

The Effect of Aphids in Parasitoid-Caterpillar-Plant Interactions

Amanda Jean Lentz

Dissertation submitted to the faculty of
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGICAL SCIENCES

Lynn S. Adler, Co-Chair
Robert H. Jones, Co-Chair
Jeffrey R. Walters
John B. Phillips
Edwin E. Lewis

19 July 2007
Blacksburg, Virginia

Keywords: *Manduca sexta*, *Cotesia congregata*, *Nicotiana tabacum*, tri-trophic interactions, herbivores, parasitoid

The Effect of Aphids in Parasitoid-Caterpillar-Plant Interactions

Amanda Jean Lentz

Abstract

The ecology and evolution of a species is often considered only within the context of pairwise interactions even though a species' distribution and abundance may be determined by interactions with many species within and between trophic levels. Multiple herbivores often share the same host and may interact indirectly by altering the relationships between herbivores, their host plants and their parasitoids. However, the relationships between parasitoids and herbivore hosts have typically been studied in isolation of other herbivore species. I examined how the outcomes of species interactions change when multiple relationships are considered.

Chapter 1 examined the potentially conflicting selection pressures *Manduca sexta* exerts on *Nicotiana tabacum* (tobacco), since *M. sexta* has pollinating adults but herbivorous larvae. I demonstrated that high nectar amino acids do not affect floral visitation, but increased oviposition of herbivores on leaves. Thus, the relative costs and benefits of nectar rewards may depend on the community of pollinators and their life histories.

In the remaining chapters I examined how feeding on tobacco by the aphid *Myzus persicae* altered the interactions between a parasitoid (*Cotesia congregata*) and its hornworm host (*M. sexta*). Chapter 2 demonstrated that aphids reduced hornworm abundance and parasitism. Changes in hornworm abundance were not due to density-dependent changes in moth oviposition, but the proportion of caterpillars attacked by

parasitoids was inversely density dependent with hornworm density. Chapter 3 examined whether changes in hornworm abundance and parasitism reflected aphid-induced changes in host plant quality or volatile emissions. Aphids increased hornworm mortality, did not affect parasitoid performance, and increased parasitoid search time. In combination with Chapter 2, results suggest that aphids can mediate parasitoid-caterpillar interactions through changes in host plants that reduce hornworm survival and alter parasitoid behavior. Chapter 4 addressed how the outcome of interactions that are altered by aphids changed with spatial scale, and found no effect spatial scale on hornworm abundance and parasitism. In this system, aphids alter parasitoid-caterpillar interactions through changes in plant quality that reduce hornworm performance and abundance, and in turn, influence parasitoid attack. This work demonstrates that the outcome of multispecies interactions may not be predictable from pairwise interactions.

Granting Agencies

Field and laboratory research was funded by the National Science Foundation's Doctoral Dissertation Improvement Grant (G2005214201, 2004), The Theodore Roosevelt Memorial Fund (American Museum of Natural History, NYC, 2003), Virginia Tech's Graduate Research Development Program (2003 & 2004), and the Virginia Tech Department of Biological Sciences.

Acknowledgements

I am truly grateful for the support of my mentor and advisor Dr. Lynn Adler. I appreciate the countless hours she has spent in reviewing my manuscripts, discussing ideas, and her encouragement to speak out. Her wisdom and guidance have enriched my experiences at Virginia Tech and taught me a great deal. I would also like to thank the members of my committee, Drs. Jones, Lewis, Walters, and Phillips, for their insight and experience that has helped to guide my research and professional development.

Many thanks to Nile Bachman, David Sharp, Matt Johnson, Dacia Leon, Roo Vandegrift, Nikki Gianni, and Mark Prince, who worked hundreds of hours counting and weighing tobacco fruits, feeding small armies of hornworms, grinding tobacco leaves, and countless other tasks. I would also like to thank Debbie Wiley for all of her help in the greenhouse, Jon Wooge for his support out at Kentland Farm, and Paul Semtner for field space at the Virginia Tech Agricultural Research and Extension Center.

Thank you to the graduate students that have facilitated critical thinking and suggestions to improve upon this work, Glen Stevens, Matt Neatrou, Travis Belote, Matt Dittler, and Kerri Huffman. I would also like to thank Johanna Barron for her insights, enthusiasm, encouragement, and great friendship.

Lastly, I would like to thank my family and friends for their unconditional love and support. I especially want to thank my loving husband, Nathan Ronning, for the months he spent in the field with me digging holes, mowing waist deep weeds, driving the tractor, removing aphids, and enduring hours of intense heat to help me collect data. I could not have finished this work without his help.

Table of Contents

Abstract.....ii
Granting Agencies.....iv
Acknowledgements.....v
Table of contents.....vi
List of Figures.....vii

Chapter 1: Nectar amino acids attract antagonists.....1
 Abstract.....1
 Introduction.....2
 Methods.....4
 Results.....9
 Discussion.....9
 Literature Cited.....13
 Figures.....17

Chapter 2: The effects of aphids in parasitoid-herbivore interactions.....18
 Abstract.....18
 Introduction.....20
 Methods.....25
 Results.....33
 Discussion.....35
 Literature Cited.....40
 Figures.....47

Chapter 3: Aphids reduce preference and performance in parasitoid-caterpillar interactions.....52
 Abstract.....52
 Introduction.....53
 Methods.....55
 Results.....62
 Discussion.....64
 Literature Cited.....69
 Figures.....72

Chapter 4: Aphids do not affect caterpillar abundance and parasitism at three spatial scales.....74
 Abstract.....74
 Introduction.....76
 Methods.....78
 Results.....82
 Discussion.....83
 Literature Cited.....87
 Figures.....93

Conclusions.....95
 Literature Cited.....97

Curriculum Vitae.....98

List of Figures

Chapter 1: Nectar amino acids attract antagonists

Figure 1. Nectar amino acids increased moth oviposition on tobacco.....17

Chapter 2: The effects of aphids in parasitoid-herbivore interactions

Figure 1. Alternative explanations of why aphids reduce parasitism.....47

Figure 2. Aphid infestation reduced (a) hornworm abundance, (b) parasitism, (c) total eggs and (d) sex ratio (proportion of females produced).....48

Figure 3. Inverse density-dependence of parasitism of *Manduca sexta* by the parasitoid *Cotesia congregata*.....49

Figure 4. The interactive effect of aphid and density on *Manduca sexta* pupation50

Figure 5. Aphid infestation reduces female plant reproduction in *Nicotiana tabacum*.....51

Chapter 3: Aphids reduce preference and performance in parasitoid-caterpillar interactions

Figure 1. The increase in hornworm mortality and reduction in 5th instar hornworm weight due to feeding on diet mixed with leaf material previously attacked by aphids.....72

Figure 2. The parasitoid *Cotesia congregata* (Say) searched longer on leaf discs previously infested with the green peach aphid, *Myzus persicae*.....73

Chapter 4: Aphids do not affect caterpillar abundance and parasitism at three spatial scales

Figure 1. Spatial configuration of experimental units.....93

Figure 2. Arrangement of aphid treatments within each experimental unit.....94

Chapter 1: Nectar amino acids attract antagonists

Abstract

Species interactions can be context-dependent, ranging from beneficial to costly depending on the traits of interacting species. Floral traits are thought to evolve in response to pollinator selection. However, pollinators such as moths are also antagonists as herbivorous larvae, resulting in the potential for conflicting selection pressures.

Manduca sexta is a nectivorous moth that lays eggs on *Nicotiana tabacum*. We examined how nectar enhanced with arginine, an essential amino acid, affects oviposition, floral visitation, and plant reproduction. Arginine-enhanced nectar increased oviposition but did not affect floral visitation or plant reproduction. Nectar amino acids may therefore increase leaf herbivory without increasing pollination. Results suggest that traits whose evolution is considered in the context of pollinator attraction, such as nectar composition, may also incur fitness costs by attracting egg-laying moths. Thus, plant-pollinator and plant-herbivore interactions are not necessarily independent, and herbivores may comprise a novel agent of natural selection on nectar composition.

Introduction

The outcome of many species interactions can be context-dependent, and range from beneficial to costly for each species. For example, cleaner fish can benefit hosts by removing parasites but may also feed on host tissue (Poulin and Grutter 1996), legume-rhizobia interactions benefit the plant when soil nutrients are scarce but are costly when soil nutrients are abundant (Ohara 2001), and ant aggression toward lycaenid predators can relate directly to the quantity and quality of nutritive rewards offered by the lycaenid (Pierce et al. 2002). Thus, the end result of many interactions considered to be mutualisms depends on the biotic and abiotic environment, and also may be mediated by traits of the interacting species. Understanding how traits determine the outcome of interactions between species whose behavior ranges from mutualist to antagonist is fundamental to predicting how such traits mediate ecological interactions and ultimately evolve in a multispecies context.

The evolution of floral traits is generally thought to be due to selection by pollinators (Grant 1949; Straw 1956; Mitchell et al. 1998; Dodd et al. 1999). For example, pollinators can exert selective pressure on floral morphology, display size, color, symmetry, and scent (Johnson and Steiner 2000). Further, pollinators have been used to explain the adaptive value of floral nectaries with respect to position in the flower, nectary structure, and origin of carbohydrates (Pacini et al. 2003). Pollinator preference has also been hypothesized to explain nectar amino acid and protein composition (Baker and Baker 1977; 1983a), nectar volume and production (Real and Rathcke 1991), and the variability of nectar composition (Lanza et al. 1995).

However, a growing number of studies suggest that floral traits may be under conflicting selection pressure to attract pollinators but deter antagonists (e.g., Brody and Mitchell 1997; Adler and Bronstein 2004; Cariveau et al. 2004). For example, changes in floral shape that deter antagonists can exact costs in pollination (Galen and Cuba 2001). Increased floral display (Brody and Mitchell 1997) and nectar volume (Real and Rathcke 1991) attract both mutualists and antagonists, and defensive compounds in floral structures can deter both antagonists and pollinators (Strauss et al. 1999; Adler and Irwin 2005). In some cases, a single species of insect can act as both a mutualist and antagonist, such as nectar-feeding adults that oviposit herbivorous offspring on their food plant. In this case, the selective advantage of floral traits depends on how the trait affects adult insect behavior, including floral visitation and oviposition. Ultimately, the selective advantage of floral signals and rewards will depend on the relative benefits of increasing pollination compared to costs of increasing herbivory or other antagonistic interactions.

Most lepidopteran-pollinated flowers have amino acids in their nectar (Baker and Baker 1973; 1983). Nectar amino acids are thought to encourage butterfly visitation since many species are protein limited (Baker and Baker 1973; 1977) and several studies provide support for this hypothesis (Alm et al. 1990; Erhardt 1991; 1992; Erhardt and Rusterholz 1998; Romeis and Waecker 2000). The concentration of amino acids varies considerably among and within plant species (Lanza et al. 1995; Gardener and Gillman 2001; 2002) and can be altered by abiotic factors such as soil nutrient availability (Gardener and Gillman 2001). In cases where pollinating Lepidoptera also function as antagonists at other life-history stages, amino acids may have a negative effect on plant fitness if higher nectar quality results in more oviposition of herbivorous larvae.

We examined these conflicting selection pressures using *Nicotiana tabacum* L. and *Manduca sexta* L. Moths consume nectar from tobacco, but also lay eggs that hatch into herbivorous larvae. We examined the role of nectar amino acids on oviposition, floral visitation, and plant reproduction.

Methods

Study system

Nicotiana tabacum L. (Solanaceae) is a domesticated hybrid tobacco species that is grown extensively in Virginia and North Carolina, USA. Due to the close proximity of the male and female reproductive structures, this species frequently self fertilizes (Paul et al. 1995). However, the out-crossing rate was 11% in one study (Paul et al. 1995) and fruits weigh more in hand-pollinated greenhouse flowers compared to unmanipulated flowers (L.S. Adler, unpublished data), suggesting that pollinators increase plant female reproduction. Tobacco flowers produce copious amounts of nectar in the field (standing crop means \pm s.e.: 50.5 ± 0.97 μ l; $n = 238$; L.S. Adler, unpublished) and are visited by a diversity of bees, wasps, butterflies, and moths, although it is uncertain which species actually pollinate. Hawkmoths are efficient pollinators in other tobacco systems (Gregory 1964; Grant 1983; Nilsson et al. 1987; Wilmott and Burquez 1996; Raguso and Willis 2002) and of other local plant species (Madden and Chamberlain 1945).

Manduca sexta L. (Lepidoptera: Sphingidae) is a strong-flying hawkmoth that feeds on the nectar of *Nicotiana tabacum* and other flowering plants species at dusk and dawn. Females in the Sphingidae have an Ovigeny Index (an index that describes the egg laying state of newly emerged moths that is determined by the number of eggs females

have ready to lay divided by the lifetime potential fecundity) of about 0.5 and may require additional amino acids as adults to complete oogenesis (Jervis et al. 2005). Female moths feed on floral nectar and then oviposit eggs that hatch into herbivorous larvae that are specialists of plants in the Solanaceae, including tobacco. Larvae consume large amounts of the host plant and can decrease plant fitness of a native congener through the removal of photosynthetic material and the induction of costly plant defense compounds (Kessler and Baldwin 2004).

Experiment

Experiments were conducted August-September 2003 in Catawba, Virginia, USA in an outdoor arbor screen tent (Wendover, USA, 4.0 m x 3.1 m x 32 m (height)) with mesh walls and a steel frame. We determined how nectar amino acids affected moth oviposition, floral visitation, and plant female reproduction by manipulating nectar amino acid composition of 60 tobacco plants that were divided evenly into two blocks in time.

In April 2003 stock NC95 tobacco seed was germinated in vermiculite in a glasshouse and transplanted 2 weeks later into 6-cell flats containing Pro-Mix BX soil (Premier Horticulture Ltd., Red Hill, Pennsylvania, USA). Seedlings were transplanted into 3.8 L pots and fertilized three times using Peter's 20-20-20 (Scotts-Sierra Horticulture Products Co., Marysville, Ohio, USA, 14.235 g/3.8 L water). Flowering tobacco plants were removed from the glasshouse and maintained in the outdoor arbor screen tent.

Plants in each block were randomly assigned to one of two nectar treatments and secured with a wooden stake into a 5 x 6 array. Plants in the first block were manipulated

for their entire flowering period and then removed before plants in the second block were placed in the tent.

We manipulated nectar composition by adding artificial nectar with and without the supplemental amino acid arginine (L-Arginine, A-5006, Sigma-Aldrich, St. Louis, Missouri, USA) to create ‘high’ and ‘low’ amino acid treatments. We did not remove the existing nectar from flowers, so our treatments can be considered an augmentation or dilution of natural nectar amino acids. ‘Low’ amino acid treatments received 30 μ l/flower of a 25 % sugar water solution in a 2:1:1 ratio of sucrose: glucose: fructose.

Lepidopteron-pollinated species including other *Nicotiana* typically have this 2:1:1 ratio (Baker and Baker 1973; Kaczorowski et al. 2005). Our ‘high’ amino acid treatment used the same sugar solution but with a 250 picomole/L supplement of arginine. Arginine is an essential amino acid found in the nectar of 90% of butterfly-pollinated plants surveyed by Baker and Baker (1973). Arginine concentration was based on the average concentration found in nectar of the congener *Nicotiana glauca* (Kaczorowski et al. 2005).

Pupae of *M. sexta* (North Carolina State Insectary) were sexed and held separately in outdoor wire mesh cages until emergence. Individual moths were marked with a paint pen (Sharpie, Sanford Corporation, Bellerou, Florida, USA) on the middle of the dorsal side of the thorax to indicate sex. Moths were then placed in a screen tent and allowed 24 hr to mate prior to observations. A total of 20 adult moths (10 male and 10 female for each block) were released into the experimental arena. Dead hawkmoths were replaced to maintain a constant density until plants stopped flowering. Hawkmoths were observed each evening at dusk (~20:00) until resting (~21:30). Nectar was added to all open flowers no more than 30 min prior to evening observations. Each day, we counted and

removed all *M. sexta* eggs oviposited on each plant from the previous night. Eggs were removed to prevent larval feeding, which alters plant quality (Karban and Baldwin 1997), increases alkaloid production in nectar (Adler et al. 2006) and induces volatiles that affect moth behavior (De Moraes et al. 2001). The number of leaves and open flowers per plant were recorded daily for use as covariates since larger plants with bigger floral displays may be more attractive to moths.

Moths were observed using a headlamp with a red light (moths cannot detect red light since they only have three classes of photoreceptors, green, violet, and UV regions and none in the red region; White et al. 1994). We recorded the number of visits (each time an insect approached and began feeding on a new plant), probes per plant (each time an insect began feeding on a new flower within the same plant), time per probe, and moth sex on a tape recorder. Individual moths were followed until they stopped foraging, at which time another active moth was followed. Observations continued each evening until moths stopped flying. Once all plants stopped flowering, they were removed from the screen tent and allowed to mature in an outdoor enclosure without herbivores. Mature fruits were removed and individually dried for 72 hr at 32.2° C. We estimated plant female reproduction as the number of fruits per plant and weight per fruit to the nearest 0.1 mg. Plant female reproduction was estimated in this manner because tobacco fruits produce thousands of seeds per fruit and this method provides a good estimate of viable seeds.

