Title

Exploiting Cucumber Beetle Behavior for Improved Management: Evaluating Attractants and Gustatory Stimulants for Targeted Pest Control

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Exploiting Cucumber Beetle Behavior for Improved Management: Evaluating Attractants and Gustatory Stimulants for Targeted Pest Control

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Table of Contents

Abstract

Western striped cucumber beetles (Acalymma trivittatum) and Western spotted cucumber beetles (Diabrotica undecimpunctata undecimpunctata) are important pests in California cucurbit production. Conventional management strategies heavily rely on broad-spectrum insecticides, which pose risks to pollinators essential for cucurbit yield. In organic systems, management options are very limited. These beetles are attracted to both floral volatiles and aggregation pheromones, which present an opportunity to exploit their attraction to these semiochemicals for pest management. Additionally, cucumber beetles are adapted to treat plant Cucurbitacin's, the bitter compounds found in cucurbit plants, as potent gustatory stimulants, potentially making these compounds a valuable tool for enhancing pest control methods by increasing the efficacy of insecticides.

In this study, we evaluated the use of synthetic floral volatiles combined with an aggregation pheromone as a targeted pest management strategy for cucumber beetles. Our goal was to minimize the attraction of non-target pollinators when these lures are deployed with traps or targeted insecticide applications to capture or eliminate beetles for effective monitoring and/or management. Field trials conducted over two years confirmed the attractiveness of synthetic floral compounds (TIC: trimethoxybenzene, indole, cinnamaldehyde), the aggregation pheromone of A. trivittatum (vittatalactone), and various combinations of the two in attracting cucumber beetles. Indole, both alone and combined with vittatalactone, effectively attracted beetles while minimizing pollinator (Apis mellifera) bycatch, making it a promising lure for selective pest management. In contrast, TIC proved highly attractive to honeybees, indicating risks for non-target attraction if used with traps or insecticide applications.

Additionally, we evaluated the potential of combining the gustatory stimulant CidetrakL (CT), derived from cucurbitacin-containing compounds, with the neonicotinoid insecticide assail (acetamiprid) to manage Western striped cucumber beetles (Acalymma trivittatum) and Western spotted cucumber beetles (Diabrotica undecimpunctata undecimpunctata). Field trials were conducted to assess whether the addition of CT to various rates of assail and indoxacarb (an alternative insecticide that has been successful in controlling other beetle species) could improve efficacy while minimizing insecticide use, thereby reducing potential negative impacts on non-target organisms such as pollinators. Laboratory bioassays were performed with assail at low concentrations combined with CidetrakL to further evaluate its effect on beetle mortality, and behavioral assays using Y-tube olfactometers and Ethovision tracking software were performed to assess the behavioral effects of combinations of CidetrakL and synthetic floral attractants. In field trials the combination of CT with reduced rates of Assail achieved levels of beetle mortality and melon damage control comparable to treatments with the full rate Assail by itself. This outcome suggests a potential pathway for reducing chemical inputs in IPM strategies. However, CT did not affect beetle behavior in lab assays, and no significant differences in feeding stimulation were observed. Our work highlights the potential of gustatory stimulants to enhance insecticide efficacy at lower rates but underscores the need for further field testing to optimize these approaches for sustainable beetle management.

Chapter 1

Evaluating Attractants for Early-Season Interception of Western Cucumber Beetles in Melon Fields

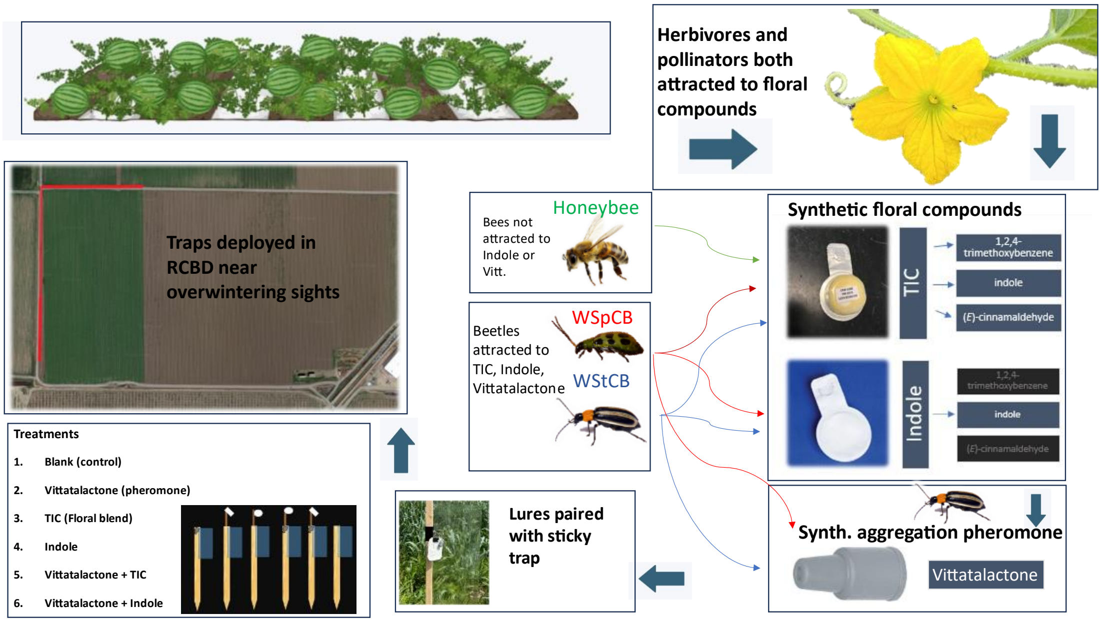

Figure 1 Visual abstract

Introduction

The functional traits of flowering plants and their pollinators have been shaped by mutualistic interactions and reciprocal selective pressures that enable each to manipulate the interacting partner. Plants have evolved a variety of traits to attract pollinators, including the production of floral scents and nectar rewards (Andrews et al., 2007). However, plants reliance on chemical signaling to attract pollinators presents an exploitable vulnerability for herbivorous insects seeking suitable food sources. Some herbivorous insects have evolved the ability to detect and exploit the same floral volatile cues used by insect pollinators, eavesdropping on plant-pollinator communication to locate host plants (Stowe et al., 1995). By intercepting these chemical signals, herbivores can locate host plants more efficiently. In an agro-ecological setting, this system of sending, receiving, and eavesdropping on signals is dynamic, as flowering crops are both targets for herbivore pests and reliant on pollinators for successful crop yield.

In addition to communicating with plants via semiochemicals, insects use chemical signals for various purposes beyond foraging, including identifying in-group and out-group members, attracting mates, and aggregating for defense or to overwhelm host-plant defenses (Bruce, 2015; Segar et al., 2019; Weber et al., 2022). For example, sex pheromones are crucial in mate attraction, while other pheromones can influence the behavior of entire colonies in social insects. Alarm pheromones can signal danger and prompt defensive behavior, while aggregation pheromones can lead to mass gatherings for collective actions such as swarming or coordinated attacks on host plants (Jones & Agrawal, 2017; Weber et al., 2014). Recognizing the diversity and specificity of chemical signals used by insect herbivores presents an opportunity to exploit them for pest management. Pheromones and plant volatiles can be harnessed to develop targeted strategies that reduce pest populations by disrupting their communication and reproduction or by luring them into traps or trap crops (Raguso, 2021; Weber et al., 2014). This approach not only offers a more sustainable alternative to chemical insecticides but also enhances the effectiveness of integrated pest management (IPM) programs, allowing for more precise and environmentally friendly control measures.

Diabroticene beetles, such as *Diabrotica undecimpunctata*, commonly known as the spotted cucumber beetle or southern corn rootworm, are well-known agricultural pests capable of feeding on a variety of crops in the United States. They can cause extensive feeding damage in the rhizosphere as larvae and on the fruits and foliage as adults (Haber et al., 2023; Gill, Goyal, & Gillett-Kaufman, 2020). In contrast to the more generalized *D. undecimpunctata*, the striped cucumber beetle *Acalymma vittatum* is an obligatory feeder on plants from the Cucurbitaceae, including cucumbers, squashes, and melons. *Acalymma vittatum* has evolved particularly effective adaptations to feeding on cucurbits and causes significant damage during both its larval and adult stages, making its management crucial for farmers growing these crops (Eben & Espinosa de Los Monteros, 2013) (Branson & Krysan, 1981).

In California, the primary pest species of cucumber beetles are the western counterparts of the striped and spotted cucumber beetles, namely the Western striped cucumber beetle (*Acalymma trivittatum;* W. striped CB) and the western spotted cucumber beetle (*Diabrotica undecimpunctata undecimpunctata;* W. spotted CB) (Haber et al., 2021).

Both species of beetles will overwinter as adults in leaf litter, tall grass, or other cover. Once temperatures warm in the springtime, the adults will emerge, with W. striped CB adults typically invading nearby cucurbit fields to lay their eggs at the base of young plants (Weber et al., 2023). The eggs will hatch, and the larvae will begin to feed on the roots before pupating and emerging as adults. As adults, the W. striped CB beetles will seek shelter within the floral whorls, under the dense cucurbit canopy, and under the developing fruit. In contrast, W. spotted CB may reproduce in both cucurbits and non-cucurbit crops, utilizing a broader range of host plants during their lifecycle (Metcalf & Lampman, 1989).

Foliar and floral damage is usually minor, but in some cases, the voracious feeding of the adults can cause seedling death via defoliation (Haber et al., 2021). Instead, feeding adults on fruit, especially by W. striped CB, is the primary cause of concern. The adults prefer to feed on the underside Of young, soft-skinned developing fruit, which causes significant scarring that becomes increasingly more visible as the melons grow in size (Haber et al., 2021). Although the damage is purely cosmetic, there is a low consumer tolerance for damage in conventionally grown melons. Typically, any melons showing scars larger than 20 mm in diameter are culled (Haber et al., 2021). Because management options are more limited in the organic market, consumer damage tolerance seems to be higher. In addition to the feeding damage, it is possible that W. striped CB can act as a vector for squash mosaic virus (Haber et al., 2021).

The damage from a single beetle can render a melon unmarketable, and this leads to low treatment thresholds in fresh market cucurbits. In conventional California melon fields, detecting a single beetle often triggers the treatment of the entire field with insecticides (Brust & Foster, 1999). However, the behavior of these beetles complicates chemical control of W. striped CB populations. For example, the beetles' habit of feeding within cucurbit flowers, under the fruit or within the dense foliar canopy limits their exposure to insecticides, reducing the effectiveness of spray coverage where beetles are frequenting (Weber et al., 2023). Additionally, the number of effective insecticide options in California is limited, which could lead to resistance issues (Capinera, 2020).

Currently, the most commonly used insecticides in California cucurbit production are neonicotinoids and pyrethroids (Capinera, 2020). These materials are non-selective and can be detrimental to pollinators, which are crucial for fruit set and overall yield. For instance, neonicotinoids can disrupt bee foraging behavior and reduce their populations. This not only affects immediate pollination but can also have long-term ecological impacts by reducing the pollinator population in the area. Although Acetamiprid (the active ingredient in Assail) is considered more pollinator-friendly compared to other neonicotinoids and its use is less restricted, it is still broad-spectrum, systemic, and can have non-target effects (Weber et al., 2023). Other neonicotinoids used in California cucurbit production (imidacloprid, thiamethoxam, clothianidin, dinotefuran) are subject to reevaluation due to their potential risk to pollinators and must follow more stringent use restrictions, including prohibitions on application during the bloom period when pollinators are most active, limitations on application methods such as foliar spraying and soil treatments, and the establishment of buffer zones to protect pollinator habitats. Pyrethroids, although not systemic like neonicotinoids, pose their own set of challenges, including broad-spectrum activity, high bee toxicity and regulatory concerns related to aquatic toxicity and water quality (Weber, 2018).

Organic melon production systems face challenges in effectively managing cucumber beetles. Current methods are often less effective, more costly, and labor-intensive compared to conventional approaches. In the organic market, the available materials, such as Spinosad and neem oil, are less harmful to pollinators but tend to be less effective and more expensive. Additionally, organic treatments often break down more quickly than conventional insecticides under field conditions, necessitating more frequent applications. Current cultural control methods include the use of physical barriers, such as row covers, netting, and plastic mulch, and crop rotation to reduce overall field populations. However, the effectiveness of these methods can also be limited. For instance, row covers and netting require considerable labor to install and maintain, and plastic mulch can increase soil temperatures and affect plant growth. Crop rotation can help disrupt the life cycle of beetles but may not be feasible for all growers due to land and economic constraints. Monitoring for W. striped CB is also labor-intensive, as fields must be scouted regularly, and plants individually checked. Effective monitoring involves inspecting flowers, leaves, and soil at the base of plants, which can be time-consuming and requires trained personnel (Alston & Worwood, 2012). There remains a need for alternative methods of monitoring and management that are both effective and sustainable. Strategies that combine chemical, biological, and cultural methods could provide a more balanced approach.

Incorporating traps with lures to monitor and/or manipulate beetle populations and using targeted insecticide applications based on trap data could reduce overall chemical use and offer new solutions in the future (Weber et al., 2023).

W. striped CB and W. spotted CB are noted for their ability to cue in on both floral and leaf volatiles to locate and invade cucurbit fields. The distinct fragrances that attract them to the plants are complex blends of volatile compounds. Among these, 1,2,4-trimethoxybenzene, trans-cinnamaldehyde, and indole have been identified as particularly attractive to these pests (Weber et al., 2023). This specific combination of floral volatiles is commonly referred to as TIC (trimethoxybenzene-indole-cinnamaldehyde). Although floral volatiles are crucial in the beetles' ability to locate host plants, leaf volatiles also play a significant role, especially in the early stages of field location (Lewis et al., 1990). These volatiles, especially floral volatiles, have long been a target for developing pest management strategies (Weber et al., 2023). There are commercially available TIC-containing lures that could be used for monitoring; however, they are also attractive to non-target organisms, particularly pollinators like honeybees (Tinslay, 2022), reducing their IPM utility.

In addition to floral cues, the male-produced aggregation pheromone vittatalactone is crucial to the rapid colonization of cucurbit fields by cucumber beetles. Originally identified as a pheromone for mate attraction in the W. striped CB, it has also been shown to attract W. spotted CB. Field studies demonstrate that both sexes of W. spotted CB are strongly attracted to vittatalactone, with significantly higher capture rates compared to un-baited traps (Haber et al., 2023). This cross-species attraction to vittatalactone suggests that it may be mediating the behavior of multiple cucurbit pests. Although vittatalactone is not yet commercialized, it presents a promising avenue for pest management, with potential applications in IPM strategies aimed at reducing pesticide use.

Scouting and Mass Trapping

Attractants are widely used in cropping systems for two main IPM strategies: scouting, which monitors pest population levels to guide action thresholds for interventions, and mass trapping, which reduces pest populations by intercepting pests as they enter fields (Smith & McSorley, 2000; Tinslay et al., 2022). Scouting is a fundamental IPM tool that informs growers when pest densities reach action thresholds, triggering interventions like pesticide applications or bio-control releases. (Cavanagh et al., 2009).

Implementing effective scouting of highly mobile pest populations, such as cucumber beetles, can be difficult. Populations of pests can fluctuate rapidly based on environmental conditions, complicating efforts to set reliable action thresholds (Branson & Krysan, 1981). Despite these challenges, scouting remains a valuable tool because it can allow for early detection and targeted management of pest "hot spots" within fields (if the fields are large enough), reducing the need for blanket insecticide applications (Tinslay et al., 2022).

Mass trapping has shown varying degrees of success across pest species and crop systems. For example, it has been effective against the Mediterranean fruit fly (Ceratitis capitata) in fruit orchards, where traps baited with food attractants significantly reduced population densities, decreasing the need for insecticide applications (Tinslay et al., 2022). Similarly, pheromone-based mass trapping has been used for the Japanese beetle (Popillia japonica) in landscapes and ornamental settings, although its success has been limited by high non-target attraction and inconsistent capture rates in some environments (Weber et al., 2022).

Mass trapping in row crops has faced significant challenges. High pest mobility and extensive field areas typical of crops such as corn and soybean make it difficult to deploy enough traps to effectively reduce pest densities (Tinslay et al., 2022). Additionally, multiple overlapping pest species can complicate efforts to target pests without impacting non-target organisms, as seen in systems using broad-spectrum floral lures (Weber et al., 2022). Effective mass trapping requires a high degree of lure specificity and placement optimization, particularly in cases where non-target attraction poses a risk to beneficial insects like pollinators (El-Sayed et al., 2006).

For cucurbit crops, attempts to use mass trapping to manage cucumber beetles have been explored, although success has varied. In field trials, floral and pheromone lure combinations have successfully attracted beetles to traps, but the effectiveness of these traps in reducing overall pest pressure remains limited, partly due to non-target capture and environmental factors (Weber et al., 2023; Tinslay, 2022). Mass trapping in cucurbit field studies indicate that the spatial arrangement and timing of trap deployment are critical for maximizing capture rates. Trap placement along field edges or within areas of high pest activity, such as overwintering sights, may improve capture rates and help intercept beetles before they spread throughout a crop (Alston & Worwood, 2012). Optimizing the trap deployment timings could boost the positive effect by attracting cucumber beetles specifically during periods of high activity, such as early spring when beetles emerge from overwintering (Weber et al., 2023).This approach may be especially beneficial in organic production systems, where chemical control options are limited, and trapping can reduce beetle densities without impacting pollinator or other beneficial insect predator populations (Smith & McSorley, 2000).

Recently, research has demonstrated that combining synthetic aggregation pheromones like vittatalactone with floral lures such as TIC could create a synergistic effect. In one study, the results showed that this combination lure attracted significantly more beetles compared to either lure alone. (Weber et al., 2023) (Figure 2). However, a key limitation of mass trapping cucumber beetles is the attractiveness of the floral lure component to pollinators, particularly honeybees. This overlap in pest-pollinator attraction requires consideration of lure formulations and deployment strategies. Lure-based traps may require modifications in lure components, concentration, trap design, or placement density to achieve reliable suppression of cucumber beetle populations. Additionally, testing lure combinations under different seasonal conditions and using scouting data to identify periods of peak beetle activity could enhance the effectiveness of this strategy. (Tinslay et al., 2022).

Figure 2
Sticky trap in field showing large numbers of cucumber beetles (mixed species) responding to attractants.
(Photo: Jasmin Ramirez-Strain)

The combination of TIC and vittatalactone aggregation pheromone lures represents a potential tool that may be used to intercept and manage Western striped and spotted cucumber beetles, by relying on the synergistic attraction of the TIC compounds and vittatalactone to lure these pests into traps to reduce their populations or providing early detection of the colonizing population invading the field.

Project Objectives

Given the significant role that floral volatiles and aggregation pheromones play in attracting cucumber beetles, our project aimed to explore the synergistic potential of combining these elements for improved pest management. The primary objective was to evaluate the attraction of cucumber beetles and pollinators to various combinations of floral attractants and a synthetic cucumber beetle pheromone. Specifically, we sought to compare the attraction of cucumber beetles and pollinators to the combined floral compounds (TIC), indole in isolation (I), and vittatalactone (P). Additionally, we aimed to test the effect on the attraction of cucumber beetles and/or pollinators when these floral lures were combined with vittatalactone. Finally, we sought to determine if season of use (spring versus fall) affects the attractiveness of these lures.

Materials and Methods

We conducted a two-year field study to assess the attractiveness of various combinations of synthetic floral compounds and the W. striped CB aggregation pheromone to both western species of cucumber beetles (W. striped CB and W. spotted CB). We also evaluated pollinator attraction, with honeybees (*Apis mellifera*) being the primary pollinator.

Site

The trials were conducted at an organic farm near Esparto, CA (38°45'27” N, 122°01'22” W). This farm is a diversified operation growing cucurbits, brassicas, and solanaceous crops, surrounded by neighboring nut orchards and alfalfa fields. All trials took place in close proximity to fields planted with cucurbits. In the spring and fall of 2022, the traps were located on the western side of the farm. In 2023, the location of the traps was shifted to another area of fields on the eastern side of the farm due to crop rotation. The spring 2022 trial began on April 15 and lasted 8 weeks, ending on June 10. The fall 2022 trial began on September 16 and lasted 10 weeks, ending on November 25. The spring 2023 trial started on April 21, and lasted 10 weeks, ending on June 30. The fall 2023 trial began on September 18 and lasted 6 weeks, ending on October 30. The fall 2023 trial ended earlier than planned due to early disking of the cucurbit field and immediate planting of another crop, the management of which interfered with trap locations.

Treatments

We compared six different treatments that included both floral volatile compounds and the aggregation pheromone vittatalactone. The treatments were as follows: a full floral lure containing trimethoxybenzene, indole, and cinnamaldehyde (TIC); indole alone (I); the aggregation pheromone vittatalactone (P); a combination of TIC and vittatalactone (TIC+P); a combination of indole and vittatalactone (I+P); and an untreated control (Control). The vittatalactone lures consisted of a small gray septum onto which 1 mg of mixed vittatalactone was loaded. The full floral lures (TIC) were provided by Trece (lure #8274) (Figure 3). Lures were deployed in plastic packaging containing a corn grit substrate loaded with TIC. The container was pierced in an "X" fashion to allow the scents to volatilize. The indole lure was similar to the above except it contained indole alone.

Figure 3
Trimethoxybenzene, indole, cinnamaldehyde (TIC) lure in packaging

Traps

We paired clear double-sided sticky traps (23cm × 30cm Pherocon trap, Trécé Inc. Adair OK, USA) with lures to assess attraction. We attached sticky traps approximately 30 cm off the ground to 1m wooden stake with staples. The lure was attached to the side of the stake ~10 cm from the top of the sticky trap, suspended using a small metal binder clip. (Figure 4)

Figure 4
Indole + pheromone lure with sticky trap

Study Design

Traps were set up in a randomized complete block design with six treatments per block and six blocks. Blocks were spaced 50 m from each other and traps were separated by 10 m. We collected and replaced the traps weekly rerandomizing treatment locations within a block each week. We replaced lures every two weeks. We covered collected sticky traps with clear plastic wrap for transport to the laboratory. The collected traps were then stored at -20°C until counting. On each trap we counted the number of W. striped CB, the number of W. spotted CB, and the number of bees.

