THE ROLE OF NATURAL ENEMIES IN PEST MANAGEMENT OF CODLING MOTH (*CYDIA POMONELLA* [L.]) IN ORGANIC APPLE ORCHARDS IN MICHIGAN

By

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ABSTRACT

THE ROLE OF NATURAL ENEMIES IN PEST MANAGEMENT OF CODLING MOTH (*CYDIA POMONELLA* [L.]) IN ORGANIC APPLE ORCHARDS IN MICHIGAN

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The codling moth (*Cydia Pomonella* [L.]) is the number one worldwide pest of apples, pears and other pome fruits. Conservation of natural enemies is one way to reduce the costs of managing this pest by reducing the need for insecticide sprays. Successful conservation of the codling moth's natural enemies in Michigan requires 1) identifying them and 2) determining major factors that affect their abundance and effectiveness in the apple agroecosystem. The first chapter of my dissertation presents a literature review of the major natural enemies of codling moth by life stage. The second chapter presents a field study using real time video observation of sentinel prey to identify codling moth's key natural enemies in Michigan apple orchards. The third chapter provides experimental data and analysis aimed at identifying major factors that may impact the efficacy of natural enemies in Michigan orchards. In chapter 4 I present the results of two laboratory investigations exploring behavioral factors affecting codling moth infection by entomopathogenic nematodes. I identified, ants, lacewings, spiders, and predatory beetles as major natural enemies of codling moth in my experimental apple orchards. Relative abundances of these organisms were very site-specific. Organically certified orchards had higher natural enemy abundance and attack rate of sentinel fifth instar codling moth compared to non-organic orchards. This was not found for codling moth egg natural enemy abundance or activity. Further investigations revealed that orchard sanitation and orchard floor vegetation management affect natural enemy abundance and effectiveness against fifth instar codling moth larvae and codling moth pupae on the orchard floor. The implications of these findings for organic pest management and for future avenues of research are also discussed in the concluding chapter.

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1.1. Introduction

The codling moth is a severe pest of apples in most pome fruit growing regions worldwide (Barnes, 1991; Lacey and Shapiro-Ilan, 2008; Simon et al., 2007; Stern et al., 1959). Apple producers in the United States in 2011 produced 4.2 million metric tons of apples worth 2.7 billion dollars (USDA-NASS, 2012a). In the same year, US apple producers applied an estimated 621 metric tons of insecticides to 84% of the total US apple bearing acreage (USDA-NASS, 2012b). Integrated pest management (IPM) and conservation biocontrol are approaches that apple growers can use to reduce the frequency of these costly insecticide applications (Jones et al., 2009; Stern et al., 1959; Van Driesche and Bellows, 1996; Zhang and Swinton, 2009).

Conservation of natural enemies has always been a key element in the theory behind IPM (Stern et al., 1959). In practice, growers can conserve natural enemies by withholding insecticide applications, using selective insecticide chemistries or application methods, and or providing resources for natural enemies (Morris and Winter, 1999). Unfortunately, codling moth directly damages the crop resulting in a low tolerance for this pest. Thus, in many apple orchards management of the codling moth still relies largely on suppression of the pest through the use of synthetic or natural toxins that have a negative impact on natural enemies (Epstein, 2010; Jones et al., 2009; Simon et al., 2010). This reliance on insecticides has limited the role of natural enemies in pest management and likely has caused an underestimation of the economic value of the contribution that natural enemies make to pest management in apple orchards (van der Geest and Evenhuis, 1991; Zhang and Swinton, 2009).

The purpose of this review was to assemble the scientific literature investigating the contribution that natural enemies make to codling moth management. Sources were limited primarily to studies that provide estimates of codling moth mortality and the causal agents of this mortality. Studies surveying natural enemy populations or diversity were not included unless they also provide data linking codling moth mortality to measured natural enemy data. References that did not provide functional information on codling moth biological control were excluded to provide conciseness and clarity. Studies that only measured the results of augmentative releases were also not included. Although there is a focus on arthropod natural enemies, I endeavored to include all natural mortality factors of the codling moth that have been studied.

The review is organized by life stage of the codling moth from egg to adult. This structure allows comparison of mortality estimates for each life stage to stage structured matrix population models, which estimate the stages of codling moth that have the greatest impact on long term population dynamics of the species (Mills, 2005b). Mortality agents are listed in the life stage in which they cause mortality not the life stage in which they infect codling moth. This is particularly relevant for the parasitoids of the codling moth that attack it before during or after the larva penetrates the apple but do not cause mortality until after the codling moth larva emerges from the apple (Mills, 2005b). There is a great deal of variation among authors in the terminology used to describe the phenological stages of the codling moth life cycle. I follow the terminology used by Geier (1963).

1.2. Major natural mortality factors of the codling moth by life stage:

1.2.1. Eggs and neonates

The egg and neonate larva (first larval instar) are two of the least protected life stages of the codling moth. Codling moth eggs are laid individually on leaves or developing apples and the survival of neonates is dependent on their ability to find a fruit, successfully penetrate the outer skin, and construct a silken shelter just beneath the fruit surface (Geier, 1963; MacLellan, 1962; Simpson, 1903). High percentages of mortality have been estimated for these life stages in the field. These estimates range from 75% (Ferro et al., 1975; Summerland and Steiner, 1943) to 25% (Jackson, 1982) total mortality from egg laying to fruit entry.

1.2.1.1. Environmental conditions

Codling moth eggs are probably not damaged significantly by the UV light frequencies that they are exposed to in the field (Caldwell et al., 2007; Paul and Gwynn-Jones, 2003). However, low humidity (45-50% RH) in a laboratory setting has been shown to reduce neonate emergence (Hagley, 1972). Jackson and Harwood (1980) performed a series of laboratory experiments to evaluate the influence of temperature and humidity on neonate codling moth survival. They found that survival times decreased as temperature increased and as humidity decreased. The maximum survival time was 300 h for larvae held at 4 °C in 100% RH. Neonate codling moth larvae survived for less than an hour at 45 °C. They also found that starved first instar larvae had a harder time penetrating apples at higher temperatures when RH was held at 100%. The duration of starvation also negatively affected the first instar larvae's abilities to penetrate apples (Jackson and Harwood, 1980). Rainfall has been reported to negatively impact neonate survival either by washing them off of foliage or by flooding their entrance tunnels (Geier, 1963; Jackson, 1982; MacLellan, 1962).

1.2.1.2. Predators and Parasitoids

Jaynes and Marucci (1947) quantified the biological control potential of the codling moth's natural enemies in unsprayed orchards in West Virginia, USA. They monitored codling moth populations in two nearby orchards, one that received insecticide sprays as needed, and another orchard, which received only a dormant oil spray, a pink spray of lime-sulfur and a petal fall spray of lime-sulfur, lime, and lead arsenate. They used sentinel eggs laid on cellophane paper and pinned in orchard trees to measure predation and parasitization of codling moth eggs. Parasitization in the sprayed orchard was considerably more variable than in the unsprayed orchard presumably because insecticide sprays reduced the populations of parasitoids temporarily. Parasitization was consistently higher in the unsprayed orchard, but this effect was much greater in the first year of the study (1938) than in the following two years. Reduced parasitization in the sprayed orchard in the first two years was attributed to insecticide sprays at the time of second-generation oviposition (August). All reported parasitization was by the egg parasitoid *Trichogramma minutum* (Riley) (Hymenoptera: Trichogrammatidae), which parasitized up to 28% of first-generation eggs.

In the same study, predation of eggs was very inconsistent and was only different between the sprayed and unsprayed orchard in one year (1939). They performed visual searches for egg predators but found very little direct evidence of egg predation in the field. The only predator that was observed feeding on codling moth eggs in the field was a predaceous thrips, *Leptothrips mali* (Fitch) (Thysanoptera: Phlaeothripidae), which migrated from senescing daisies on the orchard floor to the apple foliage where they attacked second-generation eggs. The predaceous thrips population as recorded by timed visual counts was correlated with higher predation rates of sentinel codling moth eggs in both types of orchards (16.4 – 21.9% in unsprayed, 6.4-16.6% in sprayed). In laboratory experiments, ladybeetle larvae (Coleoptera: Coccinellidae) and green lacewing larvae (Neuroptera: Chrysopidae) did not feed on codling moth eggs, but adults of the ladybeetles *Anatis quindecimpunctata* (Olivier), *Hippodamia convergens* Guerin-Meneville, and *Coccinella nevomnotata* Herbst did feed on codling moth eggs (Jaynes and Marucci, 1947).

Summerland and Steiner (1943) followed the development of 80 eggs on unsprayed trees in a 60-acre orchard May-June in Indiana, USA. Of these eggs, 37% hatched, 32% were parasitized by *T. minutum*, 19% were destroyed by predators, 5% died of unknown causes, and 7% disappeared. They identify *Chrysopa spp.* (Neuroptera: Chrysopidae) as the primary codling moth egg predator. During July-September the fate of 160 eggs was monitored on the same trees. In this time period, an average of 25% of the eggs hatched, 43% were parasitized by *T. minutum*, 23% were destroyed by predators, 4% died of unknown causes, and 5% disappeared. In total, 37% of the first brood, and 25% of the second brood eggs hatched (Summerland and Steiner, 1943).

MacLellan (1962) evaluated the importance of arthropod predators to the control of eggs and first instar codling moth in an integrated control orchard in Nova Scotia, Canada. He used weekly examination of marked fruit clusters to evaluate egg density and predation throughout the season. Predators present in the orchards throughout the season were counted and identified using a non-lethal sampling method (beating). Predators accounted for 14.4% mortality and *Trichogramma* sp. for 2.4%. The majority of predation was believed to be the work of plant bugs (Hemiptera: Miridae). However, other predators were found, including two predaceous thrips species *Haplothrips faurei* Hood and *Leptothrips mali* (Fitch). One mite, which was identified as *Anystis agilis* Banks (Acari: Anystidae), was also implicated, but believed to largely prey only on young larvae. The loss of larvae between egg hatch and fruit injury was estimated from comparisons between egg hatch data and fruit injury and was found to be about 58.5%. Codling moth populations as measured by trunk banding were low in the year of this study (0.6 larvae per foot of trunk) (MacLellan, 1962).

Geier (1963) collected branches with fruit clusters and measured the ratio of hatched eggs to deep entries in Australia's Capital Territory. He found 114 eggs to 38 entries. From these he estimated that approximately one third of 1^{st} instar codling moths are able to damage fruit after hatching. He provides no data on the causal agents of this mortality (Geier, 1963).

Glen (1975) glued codling moth eggs to leaf surfaces and apples to measure egg predation in an orchard where no insecticides were used in Long Ashton, UK. The eggs were glued singly, five per tree and checked twice weekly for signs of predation or hatching. They categorized damage as eggs disappeared, sucked out, or hatched. This was done in two years (1972, 1973). In this study three species of plant bugs (Hemiptera: Miridae) were identified as the most important agents of mortality for codling moth eggs in the field, since the majority of eggs that did not hatch had been sucked out. Disappearance of eggs was attributed to earwigs (Dermaptera: Forficulidae). However, their importance as control agents was questioned by the author because their habit of chewing holes in the apples facilitated colonization by brown rot fungus (*Monolinia* sp.), which made them more of a pest than a beneficial in those cider orchards (Glen, 1975).

Ferro et al. (1975) collected apple fruit clusters and nearby leaves to evaluate egg mortality, counted eggs, examined them for signs of predation, and reared parasitoids from them in the lab. They used data from sleeve cages to estimate first instar larval mortality by subtracting the number of fruit entries from the number of hatched eggs. They found that approximately 23% egg mortality resulted simply from non-viability of eggs, but pathogens may have been responsible for some of this mortality. They also estimated a 50% mortality of eggs from predators and parasitoids combined. After successfully hatching, they found an average of only 35% of first instars survived to damage fruit in sleeve cages (Ferro et al., 1975).

Glen (1977) used codling moths eggs glued to fruit and foliage to estimate egg predation in unsprayed orchards from 1972 -1974 Long Ashton, UK. An average of 12-86% of these eggs were “sucked out” and 3-29% were recorded as disappeared. Three species of plant bugs (Hemiptera: Miridae), a few species of predatory bug (Hemiptera: Anthocoridae), and a predatory mite (*Anystis* sp.) were found on the apple foliage in the study orchard and were able to feed on codling moth eggs in laboratory trials. The majority of egg mortality in these orchards was attributed to being attacked (sucked out) by plant bugs. Earwigs were again believed to have been responsible for the majority of egg disappearances (Glen, 1977).

Hagley et al. (1982) performed a series of laboratory experiments to determine the feeding preferences of adult ground beetles (Coleoptera: Carabidae) commonly found in Canadian apple orchards. They identified two species, *Amara aenea* De Geer and *Stenolophus comma* Fabricius that were often collected from apple foliage and branches and readily consumed early instars of codling moth in lab trials (Hagley et al., 1982).

Mills (2003; 2005b) reports that *Trichogramma platneri* Nagarkatti (Hymenoptera: Trichogrammatidae) can be an important parasitoid of codling moth eggs in California. Parasitism rates by this species in unsprayed orchards can range from 30-60% (Mills, 2003; Mills, 2005b).

1.2.2. Major natural mortality factors of the codling moth by life stage: Inside the apple (larval instars 2-4)

Inside of the apple, the codling moth larva is relatively well protected from most agents of mortality. However, mortality estimates for this life stage have ranged from 10.4% (MacLellan, 1962) to 52% (Ferro et al., 1975). MacLellan (1962), in Nova Scotia, used weekly examination of marked fruit clusters to evaluate egg density and predation throughout the season. He estimated based on these data that 10.4% mortality of larvae occurred inside the fruit. In a field study in eastern Washington (Ferro et al., 1975), mortality of larvae inside of apples ranged from 35% in early June to 52% in August. Neither of these studies provides data on the causes of mortality inside the apple.

1.2.2.1. Environmental conditions

Environmental conditions inside of the apple are not thought likely to cause mortality of codling moth larvae. For example, the relative humidity in the apple is always high, so higher external temperatures only allow the larva to grow faster (Kuhrt et al., 2005).

1.2.2.2. Predators and Parasitoids

MacLellan (1958) describes observations of a male downy woodpecker (Picoides pubescens [L.]) extracting codling moth larvae from infested fruit, but presents no data to estimate the impact of this behavior on codling moth populations (MacLellan, 1958). McAtee (1911) also cites several anecdotal reports of *P. pubescens* removing late instar codling moth larvae from infested apples.

Ascogaster quadridentata Wesmael (Hymenoptera: Braconidae)*, Elodia morio* (Fallén) (Diptera: Tachinidae)*, Hyssopus pallidus* (Askew) (Hymenoptera: Eulophidae)*,* and *Pristomerus vulnerator* Panzer (Hymenoptera: Ichneumonidae) are the most common parasitoids reported to attack codling moth larvae before, during, or after they enter the fruit. However, they do not kill the codling moth larva until after it leaves the fruit, so estimates of their impact are reported in studies of fifth instar mortality (below).

The only other reported natural enemies of the codling moth inside the apple are other codling moth larvae. Cannibalism was suspected and/or reported to occur in some early studies (Geier, 1963). However, Ferro and Harwood (1973) in a laboratory study found no evidence for cannibalism inside of the apple. They did find that intraspecific competition resulted in a developmental delay for the inferior competitor inside of apples (Ferro and Harwood, 1973).

1.2.3. Major natural mortality factors of the codling moth by life stage: Wandering larvae and overwintering larvae

The wandering larva stage is the stage that begins when the codling moth's fifth instar leaves the apple and begins to search for a location to pupate and ends when they spin the cocoon. Wandering larvae are active primarily at night (Borden, 1931; Garlick and Boyce, 1940; Geier, 1963) and may take several hours to find a suitable location for their cocoon (Garlick, 1948; Geier, 1963). Fifth instar codling moth larvae respond to a pheromone emitted by conspecifics that allows them to form aggregations of from 2-20 individuals for overwintering and pupation (Duthie et al., 2003; Jumean et al., 2009).

Cocoons can occur anywhere where conditions are relatively dry (Glen and Milsom, 1978) and they are protected from predators (Chandler, 1928; MacLellan, 1960). The majority of cocoons can be found under flakes of bark on rough barked apple trees and anywhere from the canopy to the interface with the soil surface (Chandler, 1928; Garlick, 1948; Jumean et al., 2009; Marshall, 1940). Fewer cocoons are reported found in the leaf litter and vegetation surrounding the trees (Chandler, 1928; Jaynes and Marucci, 1947; MacLellan, 1960), unless the trees have smooth bark in which case more larvae seek cocoon sites on the orchard floor (Simpson, 1903). Artificial substrates are often very attractive to codling moth wandering larvae, so barns, fence posts, trash, and apple bins have been found to serve as suitable overwintering and pupation sites (Chandler, 1928; Lacey and Chauvin, 1999; Steiner, 1929). Estimates of mortality during this life stage are highly variable, from 1.5% to 100% depending on the type of orchard, location of larvae, and time of year (Geier, 1963;

Jaques and MacLellan, 1965; Jaynes and Marucci, 1947; MacLellan, 1960; MacLellan, 1962).

1.2.3.1. Environmental conditions

Newcomer (1920) reported that during an unusual winter storm in eastern Washington, USA when temperatures reached lower than -29 ºC winter mortality of codling moth larvae approached 100%. Where the moths were protected by snow cover or where the temperature stayed above -26 ºC mortality was reportedly closer to 75% (Newcomer, 1920).

Steiner (1929) performed an experiment to determine the relative overwintering survival of larvae in cocoons in different materials on the orchard floor. He found that 2 out of 100 larvae spinning cocoons in the soil emerged the following spring. Sixty percent of larvae that had spun cocoons in plots where the orchard floor was covered in leaves emerged, in straw 38% survived, and in sod only 6% survived (Steiner, 1929). In another study, overwintering larvae were kept in an insectary in the orchard and the average percent mortality for these larvae over the five years of the study was 24.2% (Garlick, 1948). Finally, MacLellan (1962) used visual searches of tree trunks for overwintering codling moth larvae to estimate populations, and found that 3% of these larvae were killed by low temperatures (MacLellan, 1962).

1.2.3.2. Predators and Parasitoids

In an early study of overwintering codling moth mortality, Chandler (1928) undertook an investigation of the hibernation locations of codling moth larvae in January and February in southern Illinois. He states, without showing any data, that 90% of the overwintering larvae in southern Illinois orchards are removed by birds. He also states that larvae hibernating below the soil surface are much safer from bird predators than larvae on the trunk (Chandler, 1928).

Jaynes and Marucci (1947) in West Virginia, USA, used cardboard bands to collect larvae and measure predation and parasitization of this life stage, in insecticide sprayed and unsprayed orchards. The bands were examined periodically, and the numbers of larvae missing, diseased, or parasitized were recorded. Uninjured larvae and pupae in the bands were collected and kept to record emergence of parasitoids. Parasitization rates of this life stage were low (1 – 5.7%) in both orchards. The dominant parasitoid of this stage was *A. quadridentata*, but they list 16 other species that emerged from codling moth larvae and pupae collected from bands (Jaynes and Marucci, 1947).

Predation of larvae in the bands in this study was highly variable, and often the species responsible could not be identified based on codling moth remains alone (Jaynes and Marucci, 1947). Over the three years of the study seasonal average predation rates ranged from 3.6 – 14.4% in unsprayed and 4.0 – 8.68% in the sprayed orchard. One ant species, *Solenopsis molesta* (Say) (Hymenoptera: Formicidae), was reportedly responsible for the majority of predation of this life stage, but the beetle, *Tenebroides corticalis* (Melsheimer) (Coleoptera: Trogossitidae), also consumed a significant proportion of codling moth larvae in bands in some years (e.g. 14.3% in the unsprayed orchard in 1940) (Jaynes and Marucci, 1947).

Jaynes and Marucci (1947) also collected drop apples and immature fruit, and dissected them to determine whether parasitization of larvae in these locations was higher than in cardboard band traps. They found that parasitization of larvae in drop fruits was very similar to those collected from bands and did not differ between the sprayed and unsprayed orchard. The parasitism in immature fruit however was higher in an unsprayed orchard (15.9%) than an insecticide sprayed orchard (4.0%) and was considerably higher than that found in larvae taken from bands (max. 5%). The dominant parasitoid of these larvae was *A. quadridentata (Jaynes and Marucci, 1947)*.

To measure codling moth larval predation on the ground, Jaynes and Marucci (1947) also placed sentinel larvae in pieces of corrugated paper on the soil and under litter in both orchards. They combined observations in the field and lab to determine the types of remains that codling moth predators would leave behind and used this to classify the types of predation that they observed on these sentinel larvae. They found that predators destroyed almost 100% of these larvae. The majority of predation in the spring (65% in unsprayed, 69% in sprayed orchard) and fall (78.3% in unsprayed, 87.1% in sprayed) was due to the activity of carabid beetles (adults and larvae) and cantharid beetle larvae (*Chauliognathus* spp.) (Coleoptera: Cantharidae). In the summer, predation by these groups was reduced and the majority of sentinel larva mortality was caused by *Solenopsis* spp. and *Monomorium* spp., ants (Hymenoptera: Formicidae), which were responsible for 52% and 53% of predation in the unsprayed and sprayed orchard, respectively. Another notable spring predator in this study was the elaterid beetle *Conderus lividus* (De Geer) (Coleoptera: Elateridae), which consumed about 10% of the sentinel larvae in the unsprayed orchard and 5% in the sprayed orchard.

In the same study, during one winter (1940 – 41) corrugated paper strips containing larvae in October were pinned to trees and protected with hardware cloth from predation by birds. These bands were collected in April of the following year and predation rates of the cocooned larvae were estimated. This yielded figures of 5% and 1.5% predation in the unsprayed and sprayed orchards, respectively. A similar experiment in the summer showed that 25% of larvae on the tree trunks could be consumed by predators in the sprayed orchard and 28% in the unsprayed (Jaynes and Marucci, 1947).

Jaynes and Marucci (1947) also collected the top inch of soil from around apple trees in both orchards in all three years of the study and used a soil sifter to separate insects. Additionally, they used old fertilizer bags placed in the orchard and pitfall traps to catch ground beetles. These sampling methods yielded a great deal of information about the insect community in these orchards, but no clear patterns were evident. Since they found predation by ants of the mature larvae of codling moth to be the most important, they performed several experiments to determine the factors affecting the effectiveness of ants as natural control agents in their study orchards. What they found was that primarily two species of ant, *Solenopsis molesta* (Say) and *Formica subsericea* Say, were responsible for the majority of late instar codling moth mortality and that these two ant species were significantly less abundant in the sprayed orchard compared to the unsprayed orchard (Jaynes and Marucci, 1947).

Garlick (1948) performed a series of experiments using burlap bands to trap larvae as part of an investigation of codling moth larval behavior in a heavily infested, unsprayed orchard in Ontario. Over the course of this five-year-study the study trees yielded an average of 590 larvae per tree per year. The average parasitism by *A. quadridentata* during four years of the study was 18.9% (Garlick, 1948).

MacLellan (1958) used cardboard bands baited with codling moth larvae to measure the importance of woodpeckers (*Picoides* spp.) for codling moth population regulation in Nova Scotia, Canada. He also made records of woodpecker predation over several years (1950 -1956), which were based on characteristic bark punctures made by woodpeckers when they prey on codling moth under flakes of bark on apple trees. These data showed that on average woodpeckers removed 52% of the overwintering codling moth larvae in study orchards, though in individual orchards in some years the level reached 90% (MacLellan, 1958).

MacLellan (1960) traced the movements of codling moth larvae by dipping them in a radioactive solution before releasing them in orchards and then using a particle counter to find them. This was done with first instar larvae, which were recovered later in the year as mature larvae, or with mature larvae for short-term behavior experiments. He found that 94% of the larvae that made cocoons on the ground were eaten by predators (MacLellan, 1960).

MacLellan (1962) evaluated the importance of arthropod predators to the control of codling moth in an integrated control orchard. In this study visual searches of tree trunks for overwintering codling moth larvae were used to estimate populations. Using this method he found that 56% of overwintering larvae were destroyed by predators and parasitoids, 47% by woodpeckers, and 9% by *A. quadridentata* (MacLellan, 1962).

Geier (1963) noted that in Australia, larvae that spent a lot of time searching on the ground were frequently seen being attacked and killed by predators, such as ants, spiders, and earwigs, but he provides no numerical data to support these observations.

Ferro et al. (1975) found that approximately 22% of the first generation fifth instars emerging from apples were parasitized by *A. quadridentata*, whereas only about 5% of the second generation were parasitized in Washington, USA. They collected no data on predation of fifth instars but they estimated based on observations that total mortality of fifth instar codling moth larvae from the time they left the apple was approximately 30% in an unsprayed orchard. They also found that about 2.8 – 5% of pupating larvae did not successfully emerge as adults (Ferro et al., 1975).

Wearing (1975) used cobalt-58 to tag fifth instar codling moth larvae and track their mortality from the time that they left the fruit to the time that they emerged as adults the following spring (Wearing, 1975). This study was conducted for six consecutive years in an orchard of mature trees in New Zealand. The cobalt-58 tagging method produced an overall estimate of mortality of larvae from fruit exit to cocoon construction of 57.1% and a total mortality from cocoon construction to adult emergence of 63.8%. The average total mortality of larvae from fruit exit to adult emergence was 84.8%. The majority of mortality of wandering larvae was attributed to insect predators, and the majority (82%) of cocoon predation was attributed to birds. Wearing identified *Zoterops lateralis* (Latham), as the most common bird predator of codling moth in this study (Wearing, 1975).

Solomon et al. (1976) used apple logs placed in the field with fifth instar codling moth on them to measure predation of this life stage by natural enemies in Long Ashton, UK (1971-1973). They covered some of these logs with netting to exclude natural enemies and checked the logs at different intervals to record the numbers remaining and evidence of predation. They recorded an average of 95% predation of larvae on uncovered logs, which they attributed to predation by birds (Solomon et al., 1976).

Glen and Milsom (1978) used exclusion experiments in a Latin square design to determine the relative importance of ground beetles and birds to fifth instar codling moth predation in Long Ashton, UK. They found that predation was always high (90-95%) when birds were allowed access to trees regardless of whether or not beetles were excluded. In plots where ground beetles alone were allowed access to trees 56% of the codling moth larvae survived. There was no statistically significant difference between codling moth predation in plots with few beetles (beetles excluded) compared to many beetles. Blue tits (*Cyanistes caeruleus* [L.]) were found to be the most important bird predator of codling moth in these orchards. Bird predation was affected by the availability of alternative prey as well as the density of codling moth (Glen and Milsom, 1978).

Solomon and Glen (1979) observed the foraging behavior of birds in the same plots with sentinel codling moth larvae from Solomon (1976). In one of these experiments, logs with similar densities of sentinel larvae were placed in adjacent trees to see the effect that prey density had on the foraging behavior of the birds. Similar, more controlled experiments were run in an aviary with great tits (*Parus major* L.) and blue tits (*C. caeruleus*). They found evidence that codling moth larval predation by birds is consistently ~95%, but were not able to conclusively determine whether this was density dependent and or regulatory (Solomon and Glen, 1979).

