PARASITOID MEDIATED EFFECT ON HOST BEHAVIOR: IMPACTS ON INFECTION RISK BY OTHER PATHOGENS

by

SARAH ELIZABETH JOHNSON

(Under the Direction of Mike Strand)

ABSTRACT

The parasitoid wasp *Microplitis demolitor* carries the polydnavirus *Microplitis demolitor Baculovirus* (MdBV) to facilitate parasitism of the larval stages of the moth *Chrysodeixis includens*. Prior studies established that MdBV infection globally suppresses host immunity, which raised the question of whether parasitized hosts are more vulnerable to infection by other pathogens. I assessed whether parasitism altered host growth and development by measuring body weight, head capsule width, frass, and locomotor activity. I then tested host susceptibility and risk of infection by the pathogens *Bacillus thuringiensis* and *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV). Results indicated that parasitism suppressed host feeding behavior, which caused stunted host growth and development. Parasitized and non-parasitized hosts were equally as susceptible to AcMNPV and Bt when force fed. In contrast, when third instars fed naturally on AcMNPV containing diet parasitized hosts exhibited significantly lower levels of mortality, but Bt spread diet exhibited no significant mortality difference.

Keywords**:** Parasitoid, Polydnavirus, Soybean looper, Behavioral manipulation, Host-parasite coevolution, *Bacillus thuringiensis*, *Autographa californica* multicapsid nucleopolyhedrovirus

PARASITOID MEDIATED EFFECT ON HOST BEHAVIOR: IMPACTS ON INFECTION RISK BY OTHER PATHOGENS

By

SARAH ELIZABETH JOHNSON

B.S., Winthrop University, 2010

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER'S OF SCIENCE

ATHENS, GEORGIA

2013

PARASITOID MEDIATED EFFECT ON HOST BEHAVIOR: IMPACTS ON INFECTION RISK BY OTHER PATHOGENS

By

SARAH ELIZABETH JOHNSON

Major Professor: Mike Strand

Committee: Kerry Oliver
Patricia Moore

Electronic Version Approved:
Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2013

ACKNOWLEDGEMENTS

I want to thank Mike Strand, Trish Moore, and Kerry Oliver for agreeing to be on my committee and helping me write this document. If it was not for the patience and guidance of Mike Strand I would not have been able to have this amazing learning experience. I also want to thank Jena Johnson and Kavita Bitra. Thank you Jena for carrying the responsibility to rear the insects in this study and Kavita for showing me how to isolate MdBV and inject into *C. includens*. I came into this program with little experience in research, but I now feel that I will hit the ground running in my next endeavor.

TABLE OF CONTENTS

Chapter I

INTRODUCTION

Many studies in the field of parasitology focus on how parasites successfully infect their host. A group of economically important parasites that infect insects are parasitoid wasps. Many parasitoid wasps are considered beneficial species because they are used as biocontrol agents to control important agricultural pests. Insects attacked by parasitoids are also often at risk from infection by other pathogens such as bacteria, fungi, and viruses. Insects do not have an acquired immune system as vertebrates do, but they do have a well-developed innate immune system (Siva-Jothy et al., 2005). The first component of the insect immune system is a group of molecules called pattern recognition receptors (PRRs) that bind to the surface of foreign invaders. Once recognized, blood cells called hemocytes can phagocytose many microorganisms in a structure called a phagosome or encapsulate large multicellular invaders like parasitoid wasps. This innate immune response is so effective that when *Drosophila* was injected with *E.coli*, it took only 6 hours for hemocytes to remove the bacteria (Tzou et al. 2002). An example of a humoral defense response is the cascade of serine proteases that regulate the enzyme phenoloxidase (PO). PO catalyzes the production of cytotoxic molecules and melanin, which have both been implicated in the killing of parasites (Klowden 2007).

Parasitoids have evolved different tactics to invade their hosts. Parasitoids in the superfamily Ichneumonoidea are especially interesting because they rely on viruses in the family Polydnaviridae to immunosuppress their hosts. Polydnaviruses (PDVs) are divided into two genera, the *Bracovirus* and *Ichnovirus*, which are associated with wasps in the families'

Braconidae and Ichneumonidae. PDVs only replicate inside wasp ovaries and the number of PDV particles a wasp injects into a host is crucial for successful parasitism (Webb and Strand, 2005; Dupuy et al., 2006; Strand, 2009). For this reason, the PDV-wasp relationship is mutualistic. PDVs are also specific with each wasp species carrying its own genetically distinct virus (Strand, 2010; Savary et al., 1997; Wyder et al., 2002; Belle et al., 2002).

Effects of parasitism on host growth and development

Parasitoid wasps and other parasites manipulate the growth of hosts to increase their own chance for survival (Moore 2002; Thomas et al. 2005). This phenomenon is explained by Dawkins' concept of the 'extended phenotype' and the 'parasite manipulation' hypothesis. (Dawkins, 1982; Thomas et al. 2005; Cezilly et al. 2010, Cezilly et al. 2012). The idea of the extended phenotype points out that the effects of a gene are not limited to a specific biological process, but extend to all of the effects a gene has on an organism's interaction with its environment. The parasite manipulation hypothesis states that natural selection has led to parasites evolving strategies for manipulating the growth of hosts. In other words, parasites often manipulate the physiology and behavior of hosts to increase their own fitness as measured by increases in persistence in the host, and/or transmission between hosts (Moore 2002;Thomas et al. 2005; Poulin 2007; Poulin 2010). There has even been a term coined to explain 'parasite induced phenotypic alterations' (PIPAs) known as 'multidimensionality' (Cezilly and Perrot-Minnot 2005; Cezilly et al. 2012). There are countless examples of PIPAs in the animal and plant kingdoms (Kao, 2008). There have also been numerous reviews on the subject and many fascinating examples of parasite-induced host alterations. For example, the trematode *Ribeiroia ondatrae* causes its intermediate frog host *Pseudacris regilla* to suffer shorter jumping distances, slower swimming speeds, reduced endurance, and lower foraging success relative to uninfected

hosts (Goodman and Johnson 2011). This host phenotype is the result of altered leg development mediated by the parasite. Altered development renders the host more susceptible to predators and thus enhances parasite transmission (Poulin, 2005).

Another example of a PIPA is the suppression of growth and pupation of larval stage lepidopteran hosts by PDV-carrying wasps, which allows their offspring to survive(Beckage et al. 2002a; Devin et al. 2000; Strand, 2009; Beckage et al., 2002a). The altered host phenotype in this relationship is the result of the PDV's ability to suppress host feeding behavior, alter growth hormone titers, and alter metabolic reserves (Pruijssers et al., 2009; Strand, 1990; Ohnuma and Kainoh 1992; Balgopal et al., 1996). Several studies document that the maternal factors the wasp injects during oviposition, such as PDVs, cause these changes in to occur (Tanaka et al., 1987; Tanaka and Vinson, 1987; Zitnan et al., 1995; Kelly et al., 1998; Pennacchio et al.,1998; Pennacchio et al., 2001; Fallabella et al., 2003). Some PDV-carrying wasps manipulate growth by dramatically reducing the body weight and mass of hosts (Beckage, 2001). When measuring the growth hormones of these parasitized hosts, they typically exhibit reduced hemolymph ecdysteroid titers and elevated titers of juvenile hormone (JH). However, it currently remains unclear whether these alterations are due to wasp factors directly or indirectly altering hormone production by the host (Thomson 1993; Pennacchio and Strand, 2006). Some virus-induced alteration in host growth also proved signals that synchronize growth of wasp offspring with the molting cycle of their hosts (Lawrence and Lanzrein, 1993, Beckage, 2001, Beckage, 2002 and Edwards and Weaver, 2001; Beckage et al., 2002a,b). For instance, 12-24 hours before progeny from the PDV-carrying wasp *Cotesia congregata* emerge, a pre-emergence hormone appears in the host that causes wasp larvae to exit from the host's body and pupate (Beckage and Riddiford, 1982 and Gelman et al.,1998).

Effect of parasitism on host behavior

Thus far I have reviewed how parasitoids manipulate immune defenses and growth of hosts. I now will point out some interesting studies on how parasitoids alter other aspects of host behavior. In the fascinating case of the cockroach *Periplaneta americana* and the ectoparasite jewel wasp, *Ampulex compressa,* the wasp first injects venom into the base of the wing and then the head of the host. This causes the cockroach to perform an obsessive grooming behavior, which is thought to occur from a venom-induced rise in dopamine levels in the brain (Weisel-Eicher et al. 1999). The sting into the wing also induces a temporary paralysis, which facilitates the lower head sting (Moore et al., 2006). While the cockroach is temporarily paralyzed the wasp searches for a burrow to make her nest. The grooming behavior keeps the cockroach preoccupied until the wasp returns. When the jewel wasp returns she tears off both antennae and feeds from the hemolymph that spews from the fresh wounds. The cockroach does not interrupt her during this process. After she is done feeding she grabs one of the antennal stubs, while perched on the cockroach head, and directs the prey to her nest where she lays an egg on the cockroach (Fouad et al., 1994). The cockroach remains in the nest while the wasp covers up her burrow. The wasp progeny then hatches and consumes the cockroach (Liberstat and Gall 2013). Radiolabeling techniques indicate that wasp venom localizes to the midline of the sub-esophageal ganglion, the central part of the supra-esophageal ganglion, and posterior to the central complex around the mushroom bodies (Haspel et al., 2003). This shows that the wasp injects venom directly into the central nervous system to alter mechanisms of grooming behavior and locomotor activity.

Most studies on alterations in host behavior caused by PDV-carrying wasp focus on events that occur after parasitoid offspring emergence. Several studies have recorded the unusual

behavior of parasitized hosts remaining near wasp progeny that have pupated and exhibiting heightened aggression when hyperparasitoids or predators threaten the wasp pupae. These studies show that hosts can live a week or more after parasitoids emerge (Tanaka and Ohsaki 2006.2009; Grosman et al. 2008; Harvey et al 2008a,b;Janssen et al. 2010). Harvey, et al. (2011) recorded a difference between healthy and parasitized host movement and activity after a *Microplitis* sp. (Hymenoptera: Braconidae) emerged from the Oriental armyworm *Mythimna separate* (Lepidoptera: Noctuidae). They report that after emergence the parasitoid caused the posterior 4 abdominal segments of the host to be paralyzed and that the host protected the parasitoid cocoon. Brodeur and Vet (1994) concluded that parasitoids control or manipulate their host and called it the "usurpation hypothesis." This is when the host is manipulated by the effects of parasitism to "body guard" parasitoid pupae.

Microplitis demolitor- Chrysodeixis includens study system: Effects of parasitism on C. includens growth and behavior

Before I review host growth alterations mediated by *M. demolitor* parasitism, I will first review the growth mechanisms of Lepidoptera as other holometabolous insects. Larvae feed to increase their body size that eventually initiates the molting process. When larvae attain a weight threshold called 'critical weight' (Nijhout 1975), prothoracicotropic hormone (PTTH) is secreted, which stimulates the release of ecdysone from the prothoracic glands. Ecdysone is then converted to 20-hydroxyecdysone (20HE) which is the active molting hormone that stimulates apolysis and ultimately ecdysis. Since the molting process is a vulnerable time for an insect, insect larvae commonly move from their feeding location to a safer place. During apolysis the endocuticle is dissolved and reabsorbed into the integument leaving a gap between the new epicuticle and the old exo- and endocuticle. The epidermal layers along with the old tracheal

system are then shed off the caterpillar (ecdysis). Lastly, the old head capsule pops off leaving a newly ecdysed larva (Klowden 2007). The larva will continue this cycle several times until it reaches a certain weight to pupate and morph into an adult.