Statistical Analysis

All statistical analyses were conducted using R statistical software (version4.4.1. R Core Team, 2024) Counts of W. striped CB, W. spotted CB, and honeybees were each analyzed separately using linear mixed-effects models. The models were constructed using the lmer function from the lme4 package (Bates et al., 2015), with Treatment, Season, and Year included as fixed factors, along with their second- and third-order interactions. Block was treated as a random effect to account for the variability among different blocks. Due to the low counts observed in some cases, counts for each species were aggregated over the duration of trap deployment for each season to ensure sufficient data for analysis. These aggregated counts were then square root transformed to satisfy the assumptions of normality and homogeneity of variance. Model fit was assessed through diagnostic plots, including residuals and scale-location plots, to ensure the assumptions of the models were met. Model effects were evaluated using Type III Wald χ^2 tests with the Anova function from the car package (Fox, J., Weisberg, S. 2019), which provided p-values adjusted for the appropriate degrees of freedom. The emmeans package was used to explore significant interactions, conduct interaction contrasts and simple effect analyses, and generate back-transformed marginal means that were used for interpretation. In addition, planned contrasts conducted with the emmeans package were used following these analyses to test for synergistic effects compared to additive effects for the combination treatments by comparing the measured response of each combination to the sum of the responses of the individual components. Post-hoc pairwise comparisons among means were conducted where relevant using the CLD function within the emmeans package (Lenth, R. V. 2021), with p-values adjusted for multiple comparisons using the False Discovery Rate (FDR) correction method to control for Type I errors.

Results

Our results demonstrate that combinations of floral volatiles and aggregation pheromone lures (TIC+P and I+P) exhibit enhanced attraction for W. striped CB and W. spotted CB, with significant synergistic effects observed primarily in the spring season. While the treatments containing TIC effectively attracted both cucumber beetle species, they also were highly attractive to honeybees. In contrast, the indole and indole-pheromone treatments remained attractive to both species of cucumber beetles, while not attracting pollinators.

W. striped CB The three-way interaction between Treatment, Season, and Year was not significant for W. striped CB (Table 2). There was a significant interaction between Treatment and Season, but not Treatment and Year, and a marginally significant interaction between Season and Year (Table 2). The interaction between Treatment and Season was due in part to relatively minor differences in how attractive the different treatments were between spring and fall trapping periods and there was still a significant effect of Treatment within each season based on posthoc comparisons (Spring: $F_{\text{ratio}} = 49.80$, DF = 5, P < 0.001; Fall: $F_{\text{ratio}} = 26.13$, DF = 5, P < 0.001).

Overall, the relative order of treatment attractiveness to W. striped CB stayed the same, but the relative magnitude of attraction to the different treatments varied as evidenced by the significant Treatment × Season interaction (Figure 5). For the individual treatments, the aggregation pheromone was consistently very attractive across both seasons (the most attractive of the individual treatments, although the pheromone treatment was not statistically greater than indole in the fall). The TIC lure and Indole lure were less attractive and exhibited some slight seasonal variation. The TIC and Indole lures caught slightly more W. striped CB in the fall compared to spring. Although the Indole lure did attract W. striped CB across sampling periods, its performance appeared variable among weeks, especially in spring of 2023 where in some later weeks (weeks 5-10) it appeared comparable to the background capture rate observed in the control traps (no attractant) (Figure 1).

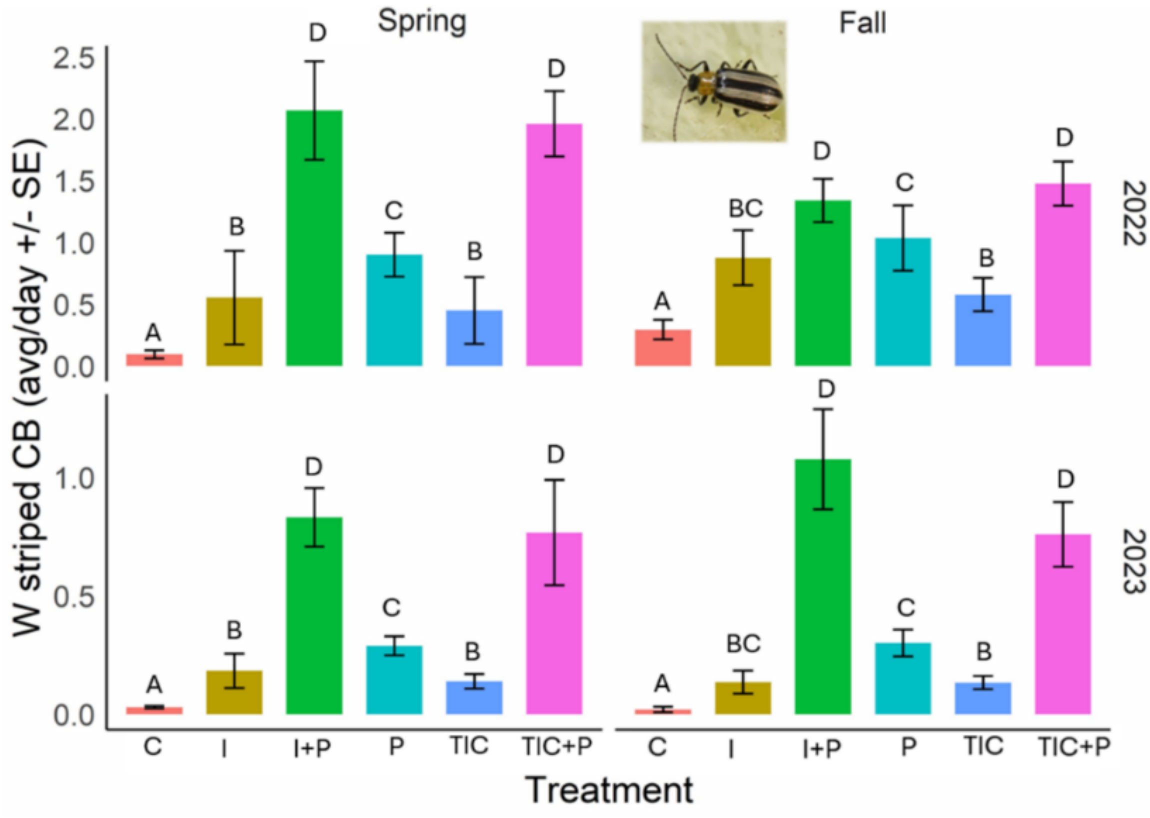

Figure 5. Captures of Western striped cucumber beetles (average daily capture) over an entire sampling period (spring/fall) for various attractants across two years(C = control, I = indole, P = pheromone, TIC = full floral lure, I + P = indole and pheromone, TIC + P = full floral lure and pheromone). Values are means across replicates ± 1 SE. Means not sharing a letter are

More pronounced seasonal differences were observed for the two combined treatments (TIC+P and I+P), which also attracted substantially more W. striped CB relative to the individual attractants in both seasons and years (Figure 1). The Treatment × Season interaction seemed to stem in large part from how the effect of the combined treatments differed between spring and fall for the magnitude of differences. In spring, traps baited with TIC+P and I+P caught 3.2 and 3.4-fold more beetles respectively than the average of each of their components (TIC/I and P). In the fall, this decreased to 2.4 and 2.3-fold more beetles. When comparing the combined treatments to their predicted additive effects, some interesting patterns emerged for W. striped CB. There was an overall synergistic effect of combining both TIC and indole with pheromone across years in the spring (TIC+P: 1.6× more than additive; I+P: 1.7× more). There were somewhat greater synergistic effects for both TIC+P and I+P in spring 2022 compared to spring 2023. However, this synergism was nonexistent across years in the fall with effects instead merely additive, although the combination treatments still caught more beetles than the individual treatments as noted.

W. spotted CB

For W. spotted CB, a significant three-way interaction effect was observed (Treatment × Season × Year) (Table 2). Posthoc comparisons revealed that the significance of the Treatment × Season interaction varied by year. In 2022, this interaction was significant (F_{ratio} = 4.9, DF1 =5 DF 2 = 100, P = <0.001) as a result of different treatment effects between spring and fall with more pronounced differences in the spring. In the spring of 2022, the Control treatment attracted the least amount of W. spotted CB, as expected (Figure 6). TIC performed better than the Control treatment, and Indole was intermediate to both TIC and the Control and not statistically different than either. The Pheromone treatment outperformed both Indole and Control. The combination treatments of TIC+P and I+P were significantly more attractive than any of the individual treatments (TIC+P: 3.5× more attractive than components, I+P: 4.1× more attractive) and were not statistically different from each other. There was a strong synergistic effect during this period for the combination treatments, with each of them greatly exceeding the sum of their individual components (TIC+P: 1.8× more than additive effect, I+P: 2.0× more than additive).

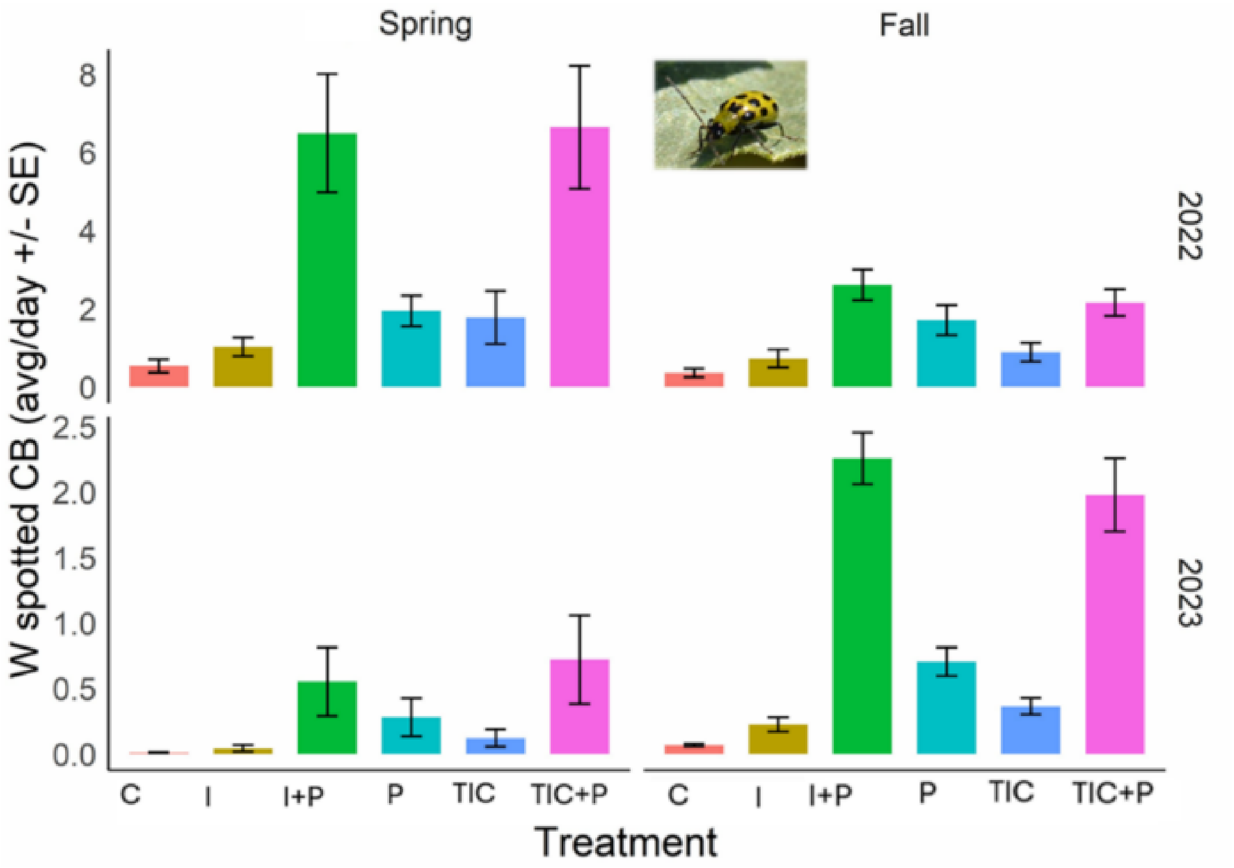

Figure 6. Captures of Western spotted cucumber beetles (average daily capture) over an entire sampling period (spring/fall) for various attractants across two years (C = control, I = indole, P = pheromone, TIC = full floral lure, I + P = indole and pheromone, TIC + P = full floral lure

and pheromone). Values are means across replicates ± 1 SE. Given the significant Treatment × Season × Year interaction, pairwise comparisons are not shown.

In the fall of 2022, the results followed a similar pattern to the spring, with a few very key differences. The individual compounds performed as above, except in this case, the Pheromone treatment performed statistically better than TIC, and it was as effective as the TIC+P combination treatment. However, the combined treatments were much less attractive in the fall compared to the spring. The Indole + P and TIC+P both generally outperformed the individual treatments (TIC+P: 1.8× more attractive than components, I+P: 2.2× more attractive), but of the two, the TIC+P was not different than the pheromone-only treatment. The combined treatments each had additive effects (Figure 2). Relative to the spring, the TIC+P was much less attractive in the fall.

In 2023, the Treatment × Season interaction was not significant ($F_{5,100}$ = .79, P = 0.6), indicating that treatment effects were more consistent across the seasons of 2023. Across seasons, the indole treatment was no different than the control. The TIC captured the next highest number of W. spotted CB, followed by the pheromone treatment. The TIC+P and I+P treatments both caught the most beetles in absolute terms (TIC+P: 1.8× more attractive than components, I+P: 2.2× more attractive). In addition, there were synergistic effects relative to the individual treatments (TIC+P: 1.8× more than additive effect, I+P: 2.3× more than additive).

Posthoc comparisons for the Treatment × Year interactions revealed that the significance of this interaction varied by season. The interaction was significant, and the effect of treatment varied by year in the spring ($F_{1,100}$ = 8.5, , P < 0.001), (Table 2). In spring of 2022, the combination treatments (TIC+P and I+P) were much more attractive than the individual treatments (same as reported for Treatment × Season; TIC+P : 3.5× > than components, I+P: 4.1×; TIC+P: 1.8× > than additive, I+P: 2.0× > than additive). During the Spring of 2023, although the combination treatments generally still outperformed the individual floral components, (TIC+P: 3.7× more attractive than components, I+P: 3.8× more attractive). they did not significantly outperform the P treatment, although the general trend was the same. Similarly, there was no synergistic effect for either combination, although the trends were similar to those in spring 2022.

In contrast, the Treatment × Year interaction was not significant for fall, indicating a more stable pattern of attraction to the treatments across years ($F_{1, 100} = 0.74$, $P = 0.60$). Across years, the Indole and TIC treatments captured more W. spotted CB than the control. The pheromone treatment captured more than both Indole and TIC, which were not statistically different from each other. The combination treatments both caught the most beetles (TIC+P: 2.3× more attractive than components, I+P: 3.1× more attractive) and were not statistically different. The two combination treatments behaved differently with regards to synergistic effect, the I+P treatment displaying a synergistic response and increase over expected captures based on the individual treatments (1.5× more than additive), while the TIC+P effect was merely additive.

Honeybees

Honeybees were primarily attracted to the TIC and TIC+P lures, but not much else (Figure 7). There was no significant three-way interaction (Treatment × Season × Year) for honeybees (Table 2); however, all two-way interactions were significant: Treatment × Season, Treatment × Year, and Season × Year (Table 2). For the Treatment × Season interaction, the effect of treatment was significant within both the spring season ($F_{5,100} = 55.12$,, $P < 0.001$) and in the fall season. ($F_{5,100} = 22.5$, $P < 0.001$. Across 2022 and 2023, the TIC and TIC+P treatments attracted the highest number of honeybees compared to all other treatments, which were not statistically different from each other, and the TIC and TIC+P were also no different from each other. Although the trends in treatments remained consistent across seasons, the difference between the seasons for how treatment influenced honeybees appeared largely due to the higher captures for the TIC and TIC+P treatments relative to the remaining treatments in spring compared to fall. In the Treatment × Year interaction, the effect of treatment was significant in both 2022 ($F_{5,100} = 56.3$, $P < 0.001$) and 2023 ($F_{5,100} = 21.7$, $P < 0.001$). The TIC and TIC+P treatments again caught more honeybees than all of the other treatments, which were themselves not statistically different from each other.

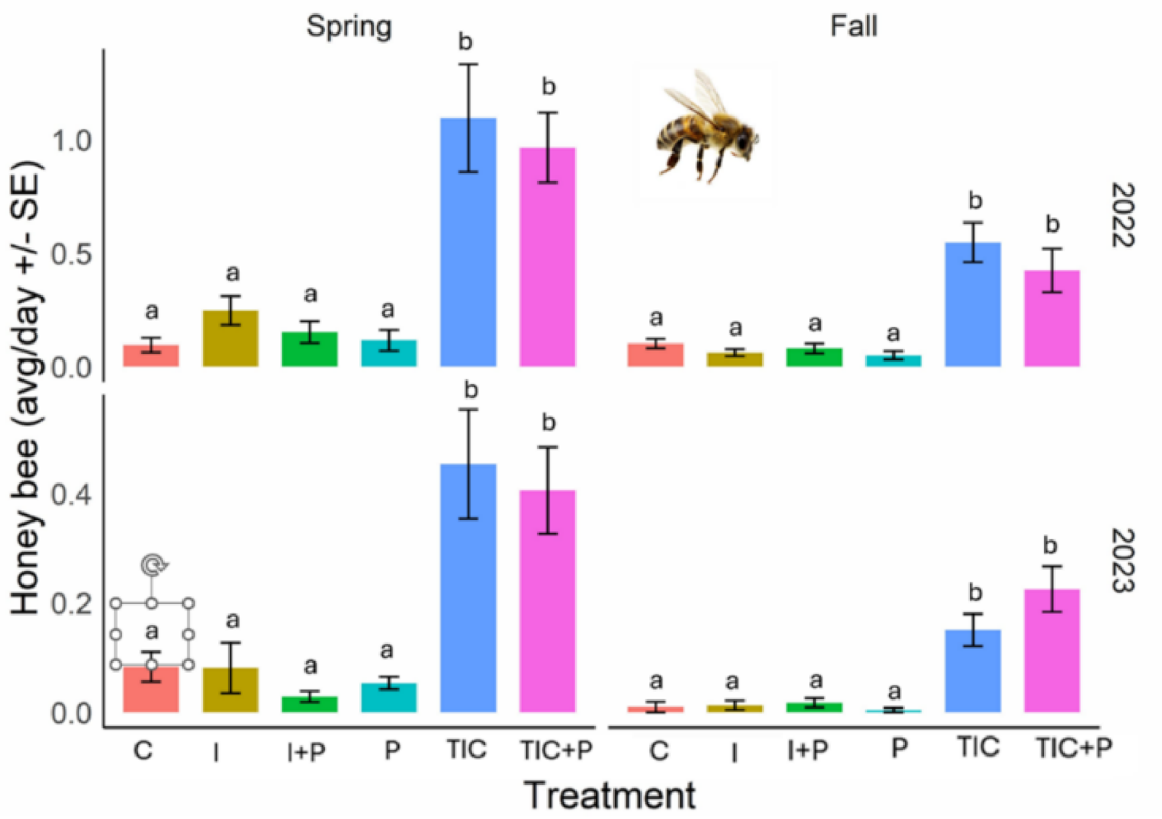

Figure 7. Captures of honeybees (average daily capture) over an entire sampling period (spring/fall) for various attractants across two years (C = control, I = indole, P = pheromone, TIC = full floral lure, I + P = indole and pheromone, TIC + P = full floral lure and pheromone). Values are means across replicates ± 1 SE. Means not sharing a letter are significantly different

based on post-hoc comparisons at α = 0.05. Both Treatment × Season and Treatment × Year interactions were significant, although results of pairwise comparisons produced identical results and are therefore not shown.

Weekly Captures

Temporal patterns in weekly captures across the study highlight seasonal fluctuations in attraction for all three species. Capture rates varied by season and year, with notable peaks aligning with beetle emergence and field disking.

W. striped CB

In 2022, captures of W. striped CB were highest during the first several weeks of the spring trial and then gradually tapered off (Figure 8). The fall of 2022 showed a pronounced spike in captures toward the end of the season. In 2023, captures in the spring exhibited two peaks, a smaller initial peak, followed by a larger spike, which gradually tapered off as the season progressed. The fall of 2023 displayed a similar pattern, with low initial captures, followed by a spike as the field was disced, although the following farm operations truncated the sampling period (Figure 8).

