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Project Title:

Identification of arthropod predators of cucumber beetles (Coleoptera: Chrysomelidae) in cucurbit agroecosystems

Investigators:

Graduate Student:

Molly Dieterich Mabin, Department of Entomology, Ohio State University

Email: dieterich.9@osu.edu

Faculty Advisors:

Mary M. Gardiner, Department of Entomology, Ohio State University

Email: gardiner.29@osu.edu

Celeste Welty, Department of Entomology, Ohio State University

Email: welty.1@osu.edu

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Abstract

The predatory arthropod assemblage often differs between organic and conventional agriculture, in terms of abundance and richness. However, this does not always result in increased biological control of a pest species. Effectively integrating conservation biological control into IPM systems depends on accurate identification of the guild of predators that provide pest suppression services. Molecular gut content analysis techniques enable researchers to accurately determine key predators of a pest; such studies allow for observations to be made within an un-manipulated system. The aim of this study was to identify the guild of predatory arthropods that consume striped cucumber beetle, *Acalymma vittatum*, using PCR-based molecular gut content analysis methods. Predatory arthropods were collected from four organic and four conventional farms located in Ohio. DNA was extracted from all samples and PCR used to amplify *A. vittatum* DNA, if present within the gut contents of a sample. In addition, predatory arthropods were sampled at each farm using pitfall traps. Of 1,215 predators tested for the presence of *A. vittatum* DNA, 139 were positive (11.4%). Approximately 40% of Coccinellidae, 14% of Opiliones and Carabidae, 6% of Lycosidae and Chilopoda, and 1% of Formicidae and Staphylinidae tested positive for *A. vittatum* DNA. A greater proportion of predators tested positive earlier in the growing season than later in the season. We found no effect of organic versus conventional management on the proportion of predators testing positive. Pitfall trap collections indicated that predatory arthropods are more abundant earlier in the season than later on organic farms, and that predator abundance does not change throughout the season on conventional farms. This study is the first to use molecular gut content methods to identify the guild of predators that consume *A. vittatum*. This identification of cucumber beetle predators is a

first step toward efficient incorporation of conservation biological control practices into any cucurbit production system.

1. Introduction

The community of predatory arthropods found within agroecosystems often differs between organic and conventional production (Bengtsson *et al.* 2005; Hole *et al.* 2005; Dieterich Mabin *et al.* in prep). Many studies have found that organic agroecosystems support predatory arthropod assemblages that are greater in abundance and richness than those found in conventional systems (Gluck & Ingrisch 1990; Feber *et al.* 1998; Bengtsson *et al.* 2005; Hole *et al.* 2005; Birkhofer *et al.* 2008; Ponce *et al.* 2011). However, results vary from studies examining the effects of predator assemblages on biological control services, and the mechanisms driving these interactions are not well understood. In a predator manipulation study, Birkhofer et al. (2008) found that enhanced spider abundance did not result in increased biological control. Additionally, a review by Kromp (1999) states that while organic management fosters predator populations, there is limited evidence for increased pest suppression in these systems. However, results from other studies suggest that increased predator abundance and richness does enhance biological control (Riechert & Lockley 1984; Letourneau *et al.* 2009; Blaauw & Isaacs 2012; Bryant *et al.* 2013; Lundgren & Fergen 2014).

The biodiversity-ecosystem function hypothesis states that trophic levels composed of a more diverse assemblage will exhibit increased resource capture (Tilman *et al.* 1997; Cardinale *et al.* 2006). Two main mechanisms have been suggested to explain this pattern: the sampling effect, in which communities with greater richness are more likely to contain a species that supplies most of the resource capture in the system, or the complementarity effect, in which resource partitioning by many species leads to increased resource capture (Loreau & Hector

2001; Cardinale *et al.* 2006; Crowder & Jabbour 2014). Letourneau et al. (2009) reminds us that while, in general, greater predator richness results in greater biological control, it is possible to see the opposite pattern if greater predator richness results in intraguild predation or competition.

Additionally, it has been suggested that factors such as increased landscape heterogeneity and complexity, as well as the incorporation of habitat management practices such as increased availability of natural edges, presence of uncultivated fields, and small field size, have more of an effect on populations of predatory arthropods than does organic or conventional production per se (Langellotto & Denno 2004; Bengtsson *et al.* 2005; Hole *et al.* 2005; Rusch *et al.* 2016). Determining the abundance and richness of predators, and correlating that with measures of biological control, provides us with some information about trophic interactions within agroecosystems (Letourneau *et al.* 2009; Schmidt *et al.* 2014; Furlong 2015). However, in order to truly integrate biological control into our pest management practices via habitat management that benefits natural enemies, we must know which predatory taxa actually consume a focal prey under natural conditions (Chisholm *et al.* 2014; Crowder & Jabbour 2014; Schmidt *et al.* 2014), because certain habitat management practices may be more beneficial to certain taxa of natural enemies (Rabb *et al.* 1976; Landis *et al.* 2000; Björkman *et al.* 2004; Bianchi *et al.* 2006; Tscharntke *et al.* 2007; Chaplin-Kramer *et al.* 2011).

Sentinel prey studies are often used to identify the predators of species of interest or to evaluate biological control, because they are relatively simple and inexpensive to conduct (Ehler 2007; Seagraves & Yeargan 2009; Smith & Gardiner 2013; Chisholm *et al.* 2014; Gardiner *et al.* 2014; Phillips & Gardiner 2016). For example, the results of two sentinel egg studies aimed at identifying predators of cucumber beetles indicated the importance of Formicidae, Opiliones, Coleoptera, and Gryllidae as above-ground egg predators (Phillips and Gardiner, 2016; Dieterich

Mabin *et al.* in prep), but it is unknown if these taxa are indeed important predators of cucumber beetles in an un-manipulated system. Generally, it is not well understood whether the guild of predators identified via sentinel prey methods corresponds to natural predation patterns (Sheppard & Harwood 2005; Chisholm *et al.* 2014).

Molecular gut content analysis techniques allow for accurate identification of the guild of predators that consume a focal prey (Sheppard & Harwood 2005; Chisholm *et al.* 2014). Many studies have been conducted using these techniques to determine trophic interactions in agroecosystems (Agustí *et al.* 2003; Greenstone & Shufran 2003; Juen & Traugott 2005; Harwood *et al.* 2007, 2009; Eitzinger & Traugott 2011; Chapman *et al.* 2013; Ekbom *et al.* 2014; Furlong *et al.* 2014; Lundgren & Fergen 2014), including identifying the respective predator guilds of the squash bug (*Anasa tristis* De Geer)) and immature western corn rootworm (*Diabrotica virgifera* LeConte) (Lundgren *et al.* 2009; Schmidt *et al.* 2014). However, molecular gut content analysis has not yet been applied to elucidate cucumber beetle food webs.

Striped and spotted cucumber beetles (*Acalymma vittatum* (Fabricius), *Diabrotica undecimpunctata howardi* Barber) are among the most abundant and damaging pest insects of cucurbit crops (Rand & Enlows 1916; Cline *et al.* 2008). In addition to direct feeding upon fruits and foliage (Brewer *et al.* 1987), these beetles are vectors of the pathogen that causes bacterial wilt, *Erwinia tracheiphila* (Rand & Enlows 1916; Necibi *et al.* 1992), which may cause widespread plant death and drastically reduced yields (Bach 1980). Additionally, bacterial wilt can spread quite quickly due to aggregation behavior – feeding by beetles results in the production of a volatile aggregation pheromone which attracts yet more beetles (Fleischer *et al.* 1999; Smyth & Hoffmann 2002, 2003).

