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Short Communication

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Field Attraction of Striped Cucumber Beetles to a Synthetic Vittatalactone Mixture

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Abstract

The striped cucumber beetle, *Acalymma vittatum* (F.) (Coleoptera: Chrysomelidae), is a key pest of cucurbits in eastern North America, rapidly colonizing young plantings and vectoring bacterial wilt of cucurbits. Its aggregation pheromone has been identified and synthesized stereospecifically, but has not been field tested to date. Here, we present field bioassays of this pheromone, using mixed vittatalactone made with a novel and cost-efficient semispecific synthesis. This mixture of eight stereoisomers of (2*R*,3*R*)-vittatalactone proved highly attractive to both sexes of striped cucumber beetle, using two different trap types and a pilot attract-and-kill combination with watermelon containing the diabroticine feeding stimulant cucurbitacin-E-glycoside, under field conditions in cucurbit vegetable plantings. Availability of mixed vittatalactones could enable highly effective and specific management of striped cucumber beetle.

Key words: aggregation pheromone, attract-and-kill, cucurbitacin, Diabroticina

The striped cucumber beetle, *Acalymma vittatum* (F.) (Coleoptera: Chrysomelidae), is a specialist herbivore on plants of the Cucurbitaceae, native to eastern and central North America, with one to three (typically two) generations per year, and is a key pest of crops in this family such as cucumbers, squash, melons, and pumpkins. In addition to significant feeding damage which may kill seedlings, adults vector *Erwinia tracheiphila*, the cause of bacterial wilt of cucurbits, which can kill susceptible crop plants of any age (Saalau Rojas et al. 2015).

Male striped cucumber beetles emit an aggregation pheromone, vittatalactone (2-methyl-3-(2,4,6,8-tetramethyloctyl)oxetan-3-one), attractive to both sexes, when feeding on a favored host (Smyth and Hoffmann 2003, Morris et al. 2005). The vittatalactone molecule has five stereogenic centers, leading to 32 possible stereoisomers; only a single isomer is emitted by the male beetle, specified in the 2 and 3 positions by Morris et al. (2005) and in the remaining positions by Schmidt and Breit (2009) as (2*R*,3*R*,4*S*,6*S*,8*S*)-vittatalactone. Several authors (Schmidt and Breit 2009, Schmidt et al. 2010, Weise et al. 2012, Yadav et al. 2011) have devised successful stereospecific syntheses of vittatalactone; however, none of these have been produced in sufficient quantities for field evaluation.

Cucurbitacins induce feeding of a number of diabroticine beetles, including *Acalymma* and *Diabrotica* species (Metcalf and Lampman 1989, Cabrera Walsh et al. 2008, Toepfer et al. 2009). Attraction of these beetles at a distance could be enhanced by deployment of their volatile pheromones, with the cucurbitacin(s) then serving as arrestants and feeding stimulants in an attract-and-kill strategy.

Chauhan and Paraselli (2017) devised and patented a synthesis that produces eight stereoisomers of vittatalactone with the ring configuration (2*R*,3*R*), including the one active isomer. This semistereospecific synthesis (i.e., that producing an isomeric mix at three chiral centers, of the five in the molecule) enables less expensive production of the *A. vittatum* aggregation pheromone in quantities useful for field testing and application. Here, we provide field results from these 'mixed vittatalactones' deployed as lures, with and without a cucurbitacin source and toxicant, to demonstrate both the attraction of the mixed vittatalactones, and a potentially effective combination of attractant, arrestant, and toxicant, for field suppression of *Acalymma*. We also discuss their deployment in potential pest monitoring and suppression in light of the availability of useful quantities of vittatalactone.

Materials and Methods

Attractants

Lures containing the eight-isomer synthetic 'mixed vittatalactones' for field studies in September 2013 were a gift from the senior author of Chauhan and Paraselli (2017). The published patent outlines the synthesis and its match to the natural vittatalactone. Here, we report replicated field trials for this material.

General Field Information

Four cucurbit fields at the USDA Agricultural Research Service's Beltsville Agricultural Research Center (BARC), Beltsville, MD, were used to test the attractiveness of the mixed vittatalactones. These fields were planted during May (winter squash) or July (other crops) of 2013 and were not treated with any insecticides or fungicides (including no seed treatments). Gray rubber septa (1-F SS 1888 GRY, West Pharmaceutical Services, Lititz, PA) were loaded with 100 µg, 1 mg, or 2 mg of mixed vittatalactones and deployed for a maximum of 10 d during September and October 2013.