Subrinprasert (1987) in Southern Sweden scraped trunk bark from trees in unsprayed orchards and measured parasitism of these larvae in the laboratory. This study found a total parasization rate of 38.3% by insect parasitoids. The dominant parasitoid was the tachinid fly *E. morio*, which accounted for 50.6% of parasitization in the study. Other important parasitoids included *P. vulnerator*, which emerged from 22.9% of the parasitized larvae, and *A. quadridentatus*, which was found in 14.7% of parasitized larvae (Subinprasert, 1987).

Hagley et al. (1982) performed a series of laboratory experiments to determine the feeding preferences of adult carabids commonly found in Canadian apple orchards. Their results indicate that, of the species tested, *Pterostichus melanarius* Illiger was the only one likely to prey on codling moth fifth instar larvae and pupae (Hagley et al., 1982). Hagley and Allen (1988) in Ontario used serological techniques to detect predation on fifth instar larvae by eight species of ground beetles. However, they were unable to correlate the density of any of these species with the number of codling moth larvae searching for pupation sites in their study orchard. They concluded that *P. melinarius* is the most important carabid predator in these orchards, but that carabids as a group were not a significant factor in the survival of codling moth larvae seeking pupation sites (Hagley and Allen, 1988).

Riddick and Mills (1994) combined data from laboratory assays and field sentinel prey studies to determine the potential importance of several species of carabid beetles as biocontrol agents of fifth instar codling moth in California apple orchards. They determined that the most important carabid predators of codling moth larvae in these orchards were *Pterostichus* spp*.* They measured a peak consumption of 60% of tethered fifth instar codling moth larvae per night during early June (Riddick, 1994).

Baumgartner (1999) measured predation of overwintering codling moth larvae for two years in two managed orchards and one abandoned orchard in the Central Coast of California. In this study the total average mortality of sentinel larvae exposed to all predators was 53.9%, but in one year in the unmanaged orchard it was as high as 98.7%. When birds were excluded the total average mortality of sentinel overwintering larvae in the study orchards was about 33%. Although it was not the intention of the study to test for density dependence, they measured an increase in predation between years from 15% to 83% when larval density was increased from one or two per tree to 14 larvae per tree (Baumgartner, 1999).

Mathews et al. (2004) used tethered codling moth fourth and fifth instars to measure nocturnal predation in a newly planted apple orchard in West Virginia. The study included four different orchard floor treatments, herbicide treated grass stubble, bare ground, composted manure, and a polyester mulch. They measured the highest predation rates (81.3%) in the herbicide plots in July. In August and September samples with human observers present they found no difference between treatments, but recorded an average predation rate of 51.93% in August and 15.42% in September. They also found that measured predation rates were significantly lower when human observers were present. Carabids and ants (Formicidae) were the only predators observed feeding on sentinel larvae by the human observers in this study (Mathews et al., 2004).

Mills (2005b) presents data from a trap band study in Europe and Central Asia from 1992 – 2003. The highest percent parasitism rates of first generation codling moth fifth instars in that study were for *Mastrus ridibundus* Gravenhorst (Hymenoptera:

Ichneumonidae) in Kazakstan (43.9%), *A. quadridentata* in Austria (42.6%), *Bassus rufipes* Nees (Hymenoptera: Braconidae) in Kazakstan (33.3%), and *P. vulnerator* in Switzerland (32.4%). Mills (2005b) also monitored the introduction of three parasitoid biological control agents of codling moth from 1992-2000 in California apple, pear, and walnut orchards. Two of the three species introduced did not establish successfully (*B. rufipes* and *Liotryphon caudatus* Ratzeburg), but one species *M. ridibundus* persisted at high levels in orchards. This species, which attacks the codling moth cocoon, was reported to reach parasitism rates as high as 56% in some unsprayed orchards and is believed to be contributing significantly to codling moth management in California (Mills, 2005a; Mills, 2005b).

1.2.3.3. Pathogens

During the course of an experiment to test the effectiveness of different formulations of *Beauveria bassiana* (Bals.-Criv.) Vuill. for codling moth control, Jaynes and Marucci (1947) measured a background *B. bassiana* infection rate of 9% on larvae collected from untreated bands (Jaynes and Marucci, 1947).

Jaques and MacLellan (1965) sampled 1 ft. wide strips of bark around the base of the tree trunks and evaluated signs of pathogen infection. They found that, from 1950 to 1958, an average of 1.7% of overwintering codling moth larvae were killed by fungal pathogens. The most common fungal pathogen isolated from these larvae was *B. bassiana* (Jaques and MacLellan, 1965).

Subrinprasert (1987) scraped trunk bark from trees in unsprayed orchards in Southern Sweden and measured pathogen infection rates of these larvae in the laboratory. They found that 20.5% of the larvae were killed by pathogens. Although they were not able to identify all of the pathogens, *B. bassiana* and *Paecilomyces farinosus* (Holmsk.) A. H. S. Br. and G. Sm., accounted for 34.4 and 29.5% of pathogen infections in that study, respectively (Subinprasert, 1987).

1.2.4. Major natural mortality factors of the codling moth by life stage: Adults

Adult mortality agents are particularly underrepresented in the literature, because adult moths are highly mobile, which makes it difficult to study them and their natural enemies under realistic conditions. Simpson (1903) lists bats, birds, and spiders as natural enemies of the adult moth, but provides no quantitative estimates for their impact on pest populations. Hagley (1972) investigated the effect of environmental conditions on adult mortality by rearing codling moth in green apples at 24 °C and 75% RH and a 16 h photoperiod. He then placed recently emerged adult pairs in oviposition cages at a range of humidity (45 – 90%) and temperatures (15.5 – 36 °C). He found that, under constant conditions of 24 °C and 60-75% RH, 76% of codling moth females died within 2 days of completing oviposition. He also found that egg viability declined significantly when females were reared at temperatures exceeding 33 °C.

1.3. Discussion: Biological control and codling moth natural mortality factors

Table 1.1 summarizes the mortality estimates provided by the studies included in this review. Some life stages of the codling moth have received more attention in the literature than others (Table 1.1). Unfortunately, in many of the cited references, sample sizes, measures of variability, experimental designs, and spatial scales are not given or are not comparable among studies and or life stages. However, over a hundred years of research has given us a great deal of useful information about the natural enemies of the codling moth. For example, we know that plant bugs are important egg predators in some parts of the world (Glen, 1975; Glen, 1977), but that predatory thrips and green lacewings can also make a contribution to egg predation where plant bugs are absent(Jaynes and Marucci, 1947; Summerland and Steiner, 1943). We also know that birds as predators of 5th instar larvae and pupae probably make the single largest contribution to pest management of the codling moth worldwide (Chandler, 1928; Glen and Milsom, 1978; MacLellan, 1958; MacLellan, 1962; Solomon et al., 1976). Parasitoids of all life stages have been particularly well studied and we have relatively good estimates of their impact on codling moth in the major apple growing regions of the world (Table 1.1). Codling moth pathogens are a group that were somewhat underrepresented by this review, because they are primarily researched in the context of augmentative applications (Lacey and Unruh, 2005) and I was restricted to studies of naturally occurring populations of natural enemies (Table 1.1).

Table 1.1. Mortality estimates for codling moth by life stage and mortality agents.

Mortality Agents:	Mortality (%)	Source	Year	Geographic region
Egg - 1st Instar				
Parasitoids				
T. minutum	28	Jaynes and Marucci	1947	West Virginia, USA
	32-43	Summerland and Steiner	1943	Indiana, USA
Trichogramma spp.	2	MacLellan	1962	Nova Scotia, Canada
T. platneri	30-60	Mills	2003	California, USA
Green lacewings				
Chrysopa spp.	19-23	Summerland and Steiner	1943	Indiana, USA
Thrips				
Leptothrips mali	6-22	Jaynes and Marucci	1947	West Virginia, USA
Plant bugs				
	12-86	Glen	1977	Long Ashton, UK
	14	MacLellan	1962	Nova Scotia, Canada
Earwigs	3-29	Glen	1977	Long Ashton, UK
Unspecified	33	Geier	1963	Australian Capital Territory
	25-50	Jackson	1982	Washington, USA
2nd - 4th Instar				
Unspecified	35-52	Ferro et al.	1975	Washington, USA
	10	MacLellan	1962	Nova Scotia, Canada

Table 1.1. (cont'd).

Mortality Agents:	Mortality (%)	Source	Year	Geographic region
5th Instar				
Birds				
Unspecified	90	Chandler	1928	Illinois, USA
	95	Solomon et al.	1976	Long Ashton, UK
Cicoides spp.	52-90	MacLellan	1958	Nova Scotia, Canada
	47	MacLellan	1962	Nova Scotia, Canada
C. caeruleus	90-95	Glen and Milsom	1978	Long Ashton, UK
Z. lateralis	24	Wearing	1975	New Zealand
Ants				
S. molesta	4 – 14	Jaynes and Marucci	1947	West Virginia, USA
Solenopsis spp. And *Monomorium* spp.	52-53	Jaynes and Marucci	1947	West Virginia, USA
Beetles				
T. corticalis	14	Jaynes and Marucci	1947	West Virginia, USA
Carabidae and Cantharidae	65-87	Jaynes and Marucci	1947	West Virginia, USA
C. lividus	5-10	Jaynes and Marucci	1947	West Virginia, USA
Carabidae	44	Glen and Milsom	1978	Long Ashton, UK
	60	Riddick and Mills	1994	California, USA
Parasitoids				
A. quadridentata	1-16	Jaynes and Marucci	1947	West Virginia, USA
	19	Garlick	1948	Ontario, Canada
	9	MacLellan	1962	Nova Scotia, Canada
	5-22	Ferro et al.	1975	Washington, USA
	15	Subrinprasert	1987	Sweden
	43	Mills	2005	Austria
E. morio	51	Subrinprasert	1987	Sweden
P. vulnerator	23	Subrinprasert	1987	Sweden
	32	Mills	2005	Switzerland

Table 1.1. (cont'd).

Mortality Agents:	**Mortality (%)**	**Source**	**Year**	**Geographic region**
5th Instar (cont'd)				
M. ridibundus	44	Mills	2005	Kazakstan
	56	Mills	2005	California, USA
B. rufipes	33	Mills	2005	Kazakstan
Unspecified predators				
	94	MacLellan	1960	Nova Scotia, Canada
	30	Ferro et al.	1975	Washington, USA
	54 - 99	Baumgartner	1999	California, USA
	57	Wearing	1975	New Zealand
	15 - 87	Mathews et al.	2004	West Virginia, USA
Pathogens				
B. bassiana	9	Jaynes and Marucci	1947	West Virginia, USA
	2	Jaques and MacLellan	1965	Nova Scotia, Canada
	7	Subrinprasert	1987	Sweden
P. farinosus	6	Subrinprasert	1987	Sweden
Adult Moth				
No data	**None**	**None**		

Unfortunately, quantitative estimates of the role of codling moth natural enemies are either lacking completely or from research performed 25+ years ago (Table 1.1) in orchards that are not representative of modern production practices. Modern orchards are trending towards higher tree densities (>1000 trees per acre) (Jackson et al., 1981; Wagenmakers, 1991) and more selective pest management practices (Blommers, 1992; Jones et al., 2009). High-density orchards have a continuous within row tree canopy, which may affect natural enemy foraging efficiency (Kareiva and Perry, 1989; Mills, 2003). Selective pest management tactics, such as mating disruption for codling moth (Witzgall et al., 2008) greatly improve the activity of natural enemies in the orchard environment (Epstein et al., 2000; Epstein et al., 2001), which is likely to have a significant impact on their contribution to pest management.

Conservation biological control aims to suppress crop pests by increasing the density or effectiveness of their natural enemies (Barbosa, 1998). In practice, natural enemy groups respond differently to conservation tactics, and conservation strategies must be tailored to fit the target pest and cropping system (Landis et al., 2000; Thomas, 1999; Thomas et al., 1991; Unruh et al., 2012; Walton, 2009). The first step in this process is to identify the natural enemies that have the greatest impact on the pest population (Mills, 2005b). This can be accomplished by building a stage structured matrix population model (Leslie, 1945) to identify the pest life stage that most contributes to population growth of the species (Caswell, 2001).

This approach can be very useful for planning biological control (Mills, 2005b) and can be used to inform pest management decision-making (Jones et al., 2009). However, analyses of this kind are dependent on the accuracy of the mortality estimates used to parameterize the model (Caswell, 2001). Extant estimates of the stage specific mortality of codling moth are highly variable or lacking entirely (Table 1) and depend on the geographic region, orchard management, and climate. Determining the optimal targets of conservation biological control of codling moth is likely to be a very regional and farming system specific process.

Future studies can contribute to present knowledge substantially by using digital video (Grieshop et al., 2012) or molecular techniques to identify mortality agents (de Roince et al., 2012) and the factors affecting their impact on codling moth populations more directly. Studies are also needed that identify the local factors (e.g. farming practices) as well as landscape-level factors that drive natural enemy impacts on codling moth.

An abundance of research has been performed concerning the non-target effects of pest management practices and the impact that naturally occurring insects have on pest control (Epstein et al., 2001; Hoyt, 1969; Kogan and Lattin, 1993; Ohnesorg et al., 2009; Van Driesche et al., 1998). However, there is still a lack of data directly linking natural enemy conservation practices to improvements in pest management in many agricultural systems (Letourneau and Bothwell, 2008; Wilby and Thomas, 2002). Understanding the major drivers of pest population dynamics in agroecosystems is essential if we are to develop a sustainable food supply for the 21st century (Thomas, 1999).

Chapter 2. Video observations of the natural enemies of eggs and fifth instar codling moth *Cydia pomonella* (L.) in Michigan apple orchards (USA)

2.1. Introduction

The conservation of natural enemies has long been fundamental to the theory and practice of Integrated Pest Management (IPM) (Jones et al., 2009; Stern et al., 1959; Vandenbosch and Stern, 1962). Numerous studies have shown that broad-spectrum insecticides can be harmful to natural enemies and thereby cause secondary pest outbreaks. (Epstein et al., 2001; Hoyt, 1969; Kogan and Lattin, 1993; Michelbacher, 1952; Ohnesorg et al., 2009; Van Driesche et al., 1998). Apple orchard agroecosystems have been a proving ground for IPM and the successful application of its guiding principles since its inception (Holdsworth Jr, 1970; Hoyt, 1969; Jones et al., 2009; Maclellan, 1972; Oatman, 1966). Yet, even in these systems very little is known about the actual contribution of natural enemies to the management of pest species (van der Geest and Evenhuis, 1991). For example, management of the codling moth in apples still relies largely on suppression of the pest through the use of synthetic or natural toxins that have a negative impact on natural enemies (Brunner et al., 2005; Epstein, 2010; Jones et al., 2009). Fortunately, recent successes in the use of selective insecticides and biopesticides (including mating disruption) have greatly increased the suitability of some apple orchards as a habitat for natural enemies (Epstein, 2010). Specifically, increased use of mating disruption for codling moth management has reduced the use of broad-spectrum insecticides in apple orchards (Jones et al., 2009; Witzgall et al., 2008). This has provided an environment where it is possible to investigate the impact of natural enemies on this pest in a setting where their potential is maximized (Epstein, 2010; Epstein et al., 2001; Gut and Brunner, 1998; Jones et al., 2009; Witzgall et al., 2008).

2.1.1. Natural enemies in organic agriculture

Organic pest management is IPM characterized by an even heavier reliance on cultural and biological pest management than conventional IPM. The pest management products available to certified organic growers in the United States include: pheromones, horticultural oils, kaolin clay, pyrethrums, spinosads, and bioinsecticides. Characteristics shared by most of these products are that they degrade quickly in the field, and or are very target specific, which reduces their impact on natural enemies (Epstein et al., 2001; Jenkins and Isaacs, 2007). Recent research suggests that organic orchards have increased biological control from natural enemies compared to conventional orchards (Crowder et al., 2010; Minarro et al., 2009), but this cannot be attributed to a reduction in synthetic insecticide use alone. Organically managed orchards generally have a low disturbance rate, high plant diversity, and are more structurally diverse than conventionally managed counterparts (Drinkwater et al., 1995). Insect communities in conventionally managed agroecosystems are dominated by the relatively few species that are adapted to broad-spectrum pest management strategies and low-diversity habitats (Crowder et al., 2010; Epstein et al., 2000; Epstein et al., 2001; Minarro et al., 2009).

In agricultural fields where broad spectrum, contact active insecticides are used frequently, arthropod communities should be limited to those species that are either good dispersers (e.g. large, strong fliers), are not active at the time insecticides are being applied (e.g. sheltered and/or in a dormant state), or that can reproduce quickly

(Brown, 1993). It remains to be seen whether this is the type of natural enemy community is less effective at suppressing pest populations than a more diverse community (Murdoch, 1975; Straub et al., 2008; van Emden and Williams, 1974).

2.1.2. Evaluating the impacts of orchard management on natural enemies of codling moth

There are a variety of methods available to researchers interested in measuring natural enemy populations and their impacts on pests in agricultural settings (Luck et al., 1988). I chose a combination of video observation of sentinel prey and relative measures of natural enemy activity (yellow sticky traps and pitfall traps). I focused on two sessile stages of the codling moth, the egg and cocoon stage. These stages are important targets for pest management from both a theoretical and practical standpoint (Geier, 1963; Jones et al., 2009; Kaya et al., 1984; Mills, 2005b).

Codling moth eggs are typically laid singly on foliage or fruit throughout the canopy of the tree and usually within 20 cm of a developing fruit (Geier, 1963; Stoeckli et al., 2008). Codling moth is an internal fruit feeder, which makes the eggs an important target for pest management aimed at preventing damage to the crop by the first instars entering the fruit (Brunner et al., 2005; Mota-Sanchez et al., 2008). Also, codling moth larvae are relatively well protected from natural enemies and insecticides once they gain entry to the fruit (Ferro et al., 1975; MacLellan, 1962), so insecticide applications targeting first-generation eggs can prevent damage by the second generation of larvae attacking the crop late in the season (Jones et al., 2009).

Fifth instar codling moth larvae leave the protection of the fruit to spin cocoons in protected locations either on the trunk of the tree or on the orchard floor (Chandler, 1928; Garlick, 1948; Garlick and Boyce, 1940; Geier, 1963). Once they have spun their cocoons they are relatively stationary as they pupate mid-summer (first generation) or overwinter as mature larvae (second generation) (Geier, 1963). During this time they are subject to attack by a variety of actively searching natural enemies (Walton, 2013a). This stage is also a good target for cultural pest management practices (Judd et al., 1997; Steiner and Ackerman, 1936), or augmentative (Lacey et al., 2006a; Mills, 2003) and classical (Mills, 2005a) biological control.

I set out to compare organic and conventionally managed apple orchards in Michigan (USA) in terms of natural enemy identity, but also in terms of their effect on codling moth sentinel prey. My principal goal was to test the hypothesis that natural enemies attack more codling moth prey in organic than conventional orchards. I tested this hypothesis by comparing attack rates of sentinel eggs and fifth instar larvae on video in three pairs of organic and conventionally managed orchards in southern Michigan.

2.2. Materials and Methods

2.2.1 Videography

Video systems used for sentinel prey observations consisted of 4-channel digital video recorders (DVRs) powered by deep-cycle12 v lead-acid batteries. I used digital cameras equipped with infrared spectrum LEDs to capture day and night insect activity. Cameras recorded continuously for 6 d (144 h), and batteries were replaced every 48 h. Video data was recorded onto removable 132 GB hard drives and transferred to 2 TB external hard drives in the laboratory. Cameras were fitted with 52 mm +10 or +4 macro lenses for egg and fifth instar predation video, respectively. A detailed description of the video system and equipment used can be found in Grieshop et al. (2012).

2.2.2 Experimental design

This study was carried out at three farms, two commercial apple orchards (42° 38.089'N 84°47.337'W and 43°1.012'N 83°54.890'W) and one research station (42° 52.563'N 85°14.900'W). At each farm, a pair of orchards was selected that were similar ages and tree densities, but differed in management regime (organic vs. conventional). All paired orchards were separated by at least 500 m and were at least 1 ha in size. In each orchard, a camera system with four cameras was placed in a tree row. Due to limitations imposed by the need to power all cameras from one centrally located battery and the need for farmers to be able to drive tractors between rows, all four cameras were located in the same tree row. Cameras were placed in separate trees with at least one tree separating them from the next camera. In general, trees with cameras in them were separated by 10 m. Each camera was focused on one sentinel prey patch. Prey patches consisted of either codling moth eggs on synthetic leaves or fifth instars in cardboard bands.

2.2.3. 2010 Experiments

2.2.3.1. Sentinel Prey

Egg patches contained 10 UV sterilized eggs that were left in place for the entire six days that video was recorded. Sentinel eggs were directly oviposited onto synthetic leaves in rearing cages in the lab. Individual gravid females were caged with synthetic leaves for one night (~12 h). Leaves with eggs were placed under a fluorescent UV-C bulb for 5 minutes to prevent hatching in the field (Calderon and Navarro, 1971). Trials in the lab found that this procedure reduced egg hatch rate to nearly zero (N.J. Walton, unpublished data).

Fifth instar patches consisted of three codling moth fifth instars in cardboard flutes. Sentinel fifth instar larvae were placed individually in plastic containers with flutes of corrugated cardboard in the lab for one night (~12 h). Flutes in which larvae had spun cocoons the following day were taken to the field and glued with cyanoacrylate glue to strips of paper that were stapled to apple tree trunks.

2.2.3.2. Data collection

Video of egg predation was recorded in each orchard for six days during June to coincide with peak egg laying for first generation codling moth. Video of fifth instar patches was recorded for six days at each orchard in August or September to coincide with the overwintering of second-generation codling moth larvae.

Unbaited yellow sticky traps were used to measure natural enemy activity in the study orchard canopies immediately following the egg video recordings. Ten of these traps were hung in the middle of the tree canopy, evenly spaced along two tree rows adjacent to the row that was used for video observations. Yellow sticky traps were in the orchards for seven days. Insect collected on yellow sticky traps were counted and categorized by functional and taxonomic group using Borror et al. (1992) and Marshall (2006).

Pitfall traps were used to measure natural enemy activity in the study orchards immediately following the fifth instar video observations in 2010. Ten pitfall traps were placed in each orchard evenly spaced along two rows adjacent to the row that was being used for the video observations. Traps were deployed for 48 hours. Pitfall traps were constructed from 1000 mL clear plastic deli containers and filled with a dilute unscented dish soap and water solution. Insects were removed from the traps and stored in 80% ethyl alcohol for storage and identification. Insects and other arthropods collected from the pitfall traps were counted and categorized by functional and taxonomic group using Borror et al. (Borror et al., 1992) and Marshall (Marshall, 2006).

2.2.4. 2011 Experiments

2.2.4.1. Sentinel Prey

In 2011, two egg predation experiments were conducted. The first was carried out to coincide with first-generation codling moth in June, and the second was conducted in August to coincide with codling moth second-generation oviposition. First-generation egg patches consisted of 3 codling moth eggs on a synthetic leaf. For the second-generation egg predation observations, codling moth eggs were not available, so I used the eggs of a closely related species, the oriental fruit moth *Grapholita molesta* Busck (Lepidoptera: Tortricidae).

Sentinel eggs were directly oviposited by female moths on green floral paper in rearing cages, which was cut and glued to synthetic leaves with cyanoacrylate glue. Eggs in 2011 were not treated with UV light to prevent hatching. Instead, these eggs were removed from the field before hatching (36 h) and replaced with new eggs.

Sentinel fifth instar larvae were placed individually in plastic containers with flutes of corrugated cardboard in the lab for one night (~12 h). Flutes in which larvae had spun cocoons the following day were taken to the field and glued with cyanoacrylate glue to strips of paper that were stapled to apple tree trunks.

2.2.4.2. Data collection

Video of egg predation was recorded for six days in June and for six days in August to coincide with peak egg laying for first and second-generation codling moth, respectively. Eggs were replaced every 36 h to prevent hatching in the field, so each camera recorded two batches of eggs for 36 h each. Video of fifth instar patches was recorded for six days at each orchard at the end of August and the beginning of September to coincide with the overwintering of second-generation codling moth larvae.

Unbaited yellow sticky traps were used to measure natural enemy activity in the study orchard canopies immediately following the egg video recordings. Twelve of these traps were hung in the middle of the tree canopy, evenly spaced along two tree rows adjacent to the row that was used for video observations. Yellow sticky traps were in the orchards for seven days. Insect collected on yellow sticky traps were counted and categorized by functional and taxonomic group using Borror et al. (1992) and Marshall (2006).

2.2.5. Video observation

Video was watched in the laboratory by paid observers. Observers viewed four video files at a time at up to 4x speed and recorded the start and end time for all video events as well as a brief description of the event. A video event was defined as beginning when an organism entered the sentinel prey patch and ending when it left the prey patch. All interactions with sentinel prey were watched by the authors for identification of the organisms and determination of whether or not predation had occurred. Sentinel prey were also collected from the field and survivorship and evidence of predation were recorded. If a determination could not be made as to whether an interaction with a sentinel prey item on video was fatal, the prey was assumed to have survived. Attacks on a prey item that had already been killed by another predator were recorded but not counted as predation events.

2.2.6. Natural enemy behavior

Timing of predation events was quantified using the video files' built in timestamps. For egg video observations, handling time was defined as the time from discovery of the prey item until feeding ceased and foraging behavior was resumed. In larval video observations, feeding could not be observed directly so handing time was defined as the time that the natural enemy spent inside of the cardboard flute. For parasitoid wasps that did not enter the cardboard flute, handing time was defined as the time spent probing the flute with the ovipositor. For all video observations, event duration was defined as the total time that an individual natural enemy spent in the sentinel prey patch. Ants from the same colony were counted as an individual natural enemy.

For characterization of diel activity patterns, dusk was defined as 2 hours before sunset. Dawn was defined as 2 hours after sunrise. Morning was the period from the end of dawn until 12 noon, and afternoon was defined as the period from 12 noon until dusk. Night began at sunset and ended at Dawn.

2.2.7. Data analysis

The response variable for video predation analysis of all experiments was the proportion of sentinel prey attacked on video per tree per 6-day observation period. Proportion values were normalized by arcsine square root transformation for statistical analysis. Video data were analyzed by mixed effects ANOVA with farm in the model as a random effect (block) and management regime as a fixed effect (*nlme*, R v2.9.2.). The

2011 fifth instar data did not meet the assumption of normality for ANOVA, so a nonparametric Kruskal-Wallis rank sum test was used to compare attack rates between organic and conventionally managed orchards (*stats*, R v.2.9.2).

Arthropod counts on yellow sticky cards and in pitfall traps were log10(x+1) transformed in order to meet the normality assumption of ANOVA. These data were also analyzed with a mixed effects model in R with farm as a random effect, management as the fixed treatment effect, and transformed natural enemy counts as the response variable (*nlme*, R v2.9.2.).

2.3. Results

2.3.1. 2010 First Generation Egg Predation

There was no effect of management on egg predation in 2010 (F=0.53, df=1,20, P=0.47). The untransformed mean proportion eggs attacked on video (+/- SEM) was 0.24 (+/- 0.10) and 0.13 (+/- 0.06) in organic and conventional orchards, respectively (Fig. 2.1).

