

ABSTRACT

Title of Thesis: USING A LIVING MULCH AND WOLF
SPIDERS TO MANAGE PEST
ARTHROPODS IN CUCUMBER

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Pest management practices that conserve beneficial organisms and improve yield are needed. This research aims to determine how: 1) red clover living mulch impacts insect herbivores, arthropod natural enemies, and cucumber quality and yield, and 2) wolf spiders influence cucumber beetles and cucumber plant attributes. Herbivore and natural enemy abundances on cucumber plants in plots with and without interplanted red clover (RC) were compared using foliage searches and sticky cards. Effects of spiders on cucumber beetle behavior, densities, and mortality was assessed in cages with and without a wolf spider and/or their cues. RC plots had fewer striped cucumber beetles and aphids, and more big-eyed bugs and minute pirate bugs. Spotted cucumber beetle densities were reduced and striped cucumber beetle mortality was higher in cages with than without spiders. These studies demonstrated that red clover living mulch and wolf spiders can be used to decrease pest arthropods in cucumber.

USING A LIVING MULCH AND WOLF SPIDERS TO MANAGE PEST
ARTHROPODS IN CUCUMBER

by

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Chapter 1: Effects of red clover living mulch on arthropod herbivores, natural enemies, and yield in cucumber

ABSTRACT

Increasing habitat complexity through cover cropping has been proposed as a method to reduce herbivore colonization of crops plants and encourage natural enemies. In order to test this, effects of red clover (*Trifolium pratense* L.) interplanted as a living mulch on insect herbivores and arthropod natural enemies associated with cucumber (*Cucumis sativus* L.) and cucumber yield were examined. In 2016 and 2017, field experiments were conducted using a randomized complete block design with two treatments: red clover interplanted as a living mulch with cucumber and cucumber grown as a monoculture. Rates of parasitism and counts of arthropod natural enemies and herbivores on cucumber plants and sticky cards were compared in cucumber monoculture and interplanted with red clover. Foliar counts indicated that two herbivores, the striped cucumber beetle (*Acalymma vittatum* (F.)) and the melon aphid (*Aphis gossypii* Glover), were reduced by the presence of red clover. Sticky card counts of the big-eyed bug, *Geocoris* spp., the minute pirate bug, *Orius* spp., and the lady beetle (Coccinellidae), were greater in cucumber interplanted with red clover than monoculture plots. Parasitism rates of cucumber beetles and cucumber yield did not differ between treatments. Potential reasons for these findings are discussed.

KEY WORDS living mulch, cucumber beetles, red clover, associational resistance, and natural enemies

Introduction

Inter-planting non-host plants can reduce pest colonization of cash crops. Ecologically, this method has been referred to as associational resistance: when a neighboring plant species is used to decrease the chance that another plant species (e.g. the cash crop) is discovered or utilized by herbivores (Barbosa et al. 2009). Two major hypotheses were proposed to explain associational resistance. Root (1973) put forward the enemies hypothesis which argues that natural enemies reduce herbivore populations in vegetative diverse plantings. These types of plantings may increase the abundance (Gupta et al. 2012, Gareau et al. 2013), survival (Gupta et al. 2012), foraging efficiency (Langer 1996, Gupta et al. 2012), or diversity (Ryan et al. 1980, Gupta et al. 2012) of natural enemies (Russell 1989, Barbosa et al. 2009). Alternatively, Tahvanainen and Root (1972) contended that insect herbivores decrease with added plant diversity, but natural enemies are not the main contributor to this reduction. It was argued that associational resistance is instead mediated by plant diversity directly affecting herbivores more than by the indirect effects of natural enemies. The research concentration hypothesis suggests that herbivores more easily find, stay in, and reproduce in monocultures of host plants than in polycultures. Applying the concept of associational resistance to agricultural systems could offer growers a sustainable alternative strategy to pesticides for managing arthropod pests.

Cucumber (*Cucumis sativus L.*; Cucurbitaceae) is a widely cultivated crop. The US produces the 6th largest amount of cucumbers by weight and market value (FAO 2012). In 2015, the market value for slicing and pickling cucumbers were approximately 177 and 173 million US dollars, respectively (NASS USDA 2016).

The Northeastern states of DE, MD, NJ, NY, and PA cultivated 7.7% of the total acreage of harvested cucurbits within the US, with a value of 146.6 million dollars (NASS USDA 2014).

Cucumbers produce a bitter compound, cucurbitacin C, that effectively repels many herbivores. However, some specialist herbivores are attracted to cucurbitacins (Chambliss and Jones 1966), which do not appear to impact their fitness (Tallamy et al. 1997). The striped (*Acalymma vittatum* (F.); Coleoptera: Chrysomelidae) and spotted cucumber beetles (*Diabrotica undecimpunctata howardi* (Barber); Coleoptera: Chrysomelidae) are predominate specialist cucumber herbivores. Cucumber beetles often cause reduction in cucumber yield and quality (Burkness and Hutchison 1998). Young cucurbit plants are especially vulnerable and often die from heavy infestations of overwintering cucumber beetle adults (Burkness and Hutchison 1998). Larvae of cucumber beetles live in the soil and feed on cucurbit roots impacting root development. Second generation cucumber beetle adults feed on plant leaves, blossoms, and fruits (Burkness et al. 1999, Diver and Hinman 2008). However, only a few studies have elucidated effects of cucumber beetles on cucumber yield (Burkness and Hutchison 1998, Snyder and Wise 1999, Barber et al. 2011). Cucumber beetles can transmit diseases to plants including bacterial wilt (*Erwinia tracheiphila*) and squash mosaic virus (Fleischer et al. 1999). Bacterial wilt can decrease plant yield and quickly kill cucurbit plants (Saalau Rojas et al. 2011, Latin 2017). Two additional cucumber pests common in Northeastern United States include the melon aphid (*Aphis gossypii*; Hemiptera: Aphididae) and the squash bug (*Anasa tristis*; Hemiptera: Coreidae) (Brust et al. 2015).

Growers have low tolerances for cucumber beetles, and they often spray pesticides five to seven times per season at the initial detection of a cucumber beetle (Burkness and Hutchison 1998, Diver and Hinman 2008). This frequent use of pesticides has caused pesticide resistance to develop in other cucumber pests such as the melon aphid (Wang et al. 2002). In addition, some pesticides being used have negative impacts on pollinators, natural enemies, and human health (Seidler et al. 1996, Ohayo-Mitoko 2000, Wesseling et al. 2002, Kamel and Hoppin 2004, Brittain et al. 2010, Whitehorn et al. 2012). Additionally, many foliar sprays do not effectively control cucumber beetles as they often inhabit the base of plants where they are protected from pesticide sprays (Brust 2017). Also, some insecticides will not impact cucumber beetle larvae in the soil. Thus, an effective management program often depends on the cucumber beetle life stage being targeted (Shelton et al. 1993). For cucurbit crops such as cucumber, it has become increasingly important to establish effective alternative management practices that pose fewer risks.

Interplanting living mulch could be a cost-effective practice that can implement associational resistance in agricultural systems. Living mulch is a type of cover crop that is planted before or after the main crop and is maintained as living ground cover throughout the crop's growing season. Compared to other forms of plant diversification (e.g. polycultures, hedge rows, row intercropping, etc.), cover crops are grown primarily to maximize several agroecosystem services. They may be used to prevent soil erosion, suppress weeds, add organic matter to the soil, and improve soil structure (Hartwig and Ammon 2002). Previous studies have shown that interplanted living mulches can reduce pests in cucurbit crops (Amirault and Caldwell

1998, Grasswitz 2013, Hinds and Hooks 2013). The presence of sunn hemp (*Crotalaria juncea* L.; Fabaceae) used as a living mulch reduced striped and spotted cucumber beetles and melon aphids in zucchini (*Cucurbita pepo* L.; Cucurbitaceae) compared with monoculture plots (Hinds and Hooks 2013). When interplanted with strips of buckwheat (*Fagopyrum esculentum* Moench; Polygonaceae), striped cucumber beetle populations were reduced by 60% on cucumber and pumpkin plants compared to plots of these crops without buckwheat (Amirault and Caldwell 1998). Pumpkin plants interplanted into a flowering mix contain reduced spotted cucumber beetles and increased big-eyed bug (*Geocoris* spp.) numbers (Grasswitz 2013).

Red clover is widely grown as a cover crop primarily because of its ability to fix nitrogen and serve as forage (Duiker and Curran 2007). Additionally, red clover is widely adaptable, and has an extended growing season, and relatively few arthropod pests (Lacefield and Ball 2010). Red clover is low-lying, and as such, when interplanted as a living mulch, does not aggressively compete with cash crops. It produces a dense flowering mat of vegetation which could decrease the visibility of host plants and subsequently reduce herbivore colonization and oviposition. For example, the turnip root fly (*Delia floralis*) oviposited less on cabbage intercropped with red clover than cabbage grown in monoculture (Björkman et al. 2010). Red clover could also provide nectar, pollen, and alternate prey and serve as structurally complex habitat that enhances natural enemy colonization.

Despite these attributes, red clover has infrequently been used as a living mulch for insect pest suppression. Furthermore, it seems that red clover as a living mulch could be a more practical living mulch option for growers compared to other

plants used to test effects of plant diversification in cucurbit systems. Unlike red clover, flowering mixes can be weedy and invasive, may not establish on agricultural land, and are often not compatible with farm machinery of larger-scale agricultural operations (Liebman and Davis 2000, Nicholls and Altieri 2013, Uyttenbroeck et al. 2016). Because buckwheat is not a legume, it cannot provide the nitrogen fixation benefit that red clover and sunn hemp can. However, while red clover is low-growing and widely available, sunn hemp living mulch grows very rapidly and needs to be clipped regularly to prevent it from shading out crop plants and sunn hemp seed is not always readily available within the United States (Hooks et al. 2007, Hinds and Hooks 2013). Although it is anticipated that red clover as a living mulch will increase yield by suppressing arthropod pests and providing nitrogen, red clover has also been demonstrated to compete with crop plants, reducing yield (Kołota and Adamczewska-Sowińska 2013). Thus, red clover's effect on yield must be considered along with effects on pest and natural enemies.

The goal of this research was to test the influence of red clover living mulch on arthropod pests, natural enemies, and yield in cucumber. I hypothesize that: 1) there would be less herbivores and more natural enemies in cucumber interplanted with red clover compared with monoculture cucumber plantings and 2) yields and quality of cucumber fruits would be higher in cucumber interplanted with red clover compared with monoculture cucumber plantings.

Materials and Methods

Experimental design. Field experiments were conducted at the Central Maryland Research and Education Center (CMREC) in Upper Marlboro, MD and

Western Maryland Research and Education Center (WMREC) in Keedysville, MD in 2016 and at CMREC only in 2017. CMREC predominately has a fine-loamy soil type, and WMREC predominately has a silt loam. The semi-dwarf cucumber variety, Fanfare (J.W. Jung Seed Company, Randolph, WI), was used as the study crop, and red clover, 'Freedom' (Lancaster Ag., Ronks, PA), was used as the interplanted living mulch. Experimental layout at each study site was a randomized complete block design with two treatments replicated four times. The two treatments were cucumber grown as a monoculture (M) or interplanted into a red clover living mulch (RC). Plot dimensions at CMREC and WMREC were 10.1 by 8.2 m and 9.1 by 7.6 m, respectively for both years. Monoculture plots remained fallow over the fall and winter and were rototilled before cucumber planting.

Red clover was seeded with a no-till drill at 18 cm row spacing in September of the prior year at a rate of 13.5 kg/ha. Red clover plots were mowed with a rotary mower and strip-tilled at a width of 46 cm using a Craftman rear-tine tiller prior to cucumber planting. Cucumbers were sowed into the center of the tilled zone one week later. Red clover strips were approximately 0.76 m wide and were cut with hand trimmers during initial weeks after cucumber planting to reduce competition for light with young cucumber seedlings. In 2016, cucumbers were direct-seeded into CMREC plots on June 06 and June 07 and into WMREC plots on June 08 and June 10. In 2017, cucumbers were seeded at the University of Maryland greenhouse on June 01 and transplanted into experimental plots on June 13. Plots at CMREC and WMREC contained eight and seven rows of cucumber in each plot, respectively. Each row contained 24 seedlings spaced 0.3 m apart within rows and 1.2 m between rows.

Cucumber seedlings that failed to establish or died were replaced weekly during the initial weeks after planting. Weeds in plots were managed by hand weeding and spot spraying with glyphosate (Roundup Powermax, Monsanto, Washington, DC).

Glyphosate was applied within only M plots on June 01, 2016 and June 12, 2017 in CMREC and on June 02, 2016 in WMREC. Due to high cucumber beetle densities present during the seedling stage at the WMREC site, Imidacloprid (Admire Pro, Bayer CropScience, Research Triangle Park, NC) was applied as a soil drench at a rate of 0.03ml/plant around each cucumber seedling (as advised by Galen Dively, pers. commun.).

Arthropod counts. Herbivore and natural enemy abundance on cucumber plants were visually surveyed weekly after cucumber planting until harvest. In 2016 fifteen 0.5 by 0.5 m and in 2017 eight 1 m long sampling areas of cucumber plants were randomly-selected from interior cucumber rows. Entire plants within sampling areas were examined, and cucumber specialists and other herbivores frequently encountered as well as arthropod natural enemies were recorded.

Yellow sticky cards (Olson Products Inc., Medina, Ohio) were used weekly throughout the cucumber growing season to help estimate natural enemies and herbivore numbers within each plot. Two yellow sticky cards (7.6 x 12.7 cm) were placed ~3.7 m apart diagonally in two central cucumber rows (rows 3 and 6), and two sticky cards were placed ~4.1 m apart diagonally in two central cucumber rows in red clover strips or bare-ground areas between cucumber rows (Fig. 2). Cards were placed just above the level of the plant canopy, and were oriented perpendicular to plant rows. Striped and spotted cucumber beetles and generalist natural enemies were

identified and counted on the sticky cards. Flies larger than 4 mm were further identified to determine whether they were tachinids (Platt et al. 1999).

Parasitism rates. In 2016, cucumber beetle parasitism was estimated on five dates from June to August similar to Smyth and Hoffmann (2010) and Lewis (2015). Randomly selected areas within each plot were searched for cucumber beetle adults until at least five were found or the plot had been searched for 10 minutes. Collected cucumber beetles were stored in 21.3g Solo[®] cups (Urbana, IL). Cups were labeled with the date, replicate, and treatment plot from which beetles were collected. In the laboratory, cups were placed in an incubator (Percival Scientific, Inc., Model #: I-35LLVL, Boone, IA) set at 26°C on a 16-8 hour light-dark cycle. Cucumber beetles were fed cucumber leaves and provided water. Cups were checked at least once a week for pupae or adults of the tachinids *Celatoria setosa* (Coquillett) or *Celatoria diabroticae* (Shimer). If cucumber beetles died prior to the emergence of a parasitoid, beetles were dissected to determine if parasitoid larvae were inside.

Cucumber yield and quality. USDA's standards for cucumber grades were used to grade and evaluate cucumber damage, shape, and marketability (USDA, Agricultural Marketing Service, Fruit and Vegetable Division 1958). Once fruits became mature, all plants from the interior rows of each plot (excluding two border rows on each side) were harvested, graded and counted to determine treatment impact on yield. Harvesting ended after cucumbers reached peak productivity and more than 50% of fruits were unmarketable from the majority of rows. Fruits in plots displaying symptoms of disease or rot were categorized accordingly. Aborted fruits were also counted if observed. In 2016, fruits were categorized, counted, weighed, and recorded

according to damage (no damage, slightly damaged, or seriously damaged) and shape (well, fair, or bad). Marketable fruit numbers and grades, Fancy, 1, or 2, were estimated from these categories. In 2017, marketable fruits were graded, and unmarketable fruit were further sorted by flaw type (scarred/injured, misshapen, yellowing, jumbo, small, or diseased). Fruits were then weighed and recorded by category, except diseased fruit which were counted and discarded. If there were signs of feeding inside the fruit (frass or tunneling), fruits were cut open and larvae collected and identified.

Statistics. For visual surveys and sticky cards, arthropod taxa that composed more than or equal to 1% of the total number of arthropods found on plants across all dates were analyzed. Counts were converted into number per meter of row and averaged over each plot for each date before analysis. Linear mixed models (“lmer”, package lme4) (R Development Core Team 2011) with repeated measures were used to compare arthropods found on cucumber plants over time. Replicate was treated as a blocking factor and plot, year, and site were treated as random effects.

Parasitism rates of the cucumber beetles were compared between treatments over time using generalized linear mixed models (“glmer”, package lme4) (R Development Core Team 2011) with repeated measures. Replicate was a blocking factor and plot and site were treated as random effects. The response variables in this model were the proportion of parasitized beetles from each treatment for each parasitoid species. These models used a binomial distribution (parasitized or not parasitized).

Number and weight of total, total marketable, Grade Fancy, Grade 1, Grade 2, and unmarketable fruit, and number of rotten and aborted fruit were compared using linear mixed effect models (“lmer”, package lme4) (R Development Core Team 2011). Generalized linear mixed models (“glmer,” package lme4) (R Development Core Team 2011) with a binomial distribution were used to compare fruit damage. Response variables were proportions of fruits, out of total number and weight, that contained scarring or feeding damage, were cull, or yellowing, or proportions of fruits out of total number that displayed symptoms of disease. Replicate was a blocking factor, while year and site were random effects in these models.

Results

Arthropod abundance - *cucumber plants*. Herbivores observed on cucumber plants that made up greater than 1% of the total population were analyzed. Those greater than 1% included striped and spotted cucumber beetles, melon aphids, squash bugs, and leafhoppers (Cicadellidae). Approximately 36% more striped cucumber beetles were found on cucumber plants in monoculture (M) compared to red clover (RC) (polyculture) plots overall. There were more striped cucumber beetles found in M than in RC plots ($\chi^2=8.53$, $df=1$, $P=0.003$; Fig. 3), and a significant treatment by date interaction ($\chi^2=15.68$, $df=4$, $P=0.003$) at CMREC in 2017. The two treatments differed on 39 ($z=3.62$, $P=0.002$) and 46 ($z=3.29$, $P=0.004$) days after planting (DAP). Overall, there were 20% more spotted cucumber beetles in M compared to RC plots. There were more spotted cucumber beetles in M compared to RC plots at WMREC in 2016 ($\chi^2=6.08$, $df=1$, $P=0.01$; Fig. 4A) and in CMREC 2016 ($\chi^2=5.45$, $df=1$, $P=0.02$; Fig. 3B). In 2016 in WMREC there were more spotted cucumber

beetles in M compared to RC plots on 40 ($z=2.77$, $P=0.009$) and 61 ($z=2.77$, $P=0.009$) DAP. In 2016 in CMREC there were more spotted cucumber beetles in M compared to RC plots on 48 ($z=2.72$, $P=0.01$) DAP. Melon aphid densities were ~34 times greater in M compared to RC plots overall. There were more melon aphids in M compared to RC plots at CMREC in 2017 ($\chi^2=10.16$, $df=1$, $P=0.001$; Fig. 5). At CMREC in 2017, more aphids were found on cucumber plants in M than RC plots at 39 DAP ($z=3.74$, $P<0.001$). The treatment by date interactions was significant for leafhoppers. There were more leafhoppers in RC compared to M plots at CMREC in 2017 ($\chi^2=4.74$, $df=1$, $P=0.03$; Fig. 6), in particular there were more leafhoppers in RC than M plots on 32 DAP ($z=-2.62$, $P=0.01$).