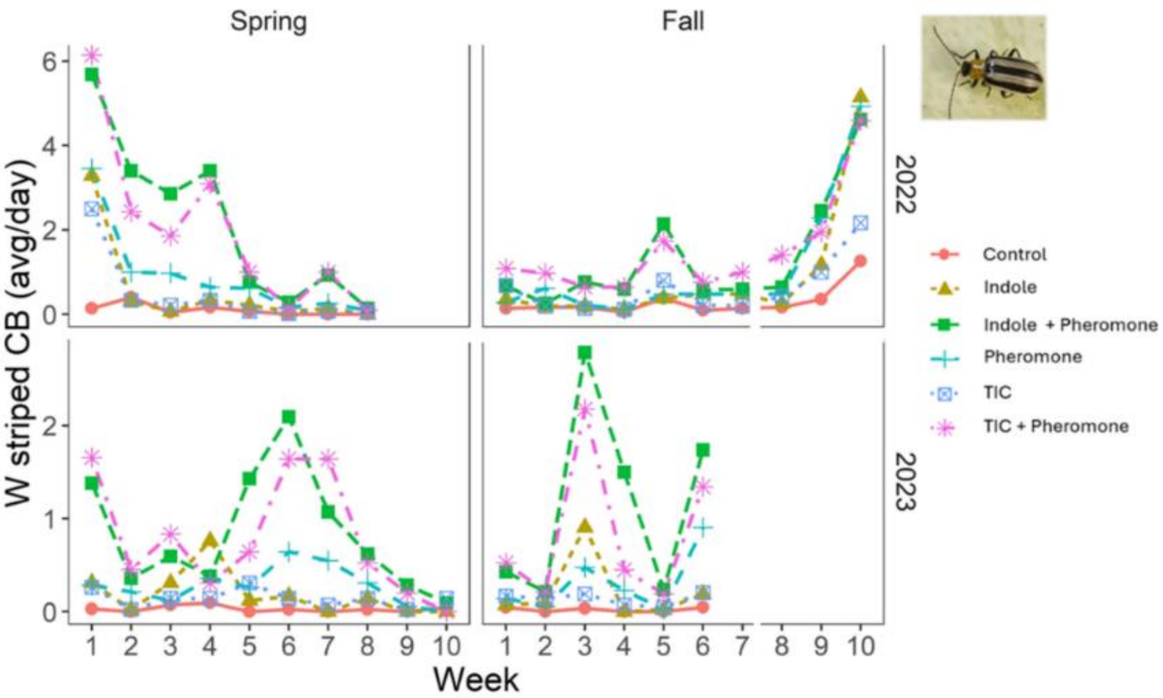

Figure 8. Captures of Western striped cucumber beetles (average daily capture) through time for various attractants for spring and fall sampling periods across two years . Values are means across replicates and weeks are the week of the sampling period. Trials started on Apr 15 and Sep 16 in 2022, and Apr 21 and Sep 18 in 2023. The nearby cucurbit fields were disked in the fall, (during week 9 in 2022 and week 3 in 2023). Weekly data were not analyzed, so no statistical results are shown

W. spotted CB

In spring 2022, W. spotted CB were already active when sampling began, leading to relatively high capture rates in week 1; conversely, 2023 had several weeks of near-zero captures, likely due to sampling starting before the beetles' initial dispersal into the field (Figure 9). Overall captures were higher in 2022 compared to 2023, both in terms of daily capture rates and during capture peaks, although this was most pronounced for the spring sampling period. Fall 2022 captures were lower and less variable than in the spring, whereas fall 2023 captures were higher than the spring and were more variable, particularly for certain treatments (Figure 9)

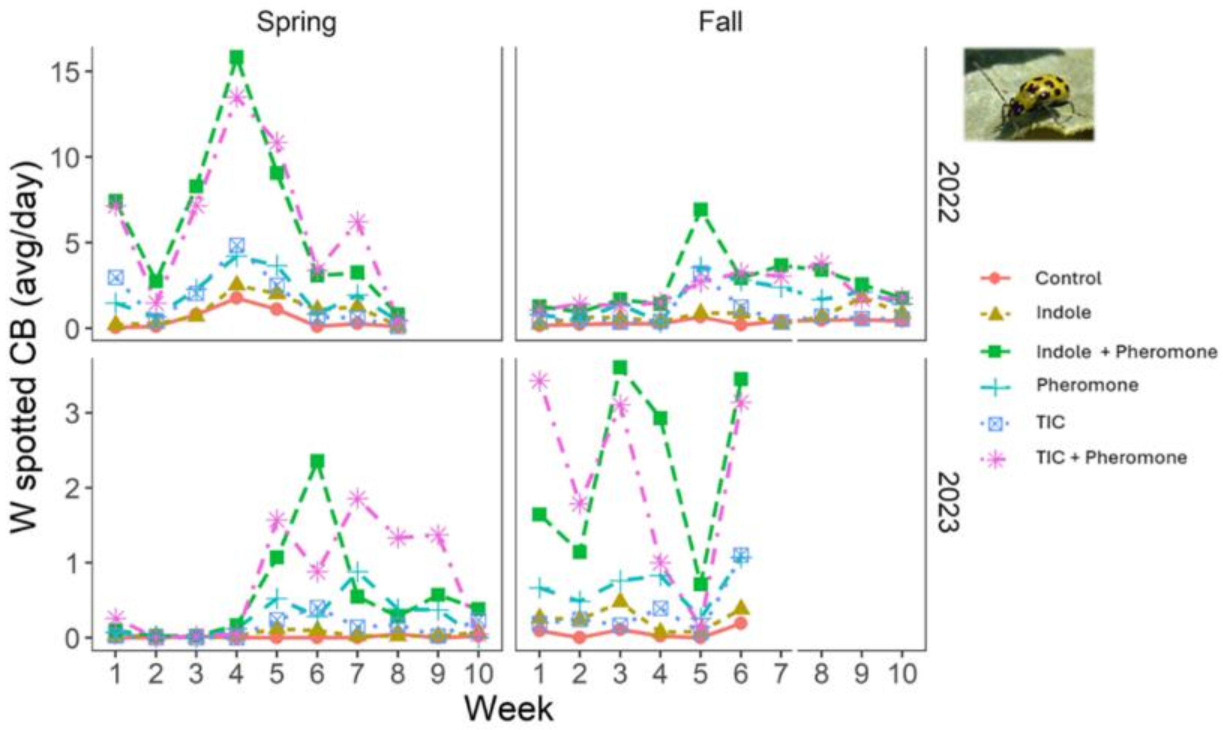

Figure 9. Captures of Western spotted cucumber beetles (average daily capture) through time for various attractants for spring and fall sampling periods across two years. Values are means across replicates, and weeks are the week of the sampling period. Trials started on Apr 15 and Sep 16 in 2022, and Apr 21 and Sep 18 in 2023. The nearby cucurbit fields were disked in the fall, (during week 9 in 2022 and week 3 in 2023). Weekly data were not analyzed, so no statistical results are shown.

Honeybees

Honeybee captures generally increased through time for the spring period and then were overall lower and slowly decreased through time for the fall period (Figure 10). There were some marked spikes and increased captures of honeybees in the spring periods, although this was only for the specific treatments that were attractive to any appreciable degree, TIC and TIC+P (Line Graph).

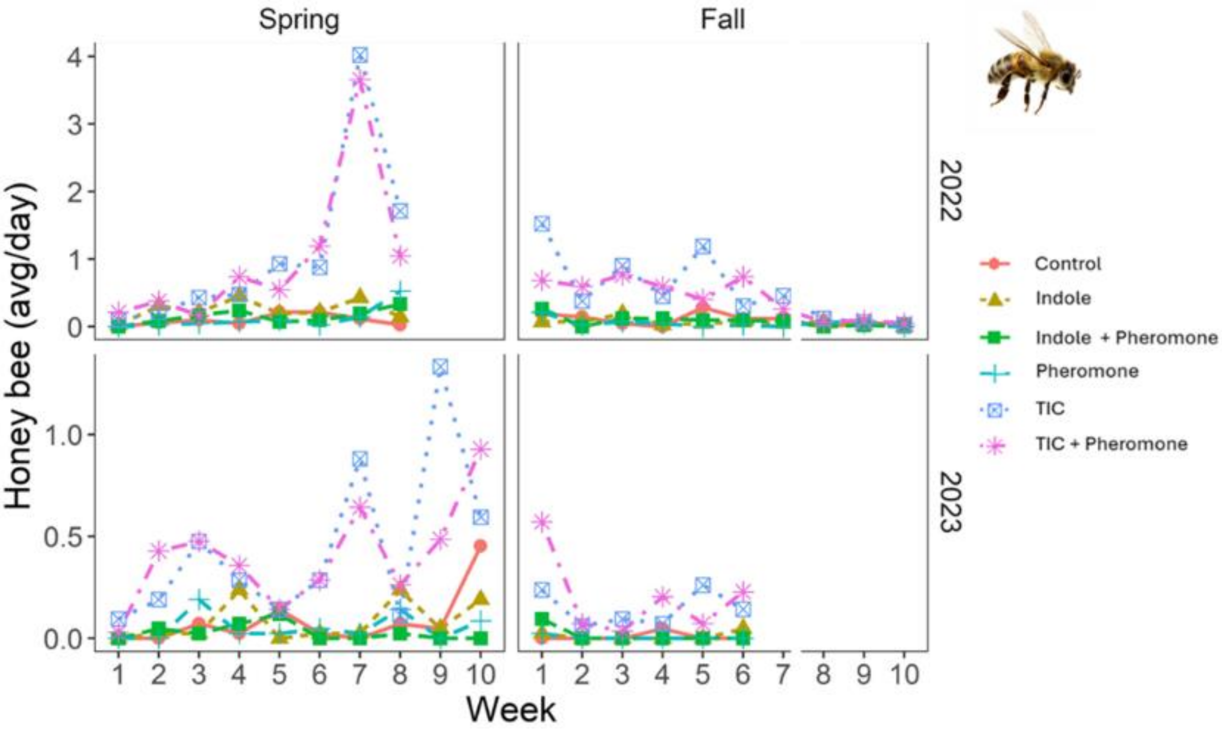

Figure 10. Captures of honeybees (average daily capture) through time for various attractants for spring and fall sampling periods across two years. Values are means across replicates, and weeks are the week of the sampling period. Trials started on Apr 15 and Sep 16 in 2022, and Apr 21 and Sep 18 in 2023. The nearby cucurbit fields were disked in the fall, (during week 9 in 2022 and week 3 in 2023). Weekly data were not analyzed, so no statistical results are shown.

Discussion

Interactions in Cucumber Beetle and Honeybee Attraction

A key finding of our study is the differential attraction of cucumber beetles and honeybees to synthetic lures. Both W. striped CB and W. spotted CB were strongly attracted to vittatalactone, indole, and TIC, while honeybees were only attracted to TIC-based lures. This selective attraction highlights a valuable opportunity for IPM: by using indole and pheromone-based lures, we can effectively target cucumber beetles while minimizing the risk of honeybee by-catch. This could help address one of the primary challenges in IPM—reducing non-target effects while maintaining strong pest control. Additionally, we found that the response of W. striped CB and W. spotted CB to plant volatiles and vittatalactone varied, with notable differences in their attraction depending on the season and year. We believe patterns emerged that may be leveraged to develop more effective IPM strategies for cucurbit pests while still limiting non-target effects.

Western striped cucumber beetle

Western striped CB was attracted to both floral scent-based lures and pheromone lures, with combination treatments increasing attraction in part due to synergistic effects. However, there was also seasonal variability in how they responded to the tested attractants. Both floral scent-based lures (TIC and Indole) were attractive, and not differently so, meaning the simpler indole-only lure could be sufficient as an attractant relative to a lure with more components. However, the pheromone tended to be more attractive overall. The relative attractiveness of the floral volatiles and pheromone was likely dependent on the tested rate given that only one rate for each was tested. Different rates could change the order of these treatments. Overall, the Indole-containing treatments (both alone and in combination with pheromone) were not different from the TIC-containing treatments, so a reduced number of attractants could be used.

The interactive effect for W. striped CB between season and the tested attractants was mostly driven by a variation in the overall magnitude of attraction of some treatments relative to the others and not changes in the overall patterns of attraction and rankings of treatments. Indeed, what seems to have driven why the effect of treatment depended on season for W. striped CB is the presence or absence of a synergistic effect for the combination treatments. In spring across years, the combination treatments (TIC+P and I+P) exhibited synergism, most strongly in 2023; however, the combination treatments only had additive effects across years in the fall. This pattern may be attributed to several moderating factors. For instance, seasonal changes in W. striped CB behavior likely played a significant role. During the spring, beetles are more actively foraging for food, especially if they recently came from overwintering areas and thus may be more responsive to plant volatiles. Their search for other beetles, both for mate and host-finding, could increase their responsiveness to vittatalactone. The combined signals from multiple attractants may have created higher levels of synergism observed during the spring. In contrast, by fall, their focus shifts towards finding overwintering sites, reducing their interest in feeding and thus the possibility of increased behavioral responses for combinations of plant volatiles and pheromones. Crop phenology could also contribute to seasonal differences in attraction for W. striped CB. In the spring, the early growth stages of cucurbits mean fewer competing volatiles, which could enhance the lures' effectiveness.

Although there was some variability in how W. striped CB responded to the lures, this work demonstrates the generally consistent attractiveness of the individual synthetic floral lures (both TIC and Indole alone), vittatalactone, and their combinations to W. striped CB, aligning with prior work on W. striped CB (Ramirez Bonilla, 2022) and its eastern congener, *A. vittatum* (Weber, 2023). Importantly, the general ranking of treatments remained relatively consistent, especially vittatalactone, and the combo treatments demonstrating synergism, especially in spring, when grower concern over W. striped CB is at its highest.

Western spotted cucumber beetle

Western spotted CB was attracted to the lures in a pattern similar to W. striped CB, but with several key differences. Like W. striped CB, there were consistencies in the ranking of attraction of spotted to the combo treatments, but the attraction to the other treatments sometimes varied based on the season or year indicating a slightly more complex dynamic in their attraction patterns. One major distinction was in the seasonal shift in performance of the combination treatments, although that also depended on year of the study. In addition, there were sometimes consistent effects across years for a given sampling season, but not for others. The largest effect of the combination treatments was in spring 2022 there were strong attraction to these treatments, with many beetles caught, and synergism between the combination of floral and pheromone attractants. However, that fall, the synergism of the lures disappeared, and the combined treatments were attractive in a merely additive manner. In 2023, captures were lower overall compared to 2022, and the combined treatments were synergistically attractive across seasons. In terms of consistency of effect across years, the effects did vary across years for spring, but were more consistent for fall, although in this case both combinations were the most attractive, synergism was apparent only for the combination involving indole, not the full component mixture.

The intricacies of how season and year affected W. spotted CB's attraction to the treatments and how they responded to the lures are more complex than for W. striped CB. They do not seem to be solely driven by shifts in magnitude of capture, but by variation in the effect of treatment as well. The floral lures, especially Indole, do not seem to be much more attractive than the control treatments in most of the trials. Meanwhile, vittatalactone remained consistently attractive, although its addition to the floral lures increased attraction, but not always in a consistent way. Nevertheless, like for W. striped CB, the attraction of W spotted CB to the combined lures in particular, and their comparable attraction to indole combined with a pheromone as well as the full TIC lure, is sufficient to be exploited in an IPM strategy and demonstrates the potential for a multi-species approach.

In addition, the synergism seen in attraction of W. spotted CB to the floral lures combined with vittatalactone is especially interesting in light of the fact that the pheromone is exclusively produced by male W. striped CB. This introduces ecological and evolutionary complexities that remain to be investigated. Vittatalactone's role in aggregating female and male W. striped CB has been studied before (Weber, 2023), However, the cross-species attraction observed in W. spotted CB is interesting in terms of potential overlap in chemical signaling or eavesdropping between these two species. One possibility is that W. spotted CB has evolved the ability to exploit this pheromone as a cue within shared agricultural habitats, particularly in areas where both species coexist. The shared environment may have driven W. spotted CB to use vittatalactone as an indirect indicator of resource availability, and this could be a form of opportunism, where W. spotted CB benefits from the presence of W. striped CB by aggregating in areas that are rich in food. Thus, vittatalactone could be leveraged in the development of multi-species traps, especially when combined with the appropriate synergistic floral compounds, simplifying pest management strategies by allowing a single combination of attractants to simultaneously monitor and manage populations of both W. striped CB and W. spotted CB.

Honeybees

The response of honeybees to lures remained generally consistent across both seasons and years, with TIC and TIC+P consistently attracting more honeybees than any other treatments. There were interactions between season and treatment, and year and treatment, but the overall pattern of attraction remained consistent. As with the cucumber beetles, the differences in nearby crop and non-crop host development related to trap deployment timing and may have influenced honeybee foraging behavior, contributing to the observed variability in captures. Nevertheless, the fact that all treatments containing TIC were highly attractive to bees, and that Indole and vittatalactone containing treatments were no different than the controls (while being highly attractive to beetles) demonstrates that Indole-based lures are a safe option and can help mitigate non-target effects of any uses of attractants for cucumber beetle management.

Implications for IPM

The strong attraction of W. striped CB and W. spotted CB to both the aggregation pheromone, the individual floral volatiles Indole and TIC, along with the synergistic effects of their combination, presents a valuable opportunity for developing IPM strategies for cucurbit growers in California. The lack of attraction to the pheromone and Indole increases the potential of these compounds, making them suitable for developing targeted, pest-specific attractants that can be used in both conventional and organic systems, where minimizing harm to beneficial insects is a priority. The fluctuations in Indole’s attraction and the inconsistent presence of synergism in the combination treatments underscore the importance of carefully timing the deployment of these tools (such as in spring, when synergism was consistently seen in the combo treatments) to maximize their effectiveness. Several potential IPM strategies can be derived from these findings.

Early-Season Monitoring and Intervention

Early-season deployment of lures could be particularly advantageous when beetles are emerging and actively seeking food and mates (Metcalf & Lampman, 1989). During this period, the reduced presence of competing natural volatiles from plants and insects likely enhances the detectability of synthetic lures (Dicke & Baldwin, 2010), and this is when we saw the highest levels of synergism in our study. These lures could serve as valuable tools for monitoring population levels and the timing of their movement into agricultural fields after they emerge from overwintering sites in the spring and then seek out crop fields. This could then inform the timing and necessity of insecticide applications, potentially reducing the overall chemical load on fields. Unfortunately, the attractants do not seem to be very effective during bloom periods, when they would be of great use to detect new movement of beetles into fields or to assess population levels.

Attract-and-Kill Strategy

Another promising management strategy is integrating these lures into an "attract-and-kill" approach, which could be employed in a few different ways by leveraging the often high levels of attraction, sometimes through synergism, of the indole and pheromone treatment for both cucumber beetle species and its lack of pollinator attraction. The attract-and-kill strategy encompasses multiple management techniques facilitated by luring pests into areas where they can be effectively controlled . Although ATK includes traditional mass trapping, where pests are killed in lethal traps, this is only one possible application of the approach, and these lures can be adapted to utilize ATK strategies such as localized area targeting or enhancing trap-crop systems. In this study, our use of small sticky traps yielded relatively low capture rates, which suggests practical limitations in this particular setup. Different types of traps vary in their propensity of capturing the two cucumber beetle species (Rijal & Gyawaly, 2023) and traps have not been optimized for this management approach. However, employing larger sticky traps, or a different, more robust trap type may yield improved results.

Lethal traps are not the sole focus within ATK; combining lures with specific field management tactics could also be highly effective. For instance, focusing on beetle “hot spots” like along field edges, or within a designated zone early in the season, lures could draw beetles away from key crop areas, and growers could treat a smaller area rather than the entire field, lowering inputs, and limiting non-target effects.

Another way to use the lures could be as a supplement to a well-performing trap crop, Blue Hubbard squash, known for its strong attraction to cucumber beetles. This sometimes variety sometimes serves as an effective trap crop positioned away from the main cucurbit fields. Supplementing this trap crop with indole and pheromone lures early in the season, could further "pull" and concentrate the first generation of beetles in this specific trap crop area, sparing the main crop from infestations. Targeted pesticide applications could then be used solely in the trap crop. Since beetles are multi-generational in the field, reducing the early colonizing population with a trap crop could lower infestation levels, alleviating beetle pressures for the entire growing season. In addition, the cucurbit fruit is most susceptible to damage when young, so even a small delay in early season damage could be significant and limiting it could lead to lower culls.

In another study, we looked at combining gustatory stimulants , such as CidetrakL, with insecticides. Incorporating attractants into an attractant-gustatory stimulant-insecticide combo treatment could offer an effective attract-and-kill solution by luring beetles to small, lethal "feeding stations" throughout the field. Such stations could be deployed within or around the field to draw in and kill beetles before the cucurbit plants flower, reducing the need for broad insecticide applications and sparing beneficial insects.

The spring period appears to be the most promising time for an attract and kill approach given the inconsistencies seen in fall seasons. Given the reduced lure attractiveness seen mid-season when there are lots of volatiles in the field (Ramirez Bonilla, 2022), attract and kill likely would provide limited benefits during this time, but there may be opportunities later in the season once the fields are disked. As W. striped CB are cucurbit specialists and appear to overwinter very near prior year’s cucurbit fields, an ATK strategy implemented during fall could help reduce overwintering beetle populations, thereby reducing the population that reemerges in the spring and lowering the initial pressure on crops, especially in CA where non-crop cucurbit hosts are limited in the agricultural landscape. This may prove more difficult for W. spotted C, which as a generalist, may move to weed hosts or migrate to other nearby crops instead of overwintering.

Although these lures have proven to be effective and there are many creative ways to employ them, it is crucial to consider factors such as pest behavior, crop phenology, and environmental conditions when planning deployment to maximize their utility in IPM strategies. This study provides an exploration into how two key pests respond to different types of attractants and how they could be exploited for selective management approaches. This work also identifies seasonal and yearly variation in the responses of these pests to these attractants and may allow us to better exploit their behavior for IPM. Developing a better understanding of the nuances of these compounds such as concentration, their interactions with varying climatic conditions, and their limitations is key to developing them into management effective tools.

Tables

Table 1. Synthetic floral compounds, synthetic aggregation pheromone, and their combinations paired tested for cucumber beetle and honeybee attraction.

Treatment number	Treatment	Compound
1	Control	-
2	Pheromone	Vittatalactone

3	TIC	Trimethoxybenzene, indole, cinnamaldehyde
4	Indole	Indole
5	TIC+P	Vittatalactone +TIC
6	I+ P	Vittatalactone + Indole

Table 2. Results of linear mixed-effects models analyzing the effects of Treatment, Season, Year, and their interactions on the capture of W. striped CB, W. spotted CB, and honeybees. Significant factors are bolded (α = 0.05).

Insect	Factor	DF	χ^2	*P*
W. striped CB	Treatment	5	226.49	**<0.001**
	Year	1	7.8	**0.001**
	Season	1	4.27	**0.043**
	Treatment × Season	5	15.48	**0.01**
	Treatment × Year	5	1.54	0.18
	Season × Year	1	3.84	**0.055**
	Treatment × Season × Year	5	5.99	0.32
W. spotted CB	Treatment	5	61.75	**<0.001**
	Year	1	8.58	**<0.006**
	Season	1	0.04	0.84
	Treatment × Season	5	4.93	**<.001**
	Treatment × Year	5	4.85	**<.001**
	Season × Year	1	0.26	0.61

	Treatment × Season × Year	5	4.39	**0.001**
Honeybee	Treatment	5	56.29	**<.001**
	Year	1	4.02	**0.047**
	Season	1	0.56	0.45
	Treatment × Season	5	2.32	**0.049**
	Treatment × Year	5	4.52	**<.001**
	Season × Year	1	4.68	**0.033**
	Treatment × Season × Year	5	1.06	0.39

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Chapter 2

Enhancing Insecticide Efficacy in Northern California Melons: An Evaluation of the Gustatory Stimulant CidetrakL in Integrated Pest Management for Cucumber Beetle Control

Introduction

In the ongoing evolutionary arms race between plants and insect herbivores, plants have developed an arsenal of chemical defenses to deter herbivory and protect themselves from damage. These compounds (secondary metabolites) are not directly involved in the basic metabolic processes of the plant, but they play a crucial role in the plant's defense strategy. (Becerra, Noge, & Venable, 2009). These chemicals can take many forms, including bitter-tasting compounds, toxic alkaloids, phenolic compounds, and terpenoids, each designed to deter herbivores in different ways. Some secondary metabolites are designed to taste unpleasant, discouraging insects from feeding on the plant (Becerra, Noge, & Venable, 2009). Others are far more potent, acting as toxins that can interfere with the nervous system, digestive processes, or other physiological functions of the insect, leading to illness or death. (Wink, 2003). Some compounds, like tannins, can reduce the digestibility of plant tissues, making them less nutritious or harder to consume (Despres 2007). Over time, these chemical defenses have evolved to target the specific feeding behaviors and physiological vulnerabilities of the plant's primary herbivores.