The main objective of this study was to identify arthropods that prey upon cucumber beetles, specifically *A. vittatum*, using PCR-based molecular gut content analysis, and to compare predator assemblages and predation of *A. vittatum* on organic and conventional farms. Predator collections were conducted within cucurbit agroecosystems on eight farms located throughout Ohio. We hypothesized that most generalist predatory arthropods present in cucurbit agroecosystems would consume *A. vittatum*, but were especially curious about Formicidae, Coleoptera, and Opiliones, because they have previously been shown to be cucumber beetle egg predators (Phillips & Gardiner 2016; Dieterich Mabin *et al.* in prep).

2. Materials and Methods

2.1 Study sites and experimental design

In 2014, predators were collected at Waterman Agricultural and Natural Resources Laboratory, a research farm owned by Ohio State University and located in Columbus, Ohio. In 2015, eight farms were selected within the U.S. state of Ohio to serve as study sites. Seven were privately managed and one was a university-owned research farm. Four of these farms were under organic management and four were under conventional management (Table 1), for a simple experimental design of two treatments (organic and conventional), with four replicates each. At each farm, our experiments were carried out within a summer squash (*Cucurbita pepo*) field; these fields ranged in size from 120 plants to 0.4 hectare of summer squash (approximately 4000 plants). Field and pest management varied based on each grower's practices, and planting dates ranged throughout the month of May. In 2016, predators for feeding trials were collected at Waterman Agricultural and Natural Resources Laboratory in Columbus, Ohio.

2.2 Field sampling of predatory arthropods and pests

In addition to active hand-collection of predatory arthropods for gut content analysis, we passively sampled for natural enemies using pitfall traps to determine the abundance, richness, diversity, and community composition of natural enemies present in our fields. Pitfall traps were deployed as sets of four, twice at each study site, for 7-day sampling periods (Table 2). Each field was divided into four equivalent sections and one trap was placed in the center of each section. Traps consisted of 946 mL clear plastic deli containers (Fabri-Kal, Kalamazoo, MI) placed in holes created using a golf course cup-cutter. Traps contained 2.5 cm of 30% propylene glycol (Old World Industries, Northbrook, IL) and were placed so that the lip of the cup was even with ground level. After collection of the trap contents, arthropods were stored in 75% ethanol and predatory taxa were identified: Chilopoda (to class), Opiliones (to order), and Araneae, Coleoptera, Orthoptera, and Formicidae (to family).

Scouting for *A. vittatum* was conducted at each site four times throughout the summer, on the same days that predators were collected (described below). Each farm was divided into four plots; within each plot ten plants were chosen at random and the number of pests present on each of those plants was counted and recorded.

2.3 Predator collection and DNA extraction

In 2014, predatory arthropods were collected between 28 July and 18 August. In 2015, predators were collected at each study site four times over the summer – twice in early summer and twice in late summer (Table 2). Each individual was collected by hand, then transferred to individual 1.5 mL plastic tubes and killed by submersion in 100% ethanol. Specimens were stored in a cooler for transport to the laboratory, where they were transferred to a -20°C freezer for long-term storage. Predatory taxa collected included Araneae: Lycosidae, Coleoptera: Carabidae, Coleoptera: Coccinellidae, Coleoptera: Staphylinidae, Hymenoptera: Formicidae,

Chilopoda: Lithobiomorpha, and Opiliones. Samples were identified to the lowest reasonable taxonomic level: species for most Opiliones, Carabidae, Coccinellidae, Formicidae, and adult Lycosidae; genus for most Staphylinidae, Lithobiomorpha, and immature Lycosidae. To minimize the potential impact of predator removal from field sites via collection for gut content analysis on the pitfall trap collection, predators were collected on the same day that pitfall traps were removed, as well as approximately two weeks later (Table 2).

DNA was extracted from crushed samples using QIAGEN DNeasy Blood and Tissue kits, per the manufacturer's animal tissue protocol (Qiagen, Valencia, CA). After extraction, sample DNA concentration was determined via spectrometry, and DNA was stored at -20°C.

2.4 Primer design, PCR optimization, and screening for predation

Two sets of primers targeting *A. vittatum* were used over the course of this experiment. The first set was used with the samples collected during 2014, but due to issues of non-specific amplification, a second set was designed for use with the samples collected during 2015 and 2016. The first set of primers included a forward primer obtained from Clark *et al.* (2001) and a reverse primer designed using the cytochrome c oxidase subunit I (COI) sequence of *A. vittatum* obtained from NCBI GenBank (accession AY533586) (forward 5'-GGAGGATTTGGAAATTGATTAGTTCC-3', T_m=54.2°C; reverse 5'-GAAAGGAGAAGCAGAAGAGCTG-3', T_m=55.5°C); this set of primers yields an amplicon size of 368 base pairs (Integrated DNA Technologies, Coralville, IA). The second set of primers were both designed using the COI sequence of *A. vittatum* (forward 5'-ATAGCTTTCCCCCGAATAAA-3', T_m=61.2°C; reverse 5'-GAAAGGAGAAGCAGAAGAGCTG-3', T_m=63.5°C); this set of primers yields an amplicon size of 320 base pairs (Sigma-Aldrich, St. Louis, MO). All primers were pre-validated in Primer

3 Plus, checked against the NCBI GenBank database, and tested by PCR for cross-amplification with relevant non-target arthropods.

Polymerase chain reaction (PCR) to amplify the target *A. vittatum* DNA was optimized to: 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 61°C for 30 seconds, and 72°C for 45 seconds, with a final extension period of 72°C for 2 minutes, then cooldown to 4°C (Szalanski *et al.* 2000; Clark *et al.* 2001; Schmidt *et al.* 2014). PCR reactions (25 µL) consisted of 12.5 µL 2X Go-Taq Green Master Mix, 1.0 µL 10 µM forward/reverse primer mix, 300 ng of sample DNA, and nuclease-free water to reach 25 µL and were processed in an Eppendorf Mastercycler™ Nexus thermocycler (Eppendorf, Hamburg, Germany). Gel electrophoresis (1.5% agarose gel stained with GelRed (Biotium Inc., Hayward, CA)) was used to visualize the amplification of prey DNA in the guts of the samples.

Extraction, PCR, and gel electrophoresis were used on all field-collected and feeding trial predator samples, described below, to identify the presence of *A. vittatum* DNA within the gut contents of these predatory arthropods. *A. vittatum* extractions were used as positive controls. All samples were tested twice to rule out false positives and negatives; in the event of a contradiction between the first two tests, a third test was processed.

