The Pennsylvania State University

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THE INFLUENCE OF CROP PHYLODIVERSITY ON HERBIVOROUS INSECTS OF

SQUASH AND THEIR NATURAL ENEMIES

A Thesis in

Entomology

by

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ABSTRACT

Herbivorous insects can be problematic for growers. Despite regular use of insecticides to manage their populations, these insects can still cause extensive damage to crops. Integrated Pest Management (IPM) was developed as an alternative to regular insecticide use to manage insects. One IPM-based tactic, intercropping, is known to reduce herbivorous insect abundance and damage in fields. Because the diet of many herbivores is restricted to only closely related taxa with similar chemistry, diverse plant communities can interfere with their ability to find suitable hosts via associational resistance. The effectiveness of intercropping against herbivorous insects may depend on the phylogenetic relatedness of neighboring crops. In my first chapter, I studied in a vegetable cropping system how evolutionary divergence times among plant species influenced the effects of associational resistance on the insect community. With butternut squash as a focal crop in a series of different intercropping combinations, I found that the phylogenetic divergence of neighboring plants had an inconsistent effect on abundance of herbivorous insects and others (predators, parasitoids, pollinators, detritivores). When considering relationships between crop plants and their insect community, my results suggest that the phylogenetic relatedness of neighboring plants might be one of the mechanisms driving associational resistance against herbivorous insects but more research is needed.

In my second chapter, I explored parasites and parasitoids of the striped cucumber beetle (*Acalymma vittatum*), an economically important pest of cucurbit crops in eastern North America. I identified several parasitoids and parasites (*Celatoria setosa, Centistes diabroticae, Howardula spp,* Nematomorpha, Gregarinasina) and revealed some details on their ecology related to date, beetle size, and beetle gender. I also found that the striped cucumber beetle and its parasites

appeared to share a density-dependent relationship to one another, and that the phylodiversity of neighboring crops may have indirectly influenced parasitism of striped cucumber beetles.

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Chapter 1

How phylogenetic relatedness affects the abundance of insect community in intercrops

Introduction

Managing pests is one of the many challenges that farmers face. Herbivorous insects are particularly problematic because they can colonize fields quickly, and in high enough populations can inflict extensive crop damage, decreasing crop quality and/or yield. Because of threats posed to crops by herbivorous insects, much of monoculture-based crop production tends to rely heavily on regular, often prophylactic, insecticide applications (Pimentel et al. 1993, Casida and Quistad 1998, Oerke and Dehne 2004). Unfortunately, despite this preventative insecticide use, outbreaks of herbivorous insect pests tend to be common in modern fields (Letourneau et al. 2011).

As an alternative to preventative insect management strategies that were popularized after World War II, entomologists developed a pest management approach that integrated ecological principles and chemical control (Smith et al. 1976). Integrated Pest Management (IPM) was meant to help farmers move away from calendar-based insecticide applications and toward an agroecological approach to combat economic damage from pest insects (Stern et al. 1959). As originally envisioned, IPM uses multiple management approaches, including cultural and biological tactics that prevent damage and foster control via natural enemies. Importantly, IPM established insecticides as a final resort should other tactics fail to keep insect populations below economic thresholds (Stern et al. 1959, Stenberg 2017). One of the principles emphasized in IPM is that the ecology of plant-insect interactions should be explored to determine if they might reveal novel management tactics (Stern et al. 1959, Kogan 1998). Planting a diversity of crop species in a field, such as in a polyculture, is the type of practice that can exploit ecological interactions to improve insect management. Well-designed polycultures can boost focal crop yield while distracting herbivorous pests and improving biological control, both of which can reduce herbivore damage (Lucas-Barbosa et al. 2011, Iverson et al. 2014, Isbell et al. 2017).

Generally speaking, most phytophagous insect species have evolved to only feed on a limited number of closely related plant taxa (Bernays 1998), which often share similar chemistry and, as a result, defenses (Erhlich and Raven 1964). Moreover, related plant species tend to have similar appearances and release similar volatile organic compounds (VOC) as a by-product of metabolism or defenses (Theis and Lerdau 2003). Having evolved specialized visual and olfactory senses (Bruce 2015), phytophagous insects can detect these cues, such as the unique VOC bouquet emitted by their host plant species and host plant shape or size (Dell'Aglio et al. 2016, Gadelha et al. 2017). Once detected, insects can track the cues to find a suitable host plant. However, cue interference from plant diversity within polycultures can complicate host plant location for herbivorous insects.

Within plant communities, interactions among plants influence insect communities. Visual and chemical cues from different plants species can interfere with host-plant location by herbivorous insect species (Zhang et al. 2016). In monocultures, high densities of host plant species can attract large numbers of phytophagous insects (Barbosa 2009, Gardner et al. 2015). According to the Resource-Concentration hypothesis, insect pests perform well in monocultures because they easily locate an abundant host plant species and are not resource constrained (Tahvanainen and Root 1973). In polycultures, however, the neighbors of a focal plant species can reduce its apparency, making it more difficult for herbivorous insects to find and reducing herbivore abundance (Price et al. 1980, Andow 1991). Specifically, insects foraging for their host-plant species can be confused by chemical and structural complexity of polycultures (Finch & Collier 2001, Randlkofer et al. 2010), masking host-plant location (Jactel et al. 2011, Castagneyrol et al. 2014b). Intercropping, an approach to polycultures in which at least two different crop species are grown together in a field, has been used by growers since ancient times because it enhances plant performance and decreases herbivore densities by associational resistance (Papendick et al. 1976, Bach 1980a., Vandermeer 1989, Hamback et al. 2000, Barbosa et al. 2009, Boudreau 2013).

Associational resistance is an interaction between nearby plants in which the presence of one plant species decreases the likelihood that its neighboring plant will be found by herbivorous insects (Price et al. 1980, Barbosa et al. 2009). Associations among neighboring plant species have been used to develop intercropping approaches that reduce pest damage on focal crops. Intercropping methods such as trap cropping and push-pull systems function by either repelling pest insects, attracting them to an alternative host, or increasing species diversity (Bach 1980b., Shelton and Badenes-Perez 2005, Gilbert and Webb 2007, Cavanagh et al. 2009, Cadoux et al. 2015, Khan et al. 2016). While many studies have explored the value of associational resistance (Barbosa et al. 2009), no studies currently appear to have explored the roles of phylogenetic relatedness for predicting its effects.

Phylogenetic diversity, or phylodiversity, describes the relatedness of species within a community, and may positively or negatively affect plant success by altering pest populations (Castillo et al. 2010). Quantifying phylodiversity between plant species using recent advances in technology have helped address community ecology questions (Sanderson et al. 2004, Cavender-Bares et al. 2009). Current studies show the potential effects of plant phylodiversity on plant-insect interactions. For example, plant communities with greater phylogenetically diversity from the perspective of an insect pollinator tend to be more pollen-limited than plant communities comprising close relatives (Sargent et al. 2011). Another study proposed that phylogenetic diversity decreased herbivory in an old field because some herbivores were phylogenetically

specialized (Dinnage 2013). However, I am not aware of any studies thus far that have addressed phylodiversity in an agricultural context as a mechanism for associational resistance.

My research explores whether associational resistance generated between neighboring crop species with known phylogenetic divergences can influence an agricultural insect community. I hypothesized that as the evolutionary divergences of neighboring plant species increased relative to a focal crop species, insect pest abundance would decrease. Moreover, I expected that the influence of phylogenetic distance would have a neutral or positive effect on other arthropod functional groups (predators, pollinators, parasitoids, detritivores) because these groups of insects may not be as closely associated to plant-produced cues as herbivorous insects. To experimentally explore the influence of phylogenetic distance on associational resistance to insect communities, I used butternut squash (*Cucurbita moschata*) as a focal crop species and surrounded it with rows of crops of different evolutionary divergence times relative to the butternut squash.

Materials and methods

Location

I conducted my two-year summer field study at The Russell E. Larson Agricultural Research Center at Rock Springs (Pennsylvania Furnace, PA). The field I used in 2017 (40.709296, -77.948777) was previously planted with wheat. Because excessive rains resulted in standing water that persisted at one end of the field for the whole summer, in 2018 I relocated my study to two adjoining fields on a different part of the farm (40.715318,-77.951797). These two fields had previously been planted with a corn and soybean rotation.

Treatments and Design

I used phylogenetic information from collaborators at Purdue University to identify crops with different divergence times (million years ago) from butternut squash (*Cucurbita moschata*), the focal crop species (Figure 1-1), and then selected two crops from each phylogenetic divergence time to use as treatments, except for crops within the Cucurbitaceae family (nine plant species total; Figure 1-2). The divergence times and plant species were [Cucurbitaceae: *Cucurbita pepo* (butternut squash), 0 mya; *Cucurbita pepo* (zucchini), 32 mya; *Citrullus lanatus* (watermelon), 147 mya], [Fabaceae: *Phaseolus vulgaris* (common bean) and *Glycine max* (soybean), 213.9 mya], [Solanaceae: *Capsicum annuum* (sweet pepper), 238.3 mya], [Asteraceae: *Helianthus annuus* (sunflower), 238.3 mya], [Poaceae: Zea mays (sweet corn) and *Avena sativa* (common oats), 376.5 mya] (Figure 1-1, 1-2). Most of these plant species were used in 2017 and 2018 with the exception of Fabaceae, which was excluded in 2018 to make the field experiment more manageable. In addition to the treatments with different plant species, I also included a "blank" treatment, which consisted of empty rows on either side of butternut squash, to test for potential resource concentration effects. As a result, the total number of treatments in 2017 was ten, whereas in 2018 it was eight.

In 2017, the experimental design was arranged within one field into 50 6.096 m plots, each five rows wide (Figure 1-3). In each plot, I spaced the rows with 2.1336 m row centers with 3.048 m between plots. In the center (or 3rd) row of each plot, I planted the focal crop, butternut squash. I used the four remaining rows (rows 1, 2, 4, and 5) for planting the treatment, which was one of the nine plant species representing a divergence time (eight "other" plant species or butternut squash planted as a monoculture) and one blank treatment in which only the center row was planted with butternut squash and the surround four rows were empty (Figure 1-4). I randomly assigned plot order with a block using a random number generator (Random.org), and this procedure was repeated for each of the five blocks. The design was slightly different in 2018 to accommodate the other location. I used 40 plots which were spaced 1.524 m between rows and 6.096 m between plots across two fields (Figure 1-5). The study site was tilled with a moldboard plough, and the five rows per plot were laid with black plastic covering planting rows, and herbicides and mowing were used to control weeds between rows and plots. A single drip irrigation line was laid in each row between the ground and black plastic. Butternut squash, watermelon, zucchini, and peppers were planted as seedling plugs. Sunflower, sweet corn, and oats were direct seeded.

Data Collection

I gathered data using scouting, sticky traps, pitfall traps, and dissections of beetles to find parasites (further details below). I scouted every other week and other methods occurred monthly. In response to weather conditions, I modified this schedule as necessary (Table 1-1). When multiple collection methods occurred during the same week, destructive methods were done last (sticky traps, pitfall traps, beetle collections). I collected data weekly from the center row (butternut squash) of each plot. To account for edge effect, 0.6096 m on either end of the butternut squash row in each plot were excluded from data collection. In 2017, due to establishment challenges associated with a wet spring and early summer, I collected data from 8 August to 20 September. In 2018, I collected data from 21 June until 30 August 30. I had difficulty managing weeds, groundhogs, and powdery mildew during our experiment and early season data were muddy because of issues with plant establishment. Plots that either failed to establish or those which died off (late season) were excluded from data collection and the analysis.

Foliage Scouting for pest abundance

I scouted butternut squash foliage to assess pest abundances in the treatments. Scouting occurred in the morning around 8:00 am. The center row of each plot was split into equal 1.61544 m subsections and labeled x, y, and z (Figure 1-6). I counted insects on the stems, leaves, and at the base of butternut squash plants in a repeating rotation schedule where week A was subsection x, week B was subsection y, and week C was subsection z. Week A followed week C to repeat the rotation. Cucurbit pest species counted included striped cucumber beetles (*Acalymma vittatum*; SCB), spotted cucumber beetles (*Diabrotica undecimpunctata*; SPCB), squash bugs (*Anasa tristis*), and western corn rootworm (*Diabrotica virgifera*; WCR).

Flower Scouting for pest and pollinator abundance

I scouted butternut squash flowers to assess pollinators and pest abundances in the treatments. Scouting occurred in the morning around 7:00 am. I counted insects in the morning when flowers were open. In each plot, for every mature flower, I counted and sexed insects, and recorded them as open (pollinator-available) or old. I counted cucurbit pest species including striped cucumber beetles (*A. vittatum*) and spotted cucumber beetles (*D. undecimpunctata*). Pollinator species counted included squash bees (*Peponapis pruinose*), bumble bees (*Bombus sp.*), and honey bees (*Apis mellifera*).