Data analysis

Individual plants were the experimental unit of replication since moths were not tracked individually. The effect of nectar treatment on moth oviposition was analyzed using Analysis of Covariance (ANCOVA) with nectar treatment and block as main effects and the number of leaves and open flowers as covariates. The effect of nectar treatment on floral visitation was analyzed using three Multiple Analyses of Covariance (MANCOVAs) due to different sample sizes for response variables. One MANCOVA analyzed total male and female visits to plants (including all plants in analysis), a second analyzed probes per visit and time per probe for male moths (including only plants visited by males), and a third analyzed probes per visit and time per probe for female moths (including only plants visited by females). All models used nectar treatment and block as main effects and the number of leaves and open flowers as covariates. Moth oviposition and the number of visits per plant were summed within plants each night and averaged across nights; plants without open treated flowers were removed from all analyses on a per-evening basis. Total probes per visit and time spent probing were averaged within plants each evening and then averaged across nights. All numerical data were $\log(x + 1)$ transformed and all proportional data were $\arcsin(\sqrt{x})$ transformed to achieve normality. The effect of nectar treatment on plant female reproduction (measured as average fruit weight and total number of fruits produced) was analyzed using MANCOVA with nectar treatment and block as main effects and the number of leaves and number of open flowers as covariates. All analyses were conducted using SAS version 9.1.

Results

Higher nectar amino acids increased oviposition. Female hawkmoths allocated significantly more eggs to tobacco plants in the high nectar amino acid treatment than the low nectar amino acid treatment ($F_{1,40} = 5.22, P = 0.03$, Fig 1). Number of leaves and open flowers did not affect oviposition ($F_{1,40} < 1.15, P > 0.29$ for both). The main effect of block was not significant ($F_{1,40} = 3.63, P = 0.06$), nor was the interaction between treatment and covariates ($F_{1,40} < 2.85, P > 0.09$ for both).

Nectar quality did not affect floral visitation. Nectar quality did not affect the total number of male and female visits (MANCOVA, treatment effect: Wilk's $\lambda = 0.90, F_{2,30} = 1.74, P = 0.19$), the number of male probes per visit or male time spent probing (MANCOVA, treatment effect: Wilk's $\lambda = 0.86, F_{2,19} = 1.54, P = 0.24$), or the number of female probes per visit or female time spent probing (MANCOVA, treatment effect: Wilk's $\lambda = 0.90, F_{2,26} = 1.47, P = 0.25$).

Nectar quality did not affect the number of fruits or average fruit weight (MANCOVA, treatment effect: Wilk's $\lambda = 0.99, F_{2,45} = 0.13, P = 0.88$).

Discussion

Our results show that floral nectar traits may play an important role in the egg-laying decisions of nectar-feeding moths. Female moths allocated significantly more eggs to tobacco plants with high compared to low nectar amino acids (Fig. 1), demonstrating that nectar quality in addition to host plant quality (Jaenike 1978; Mayhew 1997) can alter egg-laying decisions by leaf herbivores when adults are nectar feeders. One other study has tested the hypothesis that floral nectar traits can impact moth oviposition, and

showed that increased nectar volume in *Datura stramonium* flowers increased leaf oviposition by *Manduca sexta* (Adler and Bronstein 2004). These experiments support previous observations that plants growing close to floral nectar sources of different plant species are more likely oviposition sites for lepidopterans (Murphy et al. 1984). Here, we show that nectar quality impacts oviposition choices, linking behavior of floral visitors and leaf herbivores.

In this study, hawkmoth floral visitation and plant reproduction did not differ between plants with high and low nectar amino acids, indicating that amino acids did not increase pollination by this species. The lack of discrimination for high amino acid nectar in female feeding preferences has also been found in other lepidopteron species, e.g. *Battus philenor* and *Ornithoptera priamus poseidon* (Erhardt 1991; 1992), suggesting that plants with amino acids in their nectar may not gain pollinator services from all visiting species. Although the costs of herbivory were not quantified since eggs were removed prior to hatching, moths' allocating more offspring to plants with high nectar amino acids is likely to result in greater leaf herbivory and thus reduce plant fitness. One *Manduca* larva can defoliate its host by the time it pupates (McFadden 1968). Further, loss of leaf area and induction of metabolically costly plant defense compounds in response to herbivory can translate into a direct loss of resources and thus plant fitness (Karban and Baldwin 1997; Agrawal et al. 1999; Kessler and Baldwin 2004). It is important to note that the results of this experiment may have been different if larvae were allowed to hatch and feed, thereby inducing defenses in leaves and nectar that could affect subsequent oviposition decisions (Adler et al. 2006; Sharp et al. in review).

The relative costs and benefits of nectar amino acids for a particular plant species may depend on the community of pollinating insects and on the relative importance of pollination and herbivory for that species. Amino acids may increase pollinator services (Rusterholz and Erhardt 2000) and preference (Alm et al. 1990; Erhardt and Rusterholz 1998) in some species, but may not affect others. Some pollinators may prefer amino acids but not lay eggs. However, if moths also lay more eggs on plants with high amino acids in nectar, and if herbivory has a big selective impact, then nectar amino acids may be costly.

These results suggest that nectar quality may function as an ovipositional cue to nectar-feeding moths about host plant suitability for their offspring. More eggs were allocated to plants with high nectar amino acids. However, this was not due to increased floral visitation to plants with higher amino acids since nectar quality did not affect floral visitation of female moths. Essential amino acids cannot be synthesized by insects (O'Brien et al. 2002) and several species of butterflies and moths, including the sphingid *Amphion floridensis*, derive essential amino acids entirely from the larval diet (O'Brien et al. 2002; 2005). Offspring performance may be enhanced by adult moth preference for plants with high nectar amino acids, if there is a correlation between nectar and leaf amino acid content. In this case, females that allocate offspring to plants with high nectar amino acids could increase the likelihood that their offspring will feed on high protein diets.

This study demonstrates the potential costs of high quality nectar in one system, but there are many other systems where similar costs may exist. Other systems where pollinators also serve as herbivores include several Sphingids (reviewed in Adler and

Bronstein 2004), Pierids (*Pieris rapae*, Conner et al. 1995), and generalists in the Noctuidae (*Heliothis virescens* and *Helicoverpa armigera*, Cunningham et al. 1998; De Moraes et al. 2001). Furthermore, high nectar amino acids have the potential to attract floral antagonists that may not pollinate, such as some nectar robbers, florivores, and/or fungal pathogens. More studies are needed to test the generality of these results in other systems.

In conclusion, floral rewards that attract pollinators may also increase leaf herbivory. Nectar amino acids increased moth oviposition without increasing floral visitation by female or male hawkmoths. Therefore, the ultimate benefits and costs of nectar amino acids for plant reproduction may depend on the community of pollinators and the relative importance of pollination and herbivory in the system. Nectar amino acids can incur plant fitness costs by attracting egg-laying moths, suggesting that herbivores may also act as a selective agent on nectar traits. Thus, plant-pollinator and plant-herbivore interactions are not necessarily independent, and herbivores may represent a novel agent of natural selection on nectar traits.

Literature Cited

- Adler, L.S., and J. Bronstein. 2004. Attracting antagonists: does floral nectar increase leaf herbivory? *Ecology* 85: 1519-1526.
- Adler, L.S., and R.E. Irwin. 2005. Ecological costs and benefits of defenses in nectar. *Ecology* 86: 2968-2978.
- Adler, L.S., M. Wink, M. Distl, and A.J. Lentz. 2006. Leaf herbivory and nutrients increase nectar alkaloids. *Ecology Letters* 9: 960-967.
- Agrawal, A., S. Strauss, and M. Stout. 1999. Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution* 53: 1093-1104.
- Alm J., T. Ohnmeiss, J. Lanza, and L. Vriesenga. 1990. Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. *Oecologia* 84: 53-57.
- Baker, H., and I. Baker. 1973. Amino acids in nectar and their evolutionary significance. *Nature* 214: 543-545.
- Baker., H., and I. Baker. 1977. Intraspecific constancy of floral nectar amino acid complements. *Botanical Gazette* 138: 183-191.
- Baker, I., and H. Baker. 1983. Floral nectar sugar constituents in relation to pollinator type. pp 117-141 *in*: C.E. Jones and R.J. Little, editors. *Handbook of experimental pollination biology*. Science and Academic Editions, New York, USA.
- Brody, A., and R. Mitchell. 1997. Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant *Ipomopsis aggregata*. *Oecologia* 110: 86-93.
- Cariveau, D., R.E. Irwin, A.K. Brody, L.S. Garcia-Mayeya, and A. von der Ohe. 2004. Direct and indirect effects of pollinators and seed predators to selection on plant floral traits. *Oikos* 104: 15-26.
- Conner, J., and S. Rush. 1995. The effect of wild radish floral morphology on pollination efficiency by four taxa of pollinators. *Oecologia* 105: 234-245.
- Cunningham, J., S. West, and D. Wright. 1998. Learning in the nectar foraging behavior of *Helicoverpa armigera*. *Ecological Entomology* 23: 363-369.
- De Moraes, C., M. Mescher, and J. Tumlinson. 2001. Caterpillar-induced nocturnal plant volatiles repel nonspecific females. *Nature* 410: 577-580.

- Dodd, M., J. Silvertown, and M. Chase. 1999. Phylogenetic analysis of trait evolution and species diversity variation among angiosperm families. *Evolution* 53: 732-744.
- Erhardt, A. 1991. Nectar sugar and amino acid preferences of *Battus philenor* (Lepidoptera: Papilionidae). *Ecological Entomology* 16: 425-434.
- Erhardt, A. 1992. Preferences and non-preferences for nectar constituents in *Ornithoptera priamus poseidon* (Lepidoptera, Papilionidae). *Oecologia* 90: 581-585.
- Erhardt, A., and H. Rusterholz. 1998. Do peacock butterflies (*Inachis io* L.) detect and prefer nectar amino acids and other nitrogenous compounds? *Oecologia* 117: 536-542.
- Galen, C., and J. Cuba. 2001. Down the tube: pollinators, predators, and the evolution of flower shape in the Alpine Skypilot, *Polemonium viscosum*. *Evolution* 55: 1963-1971.
- Gardener, M., and M. Gillman. 2001. Analyzing variability in nectar amino acids: composition is less variable than concentration. *Journal of Chemical Ecology* 27: 2545-2553.
- Gardener, M., R. Rowe, and M. Gillman. 2002. The taste of nectar- a neglected area in pollination ecology. *Oikos* 98: 552-557.
- Grant, V. 1983. The systematic and geographical distribution of hawkmoth flowers in the temperate North American flora. *Botanical Gazette* 144: 439-449.
- Grant, V. 1949. Pollination systems as isolating mechanisms in angiosperms. *Evolution* 3: 82-97.
- Gregory, D.P. 1964. Hawkmoth pollination in the genus *Oenothera*. *Aliso* 5: 385-419.
- Jaenike, J. 1978. A hypothesis to account for maintenance of sex within populations. *Evolutionary Theory* 3: 191-194.
- Jervis, M., C. Boggs, and P. Ferns. 2005. Egg maturation strategy and its associated trade-offs: a synthesis focusing on Lepidoptera. *Ecological Entomology* 30: 359-375.
- Johnson, S.D., and K.E. Steiner. 2000. Generalization vs. specialization in plant pollination systems. *Trends in Ecology and Evolution* 15: 140-43.
- Kaczorowski, R., M. Gardener, and R. Holdsworth. 2005. Nectar traits in *Nicotiana* section *alatae* (Solanaceae) in relation to floral traits, pollinators, and mating system. *American Journal of Botany* 92: 1270-1283.

- Karban, R., and I.T. Baldwin. 1997. Induced responses to herbivory. University of Chicago Press, Chicago, USA.
- Kessler, A., and I.T. Baldwin. 2004. Herbivore-induced plant vaccination. Part I. The orchestration of plant defenses in nature and their fitness consequences in the wild tobacco *Nicotiana attenuata*. *The Plant Journal* 38: 639-649.
- Lanza, J., G. Smith, S. Sack, and A. Cash. 1995. Variation in nectar volume and composition of *Impatiens capensis* at the individual, plant, and population levels. *Oecologia* 102: 113-119.
- Madden, A., and F. Chamberlaine. 1945. Biology of the tobacco hornworm in the southern cigar tobacco district. U.S. Department of Agriculture Technical Bulletin, 896.
- Mayhew, P.J. 1997. Adaptive patterns of host-plant selection by phytophagous insects. *Oikos* 79: 417-428.
- McFadden, M. 1968. Observations on feeding and movement of tobacco hornworm larvae. *Journal of Economic Entomology* 61: 352-356.
- Mitchell, R.J., R.G. Shaw, and N.M. Waser. 1998. Pollinator selection, quantitative genetics, and predicted evolutionary responses to floral traits in *Penstemon centranthifolius* (Scrophulariaceae). *International Journal of Plant Science* 159: 331-337.
- Murphy, D.D., M.S. Menninger, and P.R. Elrich. 1984. Nectar source distribution as a determinant of oviposition host species in *Euphydryas chalcedona*. *Oecologia* 62: 269-271.
- Nilsson, L.A., L. Jonsson, L. Rason, and E. Randrianjohany. 1987. Angraecoid orchids and hawkmoths in central Madagascar: specialized pollination systems and generalist foragers. *Biotropica* 19: 310-319.
- O'Brien, D., C. Boggs, and M. Fogel. 2005. The amino acids used in reproduction by butterflies: a comparative study of dietary sources using compound-specific stable isotope analysis. *Physiological and Biochemical Zoology* 78: 819-827.
- O'Brien, D., M. Fogel, and C. Boggs. 2002. Renewable and nonrenewable resources: Amino acid turnover and allocation to reproduction in Lepidoptera. *Proceedings of the National Academy of Science* 99: 4413-4418.
- O'Hara, G.W. 2001. Nutritional constraints on root nodule bacteria affecting symbiotic nitrogen fixation: a review. *Australian Journal of Experimental Agriculture* 41: 417-433.

- Pacini, E., M. Nepi, and J. Vesprini. 2003. Nectar biodiversity: a short review. *Plant Systematics and Evolution* 238: 7-21.
- Paul, E., K. Capiou, M. Jacobs, and J. Dunwell. 1995. A study of gene dispersal via pollen in *Nicotiana tabacum* using introduced genetic markers. *The Journal of Applied Ecology* 32: 875-882
- Pierce, N.E., M.F. Braby, A. Heath, D.J. Lohman, J.R. Mathew, and D.B. and M.A. Travassos M.A. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annual Review of Entomology* 47: 733-771.
- Poulin, R. and A.S. Grutter. 1996. Cleaning symbioses: proximate and adaptive explanations. *Bioscience* 46: 512-517.
- Raguso, R.A., and M.A. Willis. 2002. Synergy between visual and olfactory cues in nectar feeding by naïve hawkmoths, *Manduca sexta*. *Animal Behavior* 64: 685-695.
- Real, L.A., and B.J. Rathcke. 1991. Individual variation in nectar production and its effect on fitness in *Kalmia latifolia*. *Ecology* 72: 149-155.
- Romeis, J., and F. Wackers. 2000. Feeding responses by female *Pieris brassicae* butterflies to carbohydrates and amino acids. *Physiological Entomology* 25: 247-253.
- Rusterholz, H., and A. Erhardt. 2000. Can nectar properties explain sex-specific flower preferences in the Adonis Blue butterfly *Lysandra bellagrus*? *Ecological Entomology* 25: 81-90.
- Strauss, S.Y., D.H. Siemens, M.B. Decher, and T. Mitchell-Olds. 1999. Ecological costs of plant resistance to herbivores in the currency of pollination. *Evolution* 53: 1105-1113.
- Straw, R.M. 1956. Adaptive morphology of the *Penstemon* flower. *Phytomorphology* 6: 112-119.
- White, R., R. Stevenson, R. Benett, and D. Cutler. 1994. Wavelength discrimination and the role of ultraviolet vision in the feeding behavior of hawkmoths. *Biotropica* 26: 427-435.
- Wilmott, A.P., and A. Burquez. 1996. The pollination of *Merremia palmeri* (Convolvulaceae): can hawkmoths be trusted? *American Journal of Botany* 83: 1050-1056.

Figure

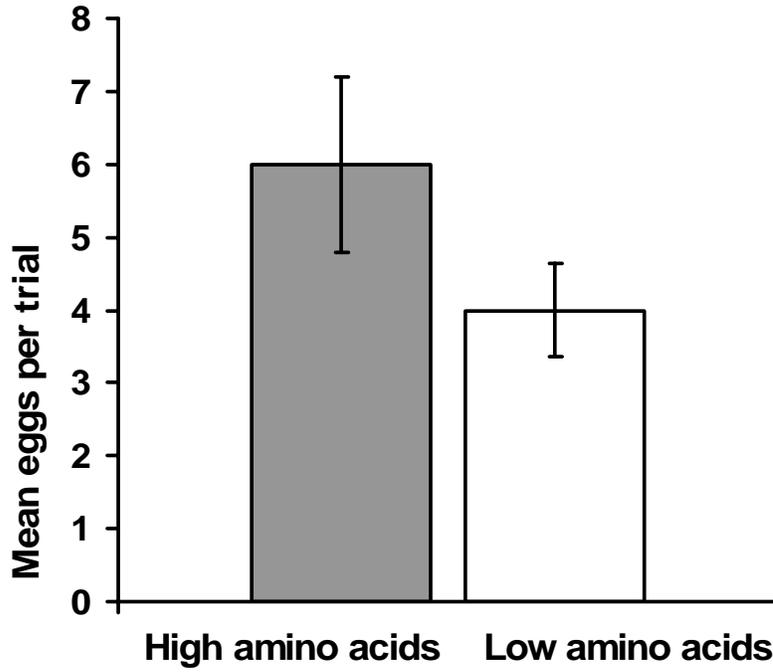


Figure 1. Nectar amino acids increased moth oviposition on tobacco. Bars represent mean \pm standard error.