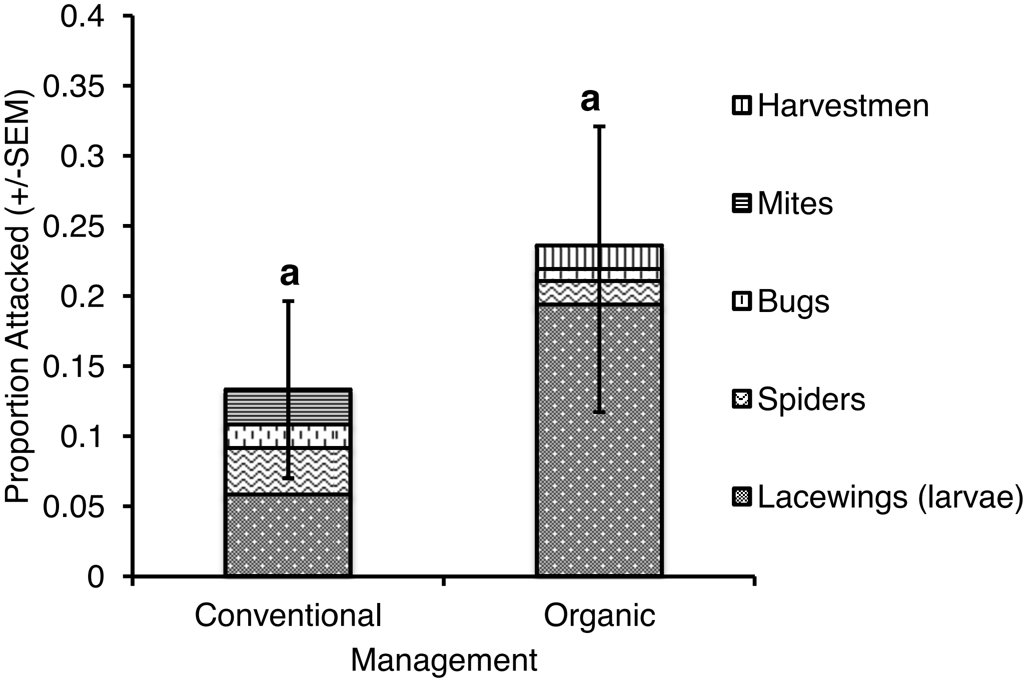

Figure 2.1. Mean proportion of sentinel codling moth eggs attacked by natural enemies on video from June 2010 in organic and conventionally managed orchards. Data shown are untransformed. Statistical analysis was performed by mixed model ANOVA (R v.2.9.2) using arcsine square root transformed proportions. Columns with the same letter are not significantly different (α=0.05).

2.3.2. 2010 Fifth Instar Predation

There were significantly more fifth instars attacked in video in organic orchards than conventional orchards in 2010 (F=7.30, df = 1,20, P=0.014). The untransformed mean proportion larvae attacked on video (+/- SEM) was 0.42 (+/- 0.12) and 0.08 (+/- 0.06) in organic and conventional orchards, respectively (Fig. 2.2).

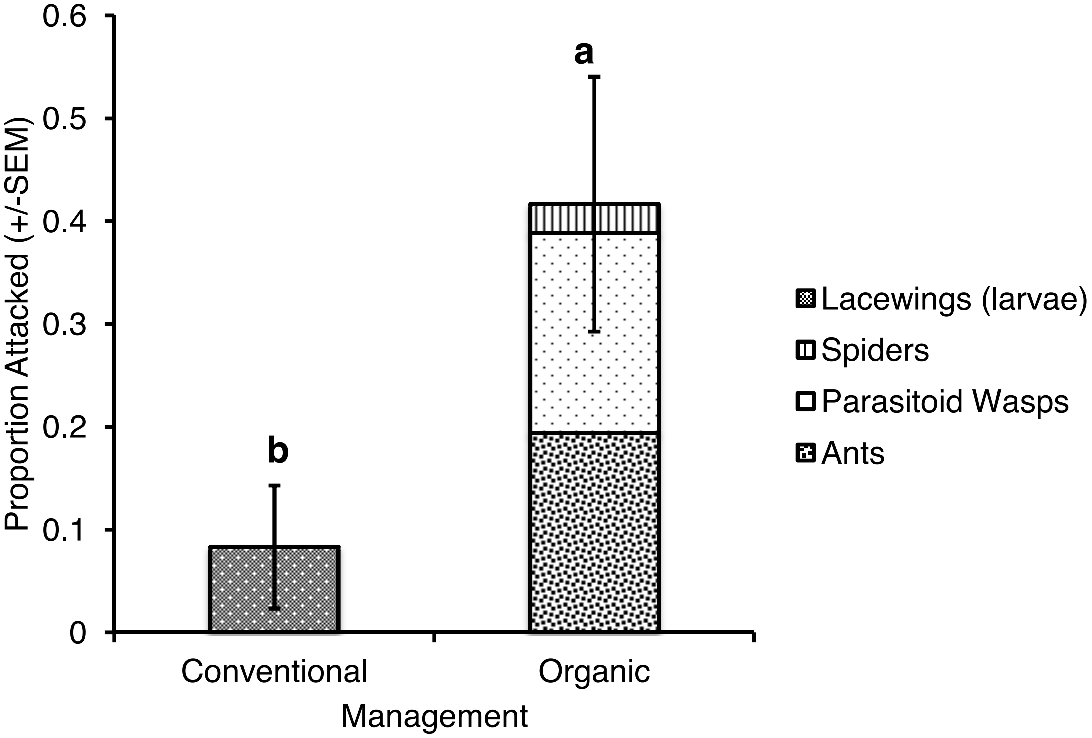

Figure 2.2. Mean proportion of sentinel codling moth fifth instars attacked by natural enemies on video from August and September 2010 in organic and conventionally managed orchards. Data shown are untransformed. Statistical analysis was performed by mixed model ANOVA (R v.2.9.2) using arcsine square root transformed proportions. Columns with the same letter are not significantly different (α=0.05).

2.3.3. 2011 First Generation Egg Predation

There was no effect of management on egg predation in June 2011 (F=0.68, df=1,20, P=0.42). The untransformed mean proportion eggs attacked on video (+/- SEM) was 0.08 (+/- 0.08) and 0.19 (+/- 0.11) in organic and conventional orchards, respectively (Fig. 2.3).

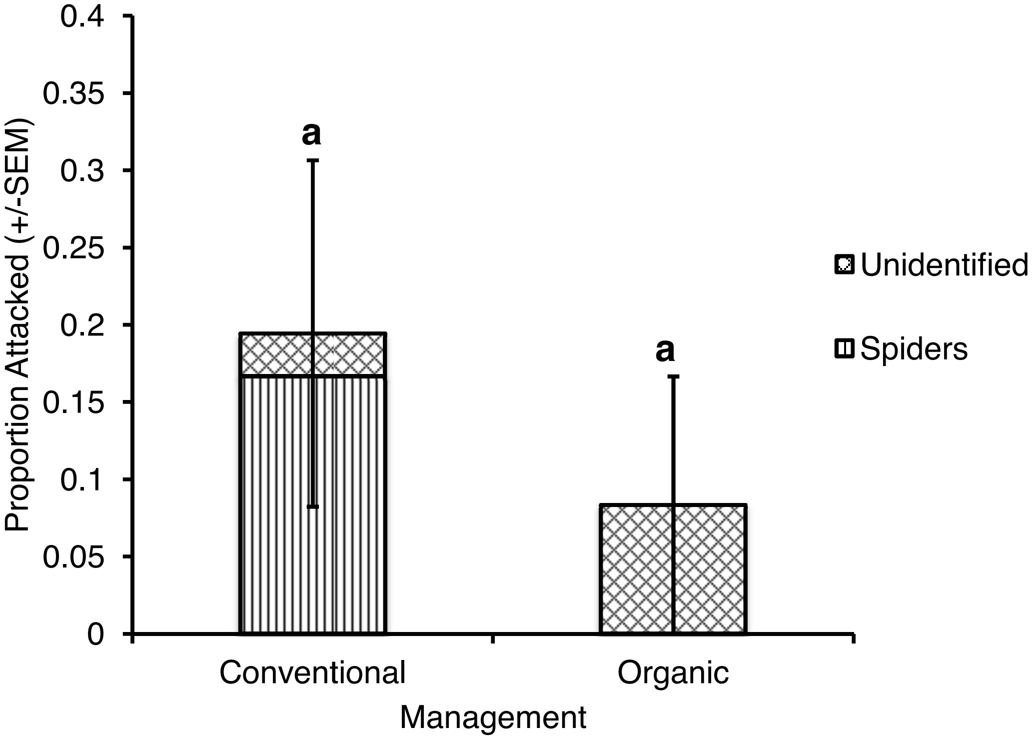

Figure 2.3. Mean proportion of sentinel codling moth eggs attacked by natural enemies on video from June 2011 in organic and conventionally managed orchards. Data shown are untransformed. Statistical analysis was performed by mixed model ANOVA (R v.2.9.2) using arcsine square root transformed proportions. Columns with the same letter are not significantly different (α=0.05).

2.3.4. 2011 Second Generation Egg Predation

There was no effect of management on egg predation in August 2011 (F=0.32, df=1,20, P=0.58). The untransformed mean proportion eggs attacked on video (+/-SEM) was 0.03 (+/- 0.03) and 0.08 (+/- 0.08) in organic and conventional orchards, respectively (Fig. 2.4).

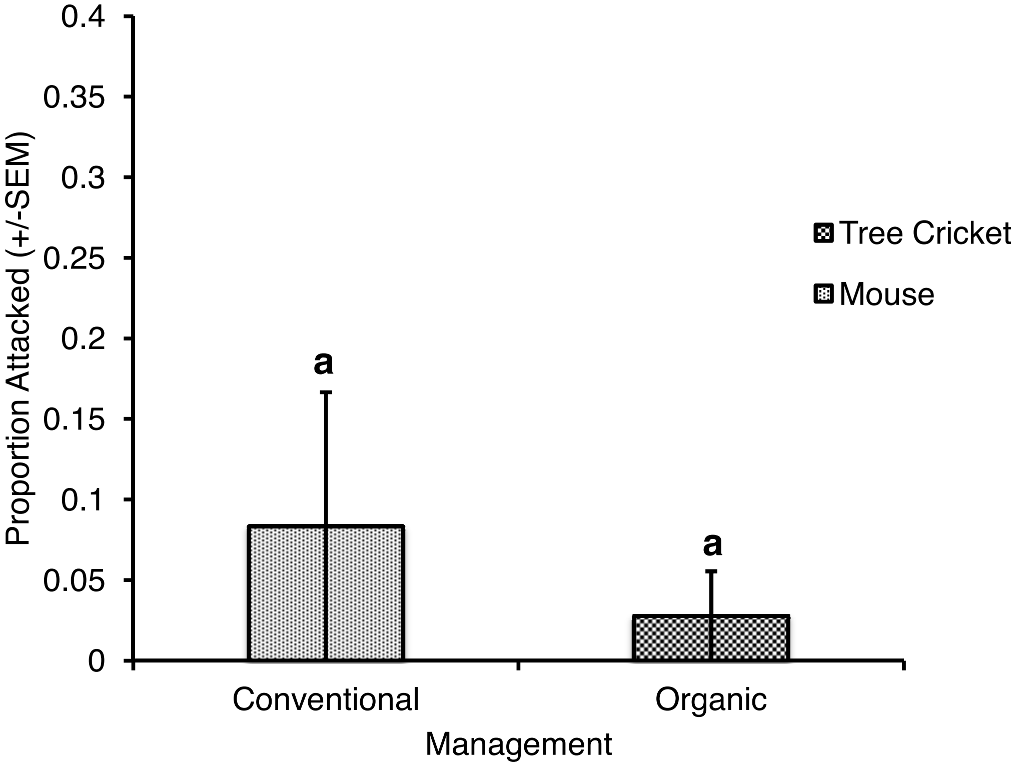

Figure 2.4. Mean proportion of sentinel oriental fruit moth eggs attacked by natural enemies on video from August 2011 in organic and conventionally managed orchards. Data shown are untransformed. Statistical analysis was performed by mixed model ANOVA (R v.2.9.2) using arcsine square root transformed proportions. Columns with the same letter are not significantly different (α=0.05).

2.3.5. 2011 Fifth Instar Predation

The effect of management on fifth instar predation in 2011 was not statistically significant (Kruskal-Wallis, X^2=3.27, df=1, P=0.079). The untransformed mean proportion larvae attacked on video (+/- SEM) was 0.11 (+/- 0.06) and 0.0 (+/- 0.0) in organic and conventional orchards, respectively (Fig. 2.5).

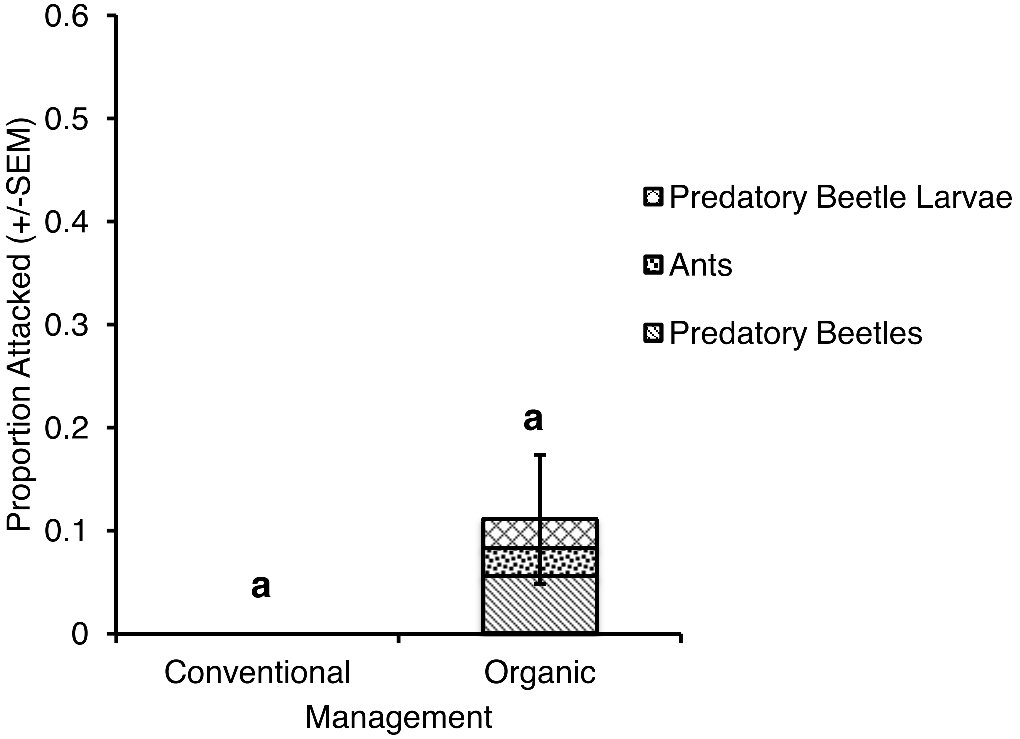

Figure 2.5. Mean proportion of sentinel codling moth fifth instars attacked by natural enemies on video from August and September 2011 in organic and conventionally managed orchards. Data shown are untransformed. A Kruskal-Wallis rank sum test was used to compare attack rates between organic and conventionally managed orchards (stats, R v.2.9.2). Columns with the same letter are not significantly different (α=0.05).

2.3.6. Yellow Sticky Traps

There were not significantly more natural enemies on yellow sticky traps in organic than conventional orchards in 2010 (t=0.61, df=50, P=0.55) (Fig. 2.6).

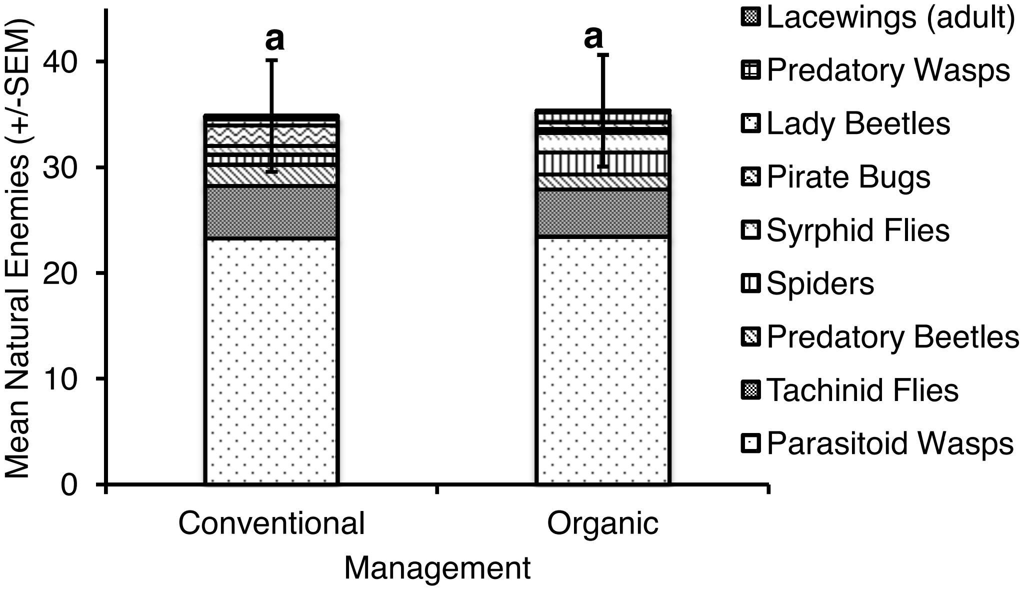

Figure 2.6. Mean number of natural enemies on unbaited yellow sticky traps in organic and conventional orchards in June 2010. Data shown are untransformed. Statistical analysis was performed by mixed model ANOVA (R v.2.9.2) using log10(x+1) transformed proportions. Columns with the same letter are not significantly different (α=0.05).

In 2010, the untransformed mean number of natural enemies on yellow sticky traps (+/- SEM) was 35.4 (+/- 5.3) and 34.9 (+/- 5.3) in organic and conventional orchards, respectively. In 2011, there were significantly more natural enemies (untransformed mean +/- SEM) in organic (98.4 +/- 20.2) than conventional orchards (43.2 +/- 18.6) (t=3.16, df=68, P=0.002) (Fig. 2.7).

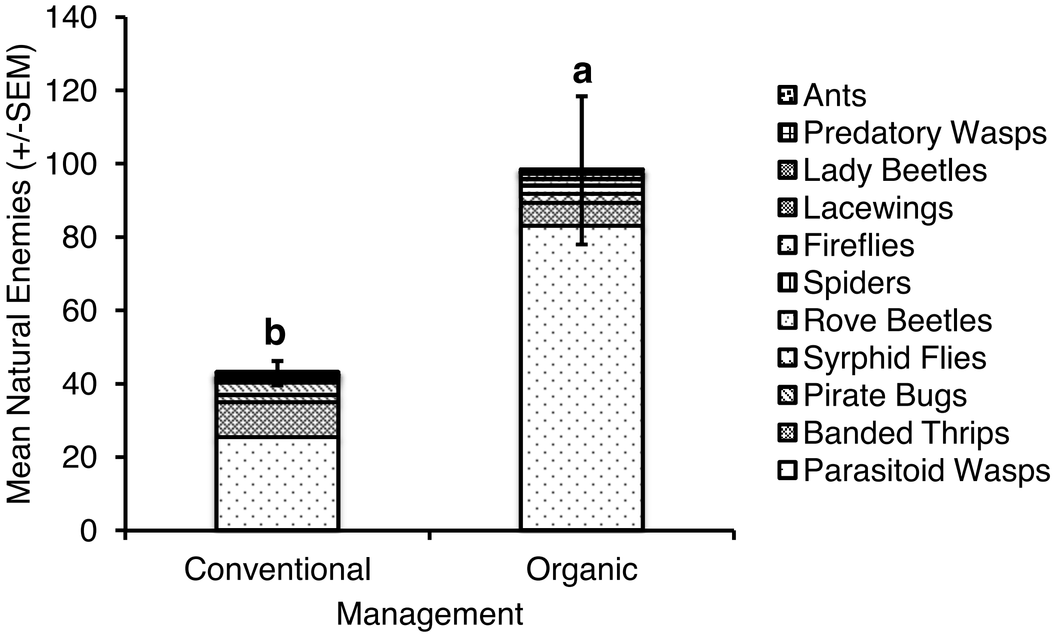

Figure 2.7. Mean number of natural enemies on unbaited yellow sticky traps in organic and conventional orchards in June 2011. Data shown are untransformed. Statistical analysis was performed by mixed model ANOVA (R v.2.9.2) using log10(x+1) transformed proportions. Columns with the same letter are not significantly different (α=0.05).

2.3.7. Pitfall Traps

When all natural enemy groups were included in the analysis, there were significantly more natural enemies in pitfall traps in organic than conventional orchards in 2010 (t=3.17, df=67, P=0.002) (Fig. 2.8). The untransformed mean number of natural enemies (+/-SEM) in pitfall traps in 2010 was 32.8 (4.9) and 17.2 (4.9) in organic and conventional orchards, respectively. No pitfall trap samples were taken in 2011.

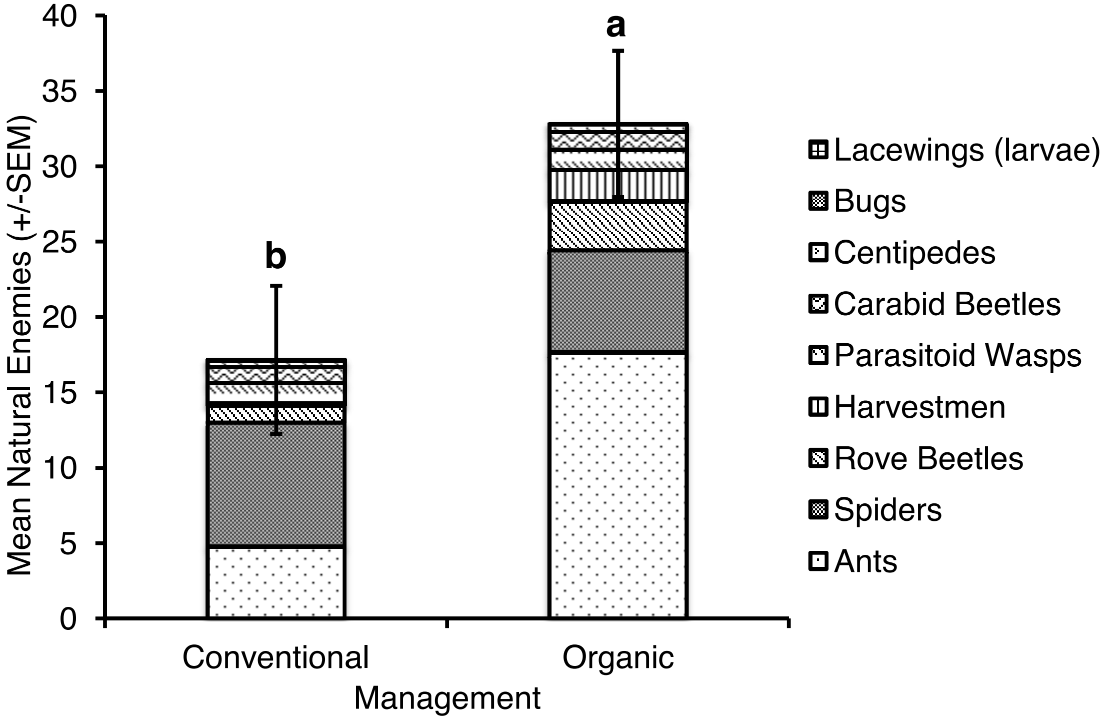

Figure 2.8. Mean number of natural enemies in pitfall traps in organic and conventional orchards in September 2010. Data shown are untransformed. Statistical analysis was performed by mixed model ANOVA (R v2.9.2) using log10(x+1) transformed proportions. Columns with the same letter are not significantly different (α=0.05).

2.3.8 Natural Enemy Behavior

The mean event duration (+/-SEM) for egg natural enemies was 16.7 m (+/-4.6). The mean handling time (+/-SEM) for egg natural enemies was 5.4 m (+/-0.9). The mean event duration for fifth instar natural enemies was 252.5 m (+/-82.4). The mean handling time (+/-SEM) for fifth instar natural enemies was 644.4 m (+/-219.9).

The mean event duration (+/-SEM) for egg natural enemies in organic and conventional orchards was 10.8 m (+/-3.7) and 25.3 m (+/-8.6), respectively. The mean handling time (+/-SEM) for egg natural enemies was 3.4 m (+/-0.5) and 7.8 m (+/-1.9) in organic and conventional orchards, respectively. The mean event duration for fifth instar natural enemies was 286.0 m (+/-94.7) and 40.4 m (+/-33.9) in organic and conventional orchards, respectively. The mean handling time (+/-SEM) for fifth instar natural enemies was 739.7 m (+/-248.2) and 40.4 m (+/-33.9) in organic and conventional orchards, respectively. Behavior data for individual natural enemy groups pooled by treatment and year are presented in Tables 2.1 and 2.2.

Table 2.1. Summary of codling moth egg natural enemy activity in video observations compared with rank abundances and mean abundances on yellow sticky traps (YSTs) pooled across years and management regimes.

Natural Enemy Group	Predation Events	YST Rank Abundance[1]	YST Abundance (±SEM)	Event duration (minutes±SEM)	Handling Time (seconds±SEM)	Active Period
Lacewings	34	7	0.2±0.1	12.7±4.8	250.3±143.1	Dusk/Dawn
Mites	6	NA	NA	14.7±3.7	492.7±332.5	Afternoon/Dusk
Spiders	6	5	1.3±0.2	60.2±32.7	702.5±553.8	Afternoon/Night
Bugs	4	NA	2.1±0.3	3.8±0.3	105.00±35.7	Night
Unidentified	4	NA	NA	17.4±10.5	657.0±1183.1	Afternoon/Night
Parasitoids	2	1	41.7±6.3	7.5±0.0	224.0±135.8	Dusk
Harvestmen	1	NA	NA	4.5±0.0	1.0±0.0	Night
Mice	1	NA	NA	0.1±0.0	4.0±0.0	Night
Tree Crickets	1	NA	NA	0.7±0.0	44.0±0.0	Night

[1] Groups were ranked based on mean abundances in yellow sticky trap counts. Natural enemies that were not counted on yellow sticky traps were not assigned a rank.

Table 2.2. Summary of codling moth fifth instar natural enemy activity in video observations compared with rank abundances and mean abundances in pitfall traps pooled across management regimes.

Natural Enemy Group	Predation Events	Pitfall Rank Abundance[1]	Pitfall Abundance (±SEM)	Event duration (minutes±SEM)	Handling Time (minutes±SEM)	Active Period
Ants	8	1	11.2±3.3	574.3±178.4	1654.3±407.2	Afternoon/Night
Parasitoids	7	4	1.4±0.3	4.2±1.4	1.5±0.7	Afternoon
Lacewings	3	9	0.03±0.02	40.4±33.9	40.4±33.9	Afternoon/Night
Beetles	2	5	1.1±0.2	30.0±0.0	30.0±0.0	Night
Beetle larvae	1	NA	NA	360.0±0.0	360.0±0.0	Night
Spiders	1	2	7.5±1.4	390.0±0.0	390.0±0.0	Night

[1] Groups were ranked based on mean abundances in pitfall trap counts. Natural enemies that were not counted in pitfall trap samples were not assigned a rank.