Sticky Traps for aerial insect abundance

I used sticky traps to assess the aerial insect community. Using binder clips, I affixed a 15.24 x 20.32 cm double-sided yellow sticky card (Trapro, Dual-Sided Yellow Sticky Traps, TR-YST-30) vertically to a (~50 cm long) thin bamboo stick and inserted one stick-card setup into

the center of each plot so the card was facing parallel to the rows. I adjusted the height of each trap so the bottom of the sticky card was just above the plant canopy. After 72 h, I collected traps, stored them in clear magazine bags (ULINE 2 mil 9 x 12 inch Industrial Poly Bags), and placed them in a freezer for later insect identification. Beneficial insects (pollinators, predators, and parasitoids) counted included feather legged flies (Tachinidae: Trichopoda pennipes), lady beetles (Coccinellidae), hover flies (Syrphidae), long-legged flies (Dolichopodidae), and parasitoids (Hymenoptera). I initially intended to include predaceous wasps (Vespidae), soldier beetles (Cantharidae: Chauliognathus pensylvanicus), big eyed bugs (Lygaeidae: Geocoris sp), and minute pirate bugs (Anthocoridae) but occurrences on the sticky traps were rare. I observed feather legged flies (Tachinidae: Trichopoda pennipes; a squash bug parasitoid), and soldier beetles (Cantharidae: Chauliognathus pensylvanicus), a cucumber beetle predator, in my plots but rarely caught them on traps. I counted the following pest insect species: striped cucumber beetles (A. vittatum), spotted cucumber beetles (D. undecimpunctata), aphids (Aphis sp.), tarnished plant bugs (Lygus lineolaris), and thrips (Thysanoptera). For counting insects, I subdivided each sticky trap into 8 equal rectangles (6.35 x 8.89 cm; 4 per side) with a marker. For each sticky card, I randomly selected one rectangle from each side to use for aphid, parasitoid, and thrips density counts. I then then multiplied these counts by four to estimate the densities for each of these insect groups on the entire card. I counted all other insects on the entire card. Due to logistical challenges, sticky trap data from 2017 is limited.

Pitfall Traps for epigeal arthropod activity density

I used pitfall traps to assess the epigeal arthropod community. I partially filled pitfall traps (8 cm tall, 11.5 cm diameter Reynolds Del Pak) with 50% propylene glycol as a killing agent and placed them in the center of each plot between row 2 and 3 in the soil (not under the

black plastic) so the tops of the traps were flush with the ground. To keep them protected from rain and debris, I covered each trap with a plastic plate elevated by a tripod of nails (8.89 cm tall). I collected traps after they were in the field for 72 hours. I then sorted, identified, and preserved the invertebrates from the traps. I discarded non-epigeal arthropods such as lightning bugs, wasps, and house flies, and micro-epigeal arthropods such as springtails and mites. Arthropods I counted included carabid beetles (Carabidae), rove beetles (Staphylinidae), spiders (Araneae, dominated by Lycosidae), harvestmen (Opiliones), millipedes (Diplopoda, dominated by *Oxidus gracilis* and Julidae), centipedes (Chilopoda), and ants (Formicidae).

Dissections for parasitism rates

In 2018, I collected and dissected striped cucumber beetles to assess parasitism. Field collections of the beetles were planned to occur shortly after scouting events because they were potentially temporarily destructive to the pest population. In each plot, I tried to collect 10 beetles from flowers and foliage by hand or with an insect vacuum. Beetles were kept alive in a refrigerator or frozen until I dissected them under a microscope in PBS 1X solution. I performed whole abdominal dissection by securing a beetle with forceps on the thorax (near the scutellum) and using a second pair of forceps to remove the abdominal cuticle and reveal organs by pulling at the pygidium (like taking a glove off; dissection method from Istvan Miko, *pers. communication*). I sexed beetles and then removed and identified any parasites or parasitoids in the abdomen. I preserved parasitic organisms and their beetle hosts on slides with glycerin and later transferred them to 80% ethanol. I reserved several parasitoid insect larvae to rear into adulthood for vouchers, but none successfully pupated. I therefore based identification of insect larvae on findings from previous studies.

Damage and yield

I intended to assess plant damage and yield of butternut squash to gauge plant health, and attempted to do so in both years. However, logistical challenges with establishment and high mortality in young plants because of weather in both years resulted in plots with mixed ages as plants were replaced, making them incomparable. Additionally, much of the observed cucurbit damage in my plots occurred on zucchini plants more than butternut squash plants, which was a treatment crop.

To assess early season feeding damage, I evaluated a subsection of each plot (same rotation as described in foliage scouting for herbivore abundance). In each sub-plot, I counted the number of fully developed leaves on plants and measured leaf surface area damage: total of each leaf using a grid printed on a 21.59x27.94 cm transparency (template from incompetech.com). The grid was made up of 1x1 cm major blocks subdivided by 0.2x0.2 cm blocks. When plants began to vine, I also counted the number of new vines. Once nearly all plants were vining, I planned to terminate early-season damage assessment.

To assess other threats to plant health, I also intended to evaluate pathogens and additional insect pests. After scouting foliage, I examined the butternut squash plants for evidence of bacterial wilt (pathogen *Erwinia tracheiphila*), powdery mildew (possible pathogens: *Erysiphe cichoracearum* and *Sphaerotheca fuliginea*), as well as insects such as squash vine borer (*Melittia satyriniformis*). Plants infected with bacterial wilt and dead plants were removed from the plots and replaced with new plants. All removed plants that exhibited wilt symptoms were also dissected at the main stem to check for squash vine borer larvae.

To assess yield, I was going to count the number of squash fruits produced by plants in sub-plots and then finally count number of marketable squash after harvest by inspecting harvested squash for pest-induced blemishes and disease.

Statistics

I used Minitab v.18 to perform all analyses. Data were modeled separately by date and where applicable, dates were pooled for season cumulative models. For all models, α = 0.05. The results of my "blank" treatment are not included in the analyses presented in my results.

In all linear regression and Poisson regression models, divergence time was a continuous variable and block/replicate was a categorical variable. When block was not significant but divergence was, block was dropped from the model. When a model contained multiple variables (i.e. divergence time, block, and number of flowers per plot), R-squared (adj) was used. When only divergence time was significant, R-squared was used. When a data set had a lot of zeros/low values/rare taxa, and for dissection data, Poisson regression was used instead of linear regression. For Poisson regressions, sometimes the model would only run when block was removed as a variable. In 2017, most dates had to be pooled into cumulative models to run because taxa were rare. Occasionally, taxa were too rare and Poisson models did not run. For all plotted linear regression and Poisson lines, the trendline includes divergence time and the response variable only.

Results

Foliage Scouting for pest abundance

In 2017, across all plots, squash bugs were the most abundant insect species I encountered, followed by spotted cucumber beetles (SPCB) and striped cucumber beetles (SCB). I found few insect herbivores on leaves and stems, and their abundances varied greatly among divergence times (Table 1-2). Because of the rarity of herbivore insects on foliage, in most cases

only models with pooled data satisfied regression assumptions. Squash bug abundance decreased as divergence time increased on August 30th (30-Aug, $\chi 2_1 = 40.18$, P = <0.0001, R-squared adj = 10.59%; Table 1-3; Figure 1-7). On the both the August 30th sampling date and cumulatively, the abundance of all herbivores pooled (squash bugs, SCB, SPCB) sign decreased as divergence time increased (30-Aug, $\chi 2_5 = 87.11$, P = <0.0001, R-squared adj = 33.65%; Cumulative, $\chi 2_5 = 75.23$, P = <0.0001, R-squared adj = 19.55%; Table 1-3; Figure 1-8).

In 2018, striped cucumber beetles (SCB) were most abundant, followed by squash bugs, western corn rootworm (WCR), and spotted cucumber beetle (SPCB) (Table 1-4). Insect abundances in June and early July were not significantly affected by divergence time (Table 1-4). Unexpectedly on July 19th, SCB abundance increased as divergence time increased (F_I = 4.69, P = 0.038, R-squared = 12.79%; Figure 1-9). Later in the field season, a pattern emerged that was consistent with my phylogenetic diversity-based hypothesis, and it appeared to be mostly driven by SCB abundance, which decreased as divergence time increased on August 6th and August 28th (6-Aug, F_I = 46.71, P = <0.0001, R-squared = 58.60%, 28-Aug; $\chi 2_I$ = 5.67, P = 0.017, R-squared = 8.29%; Table 1-5; Figure 1-9). Abundance of all herbivorous insects responded negatively to phylodiversity strongest on August 6th (F_I = 36.85, P = <0.0001, R-squared = 52.75%), followed by August 28th ($\chi 2_5$ = 40.96, P = <0.0001, R-squared adj = 33.87%; Table 1-5; Figure 1-10).

Flower scouting for pest and pollinator abundance

In 2017, cucumber beetles (SCB, SPCB) in squash flowers were very low and their abundances varied greatly among treatments (Table **1-6**). Because of the rarity of herbivore insects on foliage, in most cases only models with pooled data satisfied regression assumptions. I only counted insects in flowers for August and September due to weather delaying crop establishment. Cucumber beetle (SCB and SPCB) abundances responded to divergence time overall ($\chi 2_2 = 19.32$, $P = \langle 0.0001 \rangle$, but the explained variation was weak (5.14%) and the slope of the regression line appeared negligible (Table 1-3; Figure 1-11). Bees (squash bees, bumble bees, honey bees) were especially rare and failed regression assumptions (Table 1-6).

In 2018, beetle abundance (SCB, SPCB, and WCR) in flowers responded negatively to phylodiversity on August 6th ($\chi 2_6 = 46.32$, P = <0.0001, R-squared adj = 43.34%; Table 1-5). Although also cumulatively significant, ($\chi 2_6 = 191.53$, P = <0.0001, R-squared adj = 43.50%; Table 1-5; Table 1-7; Figure 1-12), the majority of the variation was explained by number of flowers per plot and the slope of the regression line for divergence time appeared negligible. The number of flowers in the center row of butternut squash per plot was a stronger predictor of beetle abundance than divergence time on most dates (Table 1-5). Bee abundance was low (Table 1-7). Most (64.52%) of the variation was explained by the number of flowers in the center row of butternut squash per plot, block replicate, and date ($\chi 2_8 = 207.78$, P < 0.0001) and I did not detect a significant effect of divergence time on bee abundance (Table 1-5).

Sticky Traps for abundance

In 2017, from my only bout of sampling with sticky traps, divergence time did not significantly influence abundance of natural enemies (lady beetle, long-legged fly, parasitoid wasp) or herbivorous insects (SCB, SPCB, tarnished plant bug, thrips) (Table **1-8**; Table **1-9**). Among the natural enemies, parasitoid wasps had the highest average abundance (Table **1-8**). Among the herbivores, thrips were most abundant (Table **1-9**).

In 2018, divergence time did not appear to influence abundance of natural enemies on sticky cards (Table 1-8). The long-legged fly was the only natural enemy that significantly responded to divergence time on one date, August 30th ($F_1 = 3.24$, P = 0.088, R-squared = 14.55%; Table 1-5; Figure 1-13), and its abundance unexpectedly decreased as divergence time

increased. Parasitic wasps were most abundant, followed by lady beetles, hover flies, and longlegged flies (Table 1-8). Herbivorous insects did respond to divergence time. SCB abundance decreased as divergence time increased on June 28th (F_1 = 16.94, P = <0.0001, R-squared = 33.93%) and August 30th (F_1 = 7.10, P = 0.015, R-squared = 27.20%; Table 1-5; Figure 1-14). Only thrips responded to divergence time on August 7th (F_1 = 6.80, P = 0.014, R-squared = 17.0%; Table 1-5; Figure 1-15), but the standard error was variable and they appeared to weakly decrease as divergence time increased. On August 30th, SPCB ($\chi 2_1$ = 3.45, P = 0.063, R-squared = 9.55%) abundance weakly increased as divergence time increased (Table 1-5; Figure 1-16), and tarnished plant bug (F_1 = 7.40, P = 0.014, R-squared = 28.03%) abundance decreased as divergence time increased (Table 1-5; Figure 1-16). Pooled together, the abundance for all herbivorous insects pooled together decreased as divergence time increased. (F_1 = 4.3, P = 0.041), but the explained variance was weak and standard error was high (R-squared = 4.61%; Figure 1-17). Thrips were the most abundant herbivores, followed by SCB, tarnished plant bugs, and SPCB (Table 1-9).

Pitfall traps for epigeal arthropod activity density

In 2017, divergence time did not significantly influence epigeal activity density for any groups of epigeal arthropods (Coleoptera, Arachnida, Myriapoda, and ants; Table 1-11).

In 2018, epigeal activity density occasionally responded to divergence time. Because of the rarity of some taxa, in some cases only models with pooled data satisfied regression assumptions (Table 1-11). All arthropod groups except ants responded to divergence time on June 28^{th} (Coleoptera: $\chi 2_1 = 3.72$, P = 0.054, R-squared = 8.24%; Arachnida: $F_1 = 8.01$, P = 0.008, Rsquared = 19.54%; Myriapoda: $\chi 2_1 = 4.95$, P = 0.026, R-squared = 8.27%; Table 1-5; Figure 1-18), but some of the explained variation was weak. Arachnida activity density decreased as divergence time increased, and Coleoptera and Myriapoda activity density weakly increased as divergence time increased. Both Coleoptera ($\chi 2_1 = 4.16$, P = 0.042, R-squared = 9.01%) and Myriapoda ($\chi 2_1 = 36.31$, P = 0.007, R-squared = 30.77%) activity density decreased as divergence time increased, although Coleoptera had a weak response to divergence time on July 30^{th} (Table 1-5; Figure 1-19). Cumulative Arachnida activity density decreased as divergence time increased, but with variable standard error ($F_1 = 6.56$, P = 0.012, R-squared = 6.12%; Table 1-5; Figure 1-20).