Chapter 2: The effect of aphids in parasitoid-herbivore interactions

Abstract

A single plant can support many herbivores and natural enemies of herbivores that interact as a complex system. However, tritrophic interactions are often studied with consideration of only a single species at each trophic level. This research investigated how a second herbivore species on a shared host plant affected the dynamics of a parasitoid-herbivore interaction. I manipulated aphid presence on tobacco plants in the field and demonstrated that aphids reduced hornworm abundance, the incidence of parasitism of hornworms, and parasitoid preference/performance (total eggs produced, proportion of successful larvae, and proportion of females produced). Several mechanisms that could explain these results were examined in a subsequent field season. Aphid presence and caterpillar density (egg and larval) were manipulated using diet-fed hornworms to determine if effects of aphids on hornworm populations and parasitoid behavior persisted when hornworm density and quality were controlled. Moth oviposition and caterpillar predation were not affected by aphids or egg and larval density. The proportion of caterpillars attacked by parasitoids was not affected by aphids, but was inversely-density dependent with the number of hornworm larvae on the plant. This result seemingly contradicts that of the previous year, when parasitism was lowest on aphid-infested plants that had lower hornworm abundance. An alternative explanation to the density dependent hypothesis for the reduced parasitism on hornworms on aphid-damaged plants in the previous experiment is that aphid-damaged host plants are of poorer quality than undamaged plants and therefore hornworms feeding on these plants

may also be of poorer host quality. Plant quality was controlled in the density study since hornworms were reared in the lab and fed on plant material for only 2 days, so hornworm quality was unlikely to differ as a result of aphid damage. When hornworms were reared on synthetic diet there was no effect of aphids on parasitism rates, suggesting that plant-mediated volatile release due to aphid damage does not affect parasitoid choice. Taken together, my results suggest that reduced hornworm abundance and parasitism on plants with aphids may be due to changes in host plant quality that could increase hornworm mortality and reduce parasitoid preference or performance. Thus, aphids may play an important role in structuring arthropod communities through indirect interactions on other herbivores via changes in host plant quality.

Introduction

Although the distribution and abundance of individual species may be determined by a web of interactions, the ecology and evolution of focal species is often considered only within the context of pair-wise interactions (Bjornstad et al. 2001). Indirect interactions, interactions between two species that are modified by a third (Wootton 1994), play a critical role in structuring arthropod and plant communities (Juenger and Bergelson 1998; van Zandt and Agrawal 2004; Hatcher et al. 2006; and van Veen et al. 2006). In tritrophic systems, indirect interactions are usually examined among single host plants with a focal herbivore and its natural enemy. However, plants are generally attacked by several herbivores from different feeding guilds that have numerous predators and parasitoids.

Multiple herbivore species feeding on a shared host plant can cause chemical and morphological changes in the plant that are specific to the herbivore guild. For example, phloem-feeding insects can elicit different plant defense responses than chewing insects (Broderick et al. 1997; Bronner et al. 1991; Mayer et al. 1996; van der Westhuizen et al. 1998 a; 1998 b; Walling 2000; Kessler and Baldwin 2002). Further, the effects of herbivore feeding depend on the community. For example, plant defenses induced in wild radish by one lepidopteran herbivore can have a positive, negative, or no effect on subsequent lepidopteran herbivores (Agrawal 1999; Agrawal 2000), damage by weevils to milkweed reduced the growth of monarch butterflies (Van Zandt and Agrawal 2004), weevil damage on evening primrose reduced growth and increased mortality of the beet armyworm (McGuire and Johnson 2006), and prior flea beetle damage on horsenettle increased larval development time and reduced oviposition by potato beetles (Wise and

Weinburg 2002). Herbivore damage may also induce resistance to herbivores from different feeding guilds. Corn earworm damage increased the resistance of tomato plants to aphids, mites, and other noctuid moth species (Stout et al. 1998; Bostock et al. 2001; Heil and Bostock 2002). These studies indicate that multiple herbivore species may induce changes in the host plant that can affect subsequent herbivores.

Parasitoid preference and performance can also be affected by plant defense compounds present in host herbivores (see review by Ode 2006). Larvae of *Cotesia congregata* Say suffered an increase in mortality when the secondary compound nicotine was added into a synthetic diet of its host, the tobacco hornworm. (Barbosa et al. 1991; Barbosa et al. 1986; Thurston and Fox 1972). Further, a field study demonstrated that *C. congregata* mortality increased when developing in hosts that fed on tobacco that was high in nicotine concentration (Thorpe and Barbosa 1986). Developing parasitoids (*Hyposoter exiguae*) in the corn earworm also suffer reduced eclosion rates, size, and longevity when host diet included the alkaloid tomatine (Campbell and Duffey 1979; 1981). The specialist parasitoid *Cotesia melitaearum* Wilkinson attacks larvae of the checkerspot butterfly that feeds on plants in the family Plantaginaceae that vary in the two iridoid glycosides catalpol and aucubin. In the field, caterpillars feeding on plants with high levels of iridoid glycosides were less likely to be parasitized, providing indirect evidence that parasitoids avoid or perform poorly when exposed to increased levels of secondary compounds (Nieminen et al. 2003). Further, differences in plant chemistry between plants that have been induced versus those that have not been induced have been found. For example, induction of tomato decreased the performance of *Hyposoter exiguae*, an endoparasitoid of the beet armyworm. Induction of tomato also increased

volatile production by plants, and thus parasitoid attractiveness, leading to a 2 – fold increase in parasitism in the field (Thaler 1999; 2002). Reductions in host quality due to plant chemistry can decrease parasitoid performance by retarding larval development and decreasing survival.

Sucking insects such as aphids and chewing insects such as caterpillars differ in how they affect plants, and these effects may impact natural enemies, such as parasitoids and predators. For example, some phloem-feeding herbivores induce the release of plant volatiles (Guerra et al. 1993; Micha and Wyss 1995; Du et al. 1996; Turlings et al. 1998) that differ from blends released in response to chewing herbivores (Rodriguez-Saona et al. 2002). Phloem-feeding herbivores such as aphids cause relatively little damage, and induce the pathogen-defense response that primarily defends against bacterial and fungal pathogens. The pathogen-defense response produces proteins, enhances the synthesis of secondary compounds, and produces protease inhibitors (Kombrink and Somssich 1995; Reymond and Farmer 1998). Chewing herbivores that cause more extensive cell damage activate the wound-signaling pathways, which are a part of the induced response of the plant that initiates the production of secondary compounds and wound response proteins (Bostock and Stermer 1989; Walling 2000). Thus, volatile emissions and plant defense compounds may differ depending on the herbivore community that feeds on the host plant, and altered plant chemistry may affect the attraction of natural enemies and predators.

Despite the differential effects of herbivore species on host plant quality and volatile emissions, little is known regarding how a second herbivore species affects tritrophic interactions between host plants, their herbivores, and parasitoids. Secondary

herbivore species may impact focal herbivores through the induction of the shared host plant, and affect parasitoids through changes in host plant quality and/or volatile emissions. The effects of multiple herbivores on higher trophic levels have been examined in two systems (Kessler and Baldwin 2001; 2004; Rodriguez-Saona et al. 2005). In the first, wild tobacco produced volatiles when damaged by a mirid plant bug. These volatiles attracted generalist predators that attacked tobacco hornworm eggs (Kessler and Baldwin 2004), demonstrating that higher order interactions can be altered when multiple herbivores are present. The second study examined the effect of a phloem-feeding herbivore, the potato aphid, on the preference and performance of the beet armyworm and its parasitoid *Cotesia marginiventris* on tomato. Performance of *C. marginiventris* was not affected by aphid feeding, but parasitoids were more attracted to plants that were damaged by both aphids and caterpillars in comparison to controls (Rodriguez-Saona et al. 2005). Thus, there is significant evidence that sucking insects impact higher order interactions and therefore impact community structure.

Secondary herbivores could also impact tritrophic interactions simply by altering the density of the focal herbivore if parasitism or predation is density-dependent. There are several mechanisms by which secondary herbivores could influence the density of a focal herbivore. Secondary herbivores may affect adult insect preference, where females prefer to oviposit on plants that are not infested with a secondary herbivore (Wise and Weinberg 2002). Or, secondary herbivores may also alter host plant chemistry and impact focal herbivore survival and performance (Kessler and Baldwin 2004). The density of interacting species has been shown to change the intensity and outcome of parasitoid-herbivore interactions (Lessells 1985; Walde and Murdoch 1988; Bretton and Addicott

1992; Bretton and Addicott 1992; Dubbert et al. 1998; Rotem and Agrawal 2003). Thus, the presence of secondary herbivores may influence the density of focal herbivores, and in turn may affect density-dependent rates of predation and/or parasitism on the focal herbivore.

The purpose of this study was to examine how a second herbivore on a shared host plant affects the dynamics of a plant-herbivore-parasitoid system. The green peach aphid (*Myzus persicae*) and the tobacco hornworm (*Manduca sexta*) are herbivores that feed on domestic tobacco (*Nicotiana tabacum*). The specialist parasitoid, *Cotesia congregata*, attacks larvae of *M. sexta*. This study began by first asking: 1) Do aphids affect the occurrence of hornworms and parasitoids? If so, there are several mechanisms by which this could occur. Aphids can affect hornworm abundance by affecting moth oviposition or predation rates, and parasitism through changes in parasitoid attraction and oviposition decisions. Such changes in parasitoid behavior may be explained by altered hornworm or host plant quality, or solely by hornworm density. To separate the effects of aphids and hornworm density on both moth and parasitoid behavior in this system, two additional questions were addressed: 2) Do *Manduca* density and presence of aphids affect moth oviposition? and 3) Do larval density and presence of aphids affect hornworm predation and parasitism?

Methods

Study system

Host Plant: *Nicotiana tabacum* L. (Solanaceae, NC95 line, ‘tobacco’ hereafter) is a cultivated annual species that supports a wide range of insects. One of the most damaging herbivores is the tobacco hornworm whose feeding severely impacts fitness in domestic tobacco (McFadden 1968) and wild congeners (Heil and Baldwin 2002; Kessler and Baldwin 2004). Tobacco plants respond to herbivore attack by producing an array of secondary compounds including alkaloids, and then transporting these compounds to leaf tissues (Detzel and Wink 1993; Adler et al. 2006). For example, nicotine is an alkaloid that accounts for 95 % of the alkaloids in *N. tabacum* (Sisson and Severson 1990). Tobacco plants also produce sesquiterpenoid phytoalexins (Wibberley et al. 1994) and acidic and basic forms of pathogenesis related proteins (Vidal et al. 1997) that may negatively affect herbivore performance.

Focal herbivore: *Manduca sexta* L. (Lepidoptera: Sphingidae; hereafter ‘hornworm’ or ‘moth’ as the larval and adult forms respectively), the tobacco hornworm, ranges from southern Canada to southern Brazil. Larvae feed on Solanaceous species, and tobacco is a preferred host plant (Madden and Chamberlin 1945). A single hornworm can defoliate a tobacco plant in less than 2 weeks (McFadden 1968). Hornworms have a relatively high tolerance to nicotine (Hansberry and Midalekauff 1940; Negherbon 1959; Morris 1983; 1984; Glendinning, 2002). Hornworms are attacked by a number of natural enemies including parasitic and predaceous wasps, spiders, birds, and hemipterans such as *Geocoris* spp. (McFadden 1968).

Secondary herbivore: *Myzus persicae* Sulzer (Homoptera: Aphidiidae; ‘aphid’ hereafter), the green peach or tobacco aphid, feeds on tobacco phloem. Aphid infestation can cause curling or wilting of host plants and vector plant disease (Borror et al. 1989). Nymphs secrete honeydew while feeding and are parasitized by braconid and chalcidoid wasps. Important predators include ladybird beetles, lacewings, and larvae of syrphid flies.

Parasitoid: *Cotesia congregata* Say (Hymenoptera: Braconidae; ‘parasitoid’ hereafter) is a gregarious endoparasitoid of the tobacco hornworm and other sphingid larvae (Kester and Barbosa 1991). Female parasitoids attack 3rd-instar hornworms. *Cotesia congregata* exhibits haplo-diploid reproduction; eggs fertilized with stored sperm produce diploid females and unfertilized eggs produce haploid males. Female parasitoids can directly manipulate both the number and sex of offspring (Kester and Barbosa 1991; Lentz and Kester in review). Females mate only once and are sperm limited (Freeman and Kester unpublished).

Experiment 1: Do aphids affect the occurrence of hornworms and parasitoids?

The impact of aphids on hornworm abundance and parasitism was examined in a field study in June 2003. One hundred tobacco seedlings were randomly assigned to one of two treatment groups, plants with or without aphids (n=50; n= per treatment here and throughout). Plants with aphids were inoculated using wild aphid populations in Montgomery County, VA. Plants without aphids were not inoculated, and naturally established aphids were removed bi-weekly using packaging tape (Scotch, tear by hand, USA). To control for the effects of tape residue, plants with aphids were also taped,

although to a much lesser extent. The numbers of parasitized and unparasitized hornworms were recorded weekly at the peak of the season, August 15 - Sept. 12. Parasitized hornworms were collected and held individually until parasitoid larvae egressed and pupated. The average number of hornworms was estimated by censusing plants weekly for hornworms and averaging weekly censuses for the season. Parasitism was infrequent and was assessed as yes / no over the entire season for each plant ('yes' if any parasitized hornworms were found on the plant). Parasitoid cohorts were assessed as the total number of eggs oviposited (assessed as the number of larvae that emerged and spun cocoons plus the number of larvae that died without emerging, determined by dissection), proportion of successful offspring (the number of larvae that spun cocoons divided by the total number of eggs), proportion of unsuccessful offspring (the number of larvae that did not emerge divided by the total number of eggs), and proportion of females per brood (sex ratio, the total number of females divided by the total number of wasps per brood). The values were averaged among weeks to create one value per plant for the season.

Data Analysis

Hornworm abundance was analyzed using 1-way ANOVA (SAS, 9.0, PROC GLM) and transformed with square root ($x + 0.5$) to meet parametric assumptions. Parasitism was analyzed with a chi-square test. Total eggs, proportion of successful offspring, proportion of unsuccessful offspring, and sex ratio were analyzed using one-way MANOVA. Sex ratio was transformed using arcsin square root and total eggs was

transformed using square root ($x + 0.5$) to meet parametric assumptions. A significant MANOVA was followed with univariate ANOVAs for each response.

Experiment 2: Do *Manduca* density and presence of aphids affect moth oviposition?

Changes in hornworm abundance due to aphids could result from changes in host plant quality that cause hornworm mortality or alter adult oviposition decisions.

Alternatively, changes in hornworm abundance could reflect density-dependent processes, since female moths may choose to allocate eggs to plants with conspecifics or avoid them (Figure 1). The purpose of these experiments was to examine how aphids and *Manduca* density affect moth oviposition. Two experiments were conducted, one in which egg density and aphids were manipulated (Part A) and a second in which hornworm density and aphids were manipulated (Part B).

Part A: One hundred fifty tobacco seedlings were germinated in April 2005 in vermiculite in a glasshouse and transplanted 2 weeks later into 6-cell flats containing Pro-Mix BX soil (Premier Horticulture Ltd., Red Hill, Pennsylvania, USA). On June 1 seedlings were transplanted into a 32 m x 40 m field plot at Kentland Farm, Montgomery County, Virginia, USA. Seedlings were planted in a 14 x 11 array with 2.5 m between plants in all directions. Seedlings were fertilized June 15 with Weaver Plant Food 10-10-10 (Winston Weaver Co. Inc., Winston-Salem, North Carolina, 27109, USA). Plants were randomly assigned to aphid (presence/absence) and egg density (0-7 eggs) treatments (16 treatment combinations, $n = 9$ per treatment). Aphids treatments were maintained as described previously. Egg density was manipulated by gluing caterpillar eggs (North Carolina State University Insectary) with Elmer's School Glue (Borden®, Elmer's

Products Inc., Columbus, Ohio, 43219, USA) onto randomly chosen leaves in the middle third of each plant. No more than 3 eggs were glued per tobacco leaf, using the underside of the leaf's outer edge. Egg number and position was based on previous observations of moth oviposition behavior at this site. Eggs were tracked by numbering the area on the plant leaf closest to the egg with a ball point pen. Treatment effects of glue and pen marks were controlled for by placing 7 glue spots on every plant and numbering each with a pen; only the addition of eggs differed. For two weeks, tobacco plants were censused daily for new caterpillar eggs; wild eggs were removed daily to maintain egg density treatments and counted to assess oviposition preference. Missing treatment eggs were attributed to predation and replaced. Eggs close to hatching (color change from green to yellow) were also replaced. The number of leaves per plant was recorded daily and averaged within plant for use as a covariate since larger plants may be more attractive to moths.

Part B: Aphid treatments were maintained and one month later, a second experiment was conducted using the same plants, in which larval density and aphids were manipulated to determine the effects on moth oviposition decisions. Larval density may affect moth oviposition differently than egg density since larval damage can induce changes in the host plant such as the production of volatiles and secondary plant compounds (Baldwin 1988). Larval density was manipulated using the same treatment levels as egg density. Third-instar hornworms were purchased from the North Carolina Insectary and reared on a synthetic-casein based diet (tobacco hornworm diet F9783B, Bioserv, Inc. Frenchtown, NJ, USA) until they were transferred to field tobacco plants. Hornworms were placed on the underside of tobacco leaves in the middle third portion of the tobacco plant and

allowed to move freely. Hornworm densities were maintained each day during the 48 hr of exposure. Missing larvae were replaced and all losses were recorded as predation occurrences. Tobacco plants were censused daily for moth oviposition. All wild eggs were removed and counted to assess moth oviposition. Moth oviposition was assessed for only two days in this experiment, unlike Part A where oviposition was assessed for 2 weeks, because hornworms are most susceptible to parasitoid attack as 3rd instars and leaving hornworms out for longer periods of time would greatly increase loss due to predation.