2.4. Discussion

Natural enemies in general, and in particular natural enemies of fifth instar codling moth tended to be numerically more abundant in the organic than the conventional apple orchards in this study (Figs. 2.1, 2.2, 2.5, 2.7, and 2.8). In the case of fifth instar codling moth, this trend was also reflected in the attack rates of sentinel prey on video (Figs. 2.2, 2.5, and 2.8). However, sentinel egg mortality patterns in 2011 did not follow the same trend (Figs. 2.3 and 2.4).

There is relatively little correspondence between the abundance of natural enemies on the yellow sticky card traps in both years and the abundance of codling moth egg enemies (Table 2.1; Figs. 2.1, 2.3, 2.4, 2.6, and 2.7). This illustrates the importance of direct measures of insect mortality in the field to verify conclusions based on relative measures of activity density of natural enemies in the field. Spiders and lacewings were the only groups that were detected by both sampling methods and both groups were poorly represented in the sticky trap samples (Table 2.1). Yellow sticky traps baited with herbivore-induced plant volatiles (Jones et al., 2011) or beat samples of foliage (Southwood, 1978) may be a more reliable tool for monitoring codling moth egg natural enemies in orchards.

Pitfall trap catches exhibited a closer correspondence to mortality of codling moth fifth instars on tree trunks. While the relative abundances of different natural enemy groups in pitfall traps did not reflect their importance as codling moth fifth instar predators (Table 2.2; Figs. 2.2, 2.5, and 2.8), the groups that were observed attacking codling moth were found in the pitfall traps in appreciable numbers (Table 2.2; Fig. 2.8). This provides some evidence that pitfall trap catches may continue to be a useful tool for estimating codling moth natural enemy pressure in orchard habitats (Epstein et al., 2000; Epstein et al., 2001)

2.5. Conclusion

As broad spectrum insecticides are phased out of apple production (Brunner et al., 2005; Mota-Sanchez et al., 2008) arthropod natural enemies are likely to play an increasing role in apple pest management (Epstein et al., 2000; Epstein et al., 2001; Jones et al., 2009). Quantification of this role has also become increasingly important both for pest management (Zhang and Swinton, 2009) and environmental policy decision-making (Boyd and Banzhaf, 2007; Isaacs et al., 2009; Losey and Vaughan, 2006; Luck et al., 2009). Unfortunately, previous quantitative estimates of this role in apple production systems were outdated or lacking entirely (Walton, 2013a). I have provided quantitative estimates of the mortality of two important codling moth life stages and identified the natural enemies responsible for that mortality in a small sample of southern Michigan apple orchards. The natural enemy community and their impact on codling moth varies substantially with geographic region (Walton, 2013a) and farm management practices (Walton, 2013a; Walton, 2013c), so more studies will be needed to adequately characterize the codling moth natural enemy complex in modern apple production systems.

I advocate the use of direct observation with digital video equipment to measure the important ecosystem services provide by beneficial arthropods in agricultural systems. Direct sampling of natural enemy activity with digital video systems has many advantages over other sampling methods (Frank et al., 2007; Grieshop et al., 2012; Mathews et al., 2004; Merfield et al., 2004). Of course, there are experimental limitations imposed by cost of equipment, labor needed to watch the video, and logistical considerations (see Methods above), which make it unlikely that video will completely replace other sampling methods on a large-scale in the near future. Still, video systems can be used intensively (as in this study) to calibrate or verify relative measures of arthropod activity, or they can be used more extensively to sample natural enemy activity over larger geographic areas (Grieshop et al., 2012). Reliable quantitative estimates of the impact that natural enemies have on agricultural pests can provide cost-savings to food producers and increase the sustainability of food production systems worldwide.

Chapter 3. Habitat characteristics influencing foraging success of codling moth antagonists in apple orchards

3.1. Introduction

The codling moth (*Cydia pomonella* [L].) is a severe pest anywhere that apples or pears are grown worldwide (Jones et al., 2009; van der Geest and Evenhuis, 1991). The two stages of the codling moth life-cycle that are most vulnerable to management are the egg stage and the mature larva (fifth instar) stage (Jaynes and Marucci, 1947; Mills, 2005b). Conventional pest management of codling moth in the U.S. generally relies on the use of broad-spectrum contact insecticides that are harmful to the insect natural enemies of pests (Epstein et al., 2000; Epstein et al., 2001; Giolo et al., 2009; Jones et al., 2009). Codling moth natural enemies tend to be more abundant in orchards utilizing natural enemy conservation practices in an integrated approach usually due to the use of more selective pesticide chemistries or application methods (Epstein et al., 2000; Epstein et al., 2001). As U.S. apple growers are forced to phase out their use of broad-spectrum Organophosphate insecticides under US Environmental Protection Agency (EPA) regulations, they may expect a substantial increase in the contribution of these generalist natural enemies to pest management in their orchards (Jones et al., 2009). However, the impact that these insects have on pest populations are likely influenced by factors other than the selectivity of a grower's pest management program (Norris and Kogan, 2005). I investigated the influence of two of these factors, orchard floor structural complexity and tree canopy connectivity on the mortality of codling moth in the field.

3.1.1. Orchard floor complexity and fifth instar codling moth mortality

Growers often manage the orchard floor beneath the tree canopy to optimize nutrient availability to the tree and minimize pest and disease pressure (Atucha et al., 2011; Mathews et al., 2004). Fifth-instar codling moth larvae leave the fruit and crawl from the tree canopy to the main trunk or orchard floor to seek a location spin a cocoon and pupate (mid-summer) or overwinter (fall) (Geier, 1963). In the past, the importance of the orchard floor as a location for codling moth to seek shelter for pupation and overwintering was minimal, because they are known to prefer locations under the rough bark on the trunks of traditional varieties of apple (Geier, 1963). However, with the growing acreage of dwarf apple varieties with smooth barked trunks, mature codling moth larvae are more likely to be forced to seek shelter on the orchard floor (Lacey et al., 2006a; Simpson, 1903).

Evidence from several studies of larval codling moth mortality under field conditions indicates that their survival is relatively low when they construct their cocoons on the orchard floor (Chandler, 1928; Geier, 1963; Jaynes and Marucci, 1947; MacLellan, 1960; Steiner, 1929; Steiner and Ackerman, 1936). However, these studies were largely observational and or for other reasons do not provide unequivocal tests of the hypothesis in question (Walton, 2013a). Furthermore, they were conducted in traditional low-density orchards planted with rough-barked tree cultivars, so the results may not be relevant to modern high-density orchard systems.

Known predators of fifth-instar larvae, pupae, and/or overwintering cocoons include: ground beetles, ants, birds, and lacewing larvae (MacLellan, 1958; Mills and Carl, 1991). Another important natural enemy of overwintering codling moth are entomopathogenic nematodes. Entomopathogenic nematodes are naturally occurring insect pathogens that are being investigated for use in augmentative biological control of codling moth (Kaya et al., 1984; Lacey et al., 2006a; Lacey and Unruh, 2005). For small crawling organisms such as these, with random search behavior, an orchard floor with high complexity (i.e. vegetation or litter) has a larger search area compared to an orchard floor with less structural complexity (Kostylev et al., 2005; Randlkofer et al., 2010). Therefore, the probability of an immobile prey item like a codling moth cocoon being found by a natural enemy should vary inversely with structural complexity (Holling, 1959). Orchard floor sanitation practices that reduce complexity of the orchard floor habitat thus should have a negative impact on codling moth in the presence of these natural enemies. Alternatively, structural complex orchard floors could provide alternative prey and a favorable microclimate for natural enemies (Mathews et al., 2004).

I investigated the effects of reducing complexity of the orchard floor habitat on the foraging success of codling moth natural enemies in a series of three field cage experiments. Each experiment was designed to test the hypothesis that reduced orchard floor complexity will increase the mortality of sentinel codling moth pupae or fifth instars in the presence of natural enemies. A different natural enemy was used for each experiment. The natural enemies used in the orchard floor experiments were: lacewing larvae (*Chrysoperla rufilabris,* Neuroptera: Chrysopidae), adult earwigs (*Forficula auricularia*, Dermaptera: Forficulidae), and the entomopathogenic nematode *Steinernema feltiae* (Rhabditida: Steinernematidae). I chose these organisms because they are all active searchers that have been implicated as important natural enemies of codling moth fifth instars (Epstein et al., 2000; Kaya et al., 1984) (Walton Chapter 2, this volume).

3.1.2. Canopy connectivity and mortality of codling moth eggs

The structure of the tree canopy is a characteristic of apple orchards that is changing with modern production practices (Jackson et al., 1981; Wagenmakers, 1991). In the increasingly dominant high-density orchard, dwarf apple varieties are planted at close proximity (1 – 1.5 m spacing in some orchards) and pruned to grow in a linear, “fruiting wall” along supporting trellis wire (Wagenmakers, 1991). This creates, from the perspective of small crawling insects, a continuous habitat much like a hedgerow. In contrast, under low-density apple systems, tree canopies are often distinct patches surrounded by the less favorable habitat of the orchard floor. This difference in spatial arrangement of trees is most likely to affect the movement of small, flightless arthropods with primarily random search behavior such as *Chrysoperla* spp. and other generalist natural enemies (Grieshop et al., 2008; Heinz, 1998; Kareiva and Perry, 1989; Suverkropp et al., 2010). These small crawling predators should have reduced dispersal in low-density apple plantings, which would prevent them from preying on as many prey as they would in a high-density planting (Huffaker, 1958; Kareiva and Perry, 1989).

Codling moth eggs can be found throughout the canopy of apple trees, but they are laid primarily on the upper side of the leaf in the vicinity (<20 cm) of developing fruit (Geier, 1963; Stoeckli et al., 2008). Known predators of codling moth eggs include thrips, mites, predatory bugs, and lacewing larvae (Mills and Carl, 1991).

I designed an experiment to test the hypothesis that increased canopy connectivity would increase the mortality of sentinel codling moth eggs compared to control plots in the presence of augmented numbers of flightless natural enemies. Specifically, I tested this hypothesis by releasing lacewing larvae (Chrysoperla rufilabris, Neuroptera: Chrysopidae) in experimental plots in an orchard where tree canopies overlap within tree rows (high connectivity) and an orchard where tree canopies do not overlap (low connectivity) and measuring predation of sentinel codling moth eggs. Green lacewing larvae were chosen for this experiment because they are available commercially in large quantities, and they are flightless active searching predators that are known to prey on codling moth eggs (Mills and Carl, 1991) (Walton Chapter 2 this volume).

3.2. Methods

3.2.1. Study Sites

Each experiment was conducted at one of two sites: Michigan State University's Clarksville Horticultural Experiment Station (42°52.563'N, 85°14.900'W) and AlMar orchards (43°1.012'N, 83°54.890'W), a privately owned certified organic farm. No insecticides were used while the experiments were running in either location.

3.2.2. Orchard floor complexity experiments

3.2.3. Experimental Design

For each complexity experiment, twelve rows of trees in a high-density orchard were selected to serve as blocks in a Randomized Complete Block Design. In each row, 6, 1 m by 0.3 m, field cages were placed at equal distances along the row. Each experiment had 3 levels of natural enemy treatment (described below) and 2 levels of complexity (described below) for a total of 6 treatment combinations. Within a block, one of these experimental treatment combinations was randomly assigned to each cage using a random number generator created in Microsoft Excel 14.1.0 (Microsoft Corporation 2011).

3.2.4. Natural Enemy Treatments

The three levels of natural enemy manipulation in each experiment were: *Control* (endemic natural enemies not removed, no natural enemies added), *Natural Enemies Removed*, and *Natural Enemies Added* (endemic natural enemies removed). Endemic natural enemies were removed from the cages using a modified leaf vacuum as described in Epstein et al. (Epstein et al., 2011).

3.2.5. Complexity Treatments

Orchard floor complexity was manipulated by either, using a string trimmer and leaf rake to remove vegetation and litter from beneath the trees in the orchard plots, or leaving the vegetation and litter in place. This produced two complexity levels, *Low* (vegetation removed) and *High* (vegetation undisturbed).

3.2.6. Cages

Cages were constructed in the field around 1 m by 0.3 m plots by driving a 1 m wooden stake into the corner of each plot and stapling a 1 m by 3 m sheet of 3 mil transparent plastic to each stake to make side walls. The bottom 3 cm of each plastic side wall was then buried and a 1 m by 0.3 m sheet of white nylon tulle attached with binder clips was used to cover the top of the cage.

3.2.7. Sentinel Prey

Codling moth sentinel prey were presented within each cage in small wooden shelters to simulate the protected locations where codling moth cocoons are typically found (Chandler, 1928; Steiner, 1929). Codling moth pupae and diapausing fifth instar larvae were obtained from the USDA-ARS Yakima Agricultural Research Laboratory (Wapato, WA, USA). Wooden shelters were created by cutting aged (>1 year) 4 cm diameter apple cuttings into 5 cm sections. A 0.5 cm diameter hole was drilled 2.5 cm down into the center of one of the cut ends of each section. Then, each section was split down the center with a chisel to create two shelters. The interior (split) surface of each shelter was covered with cotton gauze (affixed with hot melt glue), leaving a cavity formed by the drill-hole where the codling moth sentinel prey were inserted (pupae) or allowed to build a cocoon (fifth instars).

One day prior to each experiment, fifth instar larvae were removed from cold storage and placed individually in 500 mL polypropylene deli containers with one wooden shelter in each. On the day of the experiment shelters were inspected and those in which larvae had entered the cavity and constructed a cocoon were taken to the field to be used as sentinel prey. Four such shelters with sentinel prey in them were placed in each cage on the first day of the experiment. After 72 h in the field, shelters were collected and returned to the laboratory for mortality assessment. Any insects that were found in the shelters other than the sentinel prey were collected and preserved for identification. Beetle larvae that were found during the earwig experiment at Clarksville were treated with KAAD, preserved in 80% ethyl alcohol and identified using the taxonomic keys provided in Stehr (Stehr, 1991).

3.2.8. Experiment 1: Entomopathogenic nematodes

This experiment was conducted at AlMar orchards in August 2012 and repeated at Clarksville Horticultural Experiment Station in September 2012. At both locations, four codling moth fifth instars in wooden shelters were used as sentinel prey in the natural enemy treatment cages. In the experiment at Clarksville (September 2012), codling moth pupae were used as sentinel prey in the *Control* and *Natural Enemies Removed* cages only.

The natural enemy used in this experiment was the entomopathogenic nematode *Steinernema feltiae* (Rhabditida: Steinernematidae). After sentinel prey were introcuced to cages, nematodes were applied in water using a hand-held pressurized sprayer at a rate of 180,000 infective juveniles (IJs) in 250 mL of water per cage. Following initial application, an additional 125 mL of water was sprayed into the cages to improve survival of the IJs (Lacey et al., 2006a). No water was applied to *Control* or *Natural Enemies Removed* cages, but previous experiments have showed no detectable effect of water alone on mortality of sentinel codling moth larvae (N.J. Walton unpublished data).

3.2.9. Experiment 2: Lacewing larvae

This experiment was conducted at AlMar orchards in August 2012. The sentinel prey in this experiment were diapausing fifth instar codling moth. Wooden shelters with sentinel larvae in them were prepared as described above. The natural enemies used in this experiment were larvae of the green lacewing *C. rufilabris*. Second and 3rd instar *C. rufilabris* were supplied by Rincon Vitova Insectaries Inc., removed individually from the shipping substrate, and placed in microcentrifuge tubes for transport to the field. In the field, the microcentrifuge tubes were opened and placed in the center of the experimental cages to release the lacewing larvae. In cages that had the natural enemy treatment assigned to them, four lacewing larvae were released.

3.2.10. Experiment 3: Earwigs

This experiment was conducted in a 1 ha organic apple block at Clarksville Horticultural Experiment Station in September 2012. The natural enemy chosen for this experiment was the European earwig, *F. auricularia.* Earwigs were obtained from the Pesticide Alternatives Laboratory at Michigan State University and were wild collected in corrugated cardboard traps (Epstein et al., 2000) from an organic apple orchard. Prior to being used in this experiment they were held in 40 x 25 x 14 cm plastic containers and maintained on a diet of apples, dog food, bee pollen and water *ad libitum* with a 16:8 L:D photoperiod at 45% RH and 25 °C*.* Earwigs were starved for 24 h prior to the start of the experiment, sexed, and placed individually in individual microcentrifuge tubes. In cages that had the natural enemy treatment assigned to them, four earwigs (2 male, 2 female) were released into the center of each cage.

3.2.11. Experiment 4: Canopy connectivity

This experiment was conducted at AlMar orchards in June 2012. Tree architecture could not be manipulated directly without removing economically significant amounts of crop plant material, so experimental plots were set up within orchards with different pre-existing canopy structures. After consultation with the grower, I selected two orchards of similar size but different tree spacings. Trees in the high-connectivity orchard were planted with a 2.2 m in-row tree spacing (~400 trees per acre) whereas trees in the low-connectivity were planted at a 5 m in-row tree spacing (~150 trees per acre). The two tree densities have radically different tree architecture resulting in higher canopy connectivity for the higher density orchard. I estimated the volume of the orchard canopies in the two orchards using the tree-row-volume method developed by Manktelow and Pratt (Manktelow and Pratt, 1997). I used these canopy volume estimates to standardize the volume of canopy that was contained with each of my experimental plots, so that each plot contained approximately 200 m^3 canopy. This translated to 3 trees per plot and 18 trees per plot in the low-density and high-density orchards, respectively. Each orchard contained 10 plots per treatment arranged in a factorial treatment design with plots separated by at least 9 m between tree rows or 30 m within tree rows.

Codling moth mortality in the experimental plots was measured with sentinel codling moth eggs. Codling moth adults were reared from pupae obtained from the USDA-ARS Yakima Agricultural Research Laboratory (Wapato, WA, USA). Eggs were collected by holding male and female codling moth adults in a 27 m^3 plastic screen cage with a dilute (~5%) sugar solution provided on cotton rolls. Wax paper sheets were hung from the ceiling of the cage to serve as an oviposition substrate for the female moths. Wax paper sheets were collected daily for use in experiments. Pieces of this paper containing individual ≤24 h old eggs were cut and glued to the heads of steel pins with cyanoacrylate glue and placed in Styrofoam coolers for transport to the field. Egg bearing pins were inserted through apple leaves on the morning of the first day of the experiment. Fifteen sentinel eggs were placed in each plot. Sentinel eggs were spaced uniformly throughout the canopy of the trees in each plot. Eggs were collected from the field and returned to the lab for mortality assessment 72 h after they were placed in the field.

The natural enemy chosen for this experiment was the green lacewing *C. rufilabris*. First instar *C. rufilabris were* obtained from a commercial supplier (Rincon Vitova Insectaries, Inc.) and released at a density of 1,000 larvae per plot. Lacewing larvae were released in the canopy of the trees in the center of each plot by distributing their shipping substrate into three Bio-Boxes (Biobest Canada Ltd.), which were then hung in the tree in the center of the plot. The remaining experimental plots did not have lacewing larvae released in them (control plots). Egg mortality was assessed in the laboratory daily for one week following exposure to field conditions.

One day after all sentinel prey were removed from the orchards, the density of lacewing larvae was evaluated in the plots by beating (Southwood, 1978). Three samples were taken per plot by striking the apple foliage with a 1 m section of 3 cm PVC pipe. One such sample was taken from either end of each plot and one sample was taken from the center. Lacewing larvae were identified visually, counted, and released.

3.2.12. Statistical analysis

Orchard floor experiments were analyzed by ANOVA in R (v.2.9.2) (R-core-development-Team, 2009). Proportion sentinel prey mortality in each cage was arcsin square root transformed prior to analysis. In the final model, row was included as a blocking effect, natural enemy treatment, complexity treatment and their interaction were included as fixed effects. Preplanned comparisons of simple effects were performed using Tukey's Honest Significant Difference (HSD) (*TukeyHSD*, R v.2.9.2).

The mean numbers of predatory beetle larvae in *Low* and *High* complexity cages in the earwig experiment at Clarksville were compared using Pearson's Chi-squared test in R (*chisq.test,* R v.2.9.2). The relationship between abundance of predatory beetles and sentinel codling moth mortality in the earwig experiment was analyzed with simple linear regression (lm, R v.2.9.2) using the mean number of predatory beetle larvae and mean proportion mortality per tree row (block).

Mortality of eggs in the canopy connectivity experiment was analyzed using the Kaplan-Meier method (Kleinbaum and Klein, 2012; R-core-development-Team, 2009). Eggs on pins that could not be recovered from the field or that had been parasitized by *Trichogramma* spp. were analyzed as censored observations. Survival curves were compared using Wilcoxon (Gehan) tests (R v.2.9.2, *survival* package).

The mean number of lacewings per plot in beat samples was log10(x+1) transformed prior to analysis. These data were analyzed by ANOVA, with orchard connectivity, lacewing treatment, and their interaction included in the model as fixed effects (*aov*, R v.2.9.2).

3.3. Results

3.3.1. Entomopathogenic nematodes

The natural enemy treatment effect was statistically significant at both sites for this experiment (AlMar: F = 13.0, df = 2,55, p<0.001; Clarksville: F = 7.59, df = 2, 55, p=0.001). The complexity treatment, however was not statistically significant at either site (AlMar: F = 1.60, df = 1,55, p=0.21; Clarksville: F = 0.02, df = 1, 55, p=0.87) and neither was the interaction of natural enemy treatment and complexity (AlMar: F = 1.86, df = 2,55, p=0.17; Clarksville: F = 0.49, df = 1, 55, p=0.61).

Preplanned pairwise comparisons using Tukey’s HSD revealed some important differences between and among natural enemy treatments (Figs. 3.1 and 3.2).

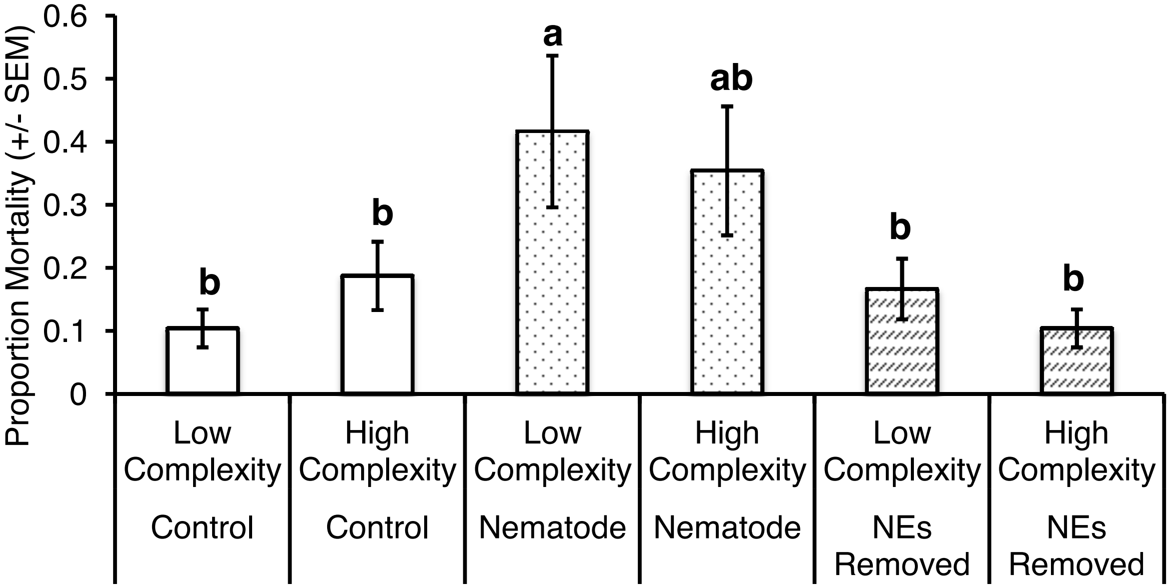

Figure 3.1. Mean proportion mortality of sentinel codling moth larvae in experimental cages at AlMar orchard in June of 2012. Control cages had no manipulation of their natural enemy communities, Nematode plots had endemic natural enemies removed and received an application of entomopathogenic nematodes. NEs removed plots had endemic natural enemies removed. Means separations were performed on arcsin square root transformed data. Tukey's HSD was used to control the experimentwise error rate at α=0.05 (R v.2.9.2). Columns with the same letter above them are not significantly different.

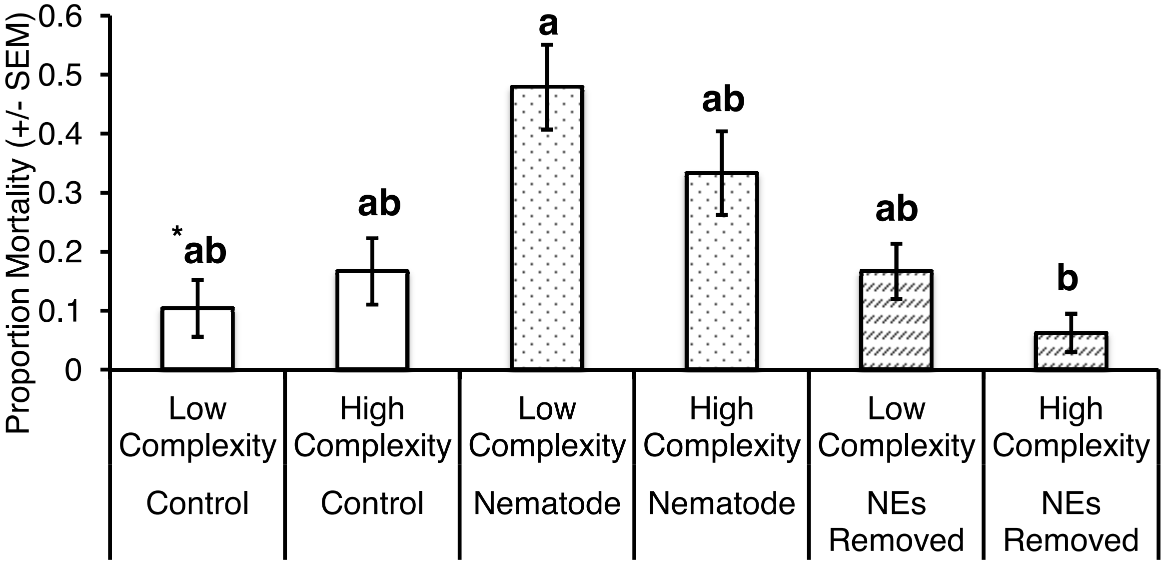

Figure 3.2. Mean proportion mortality of sentinel codling moth in experimental cages at Clarksville Horticultural Experiment Station in June of 2012. Control cages had no manipulation of their natural enemy communities, Nematode plots had endemic natural enemies removed and received an application of entomopathogenic nematodes. NEs removed plots had endemic natural enemies removed. Means separations were performed on arcsine square root transformed data. Tukey's HSD was used to control the experimentwise error rate at α=0.05 (R v.2.9.2). Columns with the same letter above them are not significantly different.

*The comparison, Low Complexity Nematode – Low Complexity Control, was marginally significant (Padj.= 0.065).

