Journal of Pest Science (2022) 95:327–338
https://doi.org/10.1007/s10340-021-01379-y

ORIGINAL PAPER

Trade-offs and synergies in management of two co-occurring specialist squash pests

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Received: 19 October 2020 / Revised: 24 March 2021 / Accepted: 16 April 2021 / Published online: 3 May 2021

Abstract

Co-occurring herbivorous pests may have shared or divergent responses to plant- and insect- derived cues, creating challenges for effective pest management in agroecosystems. We examined how behaviors of two endemic specialist herbivores of Cucurbitaceae crops, squash bugs (*Anasa tristis*, Hemiptera: Coreidae) and striped cucumber beetles (*Acalymma vittatum*, Coleoptera: Chrysomelidae) are affected by cues in the *Cucurbita pepo* agroecosystem. We evaluated plant resistance to squash bugs and beetles using cultivars that typify the two domesticated subspecies *C. p. pepo* (e.g., zucchini) and *C. p. ovifera* (e.g., straightneck summer squash), and tested how squash bugs respond to beetle aggregation and feeding. Across several field experiments, we demonstrated that squash bugs prefer to oviposit on *C. p. ovifera* over *C. p. pepo*, while beetles had the opposing preference. Nonetheless, there was no link between preference and squash bug nymphal survival or development. While squash bugs and beetles diverge in preference, we found that squash bugs positively respond to beetle-derived cues. More squash bug oviposition was observed on plants with greater beetle damage and, using both actively feeding beetles and synthetic lures, we demonstrate that bugs eavesdrop on and respond to vittatalactone, the male-produced beetle aggregation pheromone. Thus, squash bugs appear to exploit the cue of a co-occurring specialist beetle for host choice and this has implications for management: while there are trade-offs in varietal preference, synergistic trapping of both pests may be possible. By evaluating the behavior of co-occurring pests, management strategies with multi-species efficacy can be identified and applied in agroecologically-based pest management.

Keywords Anasa tristis · Acalymma vittatum · Pheromone · Vittatalactone · Cucurbita pepo · Host plant resistance · Agroecology

Communicated by Jay Rosenheim.

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Key message

- Striped cucumber beetles and squash bugs locate hosts by plant- and insect- derived cues
- These specialist herbivores of the Cucurbitaceae have opposing varietal plant preferences
- Squash bugs also eavesdrop on beetle-produced pheromone during host location
- Dual attraction to pheromone provides potential for simultaneous management of both pests

Introduction

Agroecosystems harbor diverse co-occurring plant and pest species, creating a complex information landscape with potential cues that likely affect insect host location

(Carrasco et al. 2015; Kessler 2015). In instances where the resulting insects' response to these cues are congruent (e.g., resulting in cross-resistance, Andrew et al. 2007; Kos et al. 2014), management of multiple pests may be feasible and simplified. Alternatives, however, include neutral outcomes or trade-offs between co-occurring insects (e.g., in host plant resistance, Da Costa & Jones 1971; Lankau 2007). An important implication of such outcomes for pest management is that strategies to alter the behavior of herbivores are likely to be specific (Rodriguez-Saona and Stelinski 2009), but may have compatibilities or incompatibilities with the management of other pests.

Leveraging insect and plant cues to modify behavior of insect pests is a key feature of integrated pest management. For instance, insect pheromones are used to disrupt mate location (Rodriguez-Saona and Stelinski 2009), and use of resistant (e.g., repellent) plant varieties can reduce herbivory (Stout and Davis 2009). Alternatively, use of attractive crop varieties or lures of plant, insect or microbial chemicals can be used to trap insects away from the main crop (Gregg et al. 2018). Of course, these can be used together to amplify benefits, as in push–pull cropping systems (Miller and Cowles 1990; Cook et al. 2007). However, a challenge in these approaches is that each requires significant development and is predominantly effective for a single pest species. For example, while resistance traits to numerous insect pests have been recorded in rice (*Oryza sativa*) (Heinrichs 1986; Fujita et al. 2013), cross-resistance is rare (but see, Wang et al. 2004). In some cases, the effects of a tactic to manage a pest can produce scenarios in which secondary pests become problematic. For instance, while breeding for low levels of glucosinolates in *Brassica napus* decreased damage by specialist beetle and lepidopteran pests, this led to increased damage by slugs and birds (Giamoustaris and Mithen 1995). Thus, it is imperative to assess which strategies elicit responses from multiple pest species, and how congruent effects can be applied given the vast information landscape available in agroecosystems.

Here, we addressed the potential for joint resistance to two major pests of cucurbits. The Cucurbitaceae are a global family with multiple domestications, often resulting in a shared set of characteristics (like greater fruit palatability; Chomicki et al. 2020), and well-documented dispersal of germplasm beyond native habitats for agricultural production. There is a rich history in the study of chemical ecology of cucurbit pests (Da Costa and Jones 1971; Metcalf et al. 1980; Andersen and Metcalf 1986; Smyth and Hoffmann 2003; Theis et al. 2014), and the agricultural importance of such pests is demonstrated by the multitude of cultivar screens to identify host plant resistance (Da Costa and Jones 1971; Bonjour and Fargo 1989; Hoffmann et al. 1996; Brzozowski et al. 2016). To identify cross-species interactions, we specifically examined Cucurbitaceae and herbivores with shared evolutionary history and current agricultural associations endemic to the Americas: *Cucurbita pepo* crops, and two specialist herbivores, striped cucumber beetles (*Acalymma vittatum*, Coleoptera: Chrysomelidae) (Metcalf and Lampman 1989; Haber et al. 2021) and squash bugs (*Anasa tristis*, Hemiptera: Coreidae) (Doughty et al. 2016). There were two independent domestications of *C. pepo*, *C. p. pepo* ("CPP", e.g., zucchini) and *C. p. ovifera* ("CPO", e.g., straightneck summer squash, syn. *C. p. texana*), where CPO development largely remained in the Americas, the realm of the specialist herbivores, while some CPP cultivars were bred in Europe, devoid of specialist herbivores, before returning to the Americas with European colonists (Paris 2000). These associations provide a framework for developing holistic management strategies for co-occurring and agriculturally important herbivorous pests.

