



## Ecology and Behavior

# Trapping of Crucifer-Feeding Flea Beetles (*Phyllotreta* spp.) (Coleoptera: Chrysomelidae) With Pheromones and Plant Kairomones

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## Abstract

Flea beetles (Coleoptera: Chrysomelidae) of the genus *Phyllotreta* are major pests of cole crops, canola, and related crops in the mustard family (Brassicaceae). Adults may damage seedlings or larger crop plants, impairing crop growth, rendering crops unmarketable, or killing seedlings outright. The two major North American crucifer pest species, *Phyllotreta striolata* (F.) and *Phyllotreta cruciferae* (Goeze), have male-produced pheromones attractive to both female and male adults. We tested the racemic synthetic pheromones, himachaladiene and hydroxyhimachalanone, as well as the host-plant-produced allyl isothiocyanate, alone and in combination, with experimental trapping in Maryland, Virginia, and North Dakota, using clear and yellow sticky traps and the ground-based ‘rocket’ trap (modified from boll weevil trap). *Phyllotreta striolata* was consistently attracted to the hydroxyketone, and captures were often enhanced by allyl isothiocyanate (AITC), but its response to pheromones, AITC, and trap color were variable from state to state. *Phyllotreta cruciferae* was strongly attracted to AITC, but its response to pheromone components varied by state, and this species was found rarely at the Maryland site. *Phyllotreta bipustulata* (F.) was attracted to the diene component, a new finding for this species. Several other genera of flea beetles were captured, some showing response to the semiochemicals and/or color. Results will be helpful in monitoring and possibly population suppression; however, further research is necessary to develop more efficient syntheses, optimal lure loadings, combinations, and controlled release methods.

**Key words:** monitoring, integrated pest management, chemical ecology

Flea beetles (Coleoptera: Chrysomelidae) are key pests of cole crops, canola, and related mustard family (Brassicaceae) crops in North America. Two of the most serious pest species are the crucifer flea beetle, *Phyllotreta cruciferae* (Goeze), and the striped flea beetle, *Phyllotreta striolata* (F.). By chewing numerous tiny holes in leaves, they may inflict damage on seedlings or larger crop plants that impairs crop growth, renders the crop unmarketable, or kills small plants outright (Knodel et al. 2017, Capinera 2020). Control

tactics are often needed to prevent economic damage from these pests (Mason et al. 2020). Several flea beetle species, including these two, are known to have male-produced aggregation pheromones, i.e., pheromones attractive to both male and female adults. Pesticide resistance is developing to widely used systemic neonicotinoid insecticides in North American canola for at least for one flea beetle species in North America (Knodel 2017; Knodel et al. 2017; Tansey et al. 2008, 2009), necessitating efforts to integrate effective

non-pesticidal controls in order to mitigate evolution of insecticide resistance. Semiochemical-based tactics, based on better knowledge and improved synthesis of pheromones and other attractants, could contribute to a more sustainable and integrated pest management strategy for flea beetles. Furthermore, with improved efficiency in synthesis, these attractants can be more affordable to allow widespread testing and appropriate potential adoption.

Tóth et al. (2005) discovered that (+)-(6R,7S)-2,2,6,10-tetramethylbicyclo[5.4.0]undeca-9,11-diene (or (+)-(6R,7S)-himachala-9,11-diene in terpene nomenclature) was the key pheromone component of *P. cruciferae*, and was very strongly attractive to adults when presented in combination with the mustard volatile allyl isothiocyanate (AITC) in field conditions. Furthermore, additional related male-specific compounds (C, E, and H in both Tóth et al. 2005 and Bartelt et al. 2003) 'had no obvious biological activity.' Importantly, for cost-efficient production, the (-) enantiomer of (6R,7S)-himachala-9,11-diene was not repellent (Tóth et al. 2005), thereby affording use of racemic himachala-9,11-diene, which could be produced by the method of Bartelt et al. (2003).

Beran et al. (2016) found that, for *P. striolata*, the structurally analogous compound (3S,9R,9aS)-3-hydroxy-3,5,5,9-tetramethyl-5,6,7,8,9,9a-hexahydro-1H-benzo[7]annulen-2(3H)-one [or (6R,7S)-10-hydroxyhimachalan-9-one in terpene nomenclature (hydroxyketone for short)] was the key aggregation pheromone component that attracted this species, especially with himachala-9,11-diene. To the best of our knowledge, there is no information in the literature about the use of racemic hydroxyketone and himachala-9,11-diene in field trappings of *P. striolata* and *P. cruciferae*.

Isothiocyanates are characteristic volatiles of mustard-family plants (Renwick 2002). Several investigators in North America and Europe have found allyl isothiocyanate to be attractive to crucifer-feeding *Phyllotreta* spp. (Vincent and Stewart 1984; Soroka et al. 2005; Tóth et al. 2009, 2012). Vincent and Stewart (1984) found that, using water-pan traps, both *P. cruciferae* and *P. striolata* were attracted to AITC, but attraction of *P. cruciferae* was stronger, relative to numbers sampled by D-Vac in adjacent rutabaga field, and captures in unbaited water-pan traps.

Vincent and Stewart (1986) found that color had a less strong influence than AITC on *Phyllotreta* captures, but that white- and yellow-colored sticky surfaces attracted more of both *P. cruciferae* and *P. striolata* than did green or red. Yellow sticky traps are the standard traps used for monitoring flea beetles in canola and other crops in Brassicaceae (Knodel 2017, Knodel et al. 2017). However, sticky traps saturate with high densities of captures, both target and non-target (Brown 1984, Knodel and Agnello 1990, Kuenen and Siegel 2016). They are not reusable and generate specimens that are difficult to remove and often in a degraded condition for identification. Therefore, we aimed to determine if we could improve on the yellow sticky trap by determining the effects of color (yellow versus clear) and an alternative 'rocket' trap design (Soroka et al 2005), in combination with the known attractants for the two principal North American pest species: their pheromone components and the mustard volatile AITC.

## Materials and Methods

### Chemical Synthesis and Lure Preparation

All chemicals for the pheromone syntheses and allyl isothiocyanate (95% purity) were purchased from Aldrich Chemical Company (Milwaukee, WI). TLC analyses were conducted on Whatman AL SIL G/UV plates using 20% ethanol solution of phosphomolybdic acid and/or UV for visualization of spots. Flash chromatography was carried out with 230–400 mesh silica gel from Fisher Scientific.