Squash bug and plant hopper densities were similar in RC and M plots.

Spiders and lady beetles were the only predators encountered in high enough densities during foliar surveys for analysis. Overall, lady beetle numbers were greater in M than RC plots by ~72%. The treatments significantly differed at CMREC in 2016 ($\chi^2=3.97$, $df=1$, $P=0.046$; Fig. 7), with there being more ladybugs in M than RC plots on 55 ($\chi^2=3.04$, $P=0.005$) DAP. The treatments did not significantly differ for spiders at any of the study sites.

Sticky card counts. Overall, striped cucumber beetles were approximately 83% more abundant on sticky cards in M than RC plots. The treatments significantly differed at CMREC in 2017 ($\chi^2=26.41$, $df=1$, $P<0.001$; Fig. 8), and there was a significant treatment by day interaction ($\chi^2=21.27$, $df=1$, $P<0.001$) with there being more striped cucumber beetles in M compared to RC plots at 35 ($z=3.32$, $P=0.003$), 42 ($z=3.83$, $P<0.001$), and 49 ($z=4.68$, $P<0.001$) DAP. Overall, spotted cucumber

beetles were 9% more abundant in M compared to RC plots. However, at CMREC in 2016 there were more spotted cucumber beetles in RC compared to M plots ($\chi^2=3.9$, $df=1$, $P=0.048$; Fig. 9), specifically there were more in RC plots on 50 DAP ($z=-2.51$, $P=0.02$).

Natural enemies encountered in high enough numbers on sticky cards for treatment comparisons included tachinid flies, spiders, lady beetles (Coccinellidae), big eyed bugs (*Geocoris* spp.), and minute pirate bugs (*Orius* spp.). Overall, lady beetles were ~ 60% more abundant in RC compared to M plots. In CMREC in 2017, there were more lady beetles at WMREC in 2016 ($\chi^2=6.74$, $df=1$, $P=0.009$; Fig. 10A) and CMREC in 2017 ($\chi^2=5.2$, $df=1$, $P=0.02$; Fig. 10B). There was also a significant treatment by date interaction for lady beetles at CMREC in 2017 ($\chi^2=12.42$, $df=4$, $P=0.01$). In 2016 at WMREC, they were more abundant in RC compared to M plots on 33 ($z=-2.18$, $P=0.046$) DAP, and in 2017 at CMREC there were more lady beetles in RC compared to M plots on 21 ($z=-2.16$, $P=0.04$) and 35 ($z=-3.14$, $P=0.004$) DAP. Overall, there were significantly more big-eyed bugs found in RC compared to M plots by approximately three-fold. In CMREC the abundance of big-eyed bug differed across treatment for 2016 ($\chi^2=7.18$, $df=1$, $P=0.007$; Fig. 11A) and 2017 ($\chi^2=87.8$, $df=1$, $P<0.001$; Fig. 11B), and there was a significant treatment by date interaction for both 2016 ($\chi^2=12.93$, $df=3$, $P=0.005$) and 2017 ($\chi^2=23.75$, $df=3$, $P<0.001$). In CMREC in 2016 there were more big-eyed bugs in RC than M plots on 36 ($z=-4.36$, $P<0.001$) DAP, and in 2017 there were more in RC than M plots on 21 ($z=-5.8$, $P<0.001$), 28 ($z=-3.68$, $P<0.001$), 35 ($z=-8.35$, $P<0.001$), 42 ($z=-4.1$, $P<0.001$), and 49 ($z=-2.26$, $P=0.03$) DAP. Overall, RC contained more than two-fold greater minute

pirate bugs compared to M plots. At CMREC in 2016 ($\chi^2=9.93$, $df=1$, $P=0.002$; Fig. 12A) and 2017 ($\chi^2=19.64$, $df=1$, $P<0.001$; Fig. 12B), the treatments differed. At CMREC, both 2016 ($\chi^2=61.38$, $df=4$, $P<0.001$) and 2017 ($\chi^2=50.19$, $df=4$, $P<0.001$) also had a significant treatment by date interactions. There were more minute pirate bugs in RC compared to M plots in CMREC in 2016 at 21 ($z=-7.51$, $P<0.001$) and 36 ($z=-2.39$, $P=0.03$) DAP, and in 2017 at 21 ($z=-6.75$, $P<0.001$) and 28 ($z=-4.59$, $P<0.001$) DAP. Tachinid and spider abundances did not differ significantly between treatments ($P>0.05$).

Parasitism rates. In 2016, a total of 148 cucumber beetles were dissected: 68 spotted (30 from CMREC and 38 from WMREC) and 80 striped (23 from CMREC and 57 from WMREC). Proportions of parasitized beetles from each site are given in Table 1. There were no significant differences in parasitism rate across beetle species, site, date, and treatment. On average, ~7.5% of striped cucumber beetles and ~6% of spotted cucumber beetles were parasitized. Parasitism rates were slightly higher in WMREC than CMREC. Rates of parasitism ranged from 0 to ~29% across the season for both beetle species (Fig. 13).

Cucumber yield. There were no significant differences in number and weight of marketable, grade fancy, grade 2, unmarketable, and total harvested cucumbers (Table 2). The number ($\chi^2=5.39$, $df=1$, $P=0.02$) and weight ($\chi^2=4.48$, $df=1$, $P=0.03$) of grade 1 fruits were significantly different with there being more grade 1 fruit in M plots. The proportion of damaged, cull, and yellow cucumber fruits were similar in number and weight among treatments. Most damaged fruits showed signs of

cucumber beetle feeding injury. The proportion in number of diseased fruits was also similar among treatments.

Discussion

In this study, it was hypothesized that presence of the red clover living mulch would cause a reduction of arthropod pests in cucumber plantings. As hypothesized, it was found that striped cucumber beetles at CMREC in 2017, spotted cucumber beetles at both sites in 2016, aphids at CMREC in 2017 were reduced on cucumber plants interplanted with red clover living mulch. Striped cucumber beetles on sticky cards were also reduced in plots with red clover living mulch. However, leafhoppers on cucumber plants at CMREC in 2017 and spotted cucumber beetles on sticky cards at CMREC in 2016 were increased by the presence of red clover living mulch. The presence of red clover also increased numbers of two generalist predators on sticky cards at CMREC during both years: big-eyed bugs and minute pirate bugs. These increased predators could have contributed to reductions of aphids, supporting the natural enemies hypothesis. However, factors other than natural enemies may have contributed to the observed herbivore reductions. Responses of other arthropods were more complex and differ according to sampling date or method. In general, yields were comparable between plots with and without red clover. However, grade 1 fruit numbers and weight were reduced in red clover plots.

Studies have shown that under-sown living mulch can reduce herbivores numbers due to a variety of mechanisms (Hooks et al. 1998, Hooks and Johnson 2004, Prasifka et al. 2006, Hinds and Hooks 2013). In this study, most herbivores examined, with the exception of squash bugs, were significantly reduced on

cucumber plants in red clover plots at least at one sampling location at and an herbivore was found in higher numbers on cucumber plants in RC plots only on one sampling date (leafhoppers from foliar counts on DAP 32 at CMREC in 2017), suggesting that this living mulch system has potential to suppress specialist and generalist herbivores in cucumber plantings.

As found by Hinds and Hooks (2013), striped cucumber beetle numbers were lower on cucurbit plants interplanted with a living mulch from visual and sticky card counts. Also, reduction of melon aphids were observed in this study as in Hooks et al. (1998). In the current study, colonization and establishment of cucumber beetles and melon aphids was thought to be impeded by decreased cucurbit plant apparency and unfavorable microhabitats. Striped cucumber beetles' and aphids' population peaks were lowered on 39 DAP in RC plots at CMREC in 2017. These findings suggest that red clover reduced colonization and establishment. Laboratory and field experiments assert that living mulches that are closer to crop plants more effectively reduce plant apparency by decreasing the soil:vegetation ratio that herbivores rely on to find their host plants. This subsequently exerts a greater impact on insect herbivores from both laboratory and field experiments (Finch and Collier 2001, Hooks and Johnson 2004). As such, the ability of red clover to reduce striped cucumber beetles and aphids may be due to its thick canopy which approached neighboring cucumber plants and drastically reduced the visibility of soil.

Spotted cucumber beetle abundances were impacted by presence of the living mulch in 2016 but not in 2017. This is in agreement with findings from Hinds and Hooks (2013) who found lower counts of spotted cucumber beetles on zucchini plants

interplanted with sunn hemp (*Crotalaria juncea*) during two of the three study years at two sites. The response of the spotted cucumber beetle to the presence of living mulch is not consistent in either study. However, in Hinds and Hooks (2013) in the third year the sunn hemp living mulch was mowed to a much lower height than the initial two study years and it was believed this contributed to the differences in cucumber beetle response to sunn hemp. Spotted cucumber beetles also consume red clover and other legumes (Hollingworth 2017), so red clover may have actually supported the spotted cucumber beetle populations in some years, which could explain the higher numbers of spotted cucumber beetles on sticky cards in red clover plots at CMREC in 2016.

Other mechanisms also could have contributed to reductions of the herbivores in this study. Previous studies attribute reductions in arthropod pest populations to changes in microclimate (e.g., changes in humidity and/or temperature) caused by the presence of living mulch (Hooks and Johnson 2003, 2004, Hinds and Hooks 2013). Red clover could also have reduced insect herbivores on neighboring host plants by changing host plant physiology chemically through root exudates (Theunissen 1994) or through producing volatile blends that confuse or overpower host plant volatiles that insect herbivores track (Zhang et al. 2014), as has been observed in other systems. It could also be that if insect herbivores were randomly landing the increased density of non-host plants caused insect herbivores to land on an inappropriate host, in this case the red clover, more frequently (Root 1973). Furthermore, increased natural enemy survival, foraging, or abundances in living mulch systems is another

possible explanation for the reduction of herbivores in the red clover plots, as in other systems (Prasifka et al. 2006, Hinds and Hooks 2013).

Although some natural enemies were enhanced in red clover plots, these natural enemies have not previously been reported to prey on striped cucumber beetles (Crocker and Whitcomb 1980, Snyder and Wise 1999, 2001, Grasswitz 2013, Snyder 2015), and most likely did not contribute to their reduction. However, these natural enemies are known to consume aphids and could have contributed to aphid reductions (Crocker and Whitcomb 1980, Bugg et al. 1987, Rondon et al. 2004). Numbers of big eyed bugs and minute pirate bugs were higher in red clover plots. This finding is similar to another study that found greater numbers of minute pirate bugs on pumpkin plants surrounded by a flowering plant mix compared to monoculture pumpkin (Grasswitz 2013). Big eyed bugs have been observed feeding on flowers and other plant tissue to supplement their diet (Bugg et al. 1987). For example, big-eyed bugs have been recorded feeding on crimson clover (*Trifolium incarnatum* L.; Fabaceae) (Crocker and Whitcomb 1980) which is closely related to red clover. Thus, big eyed bugs and minute pirate bugs may have built up at higher densities early in the growing season in plots with red clover (Fig. 5D and E), because they used red clover as an alternative source of nutrients. Alternatively, leafhoppers are not serious pests of cucurbits but do consume red clover (Bugg 1991), so they were probably not detrimental to cucumber plants and served as alternative prey to generalist natural enemies within red clover plots at CMREC in 2017. For example, big eyed bugs and minute pirate bugs feed on leafhoppers (York 1944, Andrews and Kuhar 2010). These extra resources could have enhanced the survival of big-eyed

bugs and minute pirate bugs, improving their ability to contribute to regulating the aphid population.

Despite having greater numbers on sticky cards in red clover plots in comparison to monoculture plots at WMREC in 2016 and CMREC in 2017, more lady beetles were found on cucumber plants in monoculture compared to red clover as a living mulch plots at CMREC in 2016. Because lady beetles consume aphids, they may have been more influenced by the presence of aphids than red clover plants (Rahman et al. 2010). Lady beetles build up higher number in monoculture plots and then relay between neighboring cover crop and cash crop (Tillman et al. 2004) likely in response to prey densities. However, lady beetle foraging of aphids can be impeded by increased plant morphological complexity (Legrand and Barbosa 2003). Thus, the red clover could impact lady beetle foraging efficiency of aphids. Sticky card counts are more accurate assessments of organism's movement (Hall 2009). This could explain why counts of lady beetles on sticky cards were in variance with counts on cucumber foliage.

Spider and tachinid numbers were not influenced by the presence of red clover. However, most spiders encountered during this study were small, and only large spider genera have been reported to consume cucumber beetles adults (Snyder and Wise 1999). The rate of parasitism of cucumber beetles by tachinids was very low compared to other studies (Smyth and Hoffmann 2010, Lewis 2015), and may have precluded detection of differences between treatments. During this study, it was confirmed that, in Maryland, *C. setosa* and *C. diabroticae* parasitize striped and spotted cucumber beetles, respectively. To our knowledge, *C. setosa* has not been

collected and identified in Maryland since their discovery in the early 1900s (Houser and Balduf 1925, Toepfer et al. 2008).

There was no impact of red clover on total yield. However, cucumber is relatively resistant to foliar pest damage (Burkness and Hutchison 1998, Snyder and Wise 1999, Barber et al. 2011) suggesting that higher counts and associated damage would be required for treatments to influence yield. Similarly, other studies examining effects of living mulch on yield have found that yield did not differ between monoculture and legume living mulch treatments in broccoli (Costello and Altieri 1995, Hooks and Johnson 2001, 2004) and corn systems (Hartwig and Ammon 2002). In other studies, yield was reduced by creeping bentgrass, red fescue, Kentucky bluegrass, and clover living mulches in cabbage systems (Andow et al. 1986, Brandsæter et al. 1998) and by oats/vetch and oats/white clover living mulch mixtures in pumpkin and cucumber systems (Amirault and Caldwell 1998). Still other studies demonstrated that living mulch can increase yield, but this increase seems to vary from year to year and is system-dependent. Hairy vetch, but not subterranean clover, living mulch increases tomato yields (Abdul-Baki and Teasdale 1993). Buckwheat and yellow mustard/weed living mulch increases zucchini yield but only in one year in a study by (Hooks et al. 1998). Andow (1991) asserted that increasing plant diversity tends to reduce plant quality via plant-plant competition. In this current study, the number and total weight of grade 1 cucumbers was lowered in plots with red clover living mulch. Likewise, in Elmstrom et al. (1988) several plant growth parameters were reduced in broccoli interplanted with white clover compared to monoculture. Establishing thinner red clover strips could improve quality and

yield. For instance, in Amirault and Caldwell (1998) plots planted in a 5:3 (cucumber/pumpkin:buckwheat) ratio produced higher cucumber and pumpkin yields compared to plots planted in a 3:5 ratio.

This study suggests that using plant diversification with a low-growing living mulch has the potential to reduce striped cucumber beetle and aphid numbers. Although there were no direct yield benefits during this study, a red clover living mulch can provide other ecosystem services that were not measured, such as suppressing weeds (Bottenberg et al. 1997, Orr et al. 1997), and reducing soil erosion (Wall et al. 1991, Duiker and Curran 2007). Additionally, legume cover crops can add nitrogen to the soil which could allow for reduction in synthetic fertilizer use (Stute and Posner 1995, Sullivan and Andrews 2012, Kołota and Adamczewska-Sowińska 2013, Caddel et al. 2017). Red clover is also known to attract pollinators and could have enhanced their numbers (Goulson 2006, Dupont et al. 2011, Rundlöf et al. 2014). As such, future studies evaluating the pest suppression benefits of red clover interplanted with a cash crop should evaluate other potential ecosystem services provided by this living mulch system.

Tables

Table 1. Table showing counts of parasitized cucumber beetles across site, beetle species, and treatment.

| Species | Site | | Grand Total |
|--------------------|-----------|-----------|-------------|
| | CMREC | WMREC | |
| Spotted | 30 | 38 | 68 |
| No | 29 | 35 | 64 |
| M | 16 | 26 | 42 |
| RC | 13 | 9 | 22 |
| Yes | 1 | 3 | 4 |
| M | 0 | 3 | 3 |
| RC | 1 | 0 | 1 |
| Striped | 23 | 57 | 80 |
| No | 23 | 51 | 74 |
| M | 18 | 14 | 32 |
| RC | 5 | 37 | 42 |
| Yes | 0 | 6 | 6 |
| M | 0 | 2 | 2 |
| RC | 0 | 4 | 4 |
| Grand Total | 53 | 95 | 148 |

Table 2: Table showing averages \pm SE, chi-square value, and p-value of yields and weights within each grade and damage category from each treatment.

| | Marketable | | | | | | Unmarketable | | | | | | | |
|---------------|----------------|---------------|------------------|------------------|------------------|----------------|-----------------|----------------|----------------|------------------|----------------|---------------|----------------|--|
| | Fancy | | 1 | | 2 | | damaged | | cull | | yellow | | diseased | |
| | amount | weight (kg) | amount | weight (kg) | amount | weight (kg) | amount | weight (kg) | amount | weight (kg) | amount | weight (kg) | amount | |
| M | 29.0 \pm 8.5 | 8.1 \pm 2.2 | 65.5 \pm 16.3 | 18.1 \pm 4.4 | 167.0 \pm 32.1 | 43.2 \pm 9.2 | 80.2 \pm 23.6 | 21.8 \pm 7.1 | 49.8 \pm 9.2 | 10.3 \pm 2.3 | 1.7 \pm 1.5 | 0.3 \pm 0.2 | 10.0 \pm 2.0 | |
| RC | 23.0 \pm 7.0 | 6.4 \pm 2.2 | 50.2 \pm 13.3 | 13.3 \pm 3.4 | 164.9 \pm 36.2 | 40.7 \pm 9.3 | 75.6 \pm 17.1 | 19.6 \pm 4.5 | 46.7 \pm 8.7 | 8.8 \pm 1.4 | 1.7 \pm 1.5 | 0.3 \pm 0.3 | 7.2 \pm 0.5 | |
| χ^2 | 2.45 | 2.48 | 5.39 | 4.48 | 0.02 | 0.38 | 0.08 | 0.2 | 0.17 | 1.04 | 0 | 0.24 | 2.51 | |
| P | 0.12 | 0.12 | 0.02* | 0.03 | 0.89 | 0.54 | 0.78 | 0.65 | 0.68 | 0.31 | 1 | 0.62 | 0.11 | |
| <u>Total:</u> | | | amount | weight (kg) | | | | | <u>Total:</u> | | amount | weight (kg) | | |
| | | M | 261.3 \pm 54.0 | 69.4 \pm 14.8 | | | | | M | 123.7 \pm 27.1 | 30.4 \pm 8.2 | | | |
| | | RC | 239.3 \pm 55.1 | 60.4 \pm 14.6 | | | | | RC | 119.1 \pm 19.8 | 27.3 \pm 5.1 | | | |
| | | χ^2 | 1.39 | 2.64 | | | | | χ^2 | 0.06 | 0.34 | | | |
| | | P | 0.24 | 0.1 | | | | | P | 0.8 | 0.56 | | | |
| | | | <u>Total:</u> | amount | weight | | | | | | | | | |
| | | | M | 385.4 \pm 77.7 | 98.9 \pm 21.7 | | | | | | | | | |
| | | | RC | 358.6 \pm 73.4 | 87.8 \pm 19.4 | | | | | | | | | |
| | | | χ^2 | 1.15 | 2.41 | | | | | | | | | |
| | | | P | 0.28 | 0.12 | | | | | | | | | |

Figures

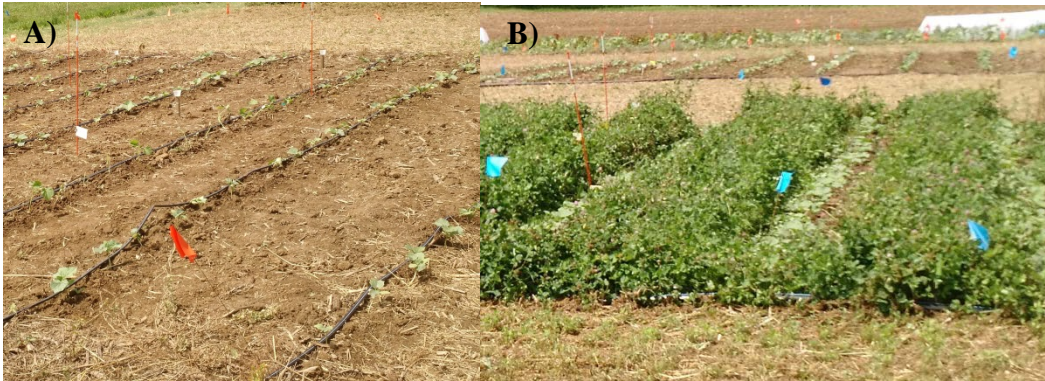


Fig. 1. Picture of: A) conventionally tilled bare ground cucumber monoculture plot and B) strip tilled cucumber plot with red clover interplanted as a living mulch.