Just as plants have evolved these defenses, many insect herbivores have developed counterstrategies. Through natural selection, some insects have evolved the ability to detoxify or sequester harmful plant secondary compounds, allowing them to feed on plants that are toxic to other species (Després, David, & Gallet, 2007). In some cases, insects have even turned these plant defenses to their advantage, using sequestered toxins to defend themselves against their own predators. For examples monarch butterflies tolerate and sequester compounds in their bodies, providing the butterflies with a defense against predators (Malcolm & Brower, 1989). This co-evolutionary process results in a continuous cycle of adaptation and counter-adaptation, with both plants and insects constantly developing new strategies to outcompete the other, creating a dynamic relationship between plants and insects. (Ehrlich & Raven, 1964)

Diabroticene beetles, like corn rootworms and cucumber beetles, are known for their ability to feed on plants that produce toxic compounds as a defense. These beetles have evolved mechanisms to detoxify or tolerate these compounds, allowing them to exploit a niche that is inaccessible to many other herbivores (Metcalf & Lampman, 1989). Striped cucumber beetles, including the eastern (*Acalymma vittatum*) and western species (*Acalymma trivittatum*), (E. striped CB and W. striped CB hereafter) specialize in feeding on cucurbits and have evolved a tolerance to cucurbitacins, bitter compounds that deter most herbivores. Both beetles not only tolerate these compounds, but also use them as gustatory stimulants, which enhance their feeding on cucurbit crops (Metcalf et al., 1980).

Cucurbitacins have been studied and leveraged for managing a variety of Diabroticene beetles across different crops, such as the Spotted cucumber beetle (*Diabrotica undecimpunctata howardi*) (Spotted CB) and the Corn rootworm (*Diabrotica virgifera*) in several different crops like maize and peanuts. One notable example is SLAM, a cucurbitacin-containing bait formulation combined with a low dose of insecticide, which has been effective in reducing beetle populations while minimizing impact on beneficial insects, such as ladybeetles and lacewings (Lewis 2005) (Pingel 2001).

These cucurbitacin-based products are often integrated with other pest management strategies, such as biological control agents like entomopathogenic nematodes or fungi, targeting multiple life stages of the beetles simultaneously (Jackson 2006). Some efforts have focused on combining them with organic-friendly insecticides to expand the tools organic growers can use to control Cucumber beetles. One promising avenue is combining cucurbitacins with Spinosad; a material derived from soil bacteria (*Saccharopolyspora spinosa*). It is approved for use in organic agriculture and has shown potential when combined with cucurbitacins to target striped cucumber beetles more effectively. Laboratory bioassays have demonstrated that cucurbitacins significantly lower the LC-50, by over 75% (Lewis 2015). Unfortunately, these results were not repeatable in the field, and Spinosad seems to be much less effective under field conditions, perhaps due to UV breakdown (Peterson 2009). Nevertheless, given the similarities in physiology and feeding behavior between corn rootworms, Spotted CBs, and Striped CBs, this approach could be adapted for more effective management of W. striped CB in California cucurbit production

In California, the management of W. striped CB is crucial due to the substantial economic damage they inflict on melon crops (Aegerter et al., n.d.). Chemical control, particularly the use of noenicitinoids, has been the predominantly relied upon method for managing these pests (Haber et al., 2021). Neonicotinoids are effective at controlling beetle populations due to their systemic properties, which allow the chemical to be absorbed and distributed throughout the plant targeting both the floral and foliar feeding adult beetles and their root-feeding larvae (Jeschke et al., 2011). As neonicotinoids are systemic, their presence in pollen and nectar poses significant threats to non-target beneficials and pollinating insects, which are vital for the pollination of many crops, including melons (Godfray et al., 2014). The frequent use of these chemicals has raised concerns about their sustainability. (Goulson, 2013). Over-reliance on limited classes of insecticides not only increases the risk of insecticide resistance development among pest populations, but it also exacerbates the negative environmental impacts associated with these chemicals. (Goulson, 2013)

In recent years, there has been growing scrutiny from regulatory bodies both in California and at the federal level concerning the use of neonicotinoids. The California Department of Pesticide Regulation has conducted a thorough review of neonicotinoid insecticides, focusing specifically on four active ingredients: imidacloprid, clothianidin, thiamethoxam, and dinotefuran, due to their potential environmental impacts, particularly concerning pollinator health. As part of these reviews, new regulations have been introduced that restrict foliar applications of these nitroguanidine neonicotinoids to mitigate risks to pollinators and other non-target species, including limitations on application rates, timing, and methods of application, especially during periods when crops are in bloom or when managed pollinators are present (Simon-Delso et al., 2015). Meanwhile, the Environmental Protection Agency has been reassessing the use of neonicotinoids under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, 1996).

Acetamiprid, the active ingredient in Assail, is less persistent in the environment compared to other neonicotinoids, breaking down more quickly and having less propensity to accumulate in the soil or water. Its shorter half-life reduces the risk of long-term exposure to non-target organisms, and its lower acute toxicity to pollinators such as bees, makes it less likely to be lethal at low levels of exposure. This combination of lower environmental persistence and reduced toxicity makes acetamiprid a more favorable option within the neonicotinoid class, particularly in agricultural practices where pollinator health is a critical concern. As a result, it was not included in the group of neonicotinoids under increased restrictions in CA, and acetamiprid remains one of the primary chemical options used against cucumber beetles in California.

Pyrethroids are another class of insecticides employed in the management of Diabroticene beetles. Pyrethroids face similar environmental concerns as they are also broad-spectrum. More importantly, a key environmental concern associated with pyrethroids is their high toxicity to aquatic organisms, like fish and invertebrates, due to the lipophilic nature of pyrethroids, causing them to accumulate in the fatty tissues of aquatic life. This has raised regulatory concerns, particularly in regions where water bodies are in close proximity to agricultural areas (Weston & Lydy, 2010).

Organic growers face additional challenges due to the limited availability of effective materials for controlling cucumber beetles. Common organic materials include neem oil, pyrethrins, and Spinosad. These materials have their own limitations; neem oil, while useful as an insect repellent and growth regulator, requires frequent applications and can be less effective under high pest pressure (Isman, 2006). Pyrethrins and Spinosad degrade rapidly under UV exposure, reducing their efficacy (Sparks & Nauen, 2015).

The heavy reliance on a limited number of chemical options that are under increasing regulatory pressures, and the lack of effective options in organic systems, highlight the urgency of developing alternative pest management strategies for W. striped CB in California. Plant-derived compounds and attractants, such as cucurbitacins and floral volatiles, offer a promising pathway for optimization of IPM strategies. By leveraging these naturally occurring substances, it may be possible to enhance the efficacy of existing control methods while reducing the environmental impact associated with conventional insecticides. Cucurbitacins could be leveraged beyond their role as mere feeding stimulants. By combining cucurbitacins with synthetic floral attractants, it may be possible to integrate them into an attract-and-kill (ATK) strategy, drawing beetles toward bait stations or treated areas. This could also be paired with the use of trap crops, where cucurbitacin-laced plants act as decoys, concentrating beetle populations in designated areas of a field. These beetle-rich zones can then be treated more efficiently with insecticides, allowing for targeted applications and reducing the overall amount of chemical input needed.

In this study, we investigated the potential of integrating CidetrakL (CT), a cucurbitacin-based gustatory stimulant, with reduced rates of the insecticide Assail (acetamiprid) to enhance the control of cucumber beetles. Our hypothesis was that combining CidetrakL with lower rates of Assail would maintain high levels of pest control while mitigating some of the negative impacts associated with high pesticide usage. We also ran a preliminary study looking at the effect of gustatory stimulants combined with attractants on W striped CB behavior. To test this hypothesis, we conducted a series of field trials, laboratory bioassays, Y-tube olfactometer tests, and behavioral assays using Ethovision software. Using these field and lab-based tests, we attempted to evaluate the potential for leveraging CidetrakL to develop sustainable pest management practices that could reduce pesticide use and minimize harm to beneficial insects and pollinators.

Methods

Field Trial

We conducted a field trial to test how insecticides, specifically acetamiprid (Assail 30SG) could be combined with the gustatory stimulant CidetrakL (CT) (Trece Inc, Adair, Oklahoma,.) for increased efficacy when CT was added to the full rate of the insecticide, or if control levels could be maintained at a reduced rate of insecticide when combined with CT. In addition, we included indoxacarb (Avaunt), also combined with CT, to evaluate if indoxacarb could serve as a viable alternative to pyrethroids and neonicotinoids. Field trials were conducted at the UC Davis Armstrong Plant Pathology Research Farm (38°31'18.9"N, 121°45'34.6"W, Davis, CA 95616) in 2022. We direct-seeded honeydew melons (Tam Dew) into 1.5m raised beds to achieve a plant density of 6 plants per square meter. The field was irrigated via subsurface drip lines and fertilized through the drip system. Broadleaf weeds were controlled using hand weeding, and a grass-selective herbicide (Arrow, clethodim) was applied to treat grassy weeds. No other pesticides were applied across the entire trial area.

The field trial was set up as a randomized complete block design with six replicates total arranged in spatial blocks. (Figure 1) The treatments are detailed in Table 1. There were three applications made, spaced fourteen days apart. The first application was on August 12th, the second on August 26th, and the final on September 9th. Treatments were applied using a backpack CO_2-powered boom sprayer at, with four 800VS2 nozzles, spaced 90cm apart. All treatments containing CT were applied at 20 gallons per acre to achieve larger droplets, while the Assail treatment not containing Cidetrak was applied at 30 gallons per acre to more closely mimic an application in a production field. The drift reducer Onsite (polyvinyl polymer, Brandt) was added to all CT containing treatments at the maximum labelled rate (10 ml/L) to help create a larger droplet size. The boom, hoses, and nozzles were cleaned via multiple rinses between each treatment to prevent cross-contamination.

We assessed W. striped CB and W. spotted CB using timed counts at multiple times post-treatment. Within each plot, we searched and counted the number of alive and dead beetles of each species for 4 minutes, counting the inner rows of each plot. The counts were completed before the first treatment application, and then three, seven, ten, and thirteen days after each application. There were three applications made, spaced fourteen days apart. The first application was on August 12th, the second on August 26th and the third on September 9th

To assess melon damage, we rated cucumber beetle damage of the melons at the end of the growing season using a scale we developed in prior insecticide efficacy work for cucumber beetles in melons. Prior to the first application, melons that could have received damage pre-treatment (> 5 cm diameter) were removed from the plots to ensure our damage assessment captured the efficacy of the treatments. Beetle damage is primarily found on the underside of the melons, so we focused our damage assessment on the hemisphere of the melon in contact with the ground. Twenty melons were randomly selected from each plot, and assigned a rating based on the amount of beetle damage on the bottom hemisphere. We assigned relatively undamaged melons getting assigned a 0 on the scale, and those with ~ 2.3 cm of linear damage (low level) a 1. Melons with a rating of 1 or higher would be considered a cull in conventional honeydew production based on prior conversations with a local collaborator who worked with conventional melon growers and pest control advisors. (Amber Vinchesi-Vahl, personal communication)

Successively higher scores had more damage, with increasingly large scarring and/or a greater number of scarred patches (detailed in Table 2).

Statistical analysis

Beetle count data

All data analyses were conducted in R (version 2023.12.1, Build 402 R Core Team 2024). We separately analyzed W. striped and W. spotted dead and alive counts. The counts were summed covering all dates to account for the variable distributions in data and low counts in some treatments following the first application. Data were analyzed using linear models (via the lm function), with the number of beetles as the response variable and Treatment and Block as fixed factors. Diagnostic plots were inspected for each model to assess model assumptions, and data were transformed using a log base 10 transformation to improve model fit. When treatment was significant, we used pairwise comparisons of means between treatment levels with the emmeans function (emmeans package, Lenth, R. V. 2021), with adjustments made for multiple comparisons using the False Discovery Rate Method.

Melon damage data

To analyze damage data, we first analyzed the number of undamaged melons (damage rating of 0) using a linear model with Treatment and Block as predictor variables. Diagnostic plots were used to assess model fit. Damage ratings for each plot were analyzed with a cumulative link mixed model (CLMM) using the clmm function of the ordinal package (Christensen 2023). In a second test, ratings of 4 and 5 were consolidated into the “4” category because of extremely low numbers of 5 ratings in this trial. Ratings for melons were used as the response, with fixed factors for Treatment and Block and a random effect of Plot. The proportional odds assumption for the CLMM models was assessed by using likelihood ratio tests to compare models with and without effects for either Treatment or Block. Posthoc comparisons between treatments were conducted using the emmeans function of the emmeans package with $\alpha = 0.05$ and a Benjamini-Hochberg correction for multiple comparisons.

Laboratory insecticide bioassay

We performed laboratory bioassays to better assess the effect of CidetrakL paired with varying rates of acetamiprid in a more controlled environment. Treatments are detailed in Table 3. Trials were conducted in 2022 and 2023 using field-collected beetles. We collected W striped CB using aspirators at the Plant Pathology Research Farm at UC Davis, and young pumpkin (Magic Lantern variety) leaves for the assays from various untreated cucurbit fields at the same location. The location was not treated with any insecticides. Beetles were kept in cages for up to a week until they were used in the assay. Before each assay, beetles were isolated in groups of 10 and starved for 24 hours. Beetles were not sexed before the trial.

For an assay, a leaf was cut into a 10×15 cm rectangle and placed into a separate plastic deli container (NYHI rectangular deli container, Amazon) on top of a piece of filter paper. We attempted to obtain droplets of approximately 1mm volume median diameter (VMD) and estimated that a droplet 0.52µL achieved this. Sixty of these droplets were applied in a grid pattern across the surface of each leaf using a pipettor (0.4 per square cm) and the droplets were then allowed to dry. Once dried, the filter paper beneath the leaf was moistened with 2 ml of deionized H2O to prevent leaf desiccation. Ten live W striped CB were then placed into each container and allowed to feed ad libitum. The containers were placed on a laboratory bench under standard fluorescent lighting and approximately at 22C. After 24 hours, the mortality of each beetle was assessed by prodding them gently with a probe up to three times; beetles were counted as dead unless they could travel 10 mm after being prodded. (Figure 1)

Statistical analysis

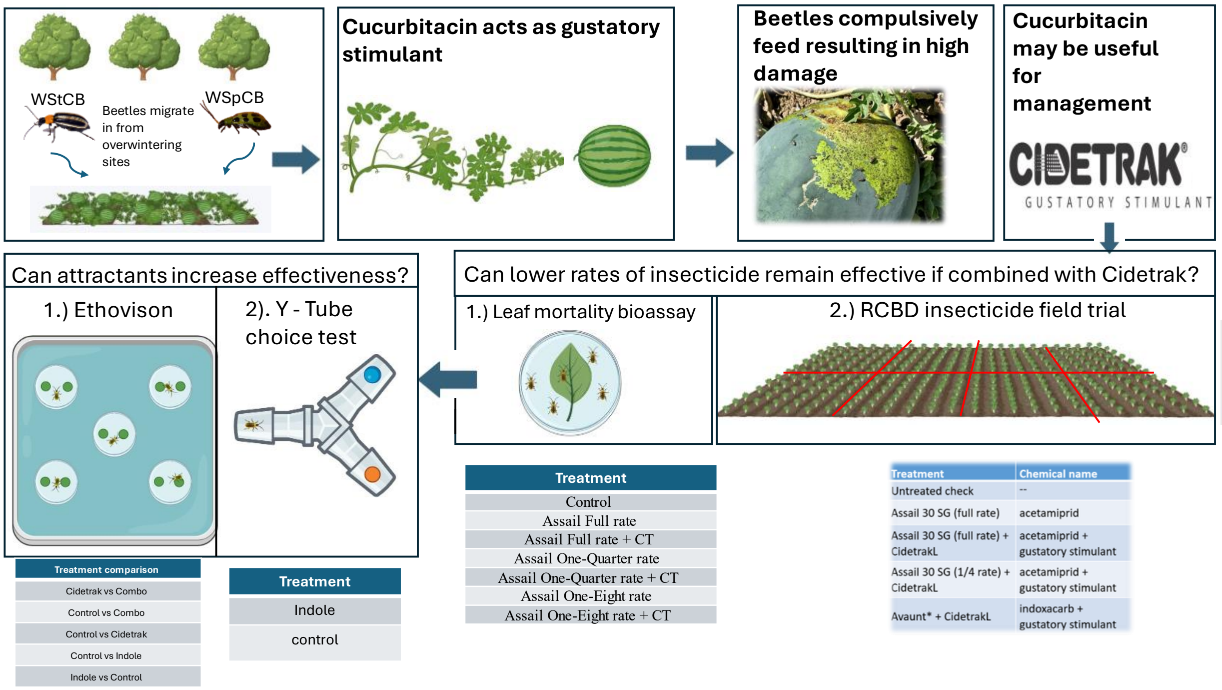
We used a linear model to analyze the effect of treatment on beetle mortality, with percent mortality as the response variable and Treatment and Trial as fixed factors. Pairwise comparisons of treatment groups were conducted using the emmeans package, with α=0.05 and a Benjamini-Hochberg correction for multiple comparisons.

Y-tube olfactometer assay

To test the attractiveness of W striped CB to CT and Indole (which we hypothesized to be unattractive and attractive respectively), we conducted two separate Y-tube olfactometer choice tests. The first tested the attractiveness of various concentrations of CidetrakL and the second tested various concentrations of Indole, both against a blank control (Table 4). The protocol for each choice test was the same (described below). Beetles were collected and stored in the same manner as the mortality bioassay above. A glass Y-tube (Sigma Scientific LLC) was set up on a lab bench and covered with a large box to block external stimuli from interfering with the experiment. The top of the box was translucent to allow ambient light to enter, and the bottom of the Y-tube was connected to a vacuum with a flow rate of 8 liters per minute.

Treatments were chosen as follows: for the CT treatments, we used an 18.76 ml/L solution. For the Indole treatment, we estimated the appropriate dosage for our experiments by referencing literature on fruit fly attraction to Indole, as the chemical properties and olfactory responses of fruit flies are well-documented (Epsky et al., 2003). We dissolved 10 mg of indole into 1 ml of acetone, resulting in a stock solution concentration of 10 mg/ml. We then diluted some of the stock solution using acetone to achieve a working solution of 0.5 mg indole/ml acetone. Treatments (Table 4) were applied via pipettor to a piece of filter paper (3 cm) and the filter papers were then randomly placed into each arm of the Y-tube. (Figure 1)

A single naive beetle was introduced into the long arm of the Y-tube and was then observed. A choice was defined as the beetle traveling more than 15cm past the junction down one of the arms. The experiment ended when a choice was made. If no choice was made within 3 minutes, the beetle was discarded and replaced with a new, naive beetle. Each test was replicated 30 times each with a new beetle. The arms were randomized after each replicate. When a choice was made, the beetle was frozen, and then its sex was identified by examining ventral anatomy under a dissecting stereoscope (White 1977)



Treatment comparison
Cidetrak vs Combo
Control vs Combo
Control vs Cidetrak
Control vs Indole
Indole vs Control

Treatment
Indole
control

Treatment
Control
Assail Full rate
Assail Full rate + CT
Assail One-Quarter rate
Assail One-Quarter rate + CT
Assail One-Eight rate
Assail One-Eight rate + CT

Treatment	Chemical name
Untreated check	--
Assail 30 SG (full rate)	acetamiprid
Assail 30 SG (full rate) + CidetrakL	acetamiprid + gustatory stimulant
Assail 30 SG (1/4 rate) + CidetrakL	acetamiprid + gustatory stimulant
Avaunt* + CidetrakL	indoxacarb + gustatory stimulant

Figure 1

Visual Abstract depicting the Insecticide field trial, leaf mortality bioassay, Y tube choice test, and Ethovision Bioassay

Statistical analysis

To assess the significance of the beetle's choice, we used the Chi-squared test of independence to compare the observed distribution of choices made in each assay to the null hypothesis of no preference (i.e., equal distribution between the treatment and control arms). Statistical significance was assessed at $\alpha = 0.05$.

Behavioral assay with feeding stimulant and attractant

We conducted a second set of bioassays using Ethovision, to determine how the gustatory stimulant CidetrakL, the attractant Indole, and their combination affect various aspects of W striped CB behavior. Beetles and leaf material were collected as described above for mortality assays. Each assay consisted of five 100 mm Petri dishes. Four 5mm vent holes spaced approximately 75mm apart were cut into the side of each petri dish and covered with mesh to allow for some airflow. Each dish was lined with filter paper, and each petri dish contained two 10 mm leaf disks (Figure 2). Each leaf disk received one treatment from Table 4. Treatments consisted of a single 0.52 µL droplet applied to the center of the leaf disk and allowed to dry. The petri dishes were placed on a white illuminated lightboard and supplemented with overhead LED lighting. A single naive beetle was placed into each petri dish and was allowed to roam freely and feed. The feeding sessions ended after 3 hours to prevent leaf desiccation. Feeding sessions were recorded, and the videos were then scored using Ethovision XT software (version 16, Noldus Information Technology). Using Ethovision, each petri dish was divided into two hemisphere zones, each containing a leaf disk. A second 2.8 cm zone was defined around each leaf disk.

Statistics (frequency of visits and time in zone) for the sum of both the dish half and the leaf disk were calculated. This was replicated 6 times. In addition, screenshots were taken of each leaf disk at the beginning and end of each assay. Using the software LeafByte (Zoe Getman-Pickering), the total amount of surface area consumed on each leaf was calculated.

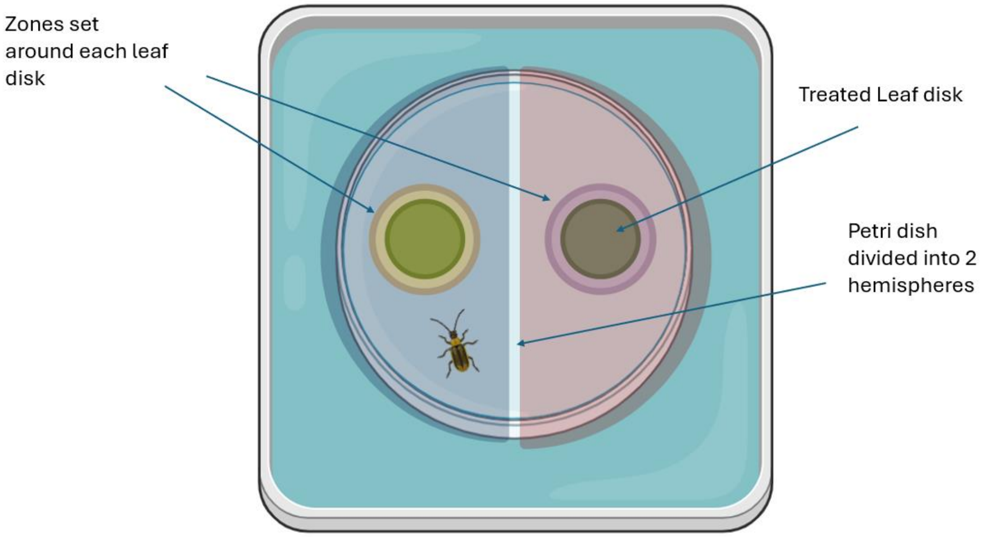


Figure 2
Petri dish arena during Ethovision bioassay. Two treated leaf disks were placed into a petri dish. The dish was then divided into two halves, and then a zone was set up around each leaf disk. The data related to the beetle’s movement and behavior around each of the four zones was recorded

Statistical analysis

Attraction: To assess the effect of treatment on beetle behavior, we conducted comparisons between leaf disks within the same petri dish using three different response variables. First, we analyzed the mean duration of time beetles spent on each leaf disk. Second, we assessed the time spent near each leaf disk, (defined as the time spent within each half of the petri dish), and third, we evaluated the number of visits to each leaf disk. For each of these comparisons, normality assumptions were assessed using the Shapiro-Wilk test. As the data deviated from normality in most cases, we employed a non-parametric Wilcoxon signed-rank test for the analysis.