GC-MS analyses were performed in electron impact (EI) ionization mode at 70 eV with an Agilent Technologies 5973 mass selective detector interfaced with 6890 N GC system and equipped with 30 m × 0.25 mm i.d. × 0.25 μm film HP-5MS Agilent J&W column. The column temperature was maintained at 40 °C for 5 min, and then raised to 190 °C at 15 °C/min for himachala-9,11-diene analysis. For hydroxyketone analysis, the column temperature was maintained at 40 °C for 5 min, then raised at 7 °C/min to 240 °C, and then again raised to 270 °C at 15 °C/min. Helium was used as a carrier gas at 1 ml/min.

The synthesis of the main component of the aggregation pheromone of *P. cruciferae*, the racemic himachala-9,11-diene (referred to as 'diene' below), was conducted following Bartelt et al. (2003). The final product consisted of 82% of desired (6R,7S/6S,7R)-himachala-9,11-diene and 18% of (6R,7S/6S,7R)-10-methylenehimachal-11-ene as expected. It was used in field studies without further purification. GC-MS of the main product (m/z, %): 204 (M<sup>+</sup>, 50), 189 (18), 161 (33), 133 (100), 119 (80), 105 (74), 93 (29), 91 (37), 77 (15), 69 (15). Mass spectral data matched those reported in Bartelt et al. (2001).

The main component of *P. striolata* aggregation pheromone, 10-hydroxyhimachalan-9-one (referred to as 'hydroxyketone' below), was synthesized by a 3-chloroperbenzoic acid oxidation of the 82:18 mixture of himachala-9,11-diene and 10-methylenehimachal-11-ene described above following Bartelt et al. (2011). From 157 mg (0.76 mmol) of starting diene, we isolated after flash chromatography on SiO<sub>2</sub> with hexane/ethyl acetate, 4:1, 54 mg of 10-hydroxyhimachalan-9-one of 73% purity as determined by GC-MS (m/z, %): 218 (M<sup>+</sup>-18, 6), 203 (10), 193 (100), 179 (6), 161 (6), 151 (6), 135 (10), 123 (11), 109 (24), 95 (15), 83 (49). R<sub>f</sub> = 0.19 (hexane/ethyl acetate, 4:1). Mass spectral data matched those reported in Bartelt et al. (2011). This hydroxyketone was used in field experiments without further purification.

Both diene and hydroxyketone lures were loaded on grey rubber septa (The West Company, Kearney, NE) at 200 μg/lure rate as described by Khrimian et al. (2008). This rate was chosen for both pheromone components because it was in the range successfully used by Soroka et al. (2005) and Tóth et al. (2005) for *P. cruciferae* as well as Beran et al. (2016) for *P. striolata*.

### Trapping experiments in North Dakota, Maryland, and Virginia

#### Experiments With Sticky Traps and Pheromone Combinations

In North Dakota in May 2018, we set up four spatial blocks of five treatments in a randomized complete block (RCB) two-way factorial: ±diene and ± hydroxyketone with clear sticky cards, and a blank yellow card treatment as a standard comparison to the blank clear sticky trap. Traps were stationed (with 10 m separation, and > 10 m between blocks) at the edge of newly-planted canola field, (48.760868 N, 98.333678 W) ~2.5 km E of Langdon (Cavalier Co.), ND. Sticky cards were collected, re-randomized, and lures and cards replaced weekly for 6 wk, start 22 May, and ending 2 July, to make a total of 24 spatiotemporal blocks.

In Virginia, in 2018, the experimental design was the same as in North Dakota, four RCB for 6 wk (24 spatiotemporal blocks), starting 5 June and ending 17 July, at Virginia Tech Kentland Farm, in Whitethorne (Montgomery Co.), Virginia, on the edge of vegetable fields (37.203478 N, 80.563166 W).

In Maryland, in 2018, we stationed sticky traps at the edge of four vegetable fields within 600 m of one another on Beltsville Agricultural Center (BARC) North Farm, Beltsville (Prince George's Co.) MD (39.033474N, 76.931520W; 39.031831N, 76.932180W;

39.031787N, 76.935147W; 39.029406N, 76.935495W), with eight treatments in a three-way factorial:  $\pm$ diene,  $\pm$ hydroxyketone, and either clear or yellow sticky trap, using four RCBs. Sticky cards were collected, re-randomized, and lures and cards replaced weekly for six weeks to make 24 spatiotemporal blocks, starting 7 June, and ending 18 July. Lures were stored in a freezer and kept frozen until placement in field for flea beetle testing at all sites. All lures were handled using a separate pair of rubber gloves for each formulation of pheromone lures. Traps within spatial blocks had 10 m separation, and 150–200 m between blocks.

#### Experiments With Rocket Traps and Pheromone Combinations With Allyl Isothiocyanate (AITC)

In Maryland and North Dakota during late spring 2020, we undertook identical 3-way factorial experiments employing rocket traps described below with  $\pm$  diene,  $\pm$ hydroxyketone, and  $\pm$  AITC. Pheromone lures were rubber septa containing 200  $\mu$ g diene or 200  $\mu$ g hydroxyketone. Allyl isothiocyanate (AITC) was dispensed in a 1.8-ml microcentrifuge tube containing 500  $\mu$ l AITC (neet) plugged with a 9.5  $\times$  6.4  $\times$  20 mm piece of folded plastic foam weatherstripping. The rocket traps were constructed from boll weevil trap bottom attached to top of 2-liter PET bottle, similar to the method of Soroka et al. (2005), but lacking the nylon net and topped with a vertical 50-ml translucent centrifuge tube, and anchored at ground level on a square (30  $\times$  30 cm) upside-down asphalt shingle. Traps were provisioned in the trap chamber with a  $\sim$ 3  $\times$  3-cm square of black mesh long-lasting insecticidal netting with 0.4% deltamethrin (ZeroFly Screen Vestergaard-Frandsen, Washington, DC) as a non-volatile, contact killing agent.

