Title

Season-long evaluation of an aggregation pheromone, vittatalactone, for two species of cucumber beetles (Coleoptera: Chrysomelidae) key pests of melons in Northern California

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Publication Date
2022

Peer reviewed|Thesis/dissertation

Season-long evaluation of an aggregation pheromone, vittatalactone, for two species of cucumber beetles (Coleoptera: Chrysomelidae), key pests of melons in northern California

By

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Entomology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2022

Abstract:

Semiochemicals like aggregation pheromones are key components of the ecology of a variety of insect species. Insects also use plant-produced volatile organic compounds as kairomones to find plant hosts. Pheromones and kairomones can also be valuable tools for developing pest management strategies. In the Northern Sacramento Valley of California, cucumber beetles - the western striped cucumber beetle (CB), *Acalymma trivittatum*, and western spotted CB, *Diabrotica undecimpunctata undecimpunctata* – are the principal pests of muskmelons, particularly for soft-rind varieties. Adults of both species feed on the fruit surface, rendering fruit unmarketable. Existing management for cucumber beetles relies heavily on broad-spectrum insecticide applications.

There is a need for alternative management tools such as semiochemical-based pest management. A novel aggregation pheromone, vittatalactone, was identified and synthesized from semiochemicals produced by the male striped cucumber beetle, *Acalymma vittatum*, the east coast congener to the western striped CB. Groundwork studies have shown that vittatalactone attracts cucumber beetles in field settings. This presents an opportunity to test vittatalactone as a tool for sampling the western species of cucumber beetle.

We conducted a two-year study to test the efficacy of vittatalactone as an attractant for the western striped and western spotted CB. We also tested if pairing a commercial floral lure with vittatalactone increased beetle captures. Clear-sticky traps attached to wooden stakes were deployed at two commercial farms in the Sacramento Valley with organic cucurbit operations with the following treatments: 1) vittatalactone alone, 2) floral lure alone (only for the second year of the study), 3) a combination of vittatalactone plus floral lure (V+F), and 4) an unbaited

control. Lastly, we measured differences in response to vittatalactone between males and females for one of the years of the study.

We determined that vittatalactone was attractive to both the western striped and western spotted CB. Furthermore, combining the floral lure with vittatalactone enhanced the attraction of both species of cucumber beetle. It also appeared that the floral and vittatalactone treatments were most attractive when the fields were not planted with a crop. We rarely observed significant interactions between treatment x month, and we found no substantial difference in attraction to vittatalactone between females and males.

Overall, our studies demonstrated that vittatalactone is attractive in a field setting to both species of cucumber beetle, while the combination of floral and vittatalactone lures are even more attractive. Additionally, the commercial floral lure was attractive on its own but greatest effects were observed mostly for the western spotted CB. In general, vittatalactone is promising as a tool for effective integrated pest management of cucumber beetles. Vittatalactone, possibly paired with a plant-odor lure, could be used as an attractant for monitoring. Similarly, these attractants could form the foundation of an attract-and-kill strategy used to remove beetles from a field or farm. Based on these data we believe that the most effective times of the year to deploy these semiochemicals would be in the early and late season of crop production prior to planting or post-harvest.

Introduction:

Semiochemicals are central to how insects communicate with conspecifics and interact with their surrounding environment (Pedigo et al. 2006; Price et al. 2011; Wyatt,2014; Tabata, 2018). They provide information that is critical to insects for finding suitable habitats, finding mates, and locating food sources. They can also be valuable tools in Integrated Pest Management (IPM) (Pedigo, 2006; Norin, 2007). The most successful and widely considered semiochemical-based tactics include monitoring, mass trapping, and various types of behavior manipulation. Monitoring is crucial for decision-making and establishing thresholds, whereas tactics like mass trapping/attract-and-kill and mating disruption can be used to directly kill the pest or reduce the ability of the pest to reproduce and infest crops.

Pheromones, including both sex and aggregation pheromones, can be very useful to IPM practitioners. Sex pheromones are typically female-produced and species-specific. They provide options for IPM, especially for many lepidopteran and coleopteran pest species (Birch, 1974; Ando et al. 2004). Sex pheromones are often used for monitoring but can also have a more direct impact via population suppression. For example, the navel orange worm, *Amyelois transitella,* is managed in almonds via mating disruption using synthetic sex pheromone deployed in lures or puffers (Burks, 2019). Similarly, larval populations of the California root borer, *Prionus californicus*, could be reduced by deploying traps with sex pheromones, achieved through some combination of attract and kill of males and mating disruption (Lyons-Yerion et al. 2018). Aggregation pheromones are often attractive to both males and females (Wyatt, 2013), and thus may be beneficial for IPM. Aggregation pheromones are often produced by insects early in the season, typically by males, and signal food and mate availability, creating aggregations of insects. One example is the brown marmorated stink bug (BMSB), *Halyomorpha halys*, which is attracted to the synthetic version of its aggregation pheromone, identified as a mixture of chemical sesquiterpene compounds (Khrimian et al. 2014; Weber et al. 2017; Morrison III et al. 2019). This pheromone is the key component in an attract-and -kill design system developed to reduce BMSB populations and decrease damage to fruit crops (Morrison II et al. 2019). In addition, more than 40 species of weevils (Coleoptera: Curculionidae) have an identified aggregation pheromone that is used in management and monitoring (Bandeira et al. 2021). Aggregation pheromones, like sex attractants, are promising tools in the hunt for semiochemicals-based IPM.

Insects have also evolved to with plants by using volatile, plant-produced semiochemicals. Insects use these volatile chemicals as kairomones to find suitable habitat and hosts. These semiochemicals are produced predominantly by blossoms or leaves of host plants and are essential in insect host selection (Metcalf, 1994; Agelopolous et al. 1999). Kairomones can be used as attractants and insect behavior modifiers. For instance, the codling moth, *Cydia pomonella* (L.), a severe pest of walnuts, apples, and pears, is highly attracted to a pear-derived kairomone that is used as an effective monitoring tool with further potential as a control tool (Light et al. 2000). Similar to insect-produced chemicals, plant-produced kairomones hold potential to help develop better and safer management alternatives to insecticides and novel monitoring tools.

Cucumber beetles (Coleoptera: Chrysomelidae) are good candidates for semiochemical-based management. The western striped cucumber beetle (CB), *Acalymma trivittatum* Mannerheim, and the western spotted cucumber beetle, *Diabrotica undecimpunctata undecimpunctata* Mannerheim, are two key pests in California cucurbits (PMSP 2016). The western striped CB is an oligophagous species that feeds on plant hosts in the family

Cucurbitaceae (Sell, 1915). In comparison, the western spotted CB is a generalist, feeding on cucurbits along with many other crop and non-crop hosts (Sell, 1915). Both species have congeners in the eastern United States, the striped cucumber beetle (*Acalymma vittatum* Fabricius) and the spotted cucumber beetle (*Diabrotica undecimpunctata howardii*, Barber), that are also pests of cucurbit crops (Haber et al. 2021).

In the western states, cucumber beetles are primarily an issue on smooth-rind varieties of muskmelons but can also damage other melons and cucurbit crops (Pedersen and Godfrey, 2009). Adult and larval stages of the western striped CB feed on the fruit surface and create cosmetic damage, leading to unmarketable fruit. The western spotted CB feeds mostly on the foliage of cucurbits and is therefore considered a less problematic pest, although it will also scar developing fruit. Adults of both species are targeted with multiple applications of broad-spectrum insecticides throughout the planting season. However, few effective insecticide modes of action are available, and there is a growing concern about insecticide resistance and non-target effects on pollinators and natural enemies (Pedersen and Godfrey, 2009). Insecticides approved for organic use have not been especially effective. Monitoring for both pests is time-consuming, as scouting typically consists of manually searching fields rather than using traps. The adults are the targeted stage for management because the larvae develop in the roots of cucurbits, which are harder to reach with standard insecticide applications. Insecticide applications targeting adults may fail to achieve sufficient coverage, particularly because adult western CB adults hide under fruit. Better monitoring and management tools would help improve IPM of these cucumber beetle species.

