FACTORS INFLUENCING NEOSEIULUS CUCUMERIS OPEN REARING IN GREENHOUSES

By

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ABSTRACT

FACTORS INFLUENCING NEOSEIULUS CUCUMERIS
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My goal was to identify and manage factors influencing open rearing of the thrips predatory mites *Neoseiulus cucumeris* Oudemans (Phytoseiidae) to improve biological control in greenhouses. I conducted a microcosm study in a greenhouse at Michigan State University to identify the potential for and effects of intraguild interactions among predators. *Atheta coriaria* (Kraatz) (Coleoptera) and *Stratiolaelaps miles* (Berlese) (Laelapidae), two predators commonly released to manage pests in greenhouses, were found to be intraguild predators that demonstrated negative effects on *N. cucumeris* populations when *N. cucumeris* were released in breeder pile open rearing systems placed on the growing media surface. I then tested an alternative release tactic —sachets in the plant canopy— for mitigating these unfavorable interactions in two trials at a commercial greenhouse in Michigan. The use of *N. cucumeris* sachets were found to reduce the effects of intraguild predation by *A. coriaria. Neoseiulus cucumeris* densities produced by different open rearing systems and how long these predators were conserved in the systems were determined in two repeated greenhouse trials. *Neoseiulus cucumeris* sachets were found to contain more mites over time compared with breeder pile open rearing systems. Furthermore, I showed that *N. cucumeris* disperse quicker from breeder piles than sachets and that sachets conserve *N. cucumeris* longer than breeder piles. I consolidated this information into an extension bulletin for distribution to greenhouse operators.

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CHAPTER 1

Introduction

Greenhouses and other forms of protected culture facilitate better growing conditions and extend the growing season thus providing more opportunities for growers to generate profit. In 2007, greenhouse grown vegetables and ornamental crops in the United States had revenue of more than $7 billion alone and $15.3 billion when combined with total nursery, greenhouse, and floriculture production (USDA 2007). The extended growing seasons and favorable growing conditions provided by greenhouses are also conducive for outbreaks of greenhouse pests (Shipp et al. 1991, Gullino et al. 2002, Brødsgaard and Albajes 2002). Fortunately, a number of pest management options are available to growers including cultural (Gullino et al. 2002), chemical, and biological tactics (Shipp et al. 1991, Berlinger et al. 2002).

Cultural tactics are used to prevent pest problems by selecting tolerant or resistant plant varieties (Cuartero et al. 2002), using pest and disease free propagation materials, and practicing crop sanitation procedures (Berlinger et al. 2002). Cultural tactics do not prevent all instances of pest pressure so other strategies such as: chemical and biological tactics are used to prevent pest levels that cause economic damage.

Chemical management of pests can provide immediate declines in pest densities. However, chemicals that are effective and available for greenhouse use pose risks to human health, have adverse effects on non-target organisms, and can be detrimental to the environment (Shipp et al. 1991, van Driesche and Bellows 1996). Furthermore, increasing costs, EPA regulations, and development of pest resistances to chemicals puts emphasis on the need for implementing alternative management or integrated approaches including biological control (Shipp et al. 1991, Duke et al. 2003, Elzen and Hardee 2003).

Biological control involves the use of one organism population to suppress the population of another (van Driesche and Bellows 1996). This form of pest management has been used extensively in field crops and has been an increasing approach in greenhouses (van Lenteren and Woets 1988, Bolckmans 2002). Biological control tactics for greenhouse pest management are an economical and attractive alternative to chemical tactics because of greatly lowered risks of phytotoxicity, worker and consumer exposure to harmful chemicals, and development of pest resistance (Shipp et al. 1991, van Lenteren 2000, Bale et al. 2008, van Lenteren 2012).

Greenhouse biological control uses multiple predator species that are released to target the spectrum of greenhouse pests inhabiting plant foliage and soil (Albajes and Alomar 2002, van Lenteren 2012). The most common approach towards greenhouse biological control is through releases of mass-produced, commercially available natural enemies (Bolckmans 2002, van Lenteren 2012). Many of these natural enemies are generalists that prey on or parasitize many greenhouse pest species (Albajes and Alomar 2002, Messelink et al. 2012).

Using generalist predators for biological control is advantageous because generalists can persist on alternative prey in the absence of the target pest (Symondson et al. 2002, Messelink et al. 2012). Unlike chemicals, natural enemies seek out prey and are not limited to the area to which they are applied (Bale et al. 2008). Although these benefits make biological control attractive, there are challenges that make implementation of biological tactics difficult (van Lenteren and Woets 1988, van Lenteren 2012).

Biological control has proven to be at least as cost efficient as and in some cases more efficient than chemical management in successful greenhouse biological control programs (van Lenteren 2000, Bolckmans 2002, Bale et al. 2008, van Lenteren 2012). However, the economic feasibility of augmentative biological control has been disputed because repeated releases of

natural enemies may be necessary and costly. Additionally, because there are many factors influencing the outcomes of biological control, failure to suppress pest densities occur and result in additional pest management approaches —e.g. pesticides.

Open rearing of natural enemies in greenhouses is one means of reducing costs associated with releasing natural enemies. Open rearing is a combination of augmentative releases and conservation biological control used to promote the persistence of natural enemies in greenhouses (Stacey 1977, Kühne 1998, Messelink et al. 2012). 'Open rearing systems' provide natural enemies released in greenhouses with supplemental food or hosts (Stacey 1977, Huang et al. 2011). The alternative food or hosts support natural enemies populations thus offering growers with better opportunities of successfully introducing natural enemies preventatively (i.e. when pest densities are low) and for maintaining natural enemy presence throughout the growing season and during transition of old to new crops.

Promoting the persistence of predators can result in interactions including competition and intraguild predation that may have positive or negative and direct or indirect effects on predators and pests (Janssen et al. 1998, Janssen et al. 2007, Messelink et al. 2012). Furthermore, these interactions are common among natural enemies released in greenhouses (Wittman and Leather 1997, Jandricic et al. 2006, Buitenhuis et al. 2009). In some cases, pest densities increase when multiple predators of the pest are present (Rosenheim 1998). In others, positive predator interactions (e.g. additive or synergistic relationships) result in increased pest suppression (Losey and Denno 1998, Rosenheim 1998). In either case, understanding how natural enemies interact is imperative for promoting successful greenhouse biological control.

Development of open rearing systems for a number of different natural enemies is on the rise (Huang et al. 2011). There are many questions, however, regarding what natural enemies can

be feasibly and economically open reared, optimal release rates, timing of introduction and replacement of individual systems, and how many natural enemies are generated from open rearing systems (Frank 2010, Huang et al. 2011). Furthermore, determining compatibility of natural enemies in open rearing systems with others that are commonly released is essential. Research addressing these topics is needed to facilitate the use of open rearing systems.

My thesis examines open rearing systems for the foliar predatory mites, *Neoseiulus* (=*Amblyseius*) *cucumeris* Oudemans. These mites are one of the most important natural enemies of thrips (Thysanoptera: Thripidae) in greenhouses (Hajek 2004) and have demonstrated efficacy against thrips in greenhouse crops (Gillespie 1989, Shipp and Wang 2003). Thrips develop quickly, are highly fecund, and are serious pests that cause damage to plants by direct feeding and disease transmission (Castañé et al. 2002). Therefore, prompt and effective management of thrips is necessary to protect greenhouse crops. Conventional application of *N. cucumeris* for thrips management involves sprinkling the contents of a carrying tube containing bran flakes, vermiculite, *N. cucumeris* predatory mites, and a low density of *Tyrophagus putrescentiae* (Shank) (Acaridae) mold mites that are eaten by *N. cucumeris,* onto greenhouse crops. More recently, this method has developed into open rearing systems that provide concentrated sources of the mite-bran mixture.

Neoseiulus cucumeris open rearing systems, named 'breeder piles,' are typically placed onto the soil of potted plants and plug trays to provide prolonged management of thrips. Breeder piles are comprised of small (1-3g) piles of a mixture of bran, *T. putrescentiae* mold mites, and *N. cucumeris* predatory mites. In breeder piles, *T. putrescentiae* feed on fungus that grows on the bran and support *N. cucumeris* (Weintraub 2007, van Lenteren 2003). The mite-bran mixture used to generate breeder piles is similar to the mixture used for the sprinkling method, but

contains a higher density of *T. putrescentiae* that sustains more feeding by *N. cucumeris* for longer periods than the sprinkling method mixture. The piles serve as local population centers for *N. cucumeris* and potentially increase the time needed between applications.

The breeder pile method places *N. cucumeris* mites onto the soil, a habitat to which they are not accustomed thus allowing for novel interactions of the mites with soil organisms. In a preliminary study that observed population dynamics of *N. cucumeris* in breeder piles, the predatory rove beetle *Atheta coriaria* (Kraatz) was found in breeder piles within 1 week of their application (Pochubay and Grieshop unpublished). *Atheta coriaria* is an effective predator of soil-dwelling thrips life stages and shore fly (Diptera: Ephydridae) and fungus gnat (Diptera: Mycetophilidae and Sciaridae) larvae (Carney et al. 2002, Birken and Cloyd 2007). It is has demonstrated negative interactions with other natural enemies used in greenhouse biological control (Jandricic et al. 2006). Therefore, the presence of *A. coriaria* in breeder piles provides evidence for potential intraguild predation of *N. cucumeris* within the piles. Chapter 2 of my thesis describes the findings of a greenhouse microcosm study that observed the effect of intraguild predation on *N. cucumeris* in breeder piles by two commercially available soil-dwelling predators, *A. coriaria* and *Stratiolaelaps miles* (Author) (Laelapidae) released to manage thrips pupae and the larvae of fungus gnats and shore flies.

Intraguild predation occurs when two organisms that share a common host or prey kill or consume each other (Polis et al. 1989). One means of promoting coexistence of intraguild predators is to increase habitat complexity (Finke and Denno 2002, Janssen et al. 2007). Slow-release sachets that contain the same mite-bran mixture used to generate breeder piles are an alternative method for open rearing mites that may protect *N. cucumeris* from intraguild

predators. Chapter 3 describes the results from an experiment that observed the use of hanging sachets to reduce intraguild predation of *N. cucumeris*.

Using sachets to protect *N. cucumeris* may influence population dynamics of the mites. Thus, mite densities in breeder piles and sachets are also observed in Chapter 3. Previous research regarding mite production and dispersal from open rearing systems is lacking. The main focus of most studies that have observed predatory mite open rearing systems measure efficacy of management and suppression of pest mites and thrips (Shipp and Wang 2003, Weintraub et al. 2003, van Houten et al. 2005). With the exception of Shipp and Wang (2003) who measured mite dispersal from slow-release sachets, the production and dispersal rates of predatory mites from breeder piles and slow-release sachets have not been observed. In a separate study outlined in chapter 3, mite dispersal from predatory mite open rearing systems is presented.

Merely conducting research into improving thrips biological control does not guarantee grower improved methodologies. Appendix A of my thesis is a bulletin generated for extension purposes that describes open rearing of natural enemies in greenhouses. This chapter outlines commercially available open rearing systems for predatory mites and hymenopteran parasitoids and provides resources to acquire those systems. There is also information regarding other open rearing methods such as the use of guardian plants in greenhouses. The concluding chapter summarizes results and implications of this research. Future research suggestions for natural enemy open rearing systems in greenhouses are also provided.

The goal of my thesis was to identify and manage factors that effect *N. cucumeris* open rearing in greenhouses to improve the use of these systems for thrips biological control. Specific objectives to accomplish this goal were to: 1) identify the potential for and effects of intraguild interactions among predators (Chapter 1), 2) mitigate unfavorable interactions (Chapter 3), 3)

determine predator mite densities produced by different open rearing systems and how long the predators are conserved in the systems (Chapter 3), and 4) provide this information to growers (Appendix A).

CHAPTER 2

Intraguild predation of *Neoseiulus cucumeris* by *Stratiolaelaps miles* and *Atheta coriaria* in greenhouse open rearing systems

1. Introduction

Augmentative biological control tactics using commercially available parasitoids and predators are commonly used in greenhouses. Many of these natural enemies are generalists that prey on or parasitize a spectrum of pests (Messelink et al. 2012). Releasing generalist predators to manage pests can be advantageous because they can persist on alternative prey in the absence of the target pest (Symondson et al. 2002, Messelink et al. 2012). Open rearing of natural enemies (i.e. a combination of augmentative releases and conservation biological control) promote the persistence of biological control agents in greenhouses (Stacey 1977, Messelink et al. 2012). However, lingering predators increase the potential for direct and indirect interactions among concurrently present biological control agents (Janssen et al. 1998, Janssen et al. 2007, Messelink et al. 2012).

Neoseiulus (=*Amblyseius*) *cucumeris* Oudemans is a foliar predator with proven efficacy against thrips (Thysanoptera: Thripidae) in greenhouse crops (Gillespie 1989, Shipp and Wang 2003). A recent development in open rearing of *N. cucumeris* in greenhouses is breeder piles. Breeder piles are small (1-3 g) piles of a mixture of bran, *Tyrophagus putrescentiae* (Shank) (Acaridae) mold mites, and *N. cucumeris* that are placed on the soil of plug trays, beds, and plant pots in greenhouses. *Tyrophagus putrescentiae* mold mites feed on fungus that grows on the bran and are an alternate food source for *N. cucumeris*. Breeder piles serve as local population centers for *N. cucumeris,* potentially increasing the time needed between applications. However, placing

breeder piles on the soil surface may increase *N. cucumeris* vulnerability to intraguild predation from other biological control agents released to manage soil-dwelling thrips pupae (e.g. *Frankliniella occidentalis* (Pergande)) and fungus gnat (Diptera: Sciaridae, Mycetophilidae) larvae and pupae.

Intraguild predation occurs when two organisms that share a common host or prey kill or consume each other (Polis et al. 1989). In some cases intraguild predation is unidirectional, meaning that one predator feeds on another (Polis et al. 1989). This has been observed among several commonly used greenhouse biological control agents such as *Orius laevigatus* Fieber (Hemiptera: Anthocoridae), *N. cucumeris*, and *Iphiseius* (*Amblyseius*) *degenerans* Berlese (Acari: Phytoseiidae), where *O. laevigatus* preyed on both *N. cucumeris* and *I. degenerans* (Wittman and Leather 1997). Intraguild predation may also be bidirectional, where predators prey upon each other (Polis et al. 1989, Rosenheim et al. 1995), as observed between *N. cucumeris* and *Amblyseius swirskii* (Athias-Henriot) (Phytoseiidae) (Buitenhuis et al. 2009). These studies observed intraguild predation among predators that generally occupy similar areas in a crop — e.g. the plant canopy. However, breeder piles introduce *N. cucumeris* mites onto the soil where they do not normally occur thus creating a situation for novel interactions among predators.

Two potential intraguild predators of *N. cucumeris* in breeder piles are the predaceous rove beetle, *Atheta coriaria* (Kraatz), and predatory mite, *Stratiolaelaps* (=*Hypoaspis*) *miles* (Berlese) (Laelapidae). Both predators are also commercially available biological control agents used in greenhouse pest management. *Atheta coriaria* is an effective predator of soil-dwelling thrips life stages and shore fly (Diptera: Ephydridae) and fungus gnat (Diptera: Mycetophilidae and Sciaridae) larvae (Carney et al. 2002, Birken and Cloyd 2007). Similarly, *S. miles* mites

inhabit the soil and are also effective for these pests (Wright and Chambers 1994, Berndt et al. 2004). *Atheta coriaria* and *Hypoaspis aculeifer* are known intraguild predators (Jandricic et al. 2006). In a preliminary study that observed population dynamics of *N. cucumeris* in breeder piles, *A. coriaria* was found in breeder piles within 1 week of their application (Pochubay and Grieshop unpublished). The presence of *A. coriaria* in breeder piles provides evidence for potential intraguild predation within the piles. Although there is little information on the intraguild predatory tendencies of *S. miles,* its polyphagous nature suggests that this interaction is likely to occur (Wright and Chambers 1994).