2.5 DNA detectability feeding trials

A digestion rate assay was conducted to determine for how long after consumption the DNA of *A. vittatum* would be detectable using the methods described above. Such assays are important with regards to interpreting molecular gut content analysis data, because it has been shown that predators digest prey at different rates (Greenstone *et al.* 2014; Renkema *et al.* 2014; Schmidt *et al.* 2014). Predators were hand-collected, using the same methods described above, during the summer of 2016 at Waterman Agricultural and Natural Resources Laboratory

(Columbus, OH). Predatory taxa were collected based on availability and perceived importance to results, thus not all predatory taxa screened for *A. vittatum* DNA were included in the digestion assay. Taxa collected included Lycosidae and Opiliones. Individuals were collected from the field and maintained in the laboratory at ambient room conditions for a 72-hour starvation period. After the starvation period, a live *A. vittatum* larva was introduced into each predator's dish and predators were given up to two hours to feed. If they had fed at the end of the two hour feeding period, this was considered time zero; if they had not, they were given another two hour feeding period. If they had not fed at the end of four hours, the *A. vittatum* larva was removed and the predator was starved for another 24 hours. Beginning at time zero, predators were again starved until a set time point after feeding (0 hr, 12 hr, 24 hr, 36 hr, 48 hr, 60 hr, 72 hr), at which time they were killed in 100% ethanol and transferred to a freezer until DNA extraction and PCR analysis (Schmidt *et al.* 2014); an unfed control group was also collected at the end of the starvation period.

2.6 Statistical analysis

To determine whether or not the proportion of predators testing positive for *A. vittatum* DNA differed between farm management (organic or conventional) or sampling time (early or late season) an analysis of variance (ANOVA) was conducted using proportion of predators testing positive as the response variable, which was arcsine square root transformed prior to analysis in order to meet the assumptions of ANOVA; family, management, and sampling time were fixed effects; all interaction terms were included in the model (PROC MIXED, SAS version 9.4).

Simple linear regressions were used to determine the relationship of *A. vittatum* density on each farm with the proportion of predators testing positive for *A. vittatum* DNA, the richness

of predators testing positive, the abundance of predators present on each farm, and the richness of predators present on each farm (stats package version 3.2.2, R version 3.2.2).

Diversity of predators captured in pitfall traps and predators testing positive for *A. vittatum* DNA was quantified using the Shannon Diversity Index. Abundance, richness, and diversity of these groups of predators were found to be non-normal via the Shapiro-Wilk normality test (stats package version 3.2.2, R version 3.2.2). A generalized linear mixed model with a gamma distribution (to maximize model fit by minimizing overdispersion) was used to determine if predator abundance, richness, or diversity differed between organic and conventional farms or between early and late season sampling (PROC GLIMMIX, SAS version 9.4). The response variable was either abundance, richness, or diversity, with independent variables farm management (organic or conventional) and sampling time (early or late); the interaction term between management and sampling time was included. Community composition of predatory arthropods was compared in R using the metaMDS and adonis functions (vegan package version 2.4-2, R version 3.2.2) to conduct non-metric multidimensional scaling analysis (NMDS). Two dimensions were used to minimize stress. Distance measures were chosen using the rankindex function in R to identify which dissimilarity indices had highest rank-order similarity with gradient separation (vegan package version 2.4-2, R version 3.2.2).

3. Results

3.1 Predatory arthropod populations

We found a significant interaction between farm management and sampling time affecting the abundance of ground-active predatory arthropods captured in pitfall traps ($F_{1,12}$=5.54, P=0.036) (Figure 1A). On organic farms, predators were more abundant during early season sampling than during late season sampling (t_{12}=4.68, P=0.0005); on conventional farms,

there was no difference in predator abundance between early and late season sampling (t_{12}=1.36, P=0.20). We found no relationship between predator abundance and *A. vittatum* density on each farm ($F_{1,14}$=2.90, P=0.11) (Figure 2A). There was a greater family richness of predator taxa on organic farms than conventional farms ($F_{1,12}$=24.62, P=0.0003), but no difference in family richness between early and late season sampling ($F_{1,12}$=4.72, P=0.05) (Figure 1BC). Additionally, we found no relationship between the predator richness found on each farm and *A. vittatum* density ($F_{1,14}$=1.69, P=0.21) (Figure 2B). Predator diversity (Shannon Diversity Index) did not differ between organic and conventional farms ($F_{1,12}$=2.6, P=0.13) or between early and late season sampling ($F_{1,12}$=0.12, P=0.74) (Figure 1DE). The community composition of predatory arthropods captured in pitfall traps did not differ between organic and conventional farms ($F_{3,4}$=1.23, P=0.30) or between early and late season sampling ($F_{3,12}$=1.18, P=0.30).

3.2 Molecular gut content analysis

A total of 1,215 predators were tested for the presence of *A. vittatum* DNA in their gut contents; 139 tested positive (11.4%). The assemblage of predators testing positive included Coccinellidae (n=58), Carabidae (n=28), Lycosidae (n=28), Opiliones (n=18), Chilopoda (n=3), Formicidae (n=3), and Staphylinidae (n=1) (Table 3, Figure 3, Supplemental Table 1). Within each family, the percentage of individuals testing positive was as follows: 42.3% of Coccinellidae, 14.8% of Opiliones, 14.0% of Carabidae, 6.7% of Lycosidae, 5.4% of Chilopoda, 1.7% of Formicidae, and 1.0% of Staphylinidae. Results of our digestion rate assay indicate that *A. vittatum* DNA is detectable within Lycosidae and Opiliones for at least 72 hours after consumption.

We found a significant effect of predator family ($F_{6,141}$=11.03, P<0.0001) on the proportion of predators testing positive for *A. vittatum* DNA within their gut contents, as well as

of sampling time (early or late season) ($F_{1,141}$=4.13, P=0.04). A greater proportion of predators tested positive early in the season than tested positive later in the season (Figure 4B); there was no effect of sampling time on *A. vittatum* density ($F_{1,124}$=0.36, P=0.55). Additionally, there was no effect of farm management on the proportion of predators testing positive ($F_{1,141}$=0.67, P=0.41) (Figure 4A), although the average density of *A. vittatum* was greater on organic farms than on conventional farms ($F_{1,124}$=18.06, P<0.0001). We found no relationship between the proportion of predators testing positive on each farm and *A. vittatum* density ($F_{1,14}$=0.90, P=0.36) (Figure 2C).

We found no effect of farm management (organic or conventional, $F_{1,23}$=2.4, P=0.14) or of sampling time (early or late season, $F_{1,23}$=2.7, P=0.11) on the abundance of predators testing positive for *A. vittatum* DNA. Additionally, there was no effect of management or sampling time on the family richness (management $F_{1,23}$=2.42, P=0.13; sampling time $F_{1,23}$=1.01, P=0.33) (Figure 4CD) or on the species richness (management $F_{1,23}$=2.67, P=0.12; sampling time $F_{1,23}$=0, P=1.0) of predators testing positive. There was a negative relationship between *A. vittatum* density on each farm and the family richness of predators testing positive ($F_{1,14}$=5.67, P=0.03) (Figure 2D); on farms with a greater richness of predators testing positive for *A. vittatum* DNA there were less *A. vittatum*. There was no effect of either management ($F_{1,15}$=0.12, P=0.73) or sampling time ($F_{1,15}$=0.12, P=0.73) on the diversity (Shannon Diversity Index) of predators testing positive (Figure 4EF).