Cucumber beetles rely on both pheromones and plant volatiles to find their cucurbit hosts and to find mates (Metcalf, 1994; Foster 1997; Pinero,2018). Early studies from the east coast led to the hypothesis that the striped CB was capable of colonizing cucurbit fields densely and rapidly due to the involvement of an aggregation pheromone (Smyth and Hoffmann, 2003), subsequently identified as vittatalactone, a novel 2,3 disubstituted beta-lactone produced by male beetles (Morris et al, 2008). Vittatalactone was then synthesized as a mixture of eight semistereospecific isomers (Paraselli and Chauhan, 2017). Field and lab studies demonstrated that the synthesized pheromone was highly attractive to the striped CB (Weber, 2017; Haber et al. 2021; Weber, unpublished data). Interestingly, the spotted CB also was attracted to vittatalactone (Weber 2017). Given the close evolutionary relationship between *A. vittatum* and *A. trivitattum*, we hypothesized that the western striped CB would also produce vittatalactone, and this has since been confirmed (Weber et al. unpublished).

Cucumber beetles detect kairomones emitted by cucurbit blossoms (Andersen & Metcalf, 1985). These kairomones are one of the primary drivers for host selection by cucumber beetles (Andersen & Metcalf, 1985; Pedersen 2009; Pinero 2017). Floral scents have been combined with aggregation pheromones in lures (Light et al. 2001; Tinzaara et al. 2007; Pineda et al. 2021), and hold potential for use in an attract-and-kill strategy. The efficacy of such a combined lure might be further enhanced by using yellow traps, as Pinero (2017) demonstrated that combining the floral lure with the color yellow increased beetle capture cucumbers and squash plantings in Missouri.

We addressed several questions using two years of field trials:

1. Is vittatalactone attractive to the western striped and spotted CB in the field?
2. Does lure attractiveness of the lures vary over the course of the season?
3. Is a floral lure attractive in isolation and in combination with vittatalactone?
4. Do males and females respond differently to vittatalactone?

Materials and Methods

Treatments and study design:

We conducted two field trials in the Sacramento Valley of California in 2020 and 2021 to assess the efficacy of vittatalactone. In Year 1 (2020) three treatments were established: 1) vittatalactone alone, 2) commercial floral lure plus vittatalactone, and 3) control (unbaited). The second treatment was added halfway through the season. In Year 2 (2021) four treatments were established: 1) vittatalactone alone, 2) commercial floral lure alone, 3) vittatalactone plus commercial floral lure (henceforth; combination V+F), and 4) unbaited (control). We deployed clear sticky cards (Pherocon trap, Trécé Inc. -Adair, OK, USA) stapled to the top of 3-ft. long wooden stakes using a completely randomized block design with four replicates per treatment at each location. Stakes were separated by 10 meters within blocks and 20 meters between blocks. All lures were replaced weekly in 2020 and every two weeks in 2021.

Lures:

Vittatalactone lures were produced at the USDA Agricultural Research Service's Beltsville Agricultural Research Center (BARC), Beltsville, MD, and consisted of gray rubber septa (1-F SS 1888 GRY, West Pharmaceutical Services, Lititz, PA) loaded with 1 mg of mixed vittatalactone. For the floral lure, we used the AgBio 5-compound lure P313-B5 (AgBio Inc., Westminster, CO; henceforth, 'Floral lure'). We used one lure per trap (or one of each type for combination treatments).

Locations

Year 1: In 2020, we used two locations in California’s Sacramento Valley for the entire sampling season. We ran trials from 25 April to 10 November. Location 1 was an organic farm near Esparto, CA (38°45'27” N, 122°01'22” W). On 6 April, the area near the traps at Location 1 was planted with mixed muskmelons and summer squash. Location 2 was an organic farm in Davis, CA (38°30'45.9” N, 121°41'34” W). On 7 July, the area near the traps at Location 2 was planted with summer squash. Prior to planting, this location was fallow.

Year 2: Location 1 in Year 2 was the same farm as Location 1 in Year 1. However, traps were placed in a different area of the farm. This organic farm was planted with summer squash on 7 June, 2020. We sampled from 26 March to 10 December, 2020.

Location 2: Due to farming operations that disrupted our sampling, we had to shift Location 2 over the course of the season to three successive locations near Davis, CA (Fig 1). From March 12th -April 25th, we used the same location that was sampled in Year 1 (38°30'45”N, 121°41'34.3”W). Then, from April 25th to June 7th, we moved the field trial 1.4 miles southeast to farm 2 (38°31'16”N, 121°40'10.2”W). Finally, we relocated to a third location 2.8 miles southeast (38°29'54”N, 121° 37' 37” W) of the previous location from June 29th – November 4th (Fig 1).

In-field data collection and lab processing:

Sticky traps were replaced every 7-14 days. Collected traps were transported to the laboratory for insect identification (western striped CB or western spotted CB) and counting. For 2020, a random subsample of up to 20 beetles per card and per species were removed to sex the beetles. If fewer than 20 individuals of a given species were present, we removed all of them. Beetles were placed into 25 mL Falcon tubes with 10-20 mL of mineral spirits to remove glue from the specimens. We identified the sex of beetles by observing their ventral anatomy with a dissecting stereoscope and searching for the dorsal supra-anal plate that is found only in males (White 1977).

Statistical Analysis:

Data analyses were conducted using R version 2022.7.1.554 (R Core Team 2022). To analyze beetle count data, we used generalized linear mixed models (GLMM) implemented with the glmmTMB package ("glmmTMB"; version 1.1.3; Brooks et al. 2017). We analyzed each species × location × year combination separately due to differences in sampling dates and farm production practices between locations. The models included fixed effects of treatment and month and their interaction and a random effect of trap ID to account for repeated sampling through time. Month was used rather than individual sampling period to allow for better fit of models and to better assess the role of time in the study. We used a negative binomial distribution with a log-link function. Analyses of count data for July and for late season in Location 2 were excluded because counts were completely zeroes for western striped CB or extremely low for western spotted CB, preventing proper model fit. Residual plots and scale-location plots were used to assess the appropriateness of each model. We used "Anova" from the car package to obtain P-values for model factors based on type III sums of squares and Wald chi-squared tests. Marginal means were estimated using the emmeams package (R package, V1.4.2, Lenth, et. al., 2019). The same package was used to conduct post-hoc multiple comparisons among lure treatments within month, with a Tukey correction for multiple comparisons.

To test for differences between male and female attraction to vittatalactone for each species of cucumber beetle, we used a general linear model with treatment and block as fixed factors and proportion of males as the response variable; counts of males and females were summed across the season for each trap. We used the lme4 package to run separate models for each species in 2020. Post-hoc tests were ran using emmeams package with a Tukey correction for multiple comparisons. We used $\alpha = 0.05$ throughout all analyses.

Results:

Western striped CB:

In 2020, the western striped CB was attracted to vittatalactone when deployed on its own or when combined with the commercial floral lure (Figure 2). The attractiveness of vittatalactone varied throughout the season, and this effect generally did not vary through time (Table 1-2; Table 9-10). Typically, the sticky traps baited with a combination (V+F) treatment caught more western striped CB than vittatalactone, although the beetle captures were not always statistically significantly different (Figure 2, Table 9- 10).