Our objective was to determine the likelihood and impact of unidirectional intraguild predation by *A. coriaria* and *S. miles* on the temporal population dynamics of *N. cucumeris* and incident thrips populations. We hypothesized that 1) *A. coriaria* and *S. miles* will have direct impacts on *N. cucumeris* populations in breeder piles and soil thus resulting in fewer *N. cucumeris* in the plant canopy, 2) these interactions will affect *T. putrescentiae* mold mite prey populations, and 3) these interactions will also impact incident thrips prey populations.

2. Materials and Methods

We conducted a 5 wk greenhouse study using caged soybean plant (*Glycine max* (L.) Merrill) microcosms to explore the potential of intraguild predation among *N. cucumeris*, *S. miles*, and *A. coriaria*. Our experiment took place in the summer of 2010 at a research greenhouse located at Michigan State University (MSU) (East Lansing, MI USA). We used a randomized complete block design with five blocks and the following treatments: Breeder pile, Breeder pile + *A. coriaria*, Breeder pile + *S. miles,* and Breeder pile + *S. miles* + *A. coriaria*. Breeder piles consisted of a 1 g mixture of bran, *T. putrescentiae* mold mites, and *N. cucumeris* mites.

2.1. Plant culture

Soybean seeds (variety: 92M33) were planted singly in 15 cm (1.33 l) pots containing potting medium. Pots were placed on trays and subirrigated every 1 to 2 days throughout the experiment. The average greenhouse temperature was 29.4ºC and ranged from averages of 27.8ºC at night to 31.7ºC in the late afternoon. No supplemental lighting or fertilizer was used throughout the experiment. We began our experiment one week after sowing the soybeans—when the hypocotyl was extended and the cotyledons were folded down.

2.2. Arthropod culture

We extracted experimental arthropods from 1 l tubes of *Amblyseius*-Breeding-System, *Hypoaspis*-System, and *Atheta*-System supplied by BioBest Biological Systems (Leamington, ON, Canada). We made 105 1 g *N. cucumeris* breeder piles by placing breeder pile material into a 59 ml diet cup. Breeder piles were held for approximately 2 hr prior to introduction into microcosms. Initial Berlese funnel extractions from breeder pile material contained 112 ± 6 (SEM) per g and 881 ± 99 (SEM) per g *N. cucumeris* mites and mold mites, respectively. For treatments containing *S. miles* we made 55 1 g piles of *Hypoaspis*-System material consisting of a mixture of peat, vermiculite, and *S. miles* mites. These piles were treated identically to the *N. cucumeris*. Initial Berlese funnel extractions from *Hypoaspis*-System material contained 18 ± 3 (SEM) *S. miles* mites per g. For treatments containing *A. coriaria* we collected 55 groups of four adult beetles from *Atheta*-System consisting of peat, vermiculite, and *A. coriaria* beetles. Beetles were carefully collected using a natural fiber paintbrush, placed into 59 ml diet cups and held for approximately 2 h prior to introduction into microcosms.

2.3. Microcosm design and set up

We constructed microcosms from potted soybean plants caged with 150 micron polyester multifilament mesh. We used a 40 cm tall 1 mm diameter wire frame to support cage material. We selected 100 healthy, 1 wk-old soybean plants for use in the experiment. The plants were randomly assigned in groups of 25 plants per treatment. The appropriate predator combinations were applied to the soil surface of the individual potted soybean plants at a rate of 1 g of *N. cucumeris* breeder pile material, 1 g of *Hypoaspis*-System and four adult *A. coriaria* beetles. The caged soybeans were placed on trays on greenhouse benches in a randomized complete block design with five blocks and five replicates of each treatment per block.

2.5. Sampling Procedure

We randomly selected five caged soybean plants per treatment per block for destructive sampling at weekly intervals over the 5 wk experimental period. Plants selected for sampling were carefully transported to the laboratory where both foliar and soil dwelling arthropods were extracted and quantified. Foliar arthropods were removed from the plant using a mite brushing machine (Leedom Engineering, Route 1, Box 325, Twain Harte, CA). Each plant was passed through the mite brush twice and arthropods collected in a Petri dish containing 20 ml of 95% ethanol solution. We extracted arthropods residing in the breeder piles and soil using Berlese funnels (Bioquip #2832, Rancho Dominguez, CA) with each sample placed into an individual funnel. We recorded initial funnel temperatures and gradually increased funnel temperatures over the first 48 hr to at least 50ºC. We maintained funnels at this temperature for an additional 102 hr. Specimens were collected into 50 ml centrifuge vials filled with 95% ethanol. Foliar and soil dwelling arthropods were identified and counted using a dissecting microscope.

2.6. Data analysis

Data were analyzed using appropriate parametric and nonparametric methods in the R statistical language (R core development team 2011). Post hoc multiple comparisons were made using Tukey's Honest Significant Difference for all ANOVA analyses.

2.6.1. Analysis of *N. cucumeris* in plant canopy

Foliar measurements of *N. cucumeris* populations were compared among treatments using a two way ANOVA with factors: block, experimental treatment, and week. Data were normalized using a $\log_{10}(x + 1)$ transformation. Main effects with $P > 0.05$ removed from the model.

2.6.2. Analysis of *N. cucumeris* in soil and breeder piles

Foliar and soil/breeder pile measurements of *N. cucumeris* populations were compared among treatments using a two way ANOVA with factors: block, experimental treatment, and week. Data were normalized using a square root $(x + 0.375)$ transformation (Kuehl 2000). Main effects with $P > 0.05$ were removed from the model.

2.6.3. Analysis of *T. putrescentiae* in soil and breeder piles

Soil/breeder pile measurements of *T. putrescentiae* mold mite populations were compared among treatments using a two way ANOVA with factors: block, experimental treatment, and week. Data were normalized using a $\log_{10}(x + 1)$ transformation. Main effects with $P > 0.05$ were removed from the model.

2.6.4. Analysis of Thrips: *F. occidentalis* and *Thrips* sp. in plant canopy

The numbers of incident thrips brushed from the plant canopy were also compared across treatments. Data could not be normalized using transformations. Therefore, data were analyzed using Kruskal-Wallis rank sum tests. The number of thrips in treatments were compared in all paired treatment combinations at each week interval.

2.6.5. *Atheta coriaria* beetles in soil and breeder piles

Soil/breeder pile measurements of *A. coriaria* populations were compared between Breeder piles + *A. coriaria* and Breeder piles + *S. miles* + *A. coriaria* treatments using a two way ANOVA with factors: block, experimental treatment, and week. Data were normalized using a square root (x + 0.375) transformation (Kuehl 2000). We removed main effects with $P > 0.05$ from the model.

We also compared *A. coriaria* beetle populations between Breeder piles + *A. coriaria* and Breeder piles + *S. miles* + *A. coriaria* treatments at each week interval. These data could not be normalized using transformations. Therefore, the numbers of *A. coriaria* in treatments at each week interval were analyzed using Kruskal-Wallis rank sum tests.

2.6.6. *Stratiolaelaps miles* mites in soil and breeder piles

Soil/breeder pile measurements of *S. miles* populations were compared between Breeder piles + *S. miles* and Breeder piles + *S. miles* + *A. coriaria* treatments. Data could not be normalized using transformations. Therefore, data were analyzed using Kruskal-Wallis rank sum tests. The numbers of *S. miles* mites in treatments were compared at each week interval.

3. Results

The most prevalent organisms extracted from soil and breeder piles samples were *T. putrescentiae* mold mites*, N. cucumeris*, *A. coriaria,* and *S. miles*. However, thrips, fungus gnat adults and larvae, collembola, and other mites were also extracted.

3.1. *Neoseiulus cucumeris* mites in plant canopy

We found significant effects for treatment, week, and the treatment by week interaction for foliar *N. cucumeris* samples ($F_{3,80} = 11.668$, $P < 0.0001$; $F_{4,80} = 181.238$, $P < 0.0001$; $F_{12,80} = 3.999$, $P < 0.0001$, respectively). Microcosms with breeder piles and lacking additional predators were found to have significantly more *N. cucumeris* than microcosms containing *A. coriaria* (Breeder pile vs. Breeder pile + *A. coriaria*, $P < 0.0001$; Breeder pile vs. Breeder pile + *S. miles* + *A. coriaria*, $P < 0.0001$) (Fig.2.1). Significant effects of week were found in all week comparisons (Fig.2.1). Significant interaction effects occurred among treatments at weeks 3, 4, and 5 (Fig.2.1). In week 3, Breeder pile alone displayed significantly higher *N. cucumeris* numbers (28.2 ± 5.24) than *N. cucumeris* numbers in other treatments (≤ 16.6 ± 5.12). In week 4, Breeder pile (70 ± 4.62) and Breeder pile + *S. miles* (69.2 ± 13.53) displayed the highest *N. cucumeris* numbers that were not significantly different from each other, but were significantly greater than numbers in treatments containing *A. coriaria* (≤ 43 ± 9.32) (Fig.2.1). In week 5, numbers of *N. cucumeris* in all treatments were significantly different from each other; Breeder pile displayed the greatest number of *N. cucumeris* (114.2 ± 19.39), followed by Breeder pile + *S. miles* (42.4 ± 6.87), Breeder pile + *S. miles* + *A. coriaria* (22.6 ± 3.09), and Breeder pile + *A. coriaria* (9.2 ± 1.88) (Fig.2.1).

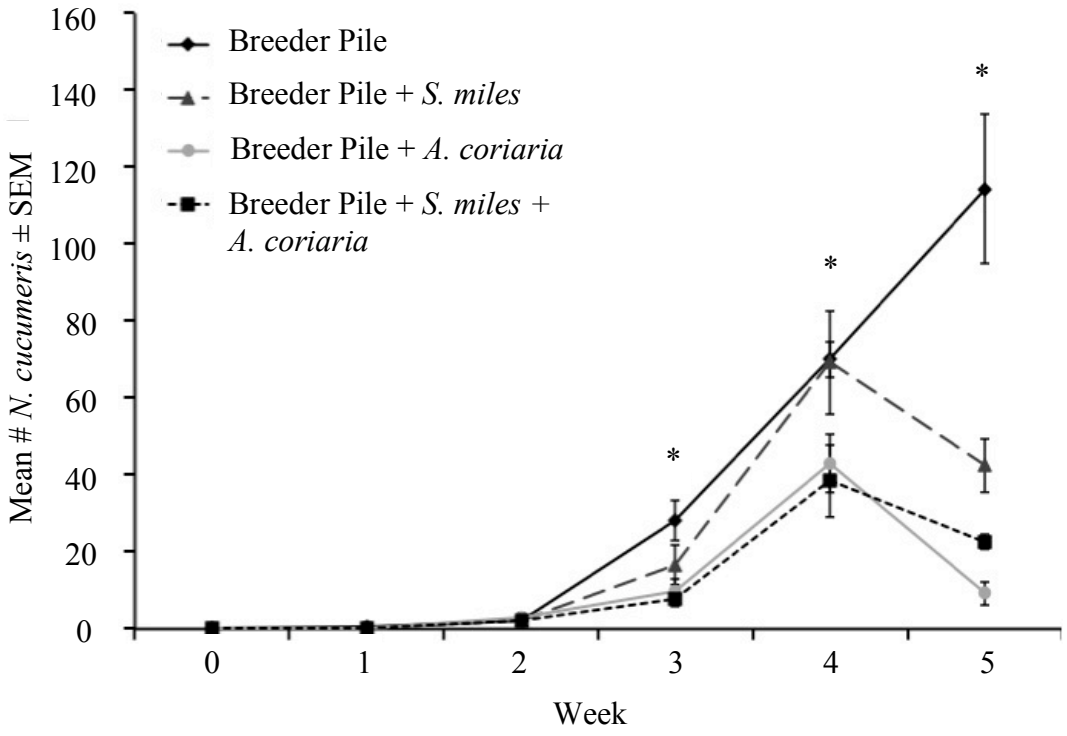

Fig. 2.1. Mean number of *N. cucumeris* (± SEM) sampled from plant canopy. * indicates significant differences among treatments in each week. Microcosms with only breeder piles had significantly more *N. cucumeris* than treatments containing *A. coriaria* (Tukey's HSD, α=0.05).

3.2. *Neoseiulus cucumeris* mites in soil and breeder piles

We found significant effects of treatment, week, and treatment by week interaction for the number of *N. cucumeris* recovered from soil samples ($F_{3,80}$ = 31.405, P < 0.0001; $F_{4,80}$ = 10.584, P < 0.0001; $F_{12,80}$ = 7.232, P < 0.0001, respectively). We also found significant differences in numbers of *N. cucumeris* observed among treatments at weeks 2 and 3 (Fig. 2.2). In week 2, Breeder pile microcosms had significantly more *N. cucumeris* (210 ± 25.6) compared to other treatments (≤ 83 ± 12.8) (Fig.2.2). In week 3, the greatest number of *N. cucumeris* were observed in Breeder pile (456 ± 90.9) compared to other treatments (≤ 127.4 ± 32.7) (Fig.2.2). The Breeder pile + *S. miles* treatment had the second highest number of *N. cucumeris* (127.4 ±

32.0) that was significantly greater than numbers of *N. cucumeris* in treatments containing *A. coriaria* (≤ 42 ± 20.96) (Fig.2.2). We did not find significant differences in numbers of *N. cucumeris* between treatments containing *A. coriaria* (Fig.2.2).

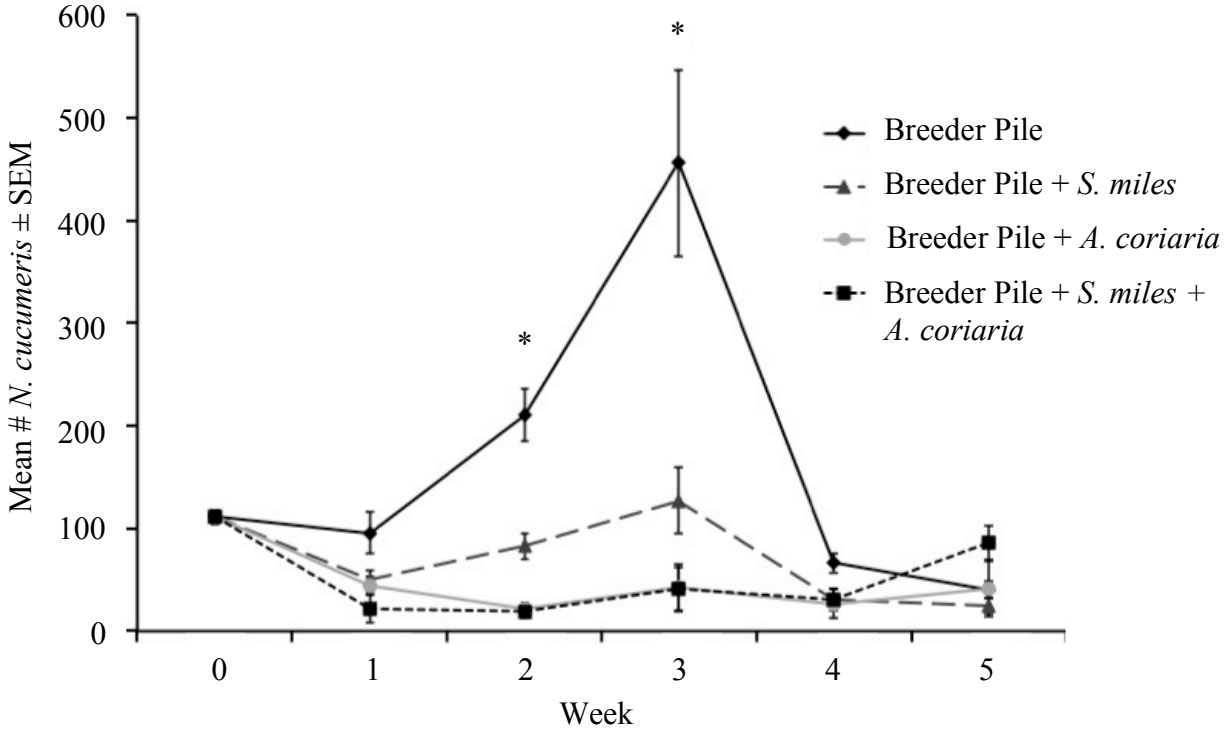

Fig. 2.2. Mean number of *N. cucumeris* (± SEM) extracted from soil and breeder piles. * indicates significant differences among treatments in each week. Microcosms with only breeder piles had significantly more *N. cucumeris* than other treatments (Tukey's HSD, α=0.05).