The beetle specialist feeds on all plant tissues and demonstrates strong preference for CPP over CPO cultivars (Hoffmann et al. 1996; Brzozowski et al. 2016), where susceptibility is conferred by cucurbitacins in cotyledons (Ferguson et al. 1983; Brzozowski et al. 2020b) and other traits in true leaves and flowers (Brzozowski et al. 2020a, b). The spatial distribution of beetle damage is largely due to a male-produced pheromone, vittatalactone, that mediates aggregation (Smyth and Hoffmann 2003), and males release vittatalactone on both CPO and CPP (Brzozowski et al. 2020a). In contrast, squash bug susceptibility lacks the same degree of characterization. Multiple studies have demonstrated variation in squash bug preference among Cucurbitaceae host species (Novero et al. 1962; Bonjour and Fargo 1989; Bonjour et al. 1990), but a relative lack of information on intraspecific preference is an impediment to informing grower cultivar choice at the field scale (but see Cornelius 2017). This need is particularly acute for squash bugs, as they cause significant damage through herbivory and vectoring cucurbit yellow vine decline (CYVD) (Doughty et al. 2016); while long-established in the southern United States, northward range expansion been identified as a threat to cucurbit production (Boucher 2005).

In this work, we examine how co-occurring beetle and squash bug pests respond to plant- and insect-derived cues in the field and explore how this could be applied in pest management. Leveraging existing knowledge of striped cucumber beetle preference in *C. pepo* germplasm, we sought to better understand host plant preference and performance of the squash bug within *C. pepo*, and the specific interactions between squash bugs and beetles. First, with field surveys and manipulative experiments, we examined squash bug oviposition preference for and nymphal performance on CPO and CPP. Then, over two years of field trials, we interrogated the connection between beetle damage, aggregation pheromone production, and squash bug oviposition preference. By examining co-occurring herbivores and plants with shared evolutionary and agricultural history, we seek to develop opportunities to streamline and augment pest management in the cucurbit agroecosystem.

Methods

Evaluating cross-resistance in *C. pepo* preference between squash bugs and beetles

We conducted a field survey of large plantings of two inbred *Cucurbita pepo* cultivars to assess squash bug (*Anasa tristis*) oviposition preference and its relationship to beetle (*Acalymma vittatum*) abundance and preference. In all experiments, unless noted otherwise, we used two cultivars representing established differences in extremes of beetle preference between *C. pepo* subspecies, *C. p. pepo* cv. Golden Zucchini, ("CPP") and *C. p. ovifera* (syn. *C. p. texana*) cv. Success PM ("CPO") (Brzozowski et al. 2016), where seeds were sourced from Cornell University seed stocks. Plants were started in late May 2019 from untreated seed in 72-cell trays at the Cornell University Agricultural Experiment Station greenhouses (Ithaca, NY, USA) in custom organic potting mix. No pest control or additional fertilizer was applied, and plants were irrigated as needed.

Seedlings were transplanted in mid-June 2019 into 0.6 m wide raised beds covered with black plastic mulch equipped with drip irrigation in certified organic fields on the Cornell University Agricultural Experiment Station's Homer C. Thompson Vegetable Research Farm (Freeville, NY, USA; 42°31′05.7″N 76°20′07.1″W). Each cultivar was grown in two 50 m rows with 1 m spacing between plants. The four rows were spaced in an east to west gradient with 3 m spacing between rows, and cultivars alternated between rows. We then observed natural infestation of squash bugs and beetles (Fig. 1). Individual plants were surveyed weekly for three weeks in July 2019 for counts of beetles (adults only), squash bugs (adults and nymphs), and squash bug egg clutches (presence and eggs per clutch). Beetle damage was recorded once during the survey on a 0–5 scale of defoliation ($0 = 0\%$ defoliation, $1 = 1 - 20\%$ defoliation, $2 = 21 - 40\%$ defoliation, etc., c.f. Brzozowski et al. 2016).

Cumulative presence or absence of squash bug adults and egg clutches on plants in total was modeled with a logistic regression model with fixed effects of cultivar, row (east to west gradient), their interaction and plant position in row (coded as numeric, to account for north to south gradient in the row) using the 'glm' function with a binomial distribution in R (R Core Team 2016). Cumulative beetle count was modeled in the same fashion but fit with a negative binomial model. In both cases, significance was determined with a likelihood ratio test. Finally, intensity of leaf damage