For the early season in 2020 and at Location 1, vittatalactone-baited traps caught more western striped CB than the unbaited traps in May, but there was no treatment effect for June or July. In comparison, at Location 2, the interaction was not significant, but there was a main effect of treatment (Table 2). Vittatalactone was much more attractive than the unbaited treatment in May and June. Later in the season, starting on the last week of July, we added the combination (V+F) to the initial two treatments. The interaction between treatment and date was not significant for both locations (Table 1-2). We observed a slight increase in captures starting in August for all treatments, including the unbaited traps, although captures were not significantly different among treatments for both locations across months (Table 9-10). In September and October at both locations, beetle counts were the highest for the combination (V+F) treatment and statistically different from at least the unbaited traps (Table 9-10). At the same time, the vittatalactone treatment was generally not statistically different from the unbaited or combination treatment for both locations, although it caught numerically more beetles than the unbaited across months (Table 9-10; Figure 2). On the first week of November at Location 1, we observed a sizeable peak of ~300 beetles per trap in the combination (V+F) treatment. However, the combination treatment did not differ from vittatalactone alone across the month of November because this peak only occurred for one sampling period. In the late season at Location 1, vittatalactone did not differ from the unbaited for the sampled months (Table 9). Differences were more pronounced at Location 2 than at Location 1 for late season, with significant differences among treatments. On one sampling period in November traps for V+ F averaged 65 beetles per trap while vittatalactone traps averaged 20 beetles per trap, with the unbaited half of that (Figure 2). The V+F treatment consistently caught the most beetles for the different months, with the vittatalactone treatment intermediate (Table 10)

In 2021, when there were four treatments (vittatalactone, floral lure, V+F, and unbaited), the interactive effects treatments and date and the main effects of treatment varied by location, although the effect of treatment was typically not influenced by month (Table 3- 4). The combination treatment was again the most attractive, followed by vittatalactone, then the floral lure (Figure 3; Table 11-12). At Location 1, vittatalactone and the floral lure generally attracted similar numbers of western striped CB (Figure 3; Table 11). These treatments typically did not differ from the unbaited treatment. Differences among treatments were most pronounced early in the season April-July, Table 11). The combination (V+F) treatment again caught the most beetles. Later in the year, captures generally did not differ among treatments aside from the combination (V+F) catching more western striped CB (Table 11). In Location 2 and at location 2.1, the effect of treatment varied by month. In March, the vittatalactone, floral, and combination (V+F) were significantly more attractive compared to the unbaited treatment, but were not different from each other. At location 2.2 (starting in May), the combination (V+F) treatment was again more attractive than the rest of the treatments, with the floral lure and vittatalactone alone more attractive than the unbaited treatment. Again (Table 12). Finally, for location 2.3 and in July and August, the combination treatment was more attractive than the rest of the treatments, with the floral lure next most attractive (Table 11). However, western striped CB captures at location 2.3 were consistently low, especially starting in September, with average counts near zero across all treatments.

Western spotted CB:

In 2020, traps baited with vittatalactone or vittatalactone in combination with the commercial floral lure were attractive to the western spotted CB. Yet, beetle captures were significantly different between vittatalactone and the combination (V+F) at different periods through the sampling season, and the interaction between treatment and date was significant for the early season at Location 1 but not later in the season (Table 5-6; Table 13-14). Usually, the sticky traps baited with the combination (V+F) treatment caught the most western spotted CB (Figure 4, Table 13-14). In the early season, the effect of treatment was influenced by date for Location 1 (Table 5). In contrast, at Location 2, there was only a main effect of treatment (Table 5-6). Vittatalactone was more attractive to the western spotted CB than the unbaited treatment in May at both sampling locations (Table 13-14). In June and July, the pattern was different for each location. At Location 1, both unbaited traps and vittatalactone-baited traps caught western spotted CB, with no differences between treatment. Meanwhile, at Location 2, the captures gradually decrease close to zero after the initial beetle captures in May.

Later in the season, the interaction between treatment and date was not significant at either location, and there were generally consistent differences among treatments across months. At Location 1, we observed an increase in captures in August for all treatments, including the unbaited traps (Table 13, Figure 4). Location 2 had an increase in captures starting in July, although captures were not as high as at Location 1. At both locations, the combination (V+F) treatment generally caught the most western spotted CB within each month (Table 13-14). For example, in September, the vittatalactone and floral lure treatment caught the most western spotted CB at both locations (Figure 4, Table 13). The vittatalactone treatment meanwhile caught comparable numbers of beetles to the unbaited. At Location 1 in October, the treatments did not differ, although general patterns were the same for captures of western spotted CB, and the combination treatment continued to have higher captures, followed by vittatalactone, numerically. At the second site, the combination (V+F) was more attractive than vittatalactone. Lastly, on the first week of November at Location 1, we observed a sizeable peak averaging approximately 900 beetles per trap, which was much higher than vittatalactone alone or the unbaited treatment. The vittatalactone treatment had intermediate captures at Location 1. Moreover, a similar event was also observed at the Location 2, with significant differences between the combination (V+F) and the rest of the treatment; combination (V+F) traps averaged 140 beetles per trap while vittatalactone traps averaged 20 beetles per trap.

In 2021, overall, the main effects of treatment and date varied across locations (Table 7-8). Additionally, date did not influence the treatment effect except for location 2.2 in the Davis area (Table 8). Nevertheless, the combination (V+F) was the most attractive treatment, numerically, for most of the season, followed by vittatalactone, then the floral lure (Figure 5; Table 15-16). At Location 1, vittatalactone and the floral lure attracted similar numbers of western spotted CB with minor differences in specific months (Figure 5; Table 15). For example, at Location 1, the captures were always the highest statistically for the combination (V+F) compared to the rest of the treatments, aside from in December when captures had decreased. However, the pattern for vittatalactone alone and floral lure alone varied throughout the season at Location 1. For example, starting in April, the floral lure and vittatalactone were similarly attractive. Then in May, the floral lure attracted twice as many western spotted CB than vittatalactone, although this difference wasn't significant (Table 15). Captures gradually decreased in June and July, and the floral lure and vittatalactone captured similar numbers of beetles. Western spotted CB captures increased in August and September for all treatments and the floral treatment caught more than the unbaited in both months, although vittatalactone only did so in September. Finally, from October to December, we observed a decrease in captures, with minimal statistical differences for the floral lure or vittatalactone alone (Table 15).

At Location 2 in 2021, we observed a similar trend where the combination treatment tended to be the most attractive. Results are not reported for location 2.1 because counts were so low. At location 2.2, captures were low and there were no differences among treatments (Table 16). Lastly, at location 2.3, captures were not different among treatments in July. In August and September, the combination treatment was generally more attractive than the rest of the treatments (but not significantly different than the floral lure treatment in August; Table 16). Vittatalactone did not differ from the unbaited at these time points. Finally, in October, treatments did not differ, although the numerical trends remained the same, with the combination continuing to have highest counts, followed by the floral lure and vittatalactone.

Sex ratios:

Western striped CB:

Proportions of male western striped CB caught were not statistically different between the unbaited and vittatalactone treatments (Location 1, $F_{4,3}= 6.75$, $P=0.07$; Location 2, $F_{4,3}= 0.56$, $P=0.71$). Captures for both types of traps were male-biased.

Western spotted CB:

The proportion of male western spotted CB caught at Location 1 was greater for the vittatalactone treatment than the unbaited treatment ($F_{4,3}= 24.94$, $P=0.012$). At Location 2, the proportion of male beetles caught did not differ between the two treatments ($F_{4,3}= 2.18$, $P=0.27$) (Table 19).

Discussion:

Vittatalactone alone, and the floral lure alone, were attractive to each species of cucumber beetle in the field. The combination of the commercial floral lure and vittatalactone enhanced captures of beetles for both species, with typically additive effects for the two attractive components. We found that the addition of vittatalactone to a trap did not attract more females and males of either beetle species.

There was not a significant interaction between date (month) and treatment in the overall analyses for most location/year combinations for each species of CB, indicating that date (month) did not substantially alter the attraction of vittatalactone or the rest of the lure treatments. Nonetheless, there were some differences and inconsistencies in how treatments affected CB attraction in the different months. There were some months for certain years and locations with very few captures of each species, in particular midseason. It is possible that populations of CB were between generations, but it also could be that the attractiveness of vittatalactone or the floral lure could be dependent on the growth stage of the crop. However, our results did not point to this consistently being the case, and our field trial design could not separate between effects of crop phenology, beetle population dynamics, or other environmental factors. Both species of CB did appear to have some of the most pronounced differences between the unbaited treatment and the treatments with vittatalactone in the early in the season or late in the season in specific post-harvest.

Overwintering populations of cucumber beetles do migrate in high numbers into planted fields once vegetation is present and temperatures are higher (Smyth and Hoffmann, 2003; Pedersen, 2009), which could drive this. Furthermore, Smyth and Hoffman (2003) suggested that male striped CB, *Acalymma vittatum* pioneered the early colonization of cucurbit fields and that these were successful at infesting fields due to olfactory cues produced by themselves -that is, the aggregation pheromone. These studies back up our findings that vittatalactone captures more beetles in the early- season or when cucurbits are not in the field.