The Maryland trial was established in four spatial blocks on the North, East, South, and West edges of a square 1-ha field on Beltsville Agricultural Research Center, South Farm, College Park (Prince George's Co.) MD (field SG9, 39.0165N, 76.9419W), that had in the previous season been planted to collard (*Brassica oleracea*) and mustard (*B. juncea*), which was completely disked under and tilled at the time of the experiment. Traps were separated by 10 m within blocks, and  $\sim$ 20 m between blocks. Traps were collected, lures replaced, and trap locations re-randomized within blocks every week for 4 wk, 29 May through 26 June 2020, to create 16 spatiotemporal blocks.

North Dakota traps were established near shelterbelts of juneberry (*Amelanchier* spp.) and cottonwood (*Populus deltoides*)  $\sim$ 2 km ESE of Langdon (Cavalier Co.) ND (48.7559N, 98.3410W and 48.7547N,  $-$ 98.3380W; two spatial blocks each, respectively). Traps were separated by 10 m within blocks and  $>$  10 m between blocks. Traps were collected, lures replaced, and trap locations re-randomized within blocks every week for 4 wk (creating 16 spatiotemporal blocks), 27 May through 24 June 2020.

#### Enumeration and Identification of Beetles Collected

Sticky cards were wrapped or bagged in plastic to secure collections, and kept in a freezer until sorting and counting. Non-sticky trap collections were placed in bags or containers and frozen until transfer to 70% ethanol. Sticky traps were enumerated in toto unless total numbers exceeded 200, in which case they were subsampled at 19.44% of the surface area to estimate total numbers (8 of 120 North Dakota 2018 samples, and 23 of 120 Virginia 2018 samples). Operational taxonomic units were determined and representative samples sent to the second author (A.S.K.) for identification, with iterative determinations in case of lumping or splitting that were inconsistent with species determinations. Voucher specimens were identified using external morphology and dissected male genitalia

with the help of available taxonomic literature (including Smith 1985 for *Phyllotreta* and White 1996 for *Chaetocnema*), and by comparison with the specimens housed in the National Insect Collection, Smithsonian National Museum of Natural History, Washington, DC. Since both target *Phyllotreta* species are invasive in the United States and native for the Palearctic, most likely Western Europe, collected specimens were compared with those from Western Europe and North America. The former were identified by F. Heikertinger, whose *Phyllotreta* species concept is universally accepted. This ensures that specimens involved in this study and those in native and non-natives ranges are conspecific.

#### Statistical Methods

To test the effect of trap color (yellow vs. clear) in the 2018 North Dakota and Virginia experiments, we used a separate generalized linear model for each experiment in proc glimmix in SAS 9.4 (SAS Institute 2016). Because we were analyzing count data (number of beetles per trap), the models used a Poisson distribution and log link function. Color was the fixed effect and block\*week (i.e., spatiotemporal block) was included as a random effect. To examine the response to diene and hydroxyketone in the 2018 North Dakota and Virginia experiments, we used generalized linear models that used a Poisson distribution and log link function. Diene (present or absent), hydroxyketone (present or absent), and their two-way interaction were included as fixed effects, and block\*week was included as a random effect. When the interaction was significant, we followed up with planned contrasts.

To test the response to diene, hydroxyketone, and trap color in the 2018 Maryland experiment, we used a separate generalized linear model for each species. The model assumed a Poisson distribution and used a log link function. Diene (present or absent), hydroxyketone (present or absent), trap color (yellow or clear), and all two- and three-way interactions were included as fixed effects, and block\*week was included as a random effect. We conducted planned contrasts on two-way interactions that were significant.

To test the responses to the different compounds used in the 2020 Maryland and North Dakota experiments, we used separate generalized linear models for each species at each location assuming a Poisson distribution and using a log link function. Diene (present or absent), hydroxyketone (present or absent), AITC (present or absent), and all two- and three-way interactions were fixed effects, and week\*block was included as a random effect. We followed up significant two-way interactions with planned contrasts.

#### Results

During the five different experiments, we captured almost 85,000 flea beetles, as shown in Table 1, with *Phyllotreta* making up over 98% of captures. *Phyllotreta striolata* was common in all three states; however, another well-known specialist on mustard family (Brassicaceae) hosts, *P. cruciferae*, while abundant at the North Dakota and Virginia sites, was encountered only rarely in Maryland. *Phyllotreta bipustulata* (F.) was also captured in Virginia and Maryland, though not commonly (Table 1; Fig. 1). Less than 2% of captured flea beetles were non-*Phyllotreta*, and some showed treatment effects, as described below.

#### Response of *P. striolata* to Pheromone Components and to Yellow Versus Clear Cards

*Phyllotreta striolata* showed a strong positive response to its principal pheromone component hydroxyketone in all experiments;

**Table 1.** Trapping experiments with flea beetles and total numbers caught during each experiment, by species

State	Year	Experiment <sup>a</sup>	Trap type(s)	<i>Phyllotreta cruciferae</i>	<i>Phyllotreta striolata</i>	<i>Phyllotreta bipunctulata</i>	<i>Dibolia borealis</i>	<i>Chaetocnema pulicaria</i>	<i>Chaetocnema confinis</i>	<i>Chaetocnema denticulata</i>	<i>Epirix</i> sp. A	<i>Epirix</i> sp. B	<i>Margaritisa atriventris</i>	<i>Systema</i> sp.	<i>Psylliodes</i> sp.	Others <sup>b</sup>
North Dakota	2018 <sup>c</sup>	diene × ketone + yellow blank	clear sticky	2,643	30,860	—	—	—	—	—	—	—	—	—	16	1
Virginia	2018 <sup>c</sup>	diene × ketone + yellow blank	clear sticky	2,254	5,105	54	484	—	—	—	—	—	—	—	—	1
Maryland	2018	diene × ketone × color	clear & yellow sticky	—	386	9	71	—	—	—	—	—	—	—	—	2
Maryland	2020	diene × ketone × allyl ITC	rocket	1	606	1	—	99	47	46	76	8	17	13	—	2
North Dakota	2020	diene × ketone × allyl ITC	rocket	42,097	549	—	—	—	—	—	—	—	—	—	—	1

Bold values had sufficient numbers for analysis of factors affecting frequency of capture.

<sup>a</sup>“x” indicates a factorial experiment; diene = himachaladiene; hydroxyketone = hydroxyhimachalanone; ITC = isothiocyanate.

<sup>b</sup>“Other” includes two occurrences *Athica* sp. and one each of *Disompha* sp. and *Orsodactne* sp.