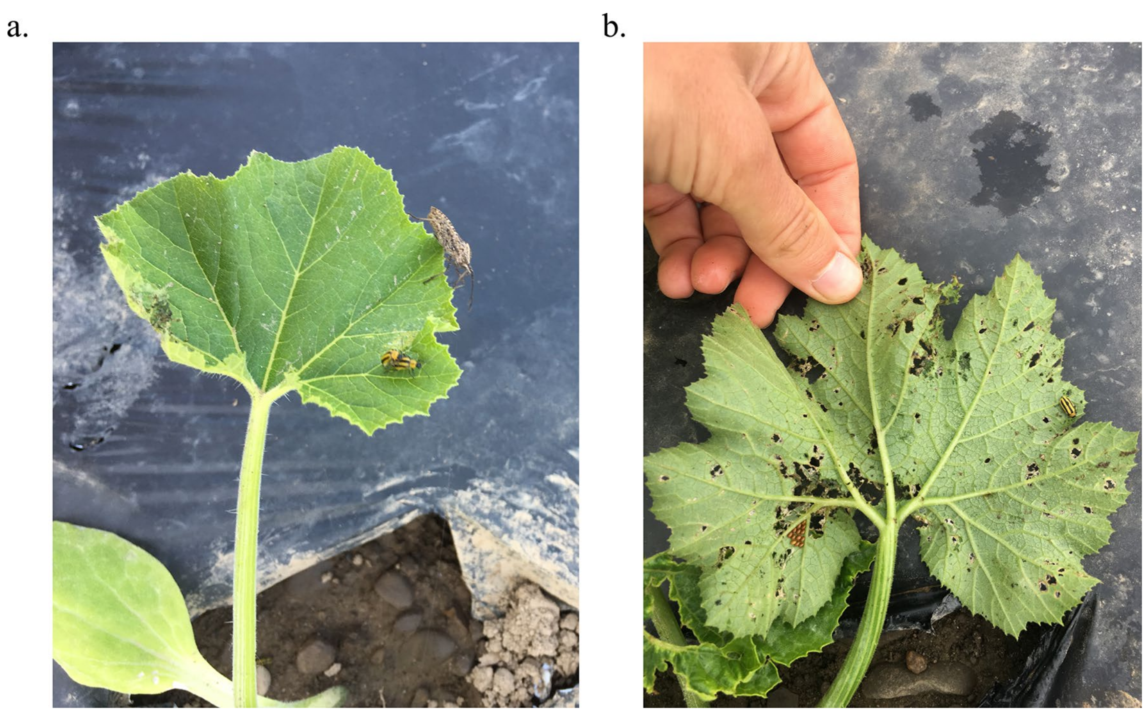

Fig. 1 Co-occurrence of **a** squash bug and striped cucumber beetle adults, and **b** squash bug egg clutch on a striped cucumber beetle damaged squash leaf

by beetles was assessed with a linear model, with the effects described above using the 'lm' function in R, and significance determined by F-tests in ANOVA.

Assessing squash bug nymph performance on *C. pepo*

We assessed differences in squash bug nymph survival and development between CPP and CPO. Plants were started in June 2019 as above and seedlings were transplanted into 1.74 L (15 cm diameter) pots after two leaves had emerged. Squash bug egg clutches were collected from the farm, and ten newly hatched nymphs (first instar) were released on to single bagged plants on the farm ($n=10$, 7 for CPP and CPO, respectively). The start date was staggered over a four day period in August 2019 due to nymph availability. Nymph counts and instar classification were assessed nine times over 30 days. Squash bugs were classified as 1st instar, 2nd instar or 3–5th instar nymphs (due to visual similarity), or adults.

To assess the effect of cultivar on nymph survival and development, we fit two separate generalized linear mixed effects models with Poisson distributions using the 'glmer' function from the R package 'lme4' (Bates et al. 2015). For survival, there were main effects of cultivar and days post infestation, and for development, there was an additional main effect of instar stage. In both cases we used random effects to match the experimental design (start date, position in field, and individual plant). Using a likelihood ratio test, we compared models with all potential fixed effects interactions to a reduced model with cultivar and all cultivar interactions removed to assess the effect of cultivar on survival and developmental timing.

Evaluating squash bug oviposition preference in response to beetle damage

To assess the response of squash bugs to beetle damage in absence of strong effects of cultivar (CPO, CPP) preference, we measured beetle damage and squash bug egg clutch frequency in inter-subspecific (CPO x CPP) lines. We used F_4 generation breeding lines selected for cotyledon (not leaf) traits, as described in (Brzozowski et al. 2020b). The field was prepared as previously described, and thirteen breeding lines were planted in June 2019 in 12 plant plots, and were replicated three times. Beetles were observed in the plots one day after planting. Beetle damage was visually estimated as leaf defoliation between 0 and 100% defoliation (in increments of 5%) and squash bug egg clutch number was recorded at the plot-level ten days after planting. Clutch number was modeled with a generalized linear mixed model using a Poisson distribution with random effects of genotype and replicate and a fixed effect of beetle damage using the 'glmer' function; significance was determined with a likelihood ratio test.

Evaluating squash bug oviposition preference in response to beetle aggregation pheromone

Within *C. pepo* cultivar (CPO, CPP), we tested squash bug oviposition preference in choice tests between cultivars with and without male beetle infestation. Plants were started from seed as above and were transplanted into 1.74 L (15 cm diameter) pots. Squash bugs and striped cucumber beetles were collected from the farm. Male beetles were identified and selected from mating pairs based on abdomen morphology (White 1977), and separated from females as exclusively male striped cucumber beetles produce the aggregation pheromone, vittatalactone (Morris et al. 2005). On plants with 3–5 leaves, five male beetles were enclosed on a single leaf with a small mesh bag on half of the plants, and an empty mesh bag was placed on the other half as controls. We then paired plants (with male beetles or control) within cultivar and placed both in a 1 m^3 field cage and released one mating pair of squash bugs (one male, one female). Squash bug eggs were counted at least every three days and summed at six days.

Assays were conducted over five temporal trials in July 2019. In each trial, there were at least two pairs of each cultivar, and a total of 30 CPO and 20 CPP pairs evaluated. However, there was no oviposition in the fifth iteration of the experiment, and that was dropped from the analysis, leaving 25 CPO and 17 CPP pairs evaluated over four times. Differences in oviposition frequency between cultivars (eggs on either treatment in the cage) were assessed with a Fisher's exact two-tailed test. Differences between treatments within cultivar was tested using a Wilcoxon signed-rank test using number of eggs on a plant as the response variable (only cages where there was oviposition were included). Both statistical analyses were conducted in R (R Core Team 2016).

Evaluating squash bug adult preference in response to beetle aggregation pheromone – attraction to active male beetle feeding

We tested the effect of plant cultivar, beetle infestation density, and beetle sex on relative attractiveness to beetles and other squash pests on the farm in June-July of 2016. In this experiment, with the same *C. pepo* cultivars (CPO, CPP) in pots, we used mesh bags to enclose male beetles feeding on plants (able to produce aggregation pheromone, and induced plant volatiles), or female beetles feeding on plants (no aggregation pheromone, but induced plant volatiles), or no beetles (plants only). Each individual plant was then surrounded by a wire cage with Tanglefoot-coated strips as sticky traps to capture incoming insects. These traps are described in detail, and pictured in Brzozowski et al. (2020a). In sum, there were 62 traps containing male beetles feeding on plants, 30 traps with female beetles feeding on plants, and 28 traps without plants only. The number of squash bugs trapped over a three day period was recorded and a William's corrected G-test was used to test if the presence of squash bugs was different between traps with male beetles and all others, male and female beetles, male beetles and no beetles, and female beetles and no beetles.