Parasitism by *M. demolitor* alters the growth and behavior of *C. includens* (Strand and Wong 1991). *Chrysodeixis includens* larvae have five instars. During the 1^{st}-4^{th} instar, larvae feed about 20 hours followed by apolysis and ecdysis that take 12-14 hours. Fifth instars feed 44-48 hours before initiating processes associated with pupation (Strand, 1990). For a fifth instar to begin the process of pupation, it must weigh at least 65 mg at ecdysis to the fifth instar and at least 130mg at 12h post-ecdysis (Strand 1990). When *M. demolitor* parasitizes *C. includens* as a third instar, it molts twice to become a fifth instar. However, its body weight is dramatically lower than non-parasitized larvae and it never pupates (Strand and Dover 1991). The wasp offspring emerge from *C. includens* 7 days post-parasitism to pupate. After the parasitoid emerges, *C. includens* also lives for an additional 2-3 days (Strand and Dover 1991). When hosts are injected with only MdBV, the same alterations in larval growth and inhibition of pupation occur (Pruijssers et al. 2009).

Some organisms exhibit illness-induced anorexia in response to infection by pathogens (Dantzer, 2004; Adamo et al., 2007; Adamo, 2008). Thus infection alone can cause hosts to reallocate metabolic reserves, which alters growth. (Anderson et al., 2004; Hotamisligil, 2006; Moret and Schmid-Hempel, 2000; Ahmed et al., 2002; Armitage et al., 2003; Dionne et al., 2006). This kind of response, however, does not explain the response of *C. includens* by *M. demolitor* because alterations in host growth only occur if transcriptionally functional MdBV infects caterpillars (Pruijssers et al. 2009). When a range of virus concentrations were injected in *C. includens* just 0.2-0.02 wasp equivalents of virus produced the same alterations in growth

that occur in parasitized hosts (Strand and Dover 1991). Work has also been done to show that even when *C. includens* is starved to cause stress the resulting metabolic alterations greatly differ from parasitized larvae (Pruijssers et al. 2009). JH titers remain elevated during parasitism, but no increase in ecdysteroid titers are observed during the 1st 92 hours. Blood sugar concentrations of infected hosts also greatly increase compared to control fed or starved larvae, while glycogen and lipid levels plummet (Pruijssers et al. 2009). Only MdBV causes these physiological alterations that benefit *M. demolitor* parasitoid by elevating nutrient levels in the blood that wasp larvae consume (Thompson, 1993; Vinson et al., 2001; Pennacchio and Strand, 2006).

Roles of MdBV in immunosuppression

As previously noted, the encapsulation response is the principal defense of hosts against endoparasitoids (Strand and Pech, 2005). *M. demolitor*, however, suppresses the encapsulation response of *C. includens*. *M. demolitor* injects an egg, venom, and *M. demolitor* bracovirus (MdBV) into *C. includens* during oviposition (Strand and Wong, 1991; Strand, 1994; Strand et al. 2006). Functional studies indicate that venom facilitates the infection of host cells by MdBV (Strand and Noda, 1991), while genes encoded by MdBV are primarily responsible for immunosuppressing *C. includens*. Within 4 h of infection hemocytes lose the ability to attach themselves to the parasitoid egg or any foreign surface. Granulocytes, a subpopulation of hemocytes, undergo apoptosis and these effects continue during the full time of parasitoid larval development (Strand, 2010). Detailed studies further show that MdBV infects virtually all hemocytes in parasitized hosts and that hosts remain immunosuppressed for the duration of parasitism (Strand, 1994; Beck et al., 2007; Bitra et al., 2011).

Problem Statement

The review of the literature shows that parasites commonly alter host immune defenses, growth, and behavior. Prior studies also show that in the case of *M. demolitor*, its viral symbiont MdBV severely immunosuppresses *C. includens* while also altering host feeding behavior, growth, and metabolism. Wasp offspring clearly benefit from MdBV-mediated immunosuppression. However, an important but unanswered question is whether immunosuppression renders parasitized hosts more susceptible to infection by other pathogens which in turn could reduce wasp fitness. Two such pathogens are the baculovirus *Autographica californica* multicapsid nucleopolyhedrovirus (AcMNPV) and the bacterium *Bacillus thuringiensis* (Bt). Both of these species infect *C. includens* larvae by per os (oral) infection. Here I tested the hypothesis that the alteration in host behavior associated with MdBV infection reduces the risk of infection by AcMNPV and Bt. In the first part of my study I compared the growth, feeding behavior, and movement of non-parasitized and parasitized larvae. I then assessed whether alterations in the feeding behavior of parasitized hosts reduced the risk of infection by AcMNPV and Bt.

Chapter II

ALTERED GROWTH, DEVELOPMENT, AND BEHAVIOR OF *C.INCLUDENS* MEDIATED BY *M. DEMOLITOR* PARASITISM

Introduction

To assess the effects of parasitism on host larval growth and molting I compared average head capsule widths and body weights of non-parasitized *C. includens* larvae to larvae parasitized by *M. demolitor* as third instars. I also measured frass accumulation to assess changes in food consumption as in Pruijssers et al. (2009), and compared the locomotor activity of parasitized and non-parasitized larvae by conducting focal observations where I monitored the location of larvae in experimental arenas at regular intervals.

Methods

Insect rearing

C. includens larvae were reared as previously described on an agar-based artificial diet in plastic rearing cups at 27±1°C with a 16 h light (L):8 h dark (D) photoperiod (lights on 10:00 h, lights off 02:00 h) (Green et al., 2006). Adults were fed a 20% sucrose solution. Host larvae used in the study were reared individually in 30-ml plastic cups half-filled with diet. *M. demolitor* was reared as outlined previously at 27 °C and 16 h light 8 h dark photoperiodic regime (Strand et al., 1988).

Insect Staging

The lepidoptera *C. includens* larvae were physiologically staged using previously established morphological characteristics (Strand, 1990). I designated the time immediately following

ecdysis to the next instar as 0 h with subsequent events recorded as hours post-ecdysis to the last instar. I refer non-parasitized *C. includens* larvae as to healthy or non-parasitized. The 0-12 h third instar *C. includens* larvae were parasitized by allowing *M. demolitor* females to oviposit only once, which prevented any possibility of superparasitism.

Head capsule, body weight, and Frass

To understand how parasitism affected growth, I measured head capsule width, wet fecal matter weight, and body weight of parasitized third instar *C. includens* relative to non-parasitized controls. Samples consisted of 25 individuals per instar and treatment. To ensure that my interactions with the insects did not affect natural circadian rhythms, I observed larvae only during the light cycle. I measured the head capsule widths of larvae at 10 AM and 9 PM every day using an ocular micrometer mounted on a stereomicroscope (Olympus) until the parasitoids emerged or the caterpillars pupated. Frass (mg) was collected from rearing cups at 2 PM every day for both treatments and weighed using an analytical balance (OHous). Body weight was determined by weighing larvae once a day at 2 PM. Molting events for non- and parasitized larvae were determined as previously outlined (Strand et al. 1988; Strand 1990).

C. includens on soybean plants

I grew organic soybean plants, *Glycine max***,** in the Entomology department's greenhouse on campus. I transported the plants to the lab's temperature and humidity controlled incubator for experimental use. Easy Log confirmed temperature and humidity readings (USB Version 5.3, Laser Electronic Inc.), which recorded a consistent daily average of 28°C and 60% relative humidity. In the incubator, I placed the plants in a tray containing about two inches of water to allow their roots to obtain water and act as a barrier to keep caterpillars on their host plant.

To control for any variation of food preference, I reared *C. includens* for two days on soybean leaves in petri dishes before placing on plants. I placed newly molted second instars on leaves in petri dishes until they molted to their third instar, which were then placed on the soybean plants for observation. I placed caterpillars on the bottom-most petiole of their own potted soybean plant. I made observations daily to calculate the amount of foliage eaten by each individual caterpillar. I categorized the plant leaves by small, medium, and large with their respected area of 550 mm^2, 1240 mm^2, and 1730 mm^2. I calculated the foliage eaten by the sum of leaf area devoured daily. I expected parasitized individuals to eat significantly less so this estimate in amount of leaves consumed would appropriately show the difference in feeding behavior mediated by parasitism.

Locomotor activity: Distance Over Time

Before measuring locomotor activity of *C. includens*, I first controlled for stress that could occur from transferring the hosts from the small diet cups to my assay arena. I used plastic containers the size of the arenas with a thin layer of diet on the surface to hold individual *C. includens* until they had quit moving and began feeding. I then cut the patch of diet around the larvae using a spatula and then gently placed the patch of diet into the middle of the assay arena. I placed healthy and newly parasitized third instar *C. includens* into acclimation containers within 12 h of ecdysis until they were relaxed and feeding.

To measure daily movement, I designed arenas using 8 oz. circular plastic containers. Concentric circles were drawn 0.5 cm apart on the lid of each container with a “Bulls Eye” in the center. I first placed one larva on the diet in the center of the arena so when I placed the lid on the arena the larva was visible under the bull's eye. I marked the location of the larva on the arena every 6 hours and distance moved was recorded every 12 h thereafter. I used dry erase

markers to mark on the outside of the arenas the position of the front half of the caterpillar during specific time points. I designed the arena to precisely measure the distance between each position mark using the bull's eye on the lid as a ruler. Movement of the larva was then recorded by measuring the distance from where the larva was located at the previous observation time. I also recorded whether the larva was on or off diet (side or top of the arena), its instar, and its physiological stage (feeding or apolysis). I monitored thirty-six non- and parasitized larvae.

Statistical Analysis

I used a repeated measures ANOVA to compare non- and parasitized *C. includens* body weight along with head capsule width and wet fecal matter weight. The time interaction factor was significant for all three comparisons so I analyzed them without time as a factor using a paired t-test or Kruskal-Wallis test (SAS Cary, NC). After 24 h into each experiment I compared non- and parasitized hosts to estimate how quickly developmental delay and suppressed feeding can be measured. I also analyzed the seventh day to compare the final weight before pupation or wasp emergence. I analyzed body weight on day one using a paired t-test and body weight on day seven with a Kruskal-Wallis test due to non-normal distribution. I analyzed head capsule width only on day seven to measure developmental delay of parasitized hosts using a Kruskal-Wallis test due to non-normal distribution. I analyzed wet frass weight on days one and seven along with total amount of soybean leaves eaten by non- and parasitized *C. includens* with a Kruskal-Wallis test due to non-normal distribution. Frass production was an indirect way to measure food consumption and amount of soybean leaves eaten was a more direct approach. I also analyzed total movement of non- and parasitized larvae as a variable to measure altered development using a Kruskal-Wallis test due to non-normal distribution. Lastly, I compared the

proportion of non- and parasitized *C. includens* on food or away from food using a Chi-square test for independence.