This study determined that both species of CB in California are attracted to vittatalactone, and our study provides a starting point for implementing semiochemicals for cucumber beetles in the Western US. The western striped CB has also been shown to produce the same aggregation pheromone as the eastern species via coupled gas chromatography - electroantennographic detection- GC-EAD (Weber et al, unpublished). Whereas the western spotted CB has not been tested we do speculate that the attraction to vittatalactone is due to biological processes such as locating hosts for food. The response of both eastern and western congeners to vittatalactone demonstrates their shared evolutionary history. Meanwhile, the western spotted CB responded to vittatalactone, an attraction that spanned across genera of cucumber beetles.

For both species, vittatalactone did not alter the sex ratio of the beetles captured on the traps. The one exception was western spotted CB at Location 1, where the catch was skewed slightly more male when vittatalactone was added to the trap. Generally, more males were caught than females overall, but this appears to because they were more abundant or more active than females. Our results align with prior studies where females and males were also subjected to preference tests over combination treatments (Smyth and Hoffmann, 2003). These preference tests assessed beetle attraction to plant and aggregation pheromones emitted by the striped CB and found no differences in between female or male preference (Smyth and Hoffmann, 2003).

The results obtained through our studies contribute to the existing research that has suggested vittatalactone, an aggregation pheromone, as a potential tool for managing cucumber beetles (Smyth & Hoffmann 2009; Weber, 2018). Using vittatalactone as a monitoring tool could improve the timing of broad-spectrum insecticides and provide a better understanding of population dynamics. Our results suggest vittatalactone likely would perform best if combined with floral volatiles. Furthermore, because there are currently no monitoring tools, vittatalactone presents an opportunity to develop a commercial aggregation pheromone and therefore provide growers with additional resources.

Incorporating vittatalactone in an attract-and-kill (ATK) strategy designed to manage cucumber beetles would offer growers another tool to mitigate CB damage on high-value cucurbits. Given our results we believe that vittatalactone alone or in combination with the floral lure would be most effective in an ATK design in the early or late in the season while cucurbits are not planted. Previous studies have suggested that the western striped CB shelters in non-crop hosts when cucurbits are not planted during the winter season, and they will feed on unmanaged weeds if necessary (Pedersen 2009, Ramirez Bonilla, unpublished). Incorporating knowledge of how cucumber beetles, especially western striped CB, use non-crop habitat of CB prior to planting and at the end of the season could be leveraged for deployment of ATK. BMSB is an example where ATK approach was proved successful by incorporation its aggregation pheromone to weekly insecticidal sprays that were perimeter driven (Morrison III et al. 2018).

In summary, vittatalactone is attractive to both the western striped and western spotted CB while the addition of a floral lure can increase beetle captures. Efficacy of each lure varied some throughout the season but is generally consistent across months. The potential of vittatalactone alone or combined with the commercial floral lure could serve as a monitoring tool or even a successful management strategy to mitigate CB damage. Further studies would help clarify the chemical ecology of these beetle species and more fully analyze the synergy between the floral scents and vittatalactone. Additionally, future research could examine incorporating floral lure and vittatalactone as components of ATK strategies and assess their efficacy at alleviating CB damage in cucurbits.

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Figures:

Figure 1. Map depicting three consecutive locations in Davis, CA, where trial from "location 2" had to be relocated due to grower logistics outside of our control. Location 1 labeled Davis 2.1— fallow field sampled from March 12 to April 25, 2021. Location 2- Davis 2.2, conventional watermelon field sampled from April 25 to June 7, 2021. Third location – Davis 2.3, organic summer squash sampled from June 29 to October 10, 2021

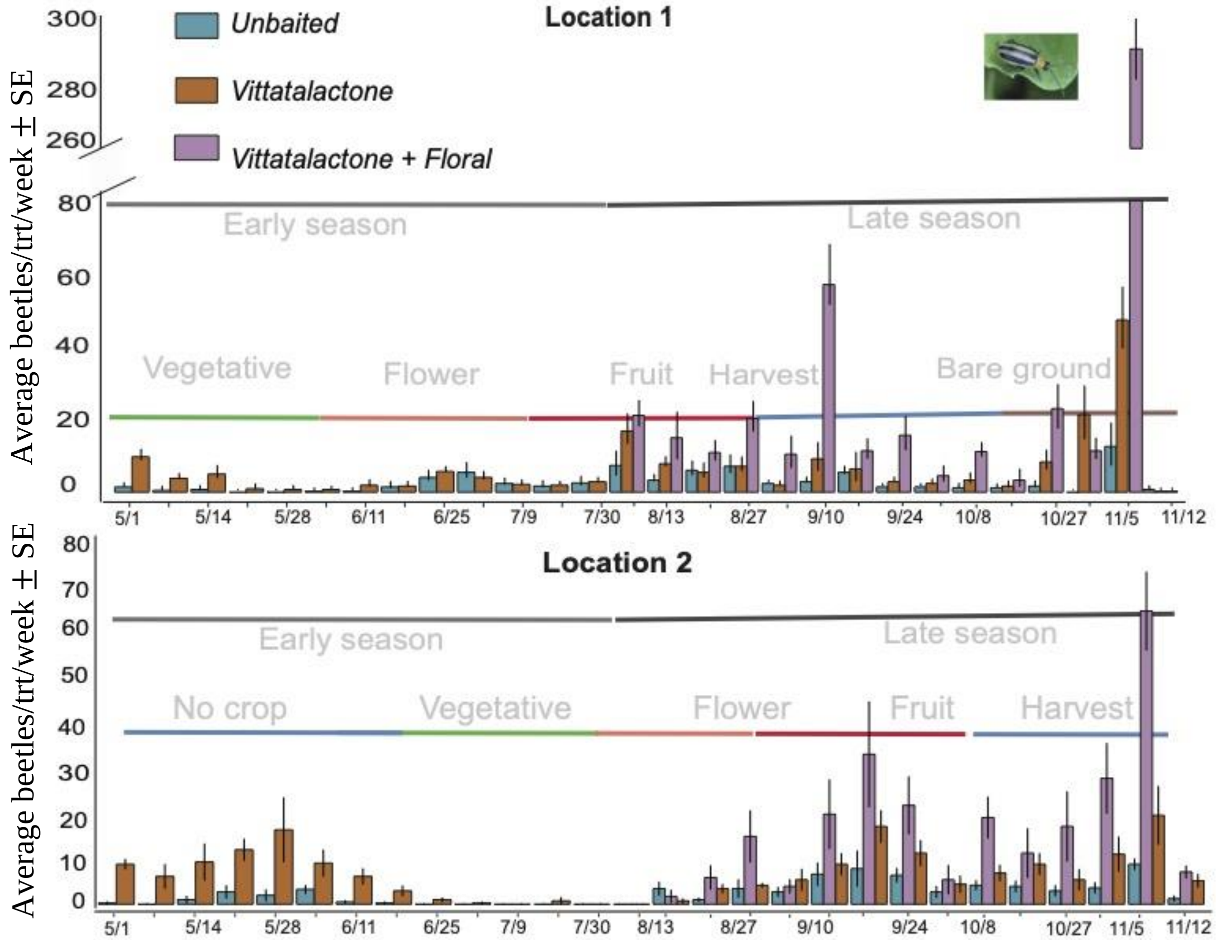

Figure 2. Average weekly counts for each lure treatment for western striped CB for A) Location 1 and b) Location 2 in Year 1 (2020). Values are means ±SE. Growth stage of the cucurbit crop for the Location 1 and location 2 are indicated