3.3. *Tyrophagus putrescentiae* mold mites in soil and breeder piles

We found significant effects of treatment and week for *T. putrescentiae* mold mites extracted from soil and breeder pile samples *($F_{3,92}$* = 18.910, $P < 0.0001$ and $F_{4,92}$ = 10.504, $P < 0.0001$, respectively). Overall, the number of mold mites extracted from Breeder pile (2428.16 ± 452.24) and Breeder pile + *S. miles* (2763.08 ± 475.71) were significantly greater than the other treatments (≤ 1049.28 ± 301.72; $P < 0.005$), but not significantly different from each other (Fig.1d). There were significant effects of week in weeks 4 and 5 ($P < 0.01$) (Fig.2.3).

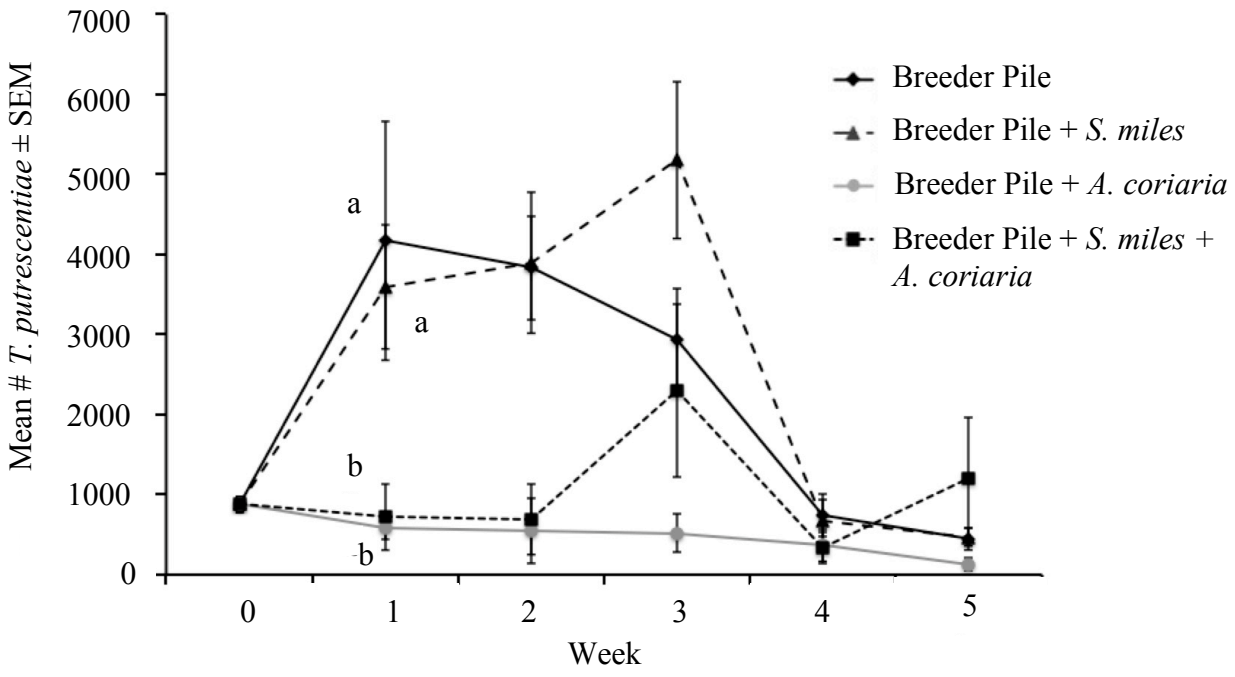

Fig. 2.3. Mean number of *T. putrescentiae* (± SEM) extracted from soil and breeder piles. Breeder pile and Breeder Pile + *S. miles* treatments had significantly more *T. putrescentiae* than Breeder Pile + *A. coriaria* and Breeder Pile + *S. mile* + *A coriaria* treatments over 5 weeks. Letters indicate significant differences among treatments overall (Tukey's HSD, α=0.05).

3.4. Thrips: *F. occidentalis* and *Thrips* sp. in plant canopy

Thrips were observed in all treatments in both brushed plant canopy samples and soil and breeder piles samples, but the number of thrips recovered from soil and breeder piles was too small for meaningful data analysis (< 4 thrips per treatment per week). Enough thrips were recovered from canopy samples for data analysis. There were marginal and significant differences in the number of thrips in the canopy at weeks 2, 3, 4, and 5 (Fig.2.4). In week 2, the number of thrips in Breeder pile + *S. miles* + *A. coriaria* (2.60 ± 0.87) was significantly greater than in Breeder pile (0.20 ± 0.20) ($df = 1$, $H = 4.513$, $P = 0.0337$) and Breeder pile + *S. miles* (0.20 ± 0.20) ($df = 1$, $H = 4.513$, $P = 0.0337$), but was not significantly greater than those in

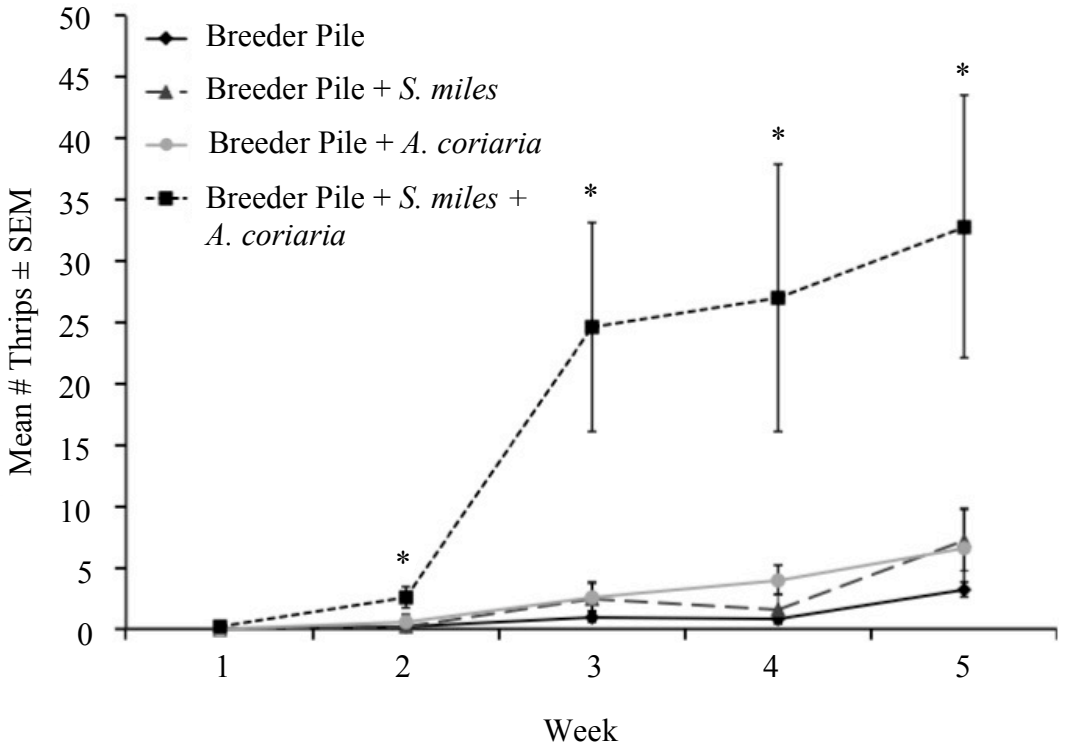

Fig. 2.4. Mean number of thrips (*Frankliniella occidentalis* and *Thrips* sp.) (± SEM) sampled from plant canopy. * indicate significant differences among treatments in that week. Significantly more thrips were observed in Breeder Pile + *S. miles* + *A. coriaria* in weeks 2, 3, 4, and 5 compared with other treatments (Kruskal-Wallis, α=0.05).

Breeder pile + *A. coriaria* (0.60 ± 0.60) (Fig.2.4). In week 3, significantly more thrips were observed in Breeder pile + *S. miles* + *A. coriaria* (24.6 ± 8.50) than in Breeder pile (1.0 ± 0.45) ($df = 1$, $H = 4.444$, $P = 0.03501$) and Breeder pile + *S. miles* (2.40 ± 1.25) ($df = 1$, $H = 4.036$, $P = 0.0445$) (Fig.2.4). In weeks 4 and 5, significantly more thrips were observed in Breeder pile + *S. miles* + *A. coriaria* (27.0 ± 10.93 and 32.80 ± 10.73, respectively) than in other treatments (≤ 7.2 ± 2.46) ($df = 1$, $H < 6.944$, $P < 0.05$) (Fig.2.4). Marginal differences were also observed in weeks 3 and 4. In week 3, marginally more thrips were found in Breeder pile + *S. miles* + *A. coriaria* (24.6 ± 8.50) compared to Breeder pile + *A. coriaria* (2.60 ± 1.21) ($df = 1$, $H = 3.556$, $P = 0.0593$). In week 4, marginally more thrips were found in Breeder pile + *A. coriaria* (4.0 ± 1.18)

compared to Breeder pile (0.8 ± 0.37) (*df* = 1, *H* = 3.272, *P* = 0.0705) (Fig.2.4).

3.5. *Atheta coriaria* in soil and breeder piles

Significantly more *A. coriaria* were recovered from microcosms where Breeder pile + *A. coriaria* were introduced than from microcosms where Breeder piles + *A. coriaria* + *S. miles* were introduced ($F_{1,48}$ = 8.826, *P* = 0.0046) (Table 2.1). We observed significantly more *A. coriaria* beetles in Breeder pile + *A. coriaria* (39.40 ± 6.03) in week 5 compared to Breeder pile + *S. miles* + *A. coriaria* (12.20 ± 5.64) (*df* = 1, *H* = 5.3778, *P* = 0.0204) and marginally more in Breeder pile + *A. coriaria* (66.40 ± 22.14) compared to Breeder pile + *S. miles* + *A. coriaria* (11.00 ± 5.89) in week 3 (*df* = 1, *H* = 3.172, *P* = 0.0749) (Table 2.1).

Table 2.1. Mean number of *A. coriaria* ± SEM for treatments containing *A. coriaria*. * indicates significant differences among treatments in that week. In week 5, significantly more *A. coriaria* were observed when *S. miles* were not present (Kruskal Wallis, α=0.05).

Week	Breeder Pile + *A. coriaria*	Breeder Pile + *S. miles* + *A. coriaria*	
1	16.00 ± 08.79	7.20 ± 04.50	
2	22.00 ± 09.82	30.20 ± 14.65	
3	66.40 ± 22.14	11.00 ± 05.89	
4	55.00 ± 16.71	14.80 ± 06.95	
5	39.40 ± 06.03	12.20 ± 05.64	*

3.6. *Stratiolaelaps miles* in soil and breeder piles

There was no effect of treatment for *S. miles* densities in Breeder pile + *S. miles* and Breeder pile + *S. miles* + *A. coriaria* at any week (Table 2.2).

4. Discussion

Previous research has demonstrated that *N. cucumeris* predatory mites can serve as either prey or predators in intraguild predatory interactions among biological control agents (Wittman and Leather 1997, Buitenhuis et al. 2009). In our study we have provided unequivocal evidence that *N. cucumeris* in breeder piles are detrimentally impacted by the presence of the soil-dwelling

predators *S. miles* and *A. coriaria*. This impact is most evident in soil and breeder piles and subtler in the plant canopy, and more detectable over time (Fig.2.1, 2.2). Thus, our findings support the hypothesis that *S. miles* and *A. coriaria* are unidirectional intraguild predators of *N. cucumeris* on the soil surface and that these interactions result in fewer *N. cucumeris* mites in the plant canopy. These findings also suggest that *A. coriaria* observed invading breeder piles in commercial greenhouses are likely consuming mites in breeder piles.

Table 2.2. Mean number of *S. miles* ± SEM for treatments containing *S. miles*. * indicates significant differences among treatments. There was no significant difference in the mean numbers of *S. miles* in treatments (Kruskal Wallis, α=0.05).

Week	Breeder Piles + *S. miles*	Breeder Pile + *S. miles* + *A. coriaria*
1	11.40 ± 5.73	1.40 ± 0.51
2	4.80 ± 1.88	8.20 ± 3.94
3	30.40 ± 9.09	10.40 ± 6.05
4	10.20 ± 5.70	2.00 ± 0.84
5	14.00 ± 7.05	13.40 ± 7.14

Although our experiment was not designed to observe *N. cucumeris* as a potential intraguild predator of *S. miles* and *A. coriaria*, previous research has shown that adult predatory mites will feed on younger mites and eggs of the other species (Wittman and Leather 1997, Buitenhuis et al. 2009). Adult mites will also feed on *A. coriaria* eggs and larvae (Jandricic et al. 2006). Therefore, bidirectional intraguild predation is possible among these predators. However, the impact of *N. cucumeris* on *S. miles* and *A. coriaria* is likely negligible because its foliar nature makes it unlikely to forage in the soil (McMurtry and Croft 1997).

The densities of *A. coriaria* and *S. miles* were monitored to indicate the presence and abundance of these predators in the microcosms. Overall, the numbers of *A. coriaria* beetles in treatments were greater when *S. miles* were not present (Table 2.1). This may indicate negative

interactions among *A. coriaria* beetles and *S. miles* as observed in previous research (Jandricic 2006). We also observed numerical differences in the numbers of *S. miles* between treatments, where in some weeks there were up to 50% fewer *S. miles* when *A. coriaria* were present (Table 2.2). However, variation in the data prevented detection of significant differences. Our objective was not to observe intraguild predation between *A. coriaria* and *S. miles*, but these results provide evidence for potential interactions that should be further investigated.

We found interesting results supporting the hypothesis that interactions among *N. cucumeris*, *S. miles*, and *A. coriaria* have direct impacts on mold mites. Similar to the effect of *A. coriaria* beetles on *N. cucumeris*, the presence of beetles had a large negative impact on mold mite populations (Fig.2.3). This suggests that *A. coriaria* is a superior intraguild competitor compared to *N. cucumeris* and thus the two predators are very unlikely to coexist (Holt and Polis 1997). It is also possible that *A. coriaria* competed with the mold mites because *A. coriaria* has been reported to feed on oatmeal and oatmeal containing mold or fungus (Birken and Cloyd 2007), similar to the food source provided for mold mites in the breeder pile system.

It is likely that *S. miles* fed on mold mites (Enkegaard et al. 1997). However, the impact of *S. miles* on mold mites was no different than that of *N. cucumeris* (Fig.2.3). Hence, low mold mite populations were largely due to *A. coriaria* when all three predators were present. Furthermore, more *N. cucumeris* and mold mites persisted in the microcosms lacking *A. coriaria*. Based on these findings *S. miles* and *N. cucumeris* breeder piles may be more compatible than *A. coriaria* and *N. cucumeris* breeder piles.