Feeding Stimulation: To analyze the gustatory stimulation of CT, we examined the effects of treatment combinations both within individual petri dishes and across multiple dishes. For within-dish comparisons, we followed the same approach as in the attraction analysis above, using the percentage of leaf disk consumed as the response variable. Since each dish contained two leaf disks exposed to the same beetle, the feeding response could be influenced by the beetle's interactions with both disks, resulting in non-independence of the observations.

To evaluate feeding stimulation across dishes, we developed a ranking system based on the number of leaf disks treated with CT within each dish. This system categorized dishes into three levels: A (no leaf disks treated with CT), B (one leaf disk treated with CT), and C (both leaf disks treated with CT). Although this analysis does not fully resolve the issue of non-independence within individual dishes, it provides a broader generalization of feeding behavior across different treatment combinations. As the data were not normally distributed, we applied a non-parametric Kruskal-Wallis (K-W) test to analyze differences across these CT levels, treating the percentage of leaf disk damage (summed within each dish) as the response variable. We repeated this analysis with the distance each beetle moved as the response variable, to see if different levels of CT had an arrestant effect on beetle movement.

Results

Field trial

W. striped CB

Prior to the first application, there was no difference in beetle abundance (live counts) among treatments at this point (df = 5,20, F = 0.27, P = 0.89,). Weekly counts of W. striped were higher earlier in the trial, with an additional peak in late August to early September (Figure 3, Table 5). W. striped CB live counts decreased as the trial progressed, while dead counts remained lower overall, with minor fluctuations (Figure 3, Figure 4). When counts of W. striped CB were summed across the entire trial, we observed differences among treatments for both live and dead beetles (Figure 5) (Table 5). Post-hoc comparisons pooled over time showed significant differences among treatments. The Avaunt + CT plots had fewer live beetles than untreated control plots, and all treatments containing Assail had fewer beetles than the Avaunt + CT plots (Figure 5). There were no differences between plots treated with the full rate of Assail, with or without the addition of CT. The quarter rate of Assail + CT had live beetle counts that were not statistically different than plots treated with the full rate of Assail. The number of dead W. striped CB was not different between the Avaunt + CT and control plots. For the Assail-treated plots, the pattern for dead beetles mirrored that of the live beetles (Figure 6). All treatments containing

Assail (full rate of Assail, full rate of Assail + CT, and quarter rate of Assail + CT) had similar numbers of dead beetles (Table 5).

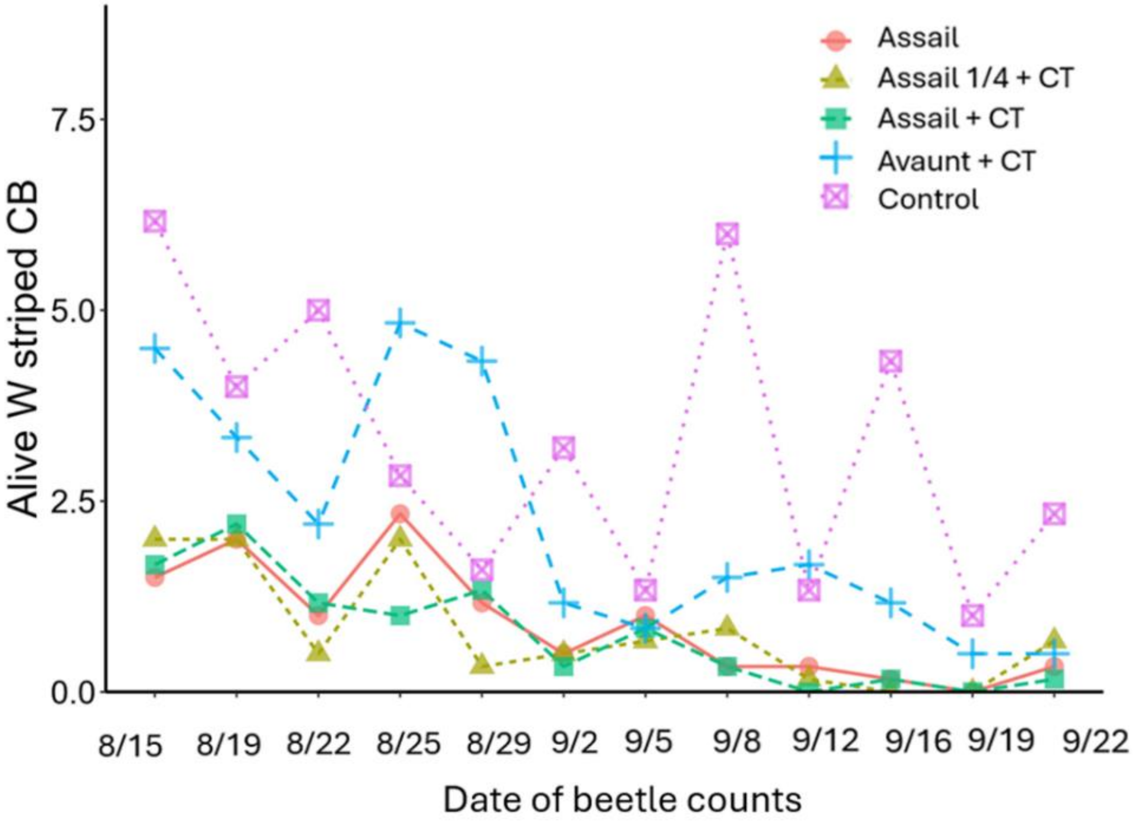


Figure 3. Weekly counts of live W. striped CB in plots treated with different rates/insecticides +/- CidetrakL. Values are means across replicates. The beetle counts were collected at consistent intervals between August 15 and September 22. Applications were made every two weeks, starting on 08/12.

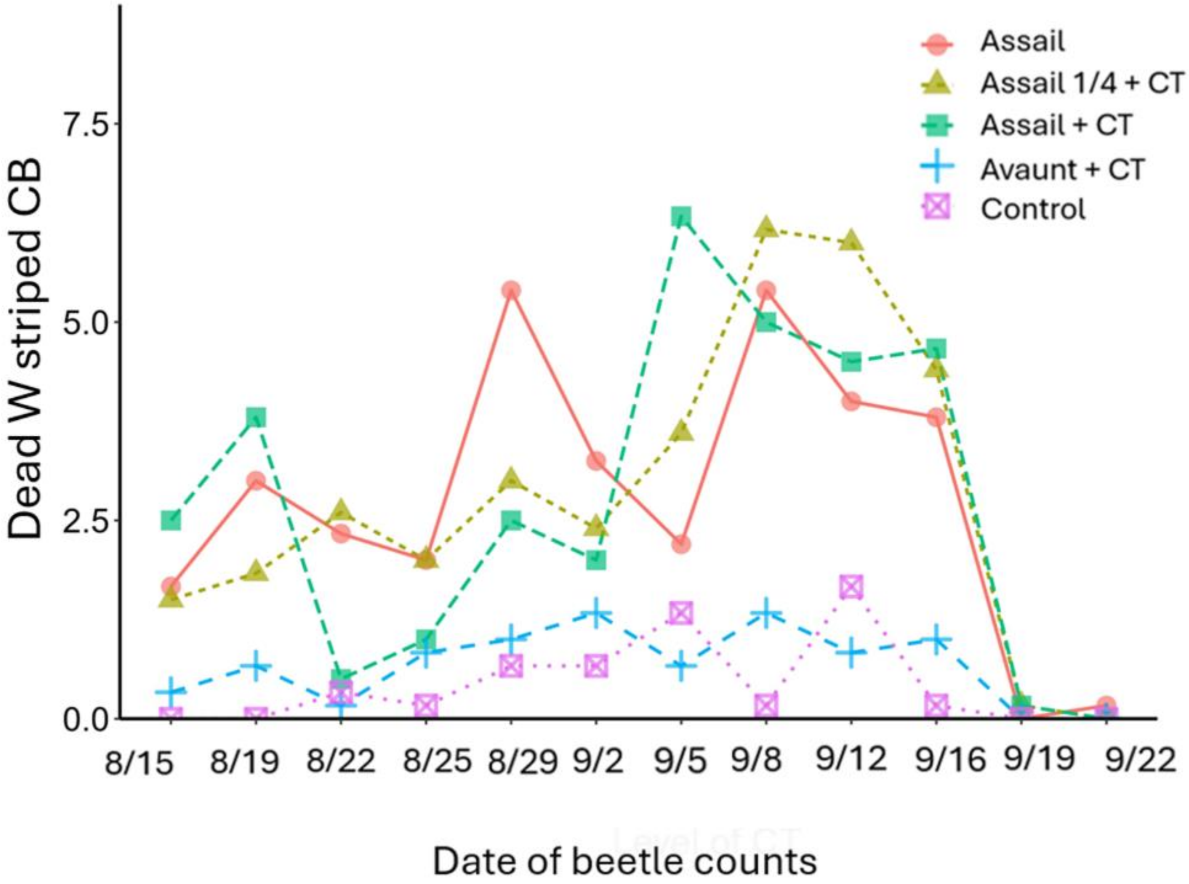


Figure 4. Weekly counts of dead W. striped CB in plots treated with different rates/insecticides +/- CidetrakL. Values are means across replicates. The beetle counts were collected at consistent intervals between August 15 and September 22. Applications were made every two weeks, starting on 08/12.

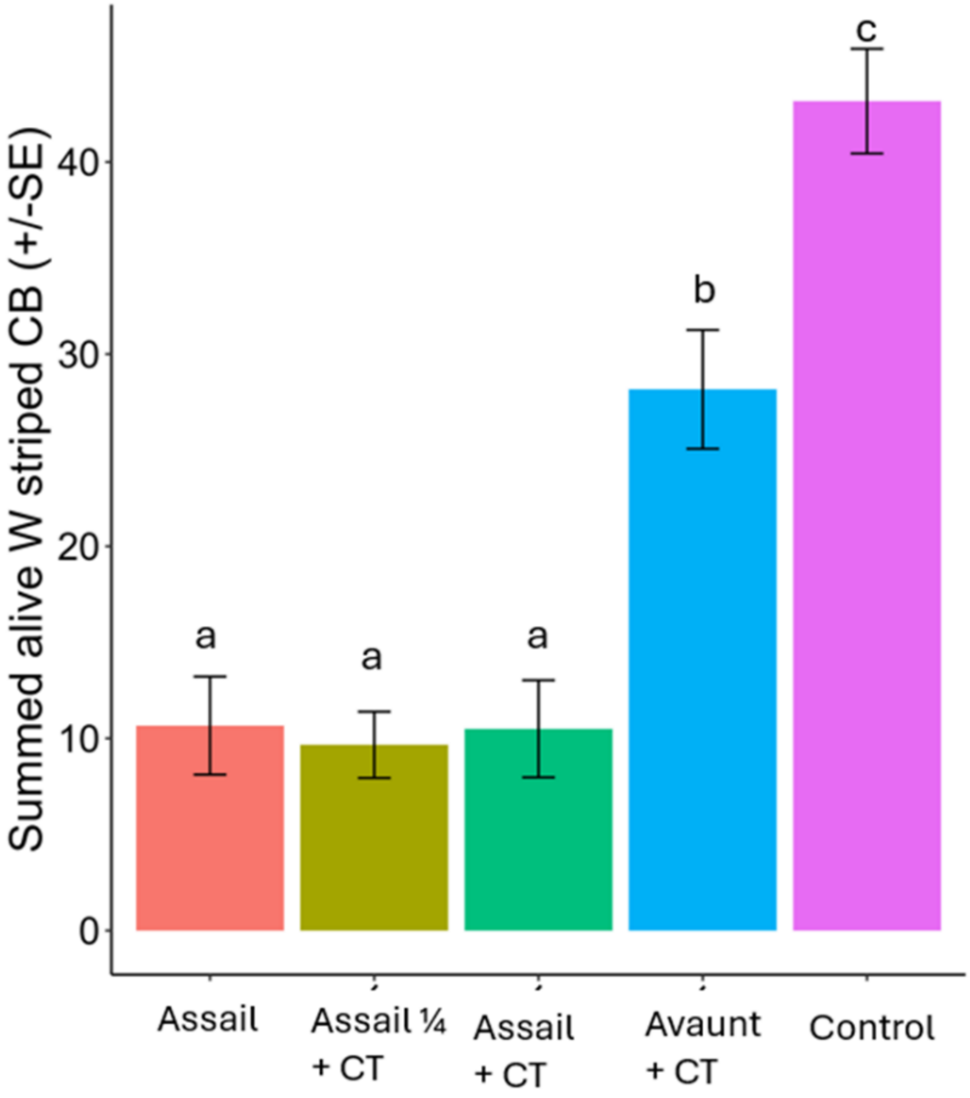


Figure 5. Summed counts of live W. striped CB collected throughout the trial, shown for different insecticide treatments. Error bars represent ± SE. Means not sharing a letter are significantly different based on post-hoc comparisons at α = 0.05

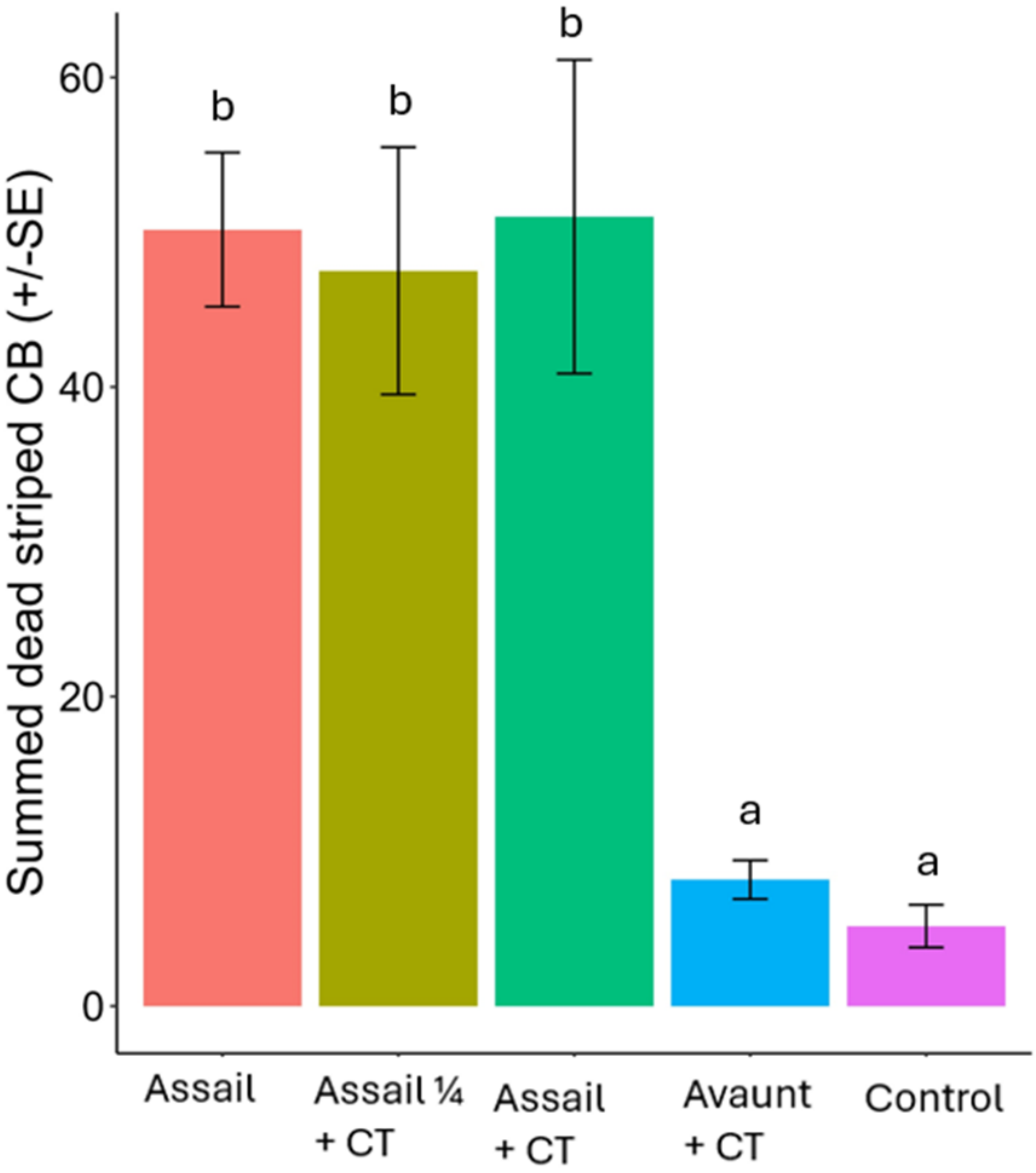


Figure 6. Summed counts of dead W. striped CB collected throughout the trial, shown for different insecticide treatments. Error bars represent ± SE Means not sharing a letter are significantly different based on post-hoc comparisons at α = 0.05

W. spotted CB

W. spotted CB abundance (live counts) did not differ among treatments prior to the start of the trial (df = 5,20 F = 0.76, P= 0.56). W. spotted CB had similar seasonal population trends as

W. striped CB, with live counts showing a second peak around early September and then decreasing (Figure 7) and dead counts lower and more consistent through the season (Figure 8). (). Post-hoc comparisons of summed counts over the season showed significant differences between treatments for W. spotted CB (Figure 9, Table 5). All plots treated with Assail had significantly lower live counts compared to the Control plots The Avaunt + CT treatment reduced beetle numbers compared to the Control. The quarter-rate of Assail + CT was not significantly different from either Avaunt + CT or either of the full-rate Assail treatments. The full rate of Assail with and without CT were comparable (Figure 9). For the dead W. spotted cucumber beetle counts, post hoc tests of pooled numbers over season also showed differences among treatments (Figure 10, Table 5). The Control and the Avaunt + CT plots had the lowest number of dead beetles, with no differences between these treatments. The quarter rate of Assail + CT, the full rate of Assail, and the full rate of Assail + CT all resulted in significantly higher beetle mortality, with no significant differences among these three treatments.

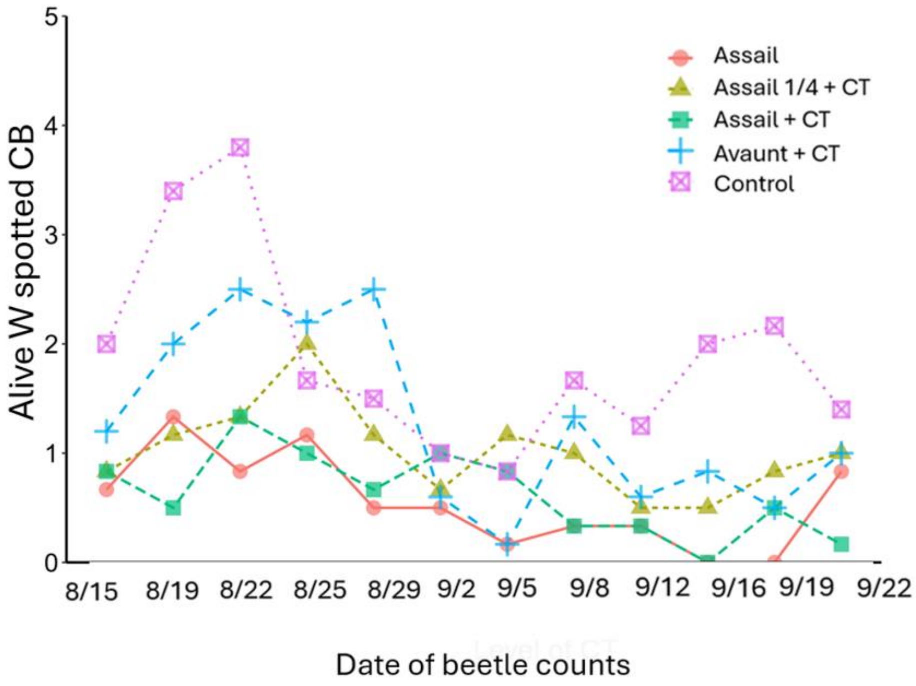


Figure 7. Weekly counts of live W. spotted CB in different treatment plots. Values are means across replicates. The beetle counts were collected at consistent intervals between August 15 and September 22. Applications were made every two weeks, starting on 08/12.