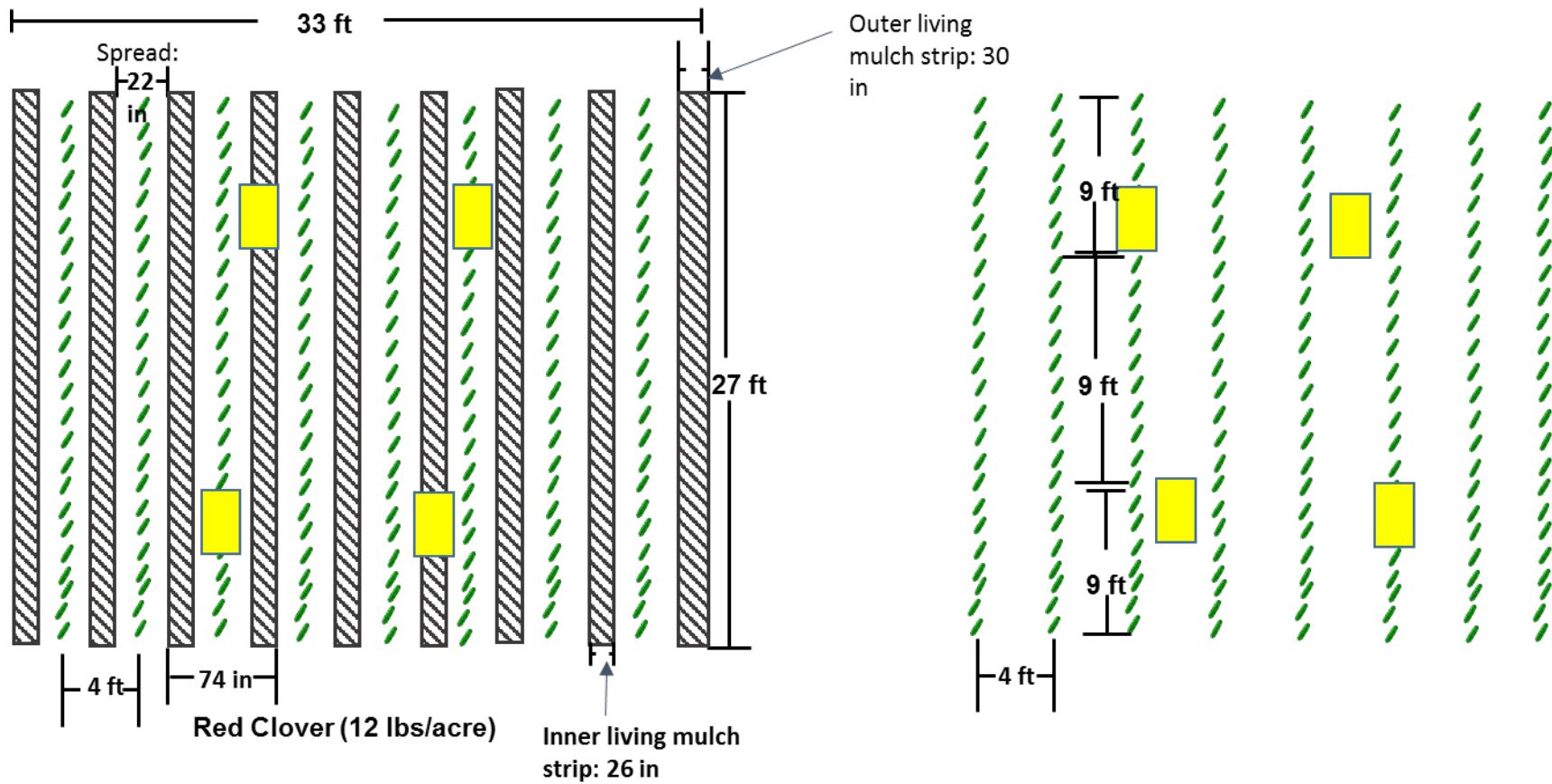


Fig. 2. Schematic diagram of the layout of the sticky cards and the plot dimensions for the RC plot (on the left) and the M plot on the right.

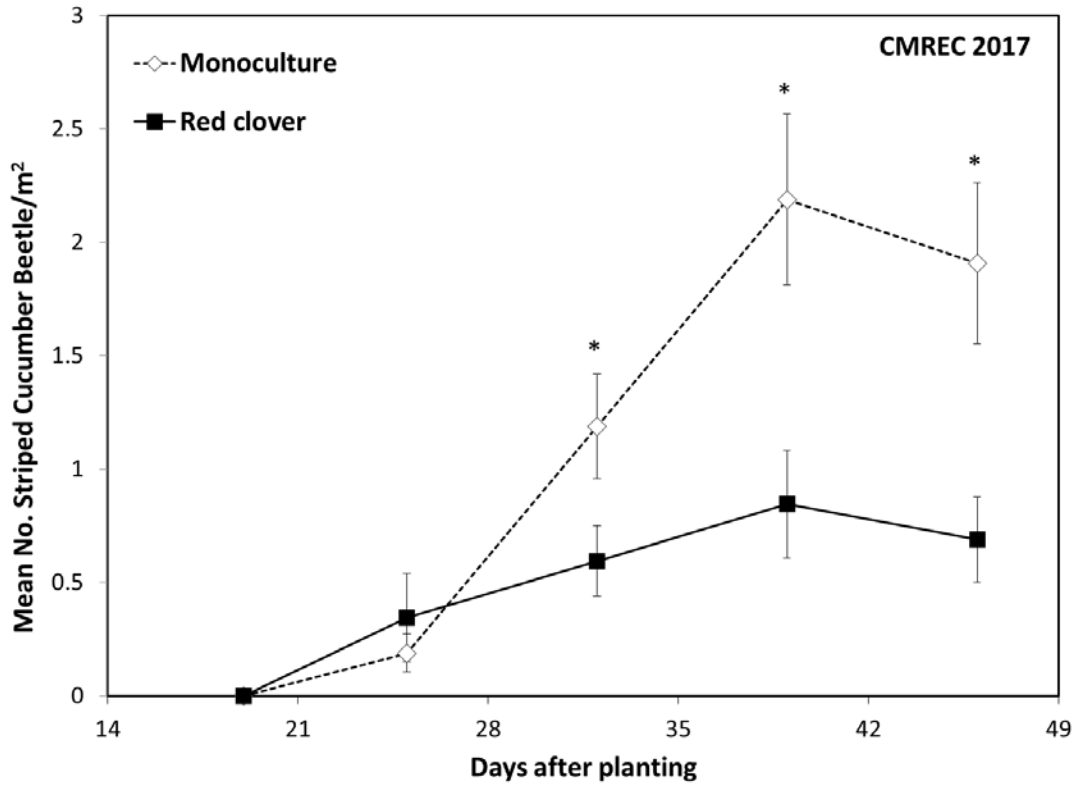


Fig. 3. Mean densities and standard error bars of striped cucumber beetles from foliar counts at CMREC from 2017. An * indicates a significant difference at $P < 0.05$.

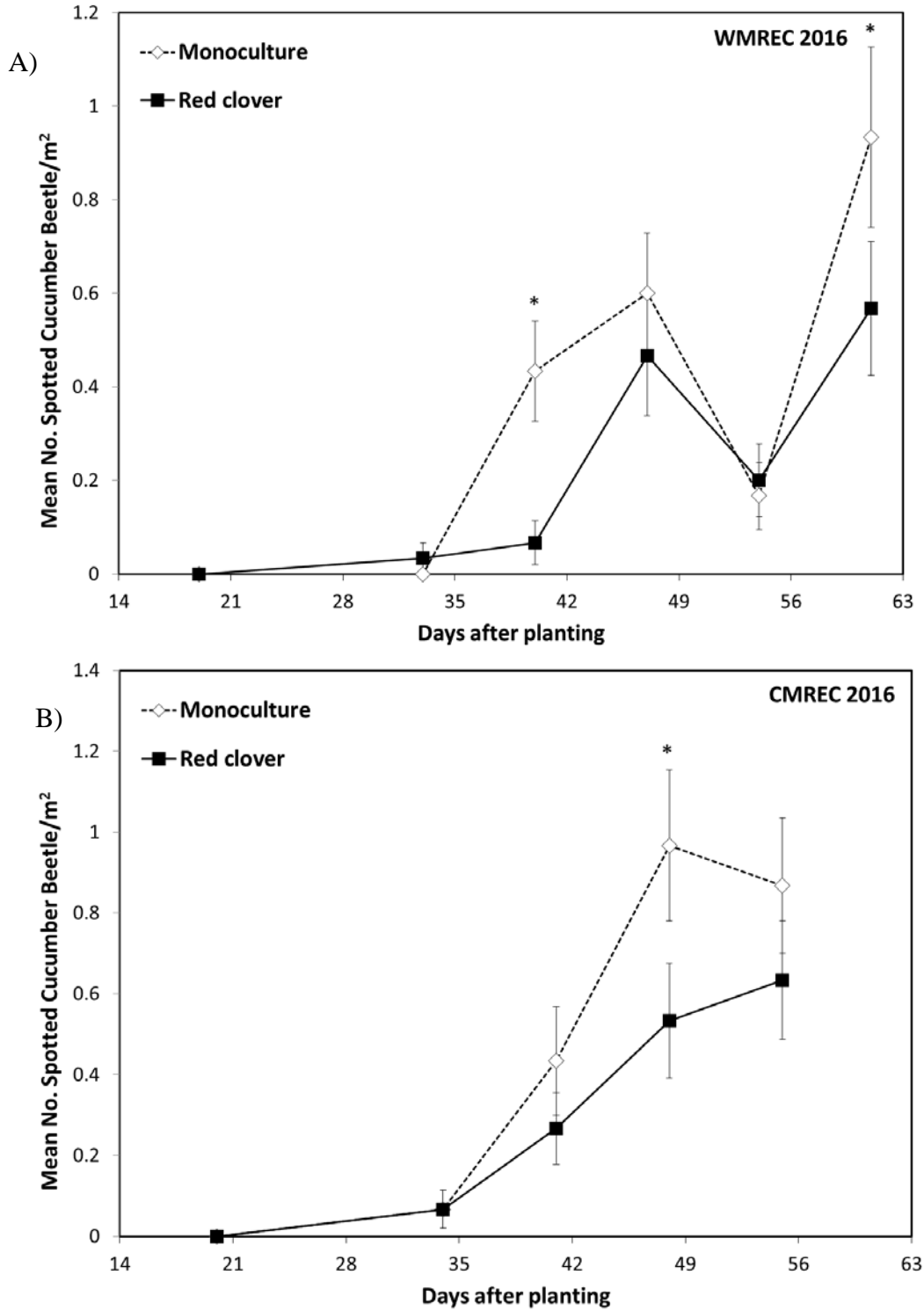


Fig. 4. Mean densities and standard error bars of spotted cucumber beetles from foliar counts at A) WMREC from 2016 and B) CMREC from 2016. An * indicates a significant difference at $P < 0.05$.

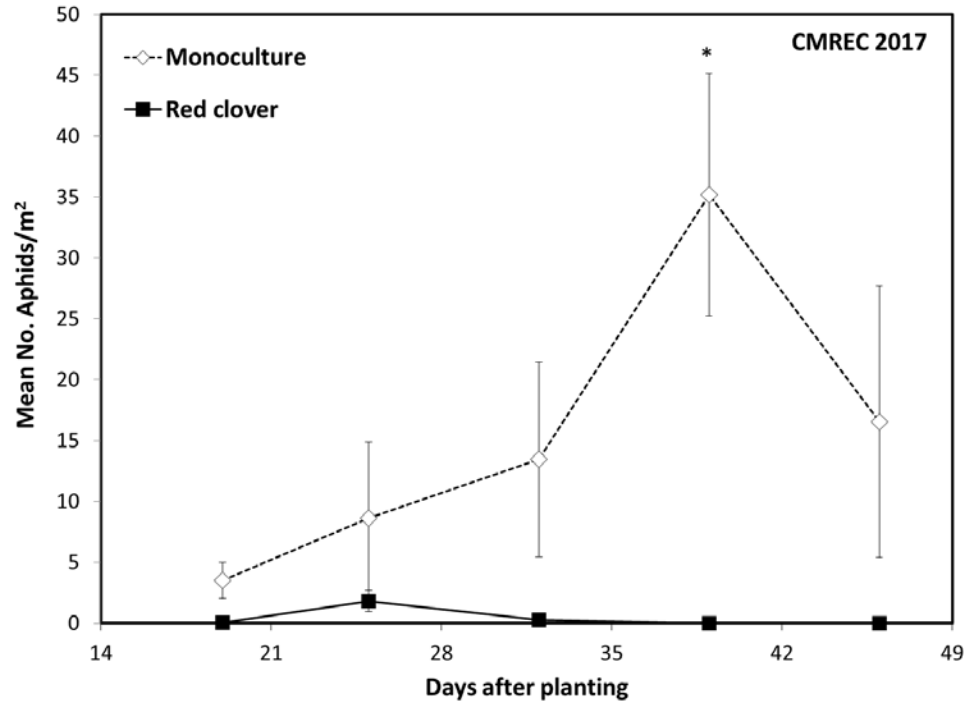


Fig. 5. Mean densities and standard error bars of aphids from foliar counts at CMREC from 2017. An * indicates a significant difference at $P < 0.05$.

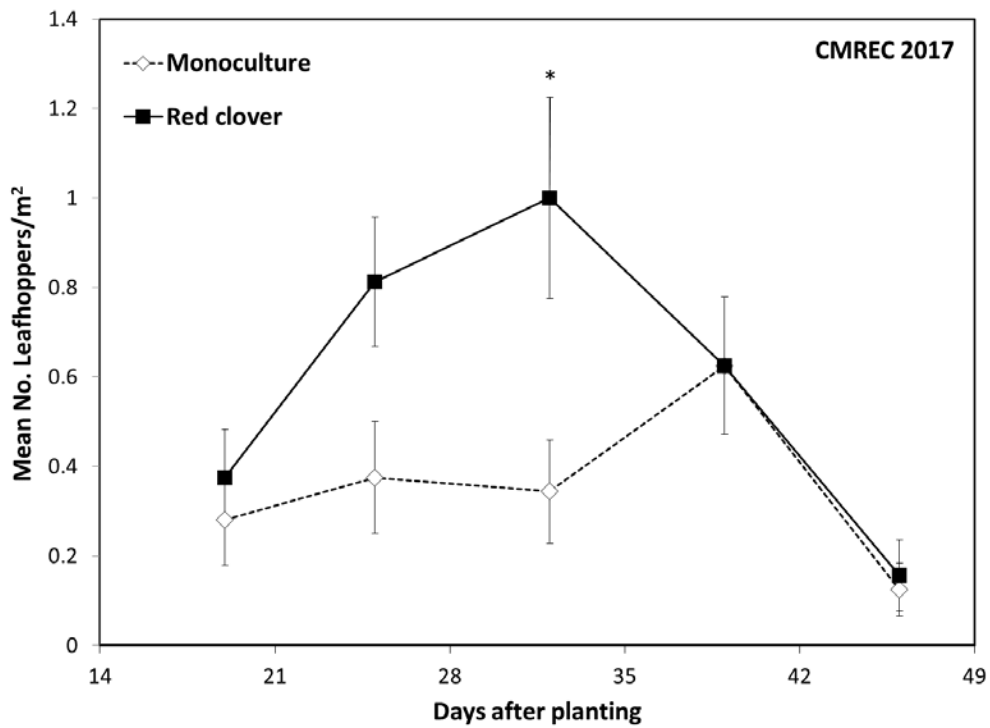


Fig. 6. Mean densities and standard error bars of leafhoppers from foliar counts at CMREC from 2017. An * indicates a significant difference at $P < 0.05$.