The community composition of predator families testing positive for *A. vittatum* DNA differed between organic and conventional farms ($F_{3,6}$=2.64, P=0.02); the community composition of predators was more variable on organic farms than on conventional farms (Figure 5). Additionally, no Chilopoda tested positive on conventional farms, while no Staphylinidae

tested positive on organic farms. A greater proportion of Opiliones and Formicidae tested positive on organic farms while a greater proportion of Coccinellidae, Carabidae, and Lycosidae tested positive on conventional farms.

4. Discussion

Organic agroecosystems often support a greater richness of predatory arthropods (Gluck & Ingrisch 1990; Feber *et al.* 1998; Bengtsson *et al.* 2005; Ponce *et al.* 2011); however, many studies have shown that this is likely due to the implementation of conservation biological control practices that often occurs on organic farms, rather than simply organic production (Langellotto & Denno 2004; Bengtsson *et al.* 2005; Hole *et al.* 2005; Rusch *et al.* 2016). In our study, predators were more abundant and had higher family richness on organic farms; however, the community composition of predators found on organic and conventional farms did not differ. This lack of difference between the predator communities on organic and conventional farms can likely be explained by this hypothesis set forth in the literature that rather than organic or conventional production practices per se, what affects the assemblage of natural enemies in an agroecosystem is landscape heterogeneity and habitat management practices (Langellotto & Denno 2004; Bengtsson *et al.* 2005; Hole *et al.* 2005). All our farms, whether organic or conventional, contained a variety of different vegetables; in fact, our cucurbit plots on every farm were bordered by at least two other vegetable crops.

Conservation biological control practices that foster populations of generalist predatory arthropods are a vital component of integrated pest management (IPM) systems (Batra, 1982; Toepfer et al., 2009; Giles et al., in press). In order to efficiently implement habitat management that will result in greater pest suppression, it is imperative to know which natural enemies provide control of the pest of interest (Landis *et al.* 2000; Bianchi *et al.* 2006; Tscharntke *et al.*

2007), and if this differs between different production strategies. For example, certain carabid species may be most affected by the presence of organic matter on the soil surface, while spiders may be more likely to inhabit fields with non-crop habitat located within their specific dispersal distance (Landis *et al.* 2000; Tscharntke *et al.* 2007).

Our study aimed to identify the guild of generalist predators that consume *A. vittatum*, a damaging pest of cucurbits, using molecular gut content analysis methods. Our results indicate that Coccinellidae, Opiliones, Carabidae, Lycosidae, and Chilopoda consume *A. vittatum*, and that Formicidae and Staphylinidae rarely prey upon *A. vittatum*. This guild has been unidentified until now, although the guild of predators that consume *Diabrotica virgifera* (western corn rootworm, a close relative of *A. vittatum*) eggs and larvae has been previously identified and includes Opiliones and Staphylinidae as the most important predators of this subterranean pest (Lundgren *et al.* 2009). Additionally, the assemblage of predators that consume *Anasa tristis* (squash bug, another pest in cucurbits) has been determined to include Coccinellidae, Geocoridae, and Lycosidae as the important predators (Schmidt *et al.* 2014).

Although this study identifies many predators that consume *A. vittatum*, we should be careful not to draw strong conclusions regarding the biocontrol efficiency of each predator as we do not have digestion rate data that allows us to accurately determine for how long cucumber beetle DNA is detectable within the gut contents of each taxa (Greenstone *et al.* 2010; Furlong *et al.* 2014). Additionally, molecular gut content analysis methods do not allow for distinction between predation, secondary predation, and scavenging (Juen & Traugott 2005; Sheppard & Harwood 2005)

However, what we are most interested in is if the predator assemblage that consumes *A. vittatum,* and the level of biological control provided, differs on organic and conventional farms.

In other words, we are interested in comparing the effects of environmental factors on biological control rather than determining biocontrol efficiency of each predator. The community composition of predator families testing positive for *A. vittatum* DNA in our study did differ between organic and conventional farms. On organic farms a greater proportion of Opiliones and Formicidae, but no Staphylinidae, tested positive. On conventional farms a greater proportion of Coccinellidae, Carabidae, and Lycosidae, but no Chilopoda, tested positive. We found no difference in the overall proportion of predators testing positive for *A. vittatum* DNA on organic and conventional farms.

We found that the abundance and richness of predatory arthropods was greater on organic farms than on conventional farms, and that there was a greater density of *A. vittatum* on organic farms. However, this did not translate into an increased proportion of predators testing positive for *A. vittatum* DNA. In other words, even though we saw enhanced predator communities and increased prey availability on organic farms, we did not see increased biological control on organic farms. Thus, out findings do not support the biodiversity-ecosystem function hypothesis, that increased richness and abundance of predators results in increased pest suppression (Cardinale *et al.* 2006).

4.1 Conclusions

Molecular gut content analysis allows researchers to clarify natural trophic interactions without manipulating prey or predator densities in the field, and to determine contributions specific predators make to biological control that may not necessarily be reflected by their abundances (Sheppard & Harwood 2005; Chisholm *et al.* 2014). Results of our molecular gut content analysis suggest that Coccinellidae, Opiliones, and Carabidae are promising biological control agents of *A. vittatum*, and that Formicidae and Staphylinidae do not regularly consume

this pest. There is some overlap between the predators testing positive for *A. vittatum* DNA and predators that have been observed consuming sentinel *D. undecimpunctata howardi* eggs (Phillips & Gardiner 2016; Dieterich Mabin *et al.* in prep); Opiliones, Coleoptera, and Formicidae are important taxa in both types of studies.

The most appropriate and effective conservation biological control practices, and the scale at which they should be implemented, may differ depending on which natural enemies are the aim of such practices, thus it is imperative to quantify the contributions of specific natural enemy taxa to biological control in order to identify the most effective natural enemies (Tscharntke *et al.* 2007; Chaplin-Kramer *et al.* 2011; Crowder & Jabbour 2014). This information is vital for vegetable growers wishing to naturally suppress pest populations by fostering populations of natural enemies that will exert necessary biological control services.

Site	Management	Summer squash field size
Sippel Family Farm *Mt. Gilead, OH*	Organic	225 plants
Whitebarn Organics *New Albany, OH*	Organic	120 plants
That Guy's Family Farm *Clarksville, OH*	Organic	300 plants (x 2 plantings)
Flying J Farm *Johnstown, OH*	Organic	300 plants
Witten Farm Market *Lowell, OH*	Conventional	0.4 hectare (x 2 plantings)
New Century CSA *Circleville, OH*	Conventional	0.4 hectare
Birney's Farm Market *Pataskala, OH*	Conventional	240 plants
Waterman Agricultural and Natural Resources Laboratory (OSU) *Columbus, OH*	Conventional	240 plants

Table 1. Location, management, and size of summer squash field at each study site used in 2015.