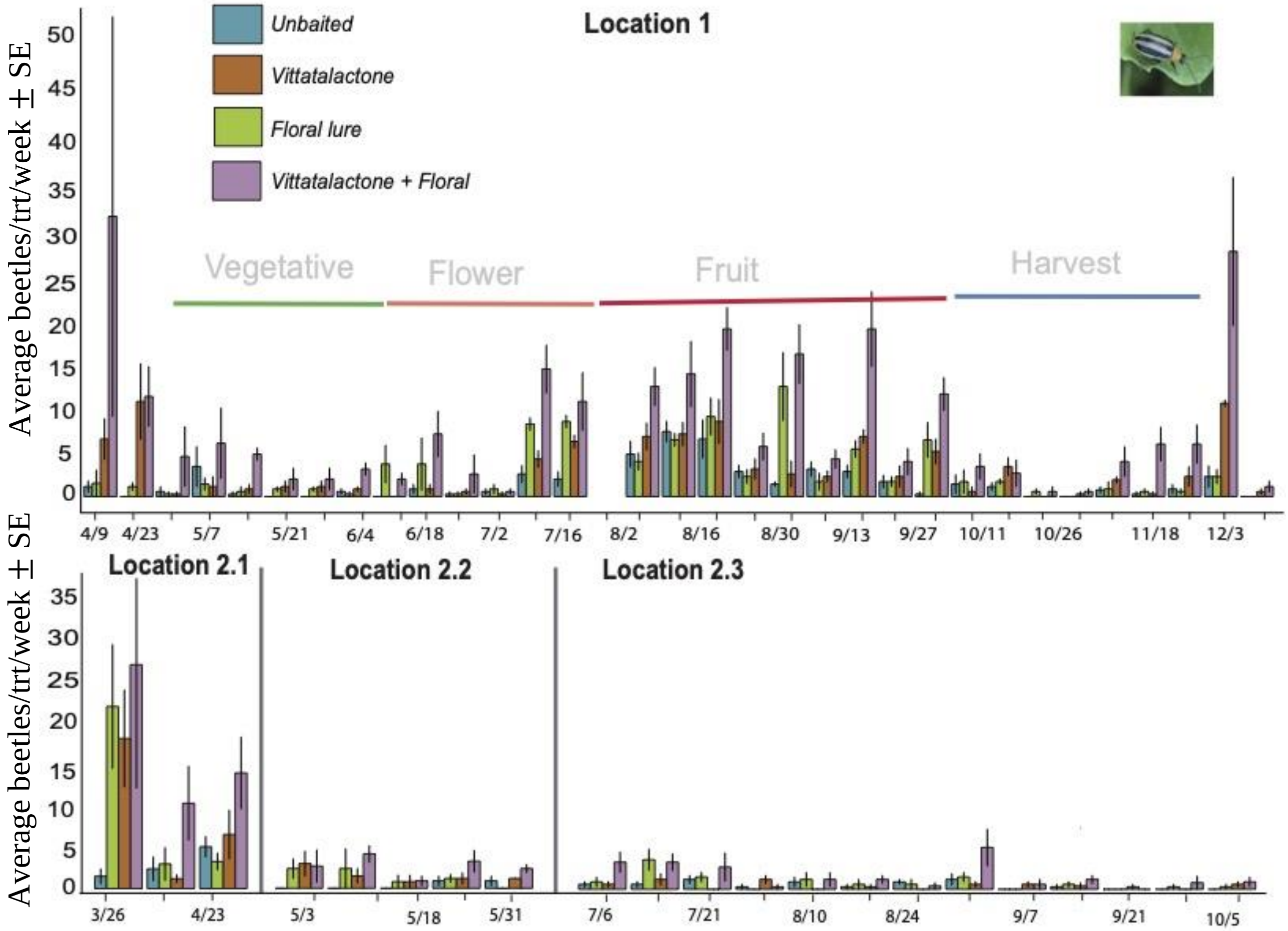

Figure 3 Average weekly counts for each lure treatment for western striped CB for both locations in Year 2 (2021). Values are means ±SE. Growth stage of the cucurbit crop for the Location 1 is indicated, but not 2-4 given the complexity with those sites. In second graph dark gray vertical lines demarcate each consecutive location for the Davis area

ge beetles/treatment/week ± SE

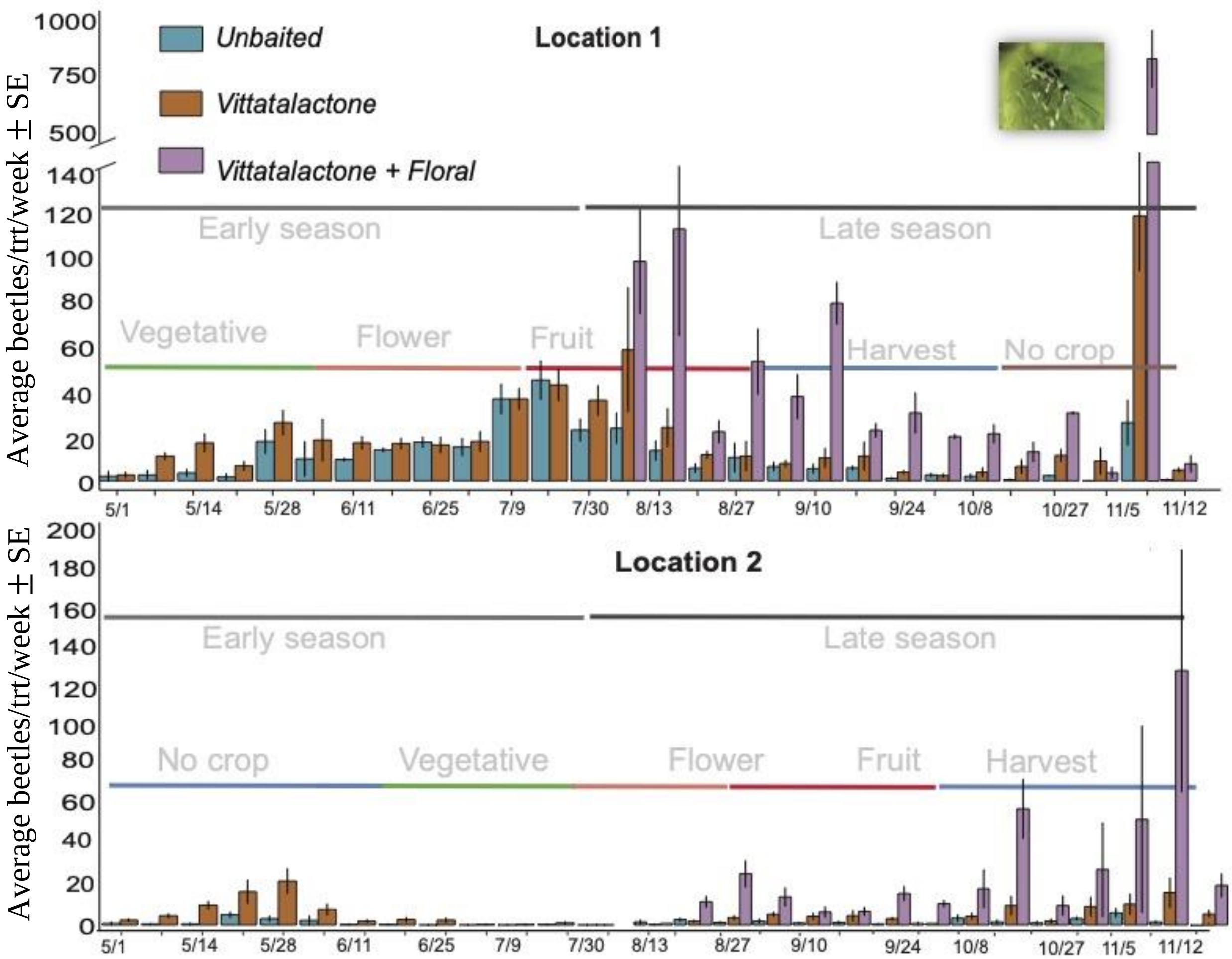

Figure 4. Average weekly counts for each lure treatment for western spotted CB for both locations in Year 1 (2020). Values are means ±SE. Growth stage of the cucurbit crop is shown in gray letters with color bars.