Dissections for parasitism rates (2018 only)

The most abundant taxa found from SCB dissections included a tachinid fly (*Celatoria setosa*), a braconid wasp (Centistes diabroticae), and a nematode (possibly *Howardula benigna*; Table **1-12**), and nematodes were the most abundant. Tachinid larvae negatively associated with divergence time on all three collection dates (July 10th: $\chi 2_1 = 3.68$, P = 0.055, R-squared = 8.26%; August 7th: $\chi 2_5 = 12.58$, P = 0.028, R-squared adj = 14.19%; August 30th: $\chi 2_1 = 3.75$, P = 0.053, R-squared = 11.11%; Figure **2-8**). Similarly, Braconid larvae and egg abundance decreased in response to divergence time on July 10th (larvae: $\chi 2_1 = 11.40$, P = 0.001, R-squared = 17.04%; eggs: $\chi 2_1 = 9.25$, P = 0.002, R-squared = 20.11%; Figure **2-9**) and August 7th (larvae: $\chi 2_5 = 24.50$, P = <0.0001, R-squared = 31.05%; eggs: $\chi 2_1 = 3.39$, P = 0.066, R-squared = 7.42%; Figure **2-9**), and displayed a stronger response than tachinid larvae. Nematode abundance had no association with divergence time.

Discussion

Within a vegetable intercropping system, I evaluated the effects of phylogeneticbased plant diversity in space (i.e., the relatedness of neighboring plant species) on the arthropod community of butternut squash. I found that plant phylogenetic diversity was an occasional predictor of herbivorous insect abundance in my focal crop, butternut squash. Phylodiversity, therefore, appears to have played an inconsistent role in my experiments structuring herbivorous insect communities, and is likely to have influenced the apparency of neighboring plants, which is derived from interactions of their chemical and visual cues. In a similar experiment exploring diversity in time (i.e., crop rotations; I explored diversity in space, i.e., intercropping) phylogenetic relatedness of plant species in a rotation were not predictive of rotational effects on a specific herbivorous insect. In a crop rotation, however, any effect of the preceding crop species on herbivores of the following crop species would have to be expressed via legacy effects on soil (Ingerslew and Kaplan 2018), rather than the mixing of cues that were operating in my field experiments.

Plant communities produce an impressive assortment of cues, mainly volatile organic compounds and visual characteristics (size, color, and structure), that change throughout the season as plants develop and reproduce (Theis and Lerdau 2003, Christensen et al. 2012, Kuper 2013, Najar-Rodriguez et al. 2013, Ishizaki et al. 2016, Pickett and Khan 2016). As phylogenetic relatedness between neighboring plants increases, their cues are less likely to be similar (Theis and Lerdau 2003) and the resulting blend of cues is expected to reduce apparency of host plant species to their herbivorous insects (Andow 1991). In my experiment, I expected that as the relatedness of the plant neighbors of butternut squash became more distant the apparency of butternut squash would decline, reducing herbivorous insect abundance. This phenomenon has

been observed in natural systems, such as the case where herbivory by the pine processionary moth was reduced by volatile cues from deciduous non-host tree (Jactel et al. 2011, Castagneyrol et al. 2014). I found some evidence that increasing phylodiversity reduced mid- to late-season herbivore abundance on butternut squash, but the effect was inconsistent and variable. For example, in 2018 striped cucumber beetle abundance decreased with increasing divergence time in late June and throughout August; Figure 1-9, 1-14). Similarly, a Canadian study that investigated how genetic relatedness of native plant communities affected the herbivory on invasive plants found that more phylogenetically isolated invasive plants had less herbivory, and the phylogenetic relationship between the native and invasive plant species was variable and only occurred later in the growing season (Hill and Kotanen 2009). A future experiment would help clarify potential mechanisms behind plant apparency and phylogenetic relatedness of neighboring plants by testing how prevalent agricultural herbivores respond to quantified visual cues (such as structure and color) and chemical cues (such as volatile bouquets emitting from intercrops). Importantly, phylogenetic diversity appeared to increase herbivore abundance in mid-July (Figure 1-9), suggesting that other factors were also influencing the herbivore populations on butternut squash.

When co-occurring in space and time, closely related plant species are likely to attract similar herbivores, potentially stressing plants and negatively influencing growth and yield (Cheplick and Kane 2004, Barbosa et al. 2009, Burns and Strauss 2011, Catola et al. 2018). I predicted that increasing phylodiversity between neighboring plants in a plot would decrease herbivorous insect abundance; however, my results did not consistently agree with this hypothesis. Instead, differences in abundance sometimes appeared variable. Other factors, such as host-plant specialization, insect life cycles, or plant composition, likely influenced plant-insect interactions and may have overshadowed the influence of phylogenetic diversity. In other systems, such as forest polycultures, variable reductions in herbivory appeared to depend on plant species relatedness and herbivore specialization, where specialists were sensitive to host abundance but generalists responded to phylodiversity of neighboring trees. Despite these differences, highly divergent mixtures generally had the least herbivore damage (Castagneyrol et all. 2014). In my vegetable polycultures, which were notably less complex than forest polycultures, I considered striped cucumber beetles and squash bugs as cucurbit specialists, but spotted cucumber beetles, thrips, and tarnished plant bugs as generalists (Wadley 1920, Bach 1980b., Metcalf et al. 1998, Capinera 2001, Skinner et al. 2014, Gardner et al. 2015). Contrary to the results with forest polycultures (Castagneyrol et all. 2014), the generalist and specialist herbivore species on butternut squash responded similarly to treatments. Despite their feeding specializations, in both 2017 and 2018 herbivores were generally negatively associated with divergence from butternut squash (Table 1-3, Table 1-5). Notably, striped cucumber beetle was the most abundant specialist herbivore I encountered, and was also more associated with divergence time than any of the other herbivores (Figure 1-9, Figure 1-14). Accordingly, while both generalist and specialist herbivorous insects appear to decrease in abundance in response to the phylodiversity of plant neighbors in a cropping system, dominant specialists might be most affected. This effect likely varies by species and ecosystem, as forest specialist herbivores did not respond to phylodiversity (Castagneyrol et all. 2014).

Like feeding specialization, insect life cycles are quite variable by species and location, affecting their relative abundance and behavior. Striped cucumber beetle, for example, overwinters as an adult, which becomes active in May, and then has one new generation per summer in Pennsylvania that emerges in the late summer and overwinters (Shelby Fleischer, Dept. of Entomology, PSU. *pers comm*). The squash bug, on the other hand, has at least one generation per summer that emerges beginning in June (depending on weather), continuously lays eggs throughout the summer, and local populations have differing host-plant preferences (Nechols 1987, Decker and Yeargan 2008, Doughty et al. 2016). Abundances for both striped

cucumber beetles and squash bugs aligned with their expected phenology, but their abundances also decreased in response to increasing phylodiversity in plots on some dates (Figure 1-3, 1-5). In 2018, I observed a peak in abundance of striped cucumber beetle on butternut squash in the first weeks of July and August. Squash bug abundances remained consistent throughout most of the season (Table 1-4, Table 1-9). The first and second peaks in striped cucumber beetle abundance were likely caused by colonization from the overwintering generation and emergence of the new generation. Based on this evidence, the emerging generation may have responded to phylogenetic differences between treatments more than the overwintered generation. Similarly, in a trapping experiment with cucumber beetles, the emerging generation of beetles was more responsive to chemicals emitted from a lure (floral volatiles plus live beetles) than the overwintered generation (Christie Shee, Dept. of Entomology, Purdue. *pers comm.*)

In addition to behaviors and life cycles of insects influencing outcomes of my experiment, the structure and life cycles of plants may have also played a role. Divergence times were represented by multiple species, and I cannot discount the possibly that species effects occurred in my experiment, although they were not directly tested. I observed that abundances of herbivorous insects shifted among crop treatments differently across dates, especially among non-cucurbit crops (Table 1-10; Figure 1-21) and suspect these shifts in abundance could be related to plant structure and growth or resource availability. For example, based on phylogeny alone (Figure 1-1, 1-2), I expected that abundance of herbivorous insects in sunflower intercrops would be lower than abundance in cucurbit intercrops, and my observations confirmed this expectation. However, monocultures of butternut squash did not always host the highest abundance of herbivorous insects. Moreover, squash surrounded by sunflower often had fewer herbivores than squash neighbored by corn or oat, contrary to my phylogenetic-relatedness hypothesis. Although not statistically significant, among all my crop species sunflower had the lowest average abundance of herbivorous insects and cucurbits had the highest (Table 1-10, Figure 1-21).

Sunflower, as the tallest crop species in my experiments, may have formed a vegetative barrier around butternut squash that physically interfered with host-finding by insects. Because herbivorous insects prefer to land on any green objects rather than soil, they are more likely to incorrectly land on large plant neighbors (i.e. more vegetative structure) than small plant neighbors near a host plant (Randlkofer et al. 2010) and often have preferences among the host plants they will attack (McGrath 2004, Hazzard et al. 2009). Despite both being 238 million years diverged from butternut squash, my pepper variety was small to medium-sized and bushy, but my sunflower variety was extra-large and tall. In both years, the cumulative average of herbivorous insects was higher in pepper plots than sunflower plots (Table 1-10). Because sunflower had more vegetative structure than pepper, it is possible that more herbivorous insects landed on sunflower. Additionally, sunflowers, which are genetically distant from many vegetable crops, are known to improve insect control and boost natural enemy diversity and abundance (Rogers 1992, Tschumi et al. 2016). In an organic sunflower-vegetable intercrop, for example, sunflowers attracted beneficial insects from 30 different families, such as big-eyed bugs, honeybees, spiders, ants, lady beetles, assassin bugs, solider beetles, and parasitic wasps, that spilled over to adjacent neighboring crops (Jones and Gillett 2005). In an agroecosystem, sunflower might be a good intercrop neighbor for many vegetable species because large sunflower varieties have a lot of vegetative structure, sunflower is phylogenetically distant from many crops, and it provides resources for beneficial insects. This concept deserves attention for future research.

Successfully managing insects in a field is one of a grower's main challenges. Not only do herbivorous insects cause direct damage to crops by feeding, but many also carry pathogens. In my system, increasing phylodiversity of neighboring plants sometimes reduced herbivore abundance, but I was unable to measure pathogen abundance or disease. Increasing diversity in a field can reduce instances of disease. In a three-year field trial to control aphid-transmitted disease, pumpkin was intercropped with either sorghum or soybean and peanut; both intercrops significantly reduced disease in pumpkin, and sorghum was most effective with 43-96% reductions (Damicone et al. 2007). In another aphid-management study, compared to bare ground buckwheat intercropped with zucchini reduced aphid densities and disease (Razze et al. 2016). The phylodiversity of intercrops might play a role in reducing pest-mediated pathogens, but there is likely to be a lot of variability among the insect vectors and pathogens involved. Several of the insect species I encountered in my plots can transmit pathogens, including viruses and bacteria, to cucurbits. For example, the most abundant herbivore specialist in my experiment, striped cucumber beetle, is a casual vector of the pathogen Erwinia tracheiphila, which causes bacterial wilt. Occurrence of bacterial wilt in cucurbits depends upon cucumber beetle density (Yao et al. 1996), and plants can become diseased when infected beetle frass comes in contact with wounded foliage or flowers (Sasu et al. 2010). In my experiment, abundance of striped cucumber beetle was sometimes negatively associated with phylodiversity, especially by mid-July both on butternut squash foliage and in the aerial space above the squash canopy. I did not, however, observe differences in beetle abundance in squash flowers. Striped cucumber beetles are attracted to squash flower fragrance (Andrews et al. 2007, Theis and Adler 2012), and it appears they were able to follow floral cues despite potentially disruptive cues from my divergent crop mixtures. Because of the disparity between beetle abundances on foliage and in flowers, it is difficult to predict how phylodiversity may have influenced the occurrence of bacterial wilt in my experiment.

My experiment and other studies show that intercropping can reduce herbivore abundance, but my design did not account for grower feasibility. Intercrops are often used in lowinput agroecosystems, but they can be labor-intensive and are not always compatible with mechanization. Low-input agriculture relies on sustainable practices like IPM to manage crops, and limits the use of high-inputs, such as fertilizers and pesticides. Low-input agroecosystems are regularly used by resource-constrained smallholder farms (Pimentel et al. 1989, Brooker et al.
2014). Because intercrops are often not compatible with mechanization, they instead rely on manual labor, which can be costly (Feike et al., 2012). For an intercrop to be economically feasible, it needs to be profitable compared to a typical monoculture system (Waddington et al 2007, Bhatti et al. 2013). Using phylogenetic relatedness and considering plant structure as methods for choosing plant neighbors in intercrops might benefit farmers, but more research is needed to understand labor input and economic impact of different vegetable intercrops. A firststep toward phylogenetically minded intercropping is to evaluate established mixtures, and then proceed toward novel mixture development if necessary. Some crop mixtures are already commonly used in forage production, such as maize and sorghum or maize and legumes. Maize and sorghum diverged about 5 million ago whereas maize and legumes diverged about 377 million years ago. Comparing them phylogenetically, maize and legumes are more divergent, so I would hypothesize this mixture should theoretically have few herbivorous insect pests. However, because phylodiversity is not the only force active in agroecosystems, formulated mixtures should also account for other interactions such as plant competition. For example, a study using buckwheat as a living fertilizer intercropped with zucchini (about 238 million years diverged) to manage aphids and whiteflies found no yield differences between some intercrop arrangements (1.06 m spaced rows, Figure A-2) and monocultures, but intercrops did reduce herbivore densities and disease and increased abundance of natural enemies. These results suggested that appropriately spaced intercrops can minimize plant-plant competition, while still experiencing benefits from the mixture and possibly increase yield (Razze et al. 2016).