Evaluating squash bug adult preference in response to beetle aggregation pheromone – attraction to synthetic beetle pheromone lures

We assessed the response of squash bugs to synthetic vittatalactone to complement assays conducted with live beetles. Lures (gray septa 1-F SS 1888 GRY, West Pharmaceutical Services, Lititz, PA) were loaded with 1 mg of mixed vittatalactones synthesized by the method of Chauhan & Paraselli (2017). This dose and lure combination was shown to be highly attractive to striped cucumber beetle in earlier field trials (Weber 2018). Clear sticky traps (Stinkbug STKY Dual Panel Adhesive Traps, Trécé Inc., Adair, OK, USA), 30 cm by 15 cm, were affixed vertically with a large metal binder clip to wooden stakes within the border of squash fields, at ~ 45 cm height (middle of trap above ground). Lures were affixed to half of the traps and hung from the large binder clip, using a small metal binder clip (Fig. 2a). Traps with and without lures were paired for each assay, and traps and lures were changed every 7 days. Trials were conducted on vegetable farms in Maryland, USA and New Hampshire, USA in August – September 2019. In each state, four pairs (blocks) of traps were stationed outside of but near to cucurbit crops (≤ 20 m), with traps separated by ≥ 10 m distance, and blocks separated by ≥ 20 m. Within blocks, traps were randomly assigned with or without the mixed vittatalactone lure, and rerandomized weekly.

In Maryland, trap blocks were positioned on the four borders of a field of senescing Yellow Crookneck Squash (CPO, Johnny's Selected Seeds, Albion, ME, USA, untreated seed), ~ 0.3 ha in area, direct-seeded 20 May 2019 and not treated with any pesticides, at the Agricultural Research Service's Beltsville Agricultural Research Station (BARC), in Beltsville, Maryland (North Farm, 39°01′33″N, 76°55′56″W). Traps were randomly assigned with or without the mixed vittatalactone lure, and rerandomized weekly. Maryland traps were installed August 9 and removed September 27, 2019.

New Hampshire traps were installed, randomized, and collected similarly in three New Hampshire locations. All were small (1.5–4.5 ha), diversified organic or pesticide-free vegetable farms, growing a range of cucurbit crops in Strafford County (Rollinsford, 43°12′30.9″N 70°49′36.4″W; Lee, 43°09′41.3″N 70°58′12.4″W; Lee, 43°09′49.8″N 70°58′58.6″W), with a total of four pairs (blocks) of traps. New Hampshire traps were installed August 20 and removed September 18, 2019.

At each site, captures of squash bugs, striped cucumber beetles and other squash-associated insects were enumerated. The data were analyzed separately for Maryland and New Hampshire, with pheromone treatment as a fixed effect

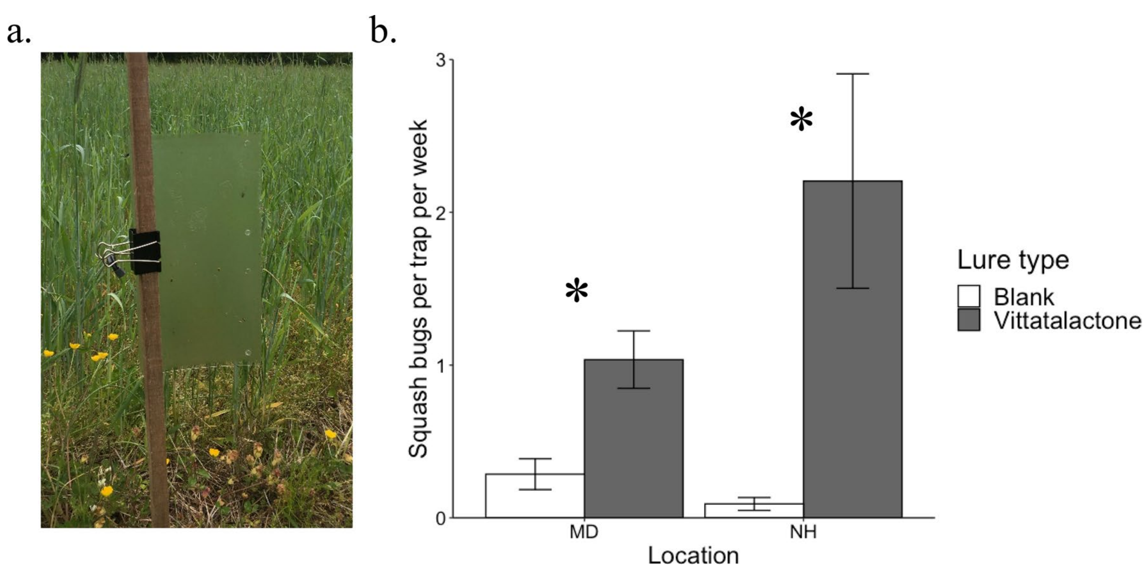

Fig. 2 **a** Experimental setup of the clear sticky trap with vittatalactone-loaded septum, and **b** Squash bug adults captured on clear sticky traps adjacent to cucurbit fields in August–September 2019 in Maryland (MD) and New Hampshire (NH), with or without mixed vittatalactone lure, where * indicates $p < 0.05$

Table 1 Analysis of deviance table for striped cucumber beetle abundance and squash bug adults and egg clutches in a field survey of the *C. p. ovifera* and *C. p. pepo* cultivars

Effect	DF	Beetle adults		Squash bug adults		Squash bug egg clutches	
		Deviance	*P*	Deviance	*P*	Deviance	*P*
Cultivar	1	239.5	**< 0.001**	17.7	**< 0.001**	4.0	**0.04**
Row (E–W)	1	18.6	**< 0.001**	3.1	0.08	6.6	**0.01**
Plant position (N–S)	1	13.3	**< 0.001**	0.3	0.60	0.001	0.97
Cultivar*row	1	25.8	**< 0.001**	3.7	0.06	1.4	0.24

Squash bug adults: Model deviance(df)–Null: 187.28(223), Residual 162.53(219) Eggs: Model deviance(df)–Null: 310.51(223), Residual 298.48(219) Beetle counts: Model deviance(df)–Null: 531.89(222), Residual 234.61(218)

The *p* value is from a likelihood ratio test

and direction or location as random effects in SAS Proc Mixed (SAS v 9.4, SAS Institute 2018).