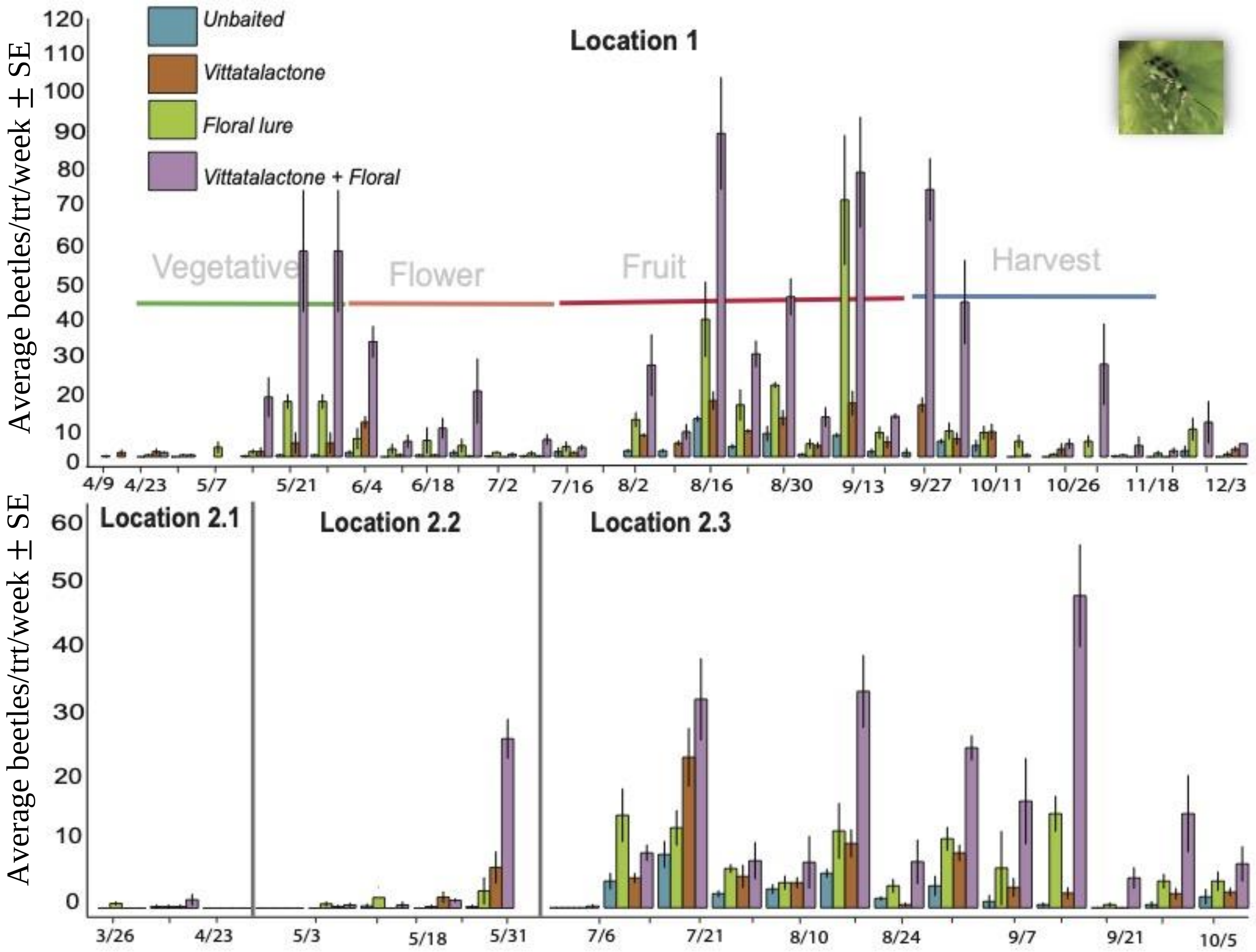

Figure 5. Average weekly counts per treatment ±SE for western spotted CB for both locations in Year 1 (2021). Graph also includes the growth stage of cucurbit crop. Growth stage of the cucurbit crop for the Location 1 is indicated, but not 2.1,2.2, and 2.3 given the complexity with those sites. In second graph dark gray vertical lines represent each consecutive location for the Davis area

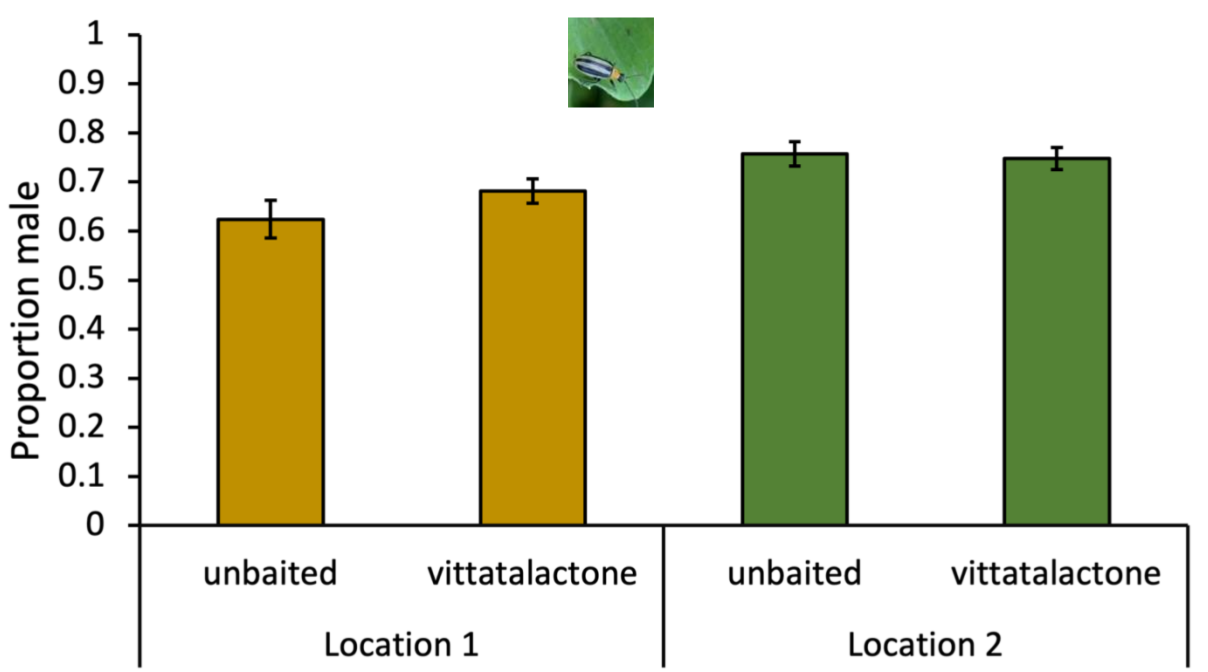

Fig 6. Western striped CB male proportions ±SE per treatment for each location in 2020.

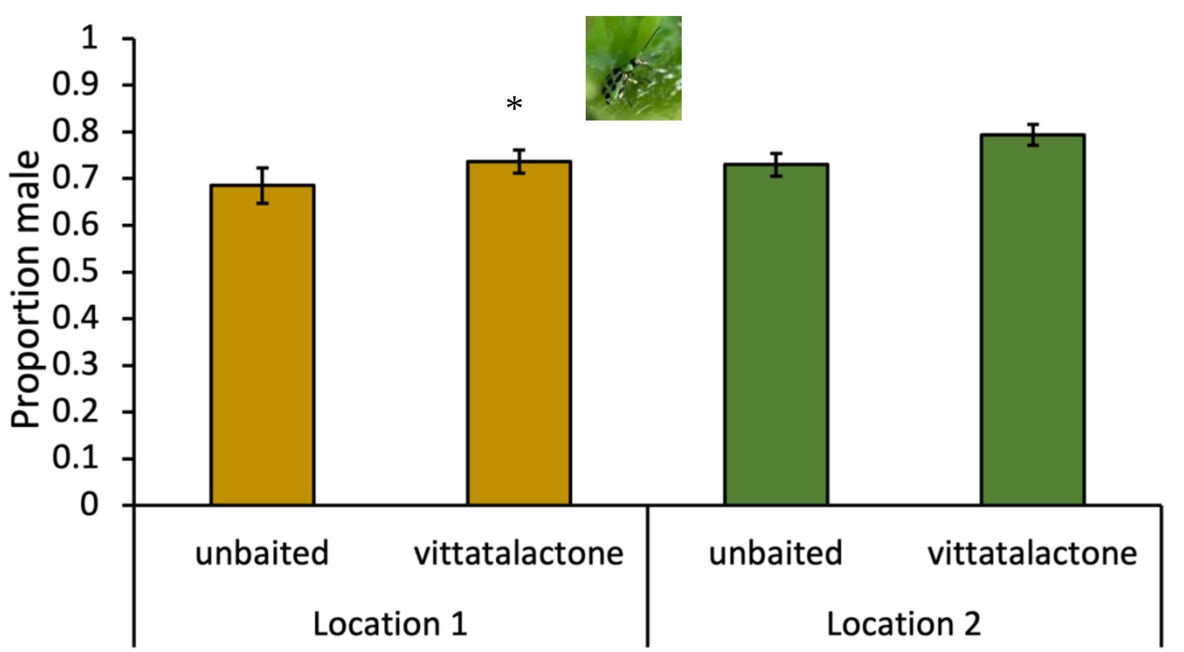

Fig 7. Western spotted CB male proportions ±SE per treatment for each location in 2020. An asterisk indicates a significant difference between treatments at $\alpha = 0.05$.