Site	Sampling time	Pitfall trap collection dates	Predator collection dates
Sippel Family Farm *Mt. Gilead, OH*	Early	June 12	June 12, June 26
	Late	July 19	July 19, August 6
Whitebarn Organics *New Albany, OH*	Early	June 22	June 22, July 1
	Late	July 23	July 23, August 14
That Guy's Family Farm *Clarksville, OH*	Early	June 19	June 19, July 1
	Late	July 22	July 22, August 13
Flying J Farm *Johnstown, OH*	Early	June 15	June 15, June 30
	Late	July 17	July 21, August 5
Witten Farm Market *Lowell, OH*	Early	June 11	June 11, June 25
	Late	July 22	July 22, August 12
New Century CSA *Circleville, OH*	Early	June 10	June 10, June 24
	Late	July 20	July 20, August 15
Birney's Farm Market *Pataskala, OH*	Early	June 30	June 23, June 30
	Late	July 17	July 17, August 5
Waterman Farm (OSU) *Columbus, OH*	Early	June 16	June 16, July 2
	Late	July 20	July 20, August 7

Table 2. Dates of pitfall trap collections and predator collections at each study site in 2015.

	Total			Organic			Conventional		
Family	Number Tested	Number Positive	Percent Positive	Number Tested	Number Positive	Percent Positive	Number Tested	Number Positive	Percent Positive
Coccinellidae	137	58	42.3	57	20	35.1	80	38	47.5
Opiliones[1]	122	18	14.8	42	9	21.4	80	9	11.3
Carabidae	200	28	14.0	69	8	11.6	131	20	15.3
Lycosidae	421	28	6.7	199	11	5.5	222	17	7.7
Chilopoda[2]	56	3	5.4	23	3	13.0	33	0	0.0
Formicidae	174	3	1.7	108	2	1.9	66	1	1.5
Staphylinidae	105	1	1.0	53	0	0.0	52	1	1.9
Total	*1215*	*139*	*11.4*	*551*	*53*	*9.6*	*664*	*86*	*13.0*

Table 3. Total number of organisms analyzed, number testing positive, and percent testing positive for *A. vittatum* DNA in each predator family; and number analyzed, number positive, and percent positive in each family on organic and conventional farms (see Appendix A for data at lower taxonomic level), for predators collected in 2014 and 2015.

[1] two families within Opiliones combined (Sclerosomatidae and Phalangiidae)

[2] all Chilopoda are in the family Henicopidae

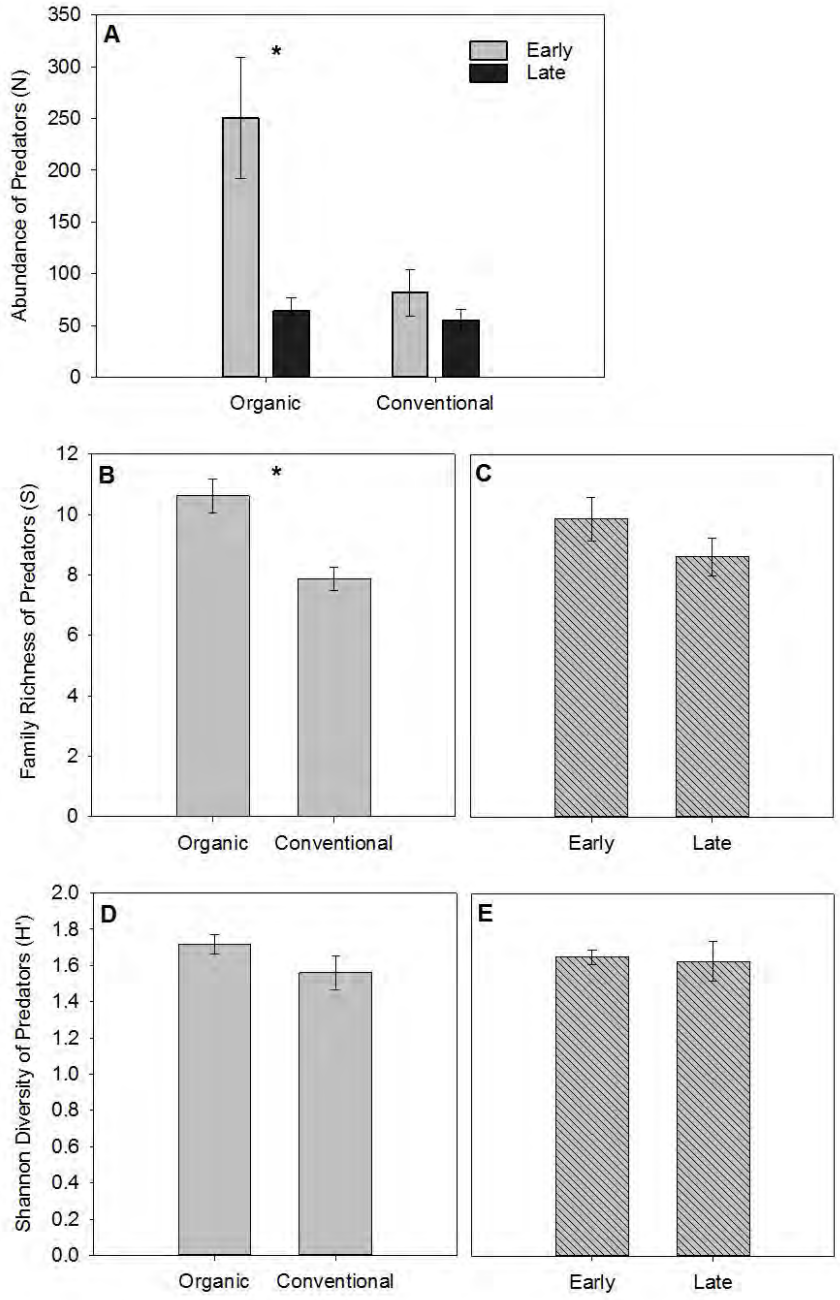

Figure 1. Abundance, family richness, and diversity of predatory natural enemies captured in pitfall traps at all sites in 2015. **A)** Abundance (mean ± SEM) of predatory natural enemies captured in pitfall traps on organic and conventional farms, early and late in the season (interaction $F_{1,12}$=5.54, P=0.036); on organic farms predators were more abundant early in the season (t_{12}=4.68, P=0.0005), while on conventional farms there was no difference in predator abundance between early and late season (t_{12}=1.36, P=0.20). Richness of predator families (mean ± SEM) on **B)** organic and conventional farms ($F_{1,12}$=24.62, P=0.0003) and **C)** early and late in the season ($F_{1,12}$=4.72, P=0.05). Shannon Diversity (mean ± SEM) of predatory natural enemies **D)** on organic or conventional farms ($F_{1,12}$=2.6, P=0.13) and **E)** early or late in the season ($F_{1,12}$=0.12, P=0.74).