Results

Cultivar-based cross-resistance in *C. pepo* preference between squash bugs and beetles

Field experiments were used to evaluate squash bug (*A. tristis*) preference for and performance on representative squash cultivars of *C. p. ovifera* (CPO) and *C. p. pepo* (CPP), and how it aligned with preference of the co-occurring pest, the striped cucumber beetle (*A. vittatum*) (Fig. 3). In our survey, almost three times as many beetles were found on CPP (4029 total) than on CPO (1432 total) (Table 1), and beetle damage was also 79% greater on CPP ($F_{1,219} = 113.1$, $p < 0.001$). In contrast, on the same plants, squash bug adults and egg clutches were more frequently found on CPO: we observed 41 adults and 154 egg clutches on CPO compared to 7 adults and 104 egg clutches on CPP (Table 1). In the cage experiment where squash bugs were presented with two plants of the same cultivar (no choice between cultivars), squash bugs also had more frequent oviposition in CPO cages (16 with, 9 without eggs) than CPP (3 with, 14 without eggs) (Fisher's exact test, $p = 0.005$). However, despite clear differences in squash bug preferences, there was no difference between CPP and CPO in squash bug nymphal survival ($\chi^2 = 2.391$, $df = 2$, $p = 0.302$) or development time ($\chi^2 = 9.7613$, $df = 8$, $p = 0.282$) (Fig. 4). In sum, these results suggest that cultivar choice presents trade-offs in agricultural management of beetles and squash bugs as cultivars have opposing susceptibility to the two herbivores.

Squash bug oviposition preference in response to beetle damage

To test the effect of beetle damage on squash bug oviposition preference independently of strong varietal preference, we used replicated plots of inter-subspecific *C. pepo* breeding lines (CPO x CPP, F_4 plots). We observed squash bug oviposition on all 13 breeding lines, and in most of the plots (22 of 39 plots). There was a maximum of five squash bug egg clutches observed in a plot, and beetle damage to plots marginally positively predicted the number of squash bug egg clutches per plot (Fig. 5; $F_{1,23} = 3.921$, $p = 0.056$).

Squash bug oviposition and adult preference in response to beetle aggregation pheromone

Using paired choice tests, we assessed whether squash bugs preferentially oviposit on plants with male beetles actively feeding–likely emitting aggregation pheromone–compared to those lacking male beetles. Across cultivars, oviposition declined over the course of the season, from oviposition in 72% of tests in the earliest trial to only 25% by the last trial. Only three egg clutches were found on CPP across all trials (two on plants with male beetles, and one on a plant lacking male beetles). For CPO, over the course of the season, there was no overall difference in oviposition on control plants versus those with male beetles (Table 2). However, there was a strong interaction with trial: squash bugs preferred CPO plants with male beetles in early trials when there was more frequent oviposition, but did not show preference in later trials when there was less frequent overall oviposition.

In a separate experiment, we tested squash bug attraction to visually masked traps of CPP and CPO with different beetle infestation treatments (Brzozowski et al. 2020a). Traps containing pheromone-producing male beetles on plants more frequently caught squash bugs than other type of trap (traps with female beetles on plants, or plants alone). Squash bugs were trapped on 14 traps with male beetles and plants (23%), as compared to five of all other trap types (9%) (William's corrected $G = 4.43$, $p = 0.035$). However, there was no difference in frequency of squash bugs trapped between traps with female beetles versus no beetles (William's corrected $G = 0.137$, $p = 0.711$). Together, these results suggest