Results and Discussion

Body Weight, Head Capsule Width, Fecal Matter

The weight of *C. includens* larvae at 24 h post parasitism did not differ from non-parasitized larvae of the same age (29 d.f, t= -0.76, P= 0.45). However, parasitized hosts 12-24 hours before wasp emergence (d7) weighed significantly less than non-parasitized larva (~15 mg vs. 105 mg) (H=22.3125, 1 d.f., P<0.0001) (Fig. 1). When I compared development by tracking changes in head capsule width, non-parasitized and parasitized *C. includens* each molted twice, but the head capsule widths of parasitized larvae as fifth instars were significantly smaller than non-parasitized fifth instars (9 vs. 17 mm by day 7) (H=22.08, 1 d.f., P<0.0001) (Fig.2). Parasitized hosts remained as fifth instars after the last molt while non-parasitized pupated. Measures of frass weight indicated that parasitized *C. includens* ate less than non-parasitized larvae just 24 h after parasitism (H=15.8,1 d.f., P<0.0001) (Fig. 3). Before pupation or wasp emergence (day 7) parasitized *C. includens* overall produced significantly less frass than non-parasitized larvae (~25mg. vs. 200mg) (H=16.8, 1 d.f. P<0.0001). Lastly, I allowed non- and parasitized *C. includens* to feed on soybean plants to measure feeding behavior directly (Fig. 4). Parasitized third instar *C. includens* ate significantly less leaf material than non-parasitized larvae when left on soybean plants until pupation or wasp emergence (1803.3 $\pm$ 222.41 vs. 9566.4 $\pm$ 678 mm^2)(H=9, 1 d.f., P=0.0027). Overall, my results clearly show that parasitism by *M. demolitor* greatly reduced growth and feeding of *C. includens* larvae.

Locomotor Activity

As reviewed in my introduction, many Lepidoptera actively move while feeding and move away from feeding sites when molting. I thus used locomotor activity as another measure for comparing development of non- parasitized and parasitized hosts. Results indicated that the total distance moved from the third instar until pupation or wasp emergence was significantly less for parasitized hosts than non-parasitized hosts ($H=23.5673$, 1 d.f., $P<0.0001$). Previous studies showed that the duration of the third stadium was one day longer for parasitized larvae than non-parasitized larvae (Strand and Dover 1991). Once the wasp emerges the host remains alive for 5-7 days and remains beside the parasitoid's cocoon even though they are perfectly capable of walking away. The reduced movement of parasitized hosts thus appears to be due to the combination of reduced feeding and delayed development.

Apolysis/Feeding Behavior in Relation to Location

I also compared the number of observations where non- and parasitized larvae were on the food verses the side/top of my assay arenas. I expected *C. includens* to be on the food when feeding and on the side/top during apolysis. I also hypothesized that parasitized *C. includens* might avoid food, which would explain suppressed growth and development. However, I detected no difference in the frequency of observations that non-parasitized and parasitized larvae times were on food ($X^2=0.0061$,d.f.=1, $P=0.9378$) or the side/top of arenas ($X^2=0.0002$, d.f.=1, $P=0.9896$) (Fig. 6). Taken together, my results indicated that parasitized *C. includens* move less but exhibit the same cyclical pattern of movement associated with feeding and molting as non-parasitized larvae. I also conclude that non-parasitized *C. includens* move more when on the food and feeding while parasitized larvae move less because feeding behavior is inhibited (data not shown).

Summary and final remarks

In summary, I have assessed multiple responses by non- parasitized and parasitized *C. includens* by *M. demolitor*. I have empirically shown how parasitism alters growth and development by measuring non- and parasitized *C. includens* head capsule width, body weight, amount of fecal matter produced, and locomotor activity My results agree with previous studies by Strand and Dover (1991) and Strand et al. (1990) on the effects of parasitism by *M. demolitor* on *C. includens* growth and development. Pruijssers et al. (2009) also found that parasitism by *M. demolitor* causes host wasting and inhibited metamorphosis. The smaller head capsule width and significantly lower body weight of parasitized hosts relative to non-parasitized hosts suggest that they are not acquiring enough nutrients to grow normally. The ability of parasitized hosts to molt regardless of suppressed growth after parasitism suggests an altered 'critical weight' threshold. As noted in my introduction MdBV infection is solely responsible for suppression of host growth (Pruijssers et al. 2009). Since the virus only replicates inside the wasp ovaries the reproductive success of the virus is reliant upon the survival of the wasp's progeny. Many parasitoids exhibit both increased and decreased rates of food consumption and often the effects seem to vary according to the developmental stage of the parasitoid (Powell, 1989; Duiodu and Antoh, 1984). Slansky (1986) stated that there is a general tendency for gregarious parasitoids to cause an increase in host food consumption while solitary species cause a decrease. Because per os transmitted pathogens are a major cause of mortality for many insects, suppressed feeding could be beneficial to *M. demolitor* by reducing the risk of the host before offspring complete their development.

For future studies, it would be interesting to use other known hosts of *M. demolitor* to measure altered growth and development by means of morphological characteristics and

locomotor activity. To further support locomotory results, a digital movement-tracking device would be useful. I measured locomotor activity during times that I predetermined to be sufficient, but the device could measure the exact amount of distance covered by non- and parasitized *C. includens* and could also measure other variables such as velocity that might be of interest. It would also be interesting to do more studies with the *M. demolitor-C. includens* study system on soybean plants. On artificial diet, parasitized *C. includens* exhibit a clear or translucent appearance due to their cleared gut from suppressed feeding. Before my soybean experiments, I hypothesized an evolutionary disadvantage that predators could easily spot parasitized hosts since they would not otherwise be camouflaged by the leaves in their gut to match the host plant. However, while parasitized hosts still exhibited suppressed growth and feeding on soybean plant, they also exhibited the same green coloration as non-parasitized hosts. In conclusion, this chapter confirms that parasitism suppressed *C. includens* feeding behavior. The next chapter assesses the risk non-parasitized and parasitized *C. includens* face of per os infection by microbial pathogens.

Graphs

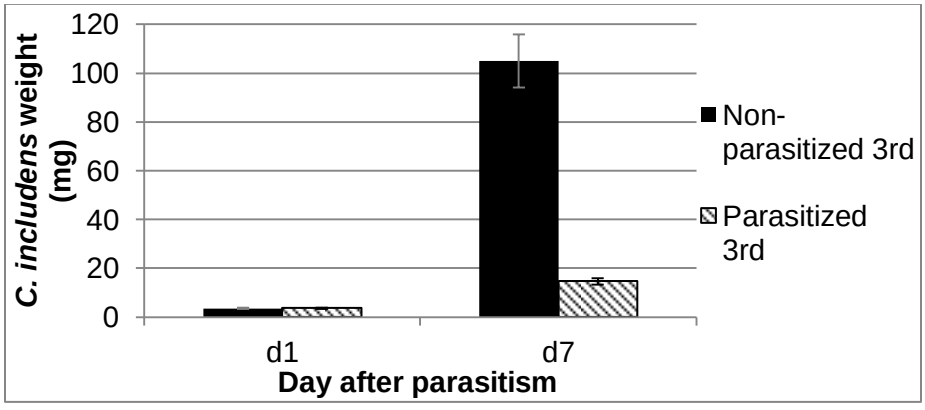

Fig. 1. Parasitized *C. includens* weigh less than non-parasitized larvae at pupation or wasp emergence (d7). I measured weight each day, but graph only shows larval weight at 24 hours after parasitism (d1) and the day of pupation or wasp emergence (d7).

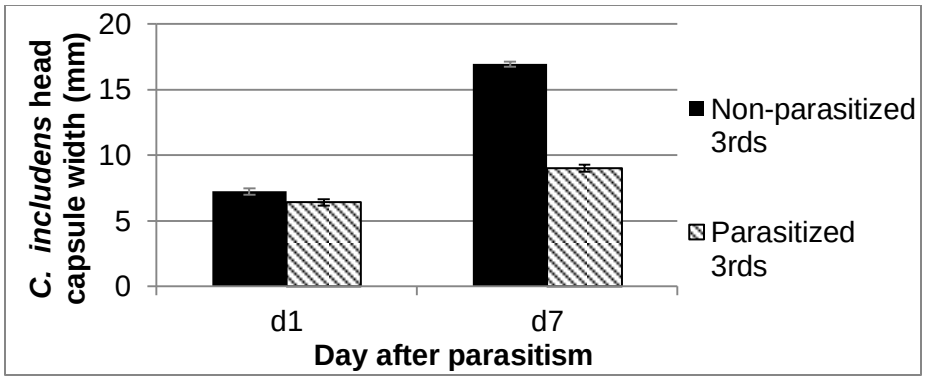

Fig. 2. Parasitized *C. includens* have smaller head capsules than non-parasitized larvae at pupation or wasp emergence (d7). I measured head capsules each day, but graph only shows larval head capsules at 24 hours after parasitism (d1) and the day of pupation or wasp emergence (d7).

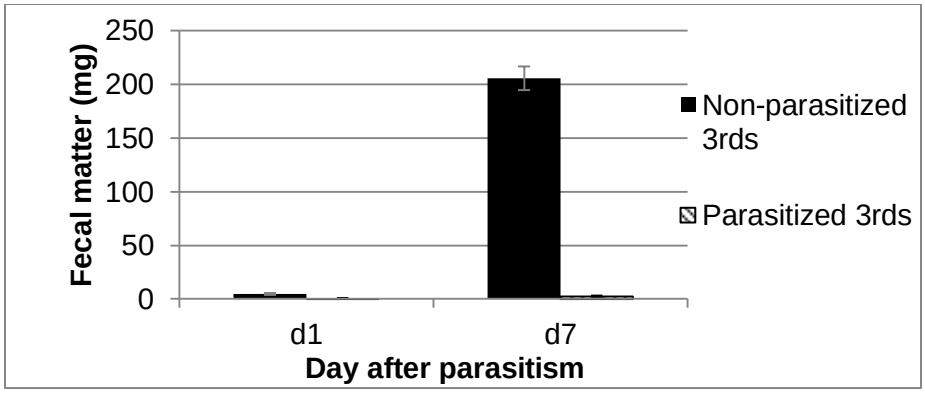

Fig. 3. Parasitized *C. includens* produce less frass than non-parasitized at pupation or wasp emergence (d7). I measured frass each day, but graph only shows larval frass 24 hours after parasitism (d1) and the day of pupation or wasp emergence (d7).

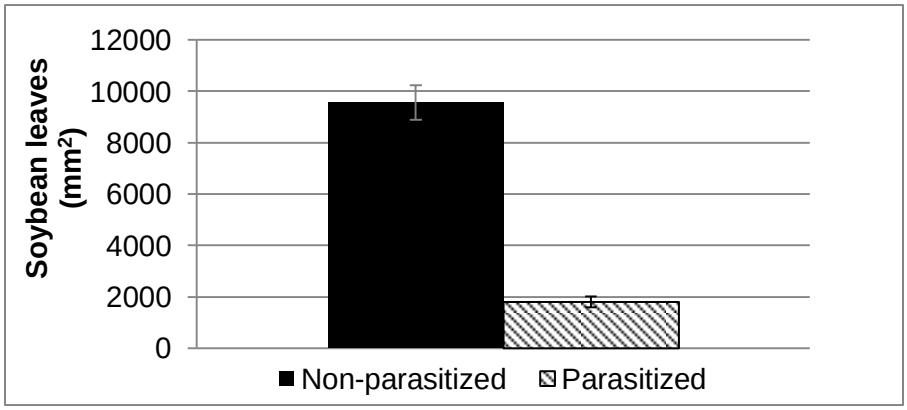

Fig. 4. Parasitized *C. includens* eat less relative to non-parasitized. Data in graph shows the average surface area of soybean leaves eaten by *C.includens* from third instar until larvae pupate or wasps emerge.