Although plants were not intentionally infested with thrips, these ubiquitous greenhouse pests (Castañá et al. 2002) were present on the soybean plants and provided a compelling outcome. Our results suggest that there was less effective thrips suppression when *N. cucumeris*

breeder piles, *S. miles*, and *A. coriaria* were concurrently present (Fig.2.4). Thrips suppression seemed to be effective when *N. cucumeris* breeder piles were alone or in conjunction with either *S. miles* or *A. coriaria* (Fig.2.4). Interestingly, there were numerically fewer predators in microcosms containing all three predators compared to the other treatments. This was probably a consequence of intraguild predation among *N. cucumeris*, *S. miles*, and *A. coriaria* where thrips were provided an opportunity to evade predation.

A future research opportunity raised by our study would be to investigate full factorial predator combinations of *N. cucumeris*, *S. miles*, and *A. coriaria* to determine compatibility of the predators and the impact of these combinations on thrips. Another possibility would be to explore methods for reducing negative interactions among these biological control agents. One approach to mitigating intraguild predation of *N. cucumeris* mites by *S. miles* and *A. coriaria* would be to increase habitat structure (Janssen et al. 2007). This could be achieved by using an alternative application method such as 'hanging sachets.' Hanging sachets are small paper or fabric envelopes that contain a similar mite-bran mixture as the breeder piles (i.e. bran, mold mites, *N.* cucumeris) and are hung into the plant canopy. Hanging sachets protect and provide refuge for *N. cucumeris*. Furthermore, partitioning the space of the predators reduces their encounters and improves *N. cucumeris* open rearing (Pochubay 2012, Chapter 3).

Our research demonstrated that intraguild predation among *N. cucumeris*, *A. coriaria*, and *S. miles* is likely to occur when *N. cucumeris* is applied using the breeder pile method, thus limiting the number of predatory mites produced by breeder piles. These findings suggest that *A. coriaria* observed invading breeder piles in commercial greenhouses (Jeanne Himmelein, personal communication) are likely consuming the mites in breeder piles. Breeder piles are intended to increase the time needed between applications of *N. cucumeris*. Unfortunately,

intraguild predation may hinder their purpose. However, predator composition influenced by predator combination and their relative densities, and the availability of shared resources influence the magnitude of intraguild predation that may occur in greenhouses. The presence of thrips in our study provided us with insight as to how predator composition may impact thrips management. Future research should continue to explore intraguild predation among generalist biological control agents and how these interactions influence pest densities at larger scales. A better understanding of these interactions and the associated impact on pest population dynamics would improve and promote biological pest management in greenhouses.

CHAPTER 3

Slow-release sachets of *Neoseiulus cucumeris* predatory mites reduce intraguild predation by *Atheta coriaria* in greenhouse biological control systems

1. Introduction

Biological control tactics for greenhouse arthropod pest management are an economical and attractive alternative to chemical tactics because of greatly lowered risks of phytotoxicity, worker and consumer exposure to harmful chemicals, and development of pest resistance (van Lenteren 2000). In greenhouse biological control, multiple predator species are often released to target the spectrum of greenhouse pests inhabiting plant foliage and soil. Natural enemy open rearing systems —*i.e.* providing natural enemies released in greenhouses with supplemental food or hosts— are used to reduce associated costs of augmentative natural enemy releases by maintaining populations of natural enemies in the greenhouse (Stacey 1977, van Steenis 1992, Huang et al. 2011). Releasing multiple predators can often result in interactions including competition and intraguild predation that may have positive or negative and direct or indirect effects on predators and pests (Janssen et al. 1998, Janssen et al. 2007, Messelink et al. 2012).

Intraguild predation is common among natural enemies released in greenhouse biological control programs (Wittman and Leather 1997, Jandricic et al. 2006, Buitenhuis et al. 2009). Studies observing intraguild predation among natural enemies primarily focus on organisms that occupy similar areas in the crop (e.g. the plant canopy or soil). However, some methods for natural enemy release and open rearing place natural enemies in unaccustomed habitats resulting in opportunities for unexpected interactions among predators. For example, *Neoseiulus cucumeris* mites are predators of early instar thrips that normally inhabit plant canopies (Wittmann and Leather 1997, Shipp and Wang 2003). Soil-dwelling predators of thrips pupae

and shore fly and fungus gnat larvae, *Atheta coriaria* and *Stratiolaelaps miles* (Wright and Chambers 1994, Carney et al. 2002, Berndt et al. 2004, Birken and Cloyd 2007), have been shown to detrimentally impact *N. cucumeris* that are placed on the soil (Pochubay and Grieshop in review). Intraguild predation has been shown to be especially intense when populations of *N. cucumeris* are maintained using "breeder pile" open rearing systems (Pochubay and Grieshop in review).

Breeder pile open rearing systems are comprised of small (1-3g) piles of a mixture of bran, *Tyrophagus putrescentiae* mold mites, and *N. cucumeris* predatory mites. Breeder piles are typically placed onto the soil of potted plants and plug trays to provide prolonged management of thrips. The bran supports *T. putrescentiae*, an alternative prey for *N. cucumeris*. Breeder piles are intended to reduce the number of *N. cucumeris* releases. Unfortunately, intraguild predation of the mites by soil-dwelling predators hinders their purpose. Therefore, alternative application methods for *N. cucumeris* that reduce the potential for intraguild predation should be investigated.

One means of promoting coexistence of intraguild predators is to increase habitat complexity (Janssen et al. 2007). Slow-release sachets —paper envelopes— that contain the same mite-bran mixture used to generate breeder piles are an alternative method for releasing mites that may protect *N. cucumeris* from intraguild predators. Furthermore, protecting *N. cucumeris* may influence population dynamics of the mites. These dynamics should be investigated to improve procedures for implementing open rearing systems and maintaining *N. cucumeris* in greenhouses.

Population dynamics of predatory mites in open rearing systems have not been thoroughly investigated. The main focus of studies that have observed predatory mite open

rearing systems measure efficacy of management and suppression of pest mites and thrips (Shipp and Wang 2003, Weintraub et al. 2003, van Houten et al. 2005). With the exception of Shipp and Wang (2003) who measured mite dispersal from slow-release sachets, the production and dispersal rates of predatory mites from breeder piles and slow-release sachets have not been observed. Different dispersal rates of mites may influence timing of releases and appropriate conditions such as optimal pest density and plant maturity for introducing predatory mite open rearing systems. Revealing the temporal production and dispersal of mites from these systems would provide us with better insight to optimize the timing of future releases and promote economical release procedures.

We conducted two experiments to address our objectives. In the first study, our objectives were to determine whether *N. cucumeris* sachets hung in the plant canopy prevent *A. coriaria* from entering the mite-bran mixture, and the abundance of *A. coriaria* in breeder piles, bran piles (without mites), sawdust piles, or hanging sachets. We also monitored population dynamics of *N. cucumeris* and mold mites in these treatments. In a second study, our objective was to observe the numbers of mites that dispersed from breeder piles and sachets when *A. coriaria* was not present.

2. Experiment One Materials and Methods

Our first experiment was conducted in spring 2011 and was repeated in fall 2011 in a 4-acre certified organic greenhouse at Elzinga and Hoeksema Greenhouses (Portage, MI). The greenhouse was climate regulated by a Hoogendoorn computer control system (Hoogendoorn Growth Management, The Netherlands) and in production of lettuce and herb plants. In this experiment we placed breeder piles, bran piles that did not contain mites, sawdust piles, and hanging sachets in a randomized complete block design on greenhouse benches containing

barley. *Atheta coriaria* beetles were released on the benches. Breeder, bran, and sawdust piles, and hanging sachets were randomly selected and destructively sampled at weekly intervals for 9 weeks. We measured and compared densities of *A. coriaria*, *N. cucumeris*, and *T. putrescentiae* in the samples.

2.1. Growing Barley

To prevent unintentional rearing of greenhouse pests, barley was selected as an experimental habitat for the experiment. Five greenhouse benches (1.68 m x 4.88 m) were lined with sheets of plastic with small drainage holes and filled with 13 cm of potting soil mix provided by Morgan Composting (Sears, MI, USA). Certified organic barley seed from Albert Lea Seed (Albert Lea, MN, USA) and Johnny's Selected Seeds (Waterville, MA, USA) was sown by hand onto each of the benches at a rate of 226.8 g per 8.2 m^2 for the first and second trial, respectively. Barley was grown under natural light for one week before introduction of treatments. Barley beds were irrigated daily using an overhead irrigation boom for the duration of the experiment.

2.2. Predator and Treatment Preparation

Two 1 l containers of Amblyseius-Breeding-System, two 1 l containers of bran (without mites), two containers of Atheta-System (100 adult *A. coriaria* per container), and mini sachets were provided by Biobest Biological Systems (Ontario, Ca.). Sawdust (i.e. small animal pet bedding) was purchased from a pet supply store in Okemos, MI, USA. Predators were stored in a refrigerator at approximately 15-16ºC for 24 hrs prior to introduction. To standardize predator release and pile sizes, 60 1.5 g of each treatment: Amblyseius-Breeding-System, bran piles, and sawdust were measured into 59 ml soufflé cups in the experimental greenhouse immediately

prior to introduction. The 1.5 g pile size was chosen because the amount of mite-bran mixture in the sachets was found to be approximately 1.5 g.

The numbers of mites in treatments were determined by Berlese funnel (Bioquip #2832, Rancho Dominguez, CA) extractions conducted in the lab on 10 samples from every treatment. Initial Berlese funnel extractions from breeder pile material contained 277 ± 18.73 (SEM) *N. cucumeris* per 1.5 g and 887 ± 65.15 (SEM) *T. putrescentiae* mold mites per 1.5 g in the first trial and 243.00 ± 24.25 (SEM) *N. cucumeris* per 1.5 g and 575.00 ± 58.11 (SEM) *T. putrescentiae* mold mites per 1.5 g in the second. Sachets contained 280.00 ± 25.23 (SEM) *N. cucumeris* per sachet and 2496.20 ± 65.45 (SEM) *T. putrescentiae* per sachet in the first trial and 482.00 ± 27.85 (SEM) *N. cucumeris* per sachet and 2980.20 ± 277.33 (SEM) *T. putrescentiae* per sachet in the second. No organisms were extracted from initial samples of sawdust and bran piles in either trial.

2.3. Experimental Design and Methodology

After one week of barley growth 50 1.5 g piles of sawdust, bran, breeder piles, and sachets were equally spaced in a randomized complete block design across barley beds. Sawdust, bran, and breeder piles were poured from soufflé cups into small piles on the soil surface of the barley beds. The sachets were hung on cardstakes. Once treatments were in place, Atheta-System containers (containing 200 adult *A. coriaria*) were evenly distributed over barley beds.

2.4. Sampling Procedure

Five piles of sawdust, bran, and breeder piles, and five hanging sachets were randomly selected at weekly intervals over a nine week period. Samples were placed into 59 ml soufflé cups, and transported to a lab at Michigan State University for processing. Organisms in piles

and hanging sachets were extracted into 95% ethanol using Berlese funnels. Week 10 samples were not taken because collecting of samples ceased when mite densities extracted in previous weeks were low and when barley senesced. Organisms extracted from samples were counted using a dissecting microscope.

2.5. Data Analysis

2.5.1. *Atheta coriaria* extracted from samples

The number of *A. coriaria* in treatments were compared at weeks 1 through 7 to detect potential preferences of *A. coriaria* in these weeks. Data analysis for weeks 8, 9, and 10 was not included due to insufficient *A. coriaria* numbers. Data could not be normalized using transformations. Thus, we compared the overall numbers of *A. coriaria* in treatments and the numbers of *A. coriaria* in treatments at each week using non-parametric Kruskal-Wallis rank sum tests in R (R core development team 2011).

2.5.2. *Neoseiulus cucumeris* and *T. putrescentiae* extracted from samples

Because initial numbers of *N. cucumeris* and *T. putrescentiae* introduced in breeder piles and sachets were dissimilar (Fig. 3.2 and 3.3) we analyzed the proportion change of each population rather than the actual count. Proportion change was calculated by subtracting the number average starting mite number from the weekly count and dividing this by the starting mite number.

This calculation resulted in percent increases and decreases of *N. cucumeris* and *T. putrescentiae* in samples. No *N. cucumeris* or *T. putrescentiae* were introduced in Bran and Sawdust piles thus this data was not included in the analysis. Data for weeks 8 and 9 in trial 1 were not analyzed due to insufficient numbers of *N. cucumeris* and *T. putrescentiae*. The

proportions of *N. cucumeris* and *T. putrescentiae* recovered in treatments could not be normalized using transformations and were compared using Kruskal-Wallis rank sum tests in R (R core development team 2011).

3. Experiment Two Materials and Methods

In this experiment we monitored mite dispersal from breeder piles and sachets when *A. coriaria* was not present. The experiment was conducted in spring 2011 and repeated in fall 2011 in a Michigan State University greenhouse (East Lansing, MI). The experimental greenhouse was computer climate regulated by ventstat (Micro Grow Greenhouse Systems, Inc., Temecula, CA) and Sunne Controls thermostat (Detroit Radiant Products Co., Warren, MI) set at 24ºC. Dispersing mites were collected on yellow sticky cards at weekly intervals for 9 weeks. We measured densities of *N. cucumeris* and *T. putrescentiae* on yellow sticky cards.

3.1. Growing Barley

Similar to the first experiment, we used barley as an experimental habitat. A total of 20 plastic containers 30 cm x 38 cm x 20 cm were filled with potting soil containing peat moss, and perlite. Organic barley seed purchased from Johnny's Selected Seeds (Waterville, MA, USA) was sown by hand onto the soil of the containers at an approximate rate of 2 g per 930 cm^2. Barley was grown under natural light. After one week of growth, 14 containers of barley were selected for use in the experiment.

3.2. Predator and Treatment Preparation

Two 1 l container of Amblyseius-Breeding-System were provided by Biobest Biological Systems (Ontario, Ca.). The Amblyseius-Breeding-System mite-bran mixture was measured into 38 1.5 g piles and placed into 59 ml diet cups. These piles were used to generate breeder piles

and hanging sachets. We fabricated hanging sachets by pouring one of the 1.5 g piles measured into the 59 ml diet cups into an empty sachet. The opening on sachets into which the mite-bran mixture was poured (i.e. not the same as the hole from which mites leave sachets) was sealed with a piece of clear plastic tape. To determine the number of mites in treatments, Berlese funnel extractions were conducted in the lab on 7 samples from breeder piles and sachets. Initial Berlese funnel extractions from breeder pile material used for breeder piles and placed in sachets contained 266 ± 15.25 (SEM) *N. cucumeris* per 1.5 g and 2803.46 ± 101.13 (SEM) *T. putrescentiae* mold mites per 1.5 g in the first trial and 130.5 ± 7 (SEM) *N. cucumeris* per g and 911.36 ± 30.24 (SEM) *T. putrescentiae* mold mites per 1.5 g in the second trial.

3.3. Experimental Design and Methodology

Containers of barley were evenly spaced and randomly placed onto a greenhouse bench. A circular yellow sticky card (16.5 cm in diameter) was placed in the center of the barley bed. A circular piece of wax paper (10.5 cm in diameter) was placed in the center of the yellow sticky card. Petri dishes (100 mm) filled with potting soil were placed onto the wax paper. The 1.5 g breeder piles were poured and sachets were randomly placed on the center of the soil in petri dishes. Overhead irrigation was simulated by watering the barley and treatments with an irrigation wand and breaker twice per day.

3.4. Sampling Procedure

We collected and replaced sticky cards at a weekly interval over a period of nine weeks for each trial. *Neoseiulus cucumeris* mites and *T. putrescentiae* on the cards were counted using a dissecting microscope.

3.5. Data Analysis

3.5.1. *Neoseiulus cucumeris* predatory mites

Data were normalized by transforming the number of *N. cucumeris* extracted from samples using $\log_{10}(x + 1)$ transformation. Normalized data were analyzed in R (R core development team 2011) using a two way ANOVA with factors: block, experimental treatment, and week. Main effects with $P > 0.05$ were removed from the model. Post hoc multiple comparisons were made using Tukey's Honest Significant Difference.