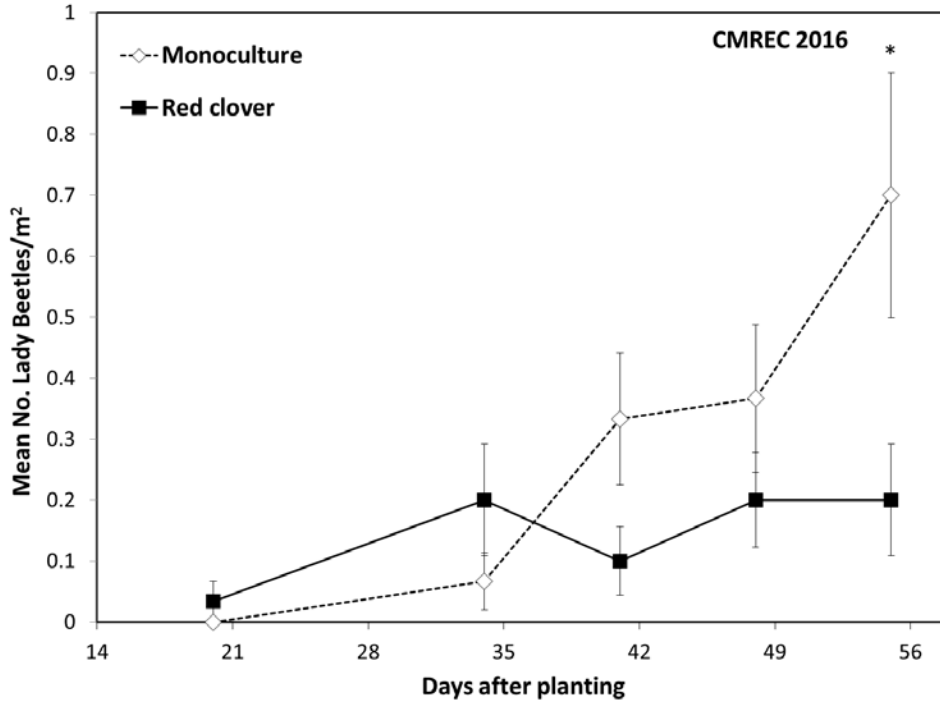


Fig. 7. Mean densities and standard error bars of lady beetles from foliar counts at CMREC from 2016. An * indicates a significant difference at $P < 0.05$.

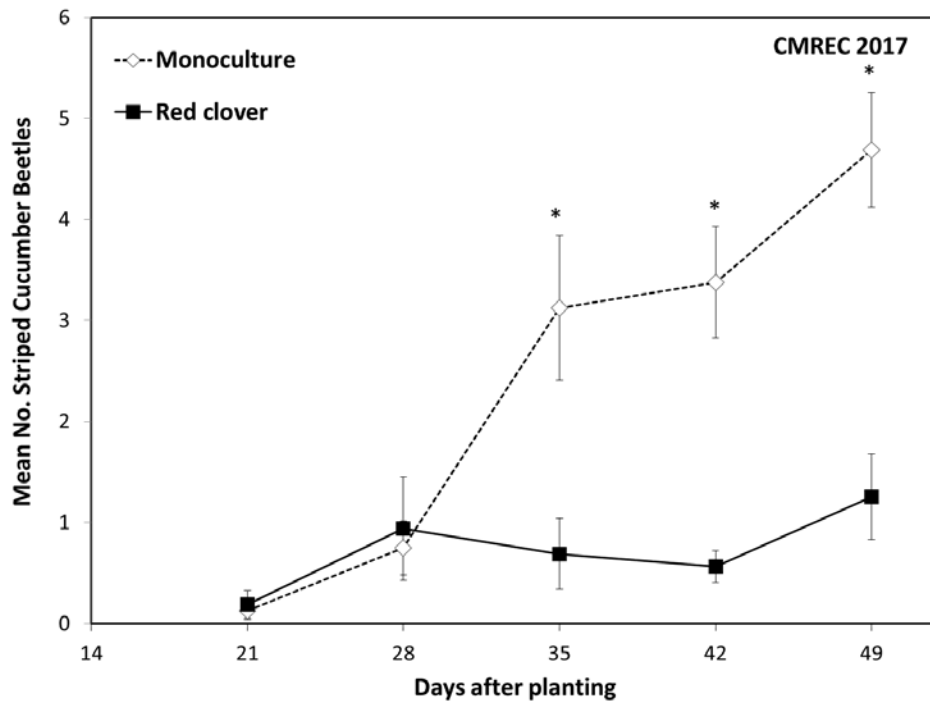


Fig. 8. Mean numbers and standard error bars of striped cucumber beetles on sticky cards at CMREC from 2017. An * indicates a significant difference at $P < 0.05$.

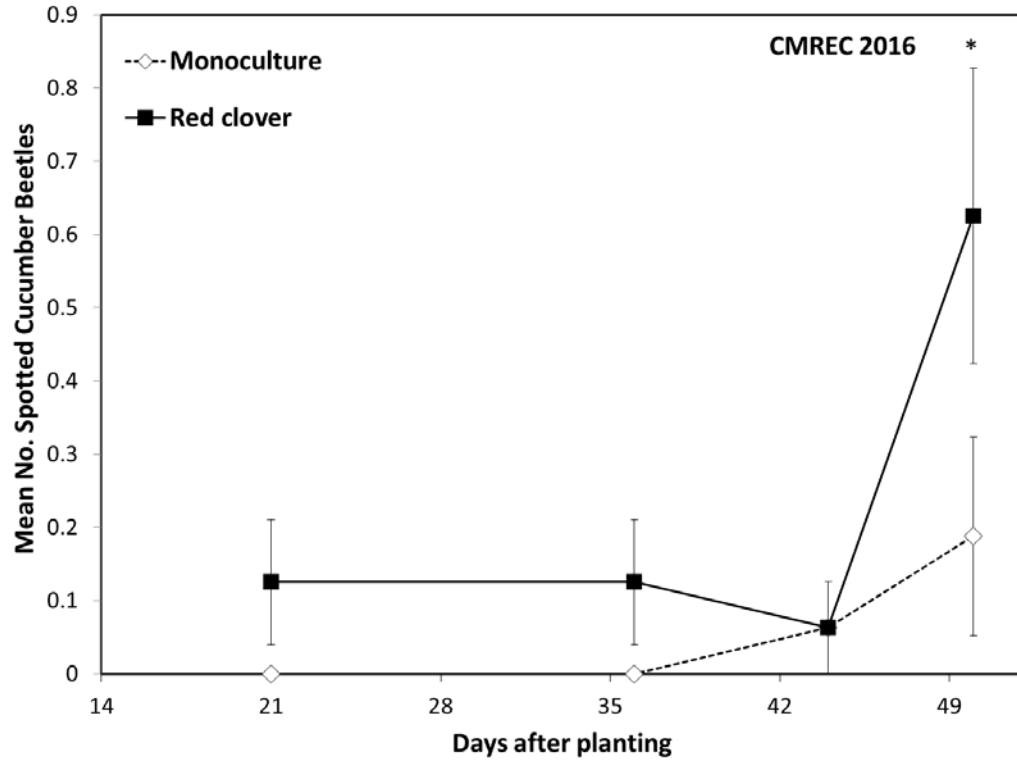


Fig. 9. Mean numbers and standard error bars of spotted cucumber beetles on sticky cards at CMREC from 2016. An * indicates a significant difference at $P < 0.05$.

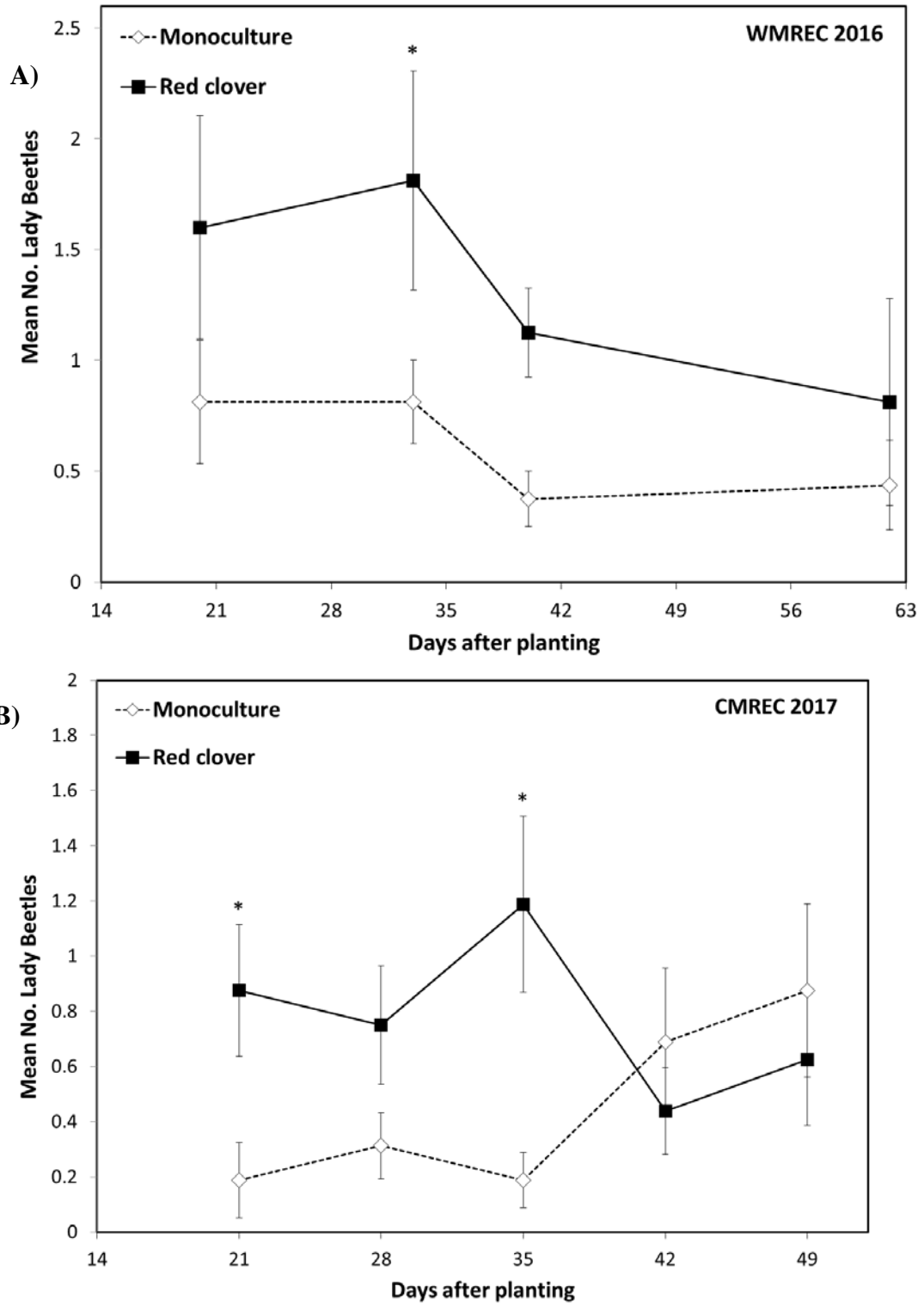


Fig. 10. Mean numbers and standard error bars of lady beetles on sticky cards at: A) WMREC from 2016 and B) CMREC from 2017. An * indicates a significant difference at $P < 0.05$.

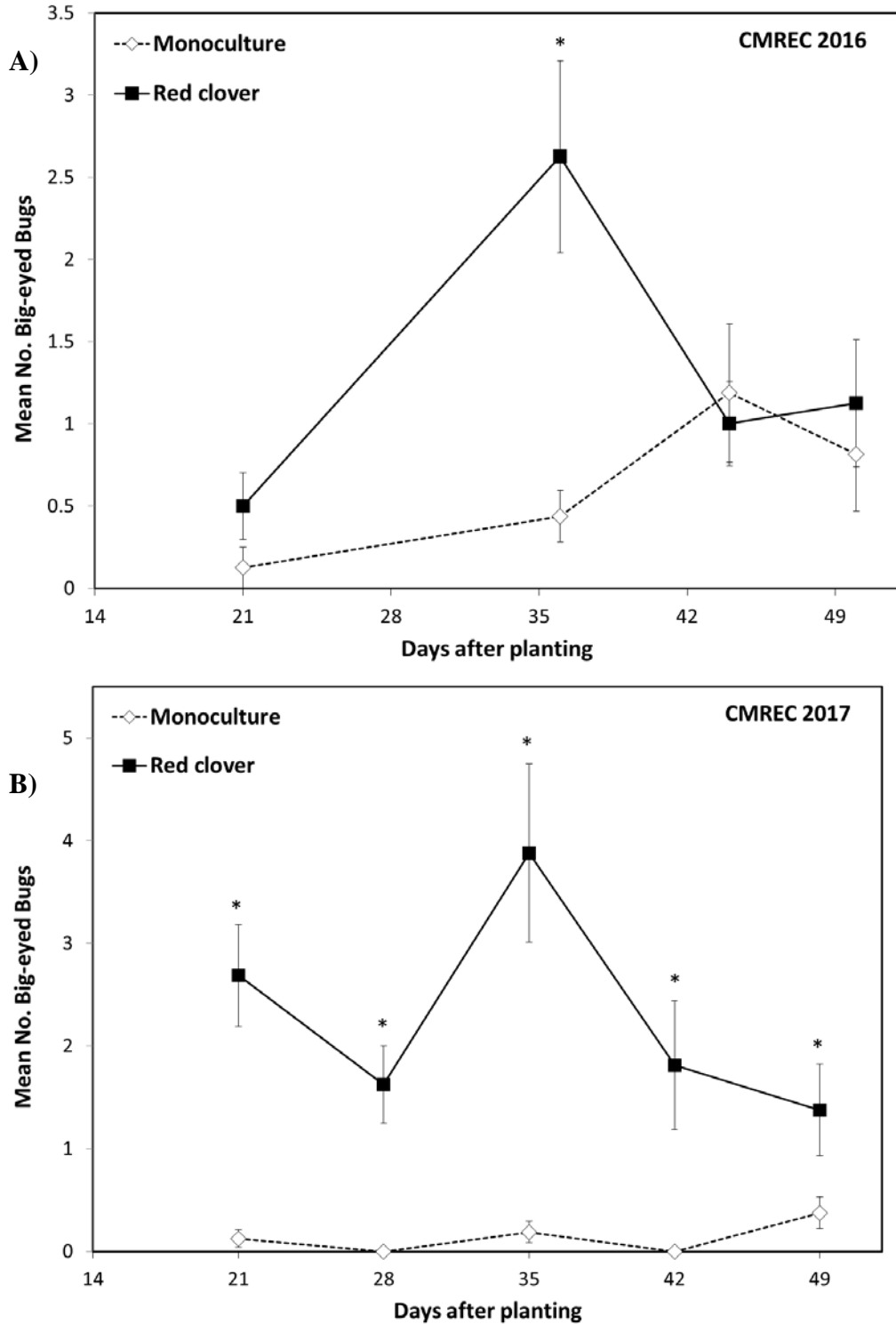


Fig. 11. Mean numbers and standard error bars of big eyed bugs on sticky cards at: A) CMREC from 2016 and B) CMREC from 2017. An * indicates a significant difference at $P < 0.05$.

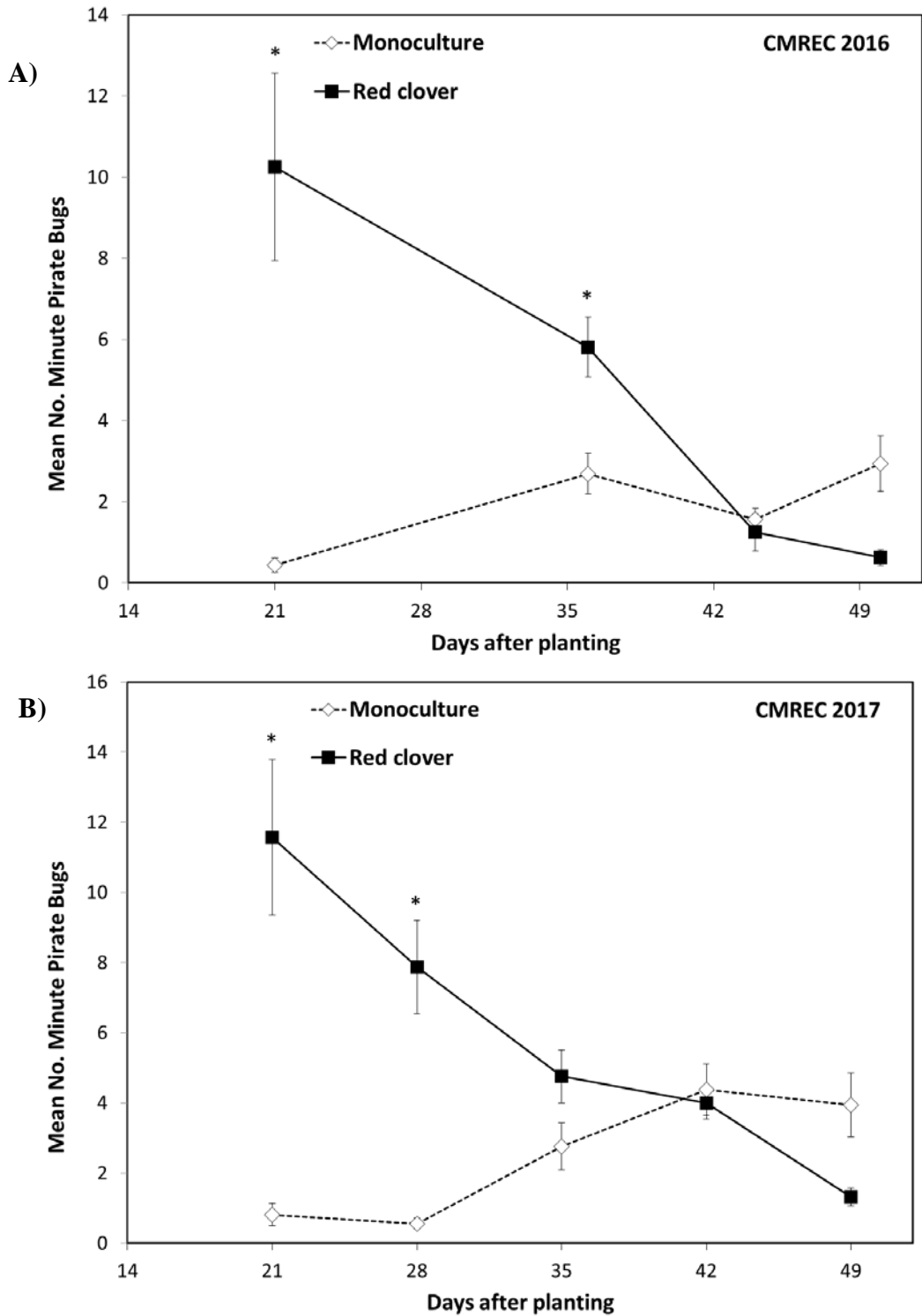


Fig. 12. Mean numbers and standard error bars of minute pirate bugs on sticky cards at: A) CMREC from 2016 and B) CMREC from 2017. An * indicates a significant difference at $P < 0.05$ level.