<u>Tables:</u>

Table 1. Western striped CB, location 1, 2020. Wald chi-squared values for main and interaction effects of treatment and date (month).

Early-season	**χ^2-value**	**df**	**p-value**
Treatment	17.06	1	<0.001
Date	9.47	2	<0.01
Date * Treatment	9.71	2	<0.01
Late-season			
Treatment	1.88	2	0.38
Date	3.75	4	0.44
Date * Treatment	3.17	8	0.92

Table 2. Western striped CB, location 2, 2020. Wald chi-squared values for main and interaction effects of treatment and date (month).

Early-season	**χ^2-value**	**df**	**p-value**
Treatment	34.14	1	<0.001
Date	0.04	2	0.98
Date * Treatment	1.32	2	0.52
Late-season			
Treatment	1.87	2	0.39
Date	3.21	3	0.36
Date * Treatment	4.12	6	0.66

Table 3. Western striped CB, location 1, 2021. Wald chi-squared values for main and interaction effects of treatment and date (month).

	χ^2-value	***df***	***p-value***
Treatment	40.20	3	<0.001
Date	45.32	8	<0.001
Date * Treatment	28.59	24	0.2

Table 4. Western striped CB, location 2, 2021. Wald chi-squared values for main and interaction effects of treatment and date (month).

Location 2.1	***χ^2-value***	***df***	***p-value***
Treatment	11.59	3	<0.01
Date	1.14	1	0.29
Date * Treatment	10.83	3	<0.05
Location 2.2			
Treatment	9.64	3	<0.05

Date	1.59	1	0.2
Date * Treatment	1.31	3	0.72
***Location* 2.3**			
Treatment	14.89	3	<0.01
Date	4.54	3	0.21
Date * Treatment	6.52	9	0.68

Table 5. Western spotted CB, location 1,2020. Wald chi-squared values for main and interaction effects of treatment and date (month).

Early season	χ^2-value	df	p-value
Treatment	11.3	1	<0.001
Date	70.11	2	<0.001
Date * Treatment	8.69	2	<0.05
Late season			
Treatment	2.47	2	0.29
Date	6.86	4	0.14
Date * Treatment	3.3	8	0.9

Table 6. Western spotted CB, location 2, 2020. Wald chi-squared values for main and interaction effects of treatment and date (month).

Early season	χ^2-value	df	p-value
Treatment	22.9	1	<0.001
Date	10.38	2	<0.01
Date * Treatment	0.68	2	0.71
Late season			
Treatment	16.59	2	0.001
Date	3.48	3	0.32
Date * Treatment	1.82	6	0.94

Table 7 Western spotted CB, location 1, 2021. Wald chi-squared values for main and interaction effects of treatment and date (month).

	χ^2-value	df	p-value
Treatment	7.75	3	0.05
Date	35.2	9	<0.001
Date * Treatment	32.97	27	0.19

Table 8 Western spotted CB, location 2, 2021. Wald chi-squared values for main and interaction effects of treatment and date (month).

Location 2.1	χ^2-value	df	p-value
Treatment	NA	NA	NA
Date	NA	NA	NA
Date * Treatment	NA	NA	NA
***Location* 2.2**			
Treatment	2.81	3	0.42
Date	0.6	1	0.43

Date * Treatment	9.97	3	<0.05
***Location* 2.3**			
Treatment	2.4	3	0.49
Date	10.69	3	<0.05
Date * Treatment	15.38	9	0.08

Table 9. Western striped CB, location 1, 2020. Mean captures based on estimated marginal means ±SE, with values back transformed from a log scale with values back-transformed from the log-scale to the response scale

Month	TRT	Mean	SE	group
May	unbaited	0.62	0.25	a
	vittatalactone	3.82	0.69	b
June	unbaited	2.07	0.49	a
	vittatalactone	3.05	0.60	a
July	unbaited	2.63	0.72	a
	vittatalactone	3.03	0.74	a
August	unbaited	12.65	3.34	a
	vittatalactone	15.00	3.75	a
	vittatalactone + floral	24.07	5.51	a
September	unbaited	9.99	2.43	a
	vittatalactone	11.38	2.81	a
	vittatalactone + floral	24.72	4.80	b
October	unbaited	5.55	2.31	a
	vittatalactone	13.60	4.10	ab
	vittatalactone + floral	22.54	6.91	b
November	unbaited	9.71	3.89	a
	vittatalactone	12.06	5.08	a
	vittatalactone + floral	23.78	8.90	a

Table 10. Western striped CB, location 2, 2020. Mean captures based on estimated marginal means ±SE, with values back transformed from a log scale with values back-transformed from the log-scale to the response scale

Month	TRT	Mean	SE	group
May	unbaited	1.20	0.42	a
	vittatalactone	10.57	1.45	b
June	unbaited	1.07	0.45	a
	vittatalactone	4.86	1.05	b
July	unbaited	NA	NA	NA
	vittatalactone	NA	NA	NA
August	unbaited	4.09	1.12	a
	vittatalactone	5.12	1.30	a
	vittatalactone + floral	6.67	1.63	a
September	unbaited	7.47	1.65	a
	vittatalactone	11.74	2.21	ab
	vittatalactone + floral	17.38	2.87	b
October	unbaited	6.31	1.54	a
	vittatalactone	9.54	1.94	ab
	vittatalactone + floral	17.27	2.94	b
November	unbaited	6.00	2.04	a
	vittatalactone	12.44	3.19	ab
	vittatalactone + floral	26.42	3.19	b

Table 11 Western striped CB, location 1, 2021. Mean captures based on estimated marginal means ±SE, with values back transformed from a log scale with values back-transformed from the log-scale to the response scale

Month	TRT	Mean	SE	group
April	unbaited	0.89	0.38	a
	Floral	1.23	0.42	ab
	Vittatalactone	3.54	0.88	b
	Vittatalactone + Floral	8.51	1.58	c
May	unbaited	0.35	0.20	a
	Floral	0.97	0.34	a
	Vittatalactone	1.27	0.41	ab
	Vittatalactone + Floral	3.03	0.69	b
June	unbaited	0.61	0.27	a
	Floral	1.32	0.47	ab
	Vittatalactone	0.61	0.27	a
	Vittatalactone + Floral	2.34	0.62	b
July	unbaited	3.13	0.81	a
	Floral	6.78	1.25	ab
	Vittatalactone	5.80	1.14	a
	Vittatalactone + Floral	11.45	1.68	b
August	unbaited	0.42	0.84	a
	Floral	6.64	1.13	a
	Vittatalactone	4.76	0.91	a
	Vittatalactone + Floral	11.97	1.50	b
September	unbaited	1.99	0.49	a
	Floral	2.98	0.64	a
	Vittatalactone	3.14	0.65	a
	Vittatalactone + Floral	6.72	1.00	b
October	unbaited	0.50	0.29	a
	Floral	1.03	0.42	a
	Vittatalactone	1.06	0.45	a
	Vittatalactone + Floral	1.45	0.58	a
November	unbaited	0.00	0.00	a

	Floral	1.25	0.41	a
	Vittatalactone	2.69	0.71	a
	Vittatalactone + Floral	7.44	1.20	b
December	unbaited	0.00	0.00	a
	Floral	0.00	0.00	a
	Vittatalactone	0.93	0.66	a
	Vittatalactone + Floral	1.28	0.85	a