Intercropping can decrease herbivore abundance, but responses from other insect groups (e.g., predators and pollinators) are less predictable (Latheef et al. 1984, Kromp 1999, Hinds and Hooks 2013, Gontijo et al. 2018). For example, intercropping reduced oviposition and abundance of the carrot rust fly (*Psila rosae*), but had no effect on predator abundance (Rämert and Ekbom 1996). Over the two years of my study, I rarely detected signals of pollinators,

predators, parasitoids, or decomposers being significantly influenced by the relatedness of neighboring crops, with some exceptions. For example, I found that abundances of tachinid larvae, braconid larvae, and braconid eggs had a negative association with divergence time (Figure **2-8**, **2-9**). Because parasitoids actively search for host insects, I suspect these were effects were related to host abundance (i.e. the negative relationship between striped cucumber beetles and increasing phylodiversity), rather than direct effects. Unlike parasitoids, nematodes did not respond. Nematode behavior is likely density-independent of plot treatments as they are distributed in the soil and wait for available hosts.

I also found several associations between abundance and phylodiversity among arthropods of the epigeal community. Arachnida (mainly spiders) activity density was negatively associated with divergence time (Figure 1-18; Figure 1-20). Rather than directly responding to phylodiversity, however, I suspect arachnids may have instead reacted to plant structure or ground cover. Arachnid communities and their dispersal are known to differ by vegetative cover and plant structure (Samu et al. 1999, Lyons et al. 2018). The closest divergence times were represented by cucurbits, vining plants with large, broad leaves that shade soil and provide nearground structure. In contrast, the most distant divergence time included grasses, which grow vertically and provide little ground cover. In 2018, other arthropods of the epigeal community, Coleoptera and Myriapoda, were positively associated with phylodiversity in late June (Figure 1-18), but negatively associated with it in late July (Figure 1-19). Phylodiversity never explained more than 9% of Coleoptera activity density, but explained up to 31% of Myriapoda activity density. Like Arachnida, perhaps Myriapoda responded to ground cover and structure rather than plant relatedness. In general, I do not surmise these significant associations between the epigeal community and phylodiversity were due to direct interactions like the herbivorous insects.

I must acknowledge there were several study limitations which could have influenced the arthropod and plant communities in my experiments and obscured phylodiversity effects.

Persistent precipitation in 2017 and 2018 and associated flooding led to many logistical challenges, such as delayed planting times, weed management, and outbreaks of powdery mildew. I was only able to collect late-season data in 2017. Part of my field was permanently flooded for the entire season and a larger portion was at least ephemerally flooded following precipitation events. My field was so wet that frogs and toads completed metamorphosis and a great blue heron occasionally came for a visit. When newly emerged anurans invaded my research plots, they could have been influencing arthropod populations. Prior to 2017, this field did not have flooding problems of this scale, and in 2018 the permanently flooded portion was discontinued for use. Flooding is known to influence arthropods. For example, a specialist pollinator of butternut squash, the squash bee *Peponapis pruinose*, often nest in the ground by cucurbit plants in agricultural fields and soil disturbances negatively affect their populations (Ullman et al. 2016). The excessively wet field in 2017 may have reduced nesting sites. Bumble bees, the most abundant pollinators in 2017, are generalists that visit squash flowers but do not depend on them (Nagamitsu et al. 2012), and therefore were presumably less affected by the flooded field conditions. I was able to collect data throughout the season in 2018 at my new location, but not as frequently as planned. Despite powdery mildew's preference for warm and dry conditions, a major disease outbreak resulted in early termination of data collection in August.

I originally planned to assess plant damage in my plots by measuring insect leaf and squash damage, and occurrence of bacterial wilt and powdery mildew. However, several logistical challenges stifled my efforts in both years. After a failed polyculture trial with cucumber as my focal crop, I was forewarned that bacterial wilt was very common at Rock Springs (Beth K. Gugino, Penn State Dept. of Plant Pathology and Microbiology, *pers. Comm.*), and instead chose butternut squash as my focal crop because of its known resistance to bacterial wilt (McGrath 2004). Although my focal crop was resistant to bacterial wilt, my zucchini treatment crop was not. Zucchini experienced high mortality from bacterial wilt in both 2017 and 2018, so I had to maintain those plots by replacing dead zucchini plants weekly. In addition to bacterial wilt being problematic for zucchini in 2017 and 2018, squash vine borers (*Melittia cucurbitae*) were also a major cause of mortality in 2017 zucchini only. I also had difficulty with plant establishment that left me with butternut squash plants of various ages, making damage and yield non-comparable. Finally, excessive wetness from rains caused fast field-wide outbreaks of powdery mildew which made it unmeasurable.

The black plastic in my plots could have been a confounding factor. When sections of black plastic showed signs of wear in August in 2018, I observed a density of squash bugs and some cucumber beetles (both species) taking refuge under it. Based on this observation, my counts of adult herbivore insects (especially of squash bugs) on foliage and flowers were likely lower than the actual populations in my plots. Squash bugs are known to aggregate and hide under black plastic and in other crevices during the day (Doughty et al. 2016). However, most eggs are also laid on plants during the day (Wadley 1920). Because squash bugs can be elusive, counting squash bug egg masses on plants might be an alternative solution to scouting adults. The eggs could also be collected and housed in petri dishes to test for egg parasitoid emergence (Wilson et al. 2017), although my results from parasitoid abundance on sticky traps and parasitoids in striped cucumber beetle abdomens suggest there probably wouldn't have been differences in squash bug egg parasitoid emergence in my study (Table 1-7, 1-9). Alternatively, black plastic could be removed completely, but this may lead to greater weed pressure in the plots which was already an issue even with the plastic. Sections of moveable weed barriers adjacent to focal plants to count squash bugs hiding underneath might work. Squash bugs are known to hide under plywood boards in fields (Doughty 2016), but this idea needs to be tested for its efficacy as a way quantify squash bugs.

My experimental scale could have also been a confounding factor. Mobile insects will adjust foraging in response to resource availability and crowding (Mazzi and Dorn 2012), and insect size and mobility influences insect community diversity (Olff and Ritchie 2002). Because my treatment plots were small, highly mobile species may have appeared unresponsive to crop relatedness. For example, carabid beetles are highly mobile (Jopp and Reuter 2009, Zoltan et al. 2014) and will respond to intercrops and vegetative structure (Hummel et al. 2012). Although I found significant differences on some dates in carabid activity density between treatments, the explained variation was low (Figure **1-18**; Figure **1-19**).

Conclusion

My findings demonstrate that increasing the phylogenetic divergence between neighboring plants can reduce pest abundance but did not directly affect other insect functional groups (predators, parasitoids, pollinators, detritivores). Evidence supporting my evolutionary divergence hypothesis was mainly found in 2018, and low abundances and insect response in 2017 may be attributed to poor field conditions. Further studies are needed to more comprehensively test this hypothesis at other locations and a larger scale. Additional experiments should help clarify specific mechanisms that could have influenced my results, such as plant volatile organic compounds.

Using phylogenetics to design intercropped vegetable systems may help ecologically minded growers to better manage pest populations in their fields. However, it will be important to continue working on understanding the effects of crop relatedness at larger scales, plant-plant interactions, insect life cycles, and considering feasibility for the grower.

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Tables

Table 1-1: Data collection by week for 2017 and 2018. In 2017, data were only collected during the late season because of flooding and rain issues. In 2018, pitfall trap and sticky trap were not possible in July because of frequent rain.

Week of		Tasks
2017		
August	6	foliage scouting, flower scouting, pitfall traps
	20	flower scouting
	27	foliage scouting, flower scouting
September	10	sticky traps
	17	foliage scouting, flower scouting
2018		
June	17	foliage scouting
	24	sticky traps, pitfall traps
July	1	foliage scouting
	8	beetle collecting
	15	foliage scouting, flower scouting
	29	pitfall traps
August	5	foliage scouting, flower scouting, sticky traps, beetle collecting
	26	foliage scouting, flower scouting, sticky traps, pitfall traps, beetle collecting

Table 1-2: Basic statistics for herbivorous insects counted on foliage in subplots for 2017. For each date and divergence time (million years ago) from butternut squash, the mean abundances of each herbivorous insect (striped cucumber beetle, spotted cucumber beetle, squash bug) are provided, along with the mean abundances and their associated standard errors for all three herbivores pooled together.

		Means			All herbiv	vores
Date 2017	Divergence (mya)	SCB	SPCB	Squash Bug	Mean	SE Mean
8-Aug	0	0	0	0	0	0
	32	0	0	0	0	0
	147	0.333	0	0	0.333	0.333
	214	0.222	0	0	0.222	0.222
	238	0	0.250	0	0.250	0.164
	376	0	0	0.167	0.167	0.167
	blank	0	0	0	0	0
30-Aug	0	0	0	7.50	7.50	7.50
	32	0	0.200	0	0.200	0.200
	147	0	0.667	0	0.667	0.333
	214	0	0.222	1.67	1.89	1.65
	238	0	0	0.500	0.500	0.327
	376	0	0	0	0	0
	blank	0	0.250	0	0.250	0.250
20-Sep	0	0	0.250	0	0.250	0.250
	32	0	0.600	0.600	1.200	0.735
	147	0	0.333	0	0.333	0.333
	214	0.111	0.111	0	0.222	0.147
	238	0.125	0.125	0	0.250	0.164
	376	0	0.333	0	0.333	0.333
	blank	0	0	0	0	0
Cumul.	0	0	0.083	2.50	2.58	2.49
	32	0	0.267	0.200	0.467	0.247
	147	0.111	0.333	0	0.444	0.176
	214	0.111	0.11	0.556	0.778	0.556
	238	0.042	0.125	0.167	0.33	0.130
	376	0	0.111	0.056	0.167	0.121
	blank	0	0.083	0	0.083	0.083

Table 1-3: Significant regression models from trap data in 2017. In 2017, Arthropod abundances were rare and most trap data had to pooled for cumulative models because they otherwise didn't satisfy model assumptions. Significances were found among herbivorous insects counted from foliage scouting and flower scouting efforts ($\alpha = 0.1$, $\alpha = 0.05^*$, $\alpha = 0.01^{**}$).

Collection method	Date (2017)	Arthropod	DF	Poisson χ2	Diver. time (mya) p-value	Block replicate p- value	Number of flowers p- value	Model p- value	% R-sq/ R-sq (adj)
Foliage scouting	30- Aug	All herbivores	5	87.11	<0.0001**	<0.0001**		<0.0001**	33.65
	Cumul.	Squash bug	1	39.06	<0.0001**			<0.0001**	15.15
		All herbivores	5	75.23	<0.0001**	<0.0001**		<0.0001**	19.55
Flower scouting	Cumul.	SCB + SPCB	2	19.32	0.002*		0.003	<0.0001**	5.14

Table 1-4: Basic statistics for herbivorous insects counted on foliage in subplots for 2018. For each date and divergence time (million years ago) from butternut squash, the mean abundances of each herbivorous insect (striped cucumber beetle, spotted cucumber beetle, squash bug, western corn rootworm) are provided, along with the mean abundances and their associated standard errors for all three herbivores pooled together.

		Means				All herbivore	5
Date 2018	Divergence (mya)	SCB	SPCB	Squash Bug	WCR	Mean	SE Mean
21-Jun	0	3.40	0	0	0	3.40	2.20
	32	0.200	0	0	0	0.200	0.20
	147	3.00	0	0	0	3.00	1.90
	238	1.600	0	0	0	1.600	0.73
	376	3.10	0.100	0	0	3.20	1.54
	blank	0.200	0	0	0	0.200	0.200
3-Jul	0	4.20	0	0.400	0	4.60	1.89
	32	7.00	Õ	0	0	7	2.49
	147	5.00	0	1.800	0	6.80	1.98
	238	6.20	0	0.500	0	6.70	2.10
	376	5.80	0	0.800	0	660	1.40
	blank	6.20	0	0	0	6.20	1.71
19-Jul	0	3 40		0.800	0.200	4 40	1 72
19 541	32	4	0	0.600	0	4.600	0.678
	147	3 20	Ő	0.400	0 200	3 80	1 20
	238	3.100	0	0.600	0	3.700	0.870
	376	6 5 5 6	Ő	0.111	ů 0	6 667	0.816
	blank	4	0	0.200	0.200	4.400	0.980
			Ŭ	0.200	0.200		0.000
6-Aug	0	6.800	0.600	0.800	1	9.200	0.53
	32	6.60	0.400	0	0.600	7.60	1.17
	147	6.20	0.600	0.400	0.800	8	1.58
	238	3.300	0	0.100	0.700	4.10	.348
	376	2.400	0.200	0.200	0.600	3.40	0.476
	blank	5.800	0.400	0.600	1	7.8	0970
28-Aug	0	1.75	1	1.250	0	4	2.31
	32	2.60	0.400	1.200	0	4.20	1.36
	147	0	0	0	0	0	0
	238	0.600	0.600	0	0	1.200	0.583
	376	0.889	0.111	0.556	0.222	1.778	0.830
	blank	1	0	1.250	0	2.25	1.03
Cumul.	0	4	0.292	0.625	0.250	5.167	0.857
	32	40.80	0.160	0.360	0.120	4.720	0.784
	147	3.480	0.120	0.520	0.200	4.320	0.848
	238	3.222	0.067	0.267	0.156	3.711	0.594
	376	3.750	0.083	0.333	0.167	4.333	0.553
	blank	3.542	0.083	0.375	0.250	4.250	0.725

Table 1-5: Significant regression models from collected data in 2018. In 2018, there were some cases where arthropod abundances were rare and most trap data had to be pooled for cumulative models because they otherwise didn't satisfy model assumptions, such as with flower scouting data. Significances were found with all collection methods except dissections. Among the arthropod groups, herbivorous insects were the most commonly significant ($\alpha = 0.1$, $\alpha = 0.05^*$, $\alpha = 0.01^{**}$). (Bees abundance with date as a predator not shown: P = <0.0001).