3.5.2. *Tyrophagus putrescentiae* mold mites

Data could not be normalized using transformations. Therefore, significant differences between the numbers of *T. putrescentiae* in treatments were detected using weekly non-parametric Kruskal-Wallis rank sum tests in R (R core development team 2011).

4. Experiment One Results

4.1. *Atheta coriaria* extracted from samples

Trial 1. We found significantly more *A. coriaria* in Bran (3.74 ± 0.85) and Breeder pile (2.37 ± 0.55) when compared with Sachet (0.37 ± 0.17) and Sawdust (0.17 ± 0.09) piles overall ($df = 1$, $H \geq 10.6622$, $P < 0.001$) (Fig. 3.1). In week 1, Bran (7.2 ± 1.685) and Breeder piles (6.6 ± 1.913) had significantly higher *A. coriaria* numbers than Sachets (0 ± 0) ($df = 1$, $H = 7.8125$, $P = 0.0052$) and Sawdust piles (0 ± 0) ($df = 1$, $H = 7.8125$, $P = 0.0052$), but were not significantly different from each other (Fig. 3.1). Bran (12.4 ± 2.9428) and Breeder piles (6.6 ± 0.5099) had significantly higher *A. coriaria* numbers than Sachets (0 ± 0) ($df = 1$, $H = 7.8125$, $P = 0.0052$) and Sawdust piles (0.6 ± 0.4) ($df = 1$, $H = 7.8125$, $P = 0.0052$), but were not significantly differ-

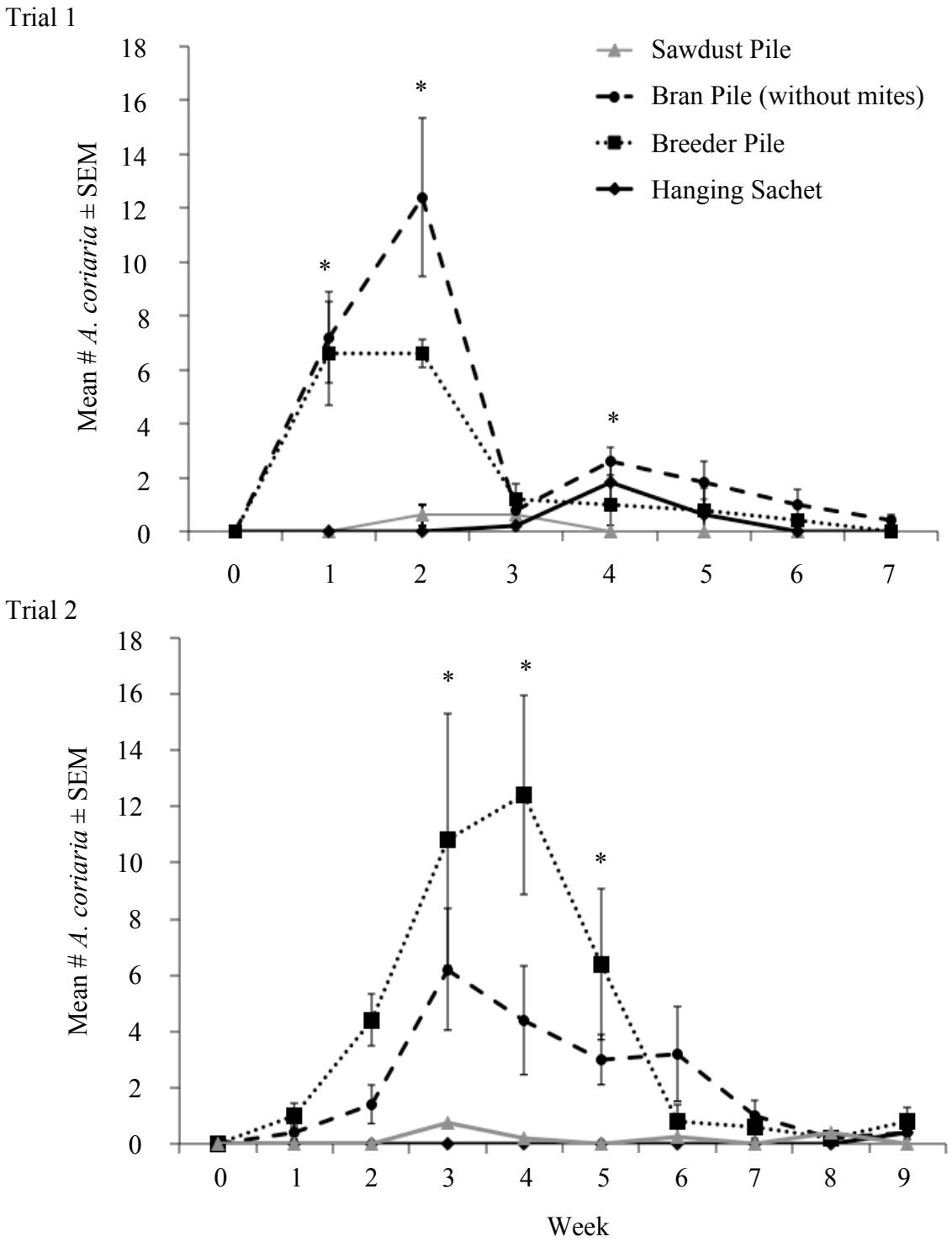

Fig. 3.1. Mean number of *A. coriaria* extracted from treatments in trial 1 and 2. * indicates significant differences among treatments in that week. Significantly more *A. coriaria* were observed in Breeder Pile and Bran Pile treatments in weeks 1 and 2 in trial 1, and in weeks 3, 4, and 5 in trial 2, compared with Sawdust Pile and Hanging Sachet treatments (Kruskal-Wallis, α=0.05).

ent from each other in week 2 ($df = 1$, $H = 7.8125$, $P < 0.009$) (Fig. 3.1). In week 4, Bran piles (2.6 ± 0.5099) and Sachets (1.8 ± 0.8602) had significantly more *A. coriaria* than Sawdust piles (0 ± 0) ($df = 1$, $H = 7.8125$, $P = 0.0052$; $df = 1$, $H = 5.5814$, $P = 0.0182$, respectively), but were not significantly different from each other (Fig. 3.1).

Trial 2. We found significantly more *A. coriaria* in Breeder (5.2 ± 0.8265) and Bran (2.80 ± 0.3470) piles than Sachets (0 ± 0) and Sawdust (0.26 ± 0.0749) piles overall ($df = 1$, $H \geq 22.22$, $P < 0.0001$) (Fig. 3.1). In week 2, Breeder piles had more *A. coriaria* (4.40 ± 0.9274) than Bran piles (1.4 ± 0.6782) ($df = 1$, $H = 4.0612$, $P = 0.04388$), Sawdust piles (0 ± 0) ($df = 1$, $H = 7.7586$, $P = 0.0053$), and Sachets (0 ± 0) ($df = 1$, $H = 7.7586$, $P = 0.0053$) (Fig. 3.1). In week 3, Breeder (10.80 ± 4.4878) and Bran (6.20 ± 2.1541) piles had significantly more *A. coriaria* than Sawdust piles (1.2 ± 0.7349) ($df = 1$, $H = 4.8699$, $P = 0.02733$; $df = 1$, $H = 4.0612$, $P = 0.04388$), and Sachets (0 ± 0) ($df = 1$, $H = 7.7586$, $P = 0.0053$; $df = 1$, $H = 7.7586$, $P = 0.0053$) (Fig. 3.1). In week 4, there were significantly more *A. coriaria* in Breeder (12.4 ± 3.5440) and Bran (4.40 ± 1.9391) piles than Sawdust piles (0.20 ± 0.2) ($df = 1$, $H = 7.3052$, $P = 0.0069$; $df = 1$, $H = 4.0783$, $P = 0.0434$), and Sachets (0 ± 0) ($df = 1$, $H = 7.8125$, $P = 0.0052$; $df = 1$, $H = 5.5814$, $P = 0.0182$) (Fig. 3.1). In week 5, there were significantly more *A. coriaria* in Breeder (6.4 ± 2.6944) and Bran (3.00 ± 0.8944) piles than Sawdust piles (0 ± 0) ($df = 1$, $H = 5.5385$, $P = 0.0186$; $df = 1$, $H = 7.8125$, $P = 0.0052$), and Sachets (0 ± 0) ($df = 1$, $H = 5.5385$, $P = 0.0186$; $df = 1$, $H = 7.8125$, $P = 0.0052$) (Fig. 3.1). In week 6, Bran piles (3.20 ± 01.6852) had significantly more *A. coriaria* than Sachets (0 ± 0) ($df = 1$, $H = 5.5814$, $P = 0.0182$) (Fig. 3.1).

4.2. Weekly proportion change of *Neoseiulus cucumeris*

We found the proportion of *N. cucumeris* in breeder piles was significantly less than those in hanging sachets in all weeks in both trials ($df = 1$, $H \geq 5.5814$, $P<0.05$) (Table 3.1 and

3.2, Fig.3.2). Furthermore, there were proportionally fewer *N. cucumeris* in breeder piles than

Table 3.1. Percentage change ± SEM in mean *N. cucumeris* densities in treatments after introduction in experiment 1, trial 1. * indicates significant differences between treatments (Kruskal-Wallis, α=0.05). The percentage change in the numbers of *N. cucumeris* in breeder piles and sachets was significantly greater in sachets compared with breeder piles in all weeks (α=0.05).

Week	Treatment	Percentage change ± SEM	*H*-value, *df* =1	*P*-value
1	Breeder pile	-92.49 ± 01.22	6.8182	0.0090*
	Hanging sachet	02.86 ± 15.21		
2	Breeder pile	-99.71 ± 00.18	7.0313	0.0080*
	Hanging sachet	99.71 ± 33.53		
3	Breeder pile	-99.86 ± 00.14	7.2581	0.0071*
	Hanging sachet	148.00 ± 40.29		
4	Breeder pile	-99.86 ± 00.09	7.0313	0.0080*
	Hanging sachet	115.07 ± 40.55		
5	Breeder pile	-99.93 ± 00.07	7.2581	0.0071*
	Hanging sachet	-59.50 ± 20.04		
6	Breeder pile	-100.00 ± 00.00	7.7586	0.0053*
	Hanging sachet	-91.14 ± 01.65		
7	Breeder pile	-100.00 ± 00.00	5.5814	0.0182*
	Hanging sachet	-98.36 ± 00.88		

Table 3.2. Percentage change ± SEM in mean *N. cucumeris* densities in treatments aft introduction in experiment 1, trial 2. * indicates significant differences between treatn (Kruskal-Wallis, α=0.05). The percentage change in the numbers of *N. cucumeris* in b piles and sachets was significantly greater in sachets compared with breeder piles in a (α=0.05).

Week	Treatment	Percentage change ± SEM	*H*-value, *df* =1	*P*-value
1	Breeder pile	-38.90 ± 06.78	6.8182	0.0090*
	Hanging sachet	30.53 ± 05.72		

Table 3.2. (cont'd)

2	Breeder pile Hanging sachet	-70.23 ± 04.92 72.87 ± 25.19	6.8182	0.0090*
3	Breeder pile Hanging sachet	-92.52 ± 03.72 69.02 ± 28.86	6.8598	0.0088*
4	Breeder pile Hanging sachet	-99.84 ± 00.10 06.89 ± 22.15	7.0313	0.0080*
5	Breeder pile Hanging sachet	-99.92 ± 00.08 66.98 ± 25.21	7.2581	0.0071*
6	Breeder pile Hanging sachet	-100.00 ± 00.00 66.49 ± 32.02	7.7586	0.0053*
7	Breeder pile Hanging sachet	-99.92 ± 00.08 15.47 ± 12.87	7.2581	0.0071*
8	Breeder pile Hanging sachet	-99.92 ± 00.08 07.55 ± 19.15	7.2581	0.0071*
9	Breeder pile Hanging sachet	-100.00 ± 00.00 -08.13 ± 10.54	7.7586	0.0053*

initially introduced in all weeks in both trials (Table 3.1 and 3.2, Fig.3.2). In contrast, the proportion of *N. cucumeris* in hanging sachets increased up to 148 ± 40.29 percent than the initial numbers of *N. cucumeris* introduced in the first trial and up to 66.98 ± 25.21 percent in the second trial ($df = 1$, $H = 7.2581$, $P=0.0071$; $df = 1$, $H = 7.2581$, $P=0.0071$, respectively) (Table 3.1 and 3.2, Fig.3.2).

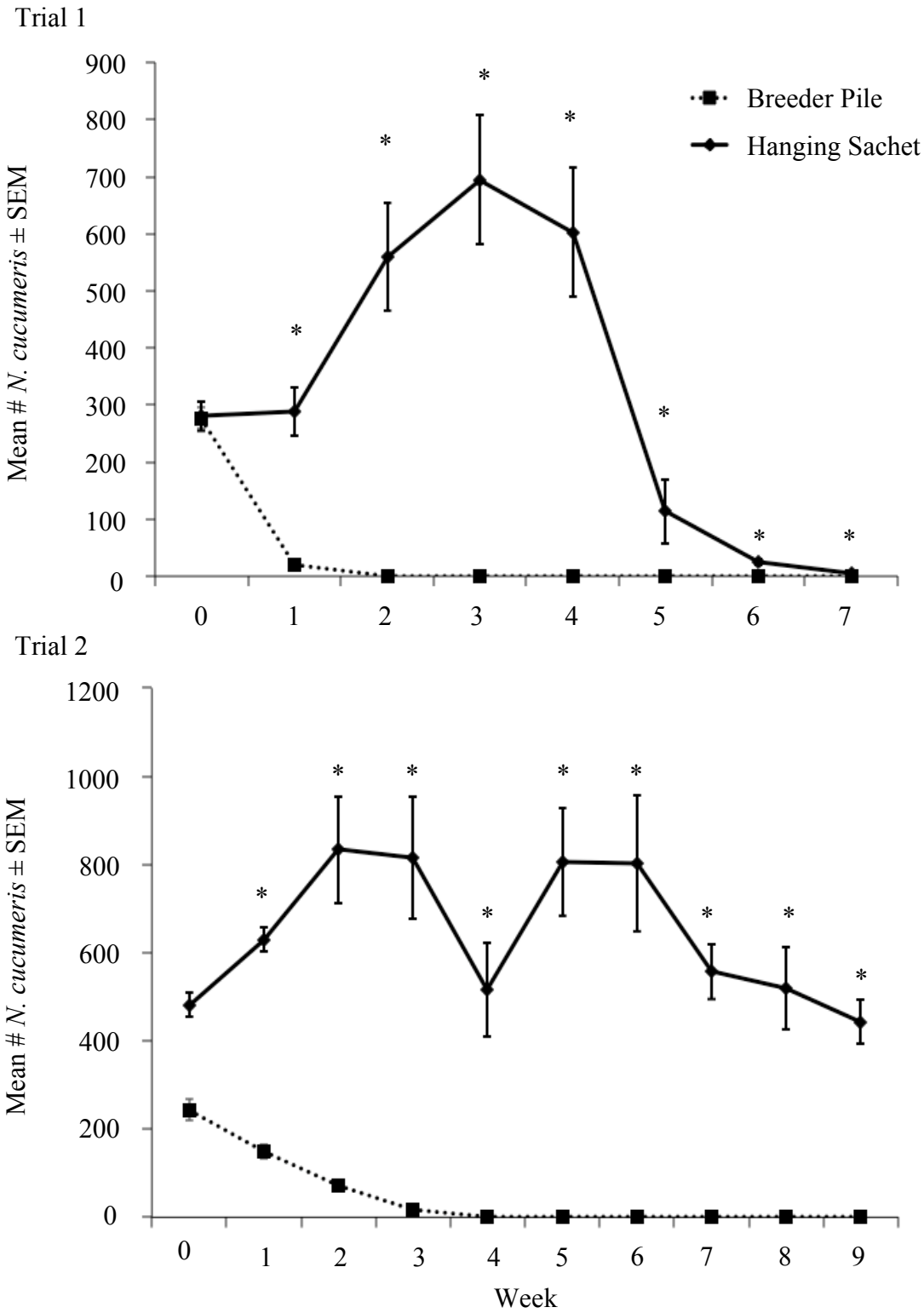

Fig. 3.2. Mean number of *N. cucumeris* extracted from treatments in trial 1 and 2. * indicates significant differences in the proportion change of mites in treatments in that week (Kruskal-Wallis, α=0.05).