The untransformed mean mortality (+/-SEM) in all nematode treated cages was 0.39 (+/- 0.06) at Clarksville, which was at least twice as high as all other natural enemy treatments (Tukey's HSD P(adj.)<0.01) (data not shown). At AlMar, the untransformed mean mortality (+/-SEM) in the nematode cages was 0.40 (+/-0.05), which was also at least twice as high as all other natural enemy treatments at that site (Tukey's HSD

P(adj.)<0.001) (data not shown). The untransformed mean (+/-SEM) mortality in natural enemies removed cages at Clarksville was 0.14 (+/-0.05) and was not significantly different from that of the control cages (0.15 +/- 0.04) regardless of complexity level (Clarksville: Tukey's HSD P(adj.)=0.95) (data not shown). The untransformed mean (+/-SEM) mortality in all natural enemies removed cages at AlMar was 0.11 (+/-0.03) and was not significantly different from that in the control cages (0.14 +/- 0.04) (Tukey's HSD P(adj.)=0.97) (data not shown).

At AlMar, the nematode treatment in the low complexity cages had higher proportion mortality (0.48 +/- 0.07) than the low (0.10 +/- 0.05) and high (0.17 +/- 0.06) complexity control cages (nematode/low - control/low: Tukey's HSD P(adj.)=0.001; nematode/low – control/high: Tukey's HSD P(adj.)=0.016) (Fig. 3.1). The nematode low complexity treatment at AlMar also had a significantly higher proportion mortality than the low complexity (0.17 +/- 0.05) and high complexity (0.06 +/- 0.03) natural enemies removed cages (nematode/low – enemies removed/low: Tukey's HSD P(adj.)=0.026; nematode/low – enemies removed/high: Tukey's HSD P(adj.)<0.001) (Fig. 3.1), but not than the nematode high complexity cages (0.33 +/- 0.07) (nematode/low – nematode/high: Tukey's HSD P(adj.)=0.65) (Fig. 3.1). Also at AlMar, the nematode treatment in the high complexity cages had higher mortality than the predators removed high complexity cages (nematode/high – predators removed/high: Tukey's HSD P(adj.)=0.026) (Fig. 3.1), but not than any other cage type (Fig. 3.1).

At Clarksville, there was significantly higher proportion mortality in the low complexity nematode cages (0.42 +/- 0.12) than the high complexity predators removed cages (0.10 +/- 0.03) (nematode/low – predators removed/high: Tukey's HSD

P(adj.)=0.046) (Fig. 3.2). The difference between proportion mortality in the nematode low complexity cages and the control low complexity cages (0.10 +/- 0.03) was also marginally significant at Clarksville (nematode/low – control/low: Tukey's HSD P(adj.)=0.066) (Fig. 3.2).

3.3.2. Lacewing larvae

The natural enemy treatment effect (F=0.93, df=2,55, P=0.39), complexity (F=0.04, df=1,55, P=0.85), or their interaction were not statistically significant in this experiment (F=1.48, df=2,55, P=0.24). Pairwise comparisons using Tukey's HSD also did not reveal any significant differences between any of the experimental cage types in this experiment (Fig. 3.3). The mean untransformed proportion mortality (+/- SEM) in lacewing treatment cages was 0.19 (+/-0.07) and 0.21 (+/- 0.06) in low complexity and high complexity cages, respectively.

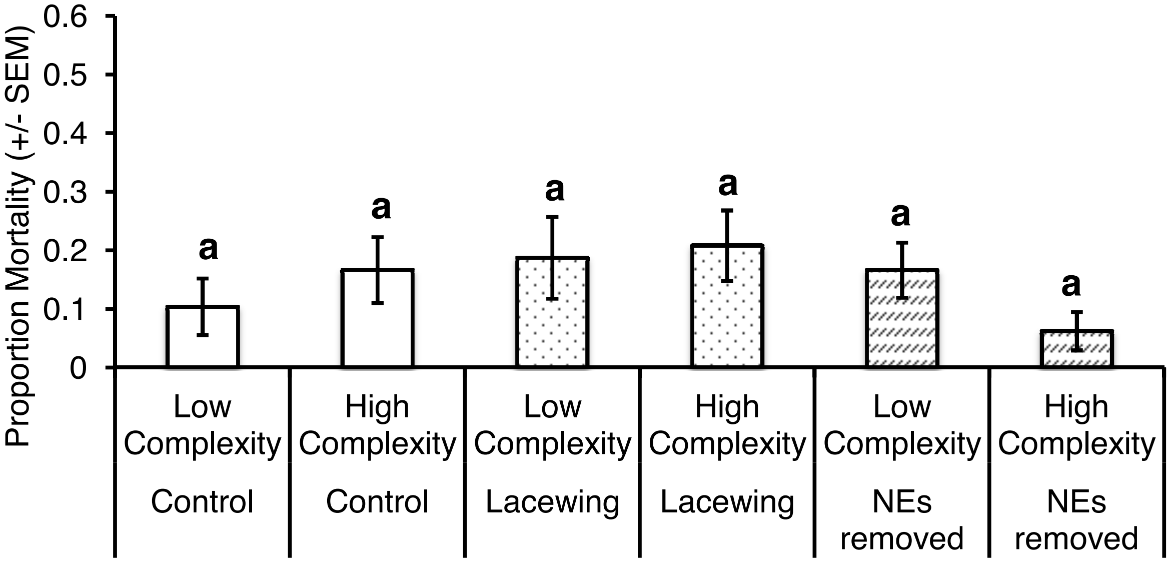

Figure 3.3. Mean proportion mortality of sentinel codling moth larvae in experimental cages at AlMar orchard in June of 2012. Control cages had no manipulation of their natural enemy communities, Nematode plots had endemic natural enemies removed and received an application of entomopathogenic nematodes. NEs removed plots had

endemic natural enemies removed. Means separations were performed on arcsine square root transformed data. Tukey's HSD was used to control the experimentwise error rate at α=0.05 (R v.2.9.2). Columns with the same letter above them are not significantly different.

3.3.3. Earwigs

The natural enemy treatment effect (F=0.30, df=2,55, P=0.75), complexity (F=0.80, df=1,55, P=0.38), or their interaction were not statistically significant in this experiment (F=1.06, df=2,55, P=35). Pairwise comparisons using Tukey's HSD also did not reveal any significant differences between any of the experimental cage types in this experiment (Fig. 3.4). The mean untransformed proportion mortality (+/- SEM) in earwig treatment cages was 0.13 (+/-0.04) and 0.23 (+/- 0.07) in low complexity and high complexity cages, respectively.

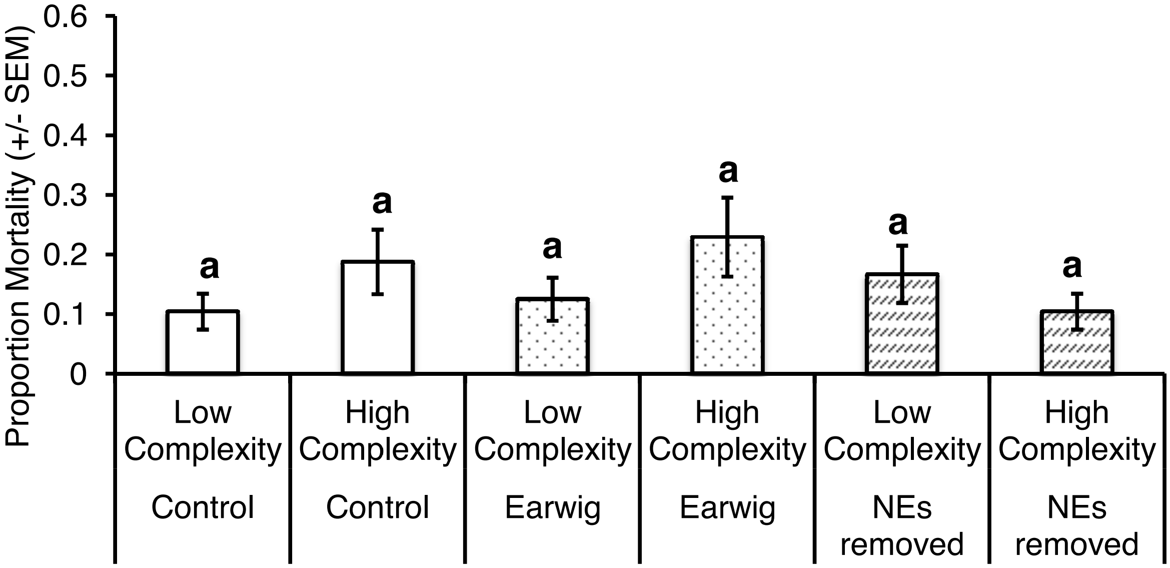

Figure 3.4. Mean proportion mortality of sentinel codling moth pupae in experimental cages at AlMar orchard in June of 2012. Control cages had no manipulation of their natural enemy communities, Nematode plots had endemic natural enemies removed

and received an application of entomopathogenic nematodes. NEs removed plots had endemic natural enemies removed. Means separations were performed on arcsine square root transformed data. Tukey's HSD was used to control the experimentwise error rate at $\alpha=0.05$ (R v.2.9.2). Columns with the same letter above them are not significantly different.

The mean predatory beetle larva density (+/-SEM) was 0.01 (+/- 0.02) and 0.06 (+/- 0.02) in low complexity and high complexity cages, respectively. The Pearson's Chi-squared test comparing predatory beetle larval density between low and high complexity cages indicated a significant difference between these two cage types ($X^2=4.63$, df=1, p=0.031) (Fig. 3.5). There was also a significant correlation between predatory beetle density and mortality of sentinel codling moth pupae in this experiment ($y=2.16x+0.14$, P=0.002, adjusted $R^2=0.58$) (Fig. 3.6).

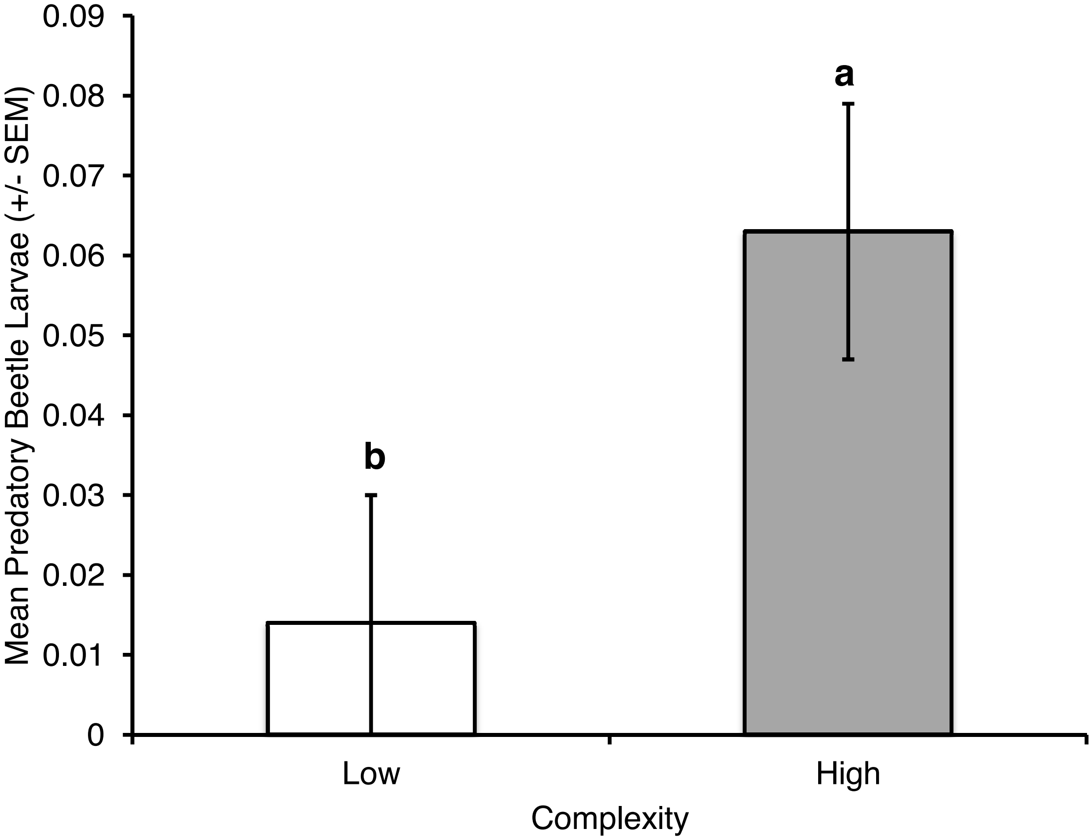

Figure 3.5. The mean number of predatory beetle larvae found preying on sentinel codling moth in the field cages at Clarksville in September 2012. Means separations were performed using Pearson’s Chi-squared test (R v.2.9.2). Columns with the same letter above them are not significantly different (α=0.05).

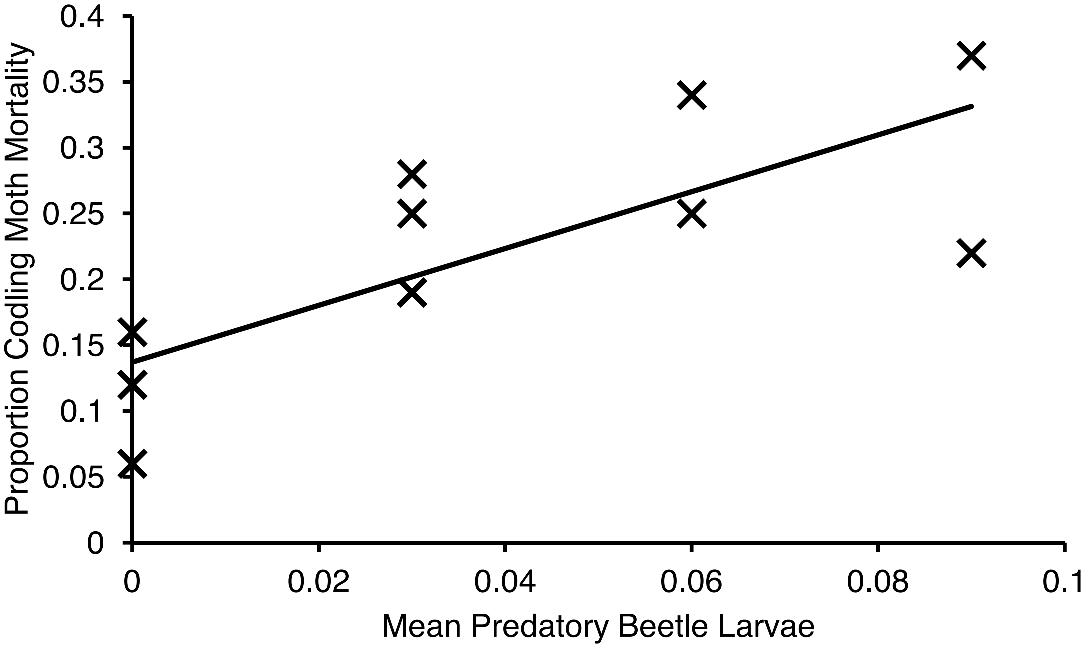

Figure 3.6. Proportion codling moth mortality versus the mean number of predatory beetle larvae in the cage experiments at Clarksville in September of 2012. Least squares regression was performed in R v.2.9.2 ($y=2.16x +0.14$, $P=0.002$, adjusted $R^2= 0.58$).

3.3.4. Canopy connectivity

The mean proportion mortality (+/- SEM) of sentinel codling moth eggs was 0.28 (+/-0.02) and 0.27 (+/-0.03) in low connectivity and high connectivity plots, respectively (data not shown). The mean proportion mortality (+/- SEM) of sentinel codling moth eggs in high connectivity plots where lacewing were released was 0.30 (+/-0.04) and it was 0.23 (+/-0.05) in high connectivity plots where they had not been released, but this difference was not statistically significant ($X^2=1.5$, df=1, $P=0.22$) (Fig. 3.7).

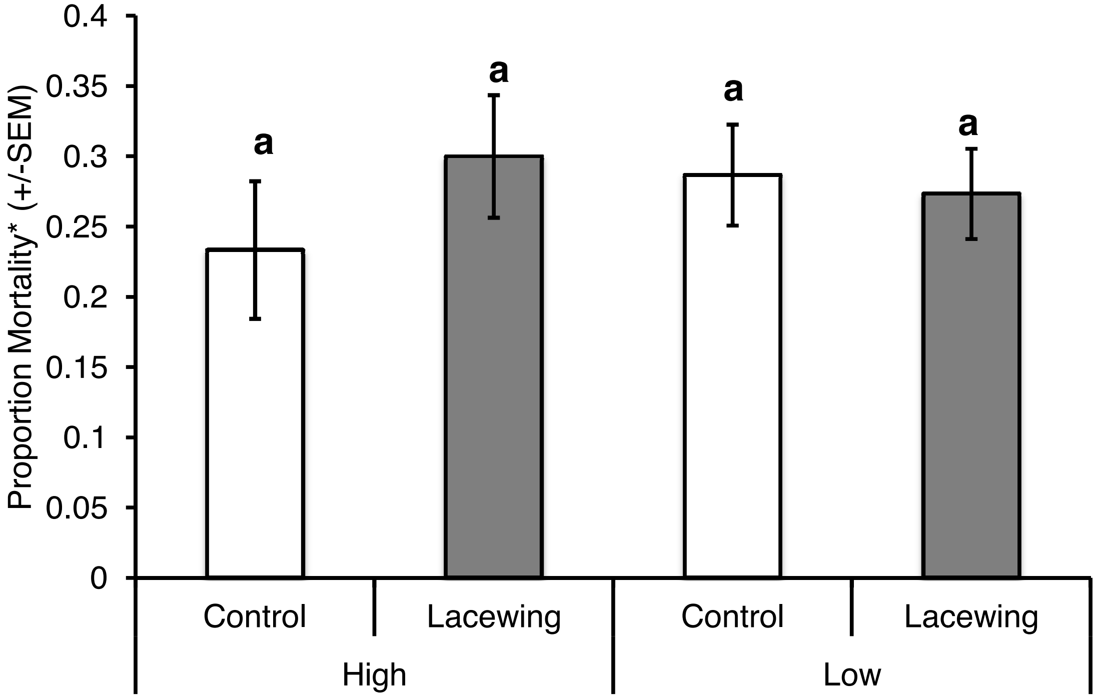

Figure 3.7. Proportion mortality of sentinel codling moth eggs in high and low connectivity orchards at AlMar in June 2012. Lacewing plots had ~1,000 first instar *C. rufilabris* released in the tree canopy at the center of the plot. Control plots had no added natural enemies. Mortality between treatments was compared using Wilcoxon (Gehan) tests (survdiff, R v.2.9.2). Columns with the same letter above them are not significantly different (α=0.05).

*Does not include mortality from parasitism by *Trichogramma* spp.

There was also no statistically significant difference in mortality of eggs in high compared to low connectivity plots where lacewing larvae were released (X^2=0.5, df=1, P=0.67) (Fig. 3.7). There was no statistically significant difference in mortality of eggs between plots that had lacewings released compared to control plots (X^2=0.5, df=1, P=0.46) (data not shown).

Analysis of log10(x+1) transformed Chrysopid counts in beat samples of trees at the edges of the plots revealed significant effects of lacewing treatment (F=6.19, df=1,36, P=0.017) and the interaction of lacewing treatment with orchard connectivity (F=8.31, df=1,36, P=0.007), but no significant effect of orchard connectivity (F=0.07, df=1,36, P=0.79). Pairwise comparisons using Tukey's HSD revealed that there were significantly more lacewing larvae (untransformed mean +/- SEM) in the edges of high connectivity plots where lacewings had been released (0.55 +/- 0.17) compared to the edges of high connectivity plots where they had not been released (0.00 +/- 0.00) (Tukey's HSD P(adj.)=0.003) (Fig. 3.8), but not significantly more in the edges of low connectivity plots where they had been released (0.20 +/- 0.09) compared to the edges of low connectivity plots where they had not been released (0.25 +/- 0.12) (Tukey's HSD P(adj.)=0.99) (Fig. 3.8). There was no significant difference between the number of lacewings in the edges of plots in the high connectivity orchard where they had been released compared to the low connectivity orchard where they had been released (Tukey's HSD P(adj.)=0.13) (Fig. 3.8).

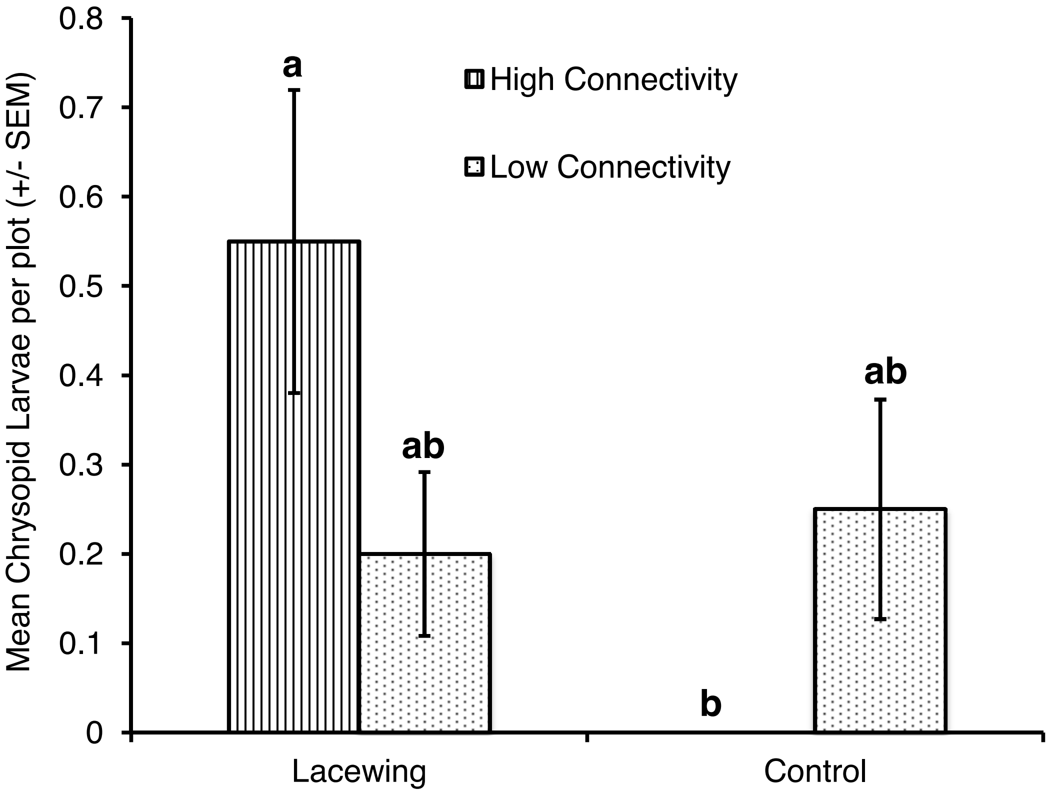

Figure 3.8. The mean number of Chrysopid larvae found in beat samples of the canopy at the edges of plots at AlMar orchards in June 2012. Lacewing plots had ~1,000 first instar *C. rufilabris* released in the tree canopy at the center of the plot. Control plots had no added natural enemies. Means separations were performed using Tukey's HSD with log10(x+1) transformed data. Columns with the same letter above them are not significantly different (α=0.05).

3.4. Discussion

3.4.1. Orchard floor complexity

My results indicate some species of entomopathogenic nematodes may have decreased foraging efficiency in complex habitats such as an orchard floor with abundant vegetation (Figs. 3.1 and 3.2). This observation is consistent with foraging theory and other studies demonstrating an inverse relationship between habitat complexity and prey mortality (Anderson, 2001; Cuddington and Yodzis, 2002; Huffaker, 1958; Keitt and Johnson, 1995; MacArthur and Pianka, 1966). Entomopathogenic nematode foraging behavior is relatively well studied in the laboratory (Lewis et al., 2006), and *S. feltiae* has been used effectively against codling moth in the field (Kaya et al., 1984; Lacey et al., 2006a). *Steinernema feltiae* exhibits a foraging mode that is considered intermediate between an ambusher (sit-and-wait) and a cruiser (search-and-destroy) (Campbell and Gaugler, 1993; Shapiro-Ilan et al., 2012), which means that infective juveniles of this species divide their time between displacement and bouts of nictation or body-waving (Campbell and Gaugler, 1993). I suspect that the proximal mechanism behind the reduced infection rate of codling moth in complex cages was reduced foraging success of the *S. feltiae* IJs caused by decreased time spent on displacement and more time spent head or body-waving (Campbell and Gaugler, 1993; Walton, 2013b).

I was not able to demonstrate a similar effect of habitat complexity on the other two species of codling moth natural enemies (Figs. 3.3 and 3.4). In the earwig and lacewing experiments, codling moth mortality was consistently low, which indicates that these natural enemies did not attack the sentinel prey in the experimental cages. This could have been caused by an inability to locate the sentinel prey, altered foraging behavior induced by the cage itself, or intraguild predation (incl. cannibalism). Unfortunately, the data do not allow us to eliminate these alternative hypotheses. I also did not detect an effect of endemic natural enemy removal in any of my experiments (Figs. 3.1 – 3.4). This provides some evidence that my natural enemy removal method did not eliminate all of the endemic natural enemies from the cages, which would have reduced my ability to detect the effect of adding natural enemies to the cages and increased the likelihood of intraguild predation. There were a variety of predatory arthropods present in the cages before they were vacuumed (N.J. Walton, personal observation) and if vacuuming failed to remove these enemies their impact may have interfered with my ability to detect the effect of added predators.

In the Clarksville earwig experiment, I was provided with strong evidence that my endemic natural enemy removal had been ineffective. When the wooden shelters were brought back to the laboratory for mortality assessment, several contained predatory beetle larvae. These larvae were primarily clerid larvae (Coleoptera: Cleridae), but a few cantharids (Coleoptera: Cantharidae) were also collected from wooden shelters. The beetles in many cases were found still feeding on codling moth pupae at the time that they were collected and signs of their feeding were seen on other pupae and fifth instar larvae in the wooden shelters at Clarksville.

Post hoc analysis of the mortality data indicated no relationship between presence of these beetle larvae and the predator removal treatment (data not shown). There was however, a clear relationship between presence of these beetle larvae and proportion mortality of codling moth sentinel prey (Fig. 3.5) and habitat complexity (Fig.

3.6). This provides evidence that at Clarksville endemic natural enemy presence was confounded with one of my experimental variables (complexity), and would have made it much more difficult to detect the predicted relationship between complexity and foraging success of the introduced natural enemies.

Additionally, I may have discovered an important natural enemy of the codling moth in Michigan. Two species of clerid beetle adults were reported to feed on codling moth in lab trials performed by Jaynes and Marucci (1947) using specimens collected from pitfall traps in apple orchards in West Virginia (USA). However, this is the first report of clerid larvae feeding on codling moth larvae in the field. These predatory beetles may be a promising new target for conservation biological control programs in apple orchards.