Data Analysis

Newly laid eggs were summed over each plant and analyzed using separate 2-way ANCOVAs (SAS v. 9.0) for Part A and Part B. Aphid presence and *Manduca* density were the main effects, and were treated as categorical variables. The aphid x density interaction and average number of leaves (covariate) were used in both models. The interactions between the covariate and the main effects were not significant in either model and were removed.

Experiment 3: Do larval density and presence of aphids affect hornworm predation and parasitism?

This experiment tested the effect of larval density and aphid presence/absence on hornworm parasitism and predation rates (Figure 1). Hornworms from Experiment 2 Part B were collected from field tobacco plants (with or without aphids) after the 48 hr exposure to parasitoids and predators in the field. *Manduca sexta* is most sensitive to

attack by *C. congregata* in the 3rd instar and it takes several days to molt to the next instar (Barbosa et al. 1991), therefore exposure time to parasitoids was optimized in this experiment since larvae of *M. sexta* were in the field during the critical stage for parasitoid attack. The effects of host plant quality on hornworms, and resulting changes in parasitoid host preference, were minimized since caterpillars were fed the same diet throughout development with the exception of the time spent out in the field. However, preference due to host plant traits, such as volatile emissions, may vary with hornworm density and affect parasitism rates. Therefore, this experiment removes the effect of caterpillar quality on parasitoid preference since all hosts were of equal quality, and tests only parasitoid preference for changes in host plant quality due to aphid attack. After collection, hornworms were reared individually on lab diet until parasitoid larvae egressed or the hornworm pupated. Individual parasitoid cohorts were assessed as described in Experiment 1, to determine the total number of eggs, proportion of successful larvae, proportion of unsuccessful larvae, and sex ratio and averaged for each plant. Missing hornworms were considered predated since 3rd -instar hornworms are unlikely to leave host plants (Van Dam et al. 2001), and predatory wasps and birds have been observed feeding on hornworms (AJL personal observations). The proportion of hornworms that were predated was determined by dividing the number of hornworms predated by the total number of hornworms put on the plant.

The impact of aphids on plant reproduction was also assessed. Aphid treatments were maintained until 90% of the tobacco fruits were mature. Tobacco inflorescences were cut from the plant and placed in individual paper bags. Mature fruits were removed

by clipping from the base of the inflorescence. Female plant reproduction was assessed as the total number of fruits produced per plant.

Data Analysis

The main effects of aphid presence, hornworm density, and their interactions were examined using MANCOVA with the number of leaves per plant as a covariate, to determine the effects on the proportion of caterpillars that were predated, parasitized, or survived to pupation. Density was treated as a categorical variable. The interaction between the covariate and the main effects were not significant in either model and were removed. A separate MANOVA with a similar model (the same with the exception of the leaf covariate) was used to determine effects on total number of eggs, proportion successful, and the proportion of females in parasitoid cohorts. A significant MANOVA was followed with univariate ANOVAs.

The effect of aphid presence and hornworm density and their interactions on female plant reproduction (number of fruits) were analyzed using 2-way ANCOVA with the number of leaves as a covariate. Female plant reproduction was not incorporated into the MANCOVA model above due to different sample sizes for the response variables. The interactions between the covariate and the main effects were not significant in both models and were removed.

Results

Experiment 1: Do aphids affect the occurrence of hornworms and parasitoids?

Aphid presence reduced the number of hornworms ($F_{1,92} = 7.02$, $p = 0.01$, Fig. 2a) and the probability that a hornworm was parasitized on a given plant ($\chi^2_1 = 8.53$, $p = 0.004$, Fig. 2b). Aphids also reduced parasitoid preference and performance (MANOVA, treatment effect: Wilk's $\lambda = 0.82$ $F_{3,64} = 4.68$; $p = 0.005$); there were significantly more eggs allocated to hosts feeding on plants without aphids (ANOVA: total eggs, treatment: $F_{1,66} = 5.14$, $p = 0.03$, Fig. 2c) and a greater proportion of larvae survived on plants without aphids (ANOVA: proportion successful, treatment: $F_{1,66} = 5.04$, $p = 0.03$). Lastly, there were significantly higher proportions of females produced from hornworms on plants without aphids compared to plants with aphids (ANOVA: sex ratio, treatment: $F_{1,66} = 7.67$, $p = 0.007$, Fig. 2d).

Experiment 2: Do *Manduca* density and presence of aphids affect moth oviposition?

The main and interactive effects of *Manduca* density and aphid presence did not affect moth oviposition in either experiment ($F < 2.5$, $P > 0.12$ for all terms). The number of leaves had a positive significant effect on moth oviposition in both experiments (egg density: ANCOVA, $F_{1,130} = 5.35$, $p = 0.02$; larval density: ANCOVA, $F_{1,133} = 8.34$, $p = 0.005$).

Experiment 3: Do larval density and presence of aphids affect hornworm predation and parasitism?

Larval density had a significant effect on hornworm fate (MANCOVA, larval density: Wilk's $\lambda = 0.71$, $p < 0.0001$). This result was driven by parasitism, not predation or pupation (ANCOVA, larval density: $F_{6, 109} = 1.78$, $p = 0.11$; $F_{6, 109} = 1.63$, $p = 0.15$, respectively). The proportion of hornworm larvae that were parasitized was inversely related to the number of hornworm larvae, with generally higher parasitism rates at lower hornworm densities (ANCOVA, $F_{6, 109} = 3.67$, $p = 0.002$, Fig. 3). Aphid presence alone did not affect parasitism, predation, or pupation (MANCOVA, aphid presence: $F_{3, 109} = 0.44$, $p = 0.72$). However, the interaction between larval density and aphid presence had a significant effect on pupation (MANCOVA, aphid presence x larval density: Wilk's $\lambda = 0.72$, $F_{18, 109} = 2.10$, $p = 0.006$; pupation: ANCOVA, $F_{6, 109} = 2.85$, $p = 0.01$, Fig. 4) but not on parasitism or predation (ANOVA, $F_{6, 109} < 1.82$, $P > 0.10$ for both). Tobacco plants with aphids and 1 larvae had a significantly higher proportion of larvae that pupated than all other treatment groups ($P < 0.03$); further, tobacco plants with aphids and 3 larvae had a significantly lower number of larvae that pupated than tobacco plants with aphids and 2 larvae and tobacco plants without aphids and 2 or 3 larvae ($P < 0.03$). There was no effect of aphid presence, hornworm density or their interactions on parasitoid performance, measured as total number of eggs, proportion successful larvae, proportion of unsuccessful larvae, and sex ratio (MANOVA, aphid: Wilk's $\lambda = 0.97$, $F_{3, 66} = 0.59$, $p = 0.63$; larval density: Wilk's $\lambda = 0.70$, $F_{21, 66} = 1.54$, $p = 0.30$; aphid presence x larval density interaction: Wilk's $\lambda = 0.85$, $F_{15, 66} = 0.73$, $p = 0.75$).

Aphids reduced plant reproduction ($F_{1, 125} = 5.16$, $p = 0.02$; aphids: 459 ± 19.6 fruits; no aphids: 518 ± 18.09 fruits) (Fig.5). Total fruits per plant was not affected by larval density ($F_{7, 125} = 0.46$, $p = 0.86$) or the interaction between aphid presence and

larval density ($F_{7, 125} = 0.41$, $p = 0.90$). This is to be expected, since larvae were only on plants for two days and caused little damage. In addition, plants with more leaves produced more fruits (ANCOVA, $F_{1, 125} = 28.76$, $p < 0.0001$).

Discussion

Tritrophic interactions between plants, herbivores and natural enemies have traditionally been examined using only a single species at each trophic level. However, in nature these interactions occur in the presence of other species. Host plants support rich faunas of herbivores and their natural enemies (Gullan and Cranston 1994) and interact as a complex system. In this study, the presence of a phloem-feeding aphid affected parasitoid-caterpillar interactions by reducing the abundance of hornworms, incidence of parasitism, and parasitoid performance (the number of larvae that survived to adulthood and the proportion of females produced; Exp. 1).

The reduction of hornworm abundance on plants with aphids was not explained by moth oviposition preferences related to aphid presence or *Manduca* density (egg or larval; Exp. 2). Moth oviposition increased with the number of leaves, which may reflect choices for greater food resources or for protection since in some systems, larger plants reduce the searching efficiency of natural enemies (Tabone et al. 2006). Predation also failed to explain the decrease in hornworm abundance on plants with aphids (Exp.3).

Although not addressed in this study, one possible mechanism that may explain reduced hornworm abundance could be the effect of aphids on host plant quality. Aphids induce the production of protease inhibitors in tomato (Rodriguez-Saona et al. 2002), vector disease in tobacco (Kanavaki et al. 2006; Srinivasan et al. 2006), and encourage

the growth of sooty mold (*Capnodium* spp.) in many plant species, including tobacco (Drees 1998). All of these changes may have a negative effect on host plant quality and reduce caterpillar performance. Laboratory experiments in this system suggest that tobacco aphids reduce host plant quality. Hornworms had higher mortality when fed diet mixed with tobacco leaves from plants with aphids compared to leaves from plants without aphids ($n = 40$, $\chi^2 = 7.67$, $p = 0.0056$, AJ Lentz, in preparation). Further, it is unlikely that larvae feeding on plants with aphids will leave to find a more suitable host plant. Larvae of *M. sexta* rarely leave the host plant as young instars since the cost of leaving (van Dam et al. 2001) and predation risks (Bernays 1997) are greater than the cost of consuming a poor quality host plant. This is also the case with the congener *M. quinquemaculata*, where oviposition site determines food choice (Kessler and Baldwin 2002). Therefore, aphids could negatively impact hornworm populations since young hornworms will consume a poor quality host plant rather than leave.

This study also demonstrated how another herbivore species can impact the third trophic level. Aphids decreased parasitism on hornworms sharing the same host plant (Exp.1). One mechanism that might explain these results is density-dependence. Parasitism was greatest on plants with 2 - 3 hornworms and lower on plants with greater numbers of hornworms (Exp. 3; Fig. 3). This result suggests that parasitoids forage in an inversely density-dependent manner. Mechanisms that explain inverse density-dependence in parasitoids include a foraging strategy that spreads eggs among patches as an adaptation to account for an unpredictable environment (Hamilton and May 1977, Kuno 1981, Levin et al. 1984, Cronin and Strong 1993, Mackauer and Volkl 1993, and Hochberg and Ives 2000), avoidance of self-superparasitism (Rosenheim and Mangel

1994), and decelerating functional responses due to handling time or group defenses (Hunter 2000).

However, aphids reduced hornworm abundance and parasitism in Experiment 1, but Experiment 3 showed that parasitism is highest when hornworms are least abundant. If parasitoids operated solely in an inversely-density dependent manner then in Experiment 1, parasitism would have been greater on plants with aphids since hornworm abundance was lowest on these plants. Instead, results of Experiment 1 suggest that host quality may be a greater factor in determining parasitoid attack than density dependence alone. In Experiment 1, hornworms fed on aphid-damaged plants and may be of poorer quality than those feeding on undamaged plants, which may have increased hornworm mortality and decreased parasitoid attack rates. In Experiment 3, hornworms were from the lab and fed on plant material for only 2 days, so hornworm quality is not likely to differ as a result of aphid damage. Therefore, even if density-dependent processes were operating, there is still evidence that suggests that there is a change in host quality due to feeding on tobacco with aphids, resulting in poorer parasitoid and hornworm performance. Thus, the impact of hornworm quality likely overrides the effect of density dependence. Further, the results of Experiment 3 provide evidence that host plant volatiles released due to aphid damage do not seem to play a role, either positive or negative, in parasitoid attack decisions (Figs. 3 and 4).

Aphids reduced tobacco female reproduction (Fig. 5). Several of the underlying mechanisms for this result may also reduce plant quality for hornworms and parasitoids. For example, decreased fruit production could result from transmission of pathogens and disease (Lojek and Orlob 1969; Kanavaki et al. 2006; Srinivasan et al. 2006), honeydew

secretions that inhibit photosynthesis and encourage the growth of sooty mold (Drees 1998) and/or nutrient loss due to aphid feeding. Caterpillar density did not reduce tobacco reproduction in this experiment, which was expected since larvae fed on plants for only 48-hrs as relatively young instars. Hornworms are most damaging in the 5th instar (McFadden 1968). Therefore, an increase in caterpillars that fed until pupation would likely reduce tobacco fitness. Aphid presence could reduce caterpillar damage since consumption of aphid-infested leaf material increases caterpillar mortality (AJ Lentz, in prep) and fewer hornworms were found on aphid-infested plants (Exp. 1). Thus, while the impact of aphids on tobacco fitness was negative in the absence of hornworms (which were removed from plants in Exp. 3), there may be some undetected benefits, such as decreased hornworm feeding, which should be examined further.

Several studies in natural systems have examined the effects of herbivory of one species on the preference and performance of another herbivore species. Damage by the flea beetle *Epitrix fuscata* on *Solanum carolinense* increased development time of *Leptinotarsa juncta*, the false potato beetle, and moths of *L. juncta* preferred to oviposit on undamaged horsenettle (Wise and Weinberg 2002). Kessler and Baldwin (2004) examined induction of *Nicotiana attenuata* by the mirid bug *Tupiocoris notatus* and found that mirid attack slowed the growth of *M. sexta* and attracted the predator *Geocoris pallens*, but did not affect plant fitness. These studies, and the present agricultural study, show a similar negative effect of multiple herbivores on chewing herbivores. Further, phloem-feeding herbivores did not impact plant fitness, or in cases such as this study where they do, the effects of reducing chewing herbivore damage may be greater, either

of which may result in an overall positive effect for the plant. Therefore, aphid presence may have some benefits in agricultural systems.

The effect of herbivore-herbivore interactions may affect herbivore community dynamics and have more of an impact on community structure than has been previously recognized (Denno et al. 1995; Wise and Weinburg 2002) not only with respect to herbivore interactions, but also in structuring the natural enemy community. This study found that aphids reduced hornworm abundance and parasitism rates. These findings were not explained by changes in moth oviposition, predation, or density-dependent processes, suggesting that host plant quality might mediate these interactions. Changes in the host plant quality due to aphid damage may lead to a reduction in hornworm quality and survival, and thus impact parasitoids indirectly through a reduction of larval host survival and hence host populations.

Literature Cited

- Adler L.S., M. Wink, M. M. Distl, and A.J. Lentz. 2006. Leaf herbivory and nutrients increase nectar alkaloids. *Ecology Letters* 9: 960-967.
- Agrawal, A.A. 1999. Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. *Ecology* 80: 1713-1723.
- Agrawal, A.A. 2000. Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. *Oikos* 89: 493-500.
- Barbosa, P., P. Gross, and J. Kemper. 1991. Influence of plant allelochemicals on the tobacco hornworm and its parasitoid, *Cotesia congregata*. *Ecology* 72: 1567-1575.
- Barbosa, P., J.A. Saunders, J. Kemper, R. Trumbule, J. Olechno, and P. Martinat. 1986. Plant allelochemicals and insect parasitoids: effects of nicotine on *Cotesia congregata* Say (Hymenoptera: Braconidae) and *Hyposoter annylipes* (Cresson) (Hymenoptera: Ichneumonidae). *Journal of Chemical Ecology* 12: 1319-1328.
- Bernays, E.A. 1997. Feeding by lepidopteran larvae is dangerous. *Ecological Entomology* 22: 121-123.
- Bjornstad, O.N., S.M. Salt, N.C. Stenseth, S.J. Thompson, and M. Begon. 2001. The impact of specialized enemies on the dimensionality of host dynamics. *Nature* 409: 1001-1006.
- Borror, D.J., C.A. Triplehorn, and N.F. Johnson. 1989. An introduction to the study of insects. 6th edition. Thompson Learning Inc., USA.
- Bostock, R.M., R. Karban, J.S. Thaler, P.D. Weyman, and D. Gilchrist. 2001. Signal interactions in induced resistance to pathogens and insect herbivores. *European Journal of Plant Pathology* 107: 103-111.
- Bostock, R.M., and B.A. Stermer. 1989. Perspectives on wound-healing in resistance to pathogens. *Annual Review of Phytopathology* 27: 343-371.
- Bretton, L.M., and J.F. Addicott. 1992. Density-dependent mutualism in an aphid-ant interaction. *Ecology* 73: 2175-2180
- Broderick, K., C. Pittock, T. Arioli, E.H. Creaser, J.J. Weinman, and B.G. Rolfe. 1997. Pathogenesis-related proteins in *Trillium subterraneum*: a general survey and subsequent characterization of a protein inducible by ethephon and redlegged earth mite attack. *Australian Journal of Plant Physiology* 24: 819-829.