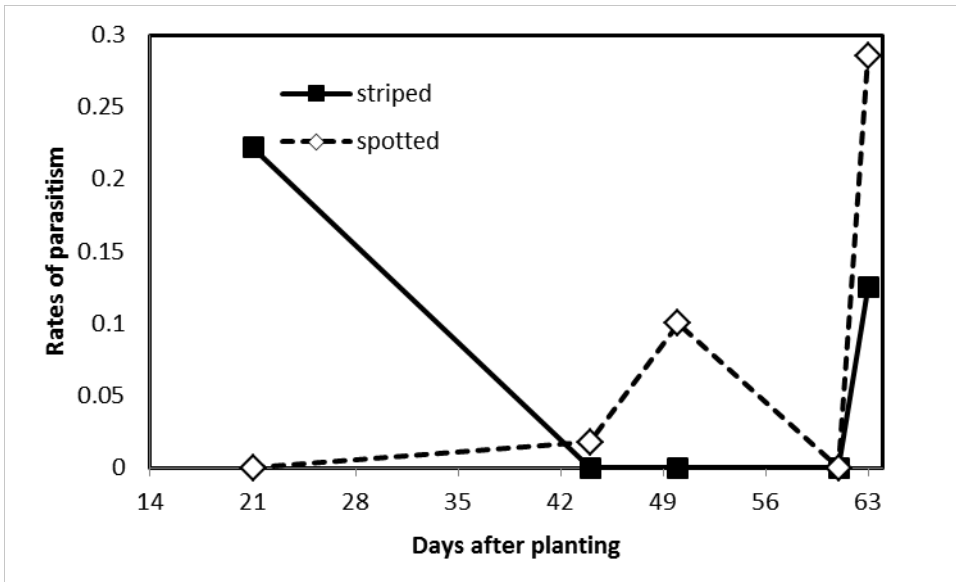


Fig. 13. Proportion of parasitized spotted and striped cucumber beetles across days after planting.

Chapter 2: Consumptive and non-consumptive effects of wolf spiders on cucumber beetles and cucumber plant damage

ABSTRACT

Biological control research often evaluates the ability of natural enemies to reduce pest densities and protect crop plants by directly consuming pests. Less studied is the ability of natural enemies to protect crop plants by altering the behavior of pest species. Field experiments were conducted to examine effects of large wolf spiders (Araneae: Lycosidae) and/or their cues (e.g., silk, excreta, or secretions) on the behavior, mortality, and densities of striped cucumber beetle (*Acalymma vittatum* (F.); Coleoptera: Chrysomelidae) and spotted cucumber beetle (*Diabrotica undecimpunctata howardi* (Barber); Coleoptera: Chrysomelidae), and growth and damage of cucumber (*Cucumis sativus* L.; Cucurbitaceae) plants. Three spotted and four striped cucumber beetles were added to caged plants with and without spiders, with spider cues (spiders removed before introduction), and with spiders and their cues present. Spotted cucumber beetle densities were reduced, and more spotted cucumber beetles disappeared in cages with a spider compared to spider-free cages. Striped cucumber beetles responded to spider cues primarily by attempting to emigrate from plants. However, cages with a spider had higher striped cucumber beetle mortality than spider-free cages. Spider presence did not enhance cucumber plant growth or reduce plant damage. These results suggest that large wolf spiders can impact cucumber beetles by consuming them and by eliciting predator-avoidance behaviors.

Introduction

Successful biological control depends on the ability of natural enemies to increase crop productivity via suppressing pest populations. Biological control has emerged as an important alternative to pesticides for controlling arthropod pests (Lewis et al. 1997, Price et al. 2011). In the United States, averted crop losses due to parasitism or predation of native herbivores by native natural enemies is calculated to be worth 4.5 billion USD annually (Losey and Vaughan 2006). To date, biological control has depended on the ability of natural enemies to consume pests and subsequently, reduce their populations (Holmes and Upadhyay 1958, Muller and Godfray 1999, Mahr et al. 2011). However, there is increasing evidence that the mere presence of predators can elicit changes in prey behavior (Lima and Dill 1990, Schmitz et al. 2004, Price et al. 2011), resulting in reduced crop damage (Sabelis et al. 2012). This occurrence is frequently termed “Trait-Mediated Indirect Interactions” (TMIIs), while direct consumption of prey is generally referred to as “Density-Mediated Indirect Interactions” (DMIIs) (Abrams 1995, Werner and Peacor 2003, Hoverman and Relyea 2012, Sabelis et al. 2012).

Inherent in TMIIs is the ability of prey to sense predators, “calculate” risk of predation, decide whether to exhibit avoidance behavior, and then act in a way that allows them to maximize their chance for survival. Studies have shown that prey can detect chemical cues of predators (Mathis and Vincent 2000, Bucher et al. 2015), and visual and tactile cues can inform prey of predator presence (Williams and Wise 2003). There is evidence that different predator cues can have various effects on prey (Stephenson 2016). Prey use several strategies to avoid predators including: hiding,

remaining motionless, running fast, flying, emigrating, dropping off, or exhibiting aggregation (Dill 1987, Lima and Dill 1990). Though predator-avoidance behaviors can lead to a decrease in numbers of prey captured by predators (Charnov et al. 1976), many of these behaviors present tradeoffs for prey (Lima and Dill 1990). For example when avoiding predators, prey may avoid ideal feeding sites, expend more energy, and eat less (Schmitz et al. 1997, Khater et al. 2016). Sensitivity and vulnerability to predators can vary across sex (Soper et al. 1976, Shine 1980, Thornhill 1980, Burk 1982), prey species (Huang and Sih 1990, Peckarsky 1996), resource availability and quality (Abrahams and Dill 1989, Peckarsky 1996), and presence of other prey (Huang and Sih 1990). Thus, multiple factors are likely to impact anti-predator behavior.

In a review of studies on TMIs and DMIs, TMIs were often more important than DMIs (Werner and Peacor 2003). However, many assessments on the value of biological control do not consider TMIs, which may lead to an underestimation of its value. Currently most studies involving TMIs are conducted in aquatic systems, partially because TMIs in aquatic systems are thought to be stronger (Preisser et al. 2005). Yet, more research on factors impacting TMIs and its relative contribution to biological control efforts in terrestrial, agricultural systems is integral to evaluating the true effectiveness of and understanding disparities in biological control efforts (Schmitz et al. 2004, Sabelis et al. 2012).

Biological control research mainly focuses on the introduction of specialist natural enemies or parasitoids. Reliance on generalist natural enemies is sometimes discouraged because they may consume non-target prey (Murdoch et al. 1985,

Howarth 1991, Henneman and Memmott 2001) and other natural enemies, disrupting effective long-term control efforts (Snyder and Ives 2001). However, a recent review of manipulative field studies found that generalist natural enemies were able to reduce pest populations significantly in ~75% of studies reviewed (Symondson et al. 2002). It is thought that multiple species of generalist natural enemies are more effective than single specialist exotic parasitoids in controlling native insect crop pests (Hawkins et al. 1999). Several studies have demonstrated that certain generalist natural enemies such as carabids (Brust 1986, Riddick and Mills 1994, Kromp 1999) and spiders (Riechert and Bishop 1990, Schmaedick and Shelton 2000, Nyffeler and Birkhofer 2017) can reduce pest populations, and subsequently decrease crop damage (Clark et al. 1994, Snyder and Wise 2000) and improve yields (Snyder and Wise 1999, 2001, Lee and Edwards 2012).

Prey can recognize and respond to cues from generalist predators. For instance, spider prey are believed to respond to spider and their cues, such as excreta and silk (Persons and Rypstra 2001, Barnes et al. 2002, Persons et al. 2002, Kortet and Hedrick 2004). In the presence of these cues prey mortality (Barnes et al. 2002, Persons et al. 2002) and food intake decreased (Persons et al. 2002). Additionally, it has been shown that spider presence and cues may have an interactive effect on prey survival (Persons et al. 2002). However, the response of prey to spiders, particularly interactive effects, remains understudied.

Previous research. The striped cucumber beetle, *Acalymma vittatum* (F.) (Coleoptera: Chrysomelidae), and spotted cucumber beetle, *Diabrotica undecimpunctata* Mannerheim (Coleoptera: Chrysomelidae) are major cucurbit pests.

Adult beetles cause significant damage to stems, leaves, flowers, and fruits, rendering fruits unmarketable and lowering yield (Houser and Balduf 1925, Hoffmann et al. 2000, Snyder 2015). Thus, growers often spray for cucumber beetles at a threshold as low as one beetle per plant (Brust and Foster 1999). Cucumber beetles sequester bitter compounds (cucurbitacins) for defense (Gould and Massey 1984, Tallamy 1998) and are relatively large pests with hard elytra (Snyder 2015). Because of these traits, they have relatively few predators (Snyder and Wise 1999). However, lycosids (Araneae), larger than 10 mm, have been shown to readily consume cucumber beetles and regulate cucumber beetles' densities (Snyder and Wise 1999). Furthermore, cucumber beetles can recognize large lycosids as threats, and their presence stimulates beetle predator-avoidance behaviors (Snyder and Wise 2000, Williams et al. 2001, Williams and Wise 2003). These anti-predator behaviors may depend on beetles' sex, as only female spotted cucumber beetles responded to spider presence (Williams et al. 2001). Because there is evidence that DMII and TMII are important for understanding the overall relationship between large wolf spiders and cucumber beetles, they offer a unique opportunity to test the relative importance and interplay of DMII and TMII for regulation of two economically important agricultural pest species. Currently, there are many gaps in research on the wolf spider-cucumber beetle interaction, and only one field study examined predator-avoidance behavior of cucumber beetles in the presence of wolf spiders (Williams and Wise 2003).

Rationale: Field experiments that consider how anti-predator behavior impacts biological control are currently lacking. It is especially important to include responses to chemical cues and changes in consumptive and non-consumptive

impacts across plant growth stage since these variables likely influence a predator's ability to regulate arthropod pests and indirectly impact crop plants.

Objectives. Study objectives include examining: 1) wolf spider efficiency at consuming cucumber beetles, 2) if wolf spider's presence and/or their chemical cues cause cucumber beetles to exhibit predator-avoidance behavior and whether this is impacted by their sex, 3) whether spider presence results in greater beetle mortality, and 4) how cucumber beetles impact plant damage and growth parameters in the presence of spiders and/or their cues.

Materials and Methods

Arthropod collection and housing. From June to September 2017, wolf spiders (greater than 10 mm) were collected primarily from dry pitfall traps placed in a neighboring cucumber planting. Wolf spiders were sexed and identified to genus, and those used as test specimens were stored in ~150 cm³ plastic cups (Solo[®], Model #: DM16, Urbana, IL). Test wolf spider genera included: *Rabidosa* spp. Roewer, *Tigrosa* spp. Brady, and *Schizocosa* spp. Chamberlin. Spiders in traps and still alive in cages at the end of the last trial were keyed out to species to get a representation of the species composition. All identified *Rabidosa* spiders were keyed out as *Rabidosa rabida* Walckenaer. *Tigrosa* spiders were keyed out as *Tigrosa helluo* Walckenaer, and *Schizocosa* spiders were keyed out as *Schizocosa avida* (Walckenaer) using Ubick et al. (2017) and (Dondale and Redner 1990). Wolf spiders and cucumber beetles were stored on different shelves in an incubator (Percival Scientific, Inc., Model #: I-35LLVL, Boone, IA) set at 26°C on a 16-8 hour light-dark cycle in the laboratory. Test spiders were provided water via moistened cotton pads, and fed field

collected and store-bought crickets (Petco Animal Supplies, Inc., San Diego, CA) every third day. Striped and spotted cucumber beetles were collected from cucurbit plantings at CMREC (e.g. cucumber, pumpkin, and squash) located at the CMREC study site and kept in 0.75 oz plastic Solo[®] cups (Model #: PO75S-1143, Urbana, IL). Test beetles were provided water via moistened cotton wicks and fed fresh cucurbit leaves and flowers from non-experimental cucumber plants.

Feeding assays. From August to early October, 2017, feeding assays were performed before cage trials to compare predation across beetle species, spider genera, and spider sex. Spider sex was included as a variable to explain consumption rates because female wolf spiders have been found to be more effective predators than male spiders (Walker and Rypstra 2002). A subset of test spiders (Table 1) were paired with additional striped and spotted cucumber beetles collected from CMREC. Beetles were added to containers with spiders that had been starved for two to three days to attempt to standardize hunger levels between spiders similar to other feeding trials that used wolf spiders (Snyder and Wise 1999). After 24 hours of exposure, containers were searched for dead beetles or their remains (e.g., elytra). If an alive beetle was not found, then it was recorded as a successful consumption. A total of 91 feeding trials were initiated with a total of 44 spiders. Of these complete information on spider genera and sex were obtained from 67 trials and thus could be used in the final analysis.

Experimental design and layout. Field experiments with mesh cages were conducted at the Central Maryland Research and Education Center (CMREC) in Upper Marlboro, MD to test objectives 2, 3, and 4. CMREC predominately consists

of a fine-loamy soil type. A semi-dwarf cucumber variety 'Fanfare' (J.W. Jung Seed Company, Randolph, WI) was used as the test crop. On August 18, 2017, cucumber was direct-seeded by hand in a 10 x 10 m plot that was rototilled prior to planting. The experimental design was a randomized complete block design with eight replicates. The plot contained eight rows, each served as a replicate, of 30 cucumber plants each, with an inter- and intra- row spacing of ~1.2 m and ~0.3 m, respectively. When seedlings germinated, 0.216 m³ mesh cages (soil emergence traps, Model No: BT2006, MegaView Science Co., Ltd., Taichung, Taiwan) were placed over every fifth plant within each row, for a total of five cages per row. Cages contained a small bottle trap at the top, and a zipper that could be opened to examine the inside of the cage (Fig. 1). Between trials, weeds that germinated on the soil surface within cages were physically removed. The plot was fertilized following recommendation of conventional practices (Brust et al. 2015).

Cage treatment designations. Three cage trials were conducted at the cotyledon (August 24, 2017), leaf (September 12, 2017), and flowering growth stages (September 26, 2017) of cucumber plants to assess changes in the cucumber beetle-spider interaction across plant growth stages. Trial 1, cotyledon-stage, persisted for six days and trial 2, leaf-stage, and trial 3, flower-stage, persisted for seven days. Six day trial periods were used in a previous cucumber beetle-spider study (Snyder and Wise 2000). The same spiders used in the feeding assay were used to establish cage treatments and the cucumber beetles were collected following the protocol in the above arthropod collection section. Test spiders were reused in some trials due to low

spider availability. Treatments of cucumber beetles and/or a spider with or without its chemical cues were assigned randomly to cages as follows (See Fig. 2):

No spider. This cage did not ever receive a spider, but did receive cucumber beetles.

Spider cues. A spider was contained on the cucumber plant. To ensure contact with the plant, the spider was placed into a mesh sleeve that covered the plant; each sleeve was composed of a 36 mesh cm^{-1} transparent fabric that was fastened around the plants' base with a nylon drawstring, (Hooks et al. 2003). All other plants within cages and flagged plants were also enclosed in sleeves (to keep conditions equal across treatments). The spider and sleeves were left for 24 hours, an adequate amount of time for spiders to deposit silk and excreta (Persons and Rypstra 2001, Barnes et al. 2002, Persons et al. 2002). At the end of 24 hours, all sleeves, along with the spider they harbored, were removed from plants, leaving a plant exposed to spider chemical cues. This same spider was also used to establish the *spider presence* treatment.

Spider presence. The spider used previously to establish the *spider cue* treatment was transferred from the *spider cue* treatment cage, to a cage previously unexposed to spiders. The spider was left to actively forage inside this cage, the *spider presence* treatment cage, for the entire trial period.

Spider presence and cues. Another test spider was contained on a caged cucumber plant for 24 hours, as done for the *spider cue* treatment. Then the spider was re-added to the same cage in which it previously established chemical cues in,

the *spider presence and cue* cage. The spider remained in that cage to actively forage for the entire trial period.

Beetle cages and trial maintenance. After actively foraging spiders were added, each cage treatment had four striped and three spotted cucumber beetles introduced individually to each caged plant. These numbers are based on maximum densities of cucumber beetles observed during previous field studies (unpublished data, Kahl, 2016). Before adding beetles, bottle traps at the top of each cage were filled to <~25% of their total volume with soapy water, which consisted of 1 drop of non-scented dish soap (365 Everyday Value[®], Canada) per gallon of water. These traps would catch beetles or spiders that attempted to emigrate from cages. Treatment assignments were kept the same across trials to avoid contamination from previous trials'. If spiders from the spider presence or spider presence and cue treatment emigrated from cages, they were replaced.

Behavioral responses. After being added to plants within each cage, beetles were observed for one minute. For each beetle, the species and initial behavioral responses (i.e., when they flew, disappeared from sight, started feeding, and time spent feeding) were recorded. If the beetle walked away under a leaf or debris where it could no longer be observed, then it was considered that the beetle disappeared from sight. After beetles were added to cages, numbers of spotted and striped cucumber beetles in traps were recorded daily over each trial period. At trial completion, specimens found in traps were collected and total number of cucumber beetles counted. Cucumber beetles and large wolf spiders still alive within cages were also counted and collected.

Influence of beetles' sex. At the end of the trial period, intact beetles found in traps and within cages were dissected to examine sex-specific genitalia to determine their sex. Presence of an extra abdominal plate was also used to identify male beetles (Krysan 1986).

Mortality. At end of each trial, the ground and edges within every cage were searched for cucumber beetle remains (e.g. elytra) to assess mortality. Spiders inside cages or traps were counted and collected. Beetles that remained unaccounted for (not found in the traps or in cages) were recorded.

Plant parameters. Along with the other established cage treatments, one uncaged plant within each row marked with flagging tape and one cage within each row without beetles added, *check* cage, were included in plant parameter comparisons to represent ambient defoliation and no beetle defoliation, respectively (Fig. 2). To examine the impact of cucumber beetles and spider cues and/or presence, flagged uncaged plants and caged plants were examined before and after each trial. Dead plants were replaced with an adjacent plant before the start of the next trial. The influence of treatments on plant growth and damage was evaluated by counting the number of and estimating the percent damage on cotyledons, leaves, or flowers (depending on the trial) for each flagged uncaged and caged plant 24 hours before the start of the trial and after trial completion.

Statistics. To compare striped and spotted cucumber beetle consumption across wolf spider genera and sex, a generalized linear model with a binomial distribution was built (“glm”, package stats) (R Development Core Team 2011). The response variable was whether a spider consumed or failed to consume a striped or

spotted cucumber beetle. Statistics were computed for the proportion of striped and spotted beetles consumed overall and across spider genera and sex.

For all analyses concerning the one-minute observations of cucumber beetle behavior, row and position within the row were used as random effects. Initial beetle behavior (whether beetles flew, disappeared from sight, or fed on plants) was compared across trial using generalized linear mixed-effects models fitted with a binomial distribution (“glmer”, package lme4) (R Development Core Team 2011). Time at which beetles flew or left sight and total time beetles spent eating were compared across treatment and trial using linear mixed-effects models (“lmer”, package lme4) (R Development Core Team 2011). In examining end of trial differences in outcomes of beetles (in traps, alive in cages, dead, or unaccounted for) linear mixed-effects models were composed with numbers of beetles that experienced each outcome as the response variables. Linear fixed effect models to examine emigration across trial period days were composed separately for each trial. Day was added as a fixed effect in these models. Also generalized linear mixed-effect models fitted with a binomial distribution were made for proportions of male and female beetles in traps and alive in cages to examine whether treatments effects varied depending on the beetle sex. For all of these models beetle species were analyzed separately, only the no spider, spider cues, spider presence, and spider presence and cues treatments were included. Models were re-run with only spider presence and absence as treatment levels.