This discovery also brings to light an important implication of my work. Even though reduced orchard floor complexity improved the effectiveness of entomopathogenic nematode releases, the removal of vegetation and other material from under the trees can have important consequences for other important natural enemies. The microclimate provided by dense vegetation (Thomas et al., 1991), resources provided by flowering plants (Begum et al., 2007), and alternative prey in understory litter (Mathews et al., 2004) can be important to beneficial insects in agricultural habitats (Isaacs et al., 2009; Landis et al., 2000). On the other hand, codling moth larvae may benefit from spatial refuge on the orchard floor as demonstrated by the effectiveness of orchard sanitation as a management tactic for this pest (Judd et al., 1997; Sherman III, 1936; Steiner and Ackerman, 1936). It has also recently been suggested that adult codling moth may use non-crop vegetation as a daytime refuge

(Epstein et al., 2011). This is certainly an area that will benefit from future research, especially as it pertains to modern apple production as beneficial arthropods become more important.

3.4.2. Canopy connectivity

My results did not provide strong support for the hypothesis that increased canopy connectivity would lead to higher levels of codling moth egg predation (Fig. 3.7). However, I was unable to manipulate the endemic natural enemy community in these orchards and the high levels of predation in control plots indicates that the effect of pre-existing codling moth egg predators in the study orchards may have overwhelmed the effect of released predators (Fig. 3.7). Visual samples of leaves while this experiment was running indicated healthy populations of other codling moth egg predators on the foliage in both study orchards (N.J. Walton, unpublished data). Results of the beat sampling did indicate that the released natural enemies were able to move farther within the high connectivity plots than the low connectivity plots (Fig. 3.8). However, this conclusion is largely based on the non-significant difference between the density of Chrysopid larvae in the edges of plots in the low connectivity orchard (Fig. 3.8). Unfortunately I did not differentiate endemic Chrysopids in beat samples from the *C. rufilabris* larvae that I released. The dispersion pattern in Figure 8 could have also resulted from a higher endemic lacewing population in that orchard and or increased intraguild predation of the introduced *C. rufilabris* larvae in the low connectivity orchard.

3.5. Conclusion

The effect that the natural enemies of a species have on that species is determined largely by the frequency of encounter and their ability to inflict mortality once an encounter has occurred (Holling, 1959). For sedentary prey and an actively searching enemy, the frequency of encounter is a function of the separation of prey individuals in space. Furthermore, when organisms are small relative to the scale of the structural heterogeneity of the habitat, separation of prey individuals in space increases disproportionately with structural complexity (Cuddington and Yodzis, 2002). I found empirical support for this simplified consumer-resource model in my experiments with entomopathogenic nematodes on the orchard floor and lacewing larvae in the tree canopy. However, I also found evidence that other important factors such as endemic natural enemy population densities are affected by the structural complexity of the orchard habitat. Future studies are needed to disentangle the effects of structural complexity on the field efficacy of natural enemies of the codling moth. Understanding the aspects of habitat structural complexity that govern the effect of natural enemies on crop pests is one of the major challenges facing practitioners of habitat manipulation for pest management (Gurr et al., 2004b; Murdoch, 1975).

Chapter 4: An exploration of how moisture and structural complexity may affect the foraging success of *Steinernema feltiae* Filipjev against codling moth (*Cydia pomonella* [L.]).

4.1. Introduction

Entomopathogenic nematodes (EPN) are one of the most promising bioinsecticides used in organic agriculture worldwide (Denno et al., 2008; Lacey and Shapiro-Ilan, 2008; Stuart et al., 2008). Entomopathogenic nematodes are obligate parasites of insects that enter into hosts through the mouth, anus, and spiracles, and release a symbiotic bacterium, *Xenorhabdus* spp., which is the agent of target pest mortality (Shapiro-Ilan et al., 2003). The codling moth (*Cydia Pomonella* [L.]) is the most serious insect pest of apple production worldwide and is of critical concern to North American apple producers (Barnes, 1991; Jones et al., 2009; Solomon, 1991). The application of entomopathogenic nematodes is a promising codling moth management option available to apple growers. Entomopathogenic nematodes are available commercially or can be reared by growers in-house and can inflict high rates of mortality on codling moth larvae (Lacey and Shapiro-Ilan, 2008).

The codling moth is most susceptible to infection by EPN in the field during the wandering larva, pupa, and overwintering larva stages (Kaya et al., 1984; Unruh and Lacey, 2001). The wandering larva stage is the stage that begins when the larva leaves an apple and begins to search for a location to pupate (summer) or overwinter (fall) and ends when they spin a cocoon in a protected location. Wandering larvae are active primarily at night (Borden, 1931; Garlick and Boyce, 1940; Geier, 1963) and may take several hours to find a suitable location for their cocoon (Garlick, 1948; Geier, 1963).

Codling moth cocoons can be found anywhere in the orchard where conditions are relatively dry (Glen and Milsom, 1978) and they are protected from predators (Chandler, 1928; MacLellan, 1960). The majority of cocoons are found under flakes of bark (on rough-barked apple trees), and in any available crack or crevice on the trunk from the canopy to the interface with the soil surface (Chandler, 1928; Garlick, 1948; Jumean et al., 2009; Marshall, 1940). In orchards with rough-barked trees, fewer cocoons are found in the leaf litter and vegetation surrounding the trees (Chandler, 1928; Jaynes and Marucci, 1947; MacLellan, 1960). Artificial substrates are often very attractive to codling moth wandering larvae, so barns, fence posts, trash, and apple bins often serve as suitable overwintering and pupation sites (Chandler, 1928; Lacey and Chauvin, 1999; Steiner, 1929).

Entomopathogenic nematode applications for codling moth management target cocooned larvae on the tree and in surface litter on the orchard floor. A study performed in the Pacific Northwest achieved between 72%-86% mortality of cocooned codling moth larvae in apple orchards (Unruh and Lacey, 2001). However, sentinel prey used in this and many similar studies are placed on the tree trunk in artificial substrates and may not reflect actual mortality rates of wild codling moth from EPN applications in the field (Kaya et al., 1984; Lacey et al., 2006a). Additionally, modern high-density (<1000 trees per acre) orchards with smooth-barked cultivars do not provide as much arboreal codling moth cocoon habitat as traditional low-density orchards with rough-barked trees (Walton, 2013c; Walton, 2013d). In the future, the availability of sheltered locations (structural complexity) on the orchard floor is likely to have an a greater impact on codling moth intergenerational survival and population dynamics than mortality on the tree trunk (Walton, 2013c).

Two of the most important abiotic factors affecting the ability of EPN IJs to kill codling moth larvae and pupae on the orchard floor are the structural complexity of that habitat (Campbell and Gaugler, 1993; Walton, 2013c) and biologically available water (Navaneethan et al., 2010). I designed two laboratory microcosm experiments to investigate the mechanisms by which these abiotic factors might affect the survival of codling moth larvae in the presence of entomopathogens. In the first experiment I manipulated the structural complexity of microcosms by varying the amount of soil surface litter and measured the infection rate by entomopathogenic nematodes in microcosms of different complexities. In the second I held structural complexity constant, varied the amount of soil moisture in the microcosms and measured the effect this had on cocoon site selection by codling moth.

4.1.2. Experiment 1: Habitat structural complexity and EPN foraging efficiency

Entomopathogenic nematodes can be classified according to foraging behavior along a spectrum from ambusher (sit-and-wait) to cruiser (search-and-destroy)(Lewis et al., 2006). There is considerable evidence that entomopathogenic nematodes with a cruiser-type foraging strategy are more effective at infecting sessile hosts in protected locations than ambushers (Campbell et al., 2003; Huey and Pianka, 1981; Lacey et al., 2006b). However, even active foragers should experience reduced foraging success in habitats with increased structural complexity (Huffaker, 1958; Janssen et al., 2007; Walton, 2013c). Unfortunately this hypothesis is difficult to test using EPNs under field conditions due to confounding variables such as temperature, soil type, alternative hosts, and predation, (Duncan et al., 2007; Ekmen et al., 2010; Greenwood et al., 2011; Stuart et al., 2008; Walton, 2013c). In addition, EPNs are essentially aquatic organisms and they are often applied in a water suspension using readily adaptable agricultural spray equipment (Kaya and Stock, 1997; Lacey et al., 2006a; Unruh and Lacey, 2001). This makes it difficult to differentiate between purely physical effects of increased habitat complexity on the orchard floor and effects caused by reduced searching efficiency of the EPNs (Unruh and Lacey, 2001; Wise et al., 2010).

In other words, is it primarily a spray penetration problem, or a structural complexity problem? I designed this experiment to answer that question using an actively searching nematode species that is commonly used in field experiments against overwintering codling moth fifth instar larvae in orchard habitats (Kaya et al., 1984; Lacey et al., 2006a; Lewis et al., 2006). The EPN species used was *Steinernema feltiae* Filipjev (Rhabditida: Steinernematidae), an EPN with a foraging mode that is intermediate between ambusher and cruiser (Campbell et al., 2003). This experiment tested the hypothesis that increasing structural complexity would reduce the infection rate of codling moth by *S. feltiae* in laboratory microcosms.

4.1.3. Experiment 2: Soil moisture and codling moth cocoon site selection

One drawback of EPNs for pest management is that nematodes are aquatic organisms and require a film of water within which to move as they search for and infect their hosts (Kaya and Stock, 1997; Lacey et al., 2006a; Lacey et al., 2006b; Lewis et al., 2006; Spence et al., 2008; Unruh and Lacey, 2001; Wallace, 1958). Codling moth have been reported to avoid moist locations as sites for overwintering or pupation (Glen and Milsom, 1978). However, no formal investigations have been made regarding moisture effects on cocoon site selection by codling moth since that report. If codling moth fifth instars actively avoid building cocoons in wet locations or leave cocoons when conditions become unfavorable (i.e. wet), EPN applications may be less effective than expected against wild populations in the field. I designed an experiment to test the hypothesis that codling moth prefer low moisture sites for cocoon construction by manipulating the moisture content of small plastic arenas filled with moist sand and corrugated cardboard flutes.

4.2. Materials and Methods

4.2.1. Experimental organisms

Sentinel fifth instar codling moth were obtained from the USDA-ARS Yakima Agricultural Research Laboratory (Wapato, WA, USA). Larvae were surface sanitized before each experiment by submerging them briefly in a 0.05% sodium hypochlorite solution. *Steinernema feltiae* infective juveniles were produced in *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) larvae using the *in vivo* method described by Kaya and Stock (Kaya and Stock, 1997). All IJs used in these experiments had emerged from hosts less than two weeks prior to the experiment.

4.2.2. Habitat structural complexity and EPN foraging efficiency

I manipulated habitat complexity and nematode density in a 3 by 3 factorial design. The three levels of nematode density were *control* (water only), *low EPN* (~12 *S. feltiae* IJs per microcosm), and *high EPN* (~28 *S. feltiae* IJs per microcosm). The three levels of complexity in the microcosms were *bare sand*, *simple, and complex*. The *bare sand* microcosms contained 500 g of play sand. Sand was washed three times to remove water soluble contaminants that might affect nematode survival and then dried in a drying oven at 100 C for 24 h to sanitize and standardize water content. Complexity was added to the other treatments with the addition of synthetic leaves made of woven polyester. Leaves were sanitized in an autoclave prior to use in the experiment. *Simple* microcosms contained 2 synthetic leaves and 500 g of sand. *Complex* microcosms contained 24 synthetic leaves and 500 g of sand. Microcosms were made of 500 mL polypropylene deli containers with lids (Fabri-Kal®, Kalmazoo, MI). A small hole was punched in the lid of each container with a push-pin to improve aeration. Containers were sanitized by soaking in a 0.1% sodium hypochlorite solution for 1 minute and then allowed to air dry. Microcosms were prepared by putting 500 g dry sanitized sand into each 500 mL container and then pipetting 20 mL boiled tap water into the center. Containers with moist sand were then left overnight to allow the water to distribute itself by capillary action. Previous experiments have shown that this procedure provides sufficient water content for survival of EPN IJs in 500 g dry sand (N.J. Walton, unpublished data).

To prepare for the experiment, 45 surface sterilized codling moth larvae were placed in microcosms designated for the *bare sand* treatment and placed in an environmental chamber overnight to spin cocoons. The remaining larvae were placed between two synthetic leaves stacked vertically in 1 oz. polystyrene soufflé cups (Solo®, Lake Forest, IL) and placed in the environmental chamber overnight to spin cocoons.

Steinernema feltiae IJs were applied in a boiled tap water suspension using a 1.75 L hand pump sprayer (Root-Lowell Manufacturing Co., Lowell, MI, USA). IJs were applied to each container in 4 mL of water at two rates: 3 IJs per mL (*Low*) and 7 IJs per mL (*High*). *Control* containers received an application of 4 mL boiled tap water only.

To begin the experiment, 75 fifth instar codling moth larvae that had spun cocoons between two synthetic leaves in the soufflé cups (above) were placed individually in the center of the microcosms that had been designated for the *simple* and *complex* treatments. *Complex* microcosms (n=30) then had 22 synthetic leaves distributed haphazardly over the surface of the sand. *Simple* (n=45) and *bare sand* (n=45) microcosms had no additional synthetic leaves added to them. Fifteen containers of each complexity level then received EPN or water applications according to the EPN treatments described above. There was no *Complex/Control* microcosm treatment. After EPN applications were made, all microcosms were randomized and placed in an environmental chamber at 13ºC and 85% RH for 48 h. To simulate field conditions for an evening application of EPN, the chamber was set to start in the dark phase of a 12:12 L:D cycle.

Following the 48 h incubation in the environmental chamber, each microcosm was opened and the codling moth larva was removed and assessed for mortality and EPN infection status. Then each larva was placed in a 60 mm petri dish containing moist filter paper and sealed with parafilm. These dishes were held in the dark at 26ºC and 45% RH for an additional 6 d and larval mortality and EPN infection status was recorded every 24 h.

4.2.3. Soil moisture and codling moth cocoon site selection

I manipulated moisture content in a completely randomized design with 20 microcosms per moisture level. The five levels of moisture were *Dry* (no water), 7 mL, 20 mL, 55 mL, and 100 mL of water. Microcosms were made of 500 mL polypropylene deli containers with lids (Fabri-Kal®, Kalmazoo, MI). A small hole was punched in the lid of each container with a push-pin to improve aeration. Containers were sanitized by soaking in a 0.1% sodium hypochlorite solution for 1 minute and then allowed to air dry. Microcosms were prepared for the experiment by putting 500 g dry sanitized sand into each 500 mL container. Before use, sand was washed three times to remove water soluble contaminants and then dried in a drying oven at 100 C for 24 h to sanitize and remove moisture. Water was boiled, cooled, and added to the center of each microcosm with a hand pipettor. After the addition of water, microcosms were left overnight to allow the water to distribute itself by capillary action. Previous experiments have shown that this procedure with 7 mL of water does not provide optimal water content (-15kPa) for infectivity of EPN IJs in 500 g dry sand but that it does for water levels >12mL (N.J. Walton, unpublished data)(Kaya and Stock, 1997). The next day, codling moth cocoon substrate was added to each microcosm by placing 24 1.75 cm by 1.75 cm squares of corrugated cardboard haphazardly on top of the sand. Then a single fifth instar codling moth was placed in the center of each container on top of the cardboard. After codling moth fifth instars were placed in all microcosms, they were randomized and placed in an environmental chamber at 13 C and 85% RH for 48 h. To simulate field conditions, the chamber lighting was set to a 12:12 L:D cycle. Following the 48 h incubation period, all microcosms were opened and searched for larvae, the locations of cocoons were recorded, and the mortality or the larvae was assessed

Cocoon locations were quantified by dividing each microcosm into 2 strata: *Cardboard* and *Sand*. A cocoon was recorded as residing in the *Cardboard* stratum if it spun its cocoon in or on a piece of cardboard and was not in contact with the sand. The *Sand* stratum was anywhere from the surface of the sand to the bottom of the container.

4.2.4. Statistical analyses

Statistical analysis for the complexity experiment was performed by survival analysis using the Kaplan-Meier method in R (*survival*, v.2.9.2)(R-core-development-Team, 2009; Therneau and Lumley, 2009). Preplanned pairwise comparisons of survival curves were performed using Gehan-Wilcoxon tests (α=0.05)(Harrington and Fleming, 1982; Therneau and Lumley, 2009).

Data for the moisture experiments were analyzed by logistic regression and Analysis of Deviance in R v2.9.2 (Dalgaard, 2002; R-core-development-Team, 2009). The response variable in the logistic regression model was a binomial variable that was equal to 1 if a larva had built its cocoon in the *Cardboard* stratum and was equal to 0 if they had not. The predictor variable was moisture treatment.

4.3. Results

4.3.1. Habitat structural complexity and EPN foraging efficiency

There was a significant overall effect of EPN treatment on codling moth survival (X^2=32.5, df=2, P<0.0001). The mean proportion EPN infected larvae (+/-SEM) in *Control* microcosms was 0 (+/-0). In *Low* and *High* microcosms the mean proportion of larvae infected with EPN was 0.40 (+/- 0.07) and 0.64 (+/- 0.07), respectively. There was no significant overall effect of complexity treatment on codling moth survival (X^2=1.3, df=2, P=0.52). The mean proportion EPN infected larvae (+/-SEM) in *Bare sand* treatments was 0.31 (+/-0.07). In *Simple* and *Complex* microcosms the mean proportion larvae infected with EPN was 0.42 (+/- 0.07) and 0.46 (+/- 0.09), respectively.

Preplanned pairwise comparisons of survival curves revealed some relevant significant differences between codling moth survival in different microcosm types (α=0.05) (Fig. 4.1). Codling moth survival in the *Low* EPN microcosms with complexity added was not significantly different from the *Control* microcosms (X^2=1.3, df=1, P=0.257) (Fig.4.1). Survival in the *Low* EPN microcosms with only two synthetic leaves added was significantly different from *Control* microcosms (X^2=12.4, df=1, P=0.0004) (Fig.1). Survival in all *High* EPN microcosms was significantly different from all *Control* microcosms (P<0.001), but not from any of the *Low* EPN microcosms (P>0.05) (Fig. 4.1).

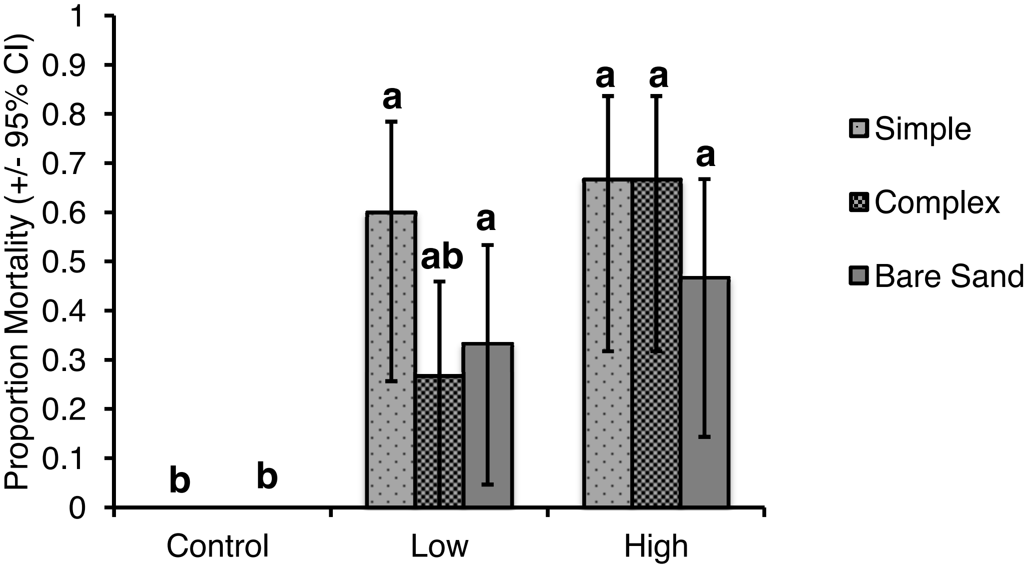

Figure 4.1. Proportion mortality of sentinel codling moth larvae in experimental microcosms. Pairwise comparisons were made using Gehan-Wilcoxon tests (α=0.05). Columns with the same letter are not significantly different. There were no Complex/Control microcosms.

4.3.2. Soil moisture and codling moth cocoon site selection

A total of 48 larvae built cocoons in the *Sand* stratum and 51 were found in cocoons in the *Cardboard* stratum. One larva from a 7 mL microcosm was unaccounted for and this microcosm was excluded from the data analysis. The results of the Analysis of Deviance indicated a significant relationship between moisture treatment and the probability that a larva would be found in the *Cardboard* stratum (df=4,94, P=0.007) (Fig. 4.2).

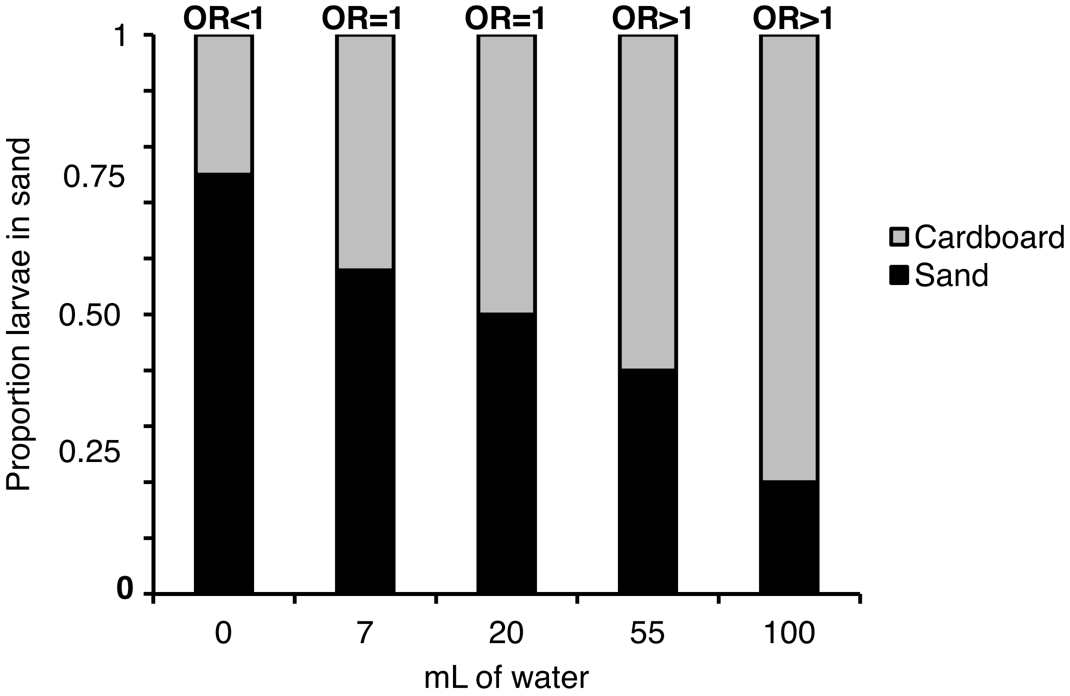

Figure 4.2. The proportion of larvae found in the Sand vs. Cardboard strata by moisture level in experimental microcosms. Odds ratio (OR) indicates results of logistic regression (α=0.05, glm, R v.2.9.2). When OR<1 larvae were more likely to be found in Sand than Cardboard. When OR=1 larvae were equally likely to be found in either stratum. When OR>1 larvae were more likely to be found in Cardboard than Sand.

The 95% confidence interval of the odds ratio for larvae in *Dry* microcosms constructing cocoons on cardboard was between 0.10 and 0.86. The odds ratio for larvae in 7 mL microcosms constructing cocoons on cardboard was between 0.57 and 9.02 and the odds ratio for larvae in 20 mL microcosms constructing cocoons on cardboard was between 0.81 and 12.24 (α=0.05). The 95% confidence interval of the odds ratio for larvae in microcosms containing 55 mL of water constructing a cocoon in the *Cardboard* stratum was from 1.22 to 18.76 and it was between 2.94 and 60.5 for larvae in the microcosms with 100 mL of water added.

4.4. Discussion

4.4.1. Habitat structural complexity and EPN foraging efficiency

In this experiment, nematode infection was higher compared to the controls when a higher density of EPN infective juveniles was added to *Complex* microcosms, but not *Bare Sand* and *Simple* microcosms (Fig. 4.1). In other words, there was no effect of increasing EPN concentration from *Low* to *High* in *Simple* or *Bare Sand* microcosms, but there was an effect of increasing EPN concentration in *Complex* microcosms. This provides evidence that EPN IJ foraging or infection success was reduced when complexity was added to microcosms.

Reduced spray penetration in the *Complex* microcosms was not likely to have caused the observed patterns. Assuming a random distribution of nematodes following application, all codling moth sentinels were well within the foraging range of S. feltiae IJs at the temporal and spatial scale of this experiment (Campbell and Gaugler, 1993; Hui and Webster, 2000; Neumann and Shields, 2006; Rolston et al., 2006). The most likely explanation for the observed difference in response to increasing nematode concentration between *Simple* and *Complex* microcosms (Fig. 4.1) is reduced foraging success of the *S. feltiae* IJs caused by increased structural complexity imposed by the addition of synthetic leaves (Cuddington and Yodzis, 2002; Holling, 1959; Huffaker, 1958; Janssen et al., 2007; Kostylev et al., 2005).

4.4.2. Soil moisture and codling moth cocoon site selection

In microcosms containing more than 55 mL water, the codling moth larvae in this experiment preferred building their cocoons in locations where they were not in contact with the moist sand (Fig. 4.2). Moist sand is considered to be an ideal foraging substrate for entomopathogenic nematode IJs (Kaya and Stock, 1997; Nielsen and Lewis, 2012). It is also known that wetting agents and humectants improve the infection rates of codling moth by *S. feltiae* by improving their ability to penetrate into the cryptic locations where codling moth construct their cocoons (Lacey et al., 2005).

Some insect larvae are known to have defensive behaviors that protect them from infection by EPN IJs (Gaugler et al., 1994; Koppenhofer et al., 2000; Renn, 1998; Schroeder et al., 1993). I did not design this experiment to determine whether or not avoidance of high moisture habitats is an adaptation of codling moth fifth instars to avoid infection by nematodes. Rather, I was simply interested in whether or not they would avoid high moisture locations when choosing where to construct their cocoons. A good next step would be to repeat this experiment with an addition of EPN to see if codling moth in the upper stratum of such microcosms exhibit lower EPN infection rates.

4.5. Conclusions

Orchardists manage the orchard floor beneath the tree canopy for optimal nutrient availability and minimized pest and disease pressure (Atucha et al., 2011; Mathews et al., 2004). The removal of vegetation, litter, and other material from under orchard trees, a practice know as orchard sanitation, has a long history of usefulness as a tactic to reduce codling moth populations (Judd et al., 1997; Sherman III, 1936; Steiner and Ackerman, 1936). My results indicate that reducing orchard floor structural complexity may improve the effectiveness of *S. feltiae* releases for codling moth management, a phenomenon that has also been demonstrated in field cages (Walton, 2013c). However, reducing the structural and vegetative complexity of the orchard floor can have non-target impacts on beneficial arthropods (Walton, 2013c).