<sup>c</sup>A limited number of sticky cards was subsampled; see text for specifications.

however, the magnitude and interactions with other factors—diene and trap color—differed from region to region.

In North Dakota 2018, sticky trap trials, *P. striolata* responded strongly to hydroxyketone (Fig. 2A) ( $F_{1,69} = 7833.8$ ;  $P < 0.0001$ ), with a 65.9-fold increase in captures in the two hydroxyketone-baited treatments, compared to the two treatments without hydroxyketone. There was also a weaker (1.26-fold) response to diene ( $F_{1,69} = 24.0$ ;  $P < 0.0001$ ), but with no significant interaction ( $P = 0.276$ ). In the separate test of blank yellow and clear sticky traps, clear traps attracted 2.29-fold more beetles, though overall numbers in unbaited traps were low, compared to traps with hydroxyketone.

In Virginia 2018 sticky trap trials (Fig. 2B), although hydroxyketone-loaded lures attracted 2.37-fold as many beetles as did unbaited traps ( $F_{1,69} = 322.6$ ;  $P < 0.0001$ ), and diene alone had no significant effect, there was a significant negative interaction of diene with hydroxyketone ( $F_{1,69} = 29.33$ ;  $P < 0.0001$ ), such that the 1.9-fold increase in the combination, compared to unbaited traps, was less than expected. Also, Virginia captures of *P. striolata* were 1.9-fold higher in yellow compared to clear unbaited sticky traps ( $F_{1,23} = 152.0$ ,  $P < 0.0001$ ), the opposite of the color effect in North Dakota.

Maryland 2018 sticky trap trials (Fig. 2C) included trap color (yellow vs. clear) as a full factorial effect. Although both hydroxyketone and trap color were both strong effects (respectively 5.7-fold,  $F_{1,161} = 110.0$ ,  $P < 0.0001$ ; and 2.5-fold,  $F_{1,161} = 30.7$ ,  $P < 0.0001$ ), there was also a significant interaction between diene and color ( $F_{1,161} = 10.1$ ,  $P = 0.0018$ ) such that in the presence of diene, the color effect was stronger (1.5-fold with diene absent; 4.3-fold with diene present).

### Response of *P. striolata* to Pheromone Components and Allyl Isothiocyanate (AITC), in Rocket Traps

As with the sticky traps, the hydroxyketone was attractive to *P. striolata* at both locations, North Dakota and Maryland, 2.1-fold and 11.8-fold, respectively, compared to blank (Fig. 3). There was a significant 3-way interaction of diene × hydroxyketone × AITC in North Dakota captures ( $F_{1,105} = 18.8$ ;  $P < 0.0001$ ). Allyl isothiocyanate significantly increased the response of North Dakota beetles to both hydroxyketone and diene, but not alone, and not in combination with hydroxyketone plus diene (Fig. 3A). In Maryland, AITC had a strong effect on all pheromone combinations, increasing them by 6.3-fold compared to traps without AITC, and 12.0-fold with AITC alone versus unbaited (Fig. 3B). AITC and hydroxyketone had a significant and positive interaction ( $F_{1,104} = 7.5$ ;  $P = 0.0072$ ) such that AITC + hydroxyketone attracted 46.0-fold more than unbaited traps. However, there was also a significant ( $F_{1,104} = 7.2$ ;  $P = 0.0085$ ) negative interaction of diene × hydroxyketone, as seen in North Dakota, both with and without AITC.

### Response of *P. cruciferae* to Pheromone Components and to Yellow Versus Clear Cards

For North Dakota *P. cruciferae* (Fig. 4A), both diene and hydroxyketone lures increased captures compared to blank clear sticky traps (2.72-fold and 2.78-fold, respectively), and beetles were strongly attracted to combined diene and hydroxyketone lures with a significant positive interaction of diene × hydroxyketone ( $F_{1,69} = 4.04$ ;  $P = 0.048$ ); the combination attracted 9.41-fold more than the unbaited clear sticky trap. With unbaited sticky traps, color had no effect.

In Virginia, the semiochemical effects were relatively weak; diene + hydroxyketone-baited traps caught only 1.54-fold that of unbaited sticky traps (Fig. 4B). The diene × hydroxyketone interaction was significant and positive ( $F_{1,69} = 8.9$ ;  $P = 0.004$ ), with a