Sticky Trap Test

White sticky traps (Pherocon II trap, Trécé Inc., Adair, OK; Supp Fig. S1A [online only]) were deployed in three fields: a 0.53-ha field of mixed summer squash (cvs. 'Yellow Crookneck' and 'Raven Zucchini,' Johnny's Seed [Albion, ME], planted July 22, BARC North Farm, 39°01′58″N, 76°55′50″W), a 0.28-ha cucumber field (cv. 'Marketmore 76,' Johnny's Seed) planted July 18, BARC North Farm, 39°01′55″N, 76°56′05″W), and a 0.65-ha field of mixed winter squash (cvs. 'Waltham Butternut,' 'Gold Nugget,' 'Blue Hubbard,' 'Sunshine,' 'Sweet Dumpling Delicata,' and 'Carnival Acorn,' Johnny's Seed, planted May 22, BARC South Farm, 39°01′07″N, 76°56′40″W). On 20 September, the traps were deployed as randomized complete blocks with three treatments (0, 100 µg, or 1 mg loading of mixed vittatalactones) in four blocks in each of the three fields (layout, Supp Fig. S2A [online only]). The traps for each block were deployed in a triangular arrangement with 13 m between traps and at least 15 m between adjacent blocks. Lures were placed inside the lure basket from a green Unitrap (International Pheromone Systems Ltd., Cheshire, United Kingdom, purchased from Great Lakes IPM, Vestaburg, MI) inside the bottom of the trap (see Supp Fig. S1A [online only]). Traps were collected on 23, 27, and 30 September, rerandomized at each collection, and all chrysomelid beetles, including striped cucumber beetle and spotted cucumber beetle *Diabrotica undecimpunctata howardi* Barber (Coleoptera: Chrysomelidae), removed and placed in vegetable oil to remove sticky material; specimens were then determined as to sex.

Bucket Trap Test

Unitraps (International Pheromone Systems Ltd.) were deployed in a factorial test of two different-colored traps and two lure loadings plus blank: three completely randomized blocks of six traps each with three all-green traps, and three traps that had white buckets, yellow funnels, and green roofs (known as 'multicolor' traps; layout shown in Supp Fig. S2B [online only]). Each of these traps was placed on the ground on 1 October 2013, in a 0.61-ha field of fruiting bitter Hawkesbury watermelon (planted 18 July) on BARC North Farm (39°01′42″N, 76°56′00″W; Supp Fig. S1B [online only]). The opening of the trap was at 20 cm height. Septa loaded with 0, 1 mg, or 2 mg of mixed vittatalactone were placed in lure baskets, and kill strips (10% dimethyl 2,2-dichlorovinyl phosphate, Hot Shot, Spectrum Brands Holdings, Middleton, WI) were placed in the bucket to kill insects that were captured beneath the funnel opening. Traps were emptied on 4 and 8 October 2013 and rerandomized between intervals.

Test With Insecticide-Laced Cucurbitacin-Rich Bait Fruit

Green Unitrap tops with lures of 0, 1, or 5 mg of mixed vittatalactone were hung approximately 10 cm above bitter Hawkesbury watermelons cut crosswise and placed upright (Supp Fig. S1C [online only]) with approximately 100 mg of Entrust 80WP formulation (AI 80.0% spinosad, Dow AgroSciences). This insecticide is registered for use on cucurbit vegetables, and although cucumber beetles are not listed as target pests, it is very effective against both species by oral ingestion induced by cucurbitacin-containing fruits or their juice (Weber, unpublished data). The 5-mg dose was provided by two 2-mg septa and one 1-mg septa. In total, six insecticide-laced melon halves were positioned on 1 October in two randomized blocks at either end of the same fruiting crop of bitter Hawkesbury watermelon in which the bucket traps, above, were deployed (Supp Fig. S2B [online only]). The number of chrysomelid beetles, including striped and spotted cucumber beetles, were counted and collected on top of, under, and near (within 20 cm of) the fruit on 4 October 2013. Due to apparently significant predation of beetles on 8 October, the numbers on this date were not counted, and the experiment was terminated.

Statistics

Trap captures were analyzed by analysis of variance (ANOVA) after conversion to block proportions, which were then transformed as arcsine of square root of the proportions (SAS Institute 1998). If the ANOVA was significant, means separation was applied as Tukey's HSD with $\alpha = 0.05$. In addition, for the bucket and melon trap tests, linear regression was applied to test for a dose response for separate captures of both species.