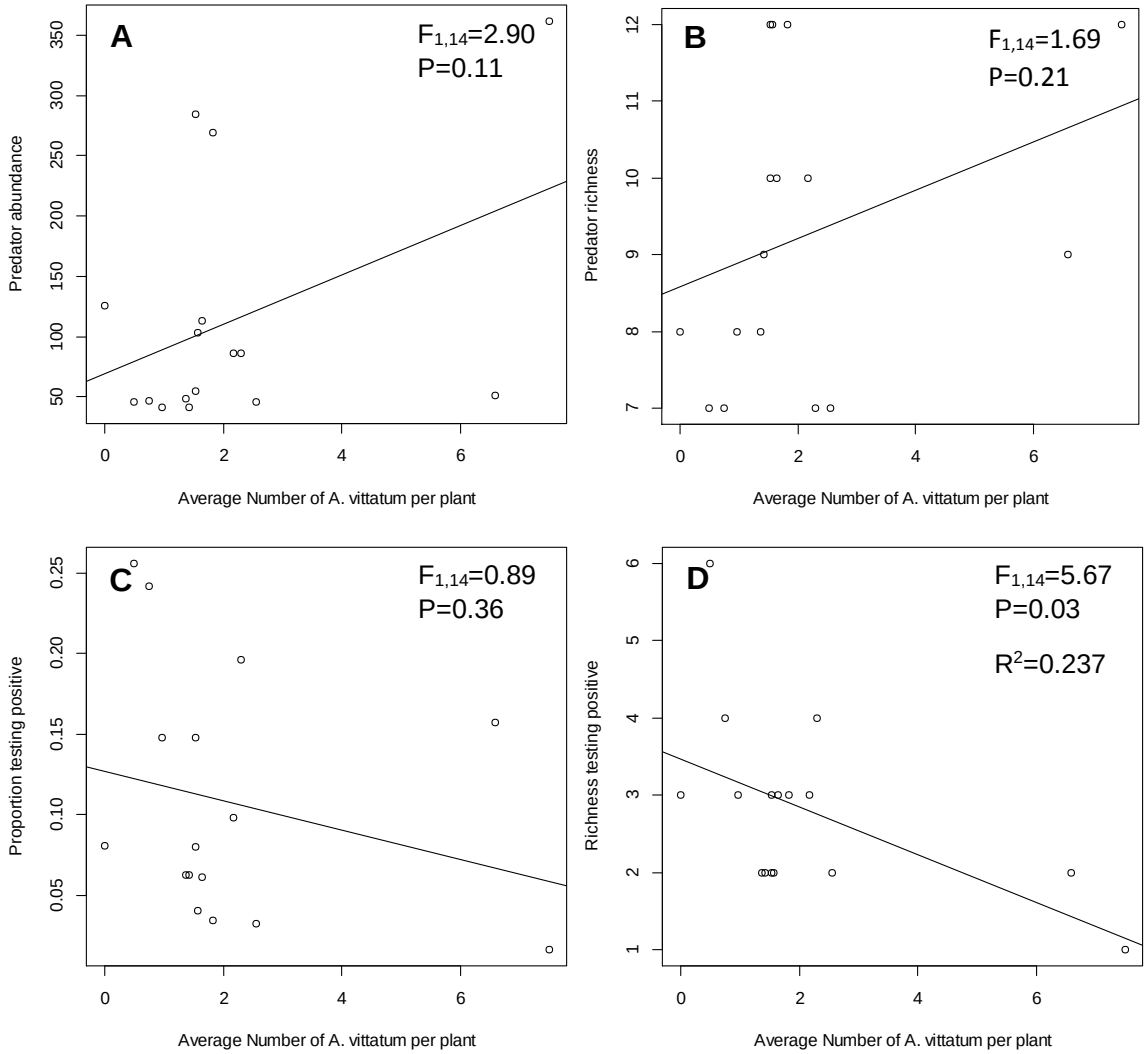

Figure 2. Relationships between predator metrics and *A. vittatum* density on each farm, 2015. **A)** Predator abundance and *A. vittatum* density, **B)** family richness of predators and *A. vittatum* density, **C)** the proportion of predators testing positive for *A. vittatum* DNA and *A. vittatum* density, and **D)** the family richness of predators testing positive for *A. vittatum* DNA and *A. vittatum* density.

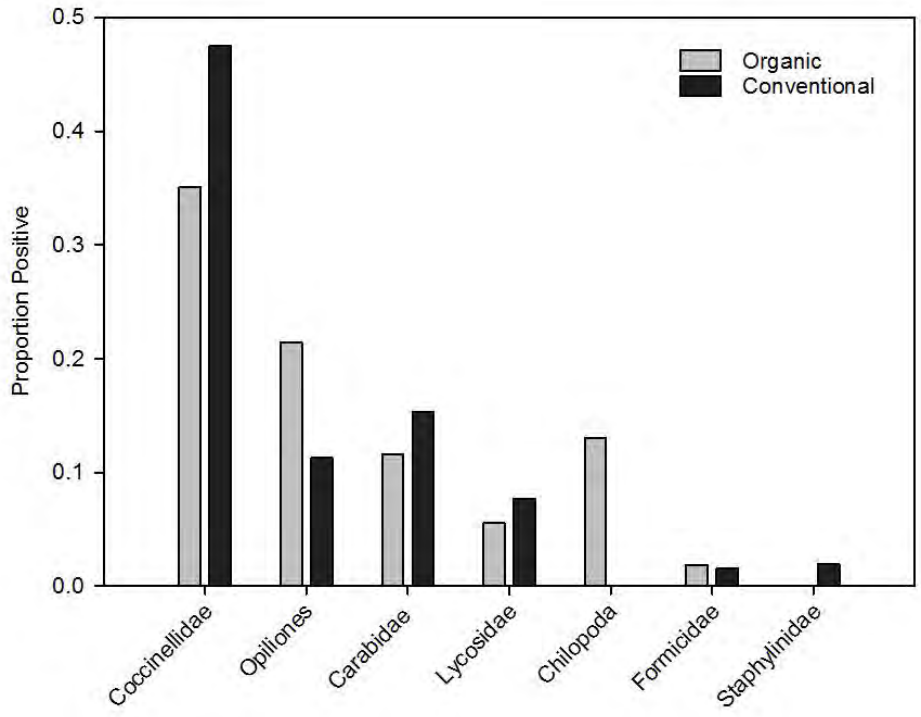

Figure 3. Proportion of individuals within each family of predators that tested positive for *A. vittatum* DNA in their gut contents from organic and conventional farms from all predators collected during 2014 and 2015.