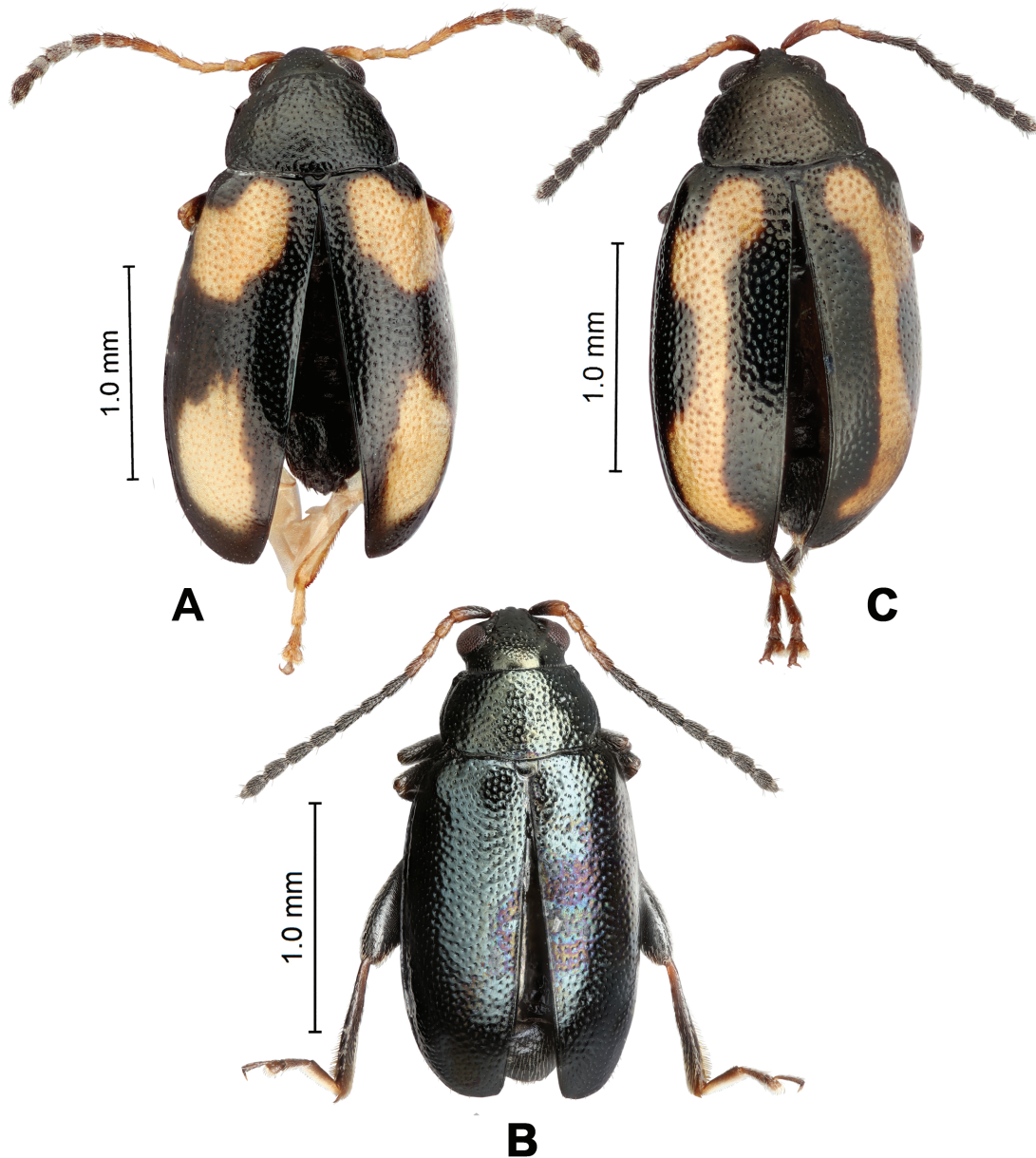


Fig. 1. Habitus figures of *Phyllotreta* species, all collected in Maryland. (A) *P. bipustulata* (F.), (B) *P. cruciferae* (Goeze), and (C) *P. striolata* (F.). Photo ASK.

significant diene effect only when hydroxyketone was present (contrast  $F_{1,69} = 7.8$ ;  $P = 0.0079$ ). A comparison of unbaited yellow and clear sticky traps showed a significant effect ( $F_{1,23} = 199.6$ ;  $P < 0.0001$ ) with yellow traps catching 2.6-fold more than clear traps.

#### Response of *P. cruciferae* to Pheromone Components and Allyl Isothiocyanate, in Rocket Traps

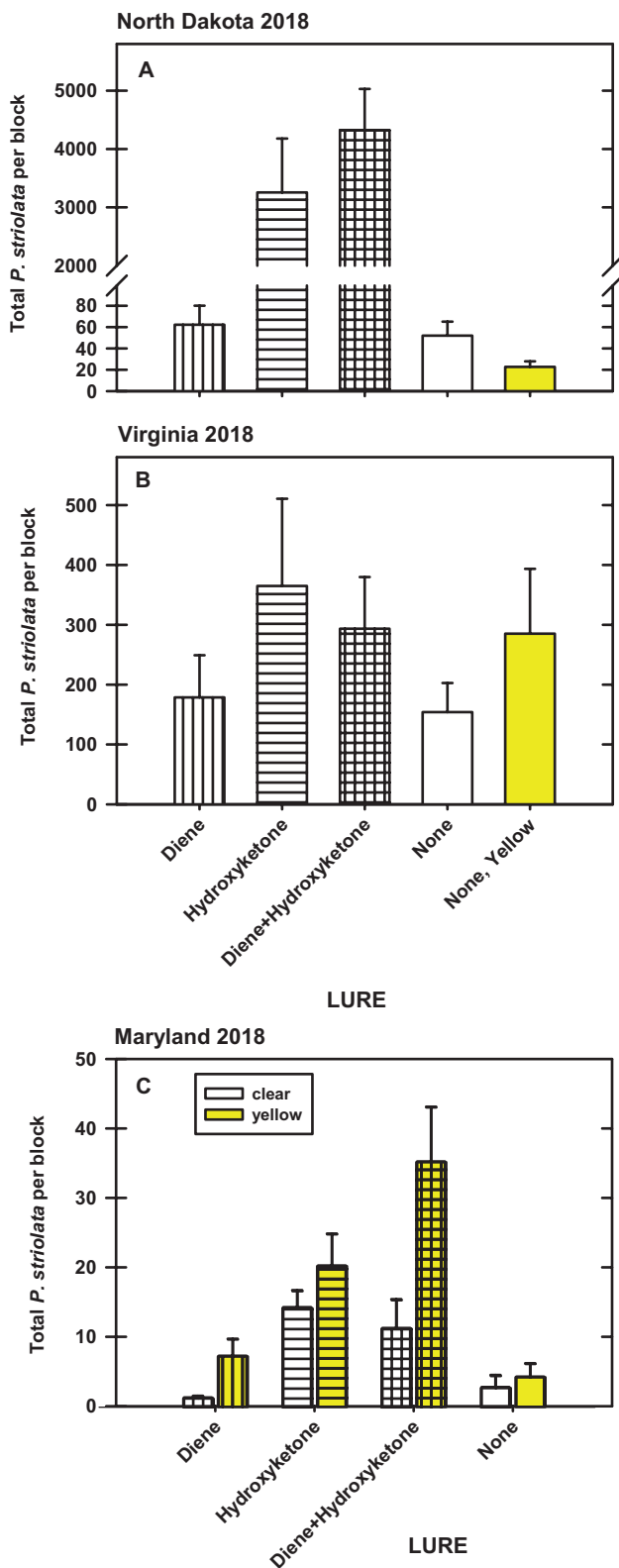
North Dakota *P. cruciferae* were extremely abundant in rocket traps in 2020 (Fig. 5). AITC was by far the most attractive factor ( $F_{1,105} = 17132.5$ ;  $P < 0.0001$ ) accounting for captures; 7.0-fold more beetles were caught in AITC-baited traps than traps without AITC. In contrast, the diene and hydroxyketone effects were both  $< 1.1$ -fold. There was a significant 3-way interaction ( $F_{1,105} = 33.1$ ;  $P < 0.0001$ ), such that both hydroxyketone and diene enhanced attraction to AITC, but interacted negatively such that diene + hydroxyketone captures were less than expected, especially in the presence of AITC (Fig. 5).