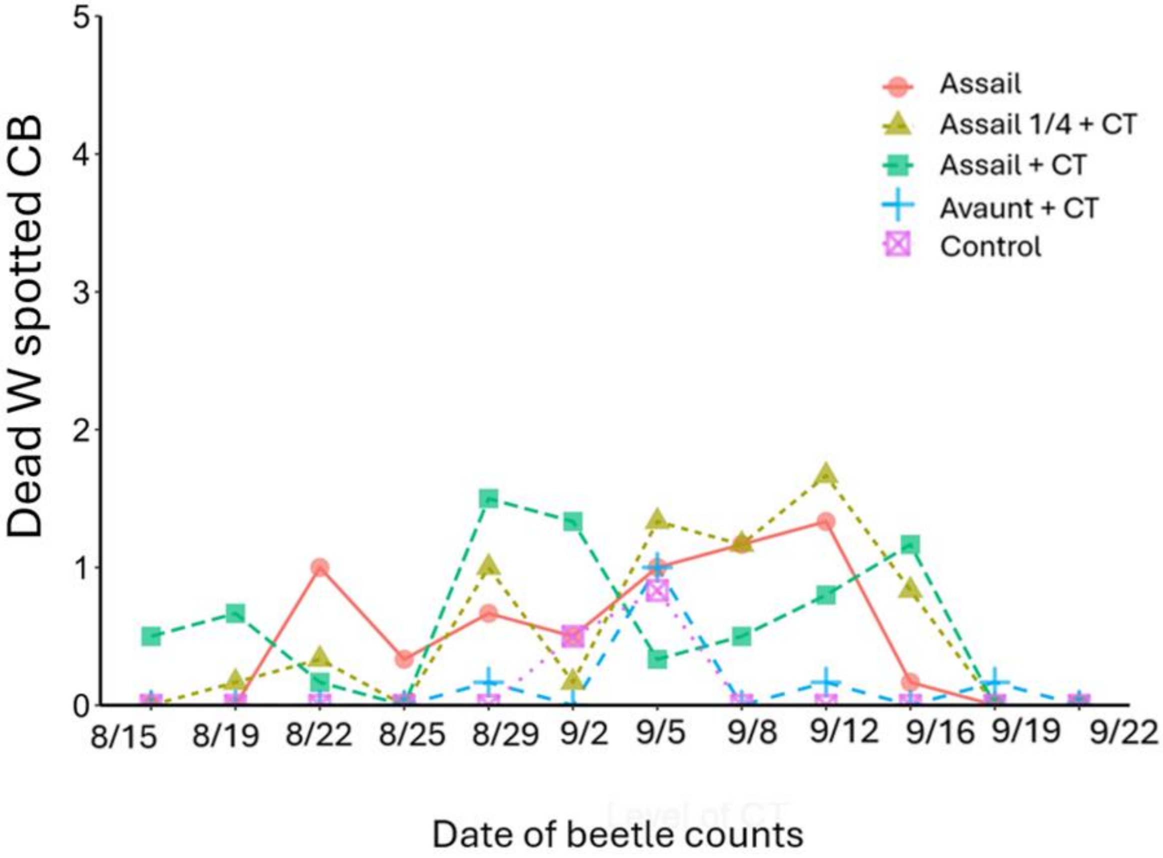


Figure 8. Weekly counts of dead W. spotted CB in different treatment plots. Values are means across replicates. The beetle counts were collected at consistent intervals between August 15 and September 22. Applications were made every two weeks, starting on 08/12.

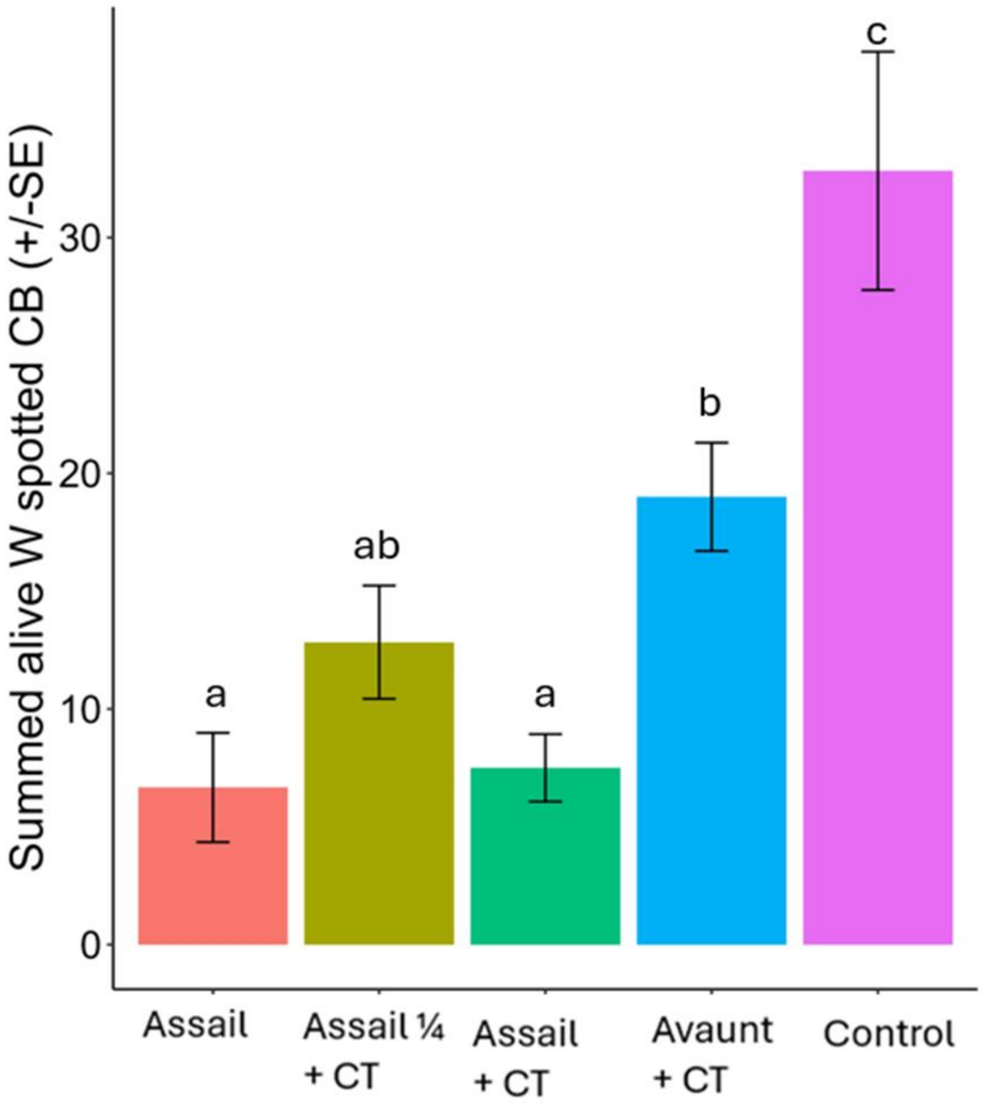


Figure 9. Summed counts of live W. spotted CB collected throughout the trial, shown for different insecticide treatments. Error bars represent ± SE. Means not sharing a letter are significantly different based on post-hoc
comparisons at α = 0.05

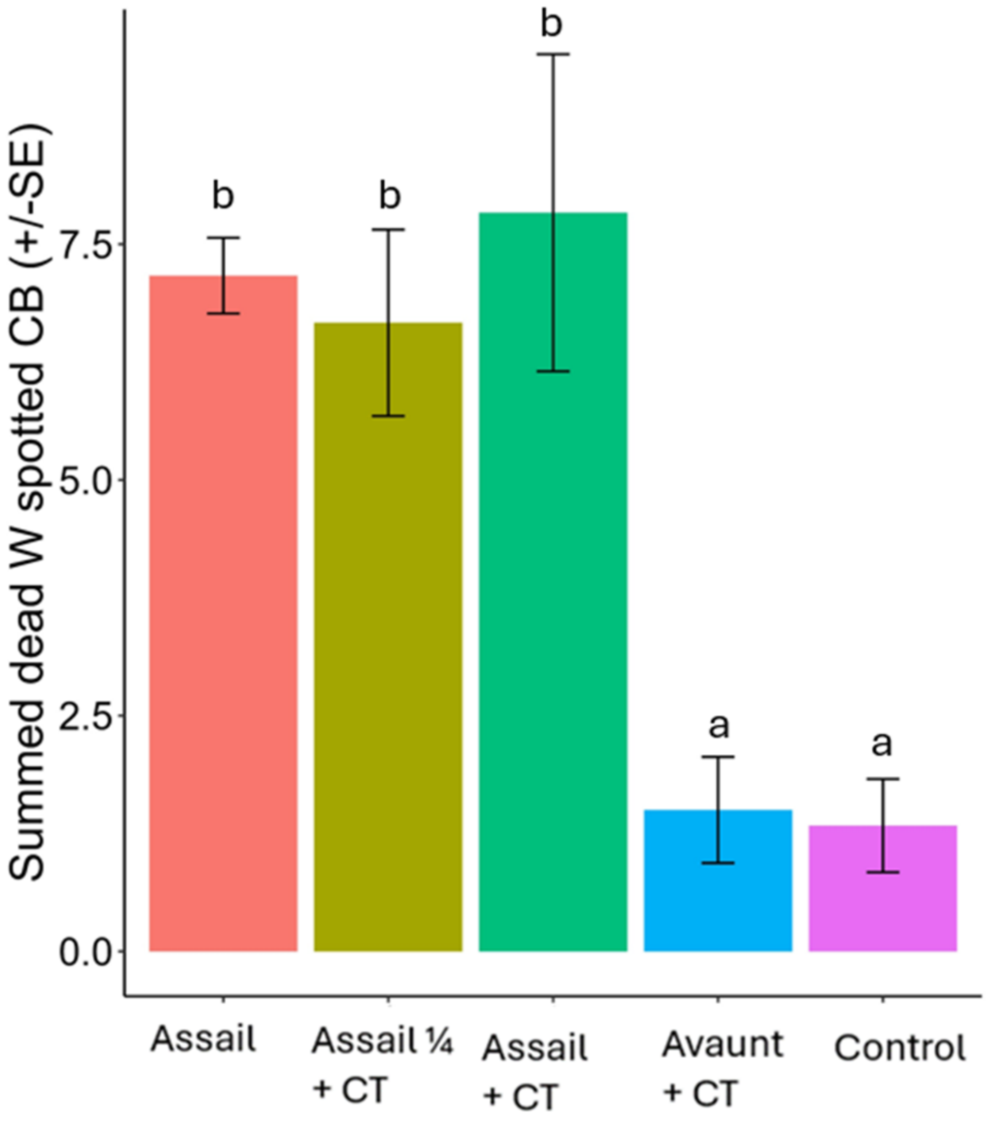


Figure 10. Summed counts of dead W. spotted CB collected throughout the trial, shown for different insecticide treatments. Error bars represent ± SE. Means not sharing a letter are significantly different based on post-hoc comparisons at α = 0.05

Melon-damage

At the end of the season, there was a marginally significant effect of treatment on the number of undamaged melons (Table 5). The damage pattern among treatments did match the effects on beetles (especially W. striped CB), with all plots that received Assail treatments having the most undamaged melons, and with Avaunt + CT and Control plots exhibiting more (and comparable) levels of cucumber beetle damage (Figure 11). Treatment also significantly affected melon damage as measured with the damage rating scale (Table 6) (df = 4,20, χ2 = 12.73). The Avaunt + CT treatment had comparable damage ratings to the control (Figure 12). The Assail full rate and the Assail quarter rate + CT had significantly reduced damage rating relative to the Control and Avaunt + CT plots (Figure 12). The Assail full rate + CT had patterns of damage intermediate to the rest of the treatments, although the damage ratings were more similar to the less damaged treatments (Figure 12).

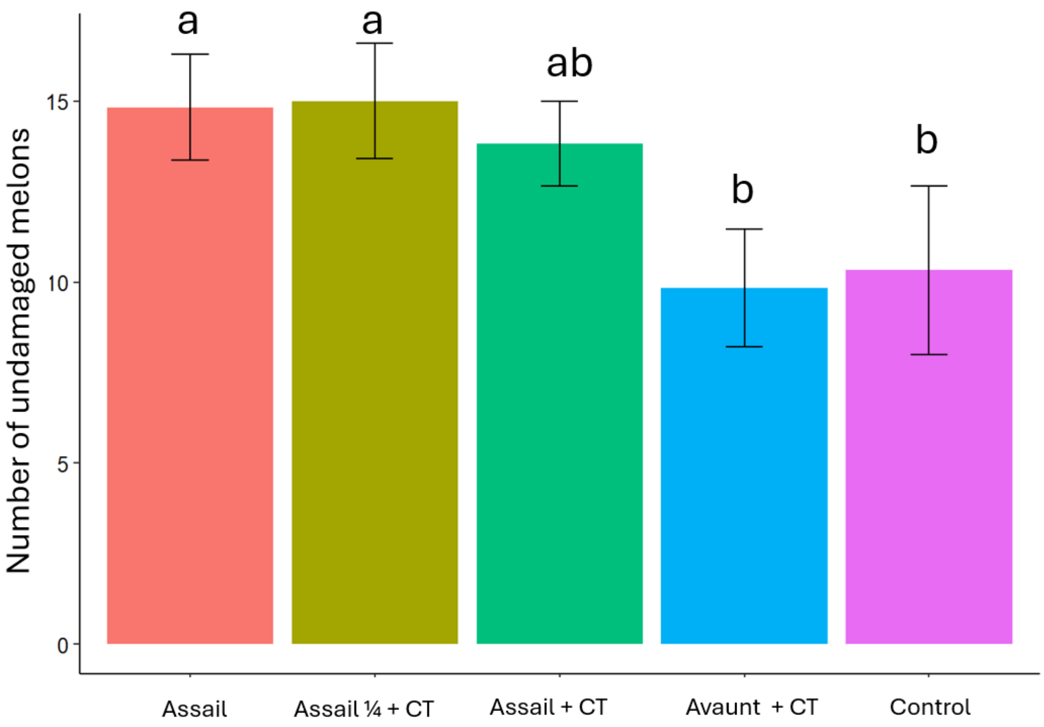


Figure 11. Number of undamaged melons across different insecticide treatments**.** Error bars represent ± SE. (α = 0.05) Means not sharing a letter are significantly different based on post-hoc comparisons at α = 0.05

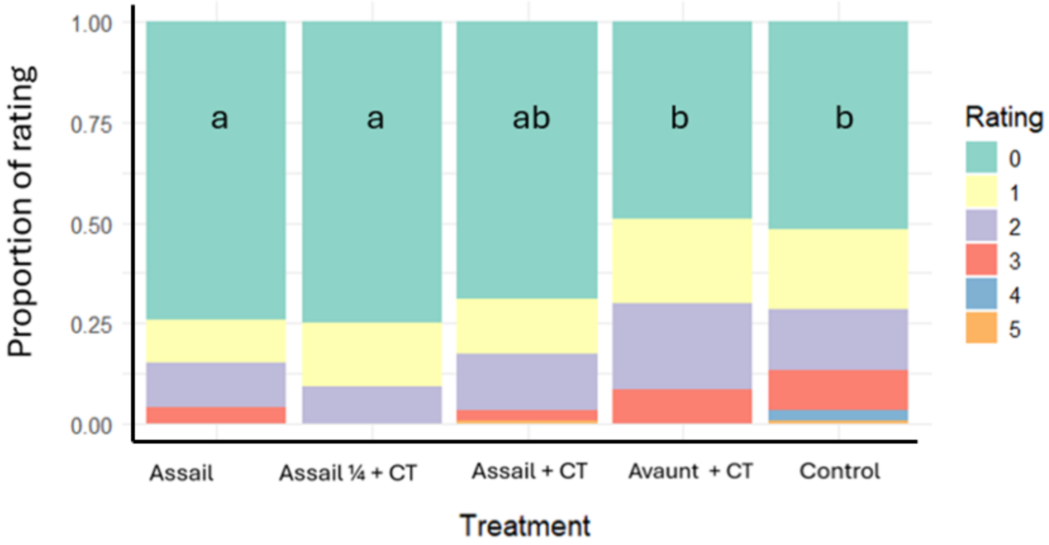


Figure 12. Proportion of melons receiving damage rating. Proportion of melons receiving the various damage ratings across different treatments. The x-axis represents the treatment, while the y-axis shows the proportion of melons that received each rating (0-5) for each treatment. Higher damage ratings indicate more significant damage on the melons. α = 0.05

Mortality Bioassay

The results of the lab bioassays complemented the findings of the field study. In the first bioassay, there was a highly significant effect of treatment on beetle mortality (Table 7). A quarter of the rate of Assail achieved only approximately 50% mortality at 24 hours, which was significantly lower than that with the full rate of Assail. However, when CT was added to the quarter rate Assail, mortality increased, and was not different from the full rate of Assail (Figure 13). Although the addition of CT to the full rate of Assail did increase mortality to nearly one hundred percent, it was not statistically different from the full rate without CT, mirroring what we saw in the field trials. (Figure 13)

The results of the second lab bioassay were generally similar, with some notable differences, in part because additional treatments were added. There was again a highly significant effect of treatment on beetle mortality (Table 7). All treatments differed from the control. (Figure 14). At the highest concentration, the full rate of Assail, both with and without CT, achieved the highest levels of mortality. Like the first bioassay, in bioassay 2, the quarter rate of Assail achieved about 50% mortality and the addition of CT to the quarter rate of Assail increased mortality to that of the full rate of Assail alone as well as the full rate of Assail + CT (although the latter was numerically higher). At the lowest rate of Assail (one-eighth rate), also resulted in approximately 50% mortality at 24 hours; although, adding CT to the one-eighth rate of Assail did not significantly increase mortality, the mortality of the one-eighth rate plus CT was comparable to the full rate of Assail alone and quarter rate plus CT, and was even not significantly different than the full rate of Assail + CT. While not always significant, there was a consistent trend of the addition of CT to the various rates of Assail and at least numerically increasing mortality.

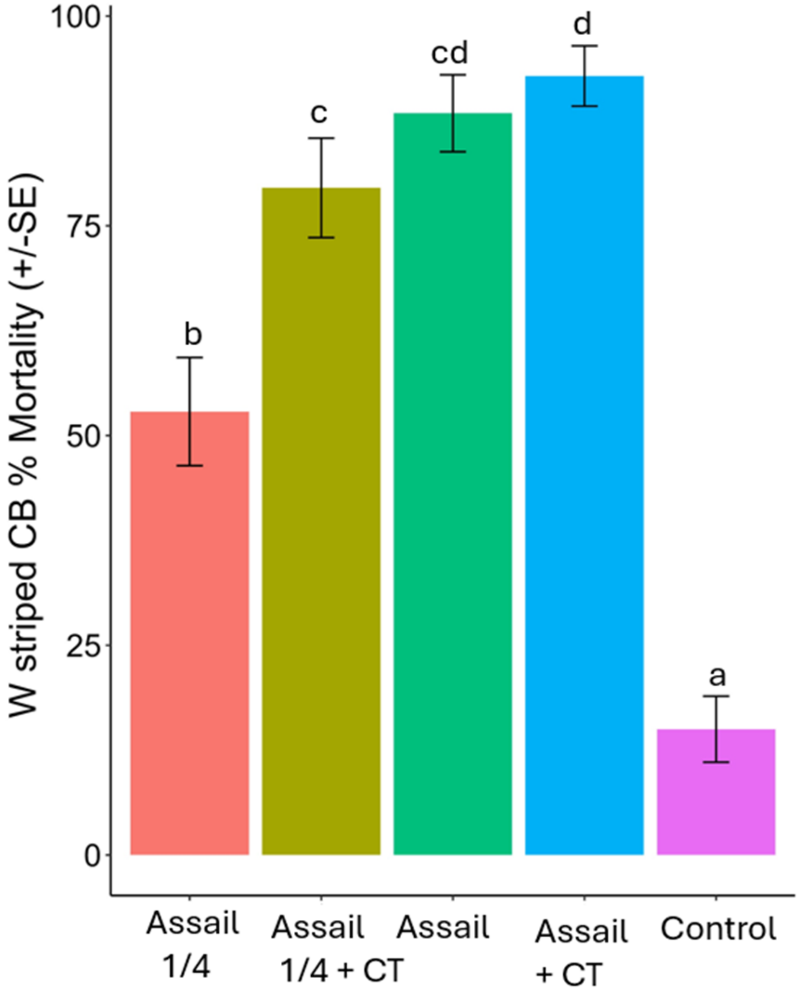


Figure 13. W. striped CB percent mortality in Bioassay 1. Means not sharing a letter are significantly different based on post-hoc comparisons at $\alpha = 0.05$. Values are means across treatments, and error bars represent ± 1 standard error (SE).

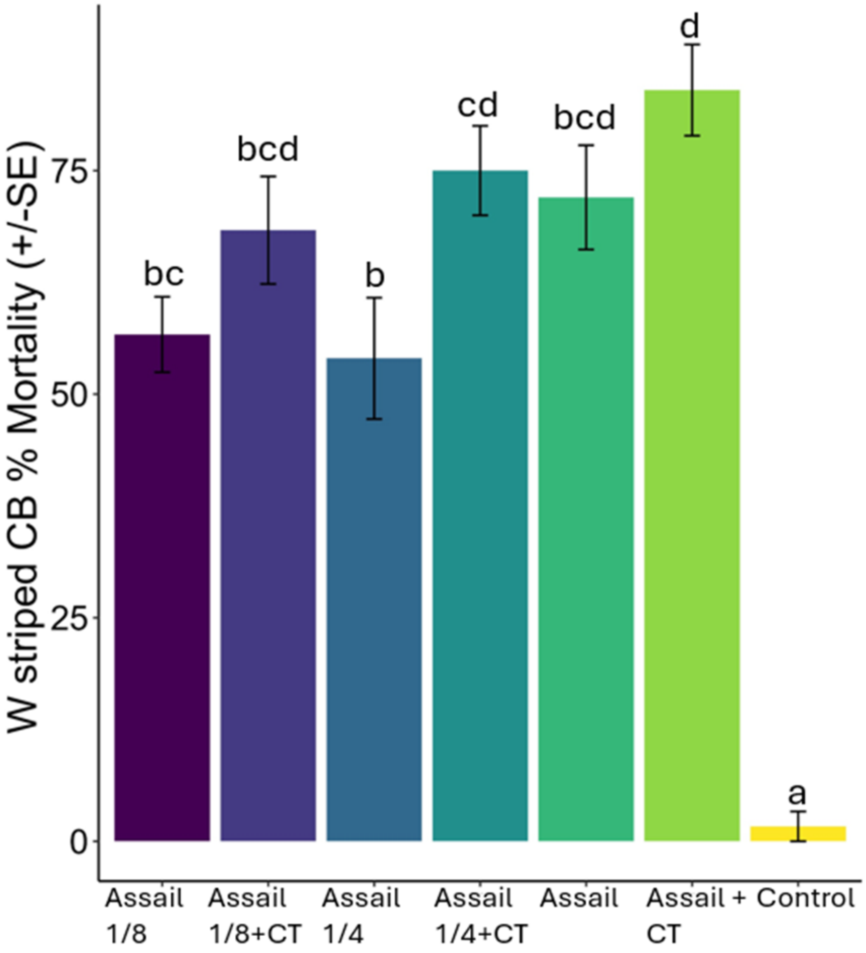


Figure 14. W. striped CB percent mortality in Bioassay 2. Means not sharing a letter are significantly different based on post-hoc comparisons at α = 0.05. Values are means across treatments, and error bars represent ± 1 standard error (SE).

Y-Tube Olfactometer

In the Y-tube assay, beetles showed no preference for the CT containing arm over the untreated control treatment arm in the first trial (df = 1, $\chi^2 = 0$, P = 1), in Trial 2 when the CT concentration was doubled (df = 1, $\chi^2 = 2.13$, P = 0.14), or in Trial 3 when it was increased to 4 times (df = 1, $\chi^2 = 0.14$, P = 0.71) (Figure 15). Striped cucumber beetles significantly preferred the Indole treated arm over the untreated control arm (df = 1, $\chi 2 = 4.8$, P = 0.028) (Figure 16), matching what we observed in an unrelated field trial (Seth Jean 2024, Jasmine Ramirez 2022). The sex ratio of field-collected beetles skewed heavily male at approximately 3:1.