Collection method	Date (2018)	Arthropod	DF	Linear F	Poisson χ2	Diver. time (mya) p- value	Block replicate p- value	Number of flowers p- value	Model p- value	% R-sq/ R-sq (adj)
Foliage	19-Jul	SCB	1	4.69		0.038*			<0.038*	12.79
securing		Squash Bug	1		2.93	0.087			0.087	8.31
	6-Aug	SCB Squash Bug	1 1	46.71	3.33	<0.0001** 0.068			<0.0001** 0.068	58.60 9.35
		All herbivores	1	36.85		<0.0001**			<0.0001**	52.75
	28-Aug	SCB	1		5.67	0.017*			0.017*	8.29
		SPCB	1		3.11	0.078			0.078	1.76
		All herbivores	5		40.96	0.003**	<0.0001**		<0.0001**	33.87
Flower scouting	6-Aug	SCB +SPCB	6		46.32	0.048*	<0.0001**	<0.0001**	<0.0001**	43.34
0	Cumul.	SCB +SPCB All Bees	6 8		191.53 207.78	0.073 0.923	0.109 <0.0001**	<0.0001** <0.0001**	<0.0001** <0.0001	43.50 64.52
Sticky trap	28-Jun	SCB	1	16.94		<0.0001**			<0.0001**	33.93
		All herbivores	1	4.01		0.054			0.054	10.83
	7-Aug	Thrips	1	6.80		0.014*			0.014*	17.08
	30-Aug	SCB	1	7.10		0.015*			0.015*	27.20
		SPCB	1		3.45	0.063			0.063	9.55
		Tarnished Plant Bug	1	7.40		0.014*			0.014*	28.03
		Long-legged fly	1	3.24		0.088			0.088	14.55
	Cumul.	All herbivores	1	4.3		0.041*			0.041*	4.61
Pitfall trap	28-Jun	Coleoptera	1		3.72	0.054			0.054	8.24
-		Arachnida	1	8.01		0.008**			0.008**	19.54
		Myriapoda	1		4.95	0.026*			0.026*	8.27
	30-Jul	Coleoptera	1		4.13	0.042*	~0.0001**		0.042*	9.01
	Cumul.	Arachnida	1	6.56		0.012*	<0.0001		0.012*	6.12
Dissections	10 - Iu1	Tachinid I	1		3.68	0.055			0.055	8 26
Dissections	10 541	Braconid L.	1		11.40	0.001**			0.001**	17.04
		Braconid E.	1		9.25	0.002**			0.002**	20.11
	7-Aug	Tachinid L.	5		12.58	0.072	0.050*		0.028*	14.19
		Braconid L.	5		24.50	0.001**	0.003**		<0.0001**	31.05
		Braconid E.	1		3.39	0.066			0.066	7.42
	30-Aug	Tachinid L.	1		3.75	0.053			0.053	11.11

Table 1-6: Basic statistics for bees and cucumber beetles counted in flowers for 2017. Abundances were very low. For each date and divergence time (million years ago) from butternut squash, the mean abundances of each bee (squash bee, bumble bee, honey bee) and cucumber beetle (striped cucumber beetle, spotted cucumber beetle) are provided, along with the mean abundances and their associated standard errors for all three bees pooled together, and then all cucumber beetles pooled together.

		Bee means						er beetle me	eans	
Date 2017	Divergence (mya)	Squash bee	Bumble bee	Honey bee	All Bees	SE Mean bees	SCB	SPCB	All beetles	SE Mean beetles
9-Aug	0	0.750	4.50	0	5.25	1.11	4.50	2	6.5	2.47
	32	0.20	2	0	2.20	102	1.4	0.200	1.600	0.748
	147	0	0.333	0	0.333	0.333	0.67	0	0.667	0.667
	214	0.333	1	0	1.333	0.782	1.444	0.111	1.556	0.973
	238	0	0.875	0	0.875	0.875	1.250	1.250	2.50	1.45
	376	0	0.833	0	0.833	0.543	1	0.500	1.500	0.957
	blank	0	3.5	0	3.50	1.26	0.500	0.250	0.750	0.479
28 4110			2		3.00	1 47		0.500	0.500	0.280
20-Aug	22	0 600	3	0	3.00	1.47	0 200	0.300	1 200	0.289
	32	0.000	5	0	5.00	2.80	0.200	0 222	1.200	0.970
	214	0 222	3 79	0	3.00	2.09	1	0.333	1.333	0.862
	214	0.333	3.70	0	4.11	2.19	0.444	0.500	1	0.800
	230	0	3	0	1.00	1.77	0.500	0.300	0.222	0.300
	570 blonk	0	4	0	4.00	0.866	0 750	0.333	0.333	1.68
	Ulalik	0	1.5	0	1.5	0.800	0.750	1.23	2.0	1.06
20-Sep	0	0.250	0.750	0.500	1.500	0.289	0.250	0.500	0.750	0.479
_	32	0	0.200	0.600	0.800	0.374	0.800	0	0.800	0.374
	147	0	1.667	0.333	2.00	1	1	0.333	1.33	1.33
	214	0	0.222	0.333	0.556	0.242	0.111	0.333	0.444	0176
	238	0	0.375	0.250	0.625	0.263	0.500	0.125	0.625	0.498
	376	0	1.167	0.333	1.500	0.428	0	0.167	0.167	0.167
	blank	0	0.500	0.500	1	0.408	0	0.500	0.500	0.289
Cumul.	0	0.333	2.75	0.167	3.250	0.730	1.583	1.00	2.58	3.92
	32	0.267	1.733	0.200	2.200	0.718	0.800	0.400	1.20	1.568
	147	0	2.33	0.111	2.44	1.12	0.89	0.222	1.11	1.537
	214	0.222	1.667	0.111	2.00	0.808	0.667	0.667	1.333	2.287
	238	0	1.417	0.083	1.500	0.673	0.750	0.625	1.375	2.871
	376	0	2	0.111	2.111	0.718	0.333	0.333	0.667	1.455
	blank	0	1.833	0.167	2.00	0.577	0.417	0.667	1.083	1.975

Table 1-7: Basic statistics for bees and cucumber beetles counted in flowers for 2018. Abundances were lowest for the 28-Aug sampling date. For each date and divergence time (million years ago) from butternut squash, the mean abundances of each bee (squash bee, bumble bee, honey bee) and cucumber beetle (striped cucumber beetle, spotted cucumber beetle, western corn rootworm) are provided, along with the mean abundances and their associated standard errors for all three bees pooled together, and then all cucumber beetles pooled together.

		Bee mea	ns				Beetle Means				
Date 2018	Divergence (mya)	Squash bee	Bumble bee	Honey bee	All Bees	SE Mean bees	SCB	SPCB	WCR	All beetles	SE Mean beetles
18-Jul	0	4.20	0.200	0.200	4.60	1.47	7.80	.600	0	8.40	1.78
	32	1.250	0250	0	1.500	0.645	1025	0	0	10.25	2.43
	147	3.60	0	0.400	4	1.45	16.80	0	0.200	17	3.89
	238	5	0	0.111	5.11	1.12	9.89	0.222	0	10.11	1.98
	376	3.70	0.100	0.300	4.10	1.18	16	0.100	0	16.10	2.42
	blank	4.20	0.200	0	4.40	1.03	15	0	0	15	3.41
6-Aug	0	4.20	0	0.200	4.40	1.29	10.600	0	0.600	11.20	1.02
	32	3.60	0	0.200	3.80	1.28	10	0.200	0.800	11	2.66
	147	5.80	0	0.600	6.40	1.75	10.40	0.200	1.400	12	5.11
	238	4.70	0.100	1.200	6	1.63	7090	0.100	0	8	1.56
	376	4	0	0.800	4.80	0.646	8090	0.200	0	9.10	1.55
	blank	7.40	0	1	8.80	2.27	10.40	0.400	0.400	11.20	2.82
28- Aug	0	0	0.667	0	0.667	0.333	9	1.33	0	10.33	2.19
_	32	0	0	0	0	0	4	0.500	0	4.50	1.50
	238	0	0	0	0	0	1.444	0	0	1.444	0.377
	376	0.250	0	0	0.200	0.200	3.400	0.200	0	3.60	1.03
	blank	0.250	0.500	0	0.750	0.479	2	0	0	0.750	0.479
Cumul.	0	3.231	0.231	0.154	3.615	0.844	9.154	0.538	0.231	9.923	0.92
	32	2.091	0.0909	0.090	2.273	0.752	9	0.182	0.364	9.55	1.60
	147	4.70	0	0.500	5.20	1.14	13.60	0.100	0.800	14.50	3.14
	238	3.0286	0.0357	0.464	3.926	0.852	6.46	0.107	0	6.57	1.08
	376	3.250	0.040	0.440	3.600	0.630	10.64	0.160	0	10.80	1.49
	blank	4.21	0.357	0.357	4.93	1.22	9.64	0.143	0.143	9.57	2.18

Table **1-8**: Basic statistics for natural enemies on sticky traps in both years. Abundances were very low in 2017. For each date and divergence time (million years ago) from butternut squash, the mean abundances of each insect (lady beetle, parasitic wasp, hover fly, long-legged fly) are provided, along with the mean abundances and their associated standard errors for all natural enemies pooled together.

Year	Date	Divergence (mya)	Lady beetle	Para. wasp	Hover fly	L.l. fly	All natural enemies	SE Mean
2017	13-Sep	0	0.250	31.25		0.500	33.25	10.66
		32	0	40		1.67	44	16.52
		147	0.222	53.8		1.222	57.3	30.5
		214	0.375	31		0.625	33.88	20.94
		238	0.600	50.4		1.20	53.6	31.9
		376	0.167	50.7		1.50	55.3	30.1
		blank	0.250	33.25		0.866	33.75	8.42
2018	28-Jun	0		36	16.80	16.80	67	10.9
		32		34.40	13.80	13.80	63.60	9.74
		147		38.67	16.33	16.33	69.33	8.30
		238		27.60	15	15	54.80	7.18
		376		35.11	13.33	13.3	60.4	8.67
		blank		25.60	1500	15.40	53.60	7.49
	7-Aug	0	8.80	28.80	3	1.40	42	3.24
	U	32	9.80	29.60	4.60	1.20	45.20	4.88
		147	7.40	31.20	1.20	0.80	40.60	6.64
		238	10.50	25.60	2	0.60	38.70	3.69
		376	10.60	33.20	3.30	0.60	47.70	3.26
		blank	5.60	36.80	2.60	0.60	45.60	7.03
	20 4112		7		5 75	0 75	665	0 76
	50-Aug	0	/ 2 22	45	5.75 11	8.73 7	00.3 48	8.70 8.08
		32 238	3.33	20.07	6.67	6	40	6.12
		376	5.222	2	8 20	4.60	54 20	0.12
		blank	6 50	36	5.20 5.75	4.00 8.25	56.5	6.20
		olulik	0.50	50	5.75	0.25	50.5	0.20
	Cumul.	0	8	36	88.71	8.07	57.93	5.50
		32	7.38	30.77	9.62	8	52.92	4.87
		147	7.40	35.27	9.45	8.18	56.27	6.88
		238	7.053	28.28	7.93	6.28	47.10	3.49
		376	8.87	34.50	8.08	5.71	53.83	4.02
		blank	6	32.57	8.07	7.07	51.57	3.97

Table 1-9: Basic statistics for herbivorous insects on sticky traps in both years. Abundances were very low in 2017. For each date and divergence time (million years ago) from butternut squash, the mean abundances of each insect (striped cucumber beetle, spotted cucumber beetle, thrips, tarnished plant bug) are provided, along with the mean abundances and their associated standard errors for all herbivorous insects pooled together.