#### Response of *P. bipustulata* to Pheromone Components, and to Yellow Versus Clear Cards

Fifty-four *P. bipustulata* (Fig. 1A) were captured in the 2018 Virginia sticky card experiment. Ten other adults were also captured in Maryland during 2018 and 2020 experiments (Table 1). In Virginia captures, the diene lure was significantly attractive ( $F_{1,69} = 10.7$ ;  $P = 0.0017$ ), with diene-baited traps attracting 3.3-fold more beetles than traps without diene. Although unbaited yellow sticky traps attracted 2.2-fold more beetles than clear sticky traps, the effect was not statistically significant ( $F_{1,23} = 2.1$ ;  $P = 0.16$ ).

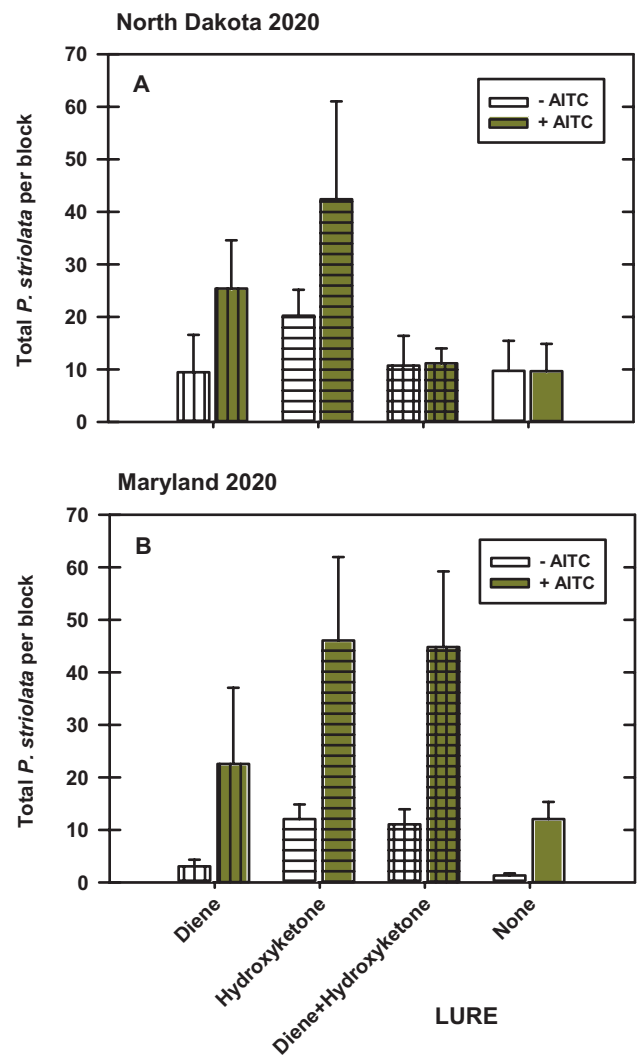
#### Response of Non-*Phyllotreta* Species

Although the non-*Phyllotreta* beetles captured were only 1.62% of total numbers captured (Table 1), we detected some significant treatment effects corresponding to positive or negative responses to color and/or semiochemical lures.



**Fig. 2.** Trap captures of *Phyllotreta striolata* in (A) North Dakota, (B) Virginia, and (C) Maryland in 2018 with clear and yellow sticky traps baited with diene and/or hydroxyketone lures. Numbers are total beetles per trap within block over 6-wk trial  $\pm$  standard error.

*Dibola borealis* Chevrolat showed a negative response to both ketone and diene lures in Virginia 2018 sticky trap tests, with 46% of beetles captured on unbaited clear sticky cards, compared to an

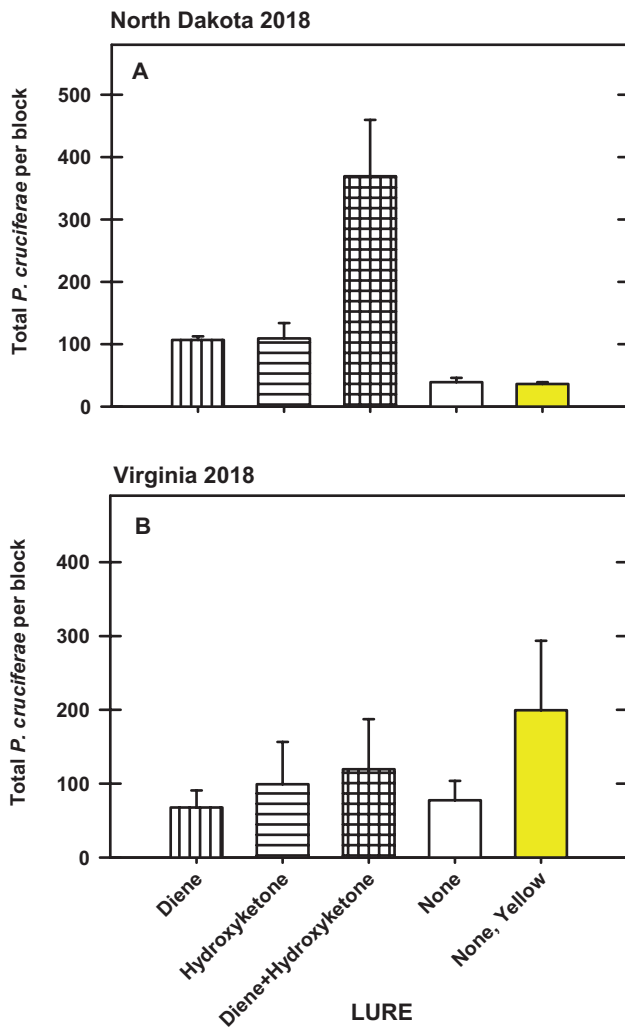


**Fig. 3.** Trap captures of *Phyllotreta striolata* in (A) North Dakota and (B) Maryland in 2020 using rocket traps baited with diene and/or hydroxyketone and/or allyl isothiocyanate (AITC) lures. Numbers are total beetles per trap within block over four-week trial  $\pm$  standard error.

expected 25% if there was no effect. There were no significant effects of lures in Maryland. However, color was a significant effect in Maryland ( $F_{1,161} = 18.0$ ;  $P < 0.0001$ ) with yellow sticky traps capturing 3.8-fold as many as clear traps. In Virginia, no color effect was detected, but this was using only unbaited traps.