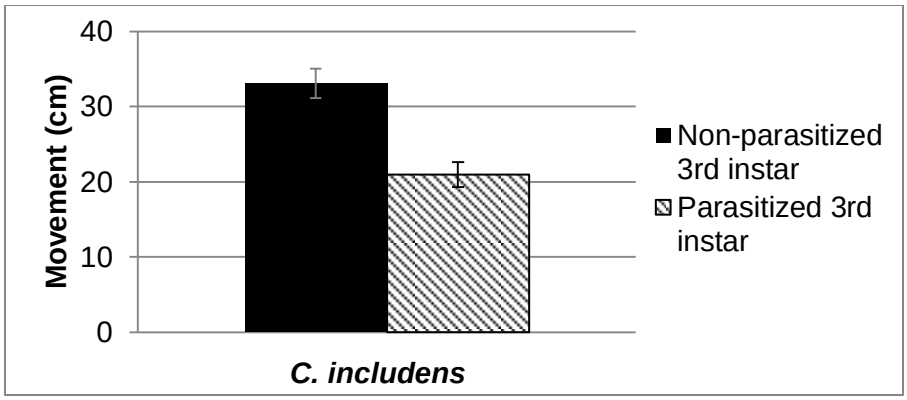

Fig. 5. Parasitized *C. includens* exhibit less locomotor activity relative to non-parasitized larvae. I assessed larval movement by measuring the distance from where the larva was located at the previous observation time. Multiple time points each day were added to calculate distance moved from third instar until larvae pupate or wasps emerge.

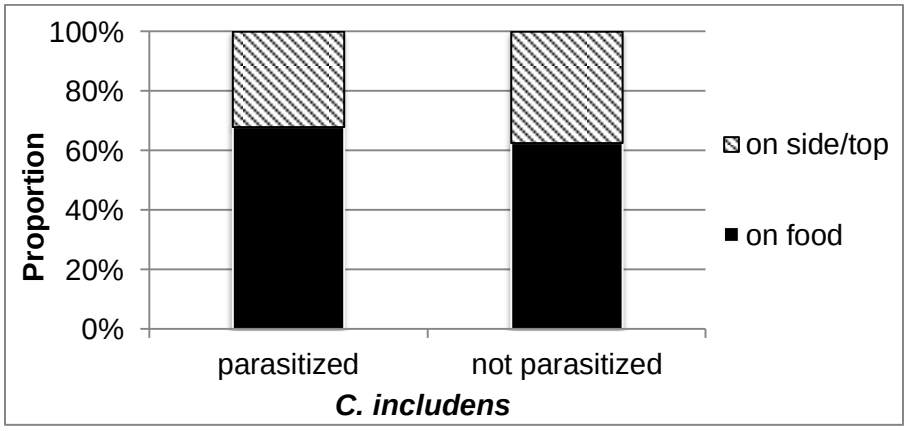

Fig.6. Non- and parasitized *C. includens* spend equal amounts of time on and away from food.

Chapter III

SUSCEPTABILITY AND RISK OF INFECTION OF *C.INCLUDENS* TO BT AND ACMNPV WHEN PARASTIZED BY *M. DEMOLITOR*

Introduction

In this chapter I examined whether the reduction of food consumption by *C. includens* after parasitism by *M. demolitor* reduced mortality risks from the per os transmitted pathogens *Bacillus thuringiensis* (Bt) and *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV). Bt produces toxins that bind to receptors on midgut cells, which damages the gut lining and leads to infection of the hemocoel (Hoffmann 1993). AcMNPV in contrast does not initially destroy the gut lining, but infects and replicates in midgut cells. Progeny or occluded virus (OB) thereafter disseminate through the hemocoel to infect other tissues, which eventually lead to lysis of host cells and death. (Rohrmann, 2009). Here I first compared the susceptibility of non-parasitized and parasitized *C. includens* when Bt or AcMNPV were fed directly to larvae. I then assessed the risk of infection after parasitism by exposing non-parasitized and parasitized larvae to each pathogen on a food source. Given my results showing that parasitism by *M. demolitor* suppresses feeding by *C. includens*, I predicted the median mortality dose for parasitized *C. includens* fed diet with a pathogen would be significantly higher than for non-parasitized *C. includens* (LD50). The LD50 or median lethal dose is commonly compared in dose –mortality assays (Merriam-Webster, 2013). Suppressed host feeding behavior would therefore protect the host and parasitoid from pathogens in the environment. Lastly, I analyzed

the mechanism behind altered risk of infection by injecting MdBV into *C. includens* and comparing mortality of injected larvae to hosts parasitized by *M. demolitor*.

Methods

Insect Rearing

I reared *C. includens* and *M. demolitor* as described in Chapter 2.

Bt and AcMNPV

I purchased a commercial formulation of Bt (Thuricide) which contained $3x10^7$ spores per µl. I kept the stock solution for experiments in a cold room covered in foil. A stock solution of occluded AcMNPV was prepared by infecting *C. includens* third instars. Occluded virus was then isolated from dead fifth instars homogenized in distilled water as outline by Lacey (1997). Stock solutions containing 1.0×10^7 occlusion bodies per µl were prepared and stored at -80◦C.

MdBV isolation

To isolate the virus, I collected MdBV from wasps by established methods (Strand et al., 1992). In the literature, the amount of MdBV collected from a single wasp equals one wasp equivalent and previous studies have shown wasps naturally infect *C. includens* with 0.1-0.01 wasp equivalent of MdBV when they oviposit (Strand et al. 1992). I injected third instar *C. includens* with 0.1 wasp equivalence of MdBV. To infect hosts, MdBV was isolated from 10 pairs of wasp ovaries that were suspended in 200 µl of PBS. Injection of PBS into larvae served as a negative control.

C. includens force fed AcMNPV and Bt assays

A standard dose-response assay was conducted to measure the susceptibility of non- and parasitized third, fourth , and fifth instar *C. includens* to AcMNPV and Bt. In brief, non-

parasitized *C. includens* were starved for 12 h and then infected by feeding a given quantity of AcMNPV or Bt in a 1µl drop from a P2 pipette (Rainin) under a stereomicroscope (Olympus). Parasitized *C. includens* were starved for 24 h and fed the same range of doses of AcMNPV or Bt. Once fed I placed the larvae individually in rearing cups and monitored then until they pupated, a wasp emerged or death.

Infectivity assays using each pathogen on artificial diet

To assess the risk of infection from natural feeding activity, I applied different concentrations of Bt or AcMNPV in water to the surface of diet at doses that ranged from 0-10^6 pathogen particles (spores or occlusion bodies) per mm^2 of diet. I predicted an LD50 within the dose range using previously recorded *C. includens* frass and body weight data along with measurements of the food arena. I placed non-parasitized and 24 h post-parasitized larvae in rearing cups. I allowed some cohorts of larvae were allowed to feed on diet with each pathogen for 24 h before transferring to diet cups without pathogens while other cohorts of larvae to feed on diet with the pathogen until pupation, wasp emergence, or death. I used 10 arenas/ concentration/ trial. I used thirty per non-parasitized or parasitized *C. includens* per instar for each dose I tested.

Mechanism behind altered phenotype and risk of infection

To highlight the mechanism behind *C. includens* suppressed feeding and lower risk of infection to AcMNPV I isolated MdBV to assess host mortality responses. I left ten *C. includens* in each trial untreated, injected with PBS, or injected with 0.1 wasp equivalent of MdBV. I then placed the different treatments on diet with 100 OB/mm^2 of AcMNPV since the AcMNPV spread diet trials established the dose produced high mortality for non-parasitized *C. includens*

and low mortality for parasitized larvae. I monitored *C. includens* until pupation, wasp emergence, or death.

Statistical Analysis

I used a logistic regression analysis to assess the susceptibility of non-parasitized and parasitized *C. includens* in my standardized infectivity assays. The logistic model was confirmed using the Goodness- of- Fit test (SAS Cary, NC). For feeding assays on artificial diet, I first compared non-parasitized vs parasitized *C. includens* when exposed to pathogen for 24 h along when exposed to the pathogens until wasp emergence or pupation. If both analyses followed the same trend, I pooled the data and compared non-parasitized vs. parasitized *C. includens*. Lastly, I used a Fisher's Exact test to compare treatments in the mechanism behind altered risk of infection assay.

Results and Discussion

C. includens susceptibility to Bt and AcMNPV

Results of my standardized infectivity assays indicated the LD50 for non-parasitized *C. includens* third instars while the LD50 for parasitized *C. includens* was 8 $x10^3$ spores (Fig.1). The LD50 for AcMNPV was 50 OB for non-parasitized and parasitized third instars (Fig.1). Non-parasitized *C. includens* exhibited high mortality when fed 1 x 10^6 Bt spores and exhibited low mortality when fed 1x 10^4 Bt spores or fewer (Fig.1). A logistic regression analysis revealed that parasitized *C. includens* were more susceptible to Bt than non-parasitized larvae (X^2=5.6846, P=0.0171) (Fig 1). Direct feeding assays with AcMNPV showed high mortality when I fed non-parasitized and parasitized larvae at least 10^2 occlusion bodies (OB) and low

mortality when fed 10 OB or less. A logistic regression analysis revealed that non-parasitized and parasitized *C. includens* are equally susceptible to AcMNPV (X^2=0.4704, P=0.4928) (Fig.2).

To assess whether susceptibility to AcMNPV changed with instar, I fed fourth and fifth instars a range of OB concentrations. Non-parasitized and parasitized fourth instar *C. includens* exhibited similar trends in susceptibility to AcMNPV as third instars (Both non- and parasitized LD50 of 3500 OB) (X^2=1.4070, P=0.2356) (Fig. 3). In contrast, fifth instars had a different mortality response such that parasitized are more susceptible to AcMNPV infection than non-parasitized larvae (Non-parasitized LD50 28,000 OB vs. 18,000 OB for parasitized) (X^2=4.6328, P=0.0314) (Fig. 4).

Overall, these data showed that AcMNPV had an LD50 of 50 OB/µl, 3500 OB/µl, and ~28,000 OB/µl in the third, fourth, and fifth instar hosts. These results provide further insight in the susceptibility of *C. includens* by body mass to AcMNPV along with effects of *M. demolitor* parasitism. Other studies have shown that the susceptibility of *C. includens* and other Lepidoptera to NPV decrease (LD50 increases) with instar (Livingston *et al.*, 1980). Viral replication has been shown to coincide with host developmental rates (Vail and Collier, 1982; Hoover et al., 1998). For example, *Spodoptera exigua* nucleopolyhedrovirus (SpeiNPV) that infected second instar *S. exigua* larvae reached a replication plateau a full day earlier post-infection than when infected as a fourth instar (Takatsuka and Kunimi, 2002).