Changes in cucumber plant attributes (number and average percent damage of cotyledons, leaves, and flowers), final measurements for the number and percent

damage of leaves and flowers, and the number of dead plants were compared across treatments using linear models with row as a random effect. Attributes of plants inside cages without beetles added and flagged plants were included as treatments in these models.

For all models, Tukey's HSD tests ("glht", package multcomp) (R Development Core Team 2011) were used to detect significant pairwise comparisons of treatment levels.

Results

Consumption. There was a significant interaction between spider genus and sex across beetle species for consumption rates ($\chi^2 = 13.46$, $df=3$, $P=0.004$), but there were no significant differences between any of the contrasts from the interaction. Less spotted (48.9%) than striped (72.1%) cucumber beetles were consumed ($\chi^2 = 6.02$, $df=1$, $P=0.01$; Table 1). *Rabidosa* males, *Schizocosa* males, and *Tigrosa* females consumed the highest proportion of striped cucumber beetles, followed by *Tigrosa* males, *Schizocosa* females, and *Rabidosa* females. *Tigrosa* spp. were the most effective at consuming striped cucumber beetles (80%). The most effective consumers of spotted cucumber beetles were *Rabidosa* males followed by *Schizocosa* males, *Tigrosa* males, *Schizocosa* females, *Tigrosa* females, and *Rabidosa* females. As a species, *Schizocosa* were most effective at consuming spotted cucumber beetles (60.9%).

Striped cucumber beetles. There was a significant treatment by plant-stage interaction for striped cucumber beetle time at flight ($\chi^2 = 15.55$, $df=6$, $P=0.02$; Fig. 3B). Striped cucumber beetles flew earlier in spider presence than no spider cages in

the flower-stage ($t=2.71$, $P=0.04$). The interaction between trial and treatment was different for times at which beetles left sight ($\chi^2=13.91$, $df=6$, $P=0.03$; Fig. 4B) but there were no significant differences between levels. There were significant differences in trial and treatment for times at which beetles started feeding ($\chi^2=19.5$, $df=5$, $P=0.002$; Fig. 5B), but there were no significant differences between levels. There was a significant difference across treatments for total time that striped cucumber beetles spent feeding ($\chi^2=18.26$, $df=5$, $P=0.003$; Fig. 5C). In trial 1, striped cucumber beetles spent more time feeding in spider presence and cue cages compared to spider presence cages ($t=-3.01$, $P=0.03$). In trial 3, striped cucumber beetles spent more time feeding in spider cue than no spider cages ($t=-3.1$, $P=0.02$) and spider presence and cue cages ($t=-3.45$, $P=0.01$). There was no significant difference in the proportion of striped cucumber beetles that flew (Fig. 3A) or left sight (Fig. 4A) across treatments ($P>0.05$).

There were no significant differences in numbers of striped cucumber beetles found in the cages at the end of each trial ($P>0.05$; Fig. 6A). There was a significant effect of treatment on the number of striped cucumber beetles found in traps at trial completion ($\chi^2=8.62$, $df=3$, $P=0.03$; Fig. 7A), but there was no significant differences between levels. Numbers of striped cucumber beetles in traps had a significant treatment by day interaction for trials 1 ($\chi^2=38.1$, $df=18$, $P=0.004$) and 2 ($\chi^2=38.48$, $df=21$, $P=0.01$) (Fig. 8A). In trial 1, there were more striped cucumber beetles in traps within spider presence and cue than in traps within no spider cages on day 1 ($t=-2.92$, $P=0.03$), 2 ($t=-2.92$, $P=0.03$), and 3 ($t=-2.92$, $P=0.03$). Also, there were more striped cucumber beetles in traps within spider cue ($t=-3.18$, $P=0.01$) than traps within no

spider cages on day 1 and marginally more on days 2 ($P=0.0502$) and 3 ($P=0.0501$). In trial 2, there were more beetles in traps within no spider than traps within spider presence cages on days 2 ($t=3.66$, $P=0.003$), 3 ($t=3.66$, $P=0.003$), 4 ($t=3.66$, $P=0.003$), 5 ($t=3.32$, $P=0.008$), and 6 ($t=2.66$, $P=0.048$), and there were more beetles in traps within spider presence and cue than in traps within spider presence cages on day 4 and 5 ($t=-2.66$, $P=0.048$). During trial 3, there were more striped cucumber beetles in traps within spider cue than traps within spider presence cages on day 1 ($t=-2.74$, $P=0.04$).

Spotted cucumber beetles. Spotted cucumber beetle responses during the one-minute observation periods differed across treatment. Spotted cucumber beetle flew later in spider presence and cue compared to no spider cages ($t=-2.7$, $P=0.04$) in trial 1 (trial by treatment interaction: $\chi^2=12.74$, $df=6$, $P=0.047$; Fig. 3B). The time at which spotted cucumber beetles left sight differed significantly between treatments ($\chi^2=27.39$, $df=3$, $P<0.001$; Fig. 4B). Spotted cucumber beetles left sight earlier in spider cue ($t=2.95$, $P=0.03$) and spider presence cages ($t=2.86$, $P=0.03$) than no spider cages. For spotted cucumber beetles, there were no significant differences across treatments ($P>0.05$) for proportions of beetles that flew (Fig. 3A), proportions of beetles that left sight (Fig. 4A), proportions of beetles that fed (Fig. 5A), times at which feeding began (Fig. 5B), and total times spent feeding (Fig. 5C).

The number of spotted cucumber beetles remaining on the plant significantly differed between cages with and without spiders across treatment ($\chi^2=6.96$, $df=2$, $P=0.03$; Fig. 6B). There were more spotted cucumber beetles in cages without than with spiders in the flower-stage ($z=2.42$, $P=0.02$). There were no significant

differences in number of spotted cucumber beetles in traps at the end of the trial ($P>0.05$; Fig. 7B). There was only a significant treatment effect for spotted cucumber beetles in traps across days for trial 1 ($\chi^2=8.33$, $df=3$, $P=0.04$; Fig. 8B). During trial 1, there were more spotted cucumber beetles in traps within spider cue cages than in traps within spider presence and cue cages on day 3 ($t=-3.04$, $P=0.02$), and on day 5, there were more spotted cucumber beetles in traps within no spider than in traps within spider presence and cue cages ($t=2.71$, $P=0.04$).

Mortality. There was a significant interaction of spider presence and trial on striped but not spotted cucumber beetle mortality. The number of dead striped cucumber beetles had a significant treatment by trial interaction ($\chi^2=7.05$, $df=2$, $P=0.03$; Fig. 9). There were more dead striped cucumber beetles in cages with than without a spider in trial 3 ($t=-2.92$, $P=0.005$). Only one dead spotted cucumber beetle was found in a spider presence cage.

There was a significant difference in number of missing striped cucumber beetles across treatment ($\chi^2=12.4$, $df=3$, $P=0.006$; Fig. 10A). There were significantly more missing striped cucumber beetles in spider presence compared to spider presence and cue cages ($t=3.29$, $P=0.02$). There was a significant treatment difference of unaccounted for spotted cucumber beetles between cages with and without spiders ($\chi^2= 4.57$, $df=1$, $P=0.03$; Fig. 10B). Significantly more missing spotted cucumber beetles were in treatments with spiders than without.

Influence of beetle sex. There were no significant differences between proportions of males or females in the cages or in the traps at the end of the trial across treatments ($P>0.05$).

TMII and DMII. Plant attributes from primary treatments (no spider, spider presence, spider cue, and spider presence and cue) did not differ significantly across all plant attributes measured. However, ambient plants, outside the cages, had significantly less flowers and leaves and more flower damage than plants inside cages (See Table 2 for significant effects and contrasts).

Discussion

In this study, non-consumptive and consumptive impacts of wolf spiders on cucumber beetles was addressed using a field experiment to increase the understanding of how generalist natural enemies can regulate arthropod pest species. It was determined that large wolf spiders consumed cucumber beetles at variable rates depending on wolf spider genus and sex. Wolf spiders consumed more striped than spotted cucumber beetles. The behavior of cucumber beetles differed across spider treatments. Striped cucumber beetles flew earlier in spider presence compared to no spider cages, spent more time eating in spider presence and cue than spider presence cages in trial 1, and spent more time eating in spider cue compared to no spider cages, spider presence cages, and spider presence and cue cages in trial 3. The presence of spiders caused spotted cucumber beetles to fly less frequently. Spotted cucumber beetles flew earlier in no spider than spider presence and spider cue cages, but they disappeared from sight (moved where they could no longer be observed) earlier in spider presence and spider cue compared to no spider cages. In trial 1 there were significantly less beetles in traps within spider presence and cue than spider cue cages on day 3 or no spider cages on day 5. Wolf spiders reduced the number of spotted cucumber beetles remaining in the cage in trial 3. There were more dead striped

cucumber beetles in cages with compared to without spiders in trial 3. Similarly, there were more unaccounted for striped cucumber beetles in spider presence than spider presence and cue cages. Also, the number of spotted cucumber beetles that were unaccounted for was higher in treatments with than without a spider.

Large wolf spiders can consume spotted and striped cucumber beetles. Other studies demonstrated that large wolf spiders are capable of consuming cucumber beetles in lab feeding trials (Snyder and Wise 1999, 2001). Spotted cucumber beetles were consumed less than striped cucumber beetles in the current study. The difference in consumption rates may be due to increased handling time that comes when consuming the larger-bodied spotted cucumber beetle. Differences in handling time due to body size differences have been reported in several studies (Griffiths 1980, Cohen and Tang 1997, Vucic-Pestic et al. 2010). However, studies that included feeding assays on cucumber beetles did not find a difference between wolf spider consumption of striped and spotted cucumber beetles possibly because they used smaller containers to perform feeding assays which made it easier for spiders to capture and subdue both species.

The finding that striped cucumber beetles were consumed at higher rates, would suggest that they may be at relatively higher risk of predation than spotted cucumber beetles in field environments containing spider genera evaluated during this study. During trials, striped cucumber beetles showed behavioral responses to wolf spider presence particularly when spider cues were present, but spiders were still able to consume them more than spotted cucumber beetles. For all trials, there was at least one day in which striped cucumber beetles left more frequently in treatments with

than without spider cues. Increased emigration in response to a predator is consistent with the suggestion that prey in high predation-risk situations often have increased escape responses (Khater et al. 2016).

There were more missing striped cucumber beetles in treatments with a spider than with spider cues. These missing beetles could have been hiding or, more likely, could have been consumed by spiders. These findings suggest that the presence of either wolf spider excreta or silk triggers anti-predator responses, primarily in the form of emigration. These responses have been documented in other studies (Persons et al. 2002, Kortet and Hedrick 2004). Cages with spiders had more dead striped cucumber beetles regardless of the presence of spider cues in trial 3. This suggests that despite the anti-predator behavior of striped cucumber beetles, wolf spiders effectively consumed them. Spiders may only be more effective at consuming striped cucumber beetles in trial 3 due to the increased structural complexity of the more mature plant, which has been shown to improve spider foraging (Snyder 2015). There was some evidence that striped cucumber beetles responded to the mere presence of wolf spiders as well: striped cucumber beetles flew earlier in spider presence than no spider cages. Another study also determined that spider presence caused striped cucumber beetles to leave plants (Williams and Wise 2003). Furthermore, there may be additive effects of having spider cues and the spider present. In trial 1, there were more beetles in traps on days 1, 2, and 3 within spider presence and cue than no spider cages. Having multiple indicators of predator presence (the spider and its cues on the plant) combined with the decreased resource availability of smaller plants in trial 1 most likely contributed to this higher emigration rate (Khater et al. 2016).

On the contrary, spotted cucumber beetle densities were reduced in cages with spiders. This was observed also in the study by Snyder and Wise (1999). Moreover, there were significantly more missing spotted cucumber beetles in cages with than without a spider. In some instances, cucumber beetle remains could have been concealed under debris in cages, making them difficult to find. Both findings suggest that it is likely that large wolf spiders prey on spotted cucumber beetles in the field environment. Reduced densities on plants and consumption of spotted cucumber beetles by spiders was also demonstrated in Williams et al. (2001). Despite the risk of predation, spotted cucumber beetles flew less frequently in cages with than without a spider, and in trial 1, flew later in spider presence, and spider presence and cue than no spider cages. However, spotted cucumber beetles did leave sight earlier in spider presence than no spider cages. Further, after one day of exposure there were more spotted cucumber beetles in cages with spider cues and spider presence and cues than cages without spiders in trial 1. Thus, spotted cucumber beetles may deploy different strategies for avoiding predators in the short- versus longer term exposure. Short-term behavior may consist of reduced movement or hiding while long-term behavior may involve emigration. This kind of graded antipredator response was observed in another study testing effects of predator cue age on *Tigrosa helluo* prey (Barnes et al. 2002) and detected in a meta-analysis that simulated behavior of prey in variable predation risk scenarios (Khater et al. 2016). Khater et al. (2016) suggested that when prey are at relatively low risk of being consumed by a specific predator, they are more likely to reduce their movement and try to maximize resource consumption. This may explain why spotted cucumber beetles did not respond to the wolf spider except for

trial 1, when plants were smallest. Alternatively, it was demonstrated that only female spotted cucumber beetle behavior was impacted by spider presence (Williams et al. 2001). Spotted cucumber beetles in this study were mostly male (less than 10% were female), which may explain the lack of certain anti-predator behavioral responses.

Growth and damage of cucumber plants were not impacted by the addition of wolf spiders. This agrees with another study that measured the effect of wolf spiders on squash and cucumber plants. In that study, only squash productivity increased in the presence of wolf spiders (Snyder and Wise 1999). Snyder and Wise (1999) suggested that cucumbers could be tolerant to cucumber beetle damage. A lack of effect on cucumber growth parameters despite increased cucumber beetle numbers was also observed in another study conducted at this same site (Chapter 1). This suggests that densities of cucumber beetles high enough to cause perceptible plant damage are required to detect the indirect effect of wolf spiders on plant productivity

This study provides evidence that spiders can impact cucumber beetles directly through consumption and indirectly through modifying cucumber beetle behavior. While wolf spiders did not decrease plant damage or increase its growth, this study provides convincing evidence that large wolf spider species can contribute to biocontrol services. These experiments revealed several avenues for future research that could improve the use of spiders in biocontrol programs. Research on whether responses of cucumber beetles to large wolf spiders vary in the presence of conspecifics or individuals of another species could reveal how interspecific competition influences anti-predator behavior as revealed in other studies (Walls et al. 2002). Previous studies have suggested that understanding the relative costs of

predator-avoidance behavior are critical to understanding indirect predator effects on overall organism fitness (Nelson et al. 2004, Hoverman et al. 2005, Khater et al. 2016). Although there were no differences found in the anti-predator response of cucumber beetles between males and females, reduced egg production and size could be an important cost of predator-avoidance behavior for cucumber beetle females and should be examined in future work. Further research in these areas would advance the understanding of consumptive and non-consumptive changes predators induce in prey populations, which could be used to improve biological control by generalist natural enemies.

Tables

Table 1. Numbers of consumed beetles and feeding attempts for striped and spotted cucumber beetles across genus and sex of large wolf spider species. The number given in the parentheses is the number of individual spiders within that category used in the feeding trial.

| Wolf spider- genera and sex | | Striped | | Spotted | |
|--------------------------------|----|----------|-----|----------|-----|
| | | attempts | ate | attempts | ate |
| <i>Rabidosa</i> spp. | fm | 3 | 1 | 3 | 0 |
| | m | 2 | 2 | 2 | 2 |
| <i>Schizocosa</i> spp. | fm | 10 | 5 | 8 | 3 |
| | m | 6 | 6 | 8 | 5 |
| <i>Tigrosa</i> spp. | fm | 7 | 7 | 8 | 3 |
| | m | 3 | 2 | 7 | 3 |
| Total | | 46 | 34 | 49 | 24 |

Table 2. Chi-square value, degrees of freedom, and p-values for plant attributes significantly affected by treatments and t-values and p-values for treatment contrasts. The values in bold are p-values for significant contrasts ($P < 0.05$).

| | Δ of leaf number | | Final leaf number | | Δ of male flower damage | | Final male flower damage | | Final female flower number | | Final female flower damage | |
|--|------------------|---------------|-------------------|---------------|-------------------------|---------------|--------------------------|------------------|----------------------------|--------------|----------------------------|---------------|
| χ^2 | 12.1 | | 20.3 | | 12.9 | | 96.15 | | 24.26 | | 11.81 | |
| df | 5 | | 5 | | 5 | | 5 | | 5 | | 5 | |
| p | 0.03 | | 0.001 | | 0.02 | | < 2.2e-16 | | 0.0002 | | 0.04 | |
| | t | P | t | P | t | P | t | P | t | P | t | P |
| no beetles vs. no spider | 0.077 | 1 | 0.645 | 0.9866 | -2.177 | 0.2599 | -1.318 | 0.773 | 1.82 | 0.464 | -0.006 | 1 |
| no beetles vs. outside | 2.705 | 0.0851 | 3.679 | 0.0093 | -2.729 | 0.0805 | -8.354 | <1e-04 | 4.10 | 0.003 | -2.863 | 0.0718 |
| no beetles vs. spider presence | 1.009 | 0.9135 | 1.484 | 0.6761 | -2.786 | 0.0701 | -0.695 | 0.981 | 0.46 | 0.997 | -0.123 | 1 |
| no beetles vs. spider presence+cues | -0.081 | 1 | 0.065 | 1 | -2.472 | 0.1448 | -0.437 | 0.998 | 0.23 | 1.000 | -0.663 | 0.9833 |
| no beetles vs. spider cues | 1.501 | 0.6642 | 2.194 | 0.266 | -0.959 | 0.9292 | -0.204 | 1 | 2.05 | 0.335 | -1.71 | 0.5215 |
| no spider vs. outside | 2.529 | 0.1277 | 3.034 | 0.0476 | -0.668 | 0.9849 | -7.134 | <1e-04 | 2.28 | 0.229 | -2.831 | 0.0767 |
| no spider vs. spider presence | 0.898 | 0.9459 | 0.839 | 0.958 | -0.609 | 0.9901 | 0.623 | 0.988 | -1.37 | 0.745 | -0.112 | 1 |
| no spider vs. spider presence+cues | -0.154 | 1 | -0.581 | 0.9917 | -0.295 | 0.9997 | 0.881 | 0.948 | -1.60 | 0.607 | -0.629 | 0.9868 |
| no spider vs. spider cues | 1.378 | 0.7394 | 1.549 | 0.636 | 1.218 | 0.8269 | 1.113 | 0.872 | 0.23 | 1.000 | -1.636 | 0.5672 |
| outside vs. spider presence | -1.667 | 0.5574 | -2.194 | 0.266 | 0.091 | 1 | 7.71 | <1e-04 | -3.65 | 0.010 | 2.826 | 0.078 |
| outside vs. spider presence+cues | -2.742 | 0.078 | -3.614 | 0.0112 | 0.388 | 0.9988 | 7.949 | <1e-04 | -3.88 | 0.006 | 2.562 | 0.1329 |
| outside vs. spider cues | -1.067 | 0.893 | -1.484 | 0.676 | 1.821 | 0.4583 | 8.164 | <1e-04 | -2.05 | 0.335 | 2.008 | 0.3487 |
| spider presence vs. spider presence+cues | -1.074 | 0.8903 | -1.42 | 0.715 | 0.314 | 0.9996 | 0.258 | 1 | -0.23 | 1.000 | -0.559 | 0.9923 |
| spider presence vs. spider cues | 0.526 | 0.995 | 0.71 | 0.9795 | 1.826 | 0.4548 | 0.49 | 0.996 | 1.60 | 0.607 | -1.643 | 0.5634 |
| spider presence+cues vs. spider cues | 1.556 | 0.6288 | 2.13 | 0.2961 | 1.513 | 0.6569 | 0.233 | 1 | 1.82 | 0.464 | -1.103 | 0.8682 |

Figures



Fig. 1. Picture of eight rows of five 0.6x0.6x0.6 m mesh cages into which spotted and striped cucumber beetles and/or large wolf spiders were added.