Results

Adult striped cucumber beetles were significantly attracted to lures deployed in the sticky traps, as shown in the results in winter squash (ANOVA $F(2,21) = 39.05$, $P < 0.0001$; Fig. 1). The cucumber and summer squash fields had much lower numbers captured; however, the trends were similar (Supp Fig. S3 [online only]). For sticky traps, zero beetles were caught in unbaited traps, whereas 159 beetles were

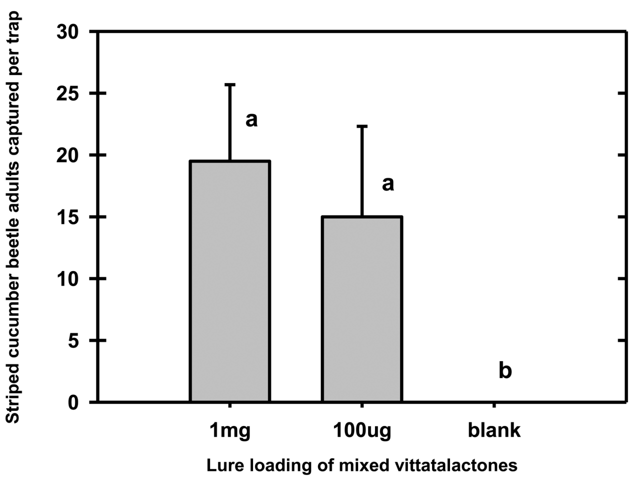

Fig. 1. Captures of *Acalymma vittatum* in white sticky traps in winter squash field, Beltsville, MD, 20–30 September 2013, means per trap over entire period. Letters indicate means which differ by Tukey's HSD.

recovered from baited sticky traps within the 10-day study period. Means comparison showed no significant dose response (difference between 100 µg and 1 mg loading). 59.5% of adults captured were males; however, the binomial 95% confidence interval (49.7, 68.7%) for this proportion was not significantly different from 1:1 at $\alpha = 0.05$. Males and females responded similarly (interaction not significant between sex and dose $F(2,18) = 0.388$, $P = 0.78$) and positively to the presence of mixed vittatalactones.

For bucket traps, the mean trap captures did not differ by funnel color. Bucket traps of yellow and green funnel color captured similar numbers of striped cucumber beetles (total 97 and 114, respectively, ANOVA $F(1,14) = 1.58$, $P = 0.23$) and far fewer spotted cucumber beetles, only two beetles each in yellow and green funnel traps, respectively. However, there was a significant interaction of funnel color and dose ($F(1,14) = 4.56$, $P = 0.05$), such that yellow-funnel traps had no significant dose response (regression $P = 0.88$), but all-green traps showed a dose response (Fig. 2) with $P = 0.038$ and a regression $y = 5.167 + 7.5x$ ($R = 0.693$).

Poisoned melons attracted comparable numbers of striped and spotted cucumber beetle adults. However, significant response to mixed vittatalactones was detected only in striped cucumber beetles (Fig. 3). Attraction to the bitter Hawkesbury slices, regardless of vittatalactone dose, is expected, because these melons typically contain approximately 0.05% cucurbitacin-E-glycoside, a powerful feeding stimulant to both species (Schroder et al. 1998), and the juice is also attractive from a distance (Cabrera Walsh et al. 2008, 2014a).

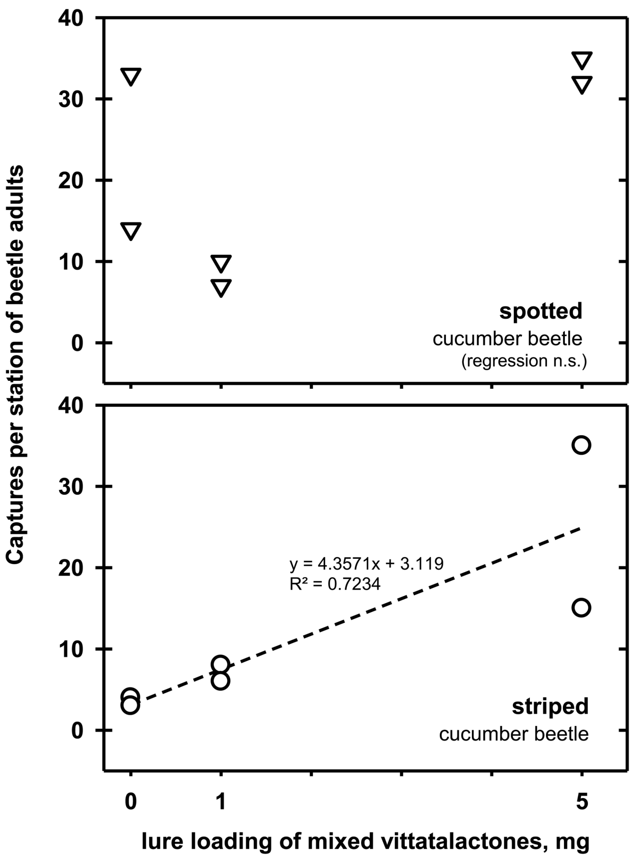

Fig. 3. Captures of spotted (top) and striped cucumber beetles (bottom) at insecticide-laced bitter Hawkesbury watermelon slices, in watermelon field, Beltsville, MD, 1–4 October 2013. Regression is significant for striped, $P = 0.032$, but not for spotted, $P = 0.215$.

