mallow bee	*Melitoma taurea*	Native	1
Blue vane trap	Hymenoptera	Apidae	Thistle longhorn bee	*Mellisodes desponsus*	Native	74
Blue vane trap	Hymenoptera	Apidae	Squash bee	*Peponapis pruinosa*	Native	398
Blue vane trap	Hymenoptera	Apidae	Sunflower longhorn bee	*Svastra obliqua*	Native	10
Blue vane trap	Hymenoptera	Apidae	Concave cuckoo bee	*Triepeolus convacus*	Native	4
Blue vane trap	Hymenoptera	Apidae	Eastern carpenter bee	*Xylocopa virginica*	Native	1
Blue vane trap	Hymenoptera	Halictidae	Bicolored sweat bee	*Agapostemon virescens*	Native	49
Blue vane trap	Hymenoptera	Halictidae	Pure green Sweat bee	*Augochlora pura*	Native	50
Blue vane trap	Hymenoptera	Halictidae	Parallel striped sweat bee	*Halictus parallelus*	Native	80
Blue vane trap	Hymenoptera	Halictidae	Ligated furrow bee	*Halictus poeyi / Halictus ligatus*	Native	72
Blue vane trap	Hymenoptera	Halictidae	Pointed sweat bee	*Lasioglossum acuminatum*	Native	7
Blue vane trap	Hymenoptera	Megachilidae	Short leafcutter bee	*Megachile brevis*	Native	3
Blue vane trap	Hymenoptera	Megachilidae	Texas mason bee	*Osmia texana*	Native	2
Yellow sticky card	Lepidoptera	Several families	Butterflies	Several species	-	284
Yellow sticky card	Diptera	Syrphidae	Hoverflies	Several species	-	21
Yellow sticky card	Coleoptera	Several families	Beetles	Several species	-	79

[a]Blue vane traps and yellow sticky cards were established as passive traps for 28 hours after fluorescent powder treatment in *Trifolium repens*-infested turfgrass experimental plots in Blacksburg, VA in 2024. Two blue vane traps and four yellow sticky cards were established for each experimental unit, with a total of 36 blue vane traps and 72 yellow sticky cards for three replications and two trials.

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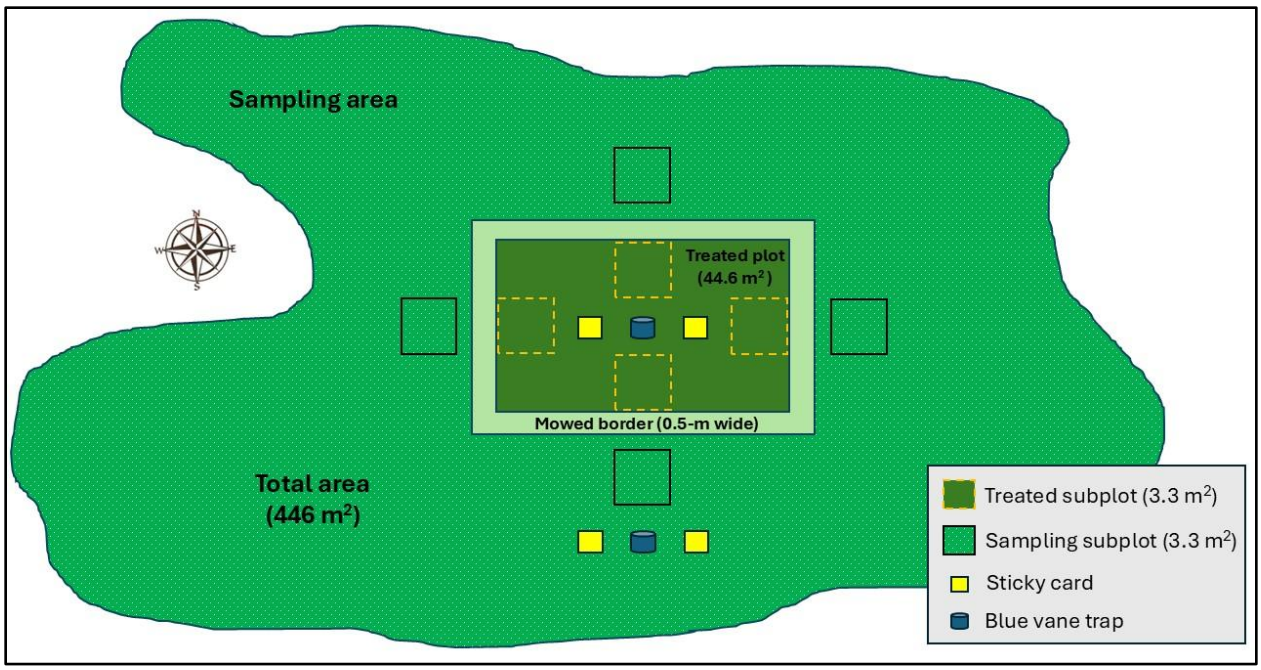

Figure 1. An experimental unit layout representing the treated plot area (44.6 m^2 - deterrent treatment followed by fluorescent powder application) and total plot area (446 m^2 composed of *Trifolium repens*-infested turfgrass with at least a floral density of 25 inflorescences m^{-2}). Four quadrants were established in each cardinal direction within and outside of the treated area for floral density and insect visitation data collection. Passive traps were randomly established in any cardinal direction 1 m away from the quadrant.