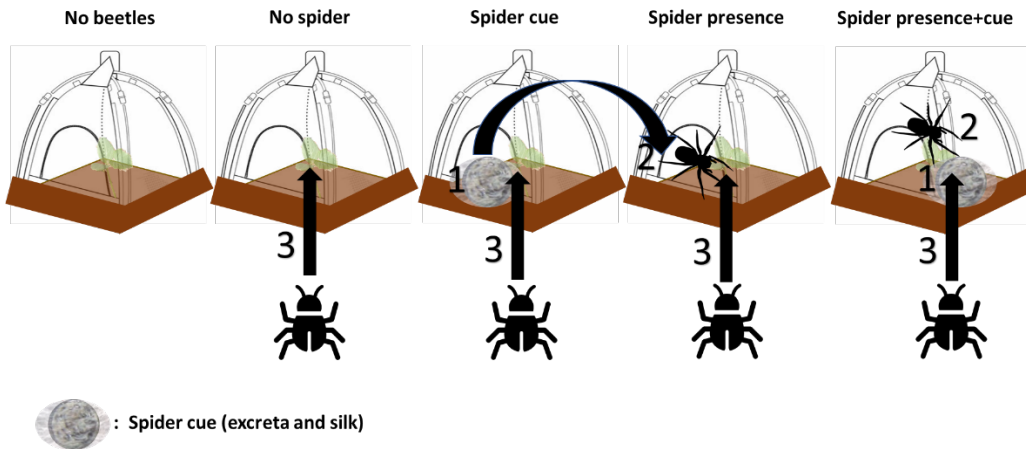


Fig. 2. Schematic diagram showing a summary of treatment designations. 1) two spiders were contained on plants within two cages and deposited cues for 24 hours; 2) one spider was moved to another cage and released and another was released within the same cage in which it deposited cues; 3) cucumber beetles were added to cages.

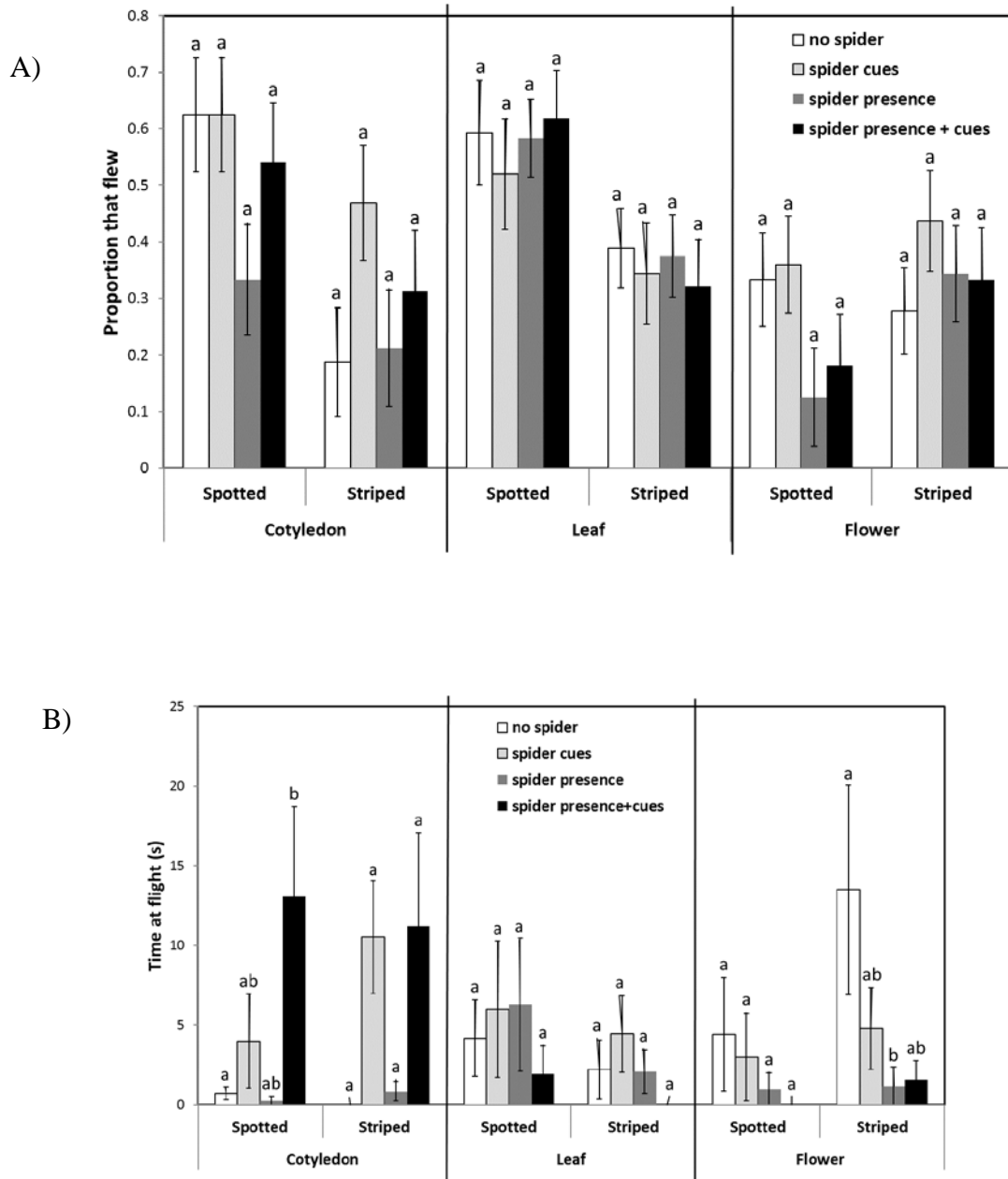


Fig. 3. The mean and standard errors for the A) proportion that flew and B) time at which flight was initiated (s) for spotted and striped cucumber beetles during the one-minute observation periods. Treatment differences were compared separately for each beetle species within trial. Different letters indicate significant differences.

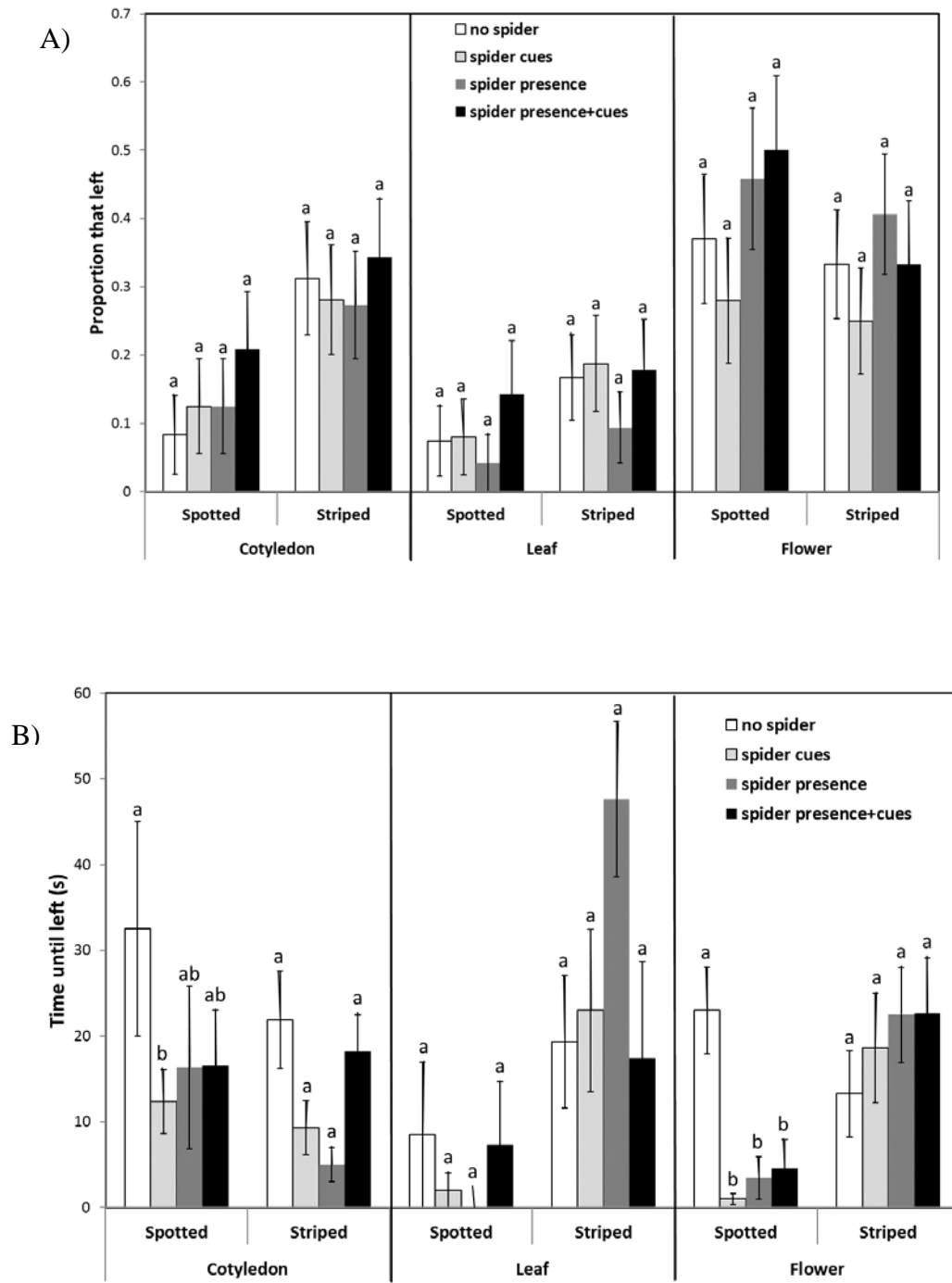
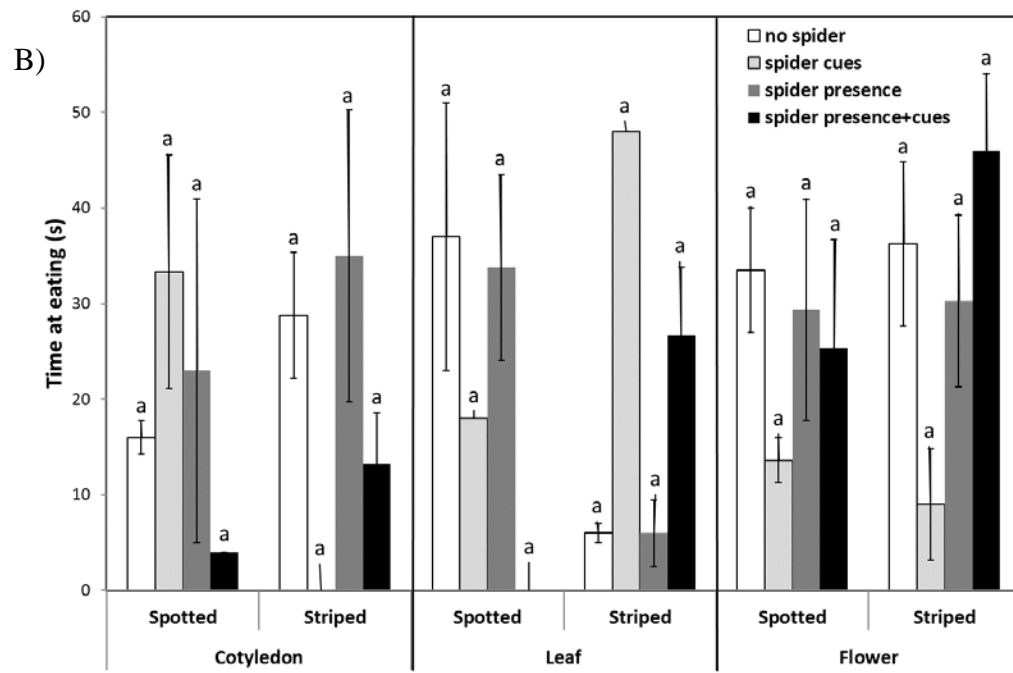
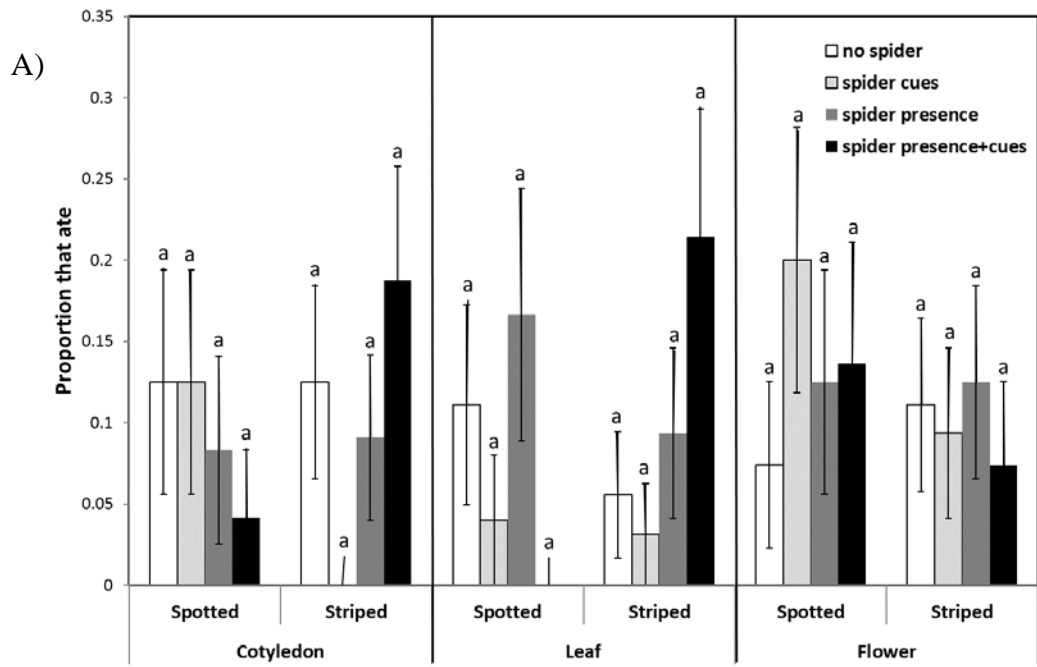


Fig. 4. The mean and standard error bars of A) proportion that left and B) time at which leaving was initiated (s) for spotted and striped cucumber beetles during the one-minute observation periods. Treatment differences were compared separately for each beetle species within trial. Different letters indicate significant differences.



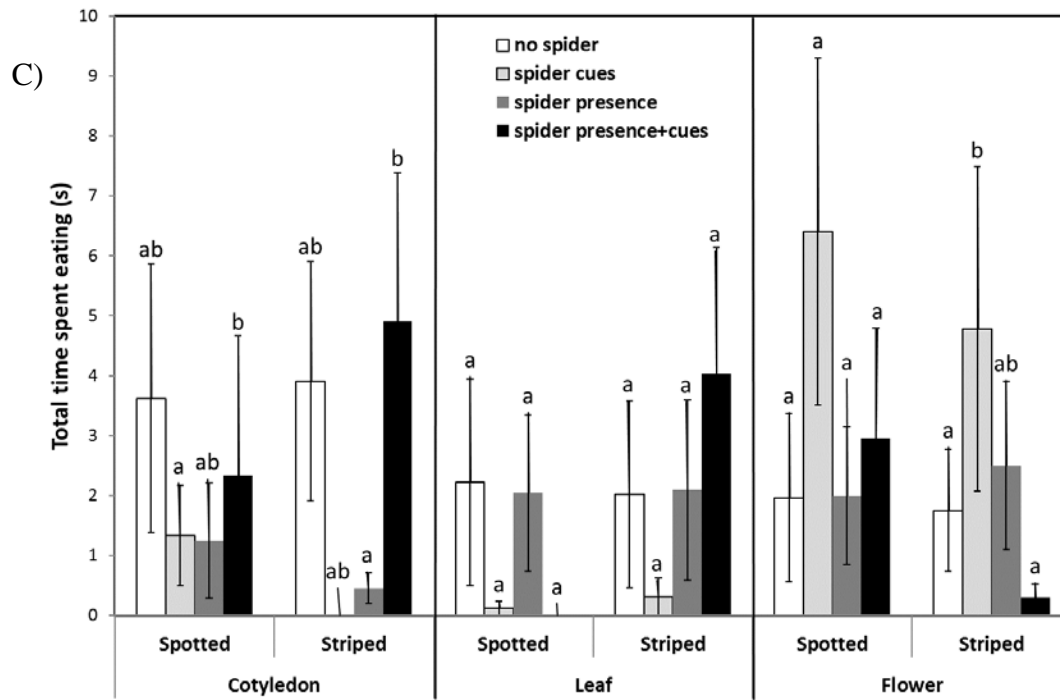


Fig. 5. Mean and standard error bars of the A) proportion that ate, B) time at which eating was initiated (s), and C) total time spent eating (s) for spotted and striped cucumber beetles during the one-minute observation periods. Treatment differences were compared separately for each beetle species within trial. Different letters indicate significant differences.