Risk of secondary infection while on artificial diet

When parasitized third instars were exposed to Bt for only 24 hours they had a higher risk of infection than non-parasitized larvae (LD50 of 1800 spores/mm^2 parasitized vs. 5000 spores/mm^2 non-parasitized) (X^2=15.8785, P<.0001) (Fig 5). When they were exposed to Bt until the time of pupation or parasitoid emergence, non- parasitized, and parasitized larvae had

similar LD50s (non- and parasitized ~1800 spores/mm^2), but a logistic regression analysis revealed that parasitized larvae overall exhibited higher mortality rates than non-parasitized larvae when exposed to Bt at densities ranging from 0-2 x 10^4 spores/mm^2 (X^2=12.2694, P=0.0005). Regardless of exposure time to Bt, parasitized *C. includens* also exhibited higher mortality rates than non-parasitized *C. includens* (X^2 =24.661, P<.0001). In contrast, assays conducted with AcMNPV showed that third instar parasitized hosts exhibited much lower mortality rates than non-parasitized larvae (LD50 of non-parasitized ~25 OB/mm^2 vs. unknown for parasitized) (X^2=114.42, P<.0001) (Fig. 6). Even when I exposed parasitized third instar hosts to 500 OB/mm^2 there was no mortality from AcMNPV infection.

Results with fourth instar hosts yielded a different mortality response to the virus spread on diet than found for third instars (Fig 7). Parasitized fourth instar *C. includens* had a lower risk to infection than non-parasitized larvae when exposed to AcMNPV for only 24 h (LD50 non-parasitized=200 OB/mm^2 vs. parasitized=800 OB/mm^2) (X^2=114.42, P<.0001). However, parasitized *C. includens* had equal risk of infection as non-parasitized larvae when continuously exposed to AcMNPV until larvae pupated, a wasp emerged, or death (LD50 non-and parasitized= ~200 OB/mm^2) (X^2=0.8758, P=0.3493) (Fig.7). From this, I concluded that parasitized fourth instar *C. includens* have a lower risk of infection than non-parasitized when exposed to AcMNPV spread diet for just a 24 h period, but equal risk to infection when left on the pathogen spread diet until larvae pupated, a wasp emerged, or death.

Parasitized fifth instar *C. includens* exhibited a lower risk of infection compared to non-parasitized larvae in 24 h and until pupation or wasp emergence (Non-parasitized LD50 ~200 OB/mm^2 vs ~1500 OB/mm^2 for parasitized) (X^2=18.2968, P<.0001) (Fig 8). Based on this

experimental design I concluded that parasitized fifth instar *C. includens* are at a lower risk of infection to AcMNPV than non-parasitized when left on the pathogen spread diet.

Previous studies have shown that the thickness and hardness of the integument in Lepidoptera can be a defense against parasitoids (Gross, 1993). I expected successful parasitism of larger hosts to be more of a challenge since they possess a thicker cuticle than younger instars along with the ability to cause more injury to the wasps (Allen, 1990). I observed all host larvae that *M. demolitor* females oviposited into to ensure *C. includens* exhibited symptoms of parasitism. I also recorded death from AcMNPV when a larva grew larger and exhibited a cloudy appearance before liquefying. However, results with fifth instar larvae are inconclusive because I couldn't clearly determine the cause of death. Since it is difficult for *M. demolitor* to parasitize fifth instar *C. includens* it is possible that some of what I assumed was successful parasitism may not have been, thus, mortality data in parasitized larvae could be incorrect.

Mechanism behind altered risk of infection

To assess directly whether MdBV infection was responsible for reducing mortality risks by AcMNPV, I injected *C. includens* with MdBV and measured host mortality when larvae were placed on diet with different densities of AcMNPV present (Fig. 9). There was no significant difference in mortality between non- and PBS injected *C. includens* without AcMNPV along with MdBV-injected *C. includens* with and without AcMNPV (0% 30% 20% 20%) ($p=0.4569$ FET). In contrast, mortality was higher in PBS-injected *C. includens* compared to MdBV-injected larvae on AcMNPV spread diet (100% vs. 20%) ($p=3.572E-.04$ FET). These results strongly suggest that MdBV is primarily responsible for *C. includens* lower risk of infection.

Summary and final remarks

It was already known before my study *M. demolitor* alters the immune response, growth, and development of *C. includens* (Strand and Wong 1991 and Strand 2010). My results show that virus-mediated host alterations also have implications for wasp fitness by reducing the risk of parasitized hosts to infection by some pathogens. MdBV mediated alteration of feeding behavior may also in part compensate for suppression of the host immune system.

My data suggest that parasitized third and fourth instar *C. includens* are equally susceptible to AcMNPV. These results challenge what is known about *M. demolitor's* effect on the host immune system. Although non-parasitized and parasitized *C. includens* are just as susceptible to AcMNPV, third and fourth instar parasitized larvae show a lower risk of infection. The MdBV mediated alteration in host feeding did indeed lower the risk of infection to AcMNPV. Parasitized third instar *C. includens* also showed a lower risk of infection when left on AcMNPV diet until larvae pupated, a wasp emerged, or death, which supports the finding that third instar *C. includens* is the optimal host for *M. demolitor*. Harvey et al. (2004) found that *M. demolitor* progeny showed the lowest mortality when oviposited in a third instar *C. includens* relative to the other developmental stages. My data along with previous studies suggest that third instar *C. includens* show the lowest mortality in *M. demolitor* progeny based on physiological and nutritional compatibility along with a lower risk to infection by per os pathogens (Harvey et al., 2004). Lastly, my final assay directly shows that altered host feeding behavior and the associated reduction in risk of infection by AcMNPV is mediated by MdBV.

Future work could test whether the diet supplied to *C. includens* affects the risk to infection by AcMNPV. Studies such as Dannon et al (2012) show how hosts on different substrates (artificial diet or on native host plant) altered life table parameters of the parasitoid,

such as reproduction and wasp emergence rate. Since my previous study showed that *M. demolitor* suppressed soybean foliage consumption in *C. includens*, I predict field studies would further correlate lab results. Other future work could test the degree that MdBV reduces *C. includens* risk to infection by utilizing different pathogens. I predict a less virulent pathogen would further provide more evidence that MdBV decreases the risk of host infection since parasitized *C. includens* would consume much less of the pathogen than non-parasitized larvae. An example is the bacterium *Serratia marcescens,* which invades the entire alimentary canal and hemolymph subsequently replacing all other gut-associated microflora (Mohan et al. 2001). A dose of 6 x 10(10) c.f.u./ml of the bacteria *S. marcescens* on diet induced 66.3% mortality of first instar *Helicoverpa armigera* larvae (Noctuidae:Lepidoptera) (Mohan et al. 2001). Other work could involve more exploration into the mechanism behind altered risk of infection. When assessing the mechanism behind altered risk of infection wasp venom and teratocytes could also be isolated and injected in combinations (Strand and Wong, 1991; Strand and Noda, 1991). This would assess any other factors that could contribute to this altered behavior and their function in the wasp calyx fluid.

Graphs

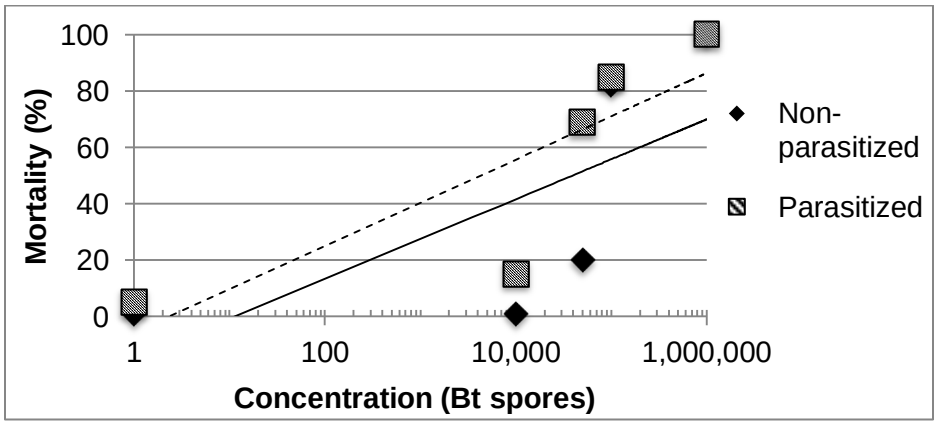

Fig.1. Parasitized third instar *C. includens* are more susceptible to Bt than non-parasitized larvae. I fed different concentrations of Bt to third instar *C. includens*. Last data points overlap.

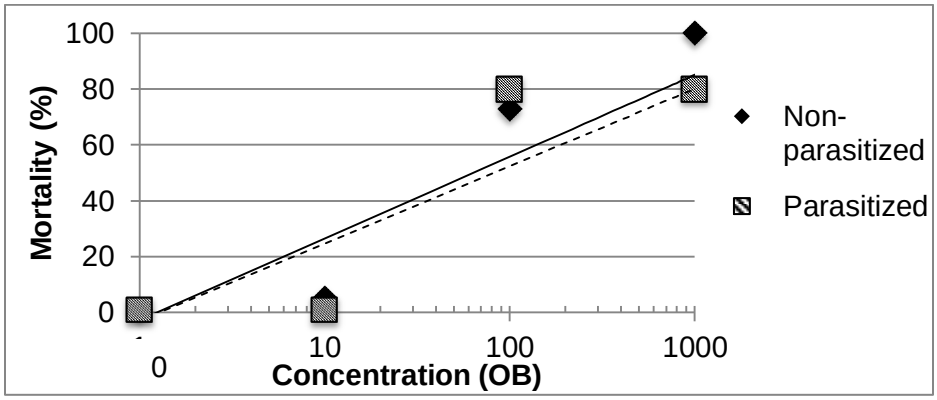

Fig.2. Parasitized third instar *C. includens* are equally susceptible to AcMNPV as non-parasitized larvae. I fed different concentrations of AcMNPV to third instar *C. includens.*

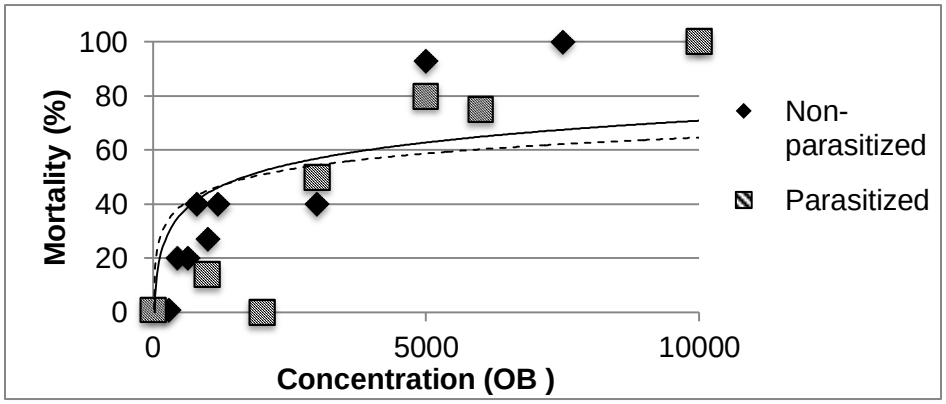

Fig.3. Parasitized fourth instar *C. includens* are equally susceptible to AcMNPV as non-parasitized larvae. I fed different concentrations of AcMNPV to fourth instar *C. includens.*