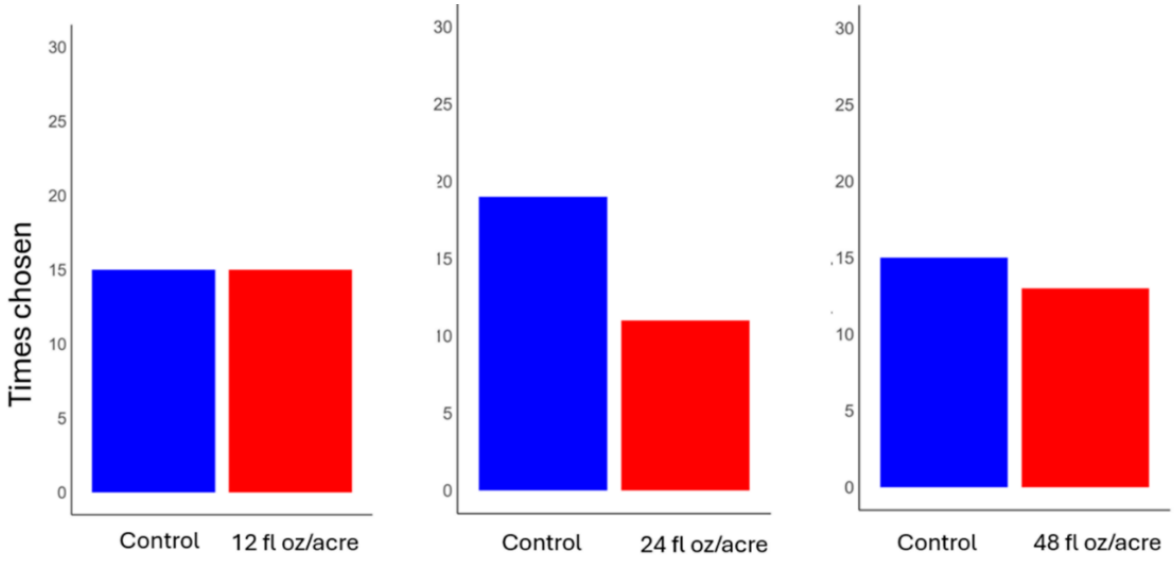


Figure 15. Y-tube choice test where W. striped CB were exposed to control and CidetrakL treatments. The test was conducted at three different concentrations of CidetrakL, as shown in the different panels of the figure. In all trials, there was no significant difference.

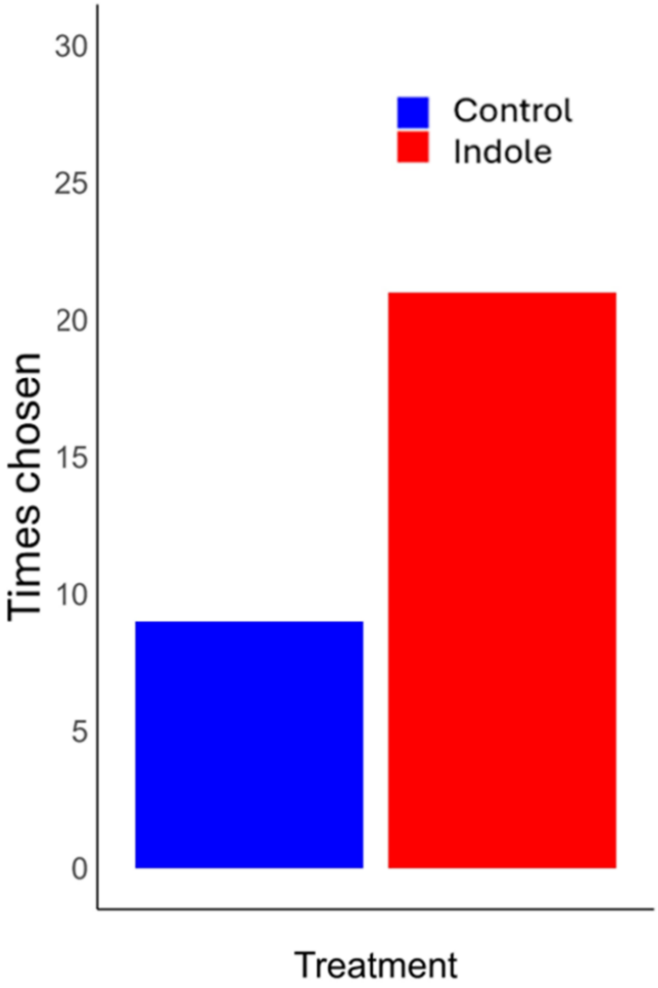


Figure 16. Indole y tube choice test with comparison between indole and control arms. W. striped CB significantly preferred the Indole-treatment over the control at α = 0.05.

Ethovison

Beetle behavior

There were no significant differences for time spent on individual leaf disks between treatments for any of the comparisons (Figure 17; Table 8), nor in the amount of time spent in in the vicinity of leaf disks (Figure 18; Table 8), or the number of times beetles visited each leaf (Figure 19; Table 8). The level of CT containing treatments within a petri dish also did not have an effect on the total distance a beetle moved. (Figure 20; Table 9)

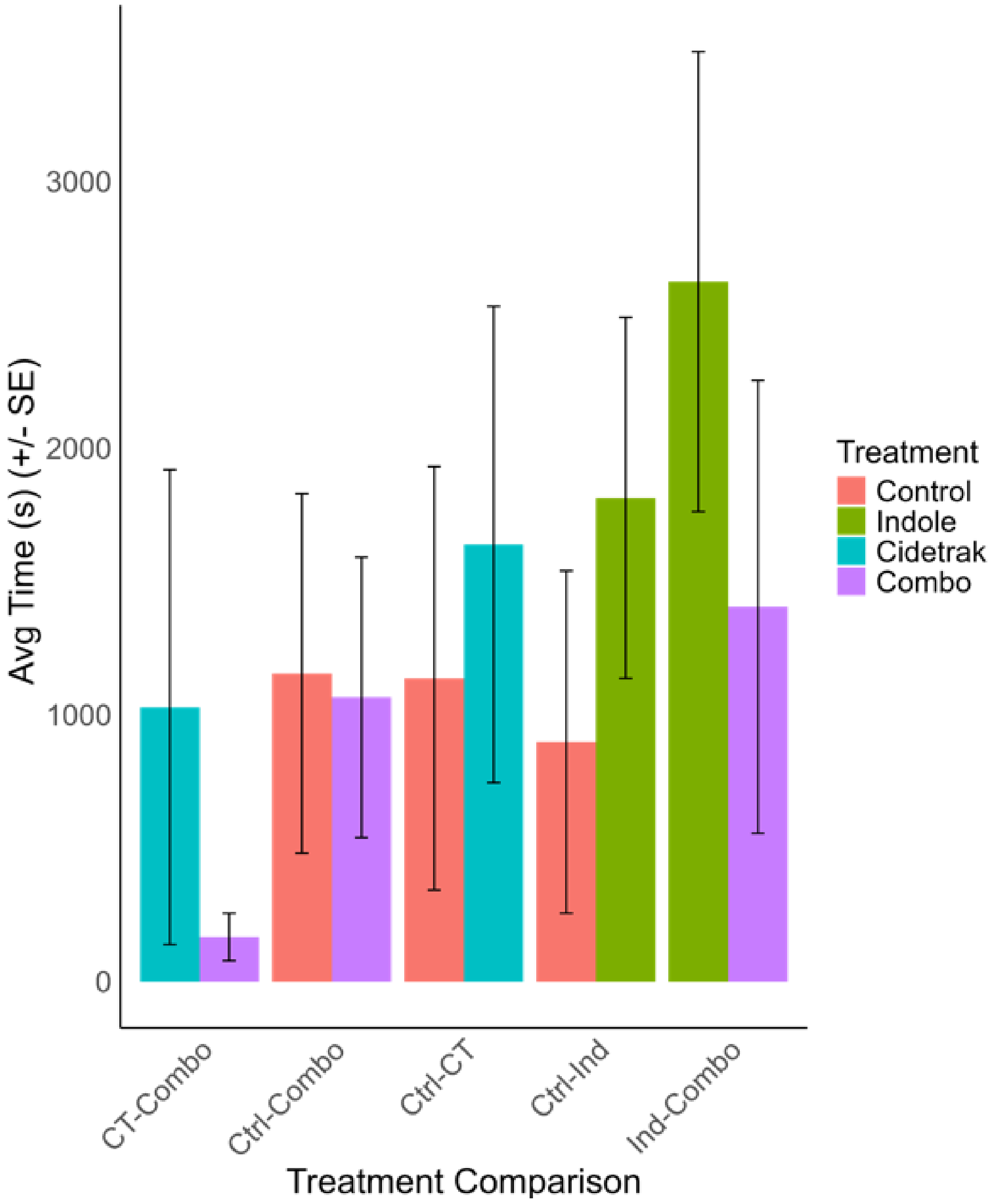


Figure 17. Average time (in seconds) W. striped CB spent on treated leaf disks during the Ethovision assay. Each pair of bars represents treatments that were compared within the same Petri dish during a choice test where a single beetle was exposed to both disks simultaneously. Treatments included Cidetrak (CT), Indole (Ind), and their combination (Combo), compared to the untreated control. No significant differences were observed in the time spent on the leaf disks across treatments. Error bars represent ± SE.

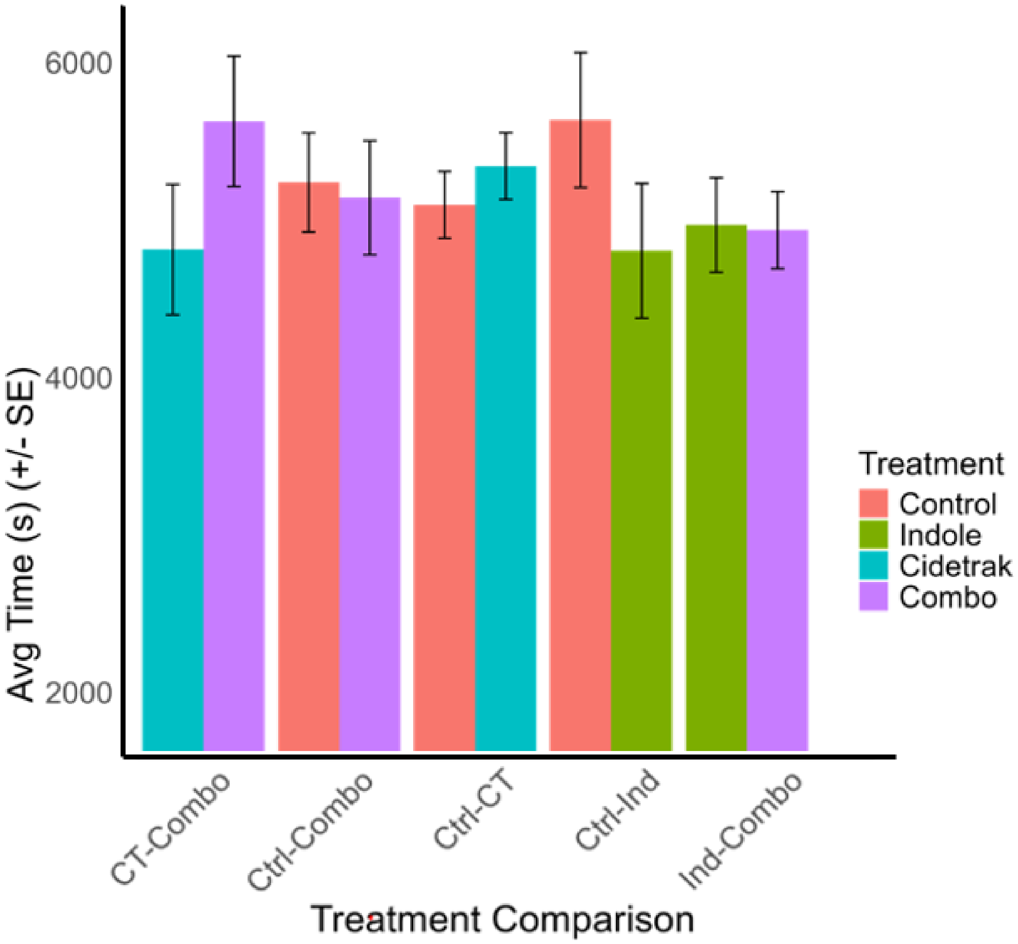


Figure 18. Average time (in seconds) W. striped CB spent in the hemisphere closest to the leaf disk during the Ethovision assay. Each pair of bars represents treatments that were compared within the same Petri dish during a choice test where a single beetle was exposed to both disks simultaneously. No significant differences were found in the amount of time spent near the leaf disks across treatments. Error bars represent ± SE

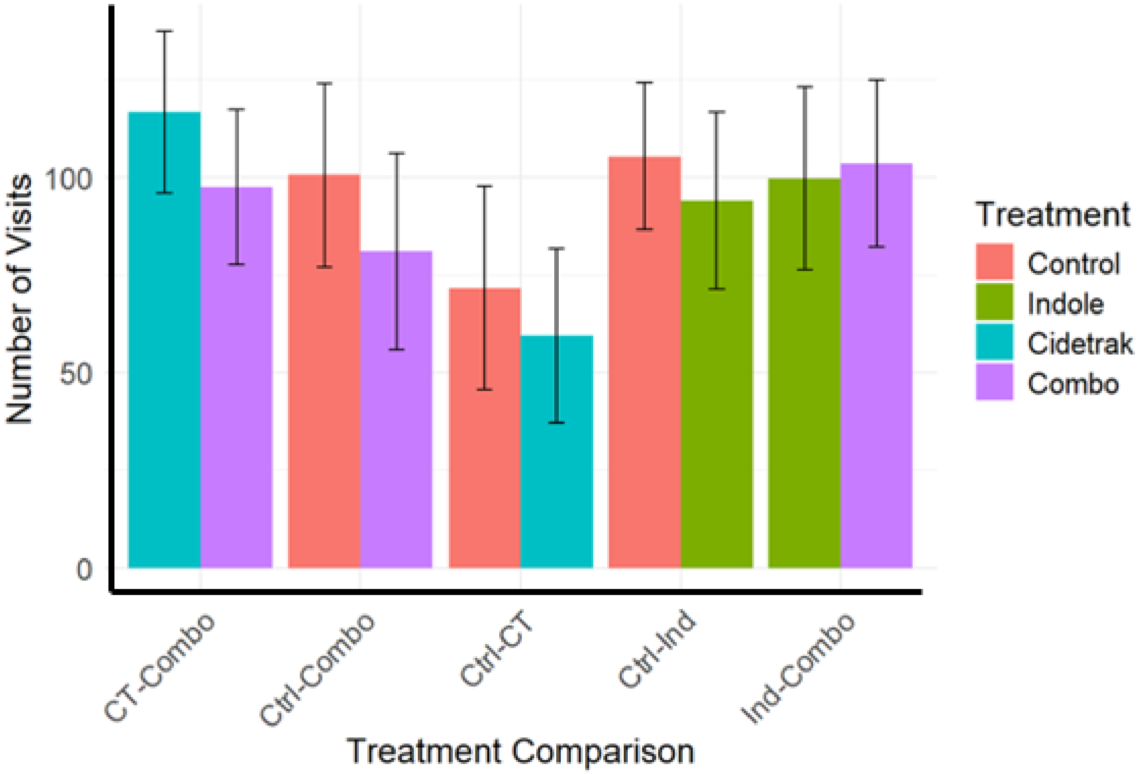


Figure 19. Visits to leaf disk Number of visits to treated leaf disks by a W. striped CB during the Ethovision assay. Each pair of bars represents treatments that were compared within the same Petri dish, where a single beetle was exposed to both disks simultaneously. No significant differences were found in the number of visits across treatments. Error bars represent ± SE.

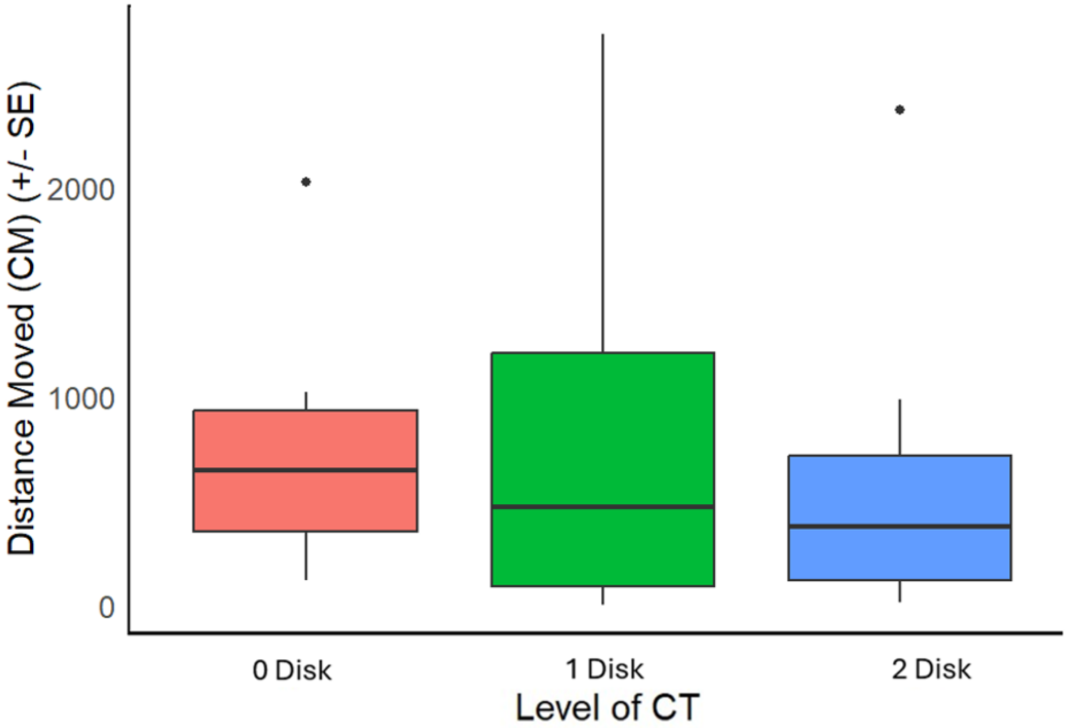


Figure 20. Distance moved by W. striped CB in Petri dishes based on levels of CT. Each level refers to how many of the two leaf disks in the dish were treated with CidetrakL. No significant differences were found in the total distance moved between the treatments, indicating that CT did not arrest beetle movement. Error bars represent ± SE.

Feeding damage

There were no differences in percentage of the leaf disk consumed when comparing treatment combinations within a petri dish. (Figure 21; Table 8) When comparing damage across the entire petri dish, the total feeding damage for the different levels of CT treatments also did not differ. (Figure 22; Table 9)

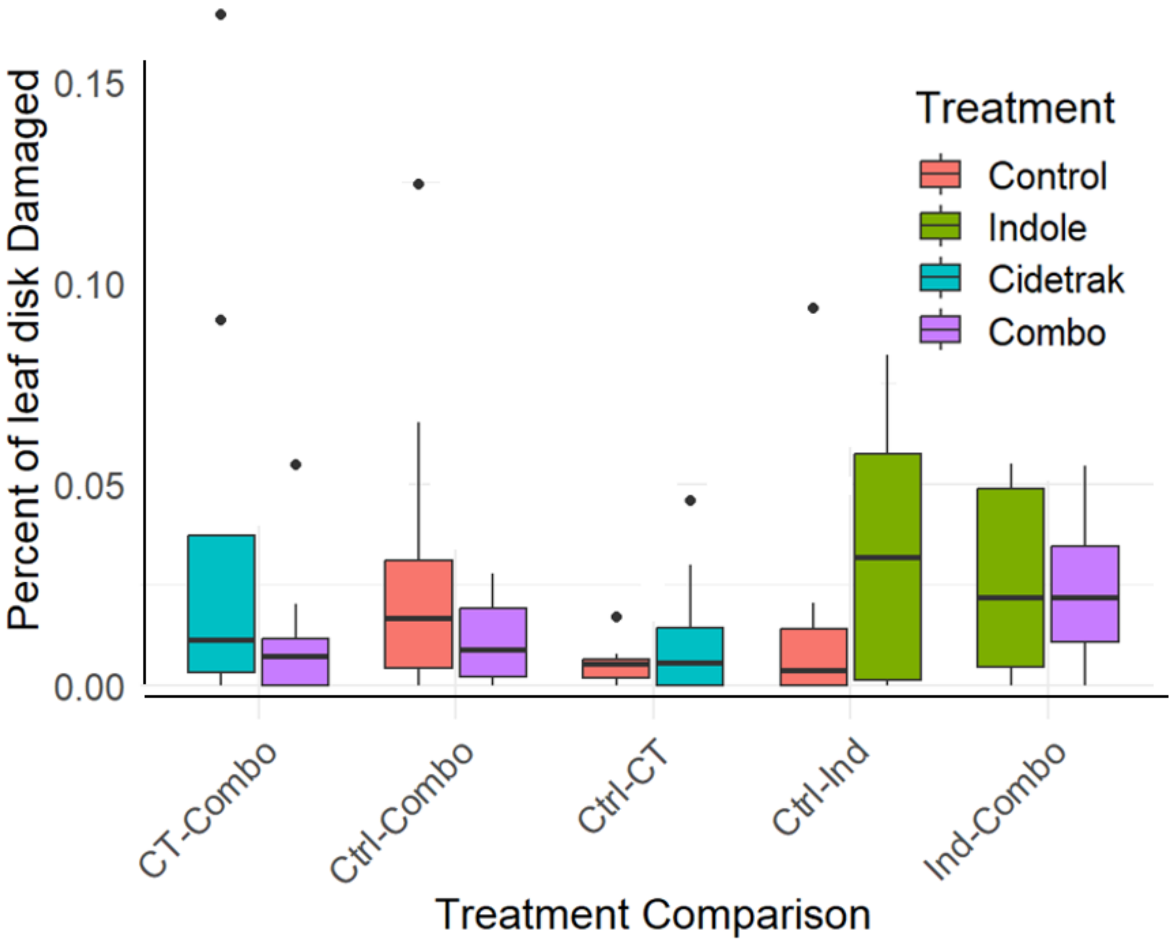


Figure 21. Percent of each leaf disk damaged by W. striped CB during the Ethovision assay, comparing treatments within the same dish. Each boxplot represents the variation in leaf damage across the different treatment combinations. No statistically significant differences in leaf damage were observed across treatments. Treatments included combinations of Cidetrak (CT), Indole (Ind), and both (Combo), compared to the untreated control

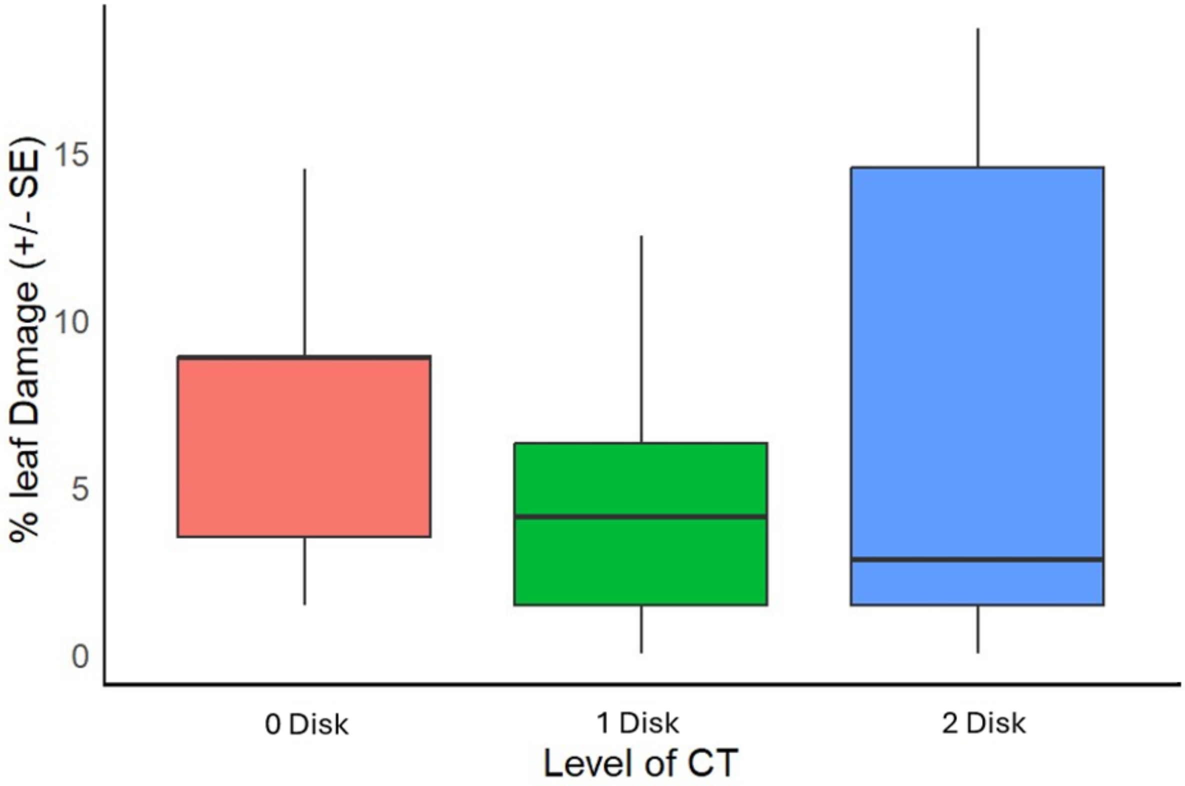


Figure 22. Percentage of leaf damage across dishes with varying levels of CT treatments. Each level represents the number of leaf disks treated with CT (0, 1, or 2) within the dish. the extent of total leaf damage in each petri dish was measured as a proxy for feeding activity. No significant differences in damage were found across the different treatment levels. Error bars represent ± SE.