4.3. Weekly proportion change of *Tyrophagus putrescentiae*

We found the proportion of *T. putrescentiae* in breeder piles was significantly less than the proportion of *T. putrescentiae* in hanging sachets in weeks 1, 2, 3, 5, and 6 in the first trial and in weeks 5,6,8, and 9 in the second trial ($df = 1$, $H \geq 6.8182$, $P < 0.05$) (Table 3.3 and 3.4, Fig.3.3). In week 3, the proportion of *T. putrescentiae* in breeder piles was significantly greater than in hanging sachets ($df = 1$, $H = 6.8182$, $P = 0.0090$) (Table 3.3 and 3.4, Fig.3.3).

Table 3.3. Percentage change ± SEM in mean *T. putrescentiae* densities in treatments after introduction in experiment 1, trial 1. * indicates significant differences between treatments (Kruskal-Wallis, α=0.05). The percentage change in the numbers of *T. putrescentaie* in breeder piles and sachets was significantly greater in sachets compared with breeder piles in all weeks except week 4 (α=0.05).

Week	Treatment	Percentage change ± SEM	*H*-value, *df* =1	*P*-value
1	Breeder pile	-92.97 ± 00.58	6.8182	0.0090*
	Hanging sachet	-12.41 ± 11.48		
2	Breeder pile	-81.10 ± 05.12	6.8182	0.0090*
	Hanging sachet	-04.89 ± 10.26		
3	Breeder pile	-91.61 ± 02.93	6.8182	0.0090*
	Hanging sachet	-36.24 ± 07.97		
4	Breeder pile	-78.02 ± 08.56	3.1527	0.0758
	Hanging sachet	-47.08 ± 12.13		
5	Breeder pile	-95.49 ± 01.81	6.8182	0.0090*
	Hanging sachet	-75.04 ± 03.97		
6	Breeder pile	-97.41 ± 01.90	6.8182	0.0090*
	Hanging sachet	-81.68 ± 02.58		
7	Breeder pile	-100.00 ± 00.00	7.7586	0.0053*
	Hanging sachet	-87.63 ± 05.44		

Table 3.4. Percentage change ± SEM in mean *T. putrescentiae* densities in treatments after introduction in experiment 1, trial 2. * indicates significant differences between treatments (Kruskal-Wallis, α=0.05). The percentage change in the numbers of *T. putrescentiae* in breeder piles and sachets was significantly greater in sachets compared with breeder piles in weeks 5, 6, 7, and 8, and significantly greater in breeder piles than sachets in week 3 (α=0.05).

Week	Treatment	Percentage change ± SEM	*H*-value, *df* =1	*P*-value
1	Breeder pile	-32.38 ± 07.85	3.1527	0.0758
	Hanging sachet	-14.23 ± 05.39		
2	Breeder pile	-16.24 ± 09.11	0.2727	0.6015
	Hanging sachet	-06.99 ± 12.23		
3	Breeder pile	72.31 ± 39.61	6.8182	0.0090*
	Hanging sachet	-46.25 ± 6.30		
4	Breeder pile	-17.15 ± 35.82	0.8836	0.3472
	Hanging sachet	-68.29 ± 02.11		
5	Breeder pile	-81.74 ± 04.17	6.8182	0.0090*
	Hanging sachet	-52.14 ± 08.39		
6	Breeder pile	-96.31 ± 01.59	6.8598	0.0088*
	Hanging sachet	-64.30 ± 04.44		
7	Breeder pile	-83.83 ± 10.36	2.4545	0.1172
	Hanging sachet	-67.65 ± 04.84		
8	Breeder pile	-97.88 ± 01.50	6.8182	0.0090*
	Hanging sachet	-75.28 ± 03.18		
9	Breeder pile	-95.44 ± 03.15	6.8182	0.0090*
	Hanging sachet	-76.48 ± 01.91		

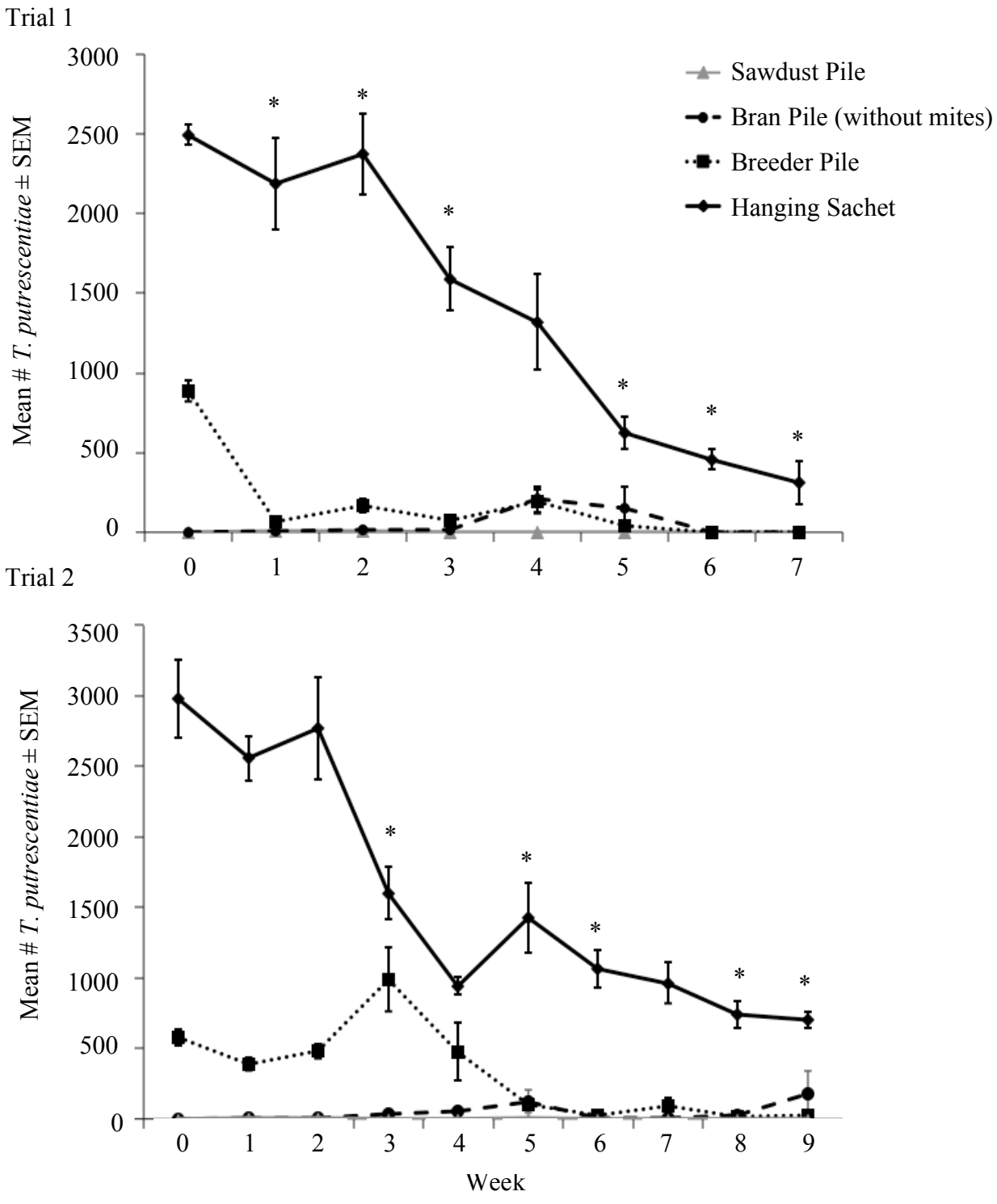

Fig. 3.3. Mean number of *T. putrescentiae* extracted from treatments in trial 1 and 2. * indicates significant differences in the proportion change of mites in treatments in that week (Kruskal-Wallis, α=0.05).

5. Experiment Two Results

5.1. *Neoseiulus cucumeris* predatory mites

Trial 1. We found significant effects of treatment, week, and the treatment by week interaction (*df* = 1, *F-value* = 22.35, $P < 0.0001$; *df* = 8, *F-value* = 29.28, $P < 0.0001$; *df* = 8, *F-value* = 13.68, $P < 0.0001$, respectively) (Fig. 3.4). There were significantly more *N. cucumeris* observed dispersing from sachets (38.587 ± 6.445) than from breeder piles (25.937 ± 5.051) overall (Fig. 3.4). Significant week effects were observed among early weeks (e.g. 1-4) and later weeks (e.g. 5-9) (Fig. 3.4). The treatment by week interaction was significant in weeks 1, 5, 6, and 7 (Fig. 3.4). In week 1, significantly more mites dispersed from breeder piles than from sachets (Fig. 3.4). Significantly more *N. cucumeris* were observed dispersing from sachets than from breeder piles in weeks 5, 6, and 7 (Fig. 3.4).

Trial 2. We found significant effects of block, week, and the treatment by week interaction ((*df* = 6, *F-value* = 2.382, $P = 0.034$; *df* = 8, *F-value* = 14.762, $P < 0.0001$; *df* = 8, *F-value* = 6.580, $P < 0.0001$, respectively) (Fig. 3.4). Significant week effects were observed among early weeks (e.g. 1-4) and later weeks (e.g. 5-9) (Fig. 3.4). A significant interaction effect was observed in week 2 where more *N. cucumeris* dispersed from breeder piles (13.2 ± 5.643) than from sachets (1.6 ± 1.6) (Fig. 3.4). A marginal significant interaction effect was observed in week 1 (Fig. 3.4).

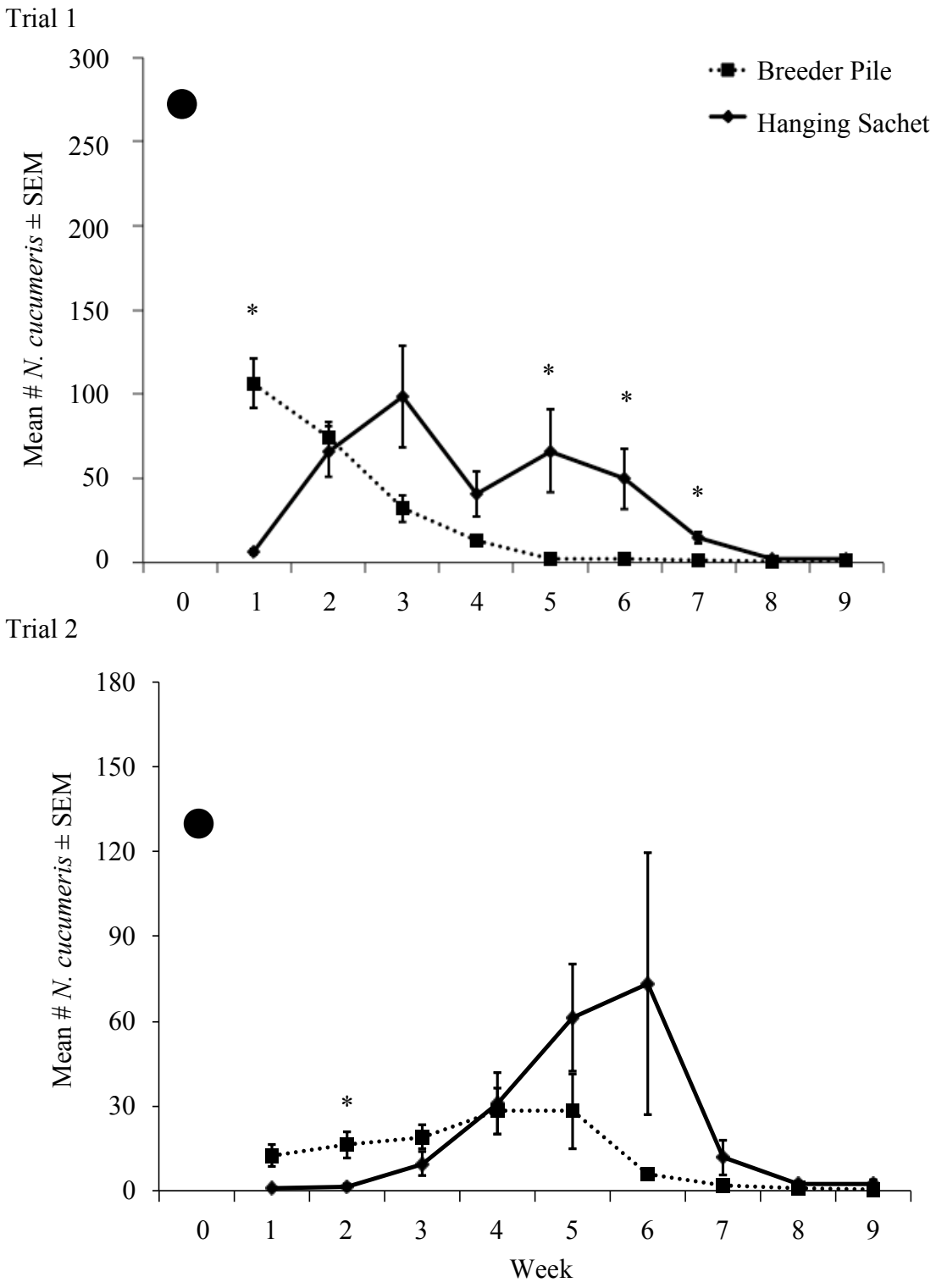

Fig. 3.4. Mean number of *N. cucumeris* dispersing from treatments in trials 1 and 2. * indicates significant differences between treatments in that week. Significantly more *N. cucumeris* dispersed from breeder piles in week 1 in trial 1 and in week 2 in trail 2, and significantly more *N. cucumeris* dispersed from sachets in weeks 5, 6, and 7 in trial 1 (Tukey's HSD, α=0.05).

5.2. *Tyrophagus putrescentiae* mold mites

Trial 1. In week 1 significantly more *T. putrescentiae* dispersed from breeder piles (109.286 ± 26.199) than from sachets (94.254 ± 22.625) ($df = 1$, $H = 9.8$, $P = 0.0017$) (Fig. 3.5). In weeks 4 and 5, significantly more *T. putrescetiae* mites dispersed from sachets than from breeder piles ($df = 1$, $H = 4.4449$, $P = 0.03501$; $df = 1$, $H = 5.6001$, $P = 0.018$, respectively) (Fig. 3.5).

Trial 2. In week 1 and 2 significantly more *T. putrescentiae* dispersed from breeder piles (40 ± 22.363 and 203 ± 46.808, respectively) than from sachets (4.2 ± 0.97 and 2.6 ± 1.435, respectively) ($df = 1$, $H = 5.0332$, $P = 0.02487$; $df = 1$, $H = 9.8216$, $P = 0.0017$, respectively) (Fig. 3.5). In week 5, significantly more *T. putrescentiae* dispersed from sachets (13.50.8 ± 857.77) than from breeder piles (127.8 ± 66.168) ($df = 1$, $H = 5.6001$, $P = 0.018$) (Fig. 3.5).