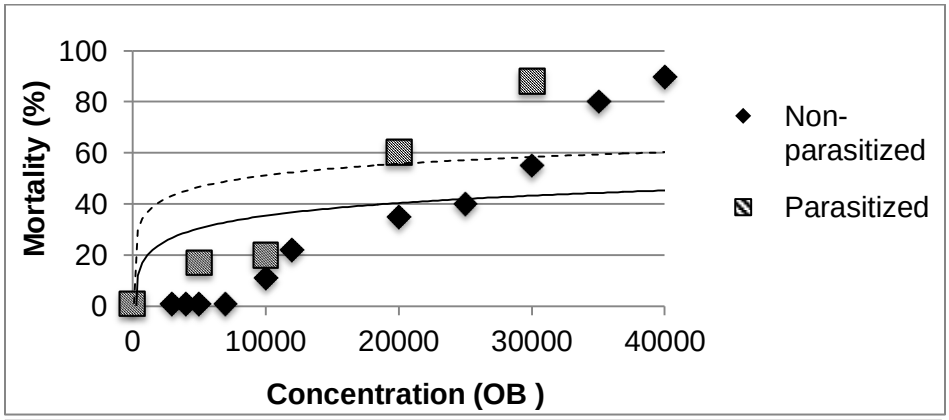

Fig.4. Parasitized fifth instar *C. includens* are more susceptible to AcMNPV relative to larvae not parasitized. I fed different concentrations of AcMNPV to fifth instar *C. includens.*

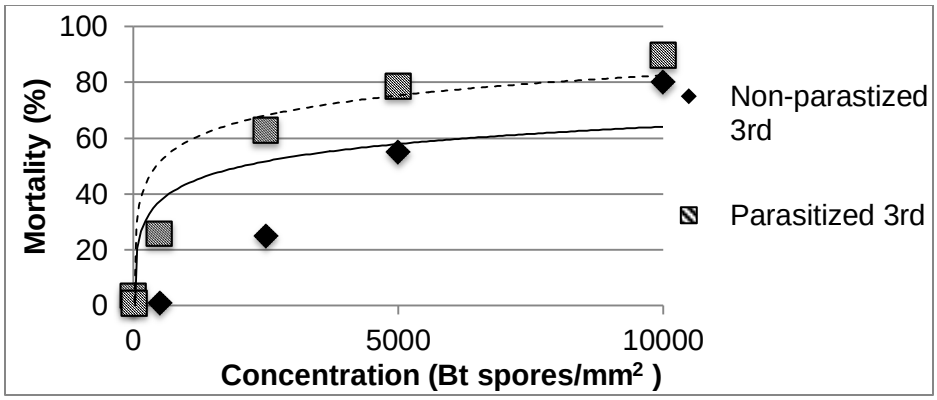

Fig.5. Third instar parasitized *C. includens* are more at risk to Bt than larvae not parasitized. I applied different concentrations of Bt to *C. includens* artificial diet and measured mortality.

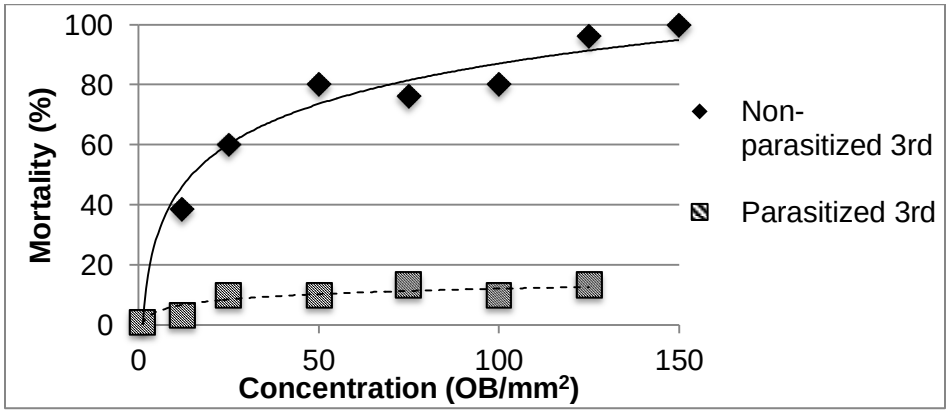

Fig.6. Parasitized third instar *C. includens* have a much lower risk of infection than larvae not parasitized. I applied different concentrations of AcMNPV to *C. includens* artificial diet and measured mortality.

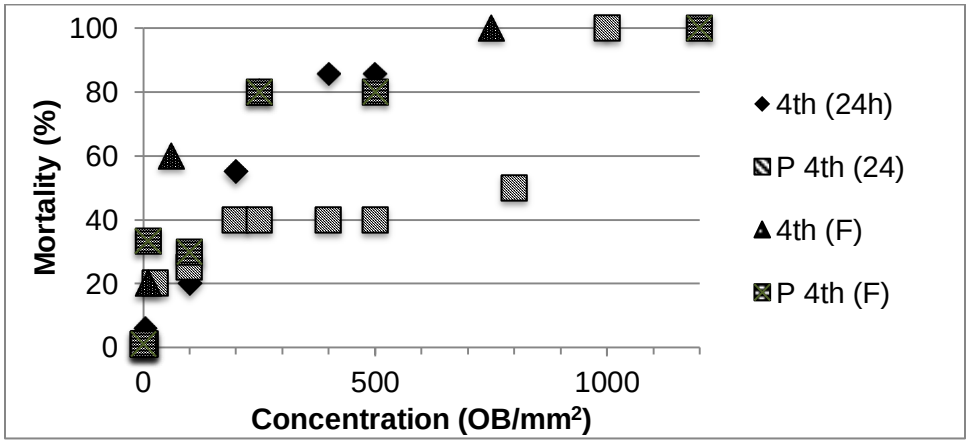

Fig.7. Parasitized fourth instar *C. includens* have lower risk of infection when exposed to AcMNPV for only 24 h. In contrast, parasitized fourth instar *C. includens* have equal risk to infection when exposed to AcMNPV spread diet until pupation or wasp emergence.

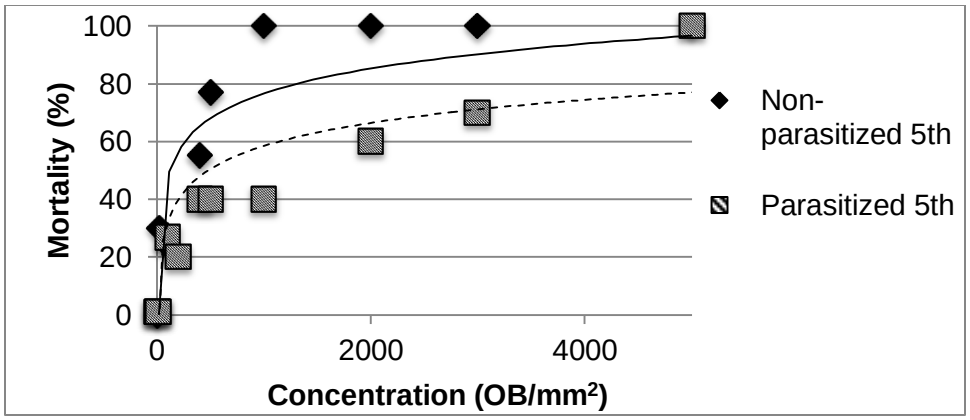

Fig.8. Parasitized fifth instar *C. includens* have a much lower risk of infection than larvae not parasitized. I applied different concentrations of AcMNPV to *C. includens* artificial diet and measured mortality.

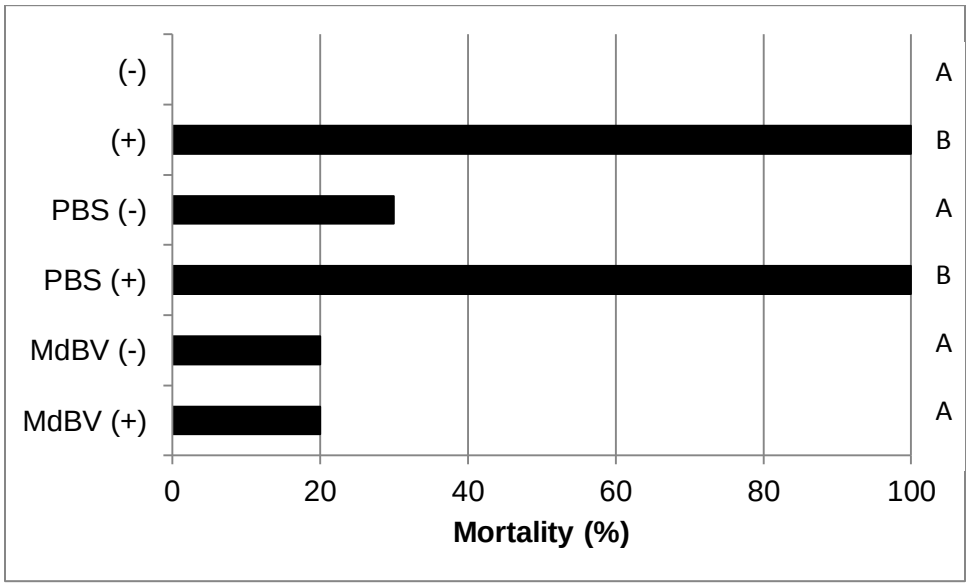

Fig. 9. *M. demolitor* bracovirus (MdBV) is responsible for suppressing host feeding behavior and reducing *C. includens* risk of infection to *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV). Figure shows non-injected, PBS injected, or MdBV injected third instar *C. includens* on water (-) or AcMNPV(+) spread diet. AcMNPV concentration on diet was 100 OB/mm^2. *C. includens* lived on the diet from third instar until pupation or wasp emergence. Similar letters represent statistical non-significance.

REFERENCES

Adamo, S. A. 2008. Bidirectional connections between the immune system and the nervous system on insects. In Insect Immunity (ed. N. E. Beckage), pp.129 -150. San Diego, CA: Academic Press.

Adamo, S. A., Fidler, T. L. and Forestell, C. A. 2007. Illness-induced anorexia and its possible function in the caterpillar, Manduca sexta. *Brain Behav. Immun*. 21,292 -300.

Ahmed, A. M., Baggott, S. L., Maingon, R. and Hurd, H. 2002. The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae.* Oikos 97, 371-377.

Allen, G.R.1990.Influence of host behavior and host size on the success of oviposition of *Cotesia urabae* and *Dolichogenidea eucalypti* (Hymenoptera: Braconidae). *J. Insect Behav.* 3, 733-749.

Andersen, S. K., Gjedsted, J., Christiansen, C. and Tonnesen, E. 2004. The roles of insulin and hyperglycemia in sepsis pathogenesis. J. Leukoc. Biol. 75, 413-421.

Armitage, S. A., Thompson, J. J., Rolff, J. and Siva-Jothy, M. T. 2003. Examining costs of induced and constitutive immune investment in *Tenebrio molitor*. *J. Evol. Biol*. 16, 1038-1044.

Audicana, M.T. and Kennedy, M.W.2008. "Anisakis Simplex: From Obscure Infectious Worm to Inducer of Immune Hypersensitivity". Clinical Microbiology Reviews 21 (2): 360–379

Barrett AJ. 2004. Bioinformatics of proteases in the MEROPS database. *Curr. Opin. Drug Discov*. Dev. 7:334–41.

Beck, M.H., Inman, R.B, and Strand, M.R. 2007. *Microplitis demolitor* bracovirus genome segments vary in abundance and are individually packaged in virions. Virology 359, 179-189.