Post-application irrigation is a recommended and effective practice that greatly improves the effectiveness of EPN applications targeting codling moth in their overwintering cocoons (Lacey et al., 2006a; Unruh and Lacey, 2001). However, most of the studies demonstrating effective EPN applications targeting codling moth have been performed in orchards in the arid west with relatively low orchard floor structural complexity and or have used sentinel codling moth larvae placed on the tree trunk (Kaya et al., 1984; Lacey et al., 2006a; Unruh and Lacey, 2001). In high complexity orchard floors such as those commonly found in certified organic orchards in the eastern United States, EPN applications targeting the overwintering stage of codling moth may be less effective than would be suggested by previous studies. If codling moth larvae actively avoid high moisture locations and increased structural complexity provides alternative cocoon sites, then they may be able to escape infection by entomopathogenic nematodes. EPN applications targeting the wandering larva stage of the codling moth may be more effective, in these types of orchards.

Chapter 5. Towards more sustainable management of codling moth (*Cydia pomonella* [L.]) in apple orchards

5.1. Introduction

The Environmental Protection Agency estimates that the United States agricultural sector spent $11.8 billion on all pesticides and $1.83 billion on insecticides and acaricides in 2007 (the latest year for which these data are available) (Grube et al., 2011). In that same year, U.S. apple producers applied an estimated 3,412.5 metric tons of insecticides (USDA-NASS, 2012b) to 97% of the total US apple bearing acreage (USDA-NASS, 2008). In 2011, U.S. apple producers applied an estimated 621 metric tons of insecticides to 84% of apple bearing acreage (USDA-NASS, 2012b). This decline in insecticide usage is expected to continue as mating disruption and other reduced risk approaches replace the broad-spectrum contact active insecticides that apple producers had previously relied on (Brunner et al., 2005; Epstein, 2010; Grube et al., 2011; Jones et al., 2009).

As dicussed previously (Walton, 2013a), this reduction in insecticide use is expected to lead to an increase in the contribution that arthropod natural enemies make to pest management in apple orchards. Table 5.1 (below) summarizes the previous estimates of codling moth mortality by life stage (Walton, 2013a). Clearly, there is a great deal of variation in estimates of the contribution that natural enemies make to codling moth management as well as the identities of the natural enemies involved (Table 5.1). Accurate quantification and identification of natural enemies of crop pests is important because it allows incorporation of this information into economic decision making tools for pest managers (Radcliffe et al., 2009; Zhang and Swinton, 2009) and environmental policy-makers (Boyd and Banzhaf, 2007; Isaacs et al., 2009; Losey and Vaughan, 2006; Luck et al., 2009).

Table 5.1. Extant estimates of codling moth mortality in the field: Summary

Stage	Estimated % Mortality	Suspected Mortality Agents	References
Egg and 1st Instar	2-86	Parasitoids, green lacewings, thrips, mites, plant bugs, earwigs	(Ferro et al., 1975; Geier, 1963; Glen, 1975; Glen, 1977; Jaynes and Marucci, 1947; MacLellan, 1962; Summerland and Steiner, 1943)
Instars 2-4	10-52	Unknown	(Ferro et al., 1975; MacLellan, 1962)
5th instar	4-100	Birds, ants, beetles, parasitoids, pathogens	(Baumgartner, 1999; Ferro et al., 1975; Glen and Milsom, 1978; Jaques and MacLellan, 1965; Jaynes and Marucci, 1947; MacLellan, 1960; MacLellan, 1962; Mathews et al., 2004; Mills, 2005b; Riddick, 1994; Solomon et al., 1976; Subinprasert, 1987)
Adult	Unknown	Birds, bats, spiders	None
Total % Mortality	16-100		

The identity of the natural enemies that make the greatest contribution to codling moth pest management is also important to producers and researchers interested in conservation biological control. Due to the diverse life histories of natural enemies it is impractical to conserve all natural enemies in a commercial orchard. Crop specific conservation practices that target the essential natural enemies (‘the right diversity’ *sensu* Landis et al. 2000) need to be developed based on rigorous scientific research. Such an effort has the potentional to increase the functional biodiversity of both organic and conventional agriculture (Letourneau and Bothwell, 2008).

Determining the optimal target for conservation biological control in apple orchards in Michigan and elsewhere will be a difficult process. Apart from determining natural enemies that are active in orchards and readily conserved, this process will also require the determination of the optimal codling moth life stage to target. Some of the natural enemies groups were observed attacking both the egg and fifth instar stages in my video study (Walton, 2013d), but it is unlikely that these were the same species and they may not have had a sufficient impact on either life stage to justify conservation efforts.

Determining the optimal life stage of codling moth to target with conservation biological control requires close examination of this pest's life history, demography, and population dynamics (Brown et al., 1978; Mills, 2005b). This information can be incorporated into a stage structured matrix model which can and has been used to plan biological control of the species (Mills, 2005b). Elasticity analysis of such a model can then provide an estimate for the percentage of mortality that is needed to bring the pest's population intrinsic growth rate (r) to less than 1 (Caswell, 2001; Mills, 2005b). More importantly it can help identify the life stage that has the greatest impact on codling moth's long-term population dynamics (Caswell, 2001). For example, in Mills' (2005) stage structured matrix model for codling moth, the adult stage has the highest elasticity value. In other words, a marginal increase in adult codling moth mortality would (given those model parameters) have a larger impact on the pest than a marginal increase in the mortality of any other life stage alone. Given this information we might conclude that the optimal target for conservation biological control of codling moth would be a natural enemy of the adult stage. In fact, this is the stage that mating disruption targets this may be why it is such an effective management strategy. Unfortunately, natural enemies of codling moth adults are poorly studied because of the difficulty of collecting data on the mortality of such a mobile stage (Walton, 2013a).

Fortunately, second to the adult in impact on long term population dynamics is the cocoon stage, followed closely by the wandering fifth instar stage and the egg stage. The model predicts that a 21.0% or a 34.6% daily mortality in the cocoon and egg stage respectively will be sufficient to bring the intrinsic growth rate of a codling moth population to less than 1. Table 5.2 (below) presents mortality rates from my field studies in Michigan for these two life stages. These rates can be crudely converted to daily mortality rates by dividing each by the number of days that sentinel prey were exposed to field conditions (Table 5.2). This yields maximum daily mortality rates (+/- SEM) of 8.7% (+/- 1.0) for sentinel eggs on pins in the canopy and 5.9% (+/- 1.5) for sentinel fifth instars in field cages on the orchard floor. These values are 28% (+/-5) and 25% (+/-4) of the critical daily mortality rates estimated by Mills (2005b) for egg and fifth instar codling moth, respectively. This suggests that natural enemies are making a contribution to pest management in the orchards that I studied in Michigan, but that on their own they would not bring codling moth population growth to a halt under current conditions. However, conservation targetted at the right natural enemies could bring this mortality rate within a range where additional economic benefits are realized (Jones et al., 2009; Zhang and Swinton, 2009).

Table 5.2. Estimates of codling moth mortality in Michigan apple orchards

Stage	% Daily Mortality (+/-SEM)	Mortality Agents	Method
Egg and 1st Instar	2.2±0.6	Lacewings, spiders, mites, bugs, parasitoids, harvestmen, tree crickets, mice	Video
Egg and 1st Instar	8.7±1.0	Lacewings, mites, predatory thrips, bugs	Sentinel prey on pins in canopy (control plots)
Instars 2-4	Unknown	Unknown	
5th instar	2.6±0.7	Ants, parasitoid wasps, spiders, beetles	Video
5th instar	5.9±1.5	Unknown, beetle larvae	Sentinel prey in control cages
Adult	Unknown	Birds, bats, spiders	None

5.2. Future Research

I have identified some of the most active natural enemies of both of these important codling moth life stages in Michigan apple orchards. However, there is still a great deal of work to be done. For example, many of the natural enemies that were observed need to be identified to species before conservation efforts can be made. Lacewings were the most active egg predators in at least one year of the study, but lacewing species differ significantly in terms of foraging habitat, phenology, and the resource needs of the adult and larval stages (McEwen et al., 2001). The same can be said of most of the other natural enemies observed in these studies.

Ants and parasitoid wasps may be two groups that will be particularly promising as targets of conservation efforts in Michigan apple orchards. Ants were one of the first organisms ever used in conservation biological control (Hajek, 2004) and in locations where a foraging colony is present they can have a significant impact on codling moth fifth instar mortality (Jaynes and Marucci, 1947; Walton, 2013d). Parasitoid wasps can also make a significant contribution to codling moth pest management in orchards and they have generally been shown to benefit from the addition of flowering habitat as a food source for the adult stage (Blaauw and Isaacs, 2012; Pfiffner et al., 2004; Walton and Isaacs, 2011a; Walton and Isaacs, 2011b). The parasitoids observed in my video studies were unidentifiable based on video images alone. One parasitoid wasp that was observed on video attacking sentinel codling moth in 2010, however, at least superficially resembled the species (*M. ridibundus)* that has become an important classical biological control introduction in California (Mills, 2005a; Walton, 2013a). Attempts to rear parasitoids from the sentinel prey that were present at that site were unsuccessful and no adult specimens were collected from the field. Adults of that species were released at AlMar orchards in 2008 (David Epstein, personal communication) and they have been collected subsequently in Michigan (Larry Gut, personal communication).

Predatory beetles should also be investigated as a possible target for conservation in apple orchards. Many predatory beetles respond positively to conservation of grassy areas within crop fields (Gurr et al., 2004a; Thomas et al., 1991; Walton, 2013c). However, these practices usually target carabid beetles, a group that was not observed preying on any life stage of codling moth in my video study. Carabids may be more important as natural enemies of codling moth wandering larvae on the orchard floor (Glen and Milsom, 1978; Jaynes and Marucci, 1947; Riddick, 1994), a life stage that I did not collect data on. Regardless, the other beetle groups that I observed attacking codling moth fifth instars in Michigan and have been observed elsewhere

(Jaynes and Marucci, 1947) may also respond positively to conservation practices that benefit carabids such as maintenance of shelter habitats(Griffiths et al., 2008).

These predatory beetles (clerids and cantharids) might also benefit from flower resources (Graham et al., 2012; Lovell, 1915; Opitz, 2002). The organic research orchard at Clarksville, MI where those beetles were observed (Walton, 2013c) is bordered by a native perenial wildflower planting and adult cantharids were observed on flowers (*Solidago* sp.) at the time that those experiments were being carried out (N.J.Walton, personal observation). Cantharid beetles and some clerid beetles are known to use flowers as a resource during their adult stages (Graham et al., 2012; Lovell, 1915; Opitz, 2002). This is a phenomenon that will hopefully be investigated further.

5.3. Trade-offs in conservation biological control of codling moth

Habitat structural complexity on the orchard floor has been shown to affect codling moth natural enemies in two opposing ways (Walton, 2013c). Increased complexity improves the effectiveness of naturally ocurring natural enemies but it reduces the effectiveness of augmentative applications of entompathogenic nematodes. This suggests the existence of a trade-off in codling moth pest management between reducing orchard floor complexity and relying on inundative augmentatiion biocontrol or increasing complexity and relying on conservation or inoculative augmentation biocontrol.

Trade-offs are not uncommon in conservation biological control (Gurr and Kvedaras, 2010; Prokopy, 1994), but there are also notable examples where apparent trade-offs have been overcome. These examples often involve the application of detailed information about the natural history of the organisms involved, especially regarding the optimal spatial and temporal scales for habitat manipulations. For example, strip management at an appropriate spatial scale has been effective in a number of systems as a means to selectively conserve natural enemies (Bugg and Waddington, 1994; Hossain et al., 2002; Thomas et al., 1991). Integration of non-crop plants into or around perrenial fruit crops has also been found to improve biological control (Brown and Mathews, 2007; Corbett and Rosenheim, 1996; Doutt and Nakata, 1973; English-Loeb et al., 2003; Thomson and Hoffmann, 2009; Thomson and Hoffmann, 2013; Unruh et al., 2012). Also, reduced mowing frequency can increase the abundance of some important natural enemies in pear orchards (Horton et al., 2003), and the use of post-emergence herbicides rather than pre-emergence herbicides has been suggested as a means to reduce weed competition while conserving soil and a structurally complex habitat (Atucha et al., 2011).

The challenge for future apple growers using habitat management to increase the impact of natural enemies on codling moth may be to find a balance between the opposing effects of habitat complexity. There may exist an optimal level of complexity that maximizes the impact of the codling moth’s natural enemies. Synergistic interactions may also exist between conservation practices and other pest management tools and these should be sought out and exploited where possible (Botto and Glaz, 2010; Grewal et al., 2005; Gurr and Kvedaras, 2010; Vreysen and Robinson, 2011).

5.4. Conclusion

Developing and implementing sustainable pest management strategies and related food production technologies will be one of the great challenges facing agriculture in the 21st century and beyond (Godfray et al., 2010; Gurr and Kvedaras, 2010). One approach to meeting this challenge is to design agricultural systems that mimic natural systems (Gurr et al., 2004a; Jackson and Jackson, 2002). Unfortunately, there is a great deal that we still do not understand about the structure and function of natural and agricultural systems. I have endeavored throughout this dissertation to provide data that will advance our understanding of both.

APPENDIX

APPENDIX

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: 2013-09

Author and Title of thesis:
Nathaniel J. Walton
The role of natural enemies in pest management of codling moth (*Cydia Pomonella* [L.]) in organic apple orchards in Michigan

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

Table A.1 List of voucher specimens.

Order*	Family*	Genus-Species	Life Stage	Quantity	Preservation
Opiliones			Adult	1	Digital
Subclass: Acari				1	Digital
Hemiptera				1	Digital
Aranae				1	Digital
Neuroptera	Chrysopidae		Larva	1	Digital
Hymenoptera	Suborder: Apocrita		Adult	1	Digital
Hymenoptera	Formicidae		Adult	1	Digital
Coleoptera	Superfamily: Cleroidea		Larva	1	Digital
Coleoptera	Superfamily: Cleroidea		Adult	1	Digital
Orthoptera	Gryllidae		Adult	1	Digital
Rodentia				1	Digital
Rhabditida	Steinernematidae	*Steinernema feltiae*	Infective Juvenile	1	Digital
Rhabditida	Steinernematidae	*Steinernema feltiae*	Adult	1	Digital
Class: Chilopoda				1	Ethanol

*Unless specified otherwise.

Table A.1 (cont'd).

Order*	Family*	Genus-Species	Life Stage	Quantity	Preservation
Opiliones				1	Ethanol
Aranae				1	Ethanol
Neuroptera	Chrysopidae		Adult	4	Ethanol
Thysanoptera	Aeolothripidae		Adult	1	Ethanol
Hemiptera	Anthocoridae		Adult	1	Ethanol
Diptera	Syrphidae		Adult	1	Ethanol
Coleoptera	Staphylinidae		Adult	1	Ethanol
Coleoptera	Superfamily: Cleridae		Larva	8	Ethanol
Coleoptera	Superfamily: Cleroidea				
Coleoptera	Cantharidae		Larva	1	Ethanol
Hymenoptera	Suborder: Apocrita		Adult	1	Ethanol
Hymenoptera	Formicidae		Adult	8	Ethanol
Lepidoptera	Tortricidae	*Cydia pomonella*	Larva	1	Ethanol
Neuroptera	Chrysopidae	*Chrysoperla rufilabris*	Larva	11	Ethanol
Dermaptera	Forficulidae	*Forficula auricularia*	Adult	12	Pinned
Diptera	Tachinidae		Adult	1	Pinned
Neuroptera	Chrysopidae	*Chrysoperla rufilabris*	Adult	7	Pinned
Lepidoptera	Tortricidae	*Grapholita molesta*	Adult	5	Pinned
Lepidoptera	Tortricidae	*Cydia pomonella*	Adult	5	Pinned
Coleoptera	Carabidae		Adult	1	Pinned
Coleoptera	Coccinellidae		Adult	1	Pinned
Coleoptera	Elateridae		Adult	1	Pinned

*Unless specified otherwise.

REFERENCES

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Anderson, T.W., 2001. Predator responses, prey refuges, and density-dependent mortality of a marine fish. Ecology 82**,** 245-257.

Atucha, A., Merwin, I.A., Brown, M.G., 2011. Long-term effects of four groundcover management systems in an apple orchard. HortScience 46**,** 1176-1183.

Barbosa, P., 1998. Conservation biological control. Academic Press, San Diego.

Barnes, M.M., 1991. Tortricids in pome and stone fruits: Codling moth occurrence host race formation and damage. In: van der Geest, L.P.S., Evenhuis, H.H., Eds.), Tortricid pests: Their biology natural enemies and control. Elsevier, New York, pp. 313-327.

Baumgartner, J.A., 1999. Birds and arthropod predation of codling moth (*Cydia pomonella*) in apple orchards. San Jose State University, pp. 75.

Begum, M., Gurr, G.M., Wratten, S.D., Hedberg, P.R., Nicol, H.I., 2007. Using selective food plants to maximize biological control of vineyard pests. Journal of Applied Ecology**,** 547-554.

Blaauw, B.R., Isaacs, R., 2012. Larger wildflower plantings increase natural enemy density, diversity, and biological control of sentinel prey, without increasing herbivore density. Ecological Entomology 37**,** 386-394.

Blommers, L., 1992. Selective package and natural control in orchard IPM. Acta Phytopathologica Et Entomologica Hungarica 27**,** 127-134.

Borden, A.D., 1931. Some field observations on codling moth behavior. Journal of Economic Entomology 24**,** 1137-1145.

Borror, D.J., Triplehorn, C.A., Johnson, N.F., 1992. An introduction to the study of insects. Harcourt Brace, New York.

Botto, E., Glaz, P., 2010. Potential for controlling codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) in Argentina using the sterile insect technique and egg parasitoids. Journal of Applied Entomology 134**,** 251-260.

Boyd, J., Banzhaf, S., 2007. What are ecosystem services? The need for standardized environmental accounting units. Ecological Economics 63, 616-626.

Brown, G.C., Berryman, A.A., Bogyo, T.P., 1978. Simulating codling moth population dynamics: Model development, validation, and sensitivity. Environmental Entomology 7, 219-227.

Brown, M.W., 1993. Resilience of the natural arthropod community on apple to external disturbance Ecological Entomology 18, 169-183.

Brown, M.W., Mathews, C.R., 2007. Conservation biological control of rosy apple aphid, *Dysaphis plantaginea* (Passerini), in Eastern North America. Environmental Entomology 36, 1131-1139.

Brunner, J.F., Beers, E.H., Dunley, J.E., Doerr, M., Granger, K., 2005. Role of neonicotinyl insecticides in washington apple integrated pest management. Part i. Control of Lepidopteran pests. Journal of Insect Science 5, 1-10.

Bugg, R.L., Waddington, C., 1994. Using cover crops to manage arthropod pests of orchards: A review. Agriculture Ecosystems & Environment 50, 11-28.

Calderon, M., Navarro, S., 1971. Effects of ultra-violet irradiation on the eggs of *Ephestia cautella* (wlk.) (Lepidoptera: Physitidae). Journal of Stored Products Research 7, 309-311.

Caldwell, M.M., Bornman, J.F., Ballare, C.L., Flint, S.D., Kulandaivelu, G., 2007. Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. Photochemical & Photobiological Sciences 6, 252-266.

Campbell, J.F., Gaugler, R., 1993. Nictation behaviour and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). Behaviour 126, 155-169.

Campbell, J.F., Lewis, E.E., Stock, S.P., Nadler, S., Kaya, H.K., 2003. Evolution of host search strategies in entomopathogenic nematodes. Journal of Nematology 35, 142-145.

Caswell, H., 2001. Matrix population models: Construction, analysis, and interpretation. Sinauer Associates Inc., Sunderland.

Chandler, S.C., 1928. Codling moth hibernation studies. Journal of Economic Entomology 21, 315-318.

Corbett, A., Rosenheim, J.A., 1996. Impact of a natural enemy overwintering refuge and its interaction with the surrounding landscape. Ecological Entomology 21, 155-164.

Crowder, D.W., Northfield, T.D., Strand, M.R., Snyder, W.E., 2010. Organic agriculture promotes evenness and natural pest control. Nature 466, 109-U123.

Cuddington, K., Yodzis, P., 2002. Predator-prey dynamics and movement in fractal environments. American Naturalist 160, 119-134.

Dalgaard, P., 2002. Logistic regression. Introductory statistics with R. Springer, New York.

de Roince, C.B., Lavigne, C., Ricard, J.M., Franck, P., Bouvier, J.C., Garcin, A., Symondson, W.O.C., 2012. Predation by generalist predators on the codling moth versus a closely-related emerging pest the oriental fruit moth: A molecular analysis. Agricultural and Forest Entomology 14, 260-269.

Denno, R.F., Gruner, D.S., Kaplan, I., 2008. Potential for entomopathogenic nematodes in biological control: A meta-analytical synthesis and insights from trophic cascade theory. Journal of Nematology 40, 61-72.

Doutt, R.L., Nakata, J., 1973. The rubus leafhopper and its egg parasitoid: An endemic biotic system useful in grape-pest management. Environmental Entomology 2, 381-386.

Drinkwater, L.E., Letourneau, D.K., Workneh, F., Bruggen, A.H.C.v., Shennan, C., 1995. Fundamental differences between conventional and organic tomato agroecosystems in California. Ecological Applications 5, 1098-1112.

Duncan, L.W., Graham, J.H., Zellers, J., Bright, D., Dunn, D.C., El-Borai, F.E., Porazinska, D.L., 2007. Food web responses to augmenting the entomopathogenic nematodes in bare and animal manure-mulched soil. Journal of Nematology 39, 176-189.

Duthie, B., Gries, G., Gries, R., Krupke, C., Derksen, S., 2003. Does pheromone-based aggregation of codling moth larvae help procure future mates? Journal of Chemical Ecology 29, 425-436.

Ekmen, Z.I., Hazir, S., Cakmak, I., Ozer, N., Karagoz, M., Kaya, H.K., 2010. Potential negative effects on biological control by *Sancassania polyphyllae* (Acari: Acaridae) on an entomopathogenic nematode species. Biological Control 54**,** 166-171.

English-Loeb, G., Rhainds, M., Martinson, T., Ugine, T., 2003. Influence of flowering cover crops on anagrus parasitoids (Hymenoptera : Mymaridae) and erythroneura leafhoppers (Homoptera : Cicadellidae) in new york vineyards. Agricultural and Forest Entomology 5**,** 173-181.

Epstein, D.L., 2010. Development and delivery of biopesticide-based pest management for Michigan apple producers. Entomology. Michigan State University, Michigan.

Epstein, D.L., Miller, J.R., Grieshop, M.J., Stelinski, L.L., Gut, L.J., 2011. Direct sampling of resting codling moth (Lepidoptera: Tortricidae) adults in apple tree canopies and surrounding habitats. Environmental Entomology 40**,** 661-668.

Epstein, D.L., Zack, R.S., Brunner, J.F., Gut, L., Brown, J.J., 2000. Effects of broad-spectrum insecticides on epigeal arthropod biodiversity in pacific northwest apple orchards. Environmental Entomology 29**,** 340-348.

Epstein, D.L., Zack, R.S., Brunner, J.F., Gut, L., Brown, J.J., 2001. Ground beetle activity in apple orchards under reduced pesticide management regimes. Biological Control 21**,** 97-104.

Ferro, D.N., Harwood, R.F., 1973. Intraspecific larval competition by the codling moth, *Laspeyresia pomonella*. Environmental Entomology 2**,** 783-790.

Ferro, D.N., Sluss, R.R., Bogyo, T.P., 1975. Factors contributing to the biotic potential of the codling moth, *Laspeyresia pomonella* (L.), in washington. Environmental Entomology 4**,** 385-391.

Frank, S.D., Wratten, S.D., Sandhu, H.S., Shrewsbury, P.M., 2007. Video analysis to determine how habitat strata affects predator diversity and predation of *Epiphyas postvittana* (Lepidoptera : Tortricidae) in a vineyard. Biological Control 41**,** 230-236.

Garlick, W.G., 1948. A five-year field study of codling moth larval habits and adult emergence. Scientific Agriculture 28**,** 273-292.

Garlick, W.G., Boyce, H.R., 1940. A note on the habits of mature codling moth larvae. The Canadian Entomologist 72**,** 87.

Gaugler, R., Wang, Y., Campbell, J.F., 1994. Aggressive and evasive behaviors in *Popillia-japonica* (Coleoptera, Scarabaeidae) larvae - defenses against entomopathogenic nematode attack. Journal of Invertebrate Pathology 64**,** 193-199.

Geier, P.W., 1963. The life history of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), in the Australian capital territory. Australian Journal of Zoology 11**,** 323.

Giolo, F.P., Medina, P., Grutzmacher, A.D., Vinuela, E., 2009. Effects of pesticides commonly used in peach orchards in brazil on predatory lacewing *Chrysoperla carnea* under laboratory conditions. BioControl 54**,** 625-635.

Glen, D.M., 1975. Effects of predators on eggs of codling moth *Cydia-pomonella*, in a cider-apple orchard in southwest england. Annals of Applied Biology 80**,** 115-119.

Glen, D.M., 1977. Predation of codling moth eggs, *Cydia pomonella*, the predators responsible and their alternative prey. Journal of Applied Ecology 14**,** 445-456.

Glen, D.M., Milsom, N.F., 1978. Survival of mature larvae of codling moth (*Cydia pomonella*) on apple trees and ground. Annals of Applied Biology 90**,** 133-146.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., Toulmin, C., 2010. Food security: The challenge of feeding 9 billion people. Science 327**,** 812-818.

Graham, E.E., Tooker, J.F., Hanks, L.M., 2012. Floral host plants of adult beetles in central Illinois: An historical perspective. Annals of the Entomological Society of America 105**,** 287-297.

Greenwood, C.M., Barbercheck, M.E., Brownie, C., 2011. Short term response of soil microinvertebrates to application of entomopathogenic nematode-infected insects in two tillage systems. Pedobiologia 54**,** 177-186.

Grewal, P.S., Ehlers, R.U., Shapiro-Ilan, D.I. Eds.), 2005. Nematodes as biocontrol agents. CABI, Cambridge, MA.

Grieshop, M.J., Flinn, P.W., Nechols, J.R., Campbell, J.F., 2008. Effects of fine-grain habitat complexity on egg parasitism by three species of trichogramma. Biological Control 45**,** 328-336.

Grieshop, M.J., Werling, B., Buehrer, K., Perrone, J., Isaacs, R., Landis, D., 2012. Big brother is watching: Studying insect predation in the age of digital surveillance. American Entomologist 58**,** 172-182.

Griffiths, G.J.K., Holland, J.M., Bailey, A., Thomas, M.B., 2008. Efficacy and economics of shelter habitats for conservation biological control. Biological Control 45**,** 200-209.

Grube, A., Donaldson, D., Kiely, T., Wu, L., 2011. Pesticide industry sales and usage: 2006 and 2007 market estimates. In: Office of Chemical Safety and Pollution Prevention, (Ed.). United States Environmental Protection Agency, Washington, DC.

Gurr, G.M., Kvedaras, O.L., 2010. Synergizing biological control: Scope for sterile insect technique, induced plant defences and cultural techniques to enhance natural enemy impact. Biological Control 52**,** 198-207.

Gurr, G.M., Wratten, S.D., Altieri, M.A., 2004a. Ecological engineering for pest management. Cornell University Press, Ithaca.