Discussion

Striped cucumber beetle populations, as well as vectored bacterial wilt, cause severe crop losses in the absence of control in eastern and central North America. Chemical control through repeated insecticidal treatments and/or systemic seed treatments is costly, environmentally risky, and often less than completely effective. Many growers incur significant added expense of transplanting greenhouse-grown cucurbits to avoid the high risk of loss in direct-seeded fields. Perimeter trap cropping with preferred high-cucurbitacin squash cultivars has shown promise in winter squash; however, this can reduce grower choice and flexibility in plantings (Cavanagh et al. 2009, 2010; Gardner et al. 2015; Brzozowski et al. 2016).

A combination of mixed vittatalactones with cucurbitacins and low-risk toxins in traps, bait stations, or attract-and-kill formulations in trap crops or main crops offers a potentially more effective and economical alternative for protection of cucurbits in a variety of settings including direct-seeded and high-tunnel crops.

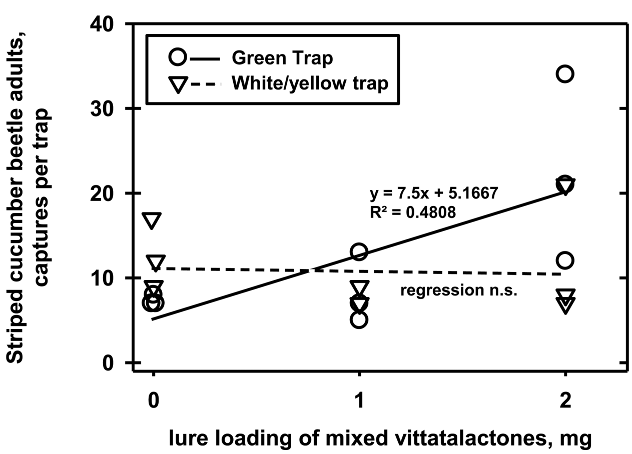

Fig. 2. Dose response of striped cucumber beetles to mixed vittatalactone-baited Unitraps (all-green and white/yellow traps), in watermelon field, Beltsville, MD, totals for 1–8 October 2013.

Use of cucurbitacins with vittatalactone has a number of benefits. The known arrestant and feeding stimulant properties of cucurbitacins, for striped cucumber beetles and other Diabroticina, including pest *Diabrotica*, is key to effectiveness of baits and also enhances trap effectiveness (Metcalf and Lampman 1989, Behle 2001, Chandler 2003). Their feeding stimulation makes feasible use of low rates of toxins and/or those that are most effective when ingested, such as the spinosad used in our pilot trial. And finally, their antifeedant properties for many mandibulate generalist feeders (Tallamy et al. 1997) would be expected to decrease nontarget effects, and these have been found negligible in bait deployment (McKenzie et al. 2002, Cabrera Walsh et al. 2014b).

Gardner et al. (2015) and others have demonstrated the importance of squash flowers for attraction of striped cucumber adults. Visual and volatile floral attractants are significantly attractive both to striped and to spotted cucumber beetles (Piñero 2018). However, floral cues should be used only after further investigation in trapping or attract-and-kill tactics with striped cucumber beetles because of possible increased capture of bees with plant volatiles (Gregg et al. 2018). In addition, certain flower-associated colors may also attract nontargets, and specifically, for the traps used here, multicolored Unitrap bucket traps, compared with identical all-green traps, caught greater numbers of both bees and coccinellids in an extensive comparative study (Spears et al. 2016). A visual stimulus clearly may not be important to striped cucumber beetle aggregation, as suggested by their remarkable attraction to visually inconspicuous cucurbit seedlings in the field.

Both attraction and arrestment are important in successful behavioral control of pest insects in the field (Hokkanen 1991, Holden et al. 2012). Combination of a long-distance attractant for both sexes with a powerful arrestant and feeding stimulant could serve to effectively mitigate both spillover and halo effects in an attract-and-kill strategy (Wallingford et al. 2018). Further experiments over the entire period of adult striped cucumber beetle activity, and in a variety of crops, will be necessary to test the value of this approach as part of an effective strategy to manage striped cucumber beetles and related species.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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