Beck, M., Strand, M.R. 2005. Glc1.8 from *Microplitis demolitor* bracovirus induces a

loss of adhesion and phagocytosis in insect high five and S2 cells. *J. Virol.* 79:1861-1870

Beckage, N.E.2001. Insect endocrine and neuroendocrine host-parasite relationships: Common themes. Found in H.J. Goos, R.K. Tastogi, H. Vaudry, R. Pierantoni (Eds.), Perspective in Comparative Endocrinology: Unity and Diversity, Monduzzi Editore, Italy (2001), pp. 333–339

Beckage, NE.2002. Parasite- and pathogen-mediated disruption of host hormones and behavior. Found in D.A. Phaff, A. Arnold, A. Etgen, S. Fahrbach, R. Rubin (Eds.), Hormones, Brain, and Behavior, Vol. 3, Academic Press, San Diego (2002), pp. 281–315

Beckage, N.E. and Riddiford, L.M. 1993. Growth and development of the endoparasitic wasp *Apantelescongregatus*: Dependance on host nutritional status and parasite load. *Phys Ento.* 8(1983): 231-241.

Beckage, N.E., Foreman, R.C., Palmatier, C.M. Tan, F.F. 2002a. Inhibition of the larval ecdysis and emergence behavior of the parasitoid *Cotesia congregata* by methoprene. J Ins Phys. (48)7:725-732

Beckage, N.E., Surratt, V., Palmatier, C., Foreman, R., Marion, K.,Park,Y.,Tan, F.F. 2002b. Immunolocalization of ecdysis-triggering hormone in the epitracheal system and silk glands of the parasitoid, and the role of the hormone in wasp ecdysis/emergence.

Belle, E., Beckage, N.E. Rousselet, J., Poirie, M., lemenunier, F., and Drezen, J.M. 2002. Visualization of polydnavirus sequences in a parasitoid wasp chromosome. J. Virol. 76, 5793-5796.

Bitra, K., Zhang, S., and Strand, M.R. 2011.Transcriptomic profiling of *Microplitis demolitor* bracovirus reveals host, tissue and stage-specific patterns of activity.*J. Gen. Vir*. 92:2060-2071

Brodeur, J., Vet, L., 1994. Usurpation of host behavior by a parasitic wasp. *Animal. Behav.* (48)1:187-19

Burk, G.R.,Strand, M.R. 2012. Deep sequencing identifies viral and wasp genes with potential roles in replication of *Microplitis demolitor* Bracovirus. *J. Virol*. (86)6:3293-3306.

Cobb, C.H., Grant, J.F., Shepard, M. 1985. Effect of parasitism by *Microplitis demolitor* (Hymenoptera: Braconidae) on foliage consumption by *Heliothis zea* (Lepidoptera: Noctuidae) larvae. *Florida Ento.* (68)3:490-492.

Dantzer, R. 2004. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. *Eur. J. Pharmacol*. 500,399 -411

Devine, G.J., Wright,D.J., and Denholm, I. 2000. A parasitic wasp (*Eretmocerus mundus Mercet*) can exploit chemically induced delays in the development rates of its whitefly host (*Bemisia tabaci Genn.*). *Bio Control.* 19(1):64-75.

Damico, V., Elkinton, J.S. 1995. Rainfall effects on transmission of gypsy moth (Lepidoptera, lymantriidae) nuclear polyhedrosis-virus. *Environ. Entomol.* (24)5: 1144-1149.

Davies, D.H., Strand, M.R. Vinson, S.B. 1987. Changes in differential haemocyte count and in vitro behavior of plasmatocytes from host *Heliothis virescens* caused by *Campoletis sonorenesis* polydnavirus. *J. Insect Physiol*. 33:143-153.

Dionne et al.2006 Dionne, M. S., Pham, L. N., Shirasu-Hiza, M. and Schneider, D. S. 2006. Akt and foxo dysregulation contribute to infection-induced wasting in *Drosophila. Curr. Biol*.310, 1977-1985

Dupuy, C., Huguet, E., Drezen, J.M., 2006. Unfolding the evolutionary story of polydnaviruses. *Virus Res.* (117)1:81-89.

Duodu, Y.A and Antoh, F.F. 1984. Effects of parasitism by *Apanteles sagax* (Hym.:Braconidae) on growth, food consumption and food utilization in *Sylepta derogate* larvae (Lep.:Pyralidae). *Entomophaga*. 29, 23-71.

Edwards, J.P. and Weaver, R. 2001. Endocrine Interactions of Insect Parasites and Pathogens, Biosis Scientific, Oxford.

Eslin P, Prévost G. 2000. Racing against host's immunity defenses: a likely strategy for passive evasion of encapsulation in *Asobara tabida* parasitoids. *J Insect Physiol*. 46:1161–1167.

Falabella, P., Varricchio, P., Gigliotti, S., Tranfaglia, A., Pennachio, F. and Malva, C. 2003. *Toxoneuron nigriceps* polydnavirus encodes a putative aspartyl protease highly expressed in parasitized host larvae. *Insect Mol. Biol.* 12, 9-17.

Fouad, K., Libersat, F. and Rathmayer, W. 1994. The venom of the cockroach hunting wasp *Ampulex compressa* changes motor thresholds: a novel tool for studying the neural control of arousal. *Zoology* 98, 23-34.

Gelman, D.B., Reed, D.A. and Beckage, N.E. 1998. Manipulation of the fifth-instar host (*Manduca sexta*) ecdysteroid levels by the parasitoid wasp *Cotesia congregata. J Ins Phys.* 44(1998):833-843.

Goodman, B.A. and Johnson, T.J. 2011. Disease and the extended phenotype: parasites control host performance and survival through induced changes in body plan. *Plos One.* 6(5):e20193.

Graham, A.L., Allen, J.E., Read, J.F. Evolutionary causes and consequences of immunopathology. *Annu Rev Ecolo S*. 36: 373-397

Green, G.L., Leppla, N.C., Dickerson, W.A. 1976. Velvet bean caterpillar: a rearing procedure and artificial medium. *J. Econ. Entomol*. 69:487-488.

Gross.1993. Insect behavioral and morphological defenses against parasitioids. *Annu. Rev. Entomol.* 38, 251-273.

Harvey, J.A., Bezemer, T.M., Elzinga, J.A., Strand, M.R. 2004. Development of the solitary endoparasitoid *Microplitis demolitor*: host quality does not increase with host age and size. *Ecol Entomol.* 29(1):35-43.

Harvey J.A., Kos M., Nakamatsu Y., Tanaka T., Dicke M., Vet L.E.M., Brodeur J., Bezemer T.M. 2008b. Do parasitized caterpillars protect their parasitoids rom hyperparasitoids? A test of the 'usurpation hypothesis'. *Anim Behav*. 76:701–708.

Haspel, G., Rosenberg, L. A. and Libersat, F. 2003. Direct injection of venom by a predatory wasp into cockroach brain. *J. Neurobiol*. 56, 287-292.

Hoover, k., Alaniz, S.A. and Yee,J.L.1998. Dietary protein and chlorogenic acid effect on baculoviral disease of noctuid (Lepidoptera : Noctuidae) larvae. *Environ Entomol.*(27)5:1264-1272.

Hoffmann, M.P., Frodsham, A.C. 1993. Natural enemies of vegetable insect pests. Cooperative Extension, Cornell University, Ithaca, NY. 63pp.

Hotamisligil, G. S. 2006. Inflammation and metabolic disorders. *Nature.* 444,860 -867.

Janssen, A., Grosman, A.H., Cordeiro, E.G., de Brito, E.F., Fonseca, J.O., Colares, F. Pallini, A., Lima, E.R., Sabelis, M.W. 2010. Context-dependent fitness effects of behavioral manipulation by a parasitoid. *Behav. Ecol.* (21)1:33-36.

Kagialis-Girard S, Mialou V, French M, Dupuis-Girod S, Pages MP, Bertrand Y. 2005. Thrombocytosis and toxocariasis: report of two pediatric cases. *Pediatr. Blood Cancer.* 44:190–92.

Kao, RH.2008. Implications of polyploidy in the host plant of a dipteran seed parasite. *West N Am Naturalist.* (68)2:225-230.

Kelly, T.J., Gelman, D.B.,Reed, D.A., and Beckage, N.E. 1998. Effects of parasitization by *Cotesia congregate* on the brain-prothoracic gland axis of its host, *Manduca sexta*. *J. Insect Physiol*. 44, 323-332.

Klowden M.J. 2007. Physiological Systems in Insects. Ed 2. pp 102-121. Burlington, MA. San Diego, California. London WC1X 8RR, UK: Elsevier Inc.

Kraaijeveld A.R. and van Alphen J.J.M. 1994. Geographical variation in resistance of the parasitoid *Asobara tabida* against encapsulation by *Drosophila melanogaster* larvae: the mechanism explored. *Physiol Entomol*. 19:9–14.

Lanot, R, Zachary D, Holder F, Meister M.2001. Postembryonic hematopoiesis in *Drosophila*. *Dev Biol*. 230:243–257.

Lavine, M.D. and Strand M.R. Insect hemocytes and their role in immunity. *Insect Biochem Mol Biol.* 2002; 32:1295–1309.

Lawrence and Lanzrein, P.O. 1993. Hormonal interactions between insect endoparasites and their host insects. Found in N.E. Beckage, S.N. Thompson, B.A. Federici (Eds.).1993. Parasites and Pathogens of Insects. vol. 1 Parasites, Academic Press, San Diego. pp. 59–86

Liberstat, F. and Gal, R. 2013. What can parasitoid wasps teach us about decision making in insects? *J Exp Bio.* 216: 47-55

Livingston, J.M., Mcleod, P.J., Yearian, W.C., Young, S.Y.1980. Laboratory and field-evaluation of a nuclear polyhedrosis-virus of the soybean looper, *Pseudoplusia-includens. J Georgia Entomolo So.* (15)2:194-199.

Lu, z., Beck, M.H., Wang, Y., Jiang, H., Strand, M.R. 2008. The viral protein Egf 1.0 is dual activity inhibitor of prophenoloxidae-activating proteinases 1 and 3 from *Manduca sexta*. J. Bio. Chem. (283)31: 21325-21333.

LyMN, SL., Usmani AS., Lambert DR. 2003. Cutaneous *Strongyloides stercoralis* infection: an unusual presentation. J. Am. Acad. Dermatol. 49:S157–60.

McKerrow, JH.2005. Cysteine proteases are required for lifecycle and virulence in a diverse group of parasites. FASEB J. (19)5:A1692-A1692.

Merriam-Webster. 2013.<Merriam-Webster.com>. Accessed 2013 Jul 20.

Mohan, M., Selevakumar, G., Sushil, S.N., Bhatt, J.C., and Gupta, H.S. 2011. Entomopathogenicity of endophytic *Serratia marcescens* strain SRM against larvae of *Helicoverpa armigera* (Noctuidae: Lepidoptera). *World J Microb Biot.* 27(11):2545-2551.

Monconduit, H. and Prevost, G. 1994. Avoidance of encapsulation by *Asobara tabida*, a larval parasitoid of Drosophila species. Norw J Agric Sci Suppl. 16:301–310.

Moore, J .2002. Parasites and the behaviour of animals. Oxford: Oxford University Press.