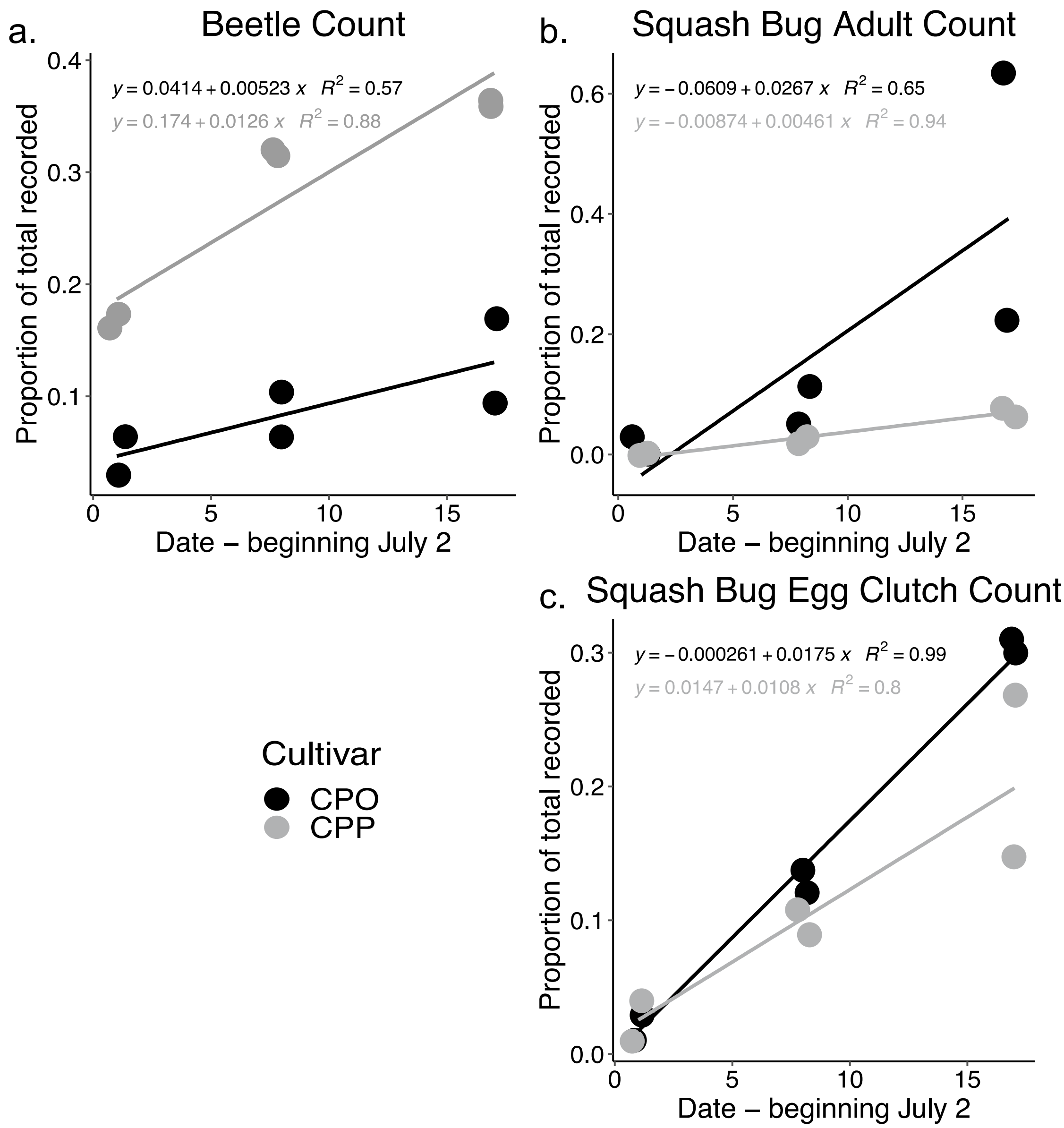

Fig. 3 Counts of **a** striped cucumber beetle adults, **b** squash bug adults and **c** squash bug egg clutches in field survey relative to total recorded over the course of the experiment. The color indicates the cultivar (*Cucurbita pepo* ssp. *pepo*, CPP; *Cucurbita pepo* ssp. *ovifera*, CPO), and the points represents each of the two rows per cultivar

that aggregation pheromone, not plant volatiles induced by beetle feeding, was responsible for squash bug attraction.

Finally, we tested squash bug attraction to isolated male beetle pheromone (vittatalactone), dispensed by synthetic lures. As expected, more striped cucumber beetle adults were captured per week on sticky traps with vittatalactone compared to traps without the pheromone (mean ± s.e., Maryland: 11.36 ± 1.21 with pheromone versus 5.36 ± 0.45 without pheromone, $F_{1,51} = 9.52$, $p = 0.003$; New Hampshire: 2.11 ± 0.57 with pheromone versus 0.03 ± 0.03 without pheromone, $F_{1,15} = 14.84$, $p = 0.002$). Traps with vittatalactone also captured substantially higher numbers of adult squash bugs than traps without pheromone (MD: $F_{1,51} = 4.49$, $p = 0.039$; NH: $F_{1,15} = 6.22$, $p = 0.025$; Fig. 2b). Nymphs were captured only in New Hampshire, and nymphal captures on traps with pheromone significantly exceeded those without the pheromone ($F_{1,4} = 16.0$, $p = 0.016$).