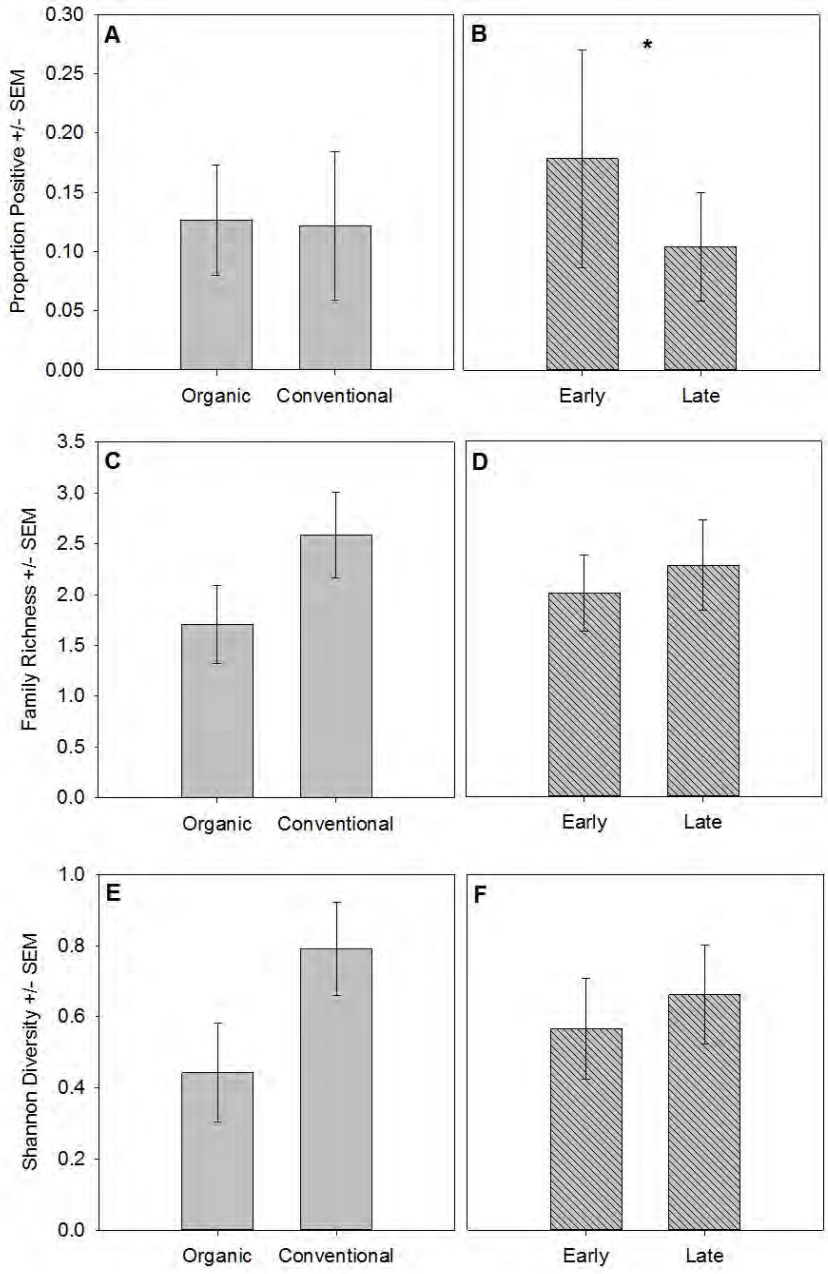

Figure 4. Proportion (± SEM) of predators testing positive for *A. vittatum* DNA within their gut contents on **A)** organic or conventional farms ($F_{1,141}$=0.67, P=0.41) and **B)** early or late in the season ($F_{1,141}$=4.13, P=0.04). Family richness (± SEM) of predators testing positive for *A. vittatum* DNA within their gut contents on **C)** organic or conventional farms ($F_{1,23}$=2.42, P=0.13) and **D)** early or late in the season ($F_{1,23}$=1.01, P=0.33). Shannon diversity (± SEM) of predators testing positive for *A. vittatum* DNA within their gut contents on **E)** organic or conventional farms ($F_{1,15}$=0.12, P=0.73) and **F)** early or late in the season ($F_{1,15}$=0.12, P=0.73) (early: collected during first two predator collections, late: collected during second two predator collections, see Table 2). Proportion positive data were analyzed using arcsine square root transformed data, raw means ± SEM are shown.

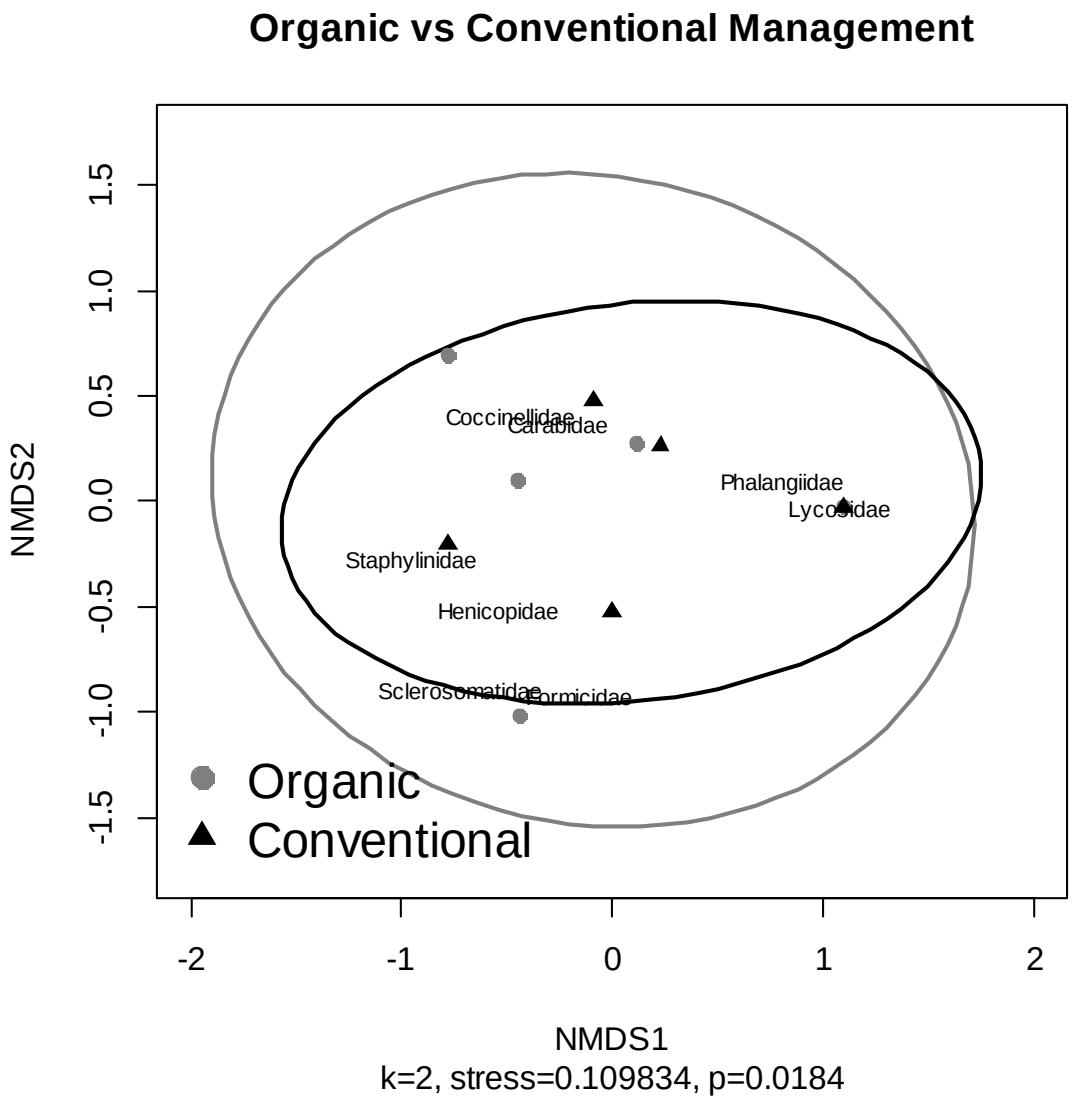

Figure 5. Non-metric multidimensional scaling analysis (NMDS) of predator families testing positive for *A. vittatum* DNA on organic and conventional farms, 2014 and 2015 data combined. Symbols represent each farm (organic (gray circles) and conventional (black triangles)); their location is weighted based on relative abundance of each taxa.

Note: There is a gray circle directly underneath the black triangle near "Lycosidae"; these sites represent the 2014 fields where only Opiliones and Lycosidae were collected.