Moore, E. L., Haspel, G., Libersat, F. and Adams, M. E. 2006. Parasitoid wasp sting: a cocktail of GABA, taurine, and beta-alanine opens chloride channels for central synaptic block and transient paralysis of a cockroach host. *J. Neurobiol*. 66, 811-820.

Moret, Y. and Schmid-Hempel, P. 2000. Survival and immunity: the price of immune system activation for bumblebee workers. *Science* 290, 1166-1168.

Nakamura-Uchiyama F, Yamasaki E, Nawa Y. 2002. One confirmed and six suspected cases of cutaneous larva migrans caused by overseas infection with dog hookworm larvae. J. Dermatol. 29:104–11.

Nijhout, H. F. 1975. A threshold size for metamorphosis in the tobacco hornworm, *Manduca sexta* (L.). *Biol. Bull.* 149.

Norton, W.N. Vinson, S.B., Stoltz, D.B: 1975. Nuclear secretory particles associated with the calyx cells of the ichneumonid parasitoid *Campoletis sonorensis*. *Cell Tissue Res*. 162, 195.

Ohnuma, Y. and Kainoh, Y. 1992. Effect of parasitism by *Ascogaster-reticulatus* (Hym, Braconidae) on growth of the host, Adoxophyes sp (Lep, Tortricidae). *Entomophaga.* (37)2:327-332.

Pennacchio, F. and Strand, M. R. 2006. Evolution of developmental strategies in parasitic Hymenoptera. *Annu. Rev. Entomol*. 51, 233-258.

Pennacchio, F., Falabella, P. and Vinson, S.B. 1998. Regulation of *Heliothis virescens* prothoracic glands by *Cardiochiles nigriceps* polydnavirus (CnPDV). *Arch. Insec. Biochem. Physiol.* 38, 1-10.

Pennacchio, F., Malva, C., Vinson, S.B. 2001. Regulation of host endocrine system by the endophagous braconid *Cardiochiles nigriceps* and its polydnavirus. In Endocrine Interaction of Insect Parasites and Pathogens (ed. J.P Edwards and R.J. Weaver) pp. 123-132. Oxford: BIOS.

Poulin, R. 1994. Meta-analysis of parasite-induced behavioural changes. Animal Behav. (48)1:137-146.

Poulin, R. 2005. "Adaptive" change in the behavior of parasitized animals: a critical review. *Int J Parasitol.* 25:1371-1383.

Poulin R . 2007. The evolutionary ecology of parasites. 2nd ed. Princeton: Princeton University Press.

Poulin R.2010. Parasite manipulation and host behavior: an update and frequently asked questions. Adv Study Behav 41: 150–186.

Powel, J.E.1989. Food consumption by tobacco budworm (Lepidoptera:Noctuidae)larvae reduced after parasitization by *Microplitis demolitor* or *M. croceipes* (Hymenoptera:Braconidae). *J. Econ. Entomol*. 82, 408-411.

Pruijssers, A.J., Falabella, P., Eum, J.H., Pennacchio, F., Brown, M.R., Strand, M.R. 2009. Infection by a symbiotic polydnavirus induces wasting and inhibits metamorphosis of the moth *Pseudoplusia includens. Exp. Biol.* 212:2998-3006

Poulin, R., Brodeur, J., and Moore, J. 1994. Parasite Manipulation of Host Behaviour: Should Hosts Always Lose? Oikos. (70)3:479-484

Rohrmann, G. 2008. The baculovirus replication cycle: Effects on cells and insects. In Baculovirus Molecular Biology. Bethesda (MD): National Center for Biotechnology Information (US).

Sakanari JA. 1990. Anisakis—from the platter to the microfuge. Parasitol. Today 6:323–27

Savary, S., Beckage, N., Tan, F., Periquet, G., and Drezen, J-M. 1997. Excision of the polydnavirus chromosomal integrated EP1 sequence of the parasitoid wasp *Cotesia congregata* (Braconidae, Microgastrinae) at potential recombinase binding sites. J. Ge. Virol. 78, 3125-3134.

Shin MH, Lee SY. 2000. Proteolytic activity of cysteine protease in excretory-secretory product of *Paragonimus westermani* newly excysted metacercariae pivotally regulate IL-8 production of human eosinophils. *Parasite Immunol*. 22:529–33.

Siva-Jothy, M.T., Moret, Y., Rolff, J., 2005. Insect immunity: an evolutionary ecology perspective. Adv. Insect Physiol. 32, 1–48.

Slansky, F. Jr. 1986. Nutritional ecology of endoparasitic insects and their hosts: An overview. *J. Insect Physiol*. 32, 255-261.

Stoltz, D. B., D. Guzo, and D. Cook. 1986. Studies of polydnavirus transmission. Virology 155:120–131.

Strand, M.R. 1990. Characterization of larval development in *Pseudoplusia includens* (Lepidoptera: Noctuidae). *Ann Entomol Soc America.* (83)3:538-544.

Strand, M.R. 1994. *Microplitis demolitor* polydnavirus infects and expresses in specific morphotypes of *Pseudoplusia includens* haemocytes. *J Gen Virol.* 75(11):3007-3020.

Strand, M.R. 2009. The interactions between polydnavirus-carrying parasitoids and their lepidopteran hosts. In Molecular Biology and Genetics of Lepidoptera, M.R. Goldsmith, and F. Marec, eds. (Boca Raton, USA: CRC Press), pp. 321-336.

Strand, M.R. 2010. Polydnaviruses. In Insect Virology, S. Asgarl, and K. Johnson, eds. (Norfolk, UK:Caister Academic Press), pp. 171-198.

Strand, M.R., Beck, M.H., Lavine, M.D., Clark, K.D. 2006. *Microplitis demolitor* bracovirus inhibits phagocytosis to hemocytes from *Pseudoplusia includens. Arch. Insect Biochem.* 61:134-145

Strand, M.R. and Dover, B.A. 1991. Developmental disruption of *Pseudoplusia includens* and *Heliothis virescens* larvae by the calyx fluid and venom of *Microplitis demolitor*. *Arch. Insect. Biochem. Physiol*. 18:131-145.

Strand M.R., Johnson J.A., Culin, J.D. 1988. Developmental interactions between the parasitoid *Microplitis demolitor* (Hymenoptera: Braconidae) and its host *Heliothis virescens* (Lepidoptera: Noctuidae*). Ann. Entomol. Soc.* Am. 81:822– 830.

Strand M.R., Johnson, J.A., Culin, J.D. 1990. Extrinsic interspecific competition between the polyembryonic parasitoid *Copidosoma floridanum* and solitary endoparasitoid *Microplitis demolitor* in *Pseudoplusia includens*. Entomol Exp Appl. 55, 275.

Strand, M.R. and Noda, T. 1991. Alterations in the haemocytes of *Pseudoplusia includens* after parasitism by *Microplitis demolitor*. *J. Insect Physiol*. 37:839–850.

Strand, M.R. and Pech, L.L. 2005. Immunological basis for compatibility in parasitoid-host relationships. Annu Rev Entomol. 40:31–56.

Strand, M.R. and Wong, E.A.1991. The growth and role of *Microplitis demolitor* teratocytes in parasitism *Pseudoplusia includes. J. Insect. Physiol.* (37)7:503-515.

Takatsuka, J. and Kunimi, V. 2002. Lethal effects of *Spodoptera exigua* nucleopolyhedrovirus isolated in Shiga Prefecture, Japan, on larvae of the beet armyworm, *Spodoptera exigua* (Lepidoptera : Noctuidae). *Appl Entomol Zool.* (37)1:93-101.

Tanaka, T. and Vinson, S.B. 1987. Depression of prothoracicotropic gland activity of *Heliothis virescens* by venom and calyx fluids from the parasitoid *Cardiochiles nigrceps*. J. Insect Physiol. 37, 137-144.

Tanaka, T., Aguai, N. and Hiruma, K. 1987. The parasitoid *Apanteles kariyai* inhibits pupation of its host, *Pseudaletia separate*, via disruption of prothoracicotropic hormone release. Gen. Comp. Endocrinol. 67, 364-374.

Tanaka, S. and Ohsaki, N. 2006. Behavioral manipulation of host caterpillars by the primary parasitoid was *Cotesia glomerata* (L.) to construct defensive webs against hyperparasitism. *Ecol. Res.* 21:570-577.

Tanaka, S. and Ohsaki, N. 2009. Does manipulation by the parasitoid wasp *Cotesia glomerata* (L.) cause attachment behavior of host caterpillars on cocoon clusters? *Ethology.*115:781-789.

Theilmann, D. A., and M. D. Summers. 1986. Molecular analysis of *Campoletis sonorensis* virus DNA in the lepidopteran host *Heliothis virescens*. J. Gen. Virol. 67:1961–1969.

Thomas, S.N. 1993. Redirection of host metabolism and effects on parasite nutrition. In Parasites and Pathogens of Insects vol. 1 (ed. N. E. Beckage, S.N. Thomas and B.A. Federici) pp. 125-144. New York: Academic Press.

Thomas, F., Adamo, S. and Moore, J. 2005. Parasitic manipulation: where are we and where should we go? *Behav Proc.* 68: 185–199.

Tzou, P.E., De Gregario, B. and Lamaitre. 2002. How *Drosophila* combats microbial infection: a model to study innate immunity and host: pathogen interactions. *Curr. Opin. Microbiol*. 5: 102-110.

Vail, P.V. and Collier, S.S. 1982. Comparative replication, mortality, and inclusion body production of the *Autographa californica* (Lepidoptera, Noctuidae) Nuclear Polyhedrosis virus in *Helitothis* sp. Ann Entomol Soc Am. (75)4:376-382.

Vinson, S.B., Pennacchio, F., Consoli, F.L. 2001. The parasitoid-host endocrine interaction from a nutritional perspective. In: Edwards JP, Weaver RJ, editors. *Endocrine Interactions of Insect Parasites and Pathogens*. BIOS Scientific Publishers Ltd; pp. 187–206.

Webb, B.A., and Strand, M.R. 2005. The biology and genomics of polydnaviruses. In Comprehensive Molecular Insect Science, Vol. 6, Gilbert, L.I., Iatrou, K. and Gill, S.S.eds., (San Diego, USA: Elsevier), pp. 323-360.

Weisel-Eichler, A., Haspel, G. and Libersat, F. 1999. Venom of a parasitoid wasp induces prolonged grooming in the cockroach. *J. Exp. Biol*. 202, 957-964.

White S.M., Burden J.P, .Maini P.K., and Hails R.S. 2012. Modeling the within-host growth of viral infections in insects. *J Theor Bio*.312 (2012) 34–43.

Wyder, S., Tschannen, A., Hochuli, A., Gruber, A., Saladin, V., Zumbach, S., and Lanzrein, B. 2002. Characterization of *Chelonus inanitus* polydnavirus segments: sequences and analysis, excision site and demonstration of clustering. *J. Gen. Virol*. 83, 247-256.

Yamakami, K., Hamajima, F., Akao, S. and Tadakuma, T. 1995. Purification and characterizationof acid cysteine protease from metacercariae of the mammalian trematode parasite *Paragonimus westermani*. *Eur. J. Biochem.* 233:490–97.

Zitnan, D., Kingan, T.G., Kramer, S.J. and Beckage, N.E. 1995. Accumulation of neuropeptides in the cerebral neurosecretory system of *Manduca sexta* larvae parasitized by the braconid was *Cotesia congregate. J. Comp. Neurol*. 356, 83-100.