Table 12. Western striped CB, location 2, 2021. Mean captures based on estimated marginal means ±SE, with values back transformed from a log scale with values back-transformed from the log-scale to the response scale

Month	Treatment	Mean	SE	group
March	unbaited	1.51	1.07	a
	Floral	16.92	5.15	b
	Vittatalactone	14.37	4.62	b
	Vittatalactone + Floral	19.52	5.7	b
April	unbaited	3.37	1.36	a
	Floral	2.93	1.22	a
	Vittatalactone	2.97	1.24	a
	Vittatalactone + Floral	9.09	2.91	a
May	unbaited	0.25	0.18	a
	Floral	1.32	0.41	ab
	Vittatalactone	1.35	0.39	ab
	Vittatalactone + Floral	2.21	0.49	b
June	unbaited	NA	NA	NA
	Floral	NA	NA	NA
	Vittatalactone	NA	NA	NA
	Vittatalactone + Floral	NA	NA	NA
July	unbaited	0.75	0.28	a
	Floral	1.66	0.43	ab
	Vittatalactone	0.51	0.24	a
	Vittatalactone + Floral	2.55	0.54	b
August	unbaited	0.64	0.20	a
	Floral	0.62	0.21	a
	Vittatalactone	0.39	0.16	a
	Vittatalactone + Floral	1.22	0.31	a
September	unbaited	0.08	0.08	a
	Floral	0.24	0.14	a
	Vittatalactone	0.32	0.16	a
	Vittatalactone + Floral	0.53	0.22	a
October	unbaited	0.00	0.00	a

	Floral	0.30	0.30	a
	Vittatalactone	0.59	0.42	a
	Vittatalactone + Floral	0.79	0.51	a

Table 13. Western spotted CB, location 1, 2020. Mean captures based on estimated marginal means ±SE, with values back transformed from a log scale with values back-transformed from the log-scale to the response scale

Month	TRT	Mean	SE	group
May	unbaited	5.00	1.04	a
	vittatalactone	15.08	3.59	b
June	unbaited	10.51	1.77	a
	vittatalactone	13.76	2.12	a
July	unbaited	28.86	4.34	a
	vittatalactone	31.48	4.47	a
August	unbaited	26.08	7.07	a
	vittatalactone	33.20	8.44	ab
	vittatalactone + floral	63.81	14.33	b
September	unbaited	17.95	4.68	a
	vittatalactone	21.89	5.50	a
	vittatalactone + floral	51.44	10.44	b
October	unbaited	10.75	4.53	a
	vittatalactone	24.63	7.49	a
	vittatalactone + floral	36.26	12.13	a
November	unbaited	19.21	7.87	a
	vittatalactone	46.42	14.83	ab
	vittatalactone + floral	98.76	26.51	b

Table 14 Western spotted CB, location 2, 2020. Mean captures based on estimated marginal means ±SE, with values back transformed from a log scale with values back-transformed from the log-scale to the response scale

Month	Treatment	Mean	SE	group
May	unbaited	2.31	0.66	a
	vittatalactone	10.43	1.58	b
June	unbaited	0.56	0.33	a
	vittatalactone	4.46	1.07	b
July	unbaited	NA	NA	NA
	vittatalactone	NA	NA	NA
August	unbaited	4.49	1.49	a
	vittatalactone	8.27	2.29	ab
	vittatalactone + floral	18.20	4.32	b
September	unbaited	2.82	1.09	a
	vittatalactone	5.39	1.74	a
	vittatalactone + floral	16.01	3.87	b
October	unbaited	4.64	1.65	a
	vittatalactone	7.98	2.36	a
	vittatalactone + floral	23.72	5.34	b
November	unbaited	7.16	2.73	a
	vittatalactone	13.76	4.40	a
	vittatalactone + floral	50.26	10.97	b

Table 15 Western spotted CB, location 1, 2021. Mean captures based on estimated marginal means ±SE, with values back transformed from a log scale with values back-transformed from the log-scale to the response scale

Month	Treatment	Mean	SE	group
April	Unbaited	0.67	0.48	a
	Floral	2.28	0.86	ab
	Vittatalactone	2.44	0.93	ab
	Vittatalactone + Floral	7.53	2.11	b
May	Unbaited	2.26	0.81	a
	Floral	10.02	2.27	b
	Vittatalactone	5.66	1.54	ab
	Vittatalactone + Floral	39.20	5.09	c
June	Unbaited	1.45	0.65	a
	Floral	4.43	1.26	ab
	Vittatalactone	1.12	0.56	a
	Vittatalactone + Floral	8.40	1.98	b
July	Unbaited	2.92	1.10	a
	Floral	6.36	1.88	ab
	Vittatalactone	4.52	1.47	ab
	Vittatalactone + Floral	10.90	2.67	b
August	Unbaited	8.48	1.92	a
	Floral	20.25	3.58	b
	Vittatalactone	13.23	2.60	ab
	Vittatalactone + Floral	36.45	4.94	c
September	Unbaited	4.68	1.21	a
	Floral	16.11	2.79	b
	Vittatalactone	11.09	2.08	b
	Vittatalactone + Floral	37.38	4.46	c
October	Unbaited	1.28	0.74	a
	Floral	6.54	1.86	ab
	Vittatalactone	4.03	1.46	ab
	Vittatalactone + Floral	7.65	2.41	b

November	Unbaited	1.27	0.64	a
	Floral	4.51	1.32	ab
	Vittatalactone	1.72	0.77	a
	Vittatalactone + Floral	8.22	1.98	b
December	Unbaited	0.00	0.00	a
	Floral	1.27	1.26	a
	Vittatalactone	5.76	2.78	a
	Vittatalactone + Floral	3.81	2.55	a

Table 16 Western spotted CB, location 2, 2021. Mean captures based on estimated marginal means ±SE, with values back transformed from a log scale with values back-transformed from the log-scale to the response scale

Month	Treatment	Mean	SE	group
March	Unbaited	0.00	0.00	NA
	Floral	0.75	0.43	NA
	Vittatalactone	0.00	0.00	NA
	Vittatalactone + Floral	0.00	0.00	NA
April	Unbaited	0.12	0.09	NA
	Floral	0.12	0.09	NA
	Vittatalactone	0.12	0.06	NA
	Vittatalactone + Floral	0.57	0.26	NA
May	Unbaited	0.13	0.13	a
	Floral	0.56	0.28	a
	Vittatalactone	0.49	0.25	a
	Vittatalactone + Floral	0.74	0.30	a
June	Unbaited	NA	NA	NA
	Floral	NA	NA	NA
	Vittatalactone	NA	NA	NA
	Vittatalactone + Floral	NA	NA	NA
July	Unbaited	4.97	1.63	a
	Floral	7.45	2.23	a
	Vittatalactone	6.74	2.08	a
	Vittatalactone + Floral	9.51	2.59	a

August	Unbaited	6.29	1.37	a
	Floral	10.15	1.92	ab
	Vittatalactone	7.32	1.60	a
	Vittatalactone + Floral	15.15	2.63	b
September	Unbaited	1.14	0.58	a
	Floral	6.30	1.69	b
	Vittatalactone	3.35	1.10	ab
	Vittatalactone + Floral	17.28	3.21	c
October	Unbaited	2.74	1.89	a
	Floral	6.10	3.14	a
	Vittatalactone	5.80	2.75	a
	Vittatalactone + Floral	7.79	3.79	a

Table 17. Western striped CB, location 1, 2020. Mean proportion male ± SE per treatment

Treatment	mean	SE	group
Unbaited	0.62	0.02	a
Vittatalactone	0.68	0.02	a

Table 18. Western striped CB, location 2, 2020. Mean proportion male ± SE per treatment.

Treatment	mean	SE	group
Unbaited	0.75	0.03	a
Vittatalactone	0.76	0.03	a

Table 19. Western spotted CB, location 1, 2020. Mean proportion male ± SE per treatment.

Treatment	mean	SE	group
Unbaited	0.68	0.01	a
Vittatalactone	0.74	0.01	b

Table 20. Western spotted CB, location 2, 2020. Mean proportion male ± SE per treatment.

Treatment	mean	SE	group
Unbaited	0.73	0.02	a
Vittatalactone	0.79	0.02	a