Year	Date	Divergence (mya)	SCB	SPCB	Thrips	T.plant bug	All herbivores	SE Mean
2017	13-Sep	0	0.250	1	11	1.250	13.50	5.25
		32	0.333	4	10.67	1.67	16.67	3.84
		147	0.222	1.444	6.22	0.444	8.33	2.49
		214	0.125	1.875	3.500	1.125	6.63	1.90
		238	0	3.60	6.40	0.800	10.80	1.16
		376	0.333	1.667	5.33	0.833	8.17	.03
		blank	0	1.750	6	0.250	8	3.16
2018	28-Jun	0	12.80	0	24		36.80	7.57
		32	9.80	0.400	20.80		31	4.53
		147	7.67	0.167	21.33		29.17	2.94
		238	590	0.200	21.60		27.70	2.34
		376	4667	0	20		24.67	4.15
		blank	6.80	0	20		26.80	2.97
-	7-Aug	0	13.80	0.200	47.20	9	70.2	10.3
	c	32	16.20	0.200	7	15	107.4	19.6
		147	17.80	0.600	63.20	19.20	100.8	12.4
		238	15.50	0	42.40	10.30	68.2	11.7
		376	18.70	0.500	34	14.50	67.70	5.68
		blank	13	0.800	61.6	10.80	86.2	13.8
-	30-Aug	0	7.75	0.750	62	11.75	82.3	24.3
	8	32	6.33	0.667	34.7	7.67	49.33	9.94
		238	5	0.889	30.22	4.778	40.89	6.39
		376	3.80	2	32.80	5.20	43.80	3.57
		blank	5	1	78	7	91	7.07
-	Cumul.	0	11.71	0.286	43.14	10.22	61.71	9.24
		32	11.46	0.385	45.23	12.25	64.6	12.4
		147	12.27	0.364	40.36	19.20	61.7	12.5
		238	8.93	0.345	31.45	7.68	45.76	5.47
		376	10.33	0.625	28.50	11.40	46.58	4389
		blank	8.50	0.571	51.43	9.11	66.36	9.60

Table 1-10: Average herbivorous insect abundance per crop in 2017 and 2018. For each trap type on each date, the crop with the lowest mean is colored. In both years, sunflower often had the lowest or one of the lowest herbivorous insect mean abundances among all treatments.

			Mean h	erbivorous	insects							
Year	Date	Method	Blank	Butter- nut	Zucc- hini	Water- melon	Garden beans	Soy- beans	Pepper	Sun- flower	Corn	oats
2017	cumulative	Sticky	31	43.75	41.2	50	35.6	39.25	49.75	19.75	46	37
		Foliage	2	5.67	2.67	1.11	0.53	2.25	1.5	0.83	2	0.78
		Flower	1.08	2.58	1.2	1.11	1.07	1.67	1.97	0.83	1.22	0.11
	8-Aug	Foliage	0	0	0.2	0.33	0.4	0	1.75	0.5	0.33	0
	9-Aug	Flower	0.75	6.5	1.6	0.67	1.6	1.5	3	2	3	0
	30-Aug	Foliage	3	14.5	3.2	2.67	0.6	4	1.25	0.5	0	1.33
	28-Aug	Flower	2	0.5	1.2	1.33	1	3.25	1.75	0.25	0.67	0
	13-Sep	Sticky	31	43.75	41.2	50	35.6	39.25	49.75	19.75	46	37
	20-Sep	Foliage	3	2.5	4.6	0.33	0.6	2.75	1.5	1.5	4	1
	20-Sep	Flower	0.5	0.75	0.8	1.333	0.6	0.25	1	0.25	0	0.33
2018	cumulative	Sticky	88.36	91.43	95.08	81			77.4	52.71	67.36	75.1
		Foliage	4.67	5.67	5.12	4.76			522	2.95	4.22	5.2
		Flower	9.57	9.92	9.54	14.5			7.54	5.73	9.71	12.18
	21-Jun	Foliage	0.2	3.4	0.2	3			1.6	1.6	2	4.4
	28-Jun	Sticky	30	54.4	35	33.17			24.2	35.2	16.6	40.8
	3-Jul	Foliage	6.8	5.4	7.4	7.4			10.4	3.8	5.6	8.4
	19-Jul	Foliage	4.8	5	5.2	4.4			5.6	2.6	6.25	8
	18-Jul	Flower	15	8.4	10.25	17			9	11	16	16.2
	7-Aug	Sticky	111	97.4	153.8	138.4			125.6	58	94.8	100.6
	6-Aug	Foliage	8.8	10.2	8.6	9			5.4	4.8	4.8	4
	6-Aug	Flower	11.2	11.2	11	12			11.4	4.6	8	10.2
	30-Aug	Sticky	133	130.25	97.33				82.4	68	96.5	85
	28-Aug	Foliage	2.25	4	4.2	0			1.67	0.5	2.5	1.2
	28-Aug	Flower	0.75	10.33	4.5				1.25	1.6	4	2

Table 1-11: Basic statistics for epigeal arthropod activity density from pitfall traps in both years. For each date and divergence time (million years ago) from butternut squash, the mean activity density for each arthropod (Coleoptera, Arachnida, Myriapoda, Ants) and its associated standard error are provided.

Year	Date	Divergence (mya)	Coleoptera	SE Mean	Arachnida	SE Mean	Myriapoda	SE Mean	Ants	SE Mean
2017	10-Aug	0	3.250	0.861	10	2.68	9.13	3.40	1.125	0.350
		32	2.600	0.636	7.90	2.80	7.70	2.61	1.60	0.340
		147	2.333	0.715	7	1.95	11.33	6.44	2.17	1.22
		214	3.1111	0.690	7.889	0.939	9	2.18	2.167	0.584
		238	3.000	0.747	9.56	3.68	6.56	2.12	0938	0.370
		376	2.167	0.601	9.33	2.10	7.33	2.84	0.583	0.193
		blank	2.750	0.701	10.8	2.46	7.13	2.77	3.13	1.41
2018	28-Jun	0	0.400	0.245	16	5.05	1.20	0.374	1.20	0.490
		32	1	0.316	17.80	5.91	1.60	1.36	1	0.632
		147	0.800	0.583	4.20	1.96	1.60	0.678	1.80	1.56
		238	1.400	0.340	6.90	2.31	2.40	0.427	2.20	0.867
		376	1.500	0.477	5.60	1.86	2.70	0.597	1	0.33
		blank	0.800	0.374	5.20	3.77	1.60	0.812	2.60	1.40
	30-Jul	0	1	3.200	3.200	0.374	5.60	2.18	1.60	0.927
		32	0.400	.400	2.400	0.748	2.80	1.62	5.40	0.812
		147	0.400	4	4	1.05	3	1.34	1.40	0.872
		238	0.500	4.100	4.100	0.623	2.90	0.504	13.60	7.42
		376	0.100	2.00	2.900	0.547	2.20	0.879	3.10	1.29
		blank	0	3.20	3.20	1.07	1.60	0.510	5.80	2.4
	30-Aug	0	2.667	0.667	4.66	0.33	1.333	0.667	0.333	0.333
		238	3.13	1.34	3.25	0.996	1.50	0.50	0.750	0.366
		376	1.60	0.927	4	0.707	1.60	0.812	2.20	1.24
		blank	8	4.34	6.25	1.70	1.75	1.18	0.500	0.289
	Cumul.	0	1.154	0.373	8.46	2.51	2.92	1.01	1.154	0.406
		32	0.700	0.213	10.10	3.80	2.20	1.0	3.20	0.879
		147	0.600	0.306	4.10	105	2.30	0.746	1.60	0.846
		238	1.571	0.456	4.857	0.918	2.321	0.287	5.86	2.81
		376	0.960	0.291	4.20	0.802	2.280	0.445	2.080	0.597
		blank	2.57	1.47	4.79	1.41	1.643	0.440	3.14	1.11

Table 1-12: Basic statistics for parasitoid and parasite mean abundances from striped cucumber beetle dissections in both years. For each date and divergence time (million years ago) from butternut squash, the mean abundance for each parasitoid and parasite (tachinid larvae, braconid larvae and eggs, nematodes) and its associated standard error are provided.

Date	Divergence	Tachinid	SE	Braconid	SE	Braconid	SE	N. (1	SE
2018	(mya)	larvae	Mean	Larvae	Mean	eggs	Mean	Nematodes	Mean
10-Jul	0	1	0.632	2.60	0.510	1.80	0.374	2.40	0.510
	32	2	0.837	1.40	0.510	1.40	0.510	1.60	0.600
	147	1.80	0.860	1	0.775	1.20	0.583	1.40	0.678
	238	1.30	0.367	1.60	0.806	1.50	0.307	1.90	0.233
	376	1.30	.559	2.40	0.600	1.50	0.401	1.70	0.367
	blank	1	0.316	2.40	0.812	2.20	0.583	2.40	0.245
7-Aug		0		0.250	0.250	0.250	0.250	2	0 707
, mug	32	0 333	0 333	0.333	0.333	0.250	0.250	1 333	0.882
	147	0.333	0.20	0.200	0.200	0.400	0.007	1.333	0.583
	238	300	0.213	0.200	0.200	0.400	0.213	2 70	0.907
	376	0.700	0.260	0.200	0.133	0.200	0.133	1.30	0.335
	blank	0.600	0.400	0.220	0.200	0.400	0.245	2	0.316
30-Aug	0	1	1	1.75	1.18	1.75	1.18	3.50	0.957
	32	0		0		2		5	
	238	0.750	0.750	0.750	0.250	0.625	0324	1.50	0.423
	376	0.500	0.500	0.833	0.307	1.167	0.477	3.667	0.955
	blank	0.250	0.250	1.50	0.645	1.250	0.479	2.50	1.50
Cumul.		0.692	0.286	.615	0.474	1.308	0.414	2.615	0.417
	32	1.22	0.547	0.889	0.351	1.222	0.364	1.889	0.564
	147	1	0.494	0.600	0.400	0.800	0.32	130	0.423
	238	0.786	0.202	0.857	0.316	0.857	0.183	2.71	0.356
	376	0.885	0.250	1.192	0.309	0.923	0.221	2	0.333
	blank	0.643	0.199	1.357	0.414	1.286	0.322	2.286	0.412





Figure 1-1: Phylogenetic relatedness of crop treatments relative to the focal crop, butternut squash



Figure 1-2: Divergence times of crop treatments relative to the focal crop, butternut squash: butternut squash (0 mya), zucchini (32 mya), watermelon (147 mya), soy and garden beans (213.9 mya), peppers and sunflowers (238.3 mya), corn and oats (376.5 mya).



Figure 1-3: Visual schematic of 2017 field layout. Each plot was randomly assigned a treatment and treatments are marked in each plot. Replicate blocks were each 10 plots long. Colored plots were flooded for the entire season and not used for data collection or analysis. Crop treatments included blank (Bl), butternut squash (Bu), zucchini squash (Z), watermelon (W), garden beans (G), soy beans (So), pepper (P), sunflower (S), corn (C), and oats (O). (not to scale)



Figure 1-4: Visual representation of plot layout. Butternut squash, the focal crop, is surrounded by four rows of a treatment. In this example, corn is the planted treatment. (Not to scale)



Figure 1-5: Visual schematic of 2018 field layout. Each plot was randomly assigned a treatment, and 8-plot sets were the block replicates. Crop treatments included blank (Bl), butternut squash (Bu), zucchini squash (Z), watermelon (W), pepper (P), sunflower (S), corn (C), and oats (O). (Not to scale)





Figure 1-6: Each crop row in a plot was about 6 m long. Butternut squash foliage was scouted for herbivorous insect abundance assessment in the treatments. The center row of each plot was split into equal 1.6 m subsections and labeled x, y, and z. They were used on a continuous rotation for subplot sampling throughout the field season. To account for edge effect, data were not collected from the outermost 0.6 m of the row on either side. (not to scale)



Figure 1-7: (+/- 1SE) Data were collected by foliage scouting. Poisson regression for average squash bug abundance by divergence time on August 30th, 2017 ($\chi 2_1 = 39.06$, P = <0.0001, R-squared adj = 15.15%, 1.408 – 0.00793 mya). Squash bug abundance decreased as divergence time increased.



Figure 1-8: (+/- 1SE) Data were collected by foliage scouting. Poisson regressions for the abundance of all herbivorous insects pooled (squash bugs, SCB, SPCB) by divergence time on August 30th, 2017 ($\chi_{25} = 87.11$, P = <0.0001, R-squared adj = 33.65%, abundance = 1.456 – 0.00736 mya) and cumulative for the field season ($\chi_{25} = 75.23$, P = <0.0001, R-squared adj = 19.55%, 0.488 – 0.00554 mya) in 2017. Herbivorous insect abundance decreased as divergence time increased.



Figure 1-9: (+/- 1SE) Data were collected by foliage scouting. Regressions for the abundance of striped cucumber beetle (SCB) by divergence time on July 19th (F_1 = 4.69, P = 0.038, R-squared = 12.79%, abundance = 2.831 + 0.00702 mya), August 6th (F_1 = 46.71, P = <0.0001, R-squared = 58.60%, abundance = 6.992 – 0.01275 mya), and August 28th ($\chi 2_1$ = 5.67, P = 0.017, R-squared = 8.29%, abundance = 0.598 – 0.00303 mya) in 2018. SCB abundance increased as divergence time increased on July 19th. SCB abundance decreased as divergence time increased on August 6th and August 28th.



Divergence time from butternut squash (mya)

Figure 1-10: (+/- 1SE) Data were collected by foliage scouting. Regressions for the abundance of all herbivorous insects pooled by divergence time on August 6th (F_1 = 36.85, P = <0.0001, R-squared = 52.75%, abundance = 8.751 – 0.01525 mya) and August 28th ($\chi 2_5$ = 40.96, P = <0.0001, R-squared adj = 33.87%, abundance = 3.216 – 0.005675 mya) in 2018. Herbivorous insect abundance decreased as divergence time increased on both dates.