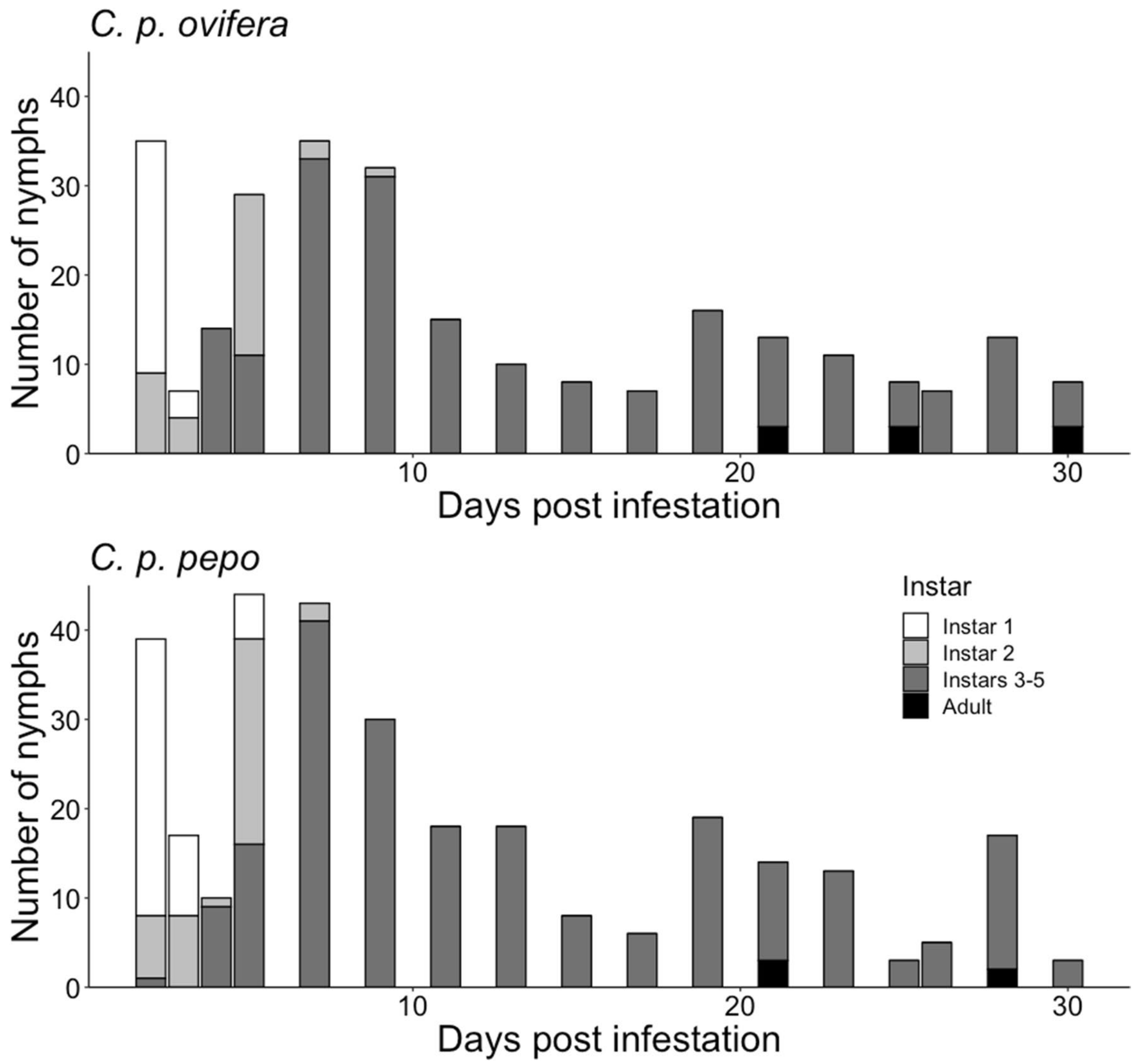

Fig. 4 Number of squash bug nymphs on *C. pepo* cultivars over time, where progressing developmental stage is indicated by darker colors

Table 2 Wilcoxon signed rank test results for total number of squash bug eggs between treatments (plants infested with male striped cucumber beetles vs. controls) on a *C. p. ovifera* cultivar from all choice tests with eggs

		Significant differences by treatment		
Trial	*N* tests with oviposition	By trial	By early, late season	Result—overall
1	7	***P*=0.0389**	***P*=0.013**	*P*=0.488
2	5	*P*=0.233		
3	1	NA	*P*=0.089	
4	3	*P*=0.149		

Discussion

The co-occurring specialized striped cucumber beetles (*Acalymma vittatum*) and squash bugs (*Anasa tristis*) respond to shared and divergent plant- and insect-derived cues in their interactions with each other and their *Cucurbita pepo* host plants. We found differences in host plant preference between the herbivores, with beetles preferentially consuming *C. p. pepo* (zucchini; CPP) and squash bugs preferentially occurring and ovipositing on *C. p. ovifera* (straightneck summer squash; CPO). In contrast, the beetle aggregation pheromone, vittatalactone, mediated positive responses from both herbivores: squash bugs preferentially laid eggs on plants with greater beetle damage and were attracted to vittatalactone. These results suggest that squash bugs eavesdrop on the pheromone of a co-occurring specialist herbivore, which could provide opportunities to develop management strategies with efficacy against both pests.

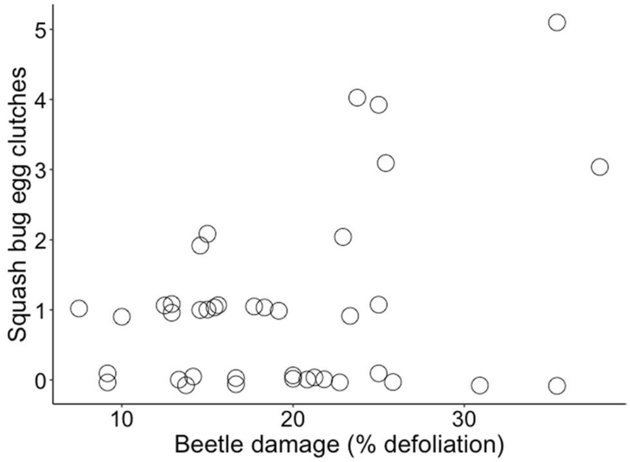

Fig. 5 Relationship between striped cucumber beetle damage to plots of *C. pepo* and number of squash bug egg clutches

Differences in host plant preference

In bioassays with *C. pepo* cultivars, we demonstrate that squash bug adults prefer CPO over CPP, while striped cucumber beetle adults prefer CPP. Adult striped cucumber beetle preference for CPP has been widely established in multi-cultivar screens (Ferguson et al. 1983; Hoffmann et al. 1996; Brzozowski et al. 2016), but there are no analogous trials for squash bugs. Recently, Cornelius (2017), demonstrated that squash bugs prefer a CPO cultivar, 'Slick Pik' over three CPP cultivars. However, lack of difference between squash bug cultivar preference between CPO and CPP (Cornelius 2017), and even preference for CPP (Novero et al. 1962) have also been reported. In addition, there are reports of rapid adaptation of squash bugs to resistant cultivars (Margolies et al. 1998). While there is a clear need for squash bug preference trials with larger sets of cultivars over multiple years, our results suggest that squash bugs and striped cucumber beetles have diverging *C. pepo* cultivar preference when measured at field scale.

While we observed differences in *C. pepo* preference, they were not tied to obvious metrics of performance for either herbivore species. Despite adult squash bug preference for CPO, we found that squash bug nymphs survived and developed equally well on both subspecies, similar to results of Bonjour and Fargo (1989) where they also noted the same rate of development on CPP and CPO, but greater survivorship on CPP. Striped cucumber beetle adults strongly prefer CPP (Brzozowski et al. 2016), and beetle larvae attain greater mass on undamaged CPP (Tallamy and Gorski 1997). While adult preference and larval performance are largely associated (Gripenberg et al. 2010), there is wide variability in the link between preference and performance even among specialist herbivores (Charlery De La Masselière et al. 2017; Hufnagel et al. 2017) .