Discussion

Our field and laboratory studies offer important insights into how gustatory stimulants and attractants might be leveraged to enhance IPM of two species of cucumber beetles by influencing their behavior. We demonstrated two important findings: first, that adding cucurbitacin to an insecticide could enhance its efficacy at label rates; and second, that this addition might enable a reduction in insecticide rates without compromising effectiveness. Our results provide some support for the potential role of gustatory stimulants in managing cucumber beetles, although the effects were varied, and there were some limitations on the conclusions we were able to draw from our various trials.

In our field trial, the addition of a gustatory stimulant containing cucurbitacin, CidetrakL, to the full rate of acetamiprid (Assail) did not further reduce either melon damage at the end of the season or counts of either species of cucumber beetle, nor did it increase beetle mortality in the lab bioassays (Table 5). Our findings are consistent with a previous study combining a different formulation of cucurbitacin (CidetrakD) with the insecticide carbaryl at both the full field rate and a reduced rate (Peterson 2009). Here, this combination of cucurbitacin-containing compounds and insecticides did not significantly improve beetle population control or reduce melon damage either in field trials or within a controlled field cage experiment. However, unlike Pedersen's study, our trials showed that it may be possible to enhance the efficacy of reduced rates of Assail when combined CidetrakL . The combination of CidetrakL and ¼ label rate of Assail achieved levels of both W. striped and W. spotted population control and melon damage reduction comparable to those of the full labeled rates of Assail alone. Unfortunately, due to spatial limitations, we were unable to test Assail at a ¼ label rate without CT and cannot definitively attribute the enhanced performance in this field trial to CT, since it is possible that acetamiprid is effective enough that a four-fold reduction in rate would not have meaningfully reduced efficacy. Nevertheless, this potential benefit of feeding stimulants could be important given California's regulatory landscape, where increased restrictions on neonicotinoids and regulatory concern with pyrethroids make limiting insecticide use important to limit non-target pollinator and environmental effects (California Department of Pesticide Regulation, 2024).

Our lab- based bioassays compared the performance of low rates of Assail both with and without CT in a controlled experiment, building on the field study and examining if there was indeed enhanced beetle mortality with feeding stimulants. We observed consistent performance improvements in Assail at low rates when CT was included. This pattern persisted, even when we tested concentrations of Assail lower than what we used in the field. Our first lab bioassay's results complimented our field observations, showing once again that Assail at ¼ rate combined with CT achieved beetle mortality comparable to the full rate of Assail. (Table 7). By contrast, the ¼ rate without CT resulted in only 50% beetle mortality in this bioassay. There was again no added benefit from combining CT with the full rate of Assail in the lab, with both treatments achieving high beetle mortality. We saw similar results in our second bioassay, where Assail at 1/8th of the labeled rate when combined with CT, produced mortality levels equivalent to the full rate of acetamiprid alone (Table 7).These effects are promising but we acknowledge they occurred where beetle exposure and treatment consistency were tightly regulated, and we only tested these treatments on W. striped CB; although they strongly support further field testing to confirm whether similar levels of control can be achieved under more variable and less controlled environments. Moreover, improvements in field trial methodology, such as testing ¼ rate Assail without CT and adding borders around each plot to prevent beetle migration, could yield more definitive results.

The consistency in effect on both W. striped CB and W. spotted CB in our trials suggests that Assail at a reduced rate (when paired with CT), could be effective for managing both species. In contrast, the indoxacarb (Avaunt) + CT combination performed poorly in our field trials, especially against W. striped CB. We were unable to test Avaunt without CT in the field and chose not to include it in the lab bioassays due to its poor performance. Despite the documented efficacy of indoxacarb (the active ingredient in Avaunt) against other Diabroticene beetle species, it appeared that it might not provide an alternative mode of action for managing

W. striped CB. However, Avaunt's success against corn root worm when combined with CT, underscores the importance of species-specific testing to get better insight on the ecological variability of cucurbitacin's gustatory stimulation. Further field or lab trials could be useful to fully evaluate the potential of a different active ingredient (like indoxacarb) paired with different formulations of cucurbitacin, for use in managing W. striped and W spotted CB.

In field conditions, achieving effective spray coverage and ensuring that pests contact insecticide-treated surfaces or plant material are critical challenges. Our lab studies demonstrated the effectiveness of Assail + CT, particularly at reduced rates, when the distribution of insecticide droplets, and thus beetle-insecticide interactions, was tightly controlled. In previous work, certain insecticides/ cucurbitacin combinations, like (Spinosad + CidetrakD) achieved high beetle mortality under lab conditions, but failed to perform as well in the field (Peterson 2009). In controlled lab settings, beetles are more consistently exposed to lethal doses of insecticide, which may not occur in field environments where various beetle behaviors limit exposure, so pest behavior, especially as it pertains to spray coverage, is a key aspect to consider. For example, W. striped CB's tendency to feed on the underside of leaves or fruit, presents a challenge for achieving optimal spray coverage. As a result, foliar sprays may be less effective unless the applications are carefully timed and targeted, or unless they are systemic. This can be especially challenging if the goal is to reduce negative effects on pollinators and natural enemies, as these systemic compounds can sometimes be present in pollen and nectar (Jeschke 2011).

There is conflicting evidence regarding Diabroticene beetle attraction to various cucurbitacin-containing compounds, which could affect how well they perform as additives to an insecticide application. Although most studies do not show beetle attraction to cucurbitacin-containing materials (Metcalf, 1987), others have found some attraction, but this may be due to the presence of other compounds and volatiles (Andersen 1986). Cucurbitacin itself is not very volatile, which may make it difficult for cucumber beetles to detect from a distance. In addition, the variability in beetle attraction to different cucurbitacin types is not well understood. Earlier formulations of feeding stimulants, such as CidetrakD, were derived from powdered bitter melon and primarily contained cucurbitacin E and I in detectable quantities (Ferguson 1983). For instance, cucurbitacin-insecticide bait combinations were tested in peanuts on Southern corn rootworm (Diabrotica undecimpunctata howardi) in a study by Barbercheck et al. (1995). In this case, yields were not improved and there was no reduction in pod damage, however this could be due to the cucurbitacin formulation. Diabroticine beetles have shown gustatory stimulation from cucurbitacin E, but cucurbitacin B is generally more effective (Eben et al. 1997). Western spotted cucumber beetles were found to be 10 times more sensitive to cucurbitacin B than to cucurbitacin E, and the striped cucumber beetle (A. vittatum) exhibited a similar sensitivity, being 10 times more responsive to cucurbitacin B compared with cucurbitacin E (Metcalf et al. 1980). The specific formulation of cucurbitacin in Barbercheck's study may have limited the bait's ability to arrest cucumber beetles long enough for them to ingest sufficient insecticides. Adding an attractant to the cucurbitacin-insecticide combination could make it more likely the pest locates and consumes the treatments, enhancing its efficacy. The formulation we used in our trials, (CidetrakL), is produced from fermented melon juice, and in our study, we also found this formulation to be unattractive to cucumber beetles; in our Y-tube assays, W. striped CB showed no preference for CT, at multiple concentrations. The apparent lack of attraction, at least to the formulation of cucurbitacin that we used, emphasizes that adding attractants to an CT–insecticide mixture could lead to greater efficacy

Attracting the pest, in this case cucumber beetles, directly to the feeding stimulant and insecticide could be key. Attracticidal baits containing insecticide, cucurbitacin, and synthetic floral attractants have been evaluated in different contexts for managing various Diabroticene species in the past, including work done on Western corn rootworm (*Diabrotica virgifera virgifera*) and Northern corn rootworm (*Diabrotica barberi*). Semiochemical based toxic baits were tested in maize fields and showed significant reductions in rootworm beetle populations (Lance & Sutter 1992). The bait formulation consisted of carbaryl and cucurbitacins in the form of powdered root of *Cucurbita foetidissima* as the feeding stimulant, along with a combination of non-pheromonal volatile compounds from cucurbit plants (eugenol, 4-methoxycinnamaldehyde, 1,2,4-trimethoxybenzene, indole, and trans-cinnamaldehyde). The attracticide was delivered via a broadcast dry, bran-based carrier. Although the bait reduced beetle populations by up to 91% in the short term, the results were less durable in the field due to inconsistent bait application to plants or being washed away. Nonetheless, this study indicated that combining attractants with insecticides and feeding stimulants can be an effective strategy, though improvements in formulation, delivery method, and active ingredients could increase the bait's longevity and field efficacy for use on W. striped CB in California.

We took preliminary steps towards investigating combining a gustatory stimulant, attractant, and insecticide by combining indole with CidetrakL. Our previous fieldwork has shown W. striped CB is attracted to indole, especially when combined with vittatalactone, a male-produced aggregation pheromone (Unpublished data, Seth Jean, Jasmin Ramirez). In addition, in this study, Y-tube assays confirmed that W striped CB was attracted to 10 mg/ml concentration of indole under lab conditions. We attempted to observe the beetle’s behavior towards attractant/feeding stimulant-treated leaf disks in Ethovision bioassays. Despite the attraction of W. striped CB to indole shown in both our field study and Y tube trials, none of the attractant/gustatory stimulant combination treated leaf disks seemed to affect beetle behavior differently, based on time spent on, or near the leaf disks, nor the number of times the leaf zones were visited (Table 8). We also did not see a significant difference in the amount of leaf area consumed between treatments though these results may be due to several limitations in our study. The length of the assays was only 3 hours, so the total time the beetles were able to feed and explore could have led to inconsistent results if beetles did not spend much time feeding or searching for food. The spatial arrangement of the arena, such as the distance between leaf disks, or ventilation holes, could have affected the beetles' behavior. In addition, our limited sample size led to very low statistical power. There is still much to explore in terms of attractant-gustatory stimulants and insecticide combinations for Diabroticene beetles like W. striped and W. spotted CB, particularly in field settings. For western cucumber beetle species, attractants of interest are indole and aggregation pheromone (vittatalactone), as this combo can be highly attractive to cucumber beetles in agricultural field conditions. (Jean, 2024; Ramirez, 2022)

Given the strong attraction of cucumber beetles to compounds like indole and vittatalactone, integrating these with insecticides such as Assail for conventional systems or Spinosad for organic farming could be particularly effective. Spinosad has demonstrated efficacy under controlled laboratory conditions (Andrew 2009). When paired with a gustatory stimulant like Cidetrak in a "attract and kill" strategy, this approach may offer an efficient solution for organic farms where control options are limited. Additionally, these attractive compounds could be adapted for use in a multi-species trap-crop strategy, where plants treated with a combination of indole and pheromones could be deployed to divert beetles away from the main crop. This strategy, focusing on beetle aggregation and targeted control, has the potential to reduce pest pressure on crops while minimizing the overall use of insecticides, but much more work needs to be done to make it viable for growers. Manipulating beetle aggregation for the purposes of targeted control has the potential to reduce pest pressure on crops while minimizing insecticide use. However, achieving long-term viability for growers will require further development. Even with successful adult beetle control, the rapid reestablishment of beetle populations can occur, especially if untreated adult beetles migrate into fields. Additionally, maintaining treatment efficacy throughout the growing season is essential due to the low damage threshold associated with cucumber beetles. In particular, treatments must remain effective until the melons reach maturity and can resist significant damage (Brandenburg et al., 1992) This could pose a problem as indole attraction, particularly to baited traps, appears to be limited mid-season (Seth Jean, 2024; Jasmin Ramirez, 2022).These limitations might be balanced by gains in other areas, such as maintaining healthy populations of pollinators and natural enemies. For example, in a study comparing attracticide-treated plots against broad-spectrum insecticides, significantly more flowers were pollinated and more early fruit were produced in control and attracticide-treated plots compared to plots sprayed with broad-spectrum insecticide. (Lance 1992) This improved pollination could be due to the preservation of pollinators, which would otherwise be disrupted by a high concentration of a broad-spectrum systemic insecticide. Alternatively, it could be due to improved maintenance of natural enemies. In another trial, attracticide-treated plots had higher yields than sprayed plots, largely because the attracticide treatments helped preserve natural enemies, which controlled an aphid outbreak that significantly reduced yields in sprayed plots. Meanwhile, the control plots, which received no pest management treatments, had even lower yields due to higher pest pressure and subsequent crop damage. (Brust et al, 1995).

Although they appear to be promising options , combining CT with attractants, such as indole, and insecticides into a useful bait may be challenging. Integrating indole with feeding stimulants like cucurbitacin requires further investigation to optimize the concentration, medium, and ratio for enhanced control. Furthermore, the lack of increased feeding stimulation by CT treated disks and the lack of behavioral response to the indole-treated leaf disks in the Ethovision study (in contrast to the results seen in the field trial, lab bioassay, Y tube and previous work) suggests that understanding the ecological interactions and behavioral responses of pests and to these compounds is crucial. Other factors, such as the concentration or dispersal method (such as type of substrate, or size of droplets) of the compounds, may need to be adjusted to achieve better results. Additionally, the effectiveness of attract-and-kill strategies can be influenced by environmental conditions, and the physiological state of pests (El-Sayed, 2006). Accounting for these factors is crucial to refine and improve the efficacy of attract-and-kill approaches for cucumber beetle management, but if successful, this approach could lead to the development of more sustainable pest management practices, minimizing insecticide use, reducing risks to pollinators, and lowering costs for growers, all while maintaining effective beetle control.

Tables

Table 1. Treatments used during the field trial to control cucumber beetles. The table lists the chemical name, application rates (per acre), and gallons per acre (GPA) for each treatment, including Assail 30 SG (acetamiprid) at full and reduced rates.

Treatment	**Chemical name**	**Rates (/ac)**	**GPA**
Untreated control	--	--	--
Assail 30SG (full rate)	acetamiprid (foliar)	5.3 oz + 12 fl oz	30
Assail 30SG (full rate) + CidetrakL	acetamiprid (foliar) + gustatory stimulant	5.3 oz + 12 fl oz	20
Assail 30SG (quarter rate) + CidetrakL	acetamiprid (foliar) + gustatory stimulant	1.325 oz + 12 fl oz	20
Avaunt + CidetrakL	indoxacarb + gustatory stimulant	6 oz + 12 fl oz	20

* Brandt On-Site no drift adjuvant was added to all treatments

Table 2. Rating system used to classify melon damage caused by cucumber beetles during the field trial. Scores range from 0 to 5, with 0 representing no damage or very minor scars, and 5 indicating extensive damage where scars or patches blend together. A rating of 1 would be a culled melon in commercial production.

Score	**Damage description**
0	None or small scars below damage rating 1
1	One 24mm diameter scars (quarter coin size) OR four 18 mm size scars
2	Two 24mm diameter size scars OR one 6 cm scar/patch of scars

3	Two 6cm patches OR one 6 cm patch + three 24mm scars OR four 24mm scars
4	Three 6cm patches OR five 24mm scars OR two 6cm patch + three 24mm scars OR extensive light spots
5	Extensive damage, blends together

Table 3. Treatments and application rates used in the laboratory mortality bioassay to evaluate the effects of Assail (acetamiprid) and its combination with CidetrakL (CT) on cucumber beetles. The treatments include varying rates of Assail, ranging from full rate (5.3 oz/acre) to one-eighth rate (0.66 oz/acre), both with and without the addition of CT at 12 fl oz/acre. A control treatment was also included, with no application of Assail or CT. Brandt On-Site no drift adjuvant was added to all treatments

Treatment	**Rate**
Control	N/A
Assail Full rate	Assail at 5.3 oz/acre
Assail Full rate + CT	Assail at 5.3 oz/acre, CT at 12 fl oz/ acre
Assail One-Quarter rate	Assail at 1.325 oz/ acre,
Assail One-Quarter rate + CT	Assail at 1.325 oz/ acre, CT at 12 fl oz/ acre
Assail One-Eight rate	Assail at 0.66 oz/ acre
Assail One-Eight rate + CT	Assail at 0.66 oz/ acre, CT at 12 fl oz/ acre

* Brandt On-Site no drift adjuvant was added to all treatments

Table 4.Y-tube olfactometer treatments used to assess the response of Western striped cucumber beetles (W. striped CB) to the gustatory stimulant CidetrakL (CT) and the attractant Indole. CidetrakL was tested at two concentrations: two times and four times the field application rate, while Indole was tested at 0.5 mg/ml concentration. A blank control was used as a comparison for all treatments.

Treatment	**Rate**	**Concentration**
Control	N/A	N/A
Cidetrak at field rate	12 fl ounce per acre	9.38 ml / L
Cidetrak 2 times field rate	24 fl ounce per acre	18.76 ml / L
Cidetrak 4 times field rate	48 fl ounce per acre	37.52 ml/L
Indole		0.5 mg/ml

Table 5. ANOVA results for the field trial evaluating the various treatments on W. striped CB and W. spotted CB populations, as well as melon damage. Significant differences (P < 0.05) were observed for all response variables across treatments

Response	**Factor**	***df***	***F***	***P***
W. striped CB (live)	Treatment	4	33.80	**<0.001**
	Block	5	1.01	0.43
W. striped CB (dead)	Treatment	4	45.12	**<0.001**
	Block	5	1.02	0.43
W. spotted CB (live)	Treatment	4	16.97	**<0.001**
	Block	5	2.47	0.06

W. spotted CB (dead)	Treatment	4	12.62	**<0.001**
	Block	5	0.27	0.92
Undamaged melons	Treatment	4	*2.83*	**.051**
	Block	5	*2.42*	0.07

Table 7. ANOVA results from laboratory bioassays evaluating the mortality of W. striped CB exposed to various treatments. Bioassay 1 included all treatments used in the field trial, plus assail at ¼ rate without CT. Bioassay 2 included the aforementioned treatments, in addition to Assail at ⅛ rate both with, and without CT. There were significant treatment effects in both bioassays

Bioassay number	**Factor**	***df***	***F***	***P***
Bioassay 1	Treatment	4	57.36	**<0.001**
Bioassay 2	Treatment	6	61.12	**<0.001**

Table 8. Comparisons of W. striped CB behavior in response to leaf disks treated with various combination of Indole and CT. Response variables include time spent in the hemisphere containing the treated leaf, time spent on the leaf itself, visits to the leaf, and the percentage of leaf area consumed. None of the comparisons showed significant differences across treatments.

Response variable	**Treatment comparison**	***V***	***P***
Time in hemisphere	CT - Combo	*7*	*0.56*
	Control -Combo	11	1.0
	Control-CT	10	1.0
	Control-Indole	15	0.44
	Indole -Combo	10	1.0
Time on Leaf	CT - Combo	15	0.44
	Control -Combo	10	1.0
	Control-CT	10	1.0
	Control-Indole	4	0.22
	Indole -Combo	15	0.44
Visits to Leaf	CT - Combo	15	0.43
	Control -Combo	17	0.22
	Control-CT	14	0.56
	Control-Indole	15	0.44
	Indole -Combo	11	1.0

Percentage of leaf damaged	CT – Combo	18	0.14
	Control -Combo	22	0.64
	Control-CT	5	0.29
	Control-Indole	7	0.27
	Indole -Combo	18	0.55

Table 9. Analysis of W. striped CB behavior after being exposed to petri dishes containing leaf disks treated with different levels of CidetrakL (CT) and attractant combination treatments. Two response variables were evaluated: the total percent of leaf area damaged within the petri dish, and the total distance moved by the beetles within the petri dish. Neither the percentage of leaf damage, nor the distance moved showed significant differences

Factor	**Response Variable**	***χ2***	***df***	***P***
Level of CT	Percent of leaf damaged	2.37	2.00	0.31
	Distance Moved	0.65	2.00	0.72

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