Gurr, G.M., Wratten, S.D., Berndt, L., Irvin, N., 2004b. Ecological engineering, habitat manipulation and pest management. In: Gurr, G.M., Wratten, S.D., Altieri, M.A., Eds., Ecological engineering for pest management. Cornell University Press, Ithaca, pp. 1-12.

Gut, L.J., Brunner, J.F., 1998. Pheromone-based management of codling moth (Lepidoptera : Tortricidae) in Washington apple orchards. Journal of Agricultural Entomology 15**,** 387-406.

Hagley, E.A.C., 1972. Observations on codling moth longevity and egg hatchability. Environmental Entomology 1**,** 123-125.

Hagley, E.A.C., Allen, W.R., 1988. Ground beetles (coleoptera, carabidae) as predators of the codling moth, *Cydia-pomonella* (L) (Lepidoptera, Tortricidae). Canadian Entomologist 120**,** 917-925.

Hagley, E.A.C., Holliday, N.J., Barber, D.R., 1982. Laboratory studies of the food preferences of some orchard carabids (Coleoptera, Carabidae). Canadian Entomologist 114**,** 431-437.

Hajek, A.E., 2004. Natural enemies: An introduction to biological control. Cambridge University Press, Cambridge.

Harrington, D.P., Fleming, T.R., 1982. A class of rank test procedures for censored survival-data. Biometrika 69**,** 553-566.

Heinz, K.M., 1998. Dispersal and dispersion of aphids (Homoptera: Aphididae) and selected natural enemies in spatially subdivided greenhouse environments. Environmental Entomology 27**,** 1029-1038.

Holdsworth Jr, R.P., 1970. Codling moth control as part of an integrated program in Ohio. Journal of Economic Entomology 63**,** 894-897.

Holling, C.S., 1959. The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. Canadian Ent 91**,** 293-320.

Horton, D.R., Broers, D.A., Lewis, R.R., Granatstein, D., Zack, R.S., Unruh, T.R., Moldenke, A.R., Brown, J.J., 2003. Effects of mowing frequency on densities of natural enemies in three pacific northwest pear orchards. Entomologia Experimentalis Et Applicata 106**,** 135-145.

Hossain, Z., Gurr, G.M., Wratten, S.D., Raman, A., 2002. Habitat manipulation in lucerne medicago sativa: Arthropod population dynamics in harvested and 'refuge' crop strips. Journal of Applied Ecology 39**,** 445-454.

Hoyt, S.C., 1969. Integrated chemical control of insects and biological control of mites on apple in Washington. Journal of Economic Entomology 62**,** 74-&.

Huey, R.B., Pianka, E.R., 1981. Ecological consequences of foraging mode. Ecology 62**,** 991-999.

Huffaker, C.B., 1958. Experimental studies on predation: Dispersion factors and predator-prey oscillations. Hilgardia 27**,** 343-383.

Hui, E., Webster, J.M., 2000. Influence of insect larvae and seedling roots on the host-finding ability of *Steinernema feltiae* (Nematoda: Steinernematidae). Journal of Invertebrate Pathology 75, 152-162.

Isaacs, R., Tuell, J., Fiedler, A., Gardiner, M., Landis, D., 2009. Maximizing arthropod-mediated ecosystem services in agricultural landscapes: The role of native plants. Frontiers in Ecology and the Environment 7, 196-203.

Jackson, D.L., Jackson, L.L., 2002. The farm as natural habitat. Island Press, Washington.

Jackson, D.M., 1982. Searching behavior and survival of 1st-instar codling moths. Annals of the Entomological Society of America 75, 284-289.

Jackson, D.M., Harwood, R.F., 1980. Survival potential of first instars of the codling moth in laboratory experiments. Annals of the Entomological Society of America 73, 160-163.

Jackson, J.E., Parry, M.S., Stephens, C.P., 1981. Intensification of tree fruit production: Current constraints, relevant research and an alternative system strategy for the 1980s. Acta Horticulturae 114, 399-406.

Janssen, A., Sabelis, M.W., Magalhaes, S., Montserrat, M., Van der Hammen, T., 2007. Habitat structure affects intraguild predation. Ecology 88, 2713-2719.

Jaques, R.P., MacLellan, C.R., 1965. Fungal mortality of overwintering larvae of the codling moth in apple orchards in Nova Scotia. Journal of Invertebrate Pathology 7, 291-296.

Jaynes, H.A., Marucci, P.E., 1947. Effect of artificial control practices on the parasites and predators of the codling moth. Journal of Economic Entomology 40, 9-25.

Jenkins, P.E., Isaacs, R., 2007. Reduced-risk insecticides for control of grape berry moth (Lepidoptera : Tortricidae) and conservation of natural enemies. Journal of Economic Entomology 100, 855-865.

Jones, V.P., Steffan, S.A., Wiman, N.G., Horton, D.R., Miliczky, E., Zhang, Q.H., Baker, C.C., 2011. Evaluation of herbivore-induced plant volatiles for monitoring green lacewings in Washington apple orchards. Biological Control 56, 98-105.

Jones, V.P., Unruh, T.R., Horton, D.R., Mills, N.J., Brunner, J.F., Beers, E.H., Shearer, P.W., 2009. Tree fruit IPM programs in the western united states: The challenge of enhancing biological control through intensive management. Pest Management Science 65**,** 1305-1310.

Judd, G.J.R., Gardiner, M.G.T., Thomson, D.R., 1997. Control of codling moth in organically-managed apple orchards by combining pheromone-mediated mating disruption, post-harvest fruit removal and tree banding. Entomologia Experimentalis Et Applicata 83**,** 137-146.

Jumean, Z., Wood, C., Gries, G., 2009. Frequency distribution of larval codling moth, *Cydia pomonella* L., aggregations on trees in unmanaged apple orchards of the Pacific Northwest. Environmental Entomology 38**,** 1395-1399.

Kareiva, P., Perry, R., 1989. Leaf overlap and the ability of ladybird beetles to search among plants. Ecological Entomology 14**,** 127-129.

Kaya, H.K., Joos, J.L., Falcon, L.A., Berlowitz, A., 1984. Suppression of the codling moth (Lepidoptera, Olethreutidae) with the entomogenous nematode, *Steinernema feltiae* (Rhabditida, Steinernematidae). Journal of Economic Entomology 77**,** 1240-1244.

Kaya, H.K., Stock, S.P., 1997. Techniques in insect nematology. In: Lacey, L.A., (Ed.), Manual of techniques in insect pathology. Academic Press, San Diego, pp. 281-324.

Keitt, T.H., Johnson, A.R., 1995. Spatial heterogeneity and anomalous kinetics - emergent patterns in diffusion-limited predatory prey interaction. Journal of Theoretical Biology 172**,** 127-139.

Kleinbaum, D.G., Klein, M., 2012. Survival analysis. Springer, New York.

Kogan, M., Lattin, J.D., 1993. Insect conservation and pest-management. Biodiversity and Conservation 2**,** 242-257.

Koppenhofer, A.M., Grewal, P.S., Kaya, H.K., 2000. Synergism of imidacloprid and entomopathogenic nematodes against white grubs: The mechanism. Entomologia Experimentalis Et Applicata 94**,** 283-293.

Kostylev, V.E., Erlandsson, J., Ming, M.Y., Williams, G.A., 2005. The relative importance of habitat complexity and surface area in assessing biodiversity: Fractal application on rocky shores. Ecological Complexity 2, 272-286.

Kuhrt, U., Samietz, J., Dorn, S., 2005. Thermoregulation behaviour in codling moth larvae. Physiological Entomology 30, 54-61.

Lacey, L.A., Arthurs, S.P., Unruh, T.R., Headrick, H., Fritts, R., 2006a. Entomopathogenic nematodes for control of codling moth (Lepidoptera : Tortricidae) in apple and pear orchards: Effect of nematode species and seasonal temperatures, adjuvants, application equipment, and post-application irrigation. Biological Control 37, 214-223.

Lacey, L.A., Chauvin, R.L., 1999. Entomopathogenic nematodes for control of diapausing codling moth (Lepidoptera : Tortricidae) in fruit bins. Journal of Economic Entomology 92, 104-109.

Lacey, L.A., Granatstein, D., Arthurs, S.P., Headrick, H., Fritts, R., 2006b. Use of entomopathogenic nematodes (Steinernematidae) in conjunction with mulches for control of overwintering codling moth (Lepidoptera : Tortricidae). Journal of Entomological Science 41, 107-119.

Lacey, L.A., Neven, L.G., Headrick, H.L., Fritts, R., 2005. Factors affecting entomopathogenic nematodes (Steinerneniatidae) for control of overwintering codling moth (Lepidoptera : Tortricidae) in fruit bins. Journal of Economic Entomology 98, 1863-1869.

Lacey, L.A., Shapiro-Ilan, D.I., 2008. Microbial control of insect pests in temperate orchard systems: Potential for incorporation into IPM. Annual Review of Entomology 53, 121-144.

Lacey, L.A., Unruh, T.R., 2005. Biological control of codling moth (*Cydia pomonella*, Lepidoptera: Tortricidae) and its role in integrated pest management, with emphasis on entomopathogens. Vedalia 12, 33 - 60.

Landis, D.A., Wratten, S.D., Gurr, G.M., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual Review of Entomology 45, 175-201.

Leslie, P.H., 1945. On the use of matrices in certain population mathematics. Biometrika 33, 183-212.

Letourneau, D.K., Bothwell, S.G., 2008. Comparison of organic and conventional farms: Challenging ecologists to make biodiversity functional. Frontiers in Ecology and the Environment 6, 430-438.

Lewis, E.E., Campbell, J., Griffin, C., Kaya, H., Peters, A., 2006. Behavioral ecology of entomopathogenic nematodes. Biological Control 38, 66-79.

Losey, J.E., Vaughan, M., 2006. The economic value of ecological services provided by insects. Bioscience 56, 311-323.

Lovell, J.H., 1915. The origin of anthophily among the Coleoptera. Psyche 22, 67-84.

Luck, G.W., Harrington, R., Harrison, P.A., Kremen, C., Berry, P.M., Bugter, R., Dawson, T.P., de Bello, F., Diaz, S., Feld, C.K., Haslett, J.R., Hering, D., Kontogianni, A., Lavorel, S., Rounsevell, M., Samways, M.J., Sandin, L., Settele, J., Sykes, M.T., van den Hove, S., Vandewalle, M., Zobel, M., 2009. Quantifying the contribution of organisms to the provision of ecosystem services. Bioscience 59, 223-235.

Luck, R.F., Shepard, B.M., Kenmore, P.E., 1988. Experimental methods for evaluating arthropod natural enemies. Annual Review of Entomology 33, 367-391.

MacArthur, R.H., Pianka, E.R., 1966. On optimal use of a patchy environment. The American Naturalist 100, 603-609.

MacLellan, C.R., 1958. Role of woodpeckers in control of codling moth in Nova Scotia. The Canadian Entomologist 90, 18-22.

MacLellan, C.R., 1960. Cocooning behaviour of overwintering codling moth larvae. The Canadian Entomologist 92, 469-479.

MacLellan, C.R., 1962. Mortality of codling moth eggs and young larvae in an integrated control orchard. The Canadian Entomologist 94, 655-666.

Maclellan, C.R., 1972. Codling moth populations under natural, integrated, and chemical control on apple in Nova-Scotia (Lepidoptera-Olethreutidae). Canadian Entomologist 104, 1397-1404.

Manktelow, D.W.L., Pratt, J.-P., 1997. The tree-row-volume spraying system and its potential use in new zealand. 50th New Zealand Plant Protection Conference, pp. 119-124.

Marshall, G.E., 1940. Some newly discovered habits of the codling moth. Journal of Economic Entomology 33**,** 200.

Marshall, S.A., 2006. Insects: Their natural history and diversity: With a photographic guide to insects of eastern north america. Firefly Books Inc., Buffalo.

Mathews, C.R., Bottrell, D.G., Brown, M.W., 2004. Habitat manipulation of the apple orchard floor to increase ground-dwelling predators and predation of *Cydia pomonella* (L.) (Lepidoptera : Tortricidae). Biological Control 30**,** 265-273.

McEwen, P., New, T.R., Whittington, F.B. Eds.), 2001. Lacewings in the crop environment. Cambridge University Press, Cambridge, UK.

Merfield, C.N., Wratten, S.D., Navntoft, S., 2004. Video analysis of predation by polyphagous invertebrate predators in the laboratory and field. Biological Control 29**,** 5-13.

Michelbacher, A.E., 1952. Walnut insect and spider-mite control in Northern California. Journal of Economic Entomology 45**,** 1020-1027.

Mills, N., 2003. Augmentation in orchards: Improving the efficacy of *Trichogramma* inundation. In: Van Driesche, R.G., (Ed.), 1st International Symposium on Biological Control of Arthropods. USDA Forest Service, pp. 130-135.

Mills, N., 2005a. Classical biological control of codling moth: The California experience. Second International Symposium on Biological Control of Arthropods. USDA Forest Service, Davos, Switzerland, pp. 126-131.

Mills, N., 2005b. Selecting effective parasitoids for biological control introductions: Codling moth as a case study. Biological Control 34**,** 274-282.

Mills, N.J., Carl, K.P., 1991. Parasitoids and predators. In: van der Geest, L.P.S., Evenhuis, H.H., Eds.), Tortricid pests. Elsevier, New York, pp. 235-252.

Minarro, M., Espadaler, X., Melero, V.X., Suarez-Alvarez, V., 2009. Organic versus conventional management in an apple orchard: Effects of fertilization and tree-row management on ground-dwelling predaceous arthropods. Agricultural and Forest Entomology 11, 133-142.

Morris, C., Winter, M., 1999. Integrated farming systems: The third way for european agriculture? Land Use Policy 16, 193-205.

Mota-Sanchez, D., Wise, J.C., Poppen, R.V., Gut, L.J., Hollingworth, R.M., 2008. Resistance of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), larvae in Michigan to insecticides with different modes of action and the impact on field residual activity. Pest Management Science 64, 881-890.

Murdoch, W.W., 1975. Diversity, complexity, stability and pest control. Journal of Applied Ecology 12, 795-807.

Navaneethan, T., Strauch, O., Besse, S., Bonhomme, A., Ehlers, R.U., 2010. Influence of humidity and a surfactant-polymer-formulation on the control potential of the entomopathogenic nematode *Steinernema feltiae* against diapausing codling moth larvae (*Cydia pomonella* L.) (Lepidoptera: Tortricidae). BioControl 55, 777-788.

Neumann, G., Shields, E.J., 2006. Interspecific interactions among three entomopathogenic nematodes, steinemema carpocapsae weiser, *S. Feltiae* Filipjev, and *Heterorhabditis bacteriophora* Poinar, with different foraging strategies for hosts in multipiece sand columns. Environmental Entomology 35, 1578-1583.

Newcomer, E.J., 1920. Winterkilling of codling moth larvae. Journal of Economic Entomology 13, 441-443.

Nielsen, A.L., Lewis, E.E., 2012. Designing the ideal habitat for entomopathogen use in nursery production. Pest Management Science 68, 1053-1061.

Norris, R.F., Kogan, M., 2005. Ecology of interactions between weeds and arthropods. Annual Review of Entomology 50, 479-503.

Oatman, E.R., 1966. Studies on integrated control of apple pests. Journal of Economic Entomology 59, 368-&.

Ohnesorg, W.J., Johnson, K.D., O'Neal, M.E., 2009. Impact of reduced-risk insecticides on soybean aphid and associated natural enemies. Journal of Economic Entomology 102**,** 1816-1826.

Opitz, W., 2002. Flower foraging behavior of the Australian species *Eleale aspera* (Newman) (Coleoptera: Cleridae: Clerinae). The Coleopterists Bulletin 56**,** 241-245.

Paul, N.D., Gwynn-Jones, D., 2003. Ecological roles of solar UV radiation: Towards an integrated approach. Trends in Ecology & Evolution 18**,** 48-55.

Pfiffner, L., Wyss, E., Gurr, G.M., Wratten, S., Altieri, M.A., 2004. Use of sown wildflower strips to enhance natural enemies of agricultural pests. Ecological engineering for pest management. Cornell University Press, Ithaca, pp. 165-186.

Prokopy, R.J., 1994. Integration in orchard pest and habitat management - a review. Agriculture Ecosystems & Environment 50**,** 1-10.

R-core-development-Team, 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Radcliffe, E.B., Hutchinson, W.D., Cancelado, R.E. Eds.), 2009. Integrated Pest Management: Concepts, tactics, strategies, and case studies. Cambridge Universiy Press, New York.

Randlkofer, B., Obermaier, E., Casas, J., Meiners, T., 2010. Connectivity counts: Disentangling effects of vegetation structure elements on the searching movement of a parasitoid. Ecological Entomology 35**,** 446-455.

Renn, N., 1998. Routes of penetration of the entomopathogenic nematode *Steinernema feltiae* attacking larval and adult houseflies (*Musca domestica*). Journal of Invertebrate Pathology 72**,** 281-287.

Riddick, E.W., Mills, N. J., 1994. Potential of adult carabids (Coleoptera: Carabidae) as predators of fifth-instar codling moth (Lepidoptera: Tortricidae) in apple orchards in California. Environmental Entomology 23**,** 1338-1345.

Rolston, A.N., Griffin, C.T., Downes, M.J., 2006. Emergence and dispersal patterns of two isolates of the entomopathogenic nematode *Steinernema feltiae*. Journal of Nematology 38**,** 221-228.

Schroeder, P.C., Villani, M.G., Ferguson, C.S., Nyrop, J.P., Shields, E.J., 1993. Behavioral interactions between japanese-beetle (Coleoptera, Scarabaeidae) grubs and an entomopathogenic nematode (Nematoda, Heterorhabditidae) within turf microcosms. Environmental Entomology 22**,** 595-600.

Shapiro-Ilan, D.I., Lewis, E.E., Campbell, J.F., Kim-Shapiro, D.B., 2012. Directional movement of entomopathogenic nematodes in response to electrical field: Effects of species, magnitude of voltage, and infective juvenile age. Journal of Invertebrate Pathology 109**,** 34-40.

Shapiro-Ilan, D.I., Stuart, R., McCoy, C.W., 2003. Comparison of beneficial traits among strains of the entomopathogenic nematode, *Steinernema carpocapsae*, for control of *Curculio caryae* (Coleoptera : Curculionidae). Biological Control 28**,** 129-136.

Sherman III, F., 1936. Eight seasons' tests in codling moth control in Michigan. Journal of Economic Entomology 29**,** 653-655.

Simon, S., Bouvier, J.C., Debras, J.F., Sauphanor, B., 2010. Biodiversity and pest management in orchard systems. A review. Agronomy for Sustainable Development 30**,** 139-152.

Simon, S., Defrance, H., Sauphanor, B., 2007. Effect of codling moth management on orchard arthropods. Agriculture Ecosystems & Environment 122**,** 340-348.

Simpson, C.B., 1903. The codling moth. Government Printing Office, Washingon D.C.

Solomon, M.E., 1991. Ecology and population dynamics. In: van der Geest, L.P.S., Evenhuis, H.H., Eds.), Tortricid pests. Elsevier, New York, pp. 209-234.

Solomon, M.E., Glen, D.M., 1979. Prey density and rates of predation by tits (*parus* spp) on larvae of codling moth (*Cydia-pomonella*) under bark. Journal of Applied Ecology 16**,** 49-59.

Solomon, M.E., Glen, D.M., Kendall, D.A., Milsom, N.F., 1976. Predation of overwintering larvae of codling moth (*Cydia pomonella* (L.)) by birds. Journal of Applied Ecology 13**,** 341-352.

Southwood, T.R.E., 1978. Ecological methods with particular reference to the study of insect populations. Chapman and Hall, London.

Spence, K.O., Lewis, E.E., Perry, R.N., 2008. Host-finding and invasion by entomopathogenic and plant-parasitic nematodes: Evaluating the ability of laboratory bioassays to predict field results. Journal of Nematology 40, 93-98.

Stehr, F.W., 1991. Immature insects. Kendall/Hunt, Dubuque.

Steiner, L.F., 1929. Miscellaneous codling moth studies. Journal of Economic Entomology 22, 648-654.

Steiner, L.F., Ackerman, A.J., 1936. Large-scale test of orchard sanitation to control codling moth Journal of Economic Entomology 29, 648-653.

Stern, V.M., Smith, R.F., van den Bosch, R., Hagen, K.S., 1959. The integrated control concept. Hilgardia 29, 81 - 101.

Stoeckli, S., Mody, K., Dorn, S., 2008. Influence of canopy aspect and height on codling moth (Lepidoptera : Tortricidae) larval infestation in apple, and relationship between infestation and fruit size. Journal of Economic Entomology 101, 81-89.

Straub, C.S., Finke, D.L., Snyder, W.E., 2008. Are the conservation of natural enemy biodiversity and biological control compatible goals? Biological Control 45, 225-237.

Stuart, R.J., El-Bopai, F.E., Duncan, L.W., 2008. From augmentation to conservation of entomopathogenic nematodes: Trophic cascades, habitat manipulation and enhanced biological control of diaprepes abbreviatus root weevils in florida citrus groves. Journal of Nematology 40, 73-84.

Subinprasert, S., 1987. Natural enemies and their impact on overwintering codling moth populations (*Laspeyresia-pomonella* L) (Lep, Tortricidae) in south Sweden. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie 103, 46-55.

Summerland, S.A., Steiner, L.F., 1943. Codling moth oviposition and fate of eggs. Journal of Economic Entomology 36, 72-75.

Suverkropp, B.P., Bigler, F., van Lenteren, J.C., 2010. Movement and host finding of *Trichogramma brassicae* on maize plants. Bulletin of Insectology 63, 115-127.

Therneau, T., Lumley, T., 2009. Survival: Survival analysis, including penalised likelihood. http://CRAN.R-project.org/package=survival.

Thomas, M.B., 1999. Ecological approaches and the development of "truly integrated" pest management. Proceedings of the National Academy of Sciences of the United States of America 96**,** 5944-5951.

Thomas, M.B., Wratten, S.D., Sotherton, N.W., 1991. Creation of 'island' habitats in farmland to manipulate populations of beneficial arthropods: Predator densities and emigration. Journal of Applied Ecology 28**,** 906-917.

Thomson, L.J., Hoffmann, A.A., 2009. Vegetation increases the abundance of natural enemies in vineyards. Biological Control 49**,** 259-269.

Thomson, L.J., Hoffmann, A.A., 2013. Spatial scale of benefits from adjacent woody vegetation on natural enemies within vineyards. Biological Control 64**,** 57-65.

Unruh, T.R., Lacey, L.A., 2001. Control of codling moth, *Cydia pomonella* (Lepidoptera : Tortricidae), with *Steinernema carpocapsae*: Effects of supplemental wetting and pupation site on infection rate. Biological Control 20**,** 48-56.

Unruh, T.R., Pfannenstiel, R.S., Peters, C., Brunner, J.F., Jones, V.P., 2012. Parasitism of leafrollers in Washington fruit orchards is enhanced by perimeter plantings of rose and strawberry. Biological Control 62**,** 162-172.

USDA-NASS, 2008. Agricultural chemical usage 2007 field crops summary. United States Department of Agriculture, pp. 120.

USDA-NASS, 2012a. Noncitrus fruits and nuts. United States Department of Agriculture - National Agricultural Statistics Service, pp. 79.

USDA-NASS, 2012b. Quick stats United States Department of Agriculture.

van der Geest, L.P.S., Evenhuis, H.H. Eds.), 1991. Tortricid pests. Elsevier, New York.

Van Driesche, R.G., Bellows, T.S., 1996. Biological control. Chapman & Hall, New York.

Van Driesche, R.G., Mason, J.L., Wright, S.E., Prokopy, R.J., 1998. Effect of reduced insecticide and fungicide use on parasitism of leafminers (*Phyllonorycter* spp.) (Lepidoptera : Gracillariidae) in commercial apple orchards. Environmental Entomology 27**,** 578-582.

van Emden, H.F., Williams, G.F., 1974. Insect stability and diversity in agro-ecosystems. Annual Review of Entomology 19**,** 455-475.

Vandenbosch, R., Stern, V.M., 1962. Integration of chemical and biological control of arthropod pests. Annual Review of Entomology 7**,** 367-&.

Vreysen, M.J.B., Robinson, A.S., 2011. Ionising radiation and area-wide management of insect pests to promote sustainable agriculture. A review. Agronomy for Sustainable Development 31**,** 233-250.

Wagenmakers, P.S., 1991. Planting systems for fruit-trees in temperate climates. Critical Reviews in Plant Sciences 10**,** 369-385.

Wallace, H.R., 1958. Movement of eelworms. Annals of Applied Biology 46**,** 74-85.

Walton, N.J., 2009. Habitat management using native flowering perennials to increase beneficial insects in Michigan highbush blueberry. Entomology. Michigan State University, Lansing, pp. 115.

Walton, N.J., 2013a. Codling moth (*Cydia pomonella* [L.]) natural enemies in apple orchards: Over a century of research. The role of natural enemies in the management of codling moth (*Cydia pomonella* [L.]) in organic apple orchards in Michigan.

Walton, N.J., 2013b. An exploration of how moisture and structural complexity may affect the foraging success of *Steinernema feltiae* Filipjev against codling moth (*Cydia pomonella* [L.]). The role of natural enemies in pest management of codling moth (*Cydia pomonella* [L.]) in organic apple orchards in Michigan.

Walton, N.J., 2013c. Habitat characteristics influencing foraging success of codling moth antagonists in apple orchards (*Cydia pomonella* [L.]) The role of natural enemies in the management of codling moth (*Cydia pomonella* [L.]) in organic apple orchards in Michigan.

Walton, N.J., 2013d. Video observations of the natural enemies of eggs and fifth instar codling moth *Cydia pomonella* (L.) in Michigan apple orchards (USA). The role of natural enemies in the management of codling moth (*Cydia pomonella* [L.]) in organic apple orchards in Michigan.

Walton, N.J., Isaacs, R., 2011a. Influence of native flowering plant strips on natural enemies and herbivores in adjacent blueberry fields. Environmental Entomology 40, 697-705.

Walton, N.J., Isaacs, R., 2011b. Survival of three commercially-available natural enemies exposed to Michigan wildflowers. Environmental Entomology 40, 1177 - 1182.

Wearing, C.H., 1975. Integrated control of apple pests in New Zealand 2. Field estimation of fifth-instar larval and pupal mortalities of codling moth by tagging with cobalt-58. New Zealand Journal of Zoology 2, 135-149.

Wilby, A., Thomas, M.B., 2002. Natural enemy diversity and pest control: Patterns of pest emergence with agricultural intensification. Ecology Letters 5, 353-360.

Wise, J.C., Jenkins, P.E., Schilder, A.M.C., Vandervoort, C., Isaacs, R., 2010. Sprayer type and water volume influence pesticide deposition and control of insect pests and diseases in juice grapes. Crop Protection 29, 378-385.

Witzgall, P., Stelinski, L., Gut, L., Thomson, D., 2008. Codling moth management and chemical ecology. Annual Review of Entomology 53, 503-522.

Zhang, W., Swinton, S.M., 2009. Incorporating natural enemies in an economic threshold for dynamically optimal pest management. Ecological Modelling 220, 1315-1324.