- Bronner, R., E. Westphal, and F. Dreger. 1991. Pathogenesis-related proteins in *Solanum ducamara* L. resistant to the fall mite *Aceria cladophthirus* Nal. *Physiological Molecular Plant Pathology* 38: 93-104.
- Campbell, B.C., and S.S. Duffey. 1979. Tomatine and parasitic wasps - potential incompatibility of plant antibiosis with biological control. *Science* 205: 700-702.
- Campbell, B.C., and S.S. Duffey. 1981. Alleviation of alpha-tomatine-induced toxicity to the parasitoid, *Hyposoter exiguae*, by phytosterols in the diet of the host, *Heliothis zea*. *Journal of Chemical Ecology* 7: 927-946.
- Cronin, J.T., and D.R. Strong. 1993. Substantially submaximal oviposition rates by a mymarid egg parasitoid in the laboratory and field. *Ecology* 74: 1813-1825.
- Denno, R.F., M.S. McClure, and J.R. Ott. 1995. Interspecific interactions in phytophagous insects - competition reexamined and resurrected. *Annual Review of Entomology* 40: 297-331.
- Detzel, A., and M. Wink. 1993. Attraction, deterrence or intoxication of bees (*Apis mellifera*) by plant allelochemicals. *Chemoecology* 4: 8-18.
- Drees, B.M., and J.A. Jackman. 1998. A field guide to common Texas insects. Gulf Publishing Company, Houston, TX.
- Du, Y.J., G.M. Poppy, and W. Powell. 1996. Relative importance of semiochemicals from first and second trophic levels in host foraging behavior of *Aphidius ervi*. *Journal of Chemical Ecology* 22: 1591-1605.
- Dubbert, M., T. Tschamtkke, and T.S. Vidal. 1998. Stem-boring insects of fragmented *Calamagrostis* habitats: herbivore-parasitoid community structure and the unpredictability of grass shoot abundance. *Ecological Entomology* 23: 271-280.
- Glendinning, J.I. 2002. How do herbivorous insects cope with noxious secondary plant compounds in their diet? *Entomologia Experimentalis et Applicata* 104: 15-25.
- Guerrieri, E., F. Pennacchio, and E. Tremblay. 1993. Flight behavior of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) in response to plant and host volatiles. *European Journal of Entomology* 90: 415-421.
- Gullan, P.J., and P.S. Cranston. 2000. *The Insects: An Outline of Entomology*. 2nd edition. Blackwell Science Ltd., Oxford.
- Hamilton, W.D., and R.M. May. 1977. Dispersal in stable habitats. *Nature* 269: 578-581.
- Hansberry, R., and W.W. Middlekauff. 1940. Toxicity of nicotine administered internally to several species of insect. *Journal of Economic Entomology* 33: 511-517.

- Hatcher, M.J., J.T.A. Dick, and A.M. Dunn. 2006. How parasites affect interactions between competitors and predators. *Ecology Letters* 9: 1253-1271.
- Heil, M. 2002. Ecological costs of induced resistance. *Current Opinion in Plant Biology* 5: 345-350.
- Heil, M., and I.T. Baldwin. 2002. Fitness costs of induced resistance: emerging experimental support for a slippery concept. *Trends in Plant Science* 7: 61-67.
- Heil, M., and R.M. Bostock. 2002. Induced systematic resistance (ISR) against pathogens in the context of induced plant defenses. *Annals of Botany* 89: 503-512.
- Hochberg, M.E., and A.R. Ives. 2000. *Parasitoid Population Biology*. Princeton University Press, Princeton.
- Hunter, M.D. 2003. Effects of plant quality on the population ecology of parasitoids. *Agricultural and Forest Entomology* 5: 1-8.
- Juenger, T., and J. Bergelson. 1998. Pairwise versus diffuse natural selection and the multiple herbivores of scarlet gilia, *Ipomopsis aggregata*. *Evolution* 52: 1583-1592.
- Kanavaki, O.M., J.T. Margaritopoulos, N.I. Katis, P. Skouras, and J.A. Tsitsipis. 2006. Transmission of Potato virus Y in tobacco plants by *Myzus persicae* nicotianae and *M. persicae* s.str. *Plant Disease* 90: 777-782.
- Kessler, A., and I.T. Baldwin. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291: 2141-2144.
- Kessler, A., and I.T. Baldwin. 2002. Plant responses to insect herbivory: the emerging molecular analysis. *Annual Review of Plant Biology* 53: 299-328.
- Kessler, A., and I.T. Baldwin. 2004. Herbivore-induced plant vaccination. Part I. The orchestration of plant defenses in nature and their fitness consequences in the wild tobacco *Nicotiana attenuata*. *The Plant Journal* 38: 639-649.
- Kester, K.M., and P. Barbosa. 1991. Postemergence learning in the insect parasitoid, *Cotesia congregata* Say (Hymenoptera: Braconidae). *Journal of Insect Behavior* 4: 727-742.
- Kombrik, E., and I.E. Somssich. 1995. Defense responses of plants to pathogens, Vol. 21: *Advances in Botanical Research* ed., pp. 1-34.
- Kuno, E. 1981. Dispersal and the persistence of populations in unstable habitats - a theoretical note. *Oecologia* 49: 123-126.

- Lessells, C.M. 1985. Parasitoid foraging: should parasitism be density dependent? *Journal of Animal Ecology* 54: 27-41.
- Levin, S.A., D. Cohen, and A. Hastings. 1984. Dispersal strategies in patchy environments. *Theoretical Population Biology* 26: 165-191.
- Mackauer, M., and W. Volkl. 1993. Regulation of aphid populations by aphidid wasps - does parasitoid foraging behavior or hyperparasitism limit impact? *Oecologia* 94: 339-350.
- Madden, A.H., and F.S. Chamberlin. 1945. Biology of the tobacco hornworm in the southern cigar-tobacco district. *Technical Bulletin* 896: 1-51.
- Mayer, R.T., T.G. McCollum, R.E. McDonald, J.E. Polston, and H. Doostdar. 1996. *Bemisia* feeding induces pathogenesis-related protein in tomato. Intercept Ltd. P., Andover.
- McFadden, M.W. 1968. Observations on feeding and movement of tobacco hornworm larvae. *Journal of Economic Entomology*. 61: 352-356
- McGuire, R.J., and M.T.J. Johnson. 2006. Plant genotype and induced responses affect resistance to herbivores on evening primrose (*Oenothera biennis*). *Ecological Entomology* 31: 20-31.
- Micha, S.G., and U. Wyss. 1995. The importance of plant odors for the host search of *Aphidius uzbekistanicus* (Hymenoptera, Aphididae), a parasitoid of the grain aphid (*Sitobion avenae*) (in German). *Gesunde Pflanzen* 47: 300-307.
- Morris, C.E. 1983. Uptake and metabolism of nicotine by the CNS of a nicotine-resistant insect, the tobacco hornworm (*Manduca sexta*). *Journal of Insect Physiology* 29: 807-817.
- Morris, C.E. 1984. Electrophysiological effects of cholinergic agents on the CNS of a nicotine-resistant insect, the tobacco hornworm (*Manduca sexta*). *Journal of Experimental Zoology* 229: 361-374.
- Negherbon, W.O. 1959. *Handbook of Toxicology*. W.B. Saunders Co, Philadelphia.
- Nieminen, M., J. Suomi, S. Van Nouhuys, P. Sauri, and M.L. Riekkola. 2003. Effect of iridoid glycoside content on oviposition host plant choice and parasitism in a specialist herbivore. *Journal of Chemical Ecology* 29: 823-844.
- Ode, P.J. 2006. Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. *Annual Review of Entomology* 51: 163-185.

- Reymond, P., and E.E. Farmer. 1998. Jasmonate and salicylate as global signals for defense gene expression. *Current Opinion in Plant Biology* 1: 404-411.
- Rodriguez-Saona, C., J.A. Chalmers, S. Raj, and J.S. Thaler. 2005. Induced plant responses to multiple damagers: differential effects on an herbivore and its parasitoid. *Oecologia* 143: 566-577.
- Rodriguez-Saona, C., S.J. Crafts-Brandner, L. Williams, and P.W. Pare. 2002. *Lygus hesperus* feeding and salivary gland extracts induce volatile emissions in plants. *Journal of Chemical Ecology* 28: 1733-1747.
- Rodriguez-Saona, C., and J.S. Thaler. 2005. The jasmonate pathway alters herbivore feeding behaviour: consequences for plant defences. *Entomologia Experimentalis et Applicata* 115: 125-134.
- Rosenheim, J.A., and M. Mangel. 1994. Patch-leaving rules for parasitoids with imperfect host discrimination. *Ecological Entomology* 19: 374-380.
- Rotem, K.A., and A.A. Agrawal. 2003. Density dependent population growth of the two spotted spider mite, *Tetranychus urticae*, on the host plant *Leonurus cardiaca*. *Oikos* 103: 559-565.
- Sisson, V.A., and R.F. Severson. 1990. Alkaloid composition of the *Nicotiana* species. *Beitrag Zur Tabakforschung International* 14: 327-339.
- Srinivasan, R., J.M. Alvarez, S.D. Eigenbrode, and N.A. Bosque-Perez. 2006. Influence of hairy nightshade *Solanum sarrachoides* (Sendtner) and Potato leafroll virus (Luteoviridae: Polerovirus) on the host preference of *Myzus persicae* (Sulzer) (Homoptera: Aphididae). *Environmental Entomology* 35: 546-553.
- Srinivasan, R., S. Utharnasamy, and N.S. Talekar. 2006. Characterization of oviposition attractants of *Helicoverpa armigera* in two solanaceous plants, *Solanum viarum* and *Lycopersicon esculentum*. *Current Science* 90: 846-850.
- Stout, M.J., K.V. Workman, R.M. Bostock, and S.S. Duffey. 1998. Specificity of induced resistance in the tomato, *Lycopersicon esculentum*. *Oecologia* 113: 74-81.
- Tabone, E., C. Bardon, B. Pintureau, and C. Alauzet. 2006. Importance of host oviposition pattern and plant size for the selection of *Trichogramma* strains to control the diamondback moth. *Entomologia Experimentalis et Applicata* 119: 47-51.
- Thaler, J.S. 1999. Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature* 399: 686-688.
- Thaler, J.S. 2002. Effect of jasmonate-induced plant responses on the natural enemies of

- herbivores. *Journal of Animal Ecology* 71.
- Thorpe, K.W., and P. Barbosa. 1986. Effects of consumption of high and low nicotine tobacco by *Manduca sexta* (Lepidoptera: Sphingidae) on survival of a gregarious endoparasitoid *Cotesia congregata* (Hymenoptera: Braconidae). *Journal of Chemical Ecology* 12: 1329-1337.
- Thurston, R., and P.M. Fox. 1972. Inhibition by nicotine of emergence of *Apanteles congregatus* from its host, the tobacco hornworm. *Annals of the Entomological Society of America* 65: 547-550.
- Turlings, T.C.J., M. Bernasconi, R. Bertossa, F. Bigler, G. Caloz, and S. Dorn. 1998. The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. *Biological Control* 11: 122-129.
- Van Dam, N.M., U. Hermenau, and I.T. Baldwin. 2001. Instar-specific sensitivity of specialist *Manduca sexta* larvae to induced defences in their host plant *Nicotiana attenuata*. *Ecological Entomology* 26: 578-586.
- van der Westhuizen, A., X.M. Qian, and A.M. Botha. 1998. 1-3-glucanases in wheat and resistance to the Russian wheat aphid. *Physiologia Plantarum* 103: 125-131.
- van der Westhuizen, A., X.M. Qian, and A. Botha. 1998. Differential induction of apoplastic peroxidase and chitinase activities in susceptible and resistant wheat cultivars by Russian wheat aphid infestation. *Plant Cell Reports* 18: 132-137.
- van Veen, F.J.F., R.J. Morris, and H.C.J. Godfray. 2006. Apparent competition, quantitative food webs, and the structure of phytophagous insect communities. *Annual Review of Entomology* 51: 187-208.
- Van Zandt, P.A., and A.A. Agrawal. 2004. Community-wide impacts of herbivore-induced plant responses in milkweed (*Asclepias syriaca*). *Ecology* 85: 2616-2629.
- Vidal, S., I.P. deLeon, J. Denecke, and E.T. Palva. 1997. Salicylic acid and the plant pathogen *Erwinia carotovora* induce defense genes via antagonistic pathways. *Plant Journal* 11: 115-123.
- Walde, S.J., and W.W. Murdoch. 1988. Spatial density dependence in parasitoids. *Annual Review of Entomology* 33: 441-466.
- Walling, L.L. 2000. The myriad plant responses to herbivores. *Journal of Plant Growth Regulation* 19: 195-216.
- Wibberley M.S., Lenton J.R., and Neill S.J. 1994. Sesquiterpenoid phytoalexins produced by hairy roots of *Nicotiana tabacum*. *Phytochemistry* 37: 349-351.

Wise, M.J., and A.M. Weinburg. 2002. Prior flea beetle herbivory affects oviposition preference and larval performance of a potato beetle on their shared host plant. *Ecological Entomology* 27: 115-122.

Wootton, T. 1994. The nature and consequences of indirect effects in ecological communities. *Annual Review of Ecological Systems* 25: 443-466.

Figures

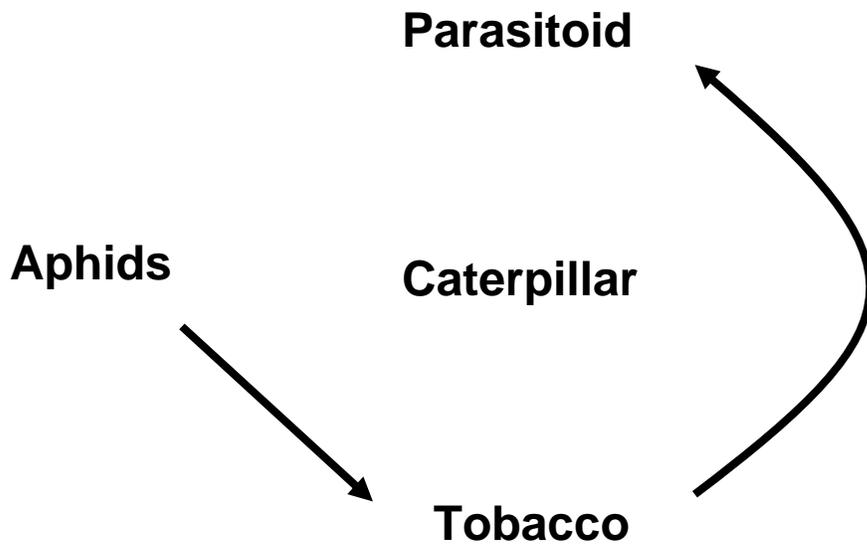
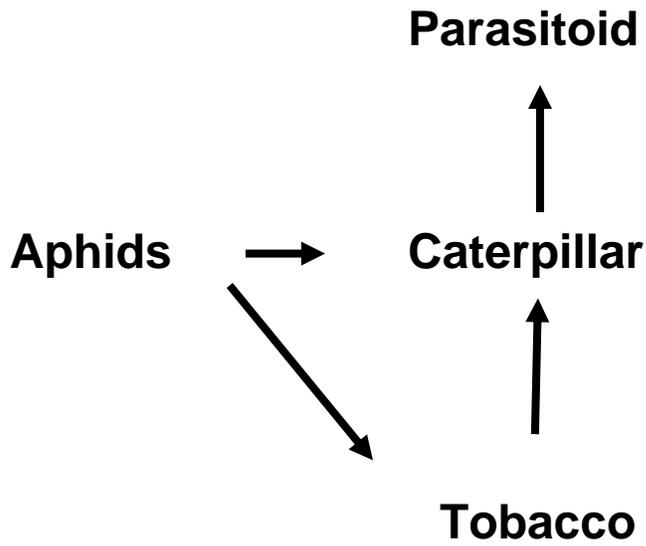


Figure 1. Alternative explanations of why aphids reduce parasitism. (A) represents a density-dependent mechanism where aphids reduce caterpillar populations via moth oviposition or host plant quality and therefore reduce parasitism. (b) represents a preference mechanism where aphids alter tobacco quality and therefore parasitoid preference.

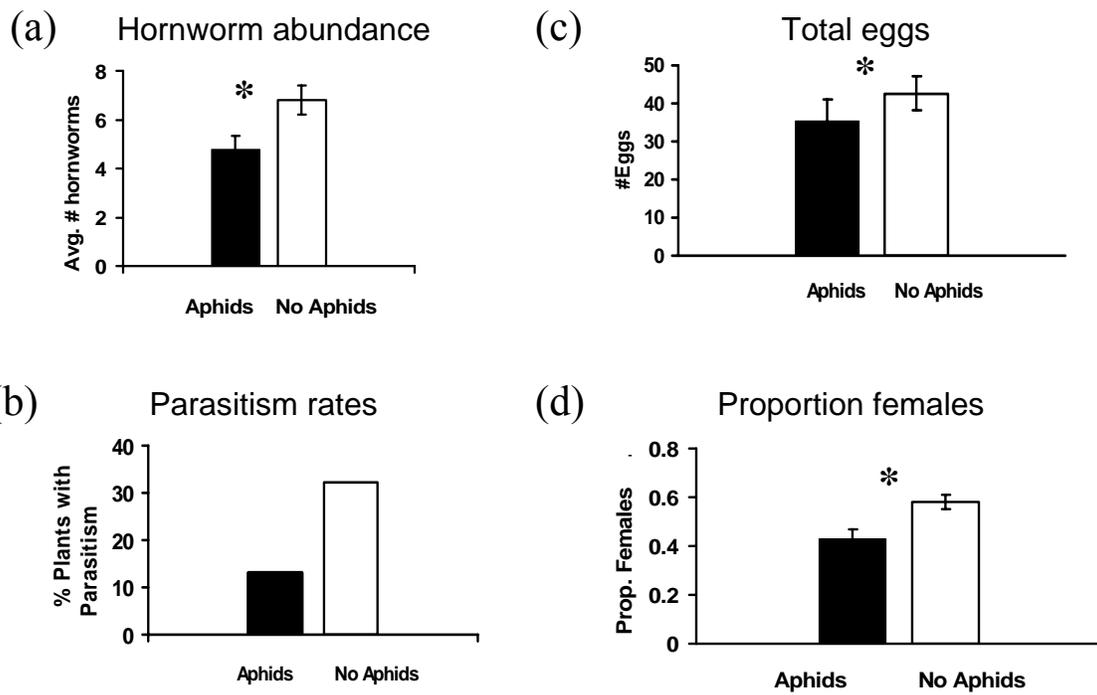


Figure 2. Aphid infestation reduced (a) hornworm abundance, (b) parasitism, (c) total parasitoid eggs and (d) sex ratio (proportion of female parasitoids) produced. Error bars represent means \pm standard error. * $P < 0.05$.