*Chaetocnema* species were captured in numbers in Maryland, a total of 192 during 2020 in the 3-way semiochemical experiments. Captures of *Chaetocnema pulicaria* Melsheimer and *Chaetocnema confinis* Crotch both showed no significant treatment effects in 2020 semiochemical (diene  $\times$  hydroxyketone  $\times$  AITC) trials with rocket traps. However, *Chaetocnema denticulata* (Illiger) was apparently repelled by AITC ( $F_{1,105} = 4.7$ ;  $P = 0.032$ ), with 2.28-fold more caught in traps without AITC, than those baited with it.

*Epitrix* species A showed a significant attraction to AITC in Maryland trials ( $F_{1,104} = 8.0$ ;  $P = 0.0055$ ), such that 2.1-fold more were captured in AITC-baited traps. However, this effect interacted negatively with the presence of the diene lure, such that AITC was only attractive in the absence of diene (contrast  $F_{1,104} = 12.3$ ;  $P = 0.0007$ ; without diene, traps with AITC were 4.0-fold more attractive than traps without).



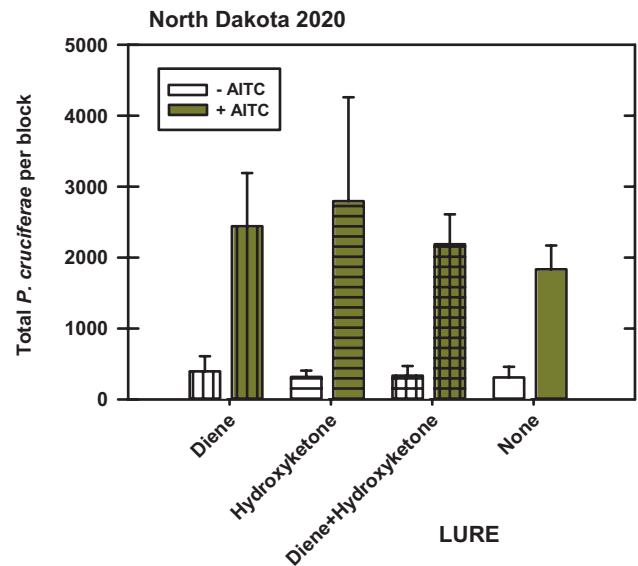
**Fig. 4.** Trap captures of *Phyllotreta cruciferae* in (A) North Dakota and (B) Virginia in 2018, with clear and yellow sticky traps baited with diene and/or hydroxyketone lures. Numbers are total beetles per trap within block over 6-wk trial  $\pm$  standard error.

The above effects show that some species of non-*Phyllotreta* flea beetles respond to AITC and/or *Phyllotreta* pheromone components, as well as yellow color, as discussed below.

## Discussion

We have demonstrated the importance of hydroxyhimachalane and himachaladiene, as well as allyl isothiocyanate (AITC), to the behavior of both *P. striolata* and *P. cruciferae* in attraction and capture in traps baited with these synthetic compounds. However, among different locations in North America (ND, MD, VA), the response was not consistent, though it must be noted that the data from Virginia encompass only 1 year.

Beran et al. (2016) cogently state that levels of AITC used in our studies, as well as other trials such as Soroka et al. (2005) and Tóth et al. (2005, 2012), exceed levels expected in the field when produced by plants or insects. Nevertheless, we found such high levels to be clearly attractive to both *Phyllotreta* species, most often in combination with pheromone components, and as such they could prove useful in monitoring or management of these key pests in agricultural systems. It is also conceivable that the receiving insect



**Fig. 5.** Trap captures of *Phyllotreta cruciferae* in North Dakota in 2020 using rocket traps baited with diene and/or hydroxyketone and/or allyl isothiocyanate (AITC) lures. Numbers are total beetles per trap within block over 4-wk trial  $\pm$  standard error.

may perceive AITC at higher ambient levels as a pheromonal (not plant kairomonal) signal given at a short range, since the beetles are capable of intrinsic sequestration of glucosinolates as well as their myrosinase-based catabolism to AITC (Beran et al. 2014). In addition, activity of other plant- or insect-produced isothiocyanates could be important in generating neuronal ‘crosstalk’ with AITC sensitivity; indeed, Tóth et al. (2012) showed the interaction of two isothiocyanates for *Phyllotreta vittula* (Redtenbacher) and *P. cruciferae* in Hungary. This is part of a larger question of influence of multiple plant volatiles on the behavior of their specialist herbivores, as Beran et al. (2016) point out.

Apparent differences in population response to semiochemicals tested probably arise from multiple causes, including:

1. Genetic differences in response, possibly selected by
  - a. range of host plants available;
  - b. management practices in agroecosystems of cultivated host plants and host weeds;
  - c. range of competing and/or semiochemically interfering congeners;
  - d. bottlenecks or other chance selection by way of introduction and invasion, since both *P. striolata* and *P. cruciferae* are not native to North America but were introduced in the past several hundred years (Knodel 2017, Knodel et al. 2017).
2. Seasonal response to pheromonal and kairomonal signals, based on reproductive and nutritional state, and environmental factors such as temperature and photoperiod.
3. Occurrence and density of conspecifics, and congeners sharing the same pheromone components.

As a possible example of selection for different responses to AITC, North Dakota *P. striolata*, unlike those in Virginia, did not respond to AITC in the absence of either the diene or the hydroxyketone pheromone component (Fig. 3A). Vincent and Stewart (1984) also found that *P. striolata* was not as strongly attracted to AITC as *P. cruciferae*. This could be due to *P. cruciferae* being a more specialized herbivore than *P. striolata* and/or *P. striolata* being less attracted to specific host plant volatiles like AITC. Another reason could be

the differences in biology between the two species with *P. striolata* emerging several weeks earlier than *P. cruciferae* in the spring, often prior to canola planting in North Dakota (Knodel 2017). Gruber et al. (2009) observed that *P. cruciferae* were most attracted to AITC in the spring and early fall, but AITC was inhibitory in the late fall. Response of adults to AITC alone in the early season might prove fatal, whereas confirmation that live male flea beetles were producing pheromones could demonstrate to the receiver that the host allowed conspecifics to survive, i.e., the host was not toxic. In the case of canola, approximately 95% of the acreage is seed-treated with systemic insecticides for management of flea beetles in North Dakota (Knodel 2017; Knodel et al. 2008). In Virginia beetles, the more diversified host range might have selected (or not selected against) use of plant kairomones such as AITC, even in the absence of pheromone signals, arising from the lower risk of toxic penalty (pesticide mortality) for sampling a potential host.