Figure 1-11: (+/- 1SE) Data were collected by flower scouting. Poisson regression for the abundance of cucumber beetles (SCB, SPCB) by divergence time cumulative for the field season ($\chi 2_2 = 19.32$, P = <0.0001, R-squared adj = 5.14%) in 2017. Cucumber beetle abundance did not appear greatly change in response to divergence time despite significance and explained variance for effect of divergence time alone was very low ($\chi 2_2 = 10.52$, P = 0.001, R-squared adj = 3.12%, abundance = 0.683 – 0.002268 mya).


Figure 1-12: (+/- 1SE) Data were collected by flower scouting. Poisson regressions for the abundance of beetles (SCB, SPCB, WCR) by divergence time on August 6th ($\chi 2_6 = 46.32$, P = <0.0001, R-squared adj = 43.34%, abundance = 2.4222 – 0.000736 mya) and cumulative for the field season, but only when number of flowers per plot was included in the model (flowers included: $\chi 2_6 = 191.53$, P = <0.0001, R-squared adj = 43.50%; flowers not included: $\chi 2_1 = 0.10$, P = 0.752, R-squared adj = 0.02%, abundance = 2.2753 – 0.000079 mya) in 2018. Beetle abundance decreased as divergence time increased on August 6th. Beetle abundance did not appear to change in response to divergence time for the season, and instead was mainly influenced by number of flowers per plot.



Figure 1-13: (+/- 1SE) Data were collected from sticky traps. Regression for the abundance of long-legged flies (various species) by divergence time on August 30^{th} ($F_1 = 3.24$, P = 0.088, R-squared = 14.55%, abundance = 8.19 - 0.00948 mya) in 2018. Long-legged fly abundance decreased as divergence time increased.



28-Jun ······ Linear (28-Jun)Linear (30-Aug) 30-Aug

Divergence time from butternut squash (mya)

Figure 1-14: (+/- 1SE) Data were collected from sticky traps. Regressions for the abundance of striped cucumber beetle by divergence time on June 28^{th} ($\hat{F}_I = 16.94$, P = <0.0001, R-squared = 33.93%, abundance = 11.14 - 0.01907 mya) and August 30^{th} ($F_1 = 7.10$, P = 0.015, R-squared = 27.20%, abundance = 7.280 - 0.00944 mya) in 2018. SCB abundance decreased as divergence time increased, and regression slope was larger on June 28th.



Figure 1-15: (+/- 1SE) Data were collected from sticky traps. Regression for the abundance of thrips by divergence time on August 7th, 2018 ($F_1 = 6.80$, P = 0.014, R-squared = 17.0%, abundance = 64.30 – 0.0788 mya). Thrips abundance weakly decreased as divergence time increased.



Divergence time from butternut squash (mya)

Figure 1-16: (+/- 1SE) Data were collected from sticky traps. Regressions for the abundance of tarnished plant bugs ($F_1 = 7.40$, P = 0.014, R-squared = 28.03%, abundance = 9.72 – 0.01580 mya) and spotted cucumber beetles ($\chi 2_1 = 3.45$, P = 0.063, R-squared = 9.55%, abundance = -0.564 + 0.00293 mya) by divergence time on August 30th, 2018. Tarnished plant bug abundance decreased as divergence time increased. SPCB abundance weakly increased as divergence time increased.



Figure 1-17: (+/- 1SE) Data were collected from sticky traps. Regression for pooled abundance of all herbivorous insects by divergence time, cumulatively ($F_1 = 4.3$, P = 0.041, R-squared = 4.61%, abundance = 63.24 – 0.0516 mya). Although variable, herbivorous insect abundance decreased as divergence time increased.



Divergence time from butternut squash (million years ago)

Figure 1-18: (+/- 1SE) Data were collected from pitfall traps. Regressions for activity density of several epigeal arthropods by divergence time on June 28th, 2018 (Coleoptera [Carabid and Rove beetles]: $\chi 2_1 = 3.72$, P = 0.054, R-squared = 8.24%, activity density = -0.367 + 0.00225 mya; Arachnida [Spiders and Harvestmen]: $F_1 = 8.01$, P = 0.008, R-squared = 19.54%, activity density = 14.89 - 0.0293 mya; Myriapoda [Millipedes and Centipedes]: $\chi 2_1 = 4.95$, P = 0.026, R-squared = 8.27%, activity density = 0.316 + 0.001911 mya). Arachnida activity density density density density as divergence time increased. Coleoptera and Myriapoda activity density weakly increased as divergence time increased.



Figure 1-19: (+/- 1SE) Data were collected from pitfall traps. Poisson regressions for activity density of Coleoptera ($\chi 2_1 = 4.16$, P = 0.042, R-squared = 9.01%, activity density = -0.213 – 0.00387 mya) and Myriapoda ($\chi 2_5 = 36.31$, P = 0.007, R-squared = 30.77%, activity density = 1.465 – 0.001851 mya) by divergence time on July 30th, 2018. Both Coleoptera ad Myriapoda activity density decreased as divergence time increased, although Coleoptera had a weaker response than Myriapoda.



Figure 1-20: (+/- 1SE) Data were collected from pitfall traps. Regression for activity density of Arachnida by divergence time, cumulatively ($F_1 = 6.56$, P = 0.012, R-squared = 6.12%, activity density = 8.42 - 0.01295 mya). Although there was some variability, Arachnida activity density decreased as divergence time increased.



Figure 1-21: 2018 average herbivorous insect abundance per plot on sticky traps (top) and foliage (bottom). In crop treatments, sunflower had the lowest average activity density. Foliar abundances were counted on the center row of butternut squash, and sticky traps were placed in the approximate center of the focal crop center row (butternut squash).

Chapter 2

An explorative survey on the parasites and parasitoids of striped cucumber beetle (*Acalymma vittatum*) in Pennsylvania

Introduction

The striped cucumber beetle (SCB; Chrysomelidae (Diabroticina): Acalymma vittatum) is a major pest of cucurbit crops in eastern North America. Early studies conservatively estimated that SCB infestations reduce yield potential by as much as 50% (Balduf 1925). Despite its economic importance, there is a poor understanding of the natural enemy species that help limit adult SCB populations, which feed upon entire cucurbit plants and transmit the serious pathogen Erwinia tracheiphila (Rojas et al. 2015). Although literature lacks details on the level of control many natural enemies provide, predators from 35 families (12 orders) are known to attack Diabroticina beetles (Balduf 1925, Toepfer et al. 2009). Some predators identified attacking SCB include insectivorous birds, solider beetles (Cantharidae: Chauliognathus pensylvanicus), ambush bugs (Reduviidae: Phymata pennsylvanica), ants, mites, spiders, and carabid beetles (Balduf 1925, Toepfer et al. 2009). In addition to predators, parasitic species, including protists, nematodes, and insects, will also attack SCB, but our understanding of the ecology of its parasitoids and parasites is limited. Gregarine protists (Apicomplexa: Eugregarinorida) can be found in adults (Balduf 1925, Toepfer et al. 2009). Generally, gregarines are believed to be a lowimpact burden on their invertebrate hosts, which may experience reduced size, longevity, mobility, and higher mortality under poor-nutrient conditions (Lange and Lord 2012, Schilder and Stewart 2019); however, their effects specifically on SCB are unknown. At least four species of

nematodes, *Howardula benigna*, *Steinernema spp.*, *Heterorhabditts* spp., and *Neoaplectana carpocapsae* (Nematoda), have been recorded infesting SCB. Nematodes, either resident or introduced, may have potential to help control multiple life stages of SCB in cucurbit fields, but field-level experiments are needed to test their establishment and mortality to *A. vittatum*. (Balduf 1925, Reed et al. 1986, Ellers-Kirk et al. 2000, Toepfer et al. 2009).

Among parasitoids of SCB, the tachinid fly *Celatoria setosa* and braconid wasp *Centistes diabroticae* are the most-studied species in North America and have been reported in Pennsylvania with up to 56% parasitism for *C. setosa* and 17% parasitism for *C. diabroticae*. Both are fatal to their hosts (Lewis 2015). More information on the ecology of these parasites is needed to help determine if they can be harnessed for biological control.

In an effort to expand our understanding of relationships between SCB and its parasitoids and parasites in Pennsylvania, I investigated (1) parasitoid and parasite abundance in SCB, (2) parasitoid and parasite seasonality, (3) parasitoid and parasite response to crop diversity, and (4) whether there is a relationship between SCB body size, SCB sex, and parasitism. I hypothesized that abundance of any parasitoids and parasites I found would vary across seasons. I also hypothesized that crop diversity would have a positive effect on parasitism abundance. Finally, I hypothesized that larger SCB would have a higher abundance of parasitoids and parasites in their abdominal cavity regardless of sex.

Materials and Methods

I investigated the ecology of parasitoids and parasites of SCB at The Russell E. Larson Agricultural Research Center at Rock Springs (40.715318,-77.951797, Pennsylvania Furnace, PA). In 2018, two neighboring fields were planted with total of 40 plots that had butternut squash (*Cucurbita moschata*) as a focal crop species that was surrounded by a second crop (Figure 1-2, **1-4**, **1-5**): butternut squash, watermelon (*Citrullus lanatus*), zucchini (*Cucurbita pepo*), sweet pepper (*Capsicum annuum*), sunflower (*Helianthus annuus*), sweet corn (*Zea mays*), and oats (*Avena sativa*). In each plot, I aimed to collect ten beetles from butternut squash plants in the center row. Depending on beetle densities on plants, I either collected beetles by hand or used an insect vacuum. Beetles were collected on three dates: 8 July, 5 August, and 26 August. After the beetles were collected, I stored them in a refrigerator until live dissections.

Under a dissecting microscope, I dissected the abdomens of SCB in PBS 1X solution to sex beetles and search for parasites and parasitoids. All parasitoids were individually counted. Nematodes and gregarines often had very high densities and instead were measured as an event (1 present, 0 absent). To relate parasite abundant to SCB body size, I measured using digital calipers the right elytron of each SCB. I reserved several parasitoid larvae for rearing to produce adults for identification and vouchers. I also mounted on slides several representative parasites and larval parasitoids as vouchers and submitted them to the Penn State's Frost Entomological Museum. To identify larvae, I referred to descriptions in Balduf (1925). To identify nematodes, I consulted nematode experts. Some nematodes were preserved in formalin and identified using morphological characteristics (Mary Barbercheck, Dept. of Entomology, Penn. State U.), and some were preserved in DI water or PBS 1X Buffer solution and sent for 18S rRNA PCR/DNA sequencing (Dee Denver, Dept. of Integrative Biology, Oregon State U.). For further details plot design and beetle collection and dissection, see Chapter 1.

Statistics

I used Minitab v.18 to perform all analyses. I modeled data separately by date and where applicable, pooled dates for models assessing cumulative abundance over the growing season. For all models, $\alpha = 0.05$. I used Poisson regression to compare crop species, crop phylodiversity, and

sampling dates for parasitoid and parasite abundance. Replicates and crops were designated as categorical variables and divergence time and number of beetles dissected per plot were designated as continuous variables. I used a two-sample *t*-test to compare the number of male and female beetles collected, and to compare beetle sex to elytron length. I used binary logistic regression to compare parasites and parasitoids with beetle sex. I used linear regression to compare elytron length with the mean total number of parasitic organisms per beetle abdomen. Because parasitism rates of newly-ecdysized SCB were much lower than mature adults, they were excluded from analyses.

Results

I collected an average of 8.5 beetles per plot throughout the season for a total of 848 beetles collected. When I dissected SCB, I found that 348 were parasitized (41.0%; 124 females, 221 males, 3 unknown). Among the parasitized SCB, 103 had at least two different species in their abdomens. I found nematodes (Figure 2-1), the most abundant parasites, in 208 beetles (~25% and 60% of collected and infested beetles, respectfully), tachinid larvae (Figure 2-2) in 76 beetles (~9% and 22%), braconid larvae (Figure 2-3) in 84 beetles (~10% and 24%), and braconid eggs (Figure 2-4) in 91 beetles (~11% and 26%). The maximum number of tachinid larvae in one SCB was five, and the maximum number of braconid larvae in one SCB was seven. The number of female and male beetles collected were different overall (Ho: $\mu_1 - \mu_2 = 0$; *T-Value* = -6.78, *DF* = 191, *P-Value* = <0.0001), with a total of 319 females and 526 males (Table 2-1, Figure 2-5). To the best of my knowledge, Figure 2-4 contains the first published digital images of a *C. diabroticae* egg.