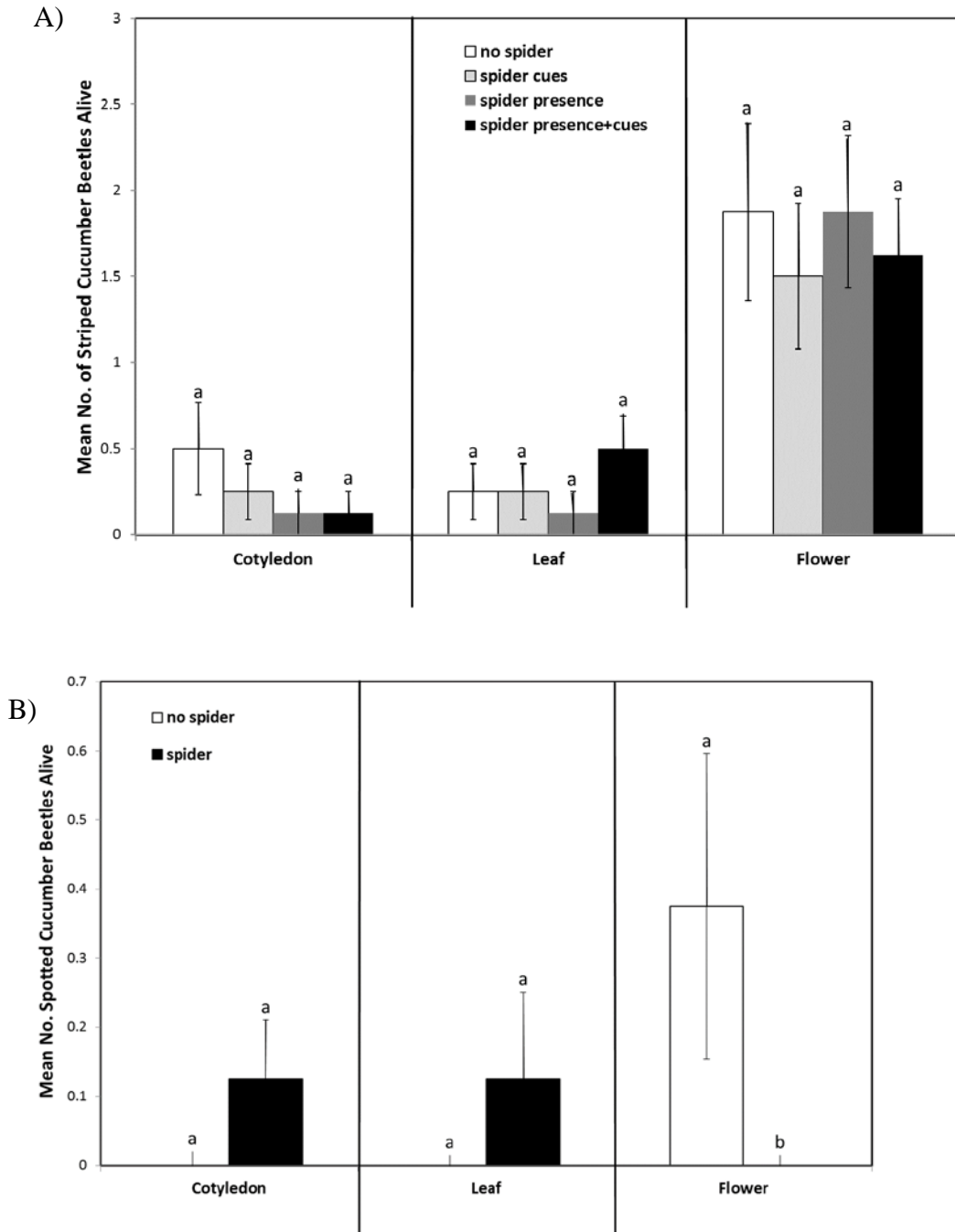


Fig. 6. Mean number and standard error bars of A) striped and B) spotted cucumber beetles remaining in the cage at the end of the trial. Spotted cucumber beetles are compared between treatments with and without spiders. Treatment differences are compared separately for each trial. Different letters indicate significant differences between treatments.

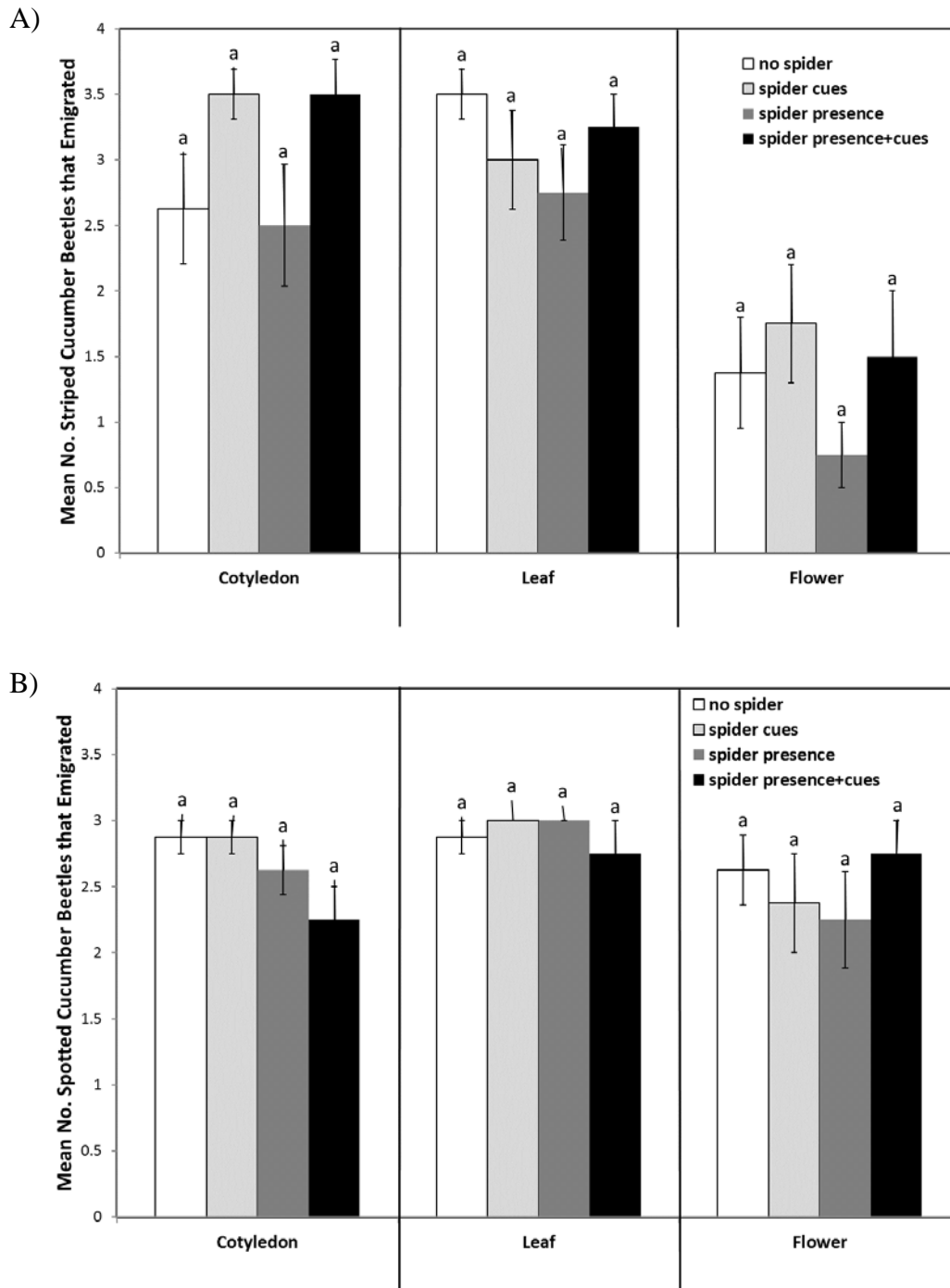


Fig 7. Mean number and standard error bars of A) striped and B) spotted cucumber beetles in the trap at the end of the week-long trial. Treatment differences are compared separately for each trial. Different letters indicate significant differences between treatments.

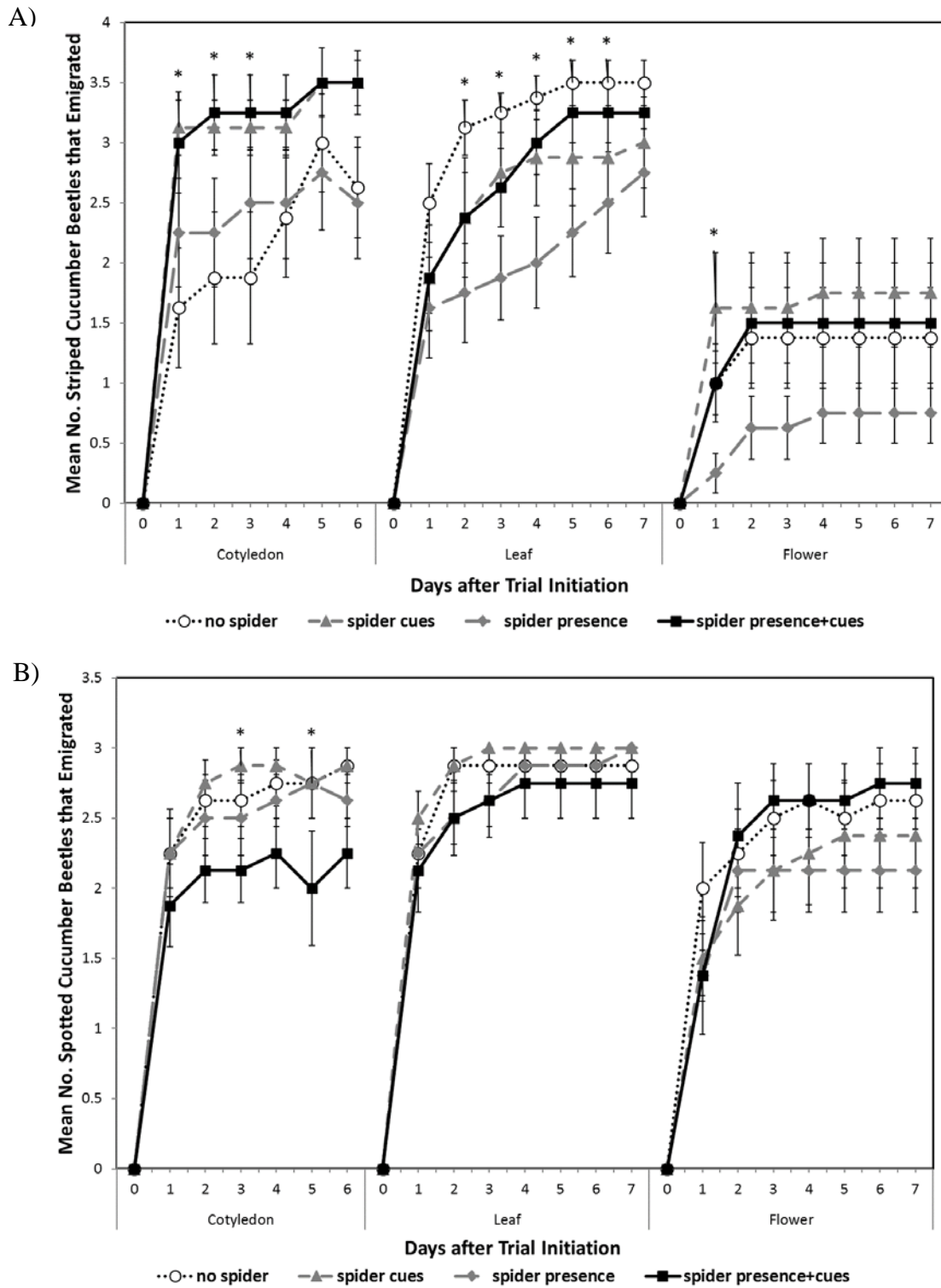


Fig. 8. Mean number and standard error bars of A) striped and B) spotted cucumber beetles in the traps across trial day. An * indicates that there are significant differences between treatment levels on that day.

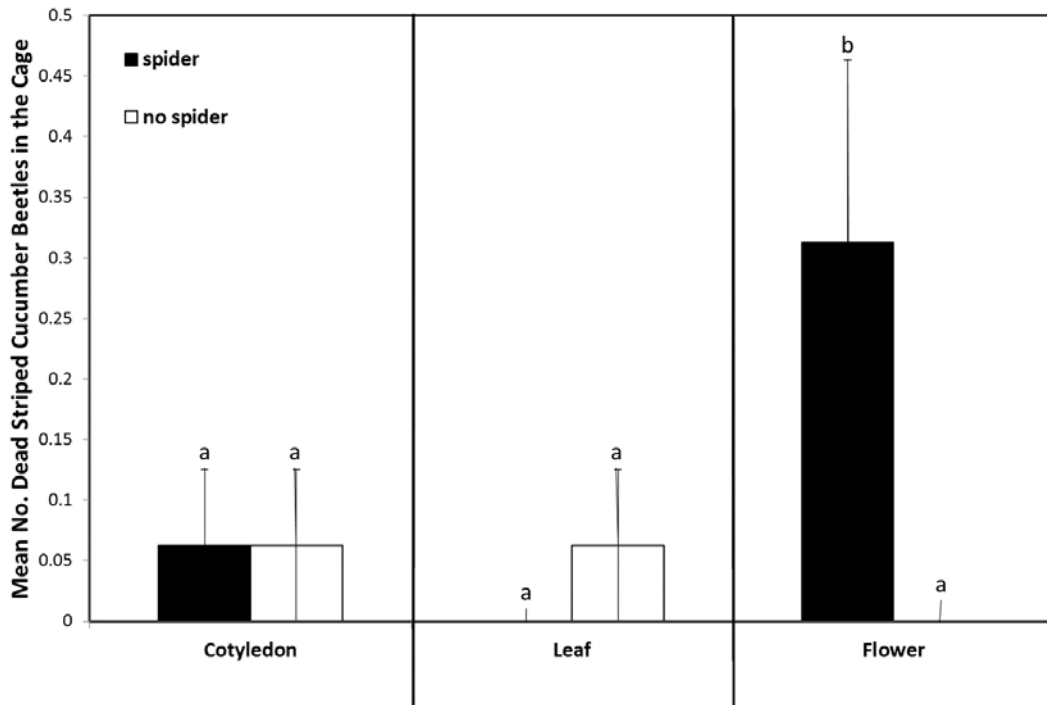


Fig. 9. Mean number and standard error bars of dead striped cucumber beetles remaining in the cage at the end of the trial between treatments. Spotted cucumber beetles are not graphed due to low numbers. Treatment differences are compared separately for each trial. Different letters indicate significant differences between treatments.

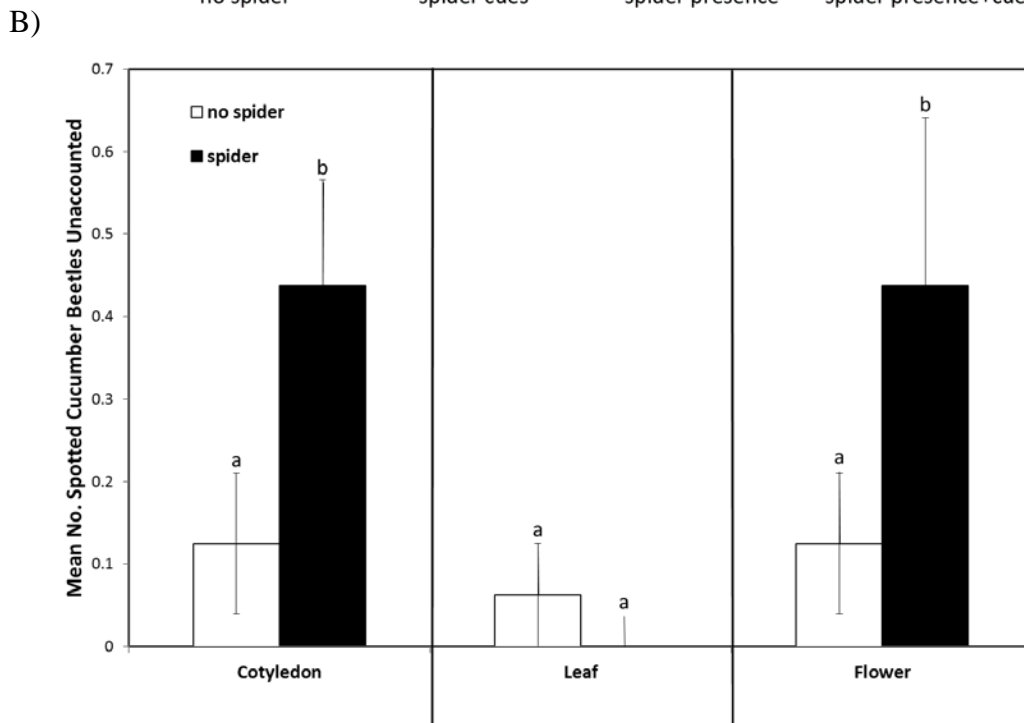
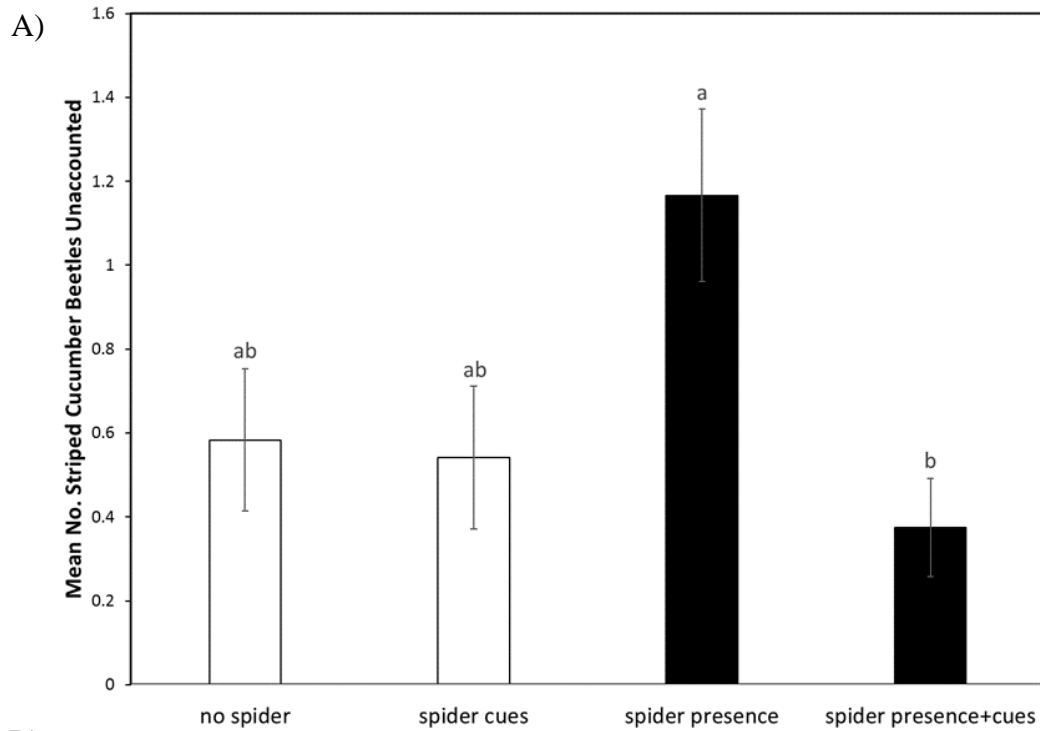


Fig. 10. Mean number and standard error bars of unaccounted for A) striped cucumber beetles across treatment and B) spotted cucumber beetles across treatment and trial. Treatment differences are compared separately for each trial. Different letters indicate significant differences between treatments.

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