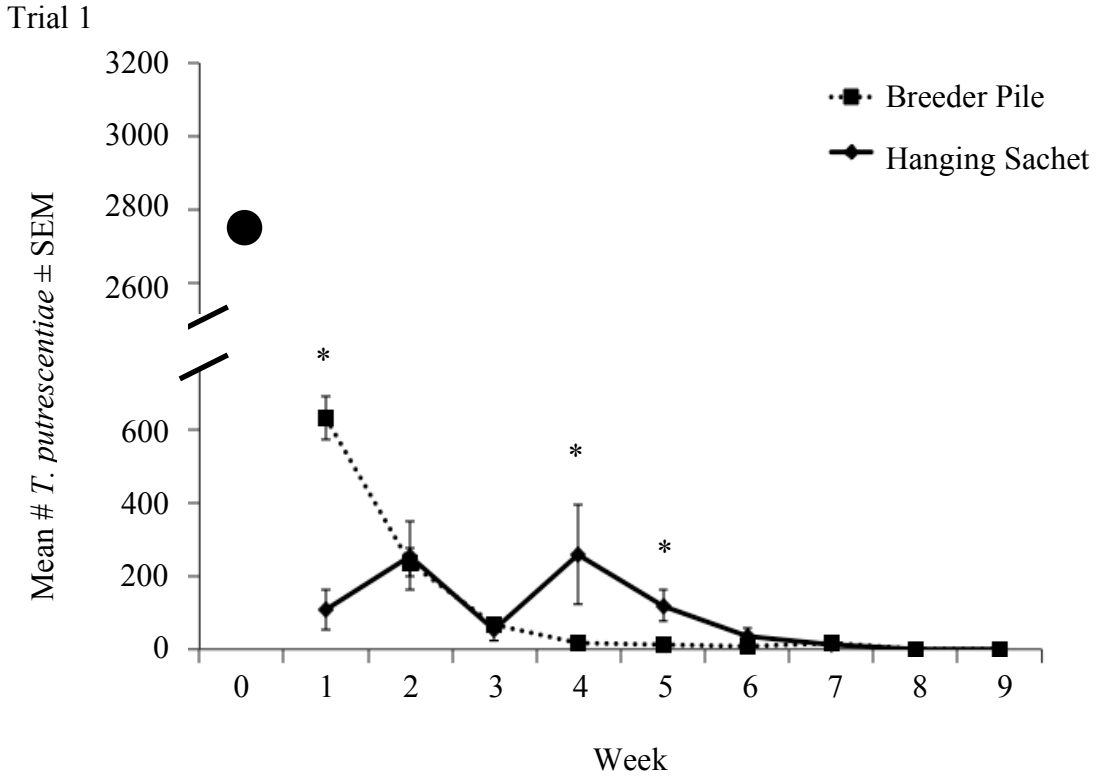

Fig.3.5. (cont'd)

Trial 2

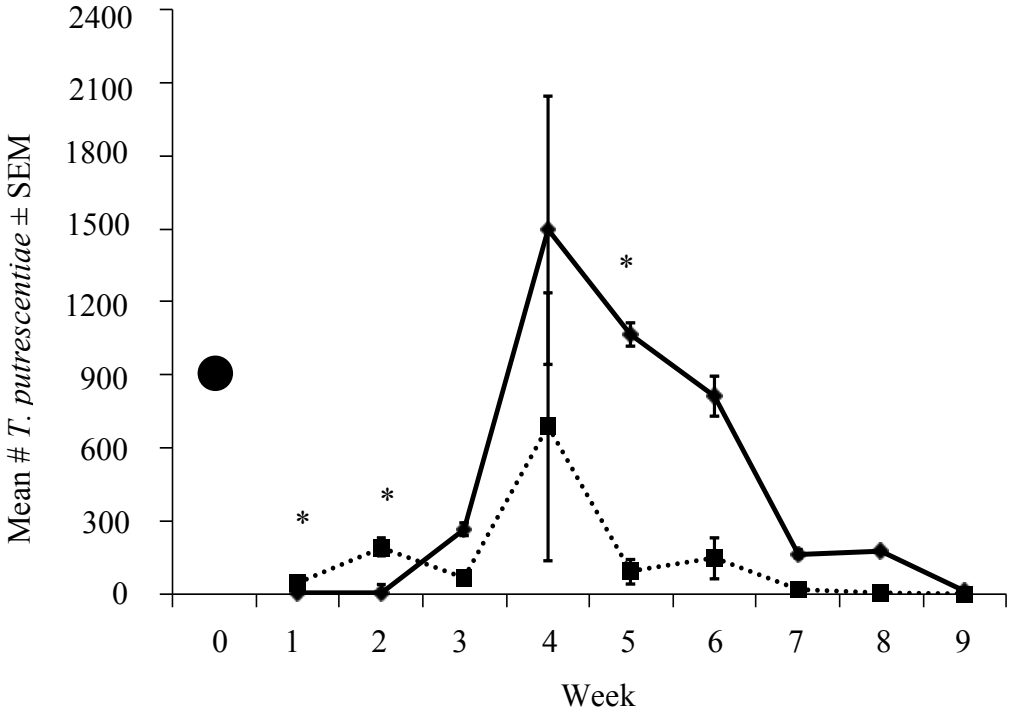

Fig. 3.5. Mean number of *T. putrescentiae* dispersing from treatments in trials 1 and 2. * indicates significant differences between treatments in that week. Significantly more *T. putrescentiae* dispersed from breeder piles in week 1 in trial 1 and in weeks 1 and 2 in trail 2, where significantly more *N. cucumeris* dispersed from sachets in weeks 4 and 5 in trial 1 and in week 5 in trial 2 (Kruskal-Wallis, α=0.05).

6. Discussion

Methods for open rearing *N. cucumeris* in greenhouses include the use of breeder piles and sachets. These open rearing systems are comprised of a mixture of bran, *T. putrescentiae* mold mites, and *N. cucumeris* predatory mites that are either applied in piles to the soil (i.e. breeder piles) or placed in a paper envelope (i.e. sachets) that can be hung in the plant canopy. Breeder piles place *N. cucumeris* onto the soil where they are vulnerable to predation by *A. coriaria* (Pochubay and Grieshop in press). Increasing habitat complexity has demonstrated positive results for reducing intraguild predation (Janssen et al. 2007). We used sachets to

increase habitat complexity and reduce intraguild predation between *N. cucumeris* and *A. coriaria*. Our results showed that sachets were an effective tool for delaying, reducing, and in some cases eliminating the invasion of *N. cucumeris* mite-bran material by *A. coriaria*. Therefore, using sachets rather than breeder piles in greenhouses where *A. coriaria* have been released would be a better open rearing approach.

Understanding reasons why *A. coriaria* invade breeder piles is the first step to mitigate invasion, if possible. Several reasons include: 1) foraging for. *N. cucumeris and T. putrescentiae* as prey resources, foraging for other prey species utilizing the pile material, foraging for the bran and fungus itself, or seeking a physical shelter or oviposition site. We hypothesized that *A. coriaria* were attracted to breeder piles for prey resources —i.e. predator and fungus mites or other arthropods or fungi growing on the bran.

Bran piles did not initially contain mites or other organisms but were invaded by other mites, collembola, and dipterans as well as *A. coriaria* within 1 week after introduction to the soil. Bran and the fungi that grow on it are likely attractive food sources for these organisms. Hence, we could not provide evidence as to what organisms or substrates may have been consumed by *A. coriaria.* However, because *A. coriaria* is polyphagous (Birken and Cloyd 2007, van Lenteren 2012) it is likely that these *A. coriaria* were consuming bran, fungi, and arthropods in the piles.

Few *A. coriaria* were recovered from sawdust piles indicating that these piles were less attractive to *A. coriaria* than bran and breeder piles. Therefore, structural aspects of the piles were not key factors influencing *A. coriaria* presence in bran and breeder piles. In general fewer organisms were observed in sawdust than in other treatments. It is likely that fewer organisms were found in sawdust because celluloses of woody plant materials are difficult for many

arthropods to digest (Klowden 2007) making the piles an inadequate food source or location for food.

The presence of more *A. coriaria* in bran and breeder piles than in sawdust piles provided evidence that *A. coriaria* were probably invading piles for food resources rather than for the physical structure of piles. Therefore, mitigating invasion of *A. coriaria* into the bran and breeder pile material is unlikely a simple scenario. Providing a barrier for breeder pile material that would allow mite dispersal, but prevent *A. coriaria* from entering is one possibility. This could be achieved by placing sachets on plug trays where young plants cannot support a hanging sachet. On the other hand, developing an open rearing system for *A. coriaria* by placing bran piles on the soil that attracts organisms for *A. coriaria* consumption could be promising and would be an interesting focus for future research.

Sachets and breeder piles are used to prolong releases of predatory mites for management of mite pests and thrips in greenhouse crops. With the exception of Shipp and Wang (2003), there has been little published work on the number of mites that are produced by and dispersing from these open rearing systems over time. Because initial densities of *N. cucumeris* and *T. putrescentiae* introduced in sachets and breeder piles were dissimilar we compared proportions of these mites. Our results demonstrated that the proportion of *N. cucumeris* in sachets was greater than in breeder piles for up to 7 weeks in the first trial and 9 weeks in the second. This trend may have continued for more weeks in the second trial as there were still *N. cucumeris* present in sachets when the experiment ended. The differences in the numbers of *N. cucumeris* observed in sachets in trials 1 and 2 could be due to the presence of *A. coriaria* in the sachets in weeks 3-5 in trial 1 (Fig. 3.2). Furthermore, the initial numbers and ratios of predator and prey

mites in the sachets differed between trials and could have played a role in the numbers of *N. cucumeris* that were produced by the sachets overtime.

Proportions of *T. putrescentiae* in sachets demonstrated mostly decreasing trends over time (Table 3.3 and 3.4, Fig. 3.3). This was also true for proportions of *T. putrescentiae* in breeder piles with exception of week 3 where there was an increase in *T. putrescentiae* density (Table 3.3 and 3.4, Fig. 3.3). In other weeks where significant differences were observed, the proportion of mites in sachets was greater than in breeder piles. These results indicate that sachets maintain more *T. putrescentiae* than breeder piles. Maintaining *T. putrescentiae* populations is an essential component for open rearing *N. cucumeris*.

Our second study was conducted in a Michigan Sate University greenhouse where *A. coriaria* had not been released to compare the numbers of mites dispersing from sachets and breeder piles in the absence of *A. coriaria*. In the first trial we found that the greatest number of *N. cucumeris* dispersed from breeder piles after one week and over time this number gradually declined. In contrast fewer *N. cucumeris* dispersed from sachets in week 1 and over time the numbers of *N. cucumeris* that dispersed from sachets varied. Overall, more *N. cucumeris* dispersed from sachets and sachets sustained greater *N. cucumeris* populations than breeder piles. In the second trial, *N. cucumeris* population dynamics differed from observations in the first trial. The trend of *N. cucumeris* that dispersed from sachets was similar to trial 1 in that fewer *N. cucumeris* dispersed in earlier weeks than in later weeks. The numbers of *N. cucumeris* that dispersed from breeder piles, however, did not gradually decline over time as observed in the first trial.

Similar to *N. cucumeris*, the number of *T. putrescentiae* mold mites that dispersed from breeder piles in the first trial was greatest during week 1 and gradually declined over time. Fewer

T. putrescentiae mold mites dispersed from sachets than from breeder piles in week 1 and over time the numbers of *T. putrescentiae* that dispersed varied. The numbers of *T. putrescentiae* that dispersed in the second trial were similar to the first in that fewer mites dispersed from sachets than from breeder piles in earlier weeks. In both trials more *T. putrescentiae* dispersed from sachets than in breeder piles in weeks 4 and 5. Overall, the numbers of *T. putrescentiae* that dispersed from sachets and breeder piles in both trials were not significantly different.

Some factors which may have lead to the inconsistencies and variability observed in experiment 2 include differences in: photoperiod, temperature, initial numbers of mites introduced, ratios of initial predator and prey mites introduced, and disturbance to treatments by cockroaches. The first trial was conducted from mid-August to early-October when photoperiod was longer and temperatures were higher, whereas photoperiod was shorter and temperatures were lower from early-December to early-February in the second. Mite development and reproduction was likely advanced due to higher temperatures and thus resulted in more mites dispersing in earlier weeks in the first trial. Whereas mite development may have been slowed due to lower temperatures and thus resulted in a delay of mite dispersal. Initial numbers of mites in the first trial were two-fold more *N. cucumeris* predatory mites and three-fold more *T. putrescentiae* mold mites than in the second trial. Differences in the numbers of mites introduced may have also contributed to slower population growth. Furthermore, cockroaches disturbed experimental units, chewed through the paper sachets, and scattered breeder piles material in the second trial. Cockroaches have not previously been observed disturbing mite-bran material, but have been reported as greenhouse pests (Fullaway 1937, Appel et al. 1990).

The findings of our experiments have generated many opportunities for future research. We observed the numbers of mites produced in open rearing systems when *A. coriaria* was

present and mite dispersal from open rearing units in the absence of *A. coriaria.* Research that observes both the numbers of mites produced by and that disperse from breeder piles and sachets would provide better insight to optimize the numbers of mites introduced for desired mite production and dispersal rates. The influence of climatic conditions such as photoperiod, temperature, and humidity on the numbers of mites produced and that disperse should be considered in such research. Optimal initial densities of predatory mites and mold mites to prolong mite production from open rearing systems should also be investigated. Our research has also opened opportunities for investigating open rearing systems for *A. coriaria.* Introducing bran piles that provide fungi and lure arthropods for *A. coriaria* consumption may be a promising tool. Furthermore, using bran piles as a means of monitoring soil arthropod presence is another possibility for research. Addressing these topics in future experiments would improve the use of open rearing systems in greenhouses.

The results from our first experiment showed that sachets protected *N. cucumeris* from intraguild predation and competition by *A. coriaria*. Therefore, *N. cucumeris* sachets, not breeder piles, should be used in greenhouses that also release *A. coriaria* in biological pest management programs. Furthermore, sachets produced and maintained more *N. cucumeris* and *T. putrescentiae* than breeder piles. The duration of mite production in sachets was also greater compared to breeder piles. These results indicate that fewer releases of *N. cucumeris* may be needed if sachets are used. Results from our second experiment showed that mite dispersal from breeder piles and sachets differed. More mites dispersed from breeder piles than sachets in earlier weeks and the opposite was true in later weeks. Therefore, breeder piles may be more appropriate for 'quick-releases' of *N. cucumeris* where as slow-release sachets are true to their name. Although both breeder pile and sachet open rearing systems should be introduced

preventatively (i.e. when pest densities are low), our results indicate that introductions of sachets should be made sooner than the recommended introduction timing for breeder piles to compensate for delayed mite dispersal.

CHAPTER 4

Conclusions and Future Research

Improving the efficacy and consistency of biological control tactics is imperative for promoting biological pest management in greenhouses. Biological control is a pest management approach with reduced risks of harming workers, consumers, crop plants, non-target organisms, and the greenhouse environment (Shipp et al. 1991, van Driesche and Bellows 1996, van Lenteren 2000, Bale et al. 2008, van Lenteren 2012). One challenge of biological control is the cost associated with repeated applications of natural enemies (Collier and Steenwyk 2004). Open rearing systems aim to reduce the number of releases thus reducing costs (Frank 2010, Huang et al. 2011). However, conserving the released natural enemies increases the potential for unfavorable predator interactions. Furthermore, information regarding the number of natural enemies produced by open rearing systems and how long natural enemies are conserved is lacking (Frank 2010). The goal of this thesis was to address these issues regarding *Neoseiulus cucumeris* Oudemans (Phytoseiidae) open rearing systems to improve their use in greenhouse biological control programs. The results of the experiments presented in this thesis were consistent with this goal.