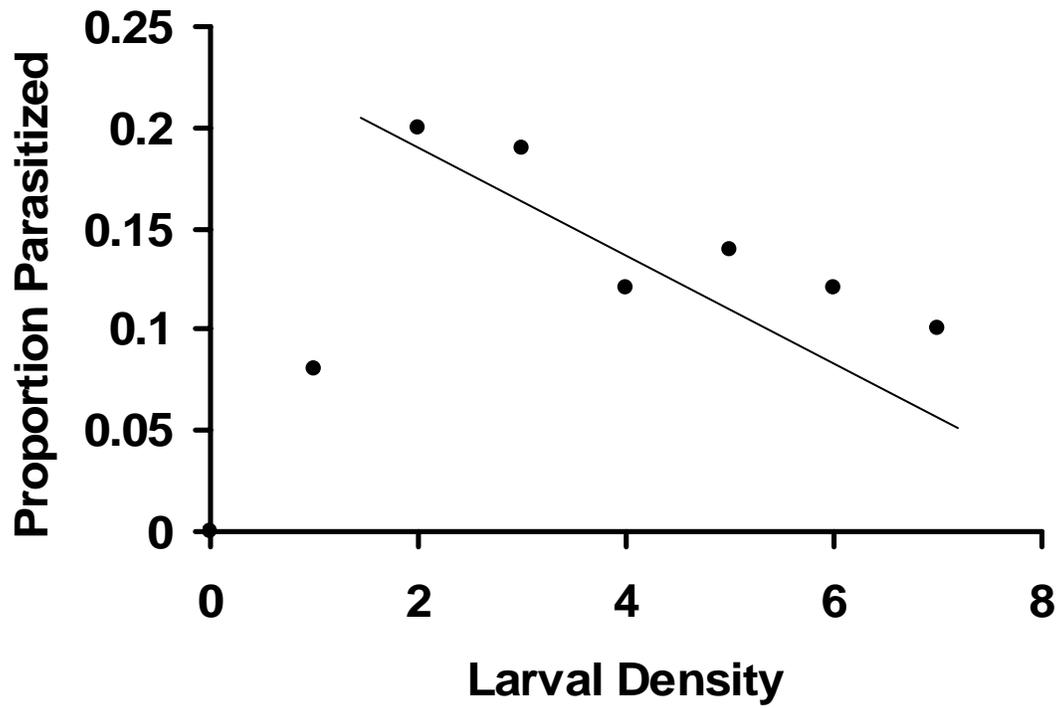


Figure 3. Inverse density-dependence of parasitism of *Manduca sexta* by the parasitoid *Cotesia congregata*.

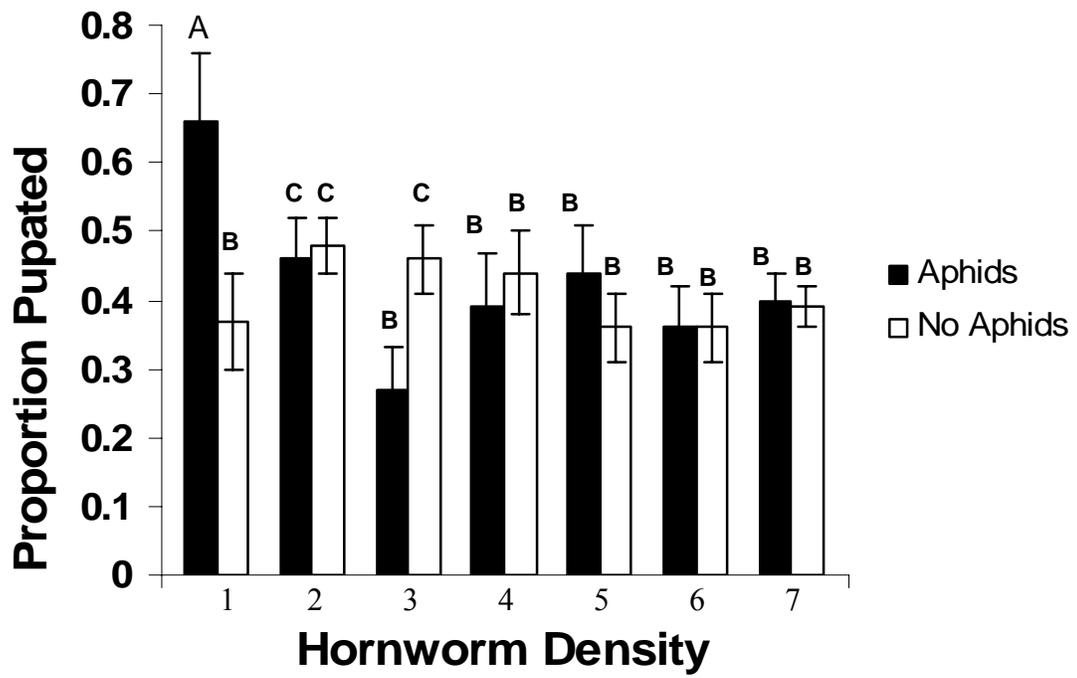


Figure 4. The interactive effect of aphid and density on *Manduca sexta* pupation. Bars with different letters represent significant differences.

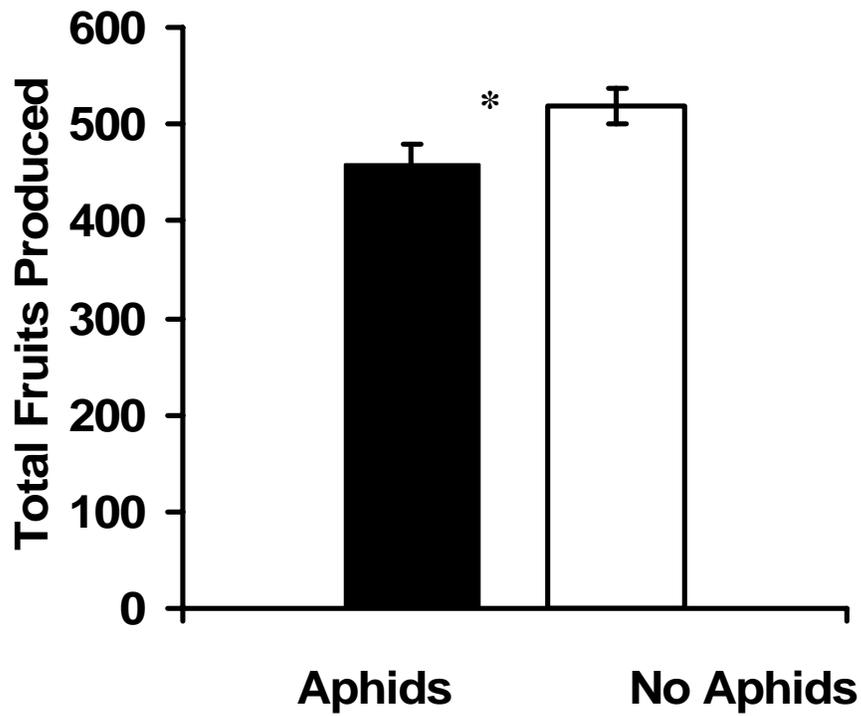


Figure 5. Aphid infestation reduces female plant reproduction in *Nicotiana tabacum*.

Error bars represent means \pm standard error. * $P < 0.05$.

Chapter 3: Aphids reduce preference and performance in parasitoid-caterpillar interactions

Abstract

Host plant quality plays a key role in mediating tritrophic interactions. Plants can influence both herbivores and natural enemies by changes in cuticular waxes, color, semiochemicals, and secondary compounds. The preference and performance of many parasitoids and their caterpillar hosts are generally examined in isolation of other species, even though the majority of host plants support complex faunas of herbivores and their natural enemies. The purpose of this study was to determine how aphids (*Myzus persicae*) feeding on tobacco (*Nicotiana tabacum*) affected the performance of a specialist herbivore, the tobacco hornworm (*Manduca sexta*), and its parasitoid (*Cotesia congregata*). Leaves from tobacco plants with or without aphids were mixed with artificial diet and fed to hornworms, a subsample of which was then parasitized by *C. congregata*. Performance parameters of hornworm and parasitoid larvae were measured. Parasitoid searching preferences were also measured by exposing females to leaf discs from tobacco leaves that were either attacked by aphids or without prior aphid attack and assessing the time spent in active antennal palpation. Aphids increased hornworm mortality and reduced weight gain in fifth instar hornworms. Aphids did not affect parasitoid performance, but parasitoids searched longer on leaf discs that had been previously attacked by aphids. Results suggest that aphids can mediate parasitoid-caterpillar interactions through changes in the host plant that reduce both hornworm survival and alter parasitoid behavior. These findings demonstrate the importance of

examining interactions in a multispecies context and emphasize the role of indirect interactions in structuring arthropod communities.

Introduction

Host plant quality plays a key role in mediating the presence and effectiveness of natural enemies. Plants can influence natural enemies through changes in morphology, such as cuticular waxes, pubescence, color or visual factors that may affect the natural enemies' ability to search or locate a host, or production of semiochemicals, such as attractants, repellents, inhibitors, and toxins, that provide cues to the natural enemy for host location (as reviewed by Bottrell et al. 1998). Plant response to herbivore damage can also induce changes that impact natural enemies, such as changes in secondary compounds that reduce natural enemy development and fitness (Barbosa et al. 1986; 1991; Havill and Raffa 2002; Thaler 2002; Hunter 2003) and changes in volatile emissions (Turlings et al. 1991; reviewed by Tumlinson et al. 1992) that influence foraging strategies.

One way in which herbivores can change plant quality is by the induction of plant defenses since different types of herbivore damage generally induce different plant defense pathways. Phloem-feeding insects induce the salicylic acid pathway (Broderick et al. 1997; Bronner et al. 1991; Mayer et al. 1996; van der Westhuizen et al. 1998 a; 1998 b; and Walling 2000) and the suite of induced plant responses differ from those elicited by chewing herbivores such as caterpillars (Stout et al. 1998a; Walling 2000). Induction of different plant pathways by multiple herbivore species may induce quantitative and qualitative changes in plant defense compounds that affect herbivores

and natural enemies differently than induction by a single herbivore species (Kessler and Baldwin 2004; Walling 2000).

Herbivores can also change plants by altering volatile emissions. Changes in volatile emissions may affect the interactions between host plants, herbivores, and natural enemies through changes in preferences or host-finding ability. Plants release volatiles in response to herbivore damage that are attractive to parasitoids (Turlings et al. 1995; Havill and Raffa 2000; review by Nordlund et al. 1988) which can increase parasitism in the field by two-fold (Thaler 1999). Volatile emissions depend on both the herbivore species and host plant species (Tumlinson et al. 1991; Shiojiri et al. 2001). For example, both maize and tobacco produce distinct volatile blends in response to damage by two closely related herbivore species, the corn earworm and tobacco budworm, and the specialist parasitoid *Cardiochiles nigriceps* can distinguish between these differing blends produced by the same plant species (De Moraes et al. 1998). Behavioral studies indicate that some phloem-feeding herbivores also induce the release of plant volatiles (Guerra et al. 1993; Micha and Wyss 1995; Du et al. 1996; Turlings et al. 1998) that differ from blends released by chewing herbivores (Rodriguez-Saona et al. 2002). Thus, volatile emissions may differ depending on a plant's herbivore community, and altered volatiles may differentially attract natural enemies.

Many studies have examined tritrophic interactions between a host plant, herbivore, and natural enemy in isolation of other species. However, attack by a single herbivore species in nature is rare, and even relatively simple insect communities consist of multiple herbivore species attacking a single host plant (Vos et al. 2001; Thaler et al. 2001; Rodriguez-Saona et al. 2005; Johnson and Agrawal 2005; Johnson et al. 2006).

Understanding the impacts of multiple herbivore species broadens our knowledge of the different factors that impact parasitoid foraging strategy and host use. Changes in foraging strategy and host use can alter community structure and our understanding of these changes can provide insight into biological control strategies.

To determine if the presence of a secondary herbivore can impact herbivore-natural enemy interactions, a field study (Lentz unpublished) manipulated the presence of aphids (*Myzus persicae*) on *Nicotiana tabacum* and demonstrated that aphids reduced abundance of the tobacco hornworm, *Manduca sexta* and parasitism by its specialist parasitoid, *Cotesia congregata*. While there are many mechanisms that might explain this result, the purpose of this study was to examine two hypotheses: aphids alter host plant quality, which in turn reduces hornworm and parasitoid fitness, and aphids alter parasitoid searching preferences. Specifically, I asked how changes in plant quality due to aphids could affect: 1) hornworm performance, 2) parasitoid performance, and 3) parasitoid search preference. The results of this study will help to determine the mechanisms that explain why aphids decrease hornworm abundance and parasitism rates on tobacco plants.

Methods

Study System: *Nicotiana tabacum* L. (Solanaceae, NC95 variety, ‘tobacco’ hereafter) is a cultivated annual species that supports a wide range of herbivorous insects (McFadden 1968). One of the most damaging herbivores is the tobacco hornworm, *Manduca sexta* L. (Lepidoptera: Sphingidae), a specialist whose feeding severely impacts wild tobacco fitness (Heil and Baldwin 2002; Kessler and Baldwin 2004). Another common herbivore

is *Myzus persicae* Sulzer (Homoptera: Aphidiidae), the green peach or tobacco aphid. Aphids are phloem-feeding herbivores that vector diseases and can cause curling or wilting of host plants (Borrer et al. 1989).

Nicotiana species are defended by a diverse group of secondary compounds, including alkaloids, phenolics, and terpenoids (Saitoh et al. 1985, Sisson and Severson 1990, Snook et al. 1986, Snook et al. 1997, Keinanen et al. 2001). Tobacco hornworms are specialists on tobacco and have a relatively high tolerance to nicotine (Hansberry and Midalekauff 1940; Negherbon 1959; Morris 1983, 1984; Glendinning 2002) and other associated compounds.

Hornworm populations can be controlled by a number of natural enemies including parasitic and predaceous wasps, spiders, and birds (McFadden 1968). *Cotesia congregata* Say (Hymenoptera: Braconidae) is a gregarious endoparasitoid of the tobacco hornworm and other sphingid larvae (Krombein et al. 1979). Its occurrence is widespread across the United States as an important parasitoid of the tobacco and tomato hornworm. Female parasitoids attack 3rd-instar hornworms which results in the death of the host. *Cotesia congregata* exhibits haplo-diploid reproduction; eggs fertilized with stored sperm produce diploid females and unfertilized eggs produce haploid males. Female parasitoids can directly manipulate both the number and sex of offspring (Kester and Barbosa, 1991; Lentz and Kester, in review).

General Methods: Stock tobacco seed was germinated in vermiculite in a glasshouse and transplanted 2 weeks later into 6-cell flats containing Pro-Mix BX soil (Premier Horticulture Ltd., Red Hill, Pennsylvania, USA) in April of 2004 and 2006. Seedlings were transplanted into 3.8 L pots and fertilized three times using Peter's 20-20-20

(Scotts-Sierra Horticulture Products Co., Marysville, Ohio, USA, 14.235 g/3.8 L water) or transplanted without fertilizer to a field plot at the Virginia Tech Agricultural Research and Extension Center in Blackstone, VA.

Plants were assigned randomly to aphid treatment groups (presence or absence). Aphid treatments were maintained on greenhouse plants by adding aphids from a wild population collected from a field site (Kentland Farm, Montgomery Co., VA) (presence) and maintained by removing aphids with water every two days (absence). In the field plants were not inoculated with aphids since natural aphid populations were extremely high. In the aphid absence treatment, naturally established aphids were removed bi-weekly using packaging tape (Scotch, tear by hand, USA), as water removal was not effective on a large scale. To control for the effects of tape residue, plants in the aphid presence treatment were also taped, although to a much lesser extent. Aphid density was not quantified since populations were extremely dense (~ 200 per leaf) on both field and greenhouse tobacco.

Hornworms (North Carolina State University Insectary) were fed on synthetic diet (tobacco hornworm diet F9783B, Bioserv, Inc. Frenchtown, NJ, USA) that consisted of 140 g of dry diet mixed with 80 g of plant material taken from greenhouse plants in the aphid presence or absence treatments. Leaf material was taken from the upper 3rd portion of several tobacco plants, aphids (when present) and leaf midribs were removed, and the leaves were blended with liquefied agar until a uniform mixture was produced. The liquid was mixed with the dry diet and allowed to cool. Diet was stored at 4 °C for a maximum of 5 days. Hornworm diet was mixed with plant material because previous attempts to maintain hornworms throughout development on greenhouse plants failed. Hornworms

from the same colony are able to reach pupation successfully on field plants. The mechanism responsible for the differences in survival on greenhouse and field plants is not known, but may reflect changes in plant material quality due to lighting and potting conditions. Feeding hornworms mixed diet is likely to decrease rather than increase the effect of aphids on hornworm performance since synthetic diet contains all of the essential nutrients for healthy insects. Thus, the greenhouse performance experiment represents a conservative test of the effect of aphids on hornworm performance.

Hornworms were not put in the field due to the difficulty of controlling the many other variables that may affect hornworm performance on field plants, such as damage by other herbivore species and attack by natural enemies. Further, the control of other herbivore species by pesticides or caging could lead to other potential artifacts.