Among the parasitized beetles, about ~36% were female and ~64% were male, and parasitized females on average were larger than parasitized males (H₀: $\mu_1 - \mu_2 = 0$; $T_{227}= 5.37$, *P*-

Value = <0.0001; Table **2-1**, Figure **2-6**). Although smaller than female beetles, male beetles had more parasitic organisms per beetle than female beetles ($\chi 2_1 = 4.53$, *P-value* = 0.033; *Y* = 0.153 + 0.301 total parasitic organisms; Figure **2-7**) and elytron length was not a significant predictor of the number of parasitic organisms per beetle host ($F_1 = 2.15$, *P-Value* = 0.144; Table **2-1**). Additional comparisons between each common parasitic organism and the beetle sex of hosts revealed that tachinids had a preference for male beetles but braconids and nematodes had no preference for either sex: tachinids: ($\chi 2_1 = 3.94$, *P-value* = 0.047; *Y* = 0.471 + 0.470 tachinids); braconids: ($\chi 2_1 = 02.42$, *P-value* = 0.120); nematodes: ($\chi 2_1 = 0.36$, *P-value* = 0.551; Figure **2-7**, Table **2-1**). I also found that elytron length was not a predictor of tachinid parasitism among parasitized beetles ($\chi 2_1 = 0.030$, *P-value* = 0.585). Tachinids and braconids responded to phylodiversity, but nematodes did not (Figure **2-8**, **2-9**; see Chapter 1).

Collection date of SCB significantly influenced abundance of all common parasitoids and parasites ($F_2 = 16.77$, *P-value* = <0.0001; Figure **2-10**). Only on 10 July did all plots have parasitized beetles: (10 July: Max 100%, Min 28.57%, average 50.72%; 7 August: Max 50%, Min 0%, average 23.07%; 30 August: Max 100%, Min 0%, average 57.08%). Seasonal abundances of total parasites and parasitoids in SCB appeared to an inverse relationship with seasonal abundances of SCB (see Chapter 1 for details on SCB data collection; Figure **2-10**).

Both the phylogenetic relatedness of neighboring plants and species of crops did not influence parasitism of SCB (Table 2-2). Overall, parasitism was highest in the butternut squash monoculture (mean = 6.20) and lowest in the watermelon intercrop (mean = 3.84), and there was a lot of variability (Table 2-2).

I was able to identify tachinid larvae (*C. setosa*) and braconid larvae (*C. diabroticae*) using Balduf (1925). I identified braconid eggs by comparing their morphology with the other known parasitoids of SCB and referred to limited descriptions (Smythe and Hoffman 2009). I also identified gregarines and horsehair worm using online resources and articles (Rueckert 2008,

Mustafa et al. 2008, Schmidt-Rhaesa and Reinhard 2001; Capinera 2017. Nematodes were morphologically identified as (Allantonematidae: *Howardula spp.*). Molecular-based identification data from Oregon State returned the nematodes unidentified, with the closest hit being *Howardula phyllotretae* from 28 samples (%ID range 96.9% to 97.6%; GenBank accession # JX291137). This indicates the nematodes I dissected from SCB are either an undescribed species or a known species whose molecular information is not in the database. Rare parasites that I identified were excluded from all statistical analyses except for the number of parasitic organisms per beetle host (Figure 2-7). I found one beetle with a possible horsehair worm (Nematomorpha), six beetles with gregarines (Gregarinasina; Figure 2-11), and three beetles with empty abdomens. None of the larvae reserved for rearing reached adulthood.

I made some additional observations related to dissections. Heavy parasite or parasitoid infection was associated with destroyed or deteriorated abdominal organs, and in extreme cases the gut was reduced to a milky liquid. In cases of parasitoid superparasitism, there often appeared to be a larger or "dominant" larva. In cases of multiparasitism, dominance was less obvious. Tachinid and braconid larvae often inhabited different areas of the beetle's abdomen when they shared a single host. While tachinids often resided in the central abdomen, braconids often stayed in the upper abdomen by the wings or lower abdomen near the anus.

Discussion

Parasitism rates are often estimated by collecting insect hosts from a field and holding them in a lab for parasitoid emergence. However, all parasitoids do not successfully emerge from their hosts and the presence of parasites might be missed without dissections (Balduf 1925). I found the overall parasitism rate at Rock Springs in 2018 was 41%, which included both parasitoids and parasites. In 2015, the average emergence rate of SCB at Rock Springs was 15.2% with *C. setosa* and *C. diabroticae* accounting for 6.5% and 8.3%, respectively (Lewis 2015). This average parasitism rate determined by emergence was similar to my average parasitism rate by dissections, which was about 19% overall (*C. setosa*: 9%; *C. diabroticae*: 10%). However, I also found that nematodes accounted for more parasitism than tachinids and braconids combined.

Although *C. setosa* and *C. diabroticae have* been some-what studied for biocontrol of beetles (Lewis 2015), I could not find any records of studies investigating entomopathogenic nematodes attacking adults in the field except for Balduf (1925). Nematodes are already used to control larvae (Ellers-Kirk 2000) and are known to cause mortality in beetles (Balduf 1925), so using them to control multiple life stages of SCB seems plausible. I also found that SCB and its parasitoids and parasites appear to be density-dependent. My observed parasite and parasitoid abundances inversely correlated with SCB abundances throughout the season (Figure **2-10**). Understanding this relationship between natural enemies and their hosts may help growers to plan when they can take advantage of natural enemies for pest control and when they might need to use insecticides. Overall, using dissections as a method to study parasitism in insect hosts is likely helpful for identifying novel natural enemies that can be used for biocontrol, and for understanding the relationships between natural enemies and their hosts.

Elytron length of SCB was not a predictor of parasitism, but beetle sex was. Although female beetles were larger than male beetles throughout the season, males on average were more likely parasitized and had more total parasitoids and parasites than females. Because this relationship is related to beetle gender but not beetle size, male SCB behavior or body condition might make it more susceptible to parasitism. Sex-biased parasitism has been observed in other insects such as damselflies (Córdoba-Aguilar 2013), whirligig beetles (Fairn et al. 2008), and scarab beetles (Walker and Allen 2013). Although braconids and nematodes did not appear to have a sex preference, tachinid larvae were significantly more abundant in male beetles, but did not respond based on elytron length. Additionally, Tachinid flies might sense specific cues that make male SCB more attractive as a host. Only male SCB are known to release aggregation pheromones and attract conspecifics (Smyth and Hoffmann 2003), so testing whether *C. setosa* responds to male SCB pheromones would help unveil why it parasitizes males over females.

Because gregarines are small and I didn't initially know the frequency or level of infection I would find, I suspect that my gregarine values might be erroneously low.

Conclusion

I found that the abundances of parasitoids and parasites of SCB vary across the season and may be inversely correlated with SCB abundance. Within abdominal cavities of SCB, I identified two parasitoids, the tachinid fly *Celatoria setosa* and the braconid wasp *Centistes diabroticae*, and three parasites, nematodes (*Howardula spp.*), gregarines (Gregarinasina), and a possible horsehair worm (Nematomorpha). Male SCB were smaller than females but more likely parasitized and had more total parasitoids and parasites. Parasitoids were negatively associated crop diversity. These findings provide an update to early studies (Badluf 1925) and introduce some novel findings on the behavior and ecology of parasitoids and parasites of SCB that may be useful for designing more ecologically minded control of SCB.

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Tables

Table 2-1: Average striped cucumber beetle elytron length, average parasitism, and average number of parasitic species per beetle by date and sex. Three beetles were not included as their sex was unknown due to empty abdominal cavities.

		Beetle Means		Parasitism Means per Beetle					
Date	Sex	abundance	elytron length	Tachinid larvae	Braconid larvae	Braconid eggs	Nematodes	Other parasites	Num. of parasitic species
8-Aug	F	3.43	4.13	0.25	0.45	0.20	0.53	0.05	1.8
	Μ	4.88	4.02	0.41	0.51	0.41	0.46	0.41	1.59
30-Aug	F	3.35	4.25	0.13	0.03	0.13	0.70	0.03	1.03
	Μ	6.05	3.83	0.20	0.13	0.16	0.71	0.0	1.18
20-Sep	F	2.65	4.16	0.09	0.23	0.21	0.71	0.06	1.26
_	Μ	4.65	3.89	0.18	0.26	0.27	0.67	0.02	1.33

Table **2-2**: Season cumulative means for parasitoids and parasites associated with different butternut squash intercrops in 2018.

Div. per.	Crop	Mean parasitoids/ parasites
0	Butternut	6.20
1	Zucchini	5.47
	Watermelon	3.84
3	Pepper	5.07
	Sunflower	4.26
4	Corn	4.80
	Oats	4.62

Figures



Figure **2-1**: Nematode (Allantonematidae: *Howardula spp.*) dissected from a SCB on 30 August. (image courtesy of Carolyn Trietsch)



Figure **2-2**: Lateral view of a Tachinid larvae *C. setosa* dissected from a SCB on 10 July. (image courtesy of Carolyn Trietsch)



Figure **2-3**: Images of a Braconid larvae *C. diabroticae* dissected from a SCB on 10 July. Lateral view (top image) of a Braconid larvae body and dorsal view (bottom image) of a Braconid larvae head. (images courtesy of Carolyn Trietsch)



Figure **2-4**: Braconid egg *C. diabroticae* dissected from a SCB on 30 August. Ventral view of egg (left image) and closeup ventral view of larval head (right image). To the best of my knowledge, these are the first published images of a *C. diabroticae* egg. (images courtesy of Carolyn Trietsch)



Figure 2-5: (+/- 1 SE; $\alpha = 0.05$) Average number of female and male beetles per plot. In a Two-Sample T-Test comparing number of female and male beetles, the average number of male and female beetles per plot was significantly different (H₀: $\mu_1 - \mu_2 = 0$; *T-Value* = -6.78, *DF* = 191, *P-Value* = <0.0001).



Figure 2-6: (+/- 1 SE; $\alpha = 0.05$) Average elytron length of female and male beetles per plot. In a Two-Sample T-Test comparing elytron length (mm) and beetle sex, the mean elytron length of male and female beetles was significantly different (H₀: $\mu_1 - \mu_2 = 0$; *T-Value* = 5.37, *DF* = 227, *P-Value* = <0.0001).



Figure 2-7: (+/- 1 SE; $\alpha = 0.05$) Average number of parasitic organisms found in a SCB. Parasitic organisms included Tachinid larvae, Braconid larvae, Braconid eggs, nematodes, and other. In a Binary Logistic Regression comparing beetle sex versus total number of parasitic organisms per beetle with male as the event (*Y*), mean total number of parasitic organisms per beetle was statistically significant ($\chi_{21} = 4.53$, *P-value* = 0.033; *Y* = 0.153 + 0.301 total parasitic organisms). Additional Binary Logistic Regressions comparing each common parasitic organism to beetle sex revealed that Tachinids had a preference for male beetles but Braconids and nematodes had no preference for either sex: Tachinids: ($\chi_{21} = 3.94$, *P-value* = 0.047; *Y* = 0.471 + 0.470 tachinids); Braconids: ($\chi_{21} = 02.42$, *P-value* = 0.120); Nematodes: ($\chi_{21} = 0.36$, *P-value* = 0.551).



Figure 2-8: (+/- 1SE) Data were collected from dissecting striped cucumber beetles. Regression for abundance of tachinid larvae inside SCB per plot, by divergence time and date (July 10th: $\chi 2_1$ = 3.68, *P* = 0.055, R-squared = 8.26%, abundance = 0.265 - 0.00257 divergence time; August 7th: $\chi 2_5$ = 12.58, *P* = 0.028, R-squared adj = 14.19%, abundance = 0.426 - 0.00233 divergence time; August 30th: $\chi 2_1$ = 3.75, *P* = 0.053, R-squared = 11.11%, abundance = 0.537 - 0.00303

divergence time). Tachinid larvae abundance decreased as divergence time increased.



Figure 2-9: (+/- 1SE) Data were collected from dissecting striped cucumber beetles. Poisson regression for abundance of braconid larvae and eggs inside SCB by divergence time and date. Braconid larvae and egg abundance decreased in response to divergence time on July 10th (larvae: $\chi 2_1 = 11.40$, P = 0.001, R-squared = 17.04%, abundance = 1.014 - 0.00333 divergence time; eggs: $\chi 2_1 = 9.25$, P = 0.002, R-squared = 20.11%, abundance = 0.741 - 0.00348 divergence time) and August 7th (larvae: $\chi 2_5 = 24.50$, P = <0.0001, R-squared = 31.05%, abundance = 0.531 - 0.00437 divergence time; eggs: $\chi 2_1 = 3.39$, P = 0.066, R-squared = 7.42%, abundance = 0.364 - 0.00256 divergence time)



Figure **2-10**: Average abundance of SCB and total parasites and parasitoids per plot with polynomial trendlines. Parasite and parasitoid abundance appeared to have an inverse relationship with SCB abundance throughout the season. It is possible that parasites and SCB have a density-dependent relationship, but I was not able to test this using my data. See Chapter 1 for details on SCB collection methods.



Figure **2-11**: Gregarinasina dissected from SCB on 30 August. A gregarine (top image) and gregarine syzygy (bottom image). (images courtesy of Carolyn Trietsch)

APPENDIX



Figure A-1: Precipitation events and totals recorded at the Rock Springs NEWA Weather Station throughout the field season for 2017 and 2018. Excessive rains caused a delay and planting in both years. In 2017, about 20% of plots were flooded throughout the season. Because of this, I relocated to another area of the research farm in 2018.



Figure A-2: Figure 1 from Razze et al. 2016. "Diagrams of the different buckwheat arrangements implemented in the intercropping field study. A) Buckwheat A, where buckwheat is planted as alternating strips on either side of the squash; B) buckwheat B, where buckwheat is planted in the middle of the squash planted on both sides of the bed; and C) buckwheat C, where buckwheat is planted continuously on both sides of the squash."