The negative interaction of diene with the hydroxyketone among 2018 captures in Virginia and Maryland *P. striolata* (Fig. 2B and C) also occurred in both North Dakota and Maryland populations in 2020, in the latter case both with and without AITC. The only case that does not show an interaction is in North Dakota in 2018, in which very large numbers of *P. striolata* occurred, but these high numbers occurred only in the first week of 6 wk of sampling. A crash in captured numbers, and presumably the population in general, corresponded with emergence of a seed-treated canola field adjacent to the trapline, probably offering few food alternatives except for the neonicotinoid-containing canola crop. In all other instances, the negative interaction of hydroxyketone with diene pheromone component may reflect the need for a ratio more near the one described and used in lab and field assays by Beran et al. (2016), which is approximately 3:1 hydroxyketone:diene. This calls into question the compatibility of precisely optimizing a combined lure and trap for both of the common species.

Regarding the response of *P. cruciferae*, the positive response of this species to the combined diene + hydroxyketone in North Dakota (Fig. 4A) is a novel finding for this species, which, so far as is known, does not produce hydroxyketone. It suggests a possible eavesdropping response to the *P. striolata* pheromone where both species occur in abundance, perhaps based on a better ability of *P. striolata* to find appropriate, untreated plant hosts, possibly reflecting the relatively wide host range of *P. striolata* within the Brassicaceae (Hicks and Tahvanainen 1974). The strong response of *P. cruciferae* to AITC is widely reported from Europe and North America, and this attraction is present in other European *Phyllotreta* species as well, as demonstrated by Tóth et al. (2005).

The finding that *P. bipustulata* was attracted to the diene pheromone lure suggests that its as-yet-unknown pheromone may contain this component as well, as hypothesized by Tóth et al. (2005) for four European congeners. This species, like *P. striolata* and *P. cruciferae*, is primarily associated with plant hosts in Brassicaceae (Barney et al. 2009), and a native species judged an 'occasional pest' by Chittenden (1927). Hicks and Tahvanainen (1974) found it primarily in shady woodland habitats, with univoltine colonization of adults to its native spring ephemeral perennial host *Dentaria diphylla*. Its presence only in Virginia and Maryland captures is probably due to lack of such habitats near the North Dakota study site.

Other flea beetle genera captured (Table 1) likely reflect the agricultural crop and weed hosts in the habitats sampled. *Dibolia borealis* feeds on plantain, *Plantago* spp. (Plantaginaceae) (Reed 1927; Barney et al. 2009). *Margaridisa atriventris* (Melsheimer) is a specialist on copperleaf, *Acalypha* spp. (Euphorbiaceae) (Barney et al. 2009). *Chaetocnema* species include specialists on corn and other grasses (*C. denticula* and *C. pulicaria*), as well as crops and weeds

in the Convolvulaceae (*C. confinis*) (Capinera 2020). *Epitrix* species generally are oligophagous on potato, eggplant, and other crops and weeds in the Solanaceae (Capinera 2020, Mason et al. 2020). In North Dakota, all flea beetles captured were known pests of crucifers: *Psylliodes* species (12 adults captured in ND 2018) [e.g., hop flea beetle, *Psylliodes punctulata* (Melsheimer)] include known pests of crucifers (Capinera 2020). The lack of non-crucifer specialized flea beetles at the North Dakota site reflects the concentration of canola cropping there. In contrast, the Virginia and particularly the Maryland site in 2020 are diversified vegetable-growing research farms with more abundant weed populations. In contrast to 2018 sticky traps, the 2020 rocket traps in Maryland allowed all specimens to be more easily extracted and identified.

Strong response of flea beetles and other herbivorous insects to combined plant- and insect-produced volatiles (including pheromone) allows the possibility to turn a host plant into a very attractive pheromone production factory (Tholl 2021). With the attractive plant kairomones already being produced, the prospect of terpenoid pheromone production for management of stink bug and flea beetle pests is well within the biochemical capability of plant pathways (Tholl 2021). Perhaps, we are not inventing something new here with the combined production of plant and insect volatiles: is this the reason perhaps for the evolution of intrinsic capability for production of pre-existing plant secondary compounds, independently in insects, as shown by Beran et al. (2016) in the case of *P. striolata*? Perhaps, the male gains added attraction to females by adding on the myrosinase pathway.

There is also the possibility in the case of captures of large numbers of beetles in either sticky or non-sticky traps that distressed or dead and decaying beetles generate release of pheromones and/or volatile breakdown products of pheromones or other biochemicals that alter subsequent arrivals and captures. This is distinct from physical-based saturation of sticky traps mentioned earlier and has been demonstrated, e.g., by Elkinton (1987) for spongy moth *Lymantria dispar* (L.) (Lepidoptera: Erebididae) carton traps and by Jactel et al. (2006) for pine processionary moth [*Thaumetopoea pityocampa* (Denis & Schiffermüller), Lepidoptera: Thaumetopoeidae].

The rocket traps used can be easily fashioned using commercially available parts in combination with the boll weevil trap base. This trap needs to be more thoroughly evaluated for possible non-target effects in a variety of settings; however, we do not suspect any significant effects on pollinators, many of which are unable or in some cases uninterested to enter through the narrow openings provided between the base and the clear inverted funnel piece.

The combination of AITC with the hydroxyketone pheromone component proved the most attractive to both key pests, *P. cruciferae* and *P. striolata*, using the rocket traps (Figs. 3 and 5). An adequate pheromone supply at reasonable cost, combined with higher-capacity trapping or baiting (attract and kill) methods, could achieve quantitative monitoring and/or population suppression in management of both of these wide-ranging pests of Brassicaceae.

There is a very clear need for wider evaluation of the pheromone components of *Phyllotreta* as well as other flea beetles predicated on improved and economical synthesis and attendant commercial availability. Using season-long experiments over the wide ranges of these near-cosmopolitan pests, researchers will be able to determine the best approaches to applying growing semiochemical knowledge about this economically and ecologically important group.

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