Hornworms were reared individually in 170 g Sweetheart cups with plastic lids (Acme Paper Supply Co., Richmond, VA, USA) and were allowed to feed *ad libitum* until pupation. Diet was changed daily to maintain freshness. Photoperiod was kept at 14.5 h L: 9.5 h D with a 1.22 m full spectrum GE Plant and Aquarium light, with supplemental indirect natural light from a nearby window. Temperature varied from 23 - 24 °C.

Wild parasitoids were collected from hornworms found on field tobacco plants in Blackstone, VA. Individual parasitoid cohorts (the group of offspring from one female) were reared separately in plastic containers supplied with honey and water. Newly emerged females were given 24 hr to mature eggs.

I. Do changes in plant quality due to aphids affect hornworm performance?

The purpose of this experiment was to determine if aphid presence decreased hornworm performance through changes in host plant quality. On August 15, 2004, eighty 2nd instar hornworms were weighed and randomly assigned to diet made with leaf material from plants with or without aphids (n=40; n= per treatment throughout). Hornworms were weighed at the beginning of each new instar (3rd – 5th) to the nearest 0.1 mg and again one day after pupation. Mortality, time until pupation, and pupal sex were noted (weight is dimorphic in this species; Madden and Chamberlain 1945).

Data Analyses: All analyses were conducted using SAS version 9.1. The effect of aphids on hornworm performance (weight of individual instars and pupal weight) was analyzed using a repeated measures 2-way MANCOVA. Aphid presence / absence and moth sex were main effects, initial hornworm weight was a covariate, and instar and pupal weight were dependent variables. A separate ANCOVA determined if time to pupation was affected by aphids. Hornworm mortality was assessed as yes / no over the experiment and analyzed using a Chi-square test. Data met the assumptions for normality.

II. Do changes in plant quality due to aphids affect parasitoid preference?

The purpose of this experiment was to examine the effect of aphid presence on the searching responses of mated female parasitoids, since aphids could affect host plant olfactory cues and thus parasitoid foraging preferences. Female parasitoids were offered leaf discs collected from field tobacco plants that had been attacked by aphids or had not

been attacked by aphids (n = 25). Aphids were removed from leaf discs prior to assaying searching responses.

Tobacco leaves were collected the day of testing from the upper 3rd portion of several field plants. Stems were placed in water and the plant material was transported in an ice-filled cooler to prevent degradation. Wild parasitoid cocoons were collected from the same site and held in emergence cages as described previously, except multiple cohorts were mixed in each cage and females were given 48 hr to mate.

Experimental design and procedures were based on those developed previously (Kester and Barbosa 1991). At the time of testing, an individual female was selected randomly from each cage and transferred to a glass shell vial. Searching responses were measured as an indication of olfactory preference (Kester and Barbosa 1991) for the volatiles that are released from plants as a result of herbivore damage. Searching responses were assayed as the time spent in active antennal palpation on the surface of a 0.8-cm leaf disc within a 2-min trial. Each female was tested once; females that did not search were excluded from analysis. Testing occurred between 10 a.m. and 12:30 p.m., a time that is peak for searching (as found in previous experiments using the same searching assay, Kester and Barbosa, 1991) with an ambient temperature of 30.5 ° C.

Data analysis: The main effect of aphid presence on parasitoid search time was assessed using ANOVA. Data were log (x + 1) transformed to achieve normality and meet parametric assumptions.

III. Do changes in plant quality due to aphids affect parasitoid performance?

The purpose of this experiment was to determine if aphids reduced parasitoid performance through changes in caterpillar-host quality. Clutch size (the total number of larvae oviposited) and time to develop were recorded on hornworm hosts that had been feeding on a diet mixed with aphid presence or absence leaf material. Clutch size was determined by adding the number of larvae that emerged from the host with the number that did not emerge, determined by dissection after parasitoid emergence. Also, the number of larvae that emerged from the host, spun cocoons, and emerged as adults, were determined for each clutch.

Sixty-eight hornworm eggs were reared on a plant-free diet until the 3rd instar to standardize host quality at the time of oviposition. The ovipositional decisions of parasitoids are affected by host weight and quality (Waage 1986; Godfray 1994) and if hornworms were fed on treatment diets they may vary in weight and quality, which could influence parasitoid preference and clutch sizes. Individual hornworms were weighed, parasitized, and randomly assigned to diet made with leaves from plants with or without aphids (n=34). At the time of testing, a naive unmated female was randomly selected from each cohort (a total of 5 cohorts with ~7 females used in each treatment) and transferred to a 3.7 mL glass shell vial with a cork stopper (cat# 8641, BioQuip, Rancho Dominguez, CA, USA) and allowed a single uninterrupted oviposition. Hornworms were reared until pupation or parasitoid larvae egressed, and then dissected to determine the number of larvae that did not emerge. Parasitoid performance was assessed as the total number of larvae oviposited, time to develop, the proportion of the total clutch size that emerged from the host, proportion of the clutch that spun cocoons, and proportion of the clutch that emerged from cocoons.

Data Analyses: The effects of aphid presence (fixed effect), larval weight (covariate), cohort (random effect) and their interactions on parasitoid larval performance were analyzed using mixed model analyses (SAS PROC MIXED, SAS-Institute 1999). Hornworm weight was used as a covariate since host weight can impact parasitoid choices and development (King 1987). Degrees of freedom for F-tests of fixed effects were estimated using the Satterthwaite approximation (SAS-Institute 1999). A likelihood-ratio χ^2 test was used for tests of random effects. The likelihood-ratio χ^2 test is a one-sided single degree of freedom test of the hypothesis that the variation caused by the random effect is greater than zero (Shaw 1987; Shaw et al. 1995; Orians et al. 1996; Roche and Fritz 1997; and Agrawal 1998). Each dependent variable was run in a separate model. Proportional data (proportion of the total that emerged from the host, proportion of the total that spun cocoons, and proportion of the total that emerged from cocoons) were transformed using arcsine(sqrt(x)) and time to develop (in days) was transformed using the log(x + 1) transformation to meet normality assumptions for parametric analyses.

Results

I. Do changes in plant quality due to aphids affect hornworm performance?

Aphid presence reduced hornworm performance. Hornworms had higher mortality when fed a synthetic diet mixed with tobacco leaves from plants attacked by aphids compared to those fed a diet mixed with leaves from plants that were not attacked by aphids ($\chi^2 = 7.67$, $p = 0.006$; with aphids 8.75 % mortality; without aphids 0.0 %

mortality) (Fig. 1a). Aphids had a significant effect on hornworm performance, but this effect changed with larval development (MANCOVA, repeated measures test for between subject effect: $F_{1,67} = 8.18$, $p = 0.006$; treatment * instar effect: Wilk's $\lambda = 0.86$, $F_{3,65} = 3.55$, $p = 0.02$). Univariate analyses showed that aphids had a significant impact on 5th instar hornworm weight (ANCOVA, treatment: $F_{1,67} = 6.93$, $p = 0.01$) (Fig. 1b) but did not affect other larval instars or pupal weight ($p > 0.11$ for all).

II. Do changes in plant quality due to aphids affect parasitoid preference?

Aphid presence had a significant effect on parasitoid searching times (ANOVA, treatment effect: $F_{1,48} = 6.01$, $p = 0.02$; Fig. 2). Female wasps searched almost twice as long on aphid damaged leaf discs as on leaf discs that had not been aphid damaged (mean \pm s.e.: aphid presence: 15.55 ± 2.48 s; aphid absence: 9.32 ± 1.46 s).

III. Do changes in plant quality due to aphids affect larval parasitoid performance?

Aphid presence did not impact parasitoid performance (individual ANCOVAs: total emerged from the host, time to develop, proportion that emerged, proportion that spun cocoons, proportion of total that emerged from cocoons, fixed effect aphid: $F < 0.59$ for all, $P > 0.42$ for all; covariate weight *aphid interaction: $F < 0.65$ for all, $P > 0.42$ for all). The weight covariate had a significant effect on the time it took parasitoids to develop (ANCOVA, weight: $F_{1,69} = 4.51$, $p = 0.04$), parasitoids developing in caterpillars weighing 0.2 – 0.3 g developed faster than other weight categories. Weight did not affect any other parasitoid performance parameter ($F < 4.01$ for all, $P > 0.05$ for all). Further, the random effect of cohort, and the interactions of cohort with the main

effect and covariate were not significant for any of the parasitoid performance parameters, indicating that genetic variation between cohorts did not influence parasitoid performance (for all variables, $dF = 1$: random effect of cohort: $\chi^2 < 0.1$ for all, $P > 0.33$ for all; cohort*aphid: $\chi^2 < 1.8$ for all, $P > 0.09$ for all; cohort*weight: $\chi^2 = 0$ for all, $P = 0.5$ for all; and cohort*aphid*weight: $\chi^2 < 0.1$ for all, $P > 0.38$ for all).

Discussion

Prior field work has shown that the aphid *Myzus persicae* has a negative impact on hornworm abundance, frequency of parasitism, and parasitoid performance (Lentz, unpublished data). One mechanism that might explain these findings is changes in host plant quality due to aphids. The results of this study demonstrate that aphids can impact hornworm-parasitoid interactions through changes in host plant quality that negatively affect hornworms but do not influence parasitoid performance. However, aphids may indirectly influence parasitoids by reducing hornworm populations.

Hornworm mortality and 5th instar larval weight were negatively affected by incorporating tobacco leaves attacked by aphids into hornworm diet. A similar reduction in hornworm performance due to phloem-feeding insects has been found in the lab with the sister species *Manduca quinquemaculata* where feeding by the mirid *Tupiocoris notatus* on wild tobacco, *Nicotiana attenuata*, reduced hornworm growth and performance (Kessler and Baldwin 2004). Further, pupal weight of the potato beetle, *Leptinotarsa juncta* (Germar), was not affected by prior flea beetle damage (*Epitrix fuscula* Crotch) on *Solanum carolinense*, but larvae on damaged plants took 8% longer to reach the pupal stage (Wise and Weinberg 2002). In contrast, feeding by the aphid

Macrosiphum euphorbiae on *Lycopersicon esculentum* (tomato) had a positive effect on larval weight, and did not affect mortality or developmental time of the beet armyworm, *Spodoptera exigua* (Rodriguez-Saona et al. 2005). Although four studies, all on Solanaceous hosts, are too few to permit generalization, these results suggest that phloem-feeding herbivores often reduce performance of chewing herbivores, as is the case in this system. A decline in hornworm survival may reduce the hornworm population, which could negatively affect both hornworms and parasitoids by reducing the availability of hosts.

Changes in hornworm survival may also have consequences for the host plant. Increased hornworm mortality due to aphids may benefit the plant since hornworms are a highly damaging herbivore that dramatically reduces plant fitness (Kessler and Baldwin 2004). However, there are costs associated with heavy aphid attack such as reducing overall parasitoid attack and tobacco fruit production (Lentz unpublished). Reduced tobacco fitness due to aphids could be caused by significant declines in gas exchange, chlorophyll fluorescence (Macedo et al. 2003), and reduced photosynthetic and transpiration rates (Shannag et al. 1998). Or, as has been found in wild parsnip (*Pastinaca sativa*) / parsnip webworm system, reduced fitness could be due to a loss of resources from herbivore feeding, or use of resources to produce secondary plant compounds (Zangerl et al. 2002). But overall, the costs of aphid feeding may be less than the benefit it provides by reducing hornworm density. Hornworms are the seemingly more damaging of the two herbivores; a single caterpillar can defoliate a full grown tobacco plant in less than two weeks time (McFadden 1968). These differences for plant fitness underscore the importance of studying plant-herbivore interactions within a

community context rather than in isolation, since different herbivore species can differentially impact one another and the host plant. Ultimately, the effect of multiple herbivore species on the host plant will depend on how each species affects host plant quality, and indirectly depend on how feeding by one herbivore species can increase or reduce feeding by another herbivore species and how these interactions affect plant fitness (Van Zandt and Agrawal 2004; Agrawal 2005; Agrawal et al. 2006).

Females of *C. congregata* searched longer on aphid-damaged leaf discs than on discs that had not been damaged. Herbivore attack can induce the production of volatile compounds that provide chemical information regarding the presence of herbivores that are feeding on a plant. Many parasitoid species are attracted to these emissions even though they are not always reliable (Dicke and Sabelis 1988; 1989; 1992; Turlings et al. 1990; 1991 a; 1991 b; 1993 a; 1993 b; 1995; Wiskerke and Vet 1994; McCall et al. 1993; 1994). Behavioral studies have also suggested that aphids induce the production of volatiles (Guerrieri et al. 1993; Micha and Wyss 1995; Du et al. 1996; Powell et al. 1998), including feeding by the aphid *Myzus persicae* on *Arabidopsis thaliana* Columbia (Brassicaceae) (Girling et al. 2006). In the present study, parasitoid attraction could be attributed to volatiles or other changes in host plant quality such as pheromones, honeydew secretions, or visual changes in the plant surface. These results seemingly contradict prior field results (AJL, unpublished), where there was an increase in parasitism on plants that did not have aphids. However, parasitoid reproduction involves a series of successive steps, beginning with the location of a suitable host through the use of host plant volatiles (Vinson 1998). Increased parasitoid attraction to leaf discs previously infested with aphids may simply reflect attraction to any herbivore damage

versus no herbivore damage. Further, even if a parasitoid locates a host on an inferior plant, it still must decide if the host is suitable (Vinson 1998) by the use of cues that are usually directly associated with the host. Therefore, increased attraction to plants previously attacked by aphids does not necessarily indicate the parasitoid will accept a host found on that plant. A foraging female can choose to reject a poor quality host, or allocate fewer offspring and females to these hosts, as may be the case for those caterpillars that feed on aphid-damaged plants. A reduction in host use, and total eggs and proportion of females allocated to hosts on aphid damaged plants has been found in the field (AJL, unpublished).

Parasitoid performance did not differ between hosts fed on diet with leaves from plants with or without aphids. Similar results were found with the endoparasitoid *Cotesia marginiventris* that attacks the beet armyworm, *Spodoptera exigua*. Parasitoid mortality, developmental time, and pupal mass did not differ between parasitoid larvae that developed on hosts feeding on leaf material previously attacked by the aphid *Macrosiphum euphorbiae* or on control leaf material (Rodriguez-Saona et al. 2005). The lack of effect of aphids on parasitoid performance in the laboratory should be interpreted cautiously. The effects of aphids on hornworm host quality may be greater when the host feeds only on leaf material throughout development. In the current experiment, caterpillars fed solely on a synthetic nutrient-rich diet until the third instar, and then a diet mixed with leaf material. Therefore, hornworm hosts were not subjected to secondary compounds as much as they would in a natural setting, particularly during the critical age of development as younger instars. Field studies have demonstrated that parasitoid preference and performance is reduced in wild caterpillars that fed only on leaf material

previously attacked by aphids during development (Lentz, unpublished), but these effects may have been mediated by host size, age, weight and parasitoid age.

There is significant evidence demonstrating that plant chemical defenses generally reduce parasitoid fitness (reviewed by Ode 2006). Larvae of *Cotesia congregata* suffered increased mortality when the secondary compound nicotine was added to synthetic diet of their host, *Manduca sexta* (Barbosa et al. 1991; Barbosa et al. 1986; Thurston and Fox 1972). In the field, *C. congregata* suffered increased mortality when developing in hosts that fed on *Nicotiana tabacum* that was high in nicotine (Thorpe and Barbosa 1986). Further, developing parasitoids (*Hyposoter exiguae*) in the host *Heliothis zea* suffer reduced eclosion rates, size, and longevity when host diet included the alkaloid tomatine (Campbell and Duffey 1979; 1981). Thus, larvae of *C. congregata* may be impacted by aphid-induced plant defenses when exposed to these compounds in as high of levels as would be experienced in the field.

Previous research has demonstrated that aphids reduced hornworm abundance and parasitism in the field (Lentz, unpublished) and the current study suggests that the mechanism responsible for these findings is changes in host plant quality. Aphids can mediate parasitoid-hornworm interactions through changes in host plant quality that negatively impact hornworm survival. Even though there were no direct effects of host plant quality on wasp performance, aphids can negatively through changes in the host population that result from the reduction in hornworm survival. These findings demonstrate the importance of examining interactions in a multispecies context and emphasize the role of indirect interactions in structuring arthropod communities.