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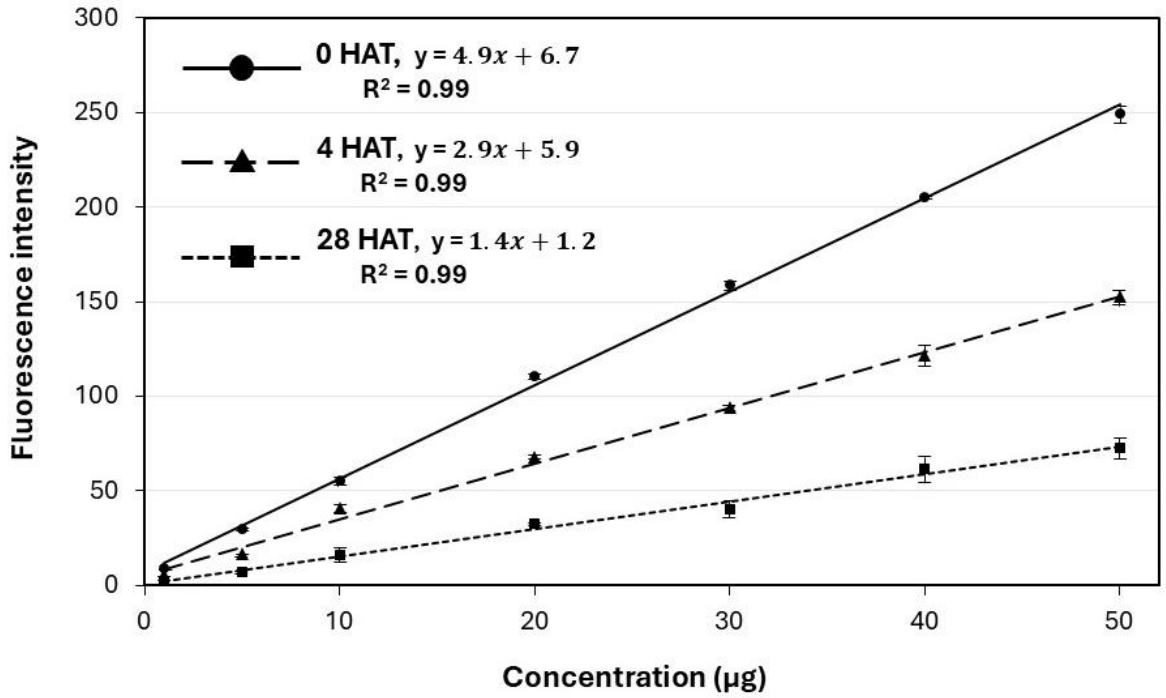

Figure 2. Standard curves were used to calculate fluorescent powder concentration based on fluorescence intensity for samples not exposed to solar radiation, exposed for 4 hours, and 28 hours after treatment (HAT) under field conditions.

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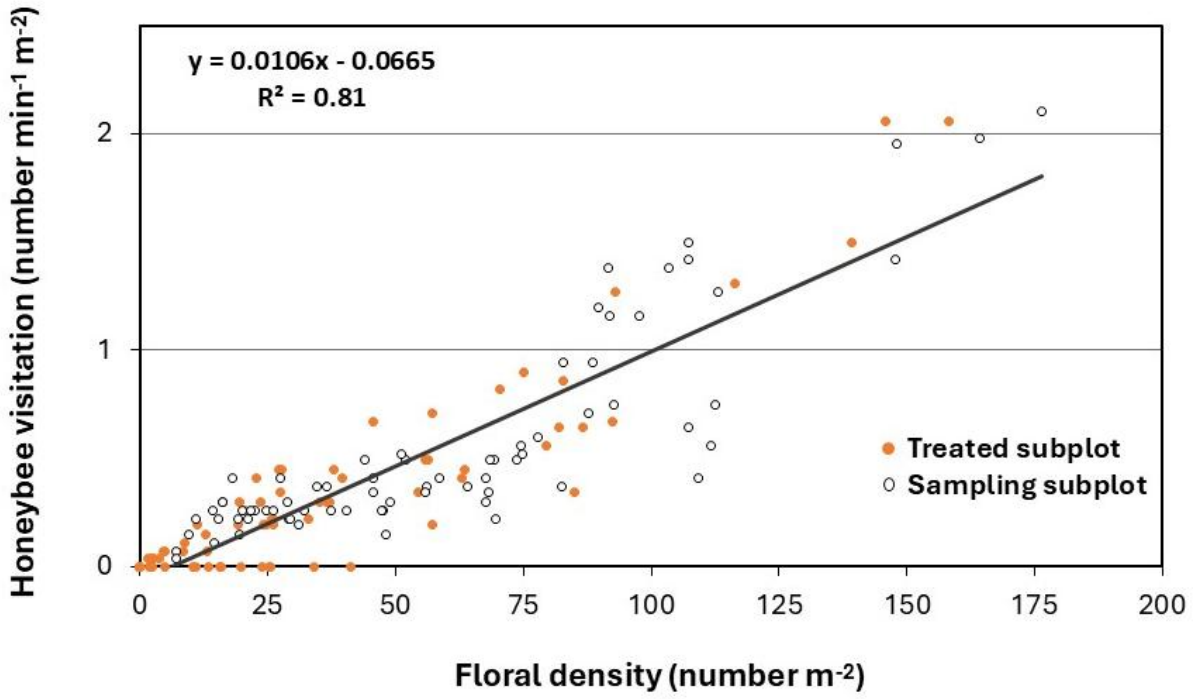

Figure 3. Relationship between honey bee visitation and *Trifolium repens* inflorescence density during the study.

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