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Extension and Outreach

Results from this research project were presented at the 2016 International Congress of Entomology meeting in Orlando, Florida, at the North Central Branch – Entomological Society of America annual meeting in Cleveland, Ohio, and at the 2017 meeting of the Ohio Produce Growers and Marketers Association Congress in Sandusky, Ohio. A publication is in progress for submission to Molecular Ecology.

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Supplemental Table 1: Number analyzed, number testing positive, and percent testing positive for *A. vittatum* DNA in each predator taxa, 2014 and 2015 data combined; and number analyzed, number positive, and percent positive in each taxa on organic and conventional farms. Taxa presented at lowest taxonomic level samples were identified to, including life stage and/or sex.

		Total			Organic			Conventional		
		Number Tested	Number Positive	Percent Positive	Number Tested	Number Positive	Percent Positive	Number Tested	Number Positive	Percent Positive
Coccinellidae	*Chilocorus* sp. larvae	1	0	0.0	1	0	0.0			
	Coccinella septempunctata adult	3	2	66.7	1	1	100.0	2	1	50.0
	Coleomegilla maculata adult	74	38	51.4	22	10	45.5	52	28	53.8
	Coleomegilla maculata larvae	39	12	30.8	23	6	26.1	16	6	37.5
	Cycloneda munda adult	3	0	0.0	1	0	0.0	2	0	0.0
	Cycloneda munda larvae	2	1	50.0	1	0	0.0	1	1	100.0
	Harmonia axyridis adult female	8	4	50.0	5	3	60.0	3	1	33.3
	Harmonia axyridis adult male	4	1	25.0	2	0	0.0	2	1	50.0
	Hippodamia variegata adult	3	0	0.0	1	0	0.0	2	0	0.0
Opiliones	*Leiobunum* sp.	4	2	50.0	3	1	33.3	1	1	100.0
	Mitopus morio	3	1	33.3	3	1	33.3			
	Opilio parietinus	71	5	7.0	20	1	5.0	51	4	7.8
	Phalangium opilio female	34	9	26.5	15	6	40.0	19	3	15.8
	Phalangium opilio male	10	1	10.0	1	0	0.0	9	1	11.1
Carabidae	*Anisodactylus carbonarius*	2	2	100.0	1	1	100.0	1	1	100.0
	Anisodactylus harrisii	1	0	0.0				1	0	0.0
	Anisodactylus sanctaecrucis	52	8	15.4	16	3	18.8	36	5	13.9
	Apristus latens	1	0	0.0	1	0	0.0			
	Bembidion anceps	1	0	0.0				1	0	0.0
	Bembidion impotens	3	0	0.0	2	0	0.0	1	0	0.0
	Bembidion quadrimaculata oppositum	2	0	0.0	2	0	0.0			
	Bembidion rapidum	9	0	0.0	4	0	0.0	5	0	0.0

	Bembidion variegatum	1	0	0.0				1	0	0.0
	Bradycellus lecontei	1	1	100.0				1	1	100.0
	Bradycellus rupestris	11	3	27.3	2	0	0.0	9	3	33.3
	Elaphropus anceps	13	0	0.0	2	0	0.0	11	0	0.0
	Elaphropus tripunctatus	1	0	0.0	1	0	0.0			
	Harpalus calinginosus	1	0	0.0				1	0	0.0
	Harpalus erraticus	1	1	100.0				1	1	100.0
	Harpalus erythropus	2	1	50.0				2	1	50.0
	Harpalus faunus	1	0	0.0				1	0	0.0
	Harpalus herbivagus	1	1	100.0	1	1	100.0			
	Harpalus pensylvanicus	15	5	33.3	1	1	100.0	14	4	28.6
	Harpalus vagans	1	0	0.0				1	0	0.0
	Lebia analis	6	0	0.0	5	0	0.0	1	0	0.0
	Lebia pumila	1	0	0.0				1	0	0.0
	Poecilus chalcites	5	2	40.0				5	2	40.0
	Poecilus lucublandus	1	0	0.0				1	0	0.0
	Pterostichus rostratus	2	0	0.0				2	0	0.0
	Stenolophus carbo	1	0	0.0				1	0	0.0
	Stenolophus comma	48	3	6.3	26	1	3.8	22	2	9.1
	Stenolophus conjunctus	1	0	0.0	1	0	0.0			
	Stenolophus lecontei	2	0	0.0				2	0	0.0
	Stenolophus ochropezus	12	1	8.3	4	1	25.0	8	0	0.0
	Stereocerus haematopus	1	0	0.0				1	0	0.0
Lycosidae	*Pardosa* sp. immature	97	5	5.2	48	2	4.2	49	3	6.1
	Pardosa sp. immature female	26	0	0.0	14	0	0.0	12	0	0.0
	Pardosa sp. immature male	37	1	2.7	21	0	0.0	16	1	6.3
	Pardosa milvina adult female	147	13	8.8	64	6	9.4	83	7	8.4
	Pardosa milvina adult male	67	6	9.0	23	0	0.0	44	6	13.6
	Pardosa pauxilla adult female	15	1	6.7	6	1	16.7	9	0	0.0
	Pardosa pauxilla adult male	18	2	11.1	13	2	15.4	5	0	0.0

	Pirata arenicola adult female	1	0	0.0	1	0	0.0			
	Pirata insularis immature	2	0	0.0	2	0	0.0			
	Pirata insularis adult	7	0	0.0	4	0	0.0	3	0	0.0
	Pirata maculatus female	1	0	0.0	1	0	0.0			
	Pirata minutus male	1	0	0.0	1	0	0.0			
	Pirata piraticus female	2	0	0.0	1	0	0.0	1	0	0.0
Chilopoda										
Henicopidae	*Lamyctes* sp.	37	2	5.4	14	2	14.3	23	0	0.0
	Henicopidae	19	1	5.3	9	1	11.1	10	0	0.0
Formicidae	*Lasius neoniger*	149	3	2.0	99	2	2.0	50	1	2.0
	Tetramorium caespitum	25	0	0.0	9	0	0.0	16	0	0.0
Staphylinidae										
Aleocharinae	*Aleochara* sp.	21	0	0.0	8	0	0.0	13	0	0.0
Oxytelinae	*Deleaster* sp.	4	0	0.0	1	0	0.0	3	0	0.0
Paederinae	*Ochthephilum* sp.	1	0	0.0	1	0	0.0			
	Paederus sp.	2	0	0.0	2	0	0.0			
Philonthina	*Belonuchus* sp.	5	0	0.0	4	0	0.0	1	0	0.0
	Bisnius sp.	1	0	0.0				1	0	0.0
	Hesperus sp.	2	0	0.0	2	0	0.0			
	Laetulonthus sp.	5	0	0.0	3	0	0.0	2	0	0.0
	Philonthus sp.	12	1	8.3	6	0	0.0	6	1	16.7
Steninae	*Stenus* sp.	37	0	0.0	20	0	0.0	17	0	0.0
Xantholinini	*Leptacinus* sp.	8	0	0.0	6	0	0.0	2	0	0.0
	Phacophallus sp.	6	0	0.0				6	0	0.0
	Xantholinini	1	0	0.0				1	0	0.0