Squash bugs respond to cues of a co-occurring beetle

We found that squash bugs positively respond to striped cucumber beetle damage and aggregation pheromone, indicating that beetle-derived cues may mediate squash bug location of host plants. While striped cucumber beetles prefer and are more frequently found on CPP (not preferred by squash bugs), beetles still occur and emit pheromone on CPO, indicating that pheromone can steer squash bugs to CPP or CPO. Intuitively, cues from the striped cucumber beetle are likely to be reliable signals for squash bugs seeking host plants: both herbivores are highly specialized on cucurbits, temporally synchronized (in temperate regions, both overwinter as adults and emerge in early summer), and have a longstanding range overlap. In agroecosystems, insects eavesdrop on cues from other species to locate a food source, with the prominent examples being parasitoids using herbivore induced plant volatiles (Turlings and Erb 2018) and pheromones (Fatouros et al. 2008) for host location. However, there are few examples of cross-attraction to pheromones between species of herbivores; we are only aware of reports for some species of stink bugs (Hemiptera: Pentatomidae) (Weber et al. 2018) and pine coneworms (Lepidoptera: Pyralidae) (Hanula et al. 1984). Nonetheless, pheromone cross-attraction among distantly related insects may be widespread. For instance, large-scale monitoring experiments using pheromone traps for three different Lepidopteran pests had significant pollinator bycatch (Spears et al. 2016; Grocock and Evenden 2020).

To further advance our understanding of the biology and the agricultural management of striped cucumber beetles and squash bugs, a key goal would be to determine if and how squash bugs benefit by using beetle aggregation pheromone as a cue. Beetle damage in *C. pepo* induces foliar volatile emission (Brzozowski et al. 2020a), and damage by lepidopteran herbivores has been shown to affect host plant resistance (Brzozowski et al. 2019), so beetle-induced changes to host plant quality may benefit squash bugs. For example, in cabbage (*Brassica oleracea*), induction by a specialist herbivore increased susceptibility to subsequent feeding by other specialists (Poelman et al. 2010). In parallel, it would be worthwhile to consider if beetle preference or performance is affected by eavesdropping squash bugs by, for instance, increased competition for food.

Implications for agricultural management

Our work has implications for management of striped cucumber beetles and squash bugs. First, knowledge of potential trade-offs in *C. pepo* cultivar preference among herbivores could allow growers to be better prepared to manage their more problematic pest. However, beyond *C. pepo*, *C. maxima* is highly preferred and *C. moschata* is less preferred for both squash bugs (Howe and Rhodes 1976; Bonjour et al. 1990; Majumdar and Price 2019) and cucumber beetles (Howe et al. 1972; Adler and Hazzard 2009; Gardner et al. 2015). The apparent consistency in preference for other *Cucurbita* ssp. beyond *C. pepo* could provide a framework for discovery of and breeding for plant traits attractive or repellent to both species of herbivores in future management strategies. For example, does the volatile blend from *C. maxima* squash blossoms that is highly attractive to striped cucumber beetles (Andersen and Metcalf 1986) and commercially available (e.g. Trécé Inc., Adair, OK, USA), also attract squash bugs?

The most compelling result from our work is that vittatalactone, the beetle produced aggregation pheromone, attracts both pest species. Vittatalactone lures are not available commercially, but using the limited quantity synthesized, vittatalactone should be further studied for use in trapping efforts to determine the timing and dosages required to trap pests at economically meaningful levels, and placement optimization to minimize unwanted vicinity effects (Wallingford et al. 2018). In addition, vittatalactone could be considered in combination with other management efforts. For instance, does vittatalactone increase the attractiveness of trap crops (like *C. maxima*) (Gardner et al. 2015; Majumdar and Price 2019), and would vittatalactone increase the efficacy of a push–pull system by further luring herbivores away from the main crop (Fair and Braman 2017; Kahn et al. 2017)? At the same time, there is growing evidence that squash bugs have their own aggregation pheromone (Weber et al., unpublished data), and it is important to examine the relative strength and response from both species. Finally, it would be interesting to examine how vittatalactone affects recruitment of biological control agents for both squash bugs (Cornelius et al. 2016; Phillips and Gardiner 2016; Wilson and Kuhar 2017) and beetles (Smyth and Hoffmann 2010). These tandem and complementary approaches are critical in applying research in chemical ecology to farms: while there are some instances of strong efficacy from a single approach (e.g. moth sex pheromone lures), in high value fruit and vegetable crops, combining approaches may be necessary to reduce injury to a degree that would be economically meaningful for growers.

Conclusions

Overall, we present strong evidence for an ecological interaction between two co-occurring specialist herbivores of *C. pepo*, with implications for pest management. We demonstrated that while there are opposing differences in host plant preference between squash bugs and striped cucumber beetles for the two *C. pepo* domesticates that may lead to trade-offs in agricultural management, we also present evidence that squash bugs respond to the striped cucumber beetle aggregation pheromone. Adults of both species were trapped at higher numbers when vittatalactone was present, and more squash bug egg clutches were observed on plants with higher beetle damage and in response to male (but not female) feeding. These results provide further incentives to explore use of vittatalactone in trapping of both species, providing an important additional avenue to explore for squash bugs, as their range expansion heightens their status as a major pest.

Acknowledgements We thank Elise He and the Cornell University Agricultural Experiment Station for assistance with field work in NY. For assistance with the lure experiments, we thank: Ashot Khrimian (coordinating the contracted synthesis of mixed vittatalactone), Filadelfo Guzman (lure preparation), Philip Brand, Tyler Murray, and Sarah Cox (assistance at New Hampshire field sites), Megan Herlihy and Alexander Bier (assistance acquiring Maryland trapping data), Mary Cornelius, George Meyers and the BARC-West farm crew (use and maintenance of Maryland squash plantings), Ariela Haber (SAS data analysis). Members of the Agrawal laboratory also provided insightful comments on earlier versions of this work.

Funding This research was partially supported by Multistate (NE-2001) and Hatch (2018–19-135) projects from the USDA National Institute of Food and Agriculture allocated through Cornell University. LB was funded by Seed Matters Graduate Student Fellowship (2015–2020).

Declarations

Conflicts of interest The authors declare that they have no conflict of interest. LB, AW, DW and AA declare they have no conflict of interest. MM is the co-founder of Row 7, an organic seed company.

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