Two main factors influencing *N. cucumeris* open rearing include the effect of intraguild predation and the type of open rearing system (e.g. breeder piles or sachets) used. Based on the results presented, intraguild predation among *N. cucumeris*, *Atheta coriaria* (Kraatz) (Staphylinidae), and *Stratiolaelaps miles* (Berlese) (Laelapidae) had direct and indirect effects on the introduced predators and on thrips (Thripidae) (Chapter 2, Fig.2.1-2.4, Table 2.1 and 2.2). Although the addition of *S. miles* reduced *N. cucumeris* populations, the strongest negative effects were observed on *N. cucumeris* populations when *A. coriaria* were present (Chapter 2,

Fig.2.1 and 2.2). Negative effects on *A. coriaria* densities were also observed when all three predator species were present. This result indicated that *S. miles* may be an intraguild predator of *A.coriaria* which agrees with previous research regarding negative effects of similar predatory mites on *A. coriaria* (Jandricic 2006).

My thesis demonstrated that breeder piles and sachets had different open rearing aspects that influenced production and dispersal of mites (Chapter 3, Fig.3.2-3.5, Tables 3.1-3.4). Sachets reduced invasion of *A. coriaria* which effected the production of mites in these systems (Chapter 3, Fig.3.1). In general the proportion of *N. cucumeris* in sachets was greater than in breeder piles when *A. coriaria* were present (Chapter 3, Table 3.1 and 3.2). Furthermore, production of *N. cucumeris* in sachets was two to three times longer than in breeder piles (Chapter 3, Fig.3.2, Table 3.1 and 3.2). Patterns of mite dispersal from these systems differed in that more *N. cucumeris* dispersed from breeder piles than sachets in earlier weeks and the numbers of *N. cucumeris* that dispersed from sachets was greater in later weeks (Chapter 3, Fig.3.4).

The results of my thesis research have been presented to and well received by the grower community. Following extension presentations, growers have asked where the information presented can be found or if they can receive a copy of the presentation. To address grower needs and achieve the second half my thesis goal (i.e. to improve the use of open rearing systems…), I generated an extension bulletin that incorporates the results of my research and overviews topics such as "what is open rearing", commercially available open rearing systems, general information regarding open rearing in greenhouses, and provides a list of suppliers for open rearing products (Appendix A). Photos of open rearing systems, natural enemies produced by the systems, and intraguild predators that invade *N. cucumeris* open rearing systems are also in the

bulletin. Thus far, my results have impacted the growers at Elzinga and Hoeksema Greenhouses (Portage, MI), who release *A. coriaria* and now use slow-release sachets in lieu of breeder piles to prevent negative effects of *A. coriaria*. Distributing the extension bulletin is a means of disseminating the results of my work to continue impacting the greenhouse community.

The findings presented in this thesis have generated many opportunities for future research. Research directly relevant to *N. cucumeris* open rearing systems includes: observing ratios of the numbers of mites produced by and that disperse from breeder piles and sachets, the influence of climatic conditions such as photoperiod, temperature, and humidity on mite production and dispersal, and optimal initial densities of predatory mites and mold mites to prolong mite production from open rearing systems. Perhaps the most pertinent to using multiple predators for thrips management in greenhouses is to investigate the effect that full factorial predator combinations of *N. cucumeris*, *S. miles*, and *A. coriaria* have on thrips. Additionally, observing the effect that these combinations have on thrips when sachets are used would provide insight to optimal predator combinations using *N. cucumeris* open rearing systems for thrips management. My results have also opened opportunities for investigating *A. coriaria* open rearing systems. Introducing bran piles that provide fungi and lure arthropods for *A. coriaria* consumption may be a promising tool. Placing bran piles onto the soil of crop plants to monitor soil arthropod presence is another possibility for research. Addressing these topics in future experiments would improve the use of multiple predators and *N. cucumeris* open rearing systems for pest management in greenhouses.

Breeder piles and sachets offer growers with *N. cucumeris* open rearing options that may be more or less suited for specific greenhouse biological control programs. Therefore, greenhouse operators intending to use *N. cucumeris* open rearing systems should generate a

biological control plan that supports these systems. The first step is to determine whether soil-dwelling predators will be used or if soil mixes contain other predatory arthropods that may effect open rearing of *N. cucumeris*.

Sachets reduce intraguild predation of *N. cucumeris* by adding complexity to the system (Janssen et al. 2007), partitioning habitats, and providing a refuge for *N. cucumeris* (Finke and Denno 2002). Hence, sachets may be a better option for open rearing *N. cucumeris* in greenhouses where soil-dwelling predators are also released. This is especially true if *Atheta coriaria* are released. If the effect of soil predators is decidedly negligible —such as may be the case with low densities of *S. miles*, it is possible that breeder piles may be effective. However, these breeder piles may not generate as many *N. cucumeris* that would otherwise be produced in the absence of *S. miles* and may require additional breeder pile applications.

If there is no concern for negative effects of other predators on *N. cucumeris*, then breeder piles or sachets may be used. The next step is to determine the desired release rate of *N. cucumeris*. When pest densities are expected to increase within a week or two, breeder piles that provide quick-releases of mites should be chosen. However, slow-releases of mites from sachets are a better option if pest pressure is not imminent. Implementing these procedures will improve the use of *N. cucumeris* open rearing systems by reducing negative predator interactions and promoting favorable conditions to maintain *N. cucumeris* in greenhouses.

APPENDICES

Appendix A

Extension Bulletin: Open Rearing Natural Enemies

1. What is Open Rearing?

Open rearing is a combination of augmentative and conservation biological control that provides natural enemies released in greenhouses with supplemental food and or hosts. These extra resources are provided to promote longevity of natural enemies thus reducing the number and cost of releases. Natural enemy open rearing methods have been developed for parasitic wasps and predatory mites and insects released to manage plant-feeding insect and mite pests.

2. Open Rearing Systems

An open rearing 'system' is the supplemental resource that provides food or hosts for natural enemies. These systems and the associated natural enemies are introduced in greenhouses when pest densities are low. Natural enemies are reared on the extra resources thereby generating a natural enemy population that prevents pest outbreaks. Using open rearing systems to introduce natural enemies before pests exceed acceptable levels is an economical approach to keep pest densities at bay. Here are some examples of commercially available open rearing systems:

2.1. Predatory Mites: Breeder Piles and Sachets

Open rearing systems for predatory mites consist of a mixture of bran, mold mites, and predatory mites such as *Neoseiulus* (=*Amblyseius*) *cucumeris*, *Amblyseius swirskii*, *Amblyseius andersoni*, and others. The bran supports mold mite populations that are an alternative food source for predatory mites. The mite-bran mixture has a specific ratio of predator and mold mites that allows rearing of the predatory mites. As predatory mite densities increase, the mites disperse onto plants and forage for prey such as pest mites and early instar thrips (Fig.A.1).

The mite-bran mixture is loose material packaged in containers or in paper envelopes called sachets (Fig.A.2). Small piles of the loose mite-bran material in containers can be placed onto potted plants and plug trays. These piles are called breeder or breeding piles. Sachets are hung in the plant canopy or placed onto potted plants and plug trays.

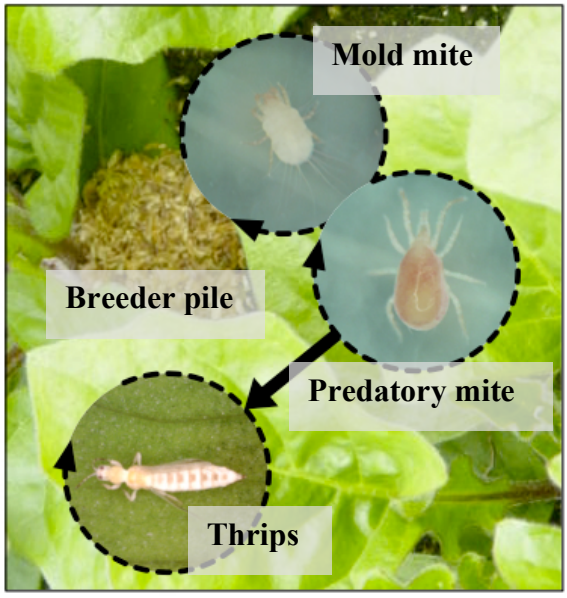

Fig.A.1. Breeder pile schematic. Breeder pile contains mold mites and predatory mites. Mold mites are reared on mold that grows on breeder pile. Predatory mites that forage on crops for thrips are reared on mold mites. (For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.)

Michigan State University and grower collaborative research has shown that more predatory mites are present in hanging sachets than in breeder piles over time. Sachets produced predatory mites for at least 5 weeks and up to 9 or more weeks. The longevity of predatory mite production is likely dependent on the number of mold mites available and favorable microclimatic conditions such as humidity and temperature.

Breeder piles produced fewer mites over time than sachets. This result was in part due to the introduction method of breeder piles. Piles are placed onto the soil where the mites are exposed to other predators. Soil-dwelling predators such as *Atheta coriaria* beetles (Fig.A.3) and *Stratiolaelaps* (=*Hypoaspis*) *miles* mites (Fig.A.3) released to manage thrips pupae and shore fly and fungus gnat larvae are two such predators that pose harm to mites in breeder piles. Therefore, breeder piles may not be as effective in greenhouses where these other predators are present or released.


Fig.A.2. Sachet

2.1.1. Release Rates

It is best to introduce predatory mites preventatively or when pest densities are low. Appropriate release rates of predatory mites in open rearing systems vary by crop and pest density at the time of introduction. Biobest Biological Systems provide recommended release rates and approaches for management of thrips on: eggplant, poinsettia, anthurium, gerbera, grape, beans, melon, rose, strawberry, sweet pepper, and in tree nurseries on their website (see Resources). Koppet Biological Systems also offer suggestions for introductions of breeder piles and sachets (see below).

Fig.A.3. *Atheta coriaria* (top). *Stratiolaelaps miles* (bottom).

Breeder piles:

- Preventative (50 mites per m^2 per 2 wk)
- Curative for low pest densities (100 mites per m^2 per 2 wk)

Sachets:

- Preventative (1 sachet per 2.5 m^2 per 6 wk)
- Curative for low pest densities (1 sachet per 2.5 m^2 per 5 wk)
- Curative for high infestations (1 sachet per 0.75 m^2 per 4 wk)

Consult a company representative for information regarding appropriate predator releasing procedures prior to introducing them.

Fig.A.4. *Aphidius colemani*

2.2. Parasitic Wasps: Banker Plants

Parasitic wasps such as *Aphidius* spp. (Fig.A.4) and *Encarsia formosa* released to manage aphids and whiteflies can be reared on alternative hosts to maintain populations in greenhouses.

Fig.A.5. Banker Plant System: bird cherry-oat aphids on barley.

Usually alternative hosts are not pests of the greenhouse crop and non-crop plants are used to support them. The non-crop plant supporting alternative hosts is called a banker plant. Banker plants are introduced into greenhouses shortly before or after parasitic wasps are released.

Banker plants for aphid parasitic wasps consist of a cereal grass such as barley or wheat that is infested with bird cherry-oat aphids or *Sitobion avenae* aphids, respectively (Fig.A.5). These aphids pose no threat to greenhouse vegetables, herbs, and flowers.

Some growers maintain colonies of alternative host aphids by growing barley or wheat in cages and introducing aphids free of parasites to the new plants (Fig.A.6). Cages are used to prevent attacks on aphids from predators and parasites while the aphids establish. After aphids establish, new banker plants replace old ones. Parasitic wasps persisting in the greenhouse parasitize the aphids on the newly introduced banker plants.

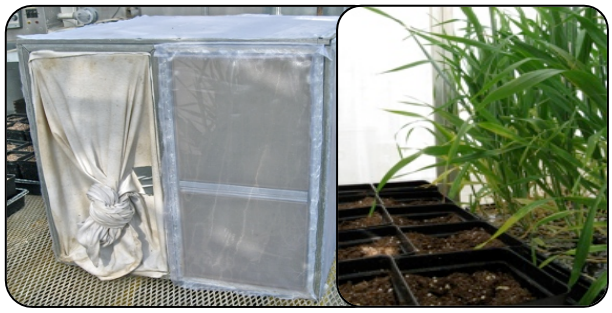

Fig.A.6. Cage excludes aphid parasites (left). Barley in cage hosts bird cherry-oat aphids (right).

2.2.1. Release Rates

Wasps and flies are highly mobile flying insects that disperse into greenhouse crops faster than crawling predators. Michigan State University and grower collaborative research has demonstrated that *Aphidius colemani* parasitic wasps reared using banker plants can locate an aphid host at least 100 feet from a banker plant. However, the distance that these parasitoids fly

may depend on the availability and proximity of aphids on crops. Koppert Biological Systems recommends preventative introductions at 5 banker plants per 2.5 acres for aphid management.

3. Product Awareness

Commercially available open rearing systems among different natural enemies may have different release methods, rates, recommended crops and maintenance requirements necessary for optimal results. Be sure to follow recommendations provided by supply companies and consult representatives with any questions regarding products.

4. Additional Information

Currently, commercially available products and science-based recommendations for open rearing are limited. Even so, researchers and growers continue to explore open rearing concepts and are developing their own methods. For example, growers and researchers have generated open rearing systems similar to banker plants by placing plant varieties that are more susceptible to arthropod pest invasions among crops in greenhouses. These plants, called 'guardian plants' attract pests and natural enemies that feed on or parasitize the pests thus supporting the natural enemy population. Although this approach may be effective, proper introduction, maintenance, and removal of heavily infested plants is necessary**.** Find more information regarding guardian plants on the IPM Laboratories website (see Resources) under the 'Guardian Plants' tab.

Another tactic that has demonstrated positive results is to provide pollen to natural enemies. Pollen is a valuable protein source that many insects eat. Plants that have available pollen resources or pollen itself distributed on greenhouse crops can be provided. Similarly, sterilized eggs (available through biological supply companies) are another source of protein that

can be distributed on the crop. This approach does not introduce individual open rearing units, but instead generates a greenhouse expansive open rearing system.

5. Future Projects

There is still much to learn about open rearing in greenhouses. The Organic Pest Management Lab at Michigan State University is currently researching methods for open rearing entomopathogenic nematodes to manage soil-dwelling pests such as thrips pupae, shore fly larvae, and fungus gnat larvae in greenhouses. Please check the OPM Lab website (www.opm.msu.edu) for more information on the progress of open rearing projects.

Want to be a grower collaborator? Contact:

Dr. Matt Grieshop
Center for Integrative Plant Systems
578 Wilson Rd Rm 205
East Lansing, Michigan 48824
Tel: 517-432-8034
grieshop@msu.edu

6. Resources

Below is a list of a few major suppliers of natural enemies and open rearing systems. We recommend searching online for local suppliers and distributors. Buying local can reduce stress on natural enemies by reducing their time spent in transit. It is also advised to purchase from companies that guarantee quality product.

Biobest Biological Systems
2020 Fox Run Road, RR 4
Leamington, Ontario N8H 3V7
Tel: +1 519-322-2178
e-mail: info@biobest.ca
www.biobest.ca
Products: Amblyseius-Breeding-System, Swirskii-Breeding-System, predatory mite sachets

IPM Laboratories, Inc.
980 Main Street
PO Box 300
Locke, New York 13092
Tel: +1 315-497-2063
www.ipmlabs.com
Products: Aphid Guard

Koppert Biological Systems
1502 Old US-23
Howell, MI 48843
Tel: +1 810-632-8750
E-mail: asktheexpert@koppertonline.com
Web: www.koppert.com
Products: THRIPEX, THRIPEX-PLUS (sachets), ERVIBANK

Appendix B

Record of Deposition of Voucher Specimens

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: _____2012-07_____

Author and Title of thesis:
Emily A. Pochubay
Factors influencing *Neoseiulus cucumeris* open rearing in greenhouses

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Staphylinidae	*Atheta coriaria*	adult	10	pinned
Phytoseiidae	*Neoseiulus cucumeris*	adult	3	slide
Acaridae	*Tyrophagus putrescentiae*	adult	3	slide
Laelapidae	*Stratiolaelaps miles*	adult	3	slide
Thripidae	*Frankliniella occidentalis*	adult	3	slide
Thripidae	*Thrips* sp.	adult	3	slide

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