Literature Cited

- Barbosa P., P. Gross, and J. Kemper. 1991. Influence of plant allelochemicals on the tobacco hornworm and its parasitoid, *Cotesia congregata*. *Ecology* 72: 1567-1575.
- Barbosa P., J.A. Saunders, J. Kemper, R. Trumbule, J. Olechno, and P. Martinat. 1986. Plant allelochemicals and insect parasitoids: effects of nicotine on *Cotesia congregata* Say (Hymenoptera: Braconidae) *Hyposter Annulipes* (Cresson) (Hymenoptera: Ichneumonidae). *Journal of Chemical Ecology* 12: 1319-1328.
- Bottrell, D.G., P. Barbosa, and F. Gould. 1998. Manipulating natural enemies by plant variety selection and modification: a realistic strategy? *Annual Review of Entomology* 43: 347-367.
- Havill, N.P., and K.F. Raffa. 2002. Effects of gypsy moth (Lepidoptera: Lymantriidae) laboratory strain and crowding on emergence of the parasitoid *Cotesia melanoscelus* (Hymenoptera: Braconidae). *Entomological News* 113: 197-202.
- Hunter, M.D. 2003. Effects of plant quality on the population ecology of parasitoids. *Agricultural and Forest Entomology* 5: 1-8.
- Thaler, J.S. 2002. Effect of jasmonate-induced plant responses on the natural enemies of herbivores. *Journal of Animal Ecology* 71: 141-150.
- Thaler, J.S., A.L. Fidantsef, S.S. Duffey, and R.M. Bostock. 1999. Trade-offs in plant defense against pathogens and herbivores: A field demonstration of chemical elicitors of induced resistance. *Journal of Chemical Ecology* 25: 1597-1609.
- Thaler, J.S., M.J. Stout, R. Karban, and S.S. Duffey. 2001. Jasmonate-mediated induced plant resistance affects a community of herbivores. *Ecological Entomology* 26: 312-324.
- Thorpe, K.W., and P. Barbosa. 1986. Effects of consumption of high and low nicotine tobacco by *Manduca sexta* (Lepidoptera: Sphingidae) on survival of the gregarious endoparasitoid *Cotesia congregata* (Hymenoptera: Braconidae). *Journal of Chemical Ecology* 12: 1329-1337.
- Thurston, R., and P.M. Fox. 1972. Inhibition by nicotine of emergence of *Apanteles congregatus* from its host, the tobacco hornworm. *Annals of the Entomological Society of America* 65: 547-550.
- Tumlinson, J.H. 1992. Semiochemicals that increase the efficiency of beneficial insect parasitoids. *Abstracts of Papers of the American Chemical Society* 203: 174-AGRO.

- Tumlinson, J.H., T.C.J. Turlings, and W.J. Lewis. 1991. The semiochemical complexes that mediate insect parasitoid foraging. *Agricultural Zoology Reviews* 5: 221-253.
- Tumlinson, J.H., T.C.J. Turlings, and W.J. Lewis. 1993. Semiochemically mediated foraging behavior in beneficial parasitic insects. *Archives of Insect Biochemistry and Physiology* 22: 385-391.
- Turlings, T.C.J., and B. Benrey. 1998. Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience* 5: 321-333.
- Turlings, T.C.J., M. Bernasconi, R. Bertossa, F. Bigler, G. Caloz, and S. Dorn. 1998a. The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. *Biological Control* 11: 122-129.
- Turlings, T.C.J., U.B. Lengwiler, M.L. Bernasconi, and D. Wechsler. 1998b. Timing of induced volatile emissions in maize seedlings. *Planta* 207: 146-152.
- Turlings, T.C.J., J.H. Loughrin, P.J. McCall, U.S.R. Rose, W.J. Lewis, and J.H. Tumlinson. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America* 92: 4169-4174.
- Turlings, T.C.J., P.J. McCall, H.T. Alborn, and J.H. Tumlinson. 1993. An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *Journal of Chemical Ecology* 19: 411-425.
- Turlings, T.C.J., J.W.A. Scheepmaker, L.E.M. Vet, J.H. Tumlinson, and W.J. Lewis. 1990a. How contact foraging experiences affect preferences for host-related odors in the larval parasitoid *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae). *Journal of Chemical Ecology* 16: 1577-1589.
- Turlings, T.C.J., and J.H. Tumlinson. 1991. Do parasitoids use herbivore-induced plant chemical defenses to locate hosts? *Florida Entomologist* 74: 42-50.
- Turlings, T.C.J., J.H. Tumlinson, F.J. Eller, and W.J. Lewis. 1991. Larval-damaged plants - source of volatile synomes that guide the parasitoid *Cotesia marginiventris* to the microhabitat of its hosts. *Entomologia Experimentalis et Applicata* 58: 75-82.
- Turlings, T.C.J., J.H. Tumlinson, and W.J. Lewis. 1990b. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251-1253.
- van der Westhuizen, A.J., X.M. Qian, and A.M. Botha. 1998a. Beta-1, 3-glucanases in wheat and resistance to the Russian wheat aphid. *Physiologia Plantarum* 103: 125-131.

- van der Westhuizen, A.J., X.M. Qian, and A.M. Botha. 1998b. Differential induction of apoplastic peroxidase and chitinase activities in susceptible and resistant wheat cultivars by Russian wheat aphid infestation. *Plant Cell Reports* 18: 132-137.
- Van Zandt, P.A., and A.A. Agrawal. 2004. Community-wide impacts of herbivore-induced plant responses in milkweed (*Asclepias syriaca*). *Ecology* 85: 2616-2629.
- Vos, M., S.M. Berrocal, F. Karamaouna, L. Hemerik, and L.E.M. Vet.. 2001. Plant-mediated indirect effects and the persistence of parasitoid-herbivore communities. *Ecology Letters* 4: 38-45.
- Waage J.K. 1986. *Insect Parasitoids*. Academic Press, London.
- Walling, L.L. 2000. The myriad plant responses to herbivores. *Journal of Plant Growth Regulation* 19: 195-216.
- Wise, M.J., and A.M. Weinberg. 2002. Prior flea beetle herbivory affects oviposition preference and larval performance of a potato beetle on their shared host plant. *Ecological Entomology* 27: 115-122.
- Wiskerke, J.S.C., and L.E.M. Vet. 1994. Foraging for solitary and gregariously feeding caterpillars- a comparison of 2 related parasitoid species (Hymenoptera: Braconidae). *Journal of Insect Behavior* 7: 585-603.
- Zangerl, A.R., J.G. Hamilton, T.J. Miller, A.R. Crofts, K. Oxborough, M.R. Berenbaum, and E.H. de Lucia. 2002. Impact of folivory on photosynthesis is greater than the sum of its holes. *Proceedings of the National Academy of Sciences of the United States of America* 99: 1088-1091.

Figures

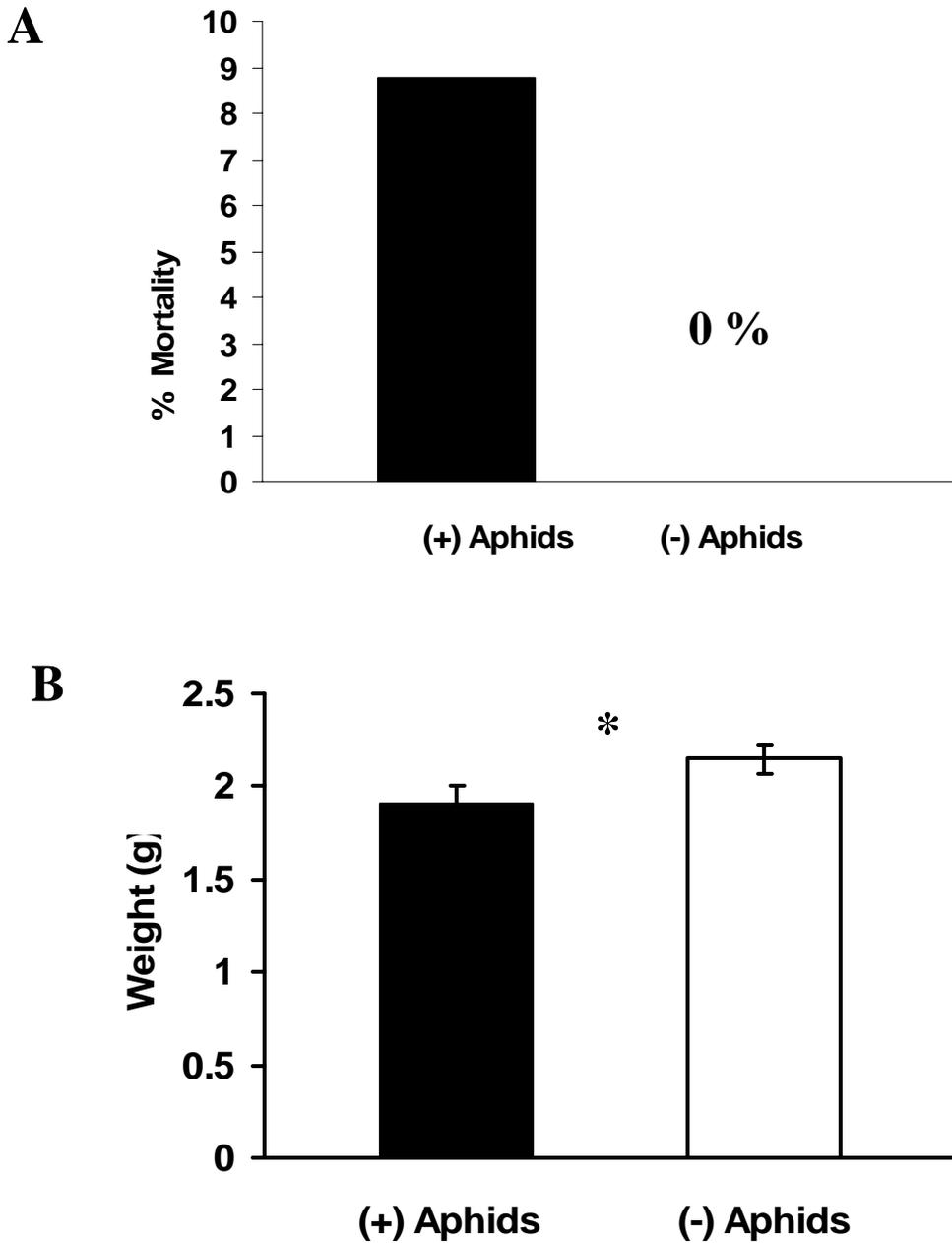


Figure 1. The increase in hornworm mortality (Fig. 1 A) and reduction in 5th instar hornworm weight (Fig. 1 B) due to feeding on diet mixed with leaf material previously attacked by aphids. Bars represent percent mortality (Fig. 1 A) and error bars represent means \pm standard error. * $P < 0.05$.

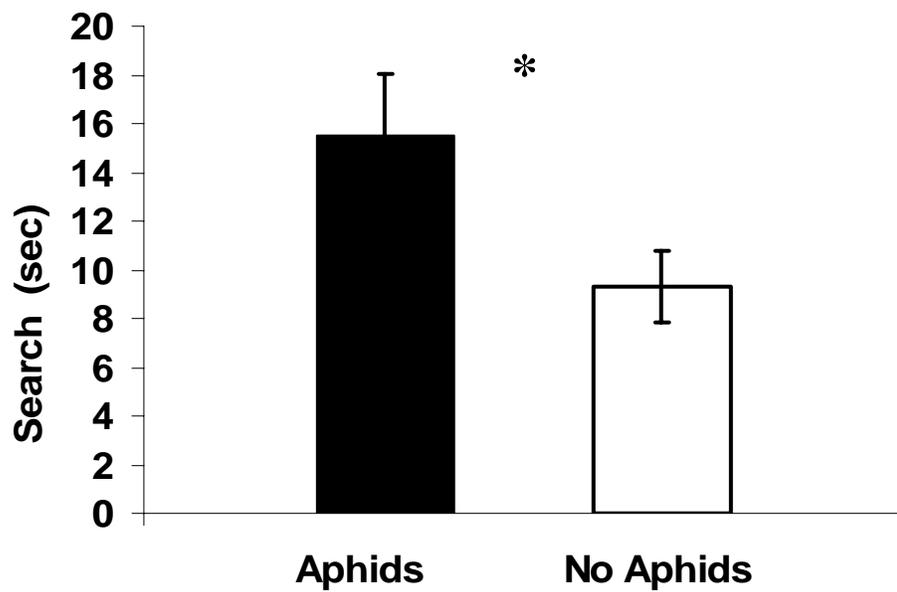


Figure 2. The parasitoid *Cotesia congregata* Say searched longer on leaf discs previously infested with the green peach aphid, *Myzus persicae*. Bars represent means \pm SE. * = $P < 0.05$.

Chapter 4: Aphids do not affect caterpillar abundance and parasitism on tobacco plants arrayed at three spatial scales

Abstract

The strength or outcome of interactions between species that are observed at one spatial scale can change when observed at another scale. Prior research has shown that the aphid *Myzus persicae* reduced the abundance of the tobacco hornworm, *Manduca sexta*, and parasitism by its specialist parasitoid, *Cotesia congregata*. The purpose of this research was to determine if changes in spatial scale alter the effect of aphids on hornworm-parasitoid interactions. For distances up to several miles, spatial scale should not affect the ovipositional decisions of hawkmoths since they are strong flying adults and can easily move to locate more suitable plants. Thus, if moths avoid plants with aphids, they should do so even at larger spatial scales. Parasitoids are not strong flying insects and moving long distances to locate another more suitable plant may be energetically costly. Therefore, at small spatial scales parasitoids should avoid plants with aphids, but at larger scales they should not discriminate between plants with or without aphids. To examine the potential influences of spatial scale, tobacco plants (*Nicotiana tabacum*) were arranged in square arrays within a field bordered by an oak dominated forest. A plant was placed in the corner of each square and was spaced 5, 10, or 25 m apart from the remaining three plants in the array. Tobacco plants within each square were assigned to one of two aphid treatments (presence or absence). Hornworm abundance, parasitism, and plant female fitness was assessed for each plant. These responses were not affected

by spatial treatment, aphids, or their interactions. Hornworm abundance was distributed evenly throughout the plot and parasitism was greatest on plants closest to the woods. It is important to note that parasitism attack was extremely low and these results may not accurately reflect the ability to test for an effect of spatial scale. Nevertheless, it appeared that parasitoids do not disperse as well as their herbivorous hosts, since attack was limited to areas closest to the areas near the woods, which serve as a source of parasitoids. The resulting habitat patchiness may lead to higher herbivory in isolated plant patches that are further away from the woods due to the absence of the third trophic level and the ability of moths to utilize these isolated patches.

Introduction

Ecologists have become increasingly interested in patterns and processes that are scale dependent, since patterns at one scale can change when observed at another scale (Wiens 1989; Levin 1992; Schneider 1994; Tenhumberg et al. 2001). The importance of spatial scale in ecological processes such as host-parasitoid interactions have been appreciated for several decades now (Weins 1989; Levin 1992; Schneider 1994; Norowi et al. 2000), and a growing body of literature has demonstrated that larger scale approaches are essential for understanding population and community ecology (Cronin and Reeve 2005).

Large scale studies on host and parasitoid spatial population structure have examined classic metapopulation dynamics (Eber and Brandl 1994; 1996; 1997; Eber 2001; Weisser 2000; van der Meijden and van der Veen-van Wijk 1997; Lei and Hanski 1997; van Nouhuys and Hanski 1999; 2000; van Nouhuys and Ehrnsten 2004; Kankare et al. 2005), such as the movement and spatial population structure of a host and its parasitoid among spatially discrete habitat patches (Cronin and Reeve 2005). Large scale studies have also examined other approaches, such as mainland-island populations (Cronin 2003 a; 2003 b; 2004; Cronin and Hayes 2004), and patchy populations with stable or unstable local dynamics (Dempster et al. 1995 a; 1995 b) (Cronin and Reeve 2005). Studies have also focused on the effect of scale on density-dependence (Hopper et al. 1991; Rothman and Darling 1991; Ives et al. 1993; Jarosik and Lapch 1996; Ray and Hastings 1996; Schooler et al. 1996; Roland and Taylor 1997; Norowi et al. 2000; Williams and Liebold 2000; and Umbanhowe et al. 2003) and the effects of spatial scale and patch size on parasitoid-host dynamics (Bukovinsky et al. 2005; Legaspi and Legaspi

2005). These studies illustrate contrasting examples regarding the actual dispersal abilities and spatial patterns of the occurrence of insect species.

Spatial scale may alter the strength or outcome of interactions between species. Population densities and dynamics, trophic interactions, and community structure within a habitat may depend on processes that vary at scales larger or smaller than the spatial extent of a local population scale (Gering et al. 2003; Kareiva et al. 1995; Tscharnike and Brandl 2004). Increasing spatial scale can diminish the ability of insects to “choose” between simple and diversified plots (Bommarco and Banks 2003) and scale does not influence all species equally (Kareiva 1990; Holt 1996; With et al. 1999; 2002; Thies et al. 2003). The perception of spatial scale is species specific and can facilitate or impede movement among habitat patches depending on the ability to permeate the landscape (Taylor et al. 1993; Weins et al. 1997; Thies et al. 2003). Further, the foraging range and dispersal ability of a species determines which landscape elements contribute to population dynamics and trophic interactions (den Boer 1990; Eber 2001; Fahrig 2001; Tscharnike and Brandl 2004). For example, similarly patchy distributions of the host plant can promote contrasting dynamics of the herbivore, depending on plant type, herbivore habitat preferences, and the patch dynamics of the plant (Eber 2001).

Prior research has shown that the presence of a secondary herbivore can impact parasitoid-herbivore interactions. In a field study that manipulated the presence of aphids (*Myzus persicae*) on *Nicotiana tabacum*, aphids reduced abundance of the tobacco hornworm, *Manduca sexta*, and parasitism by its specialist parasitoid, *Cotesia congregata* (Lentz unpublished). Further laboratory investigations suggest that aphids mediate parasitoid-caterpillar interactions through changes in the host plant that reduce

hornworm survival and alter parasitoid searching behavior (Lentz unpublished). In an effort to understand the role of multiple herbivores in altering the outcome of interactions, the effects of spatial scale must be considered since choices made at varying spatial scales may differ for herbivores and parasitoids. The purpose of this study was to determine if changes in spatial scale alter the effect of aphids on plant-hornworm-parasitoid interactions. Community level studies suggest that parasitoids tend to have limited dispersal abilities, on the order of tens of meters, compared to their hosts (Roland 1993; Elzinga et al. 2007). Therefore, spatial scale should not affect the ovipositional decisions of hawkmoths since they are strong flying adults and can easily move to locate more suitable plants. Thus, if moths avoid plants with aphids, they should do so at all spatial scales. However, parasitoids are not strong flying insects and moving long distances to locate another more suitable plant may be energetically costly. At small spatial scales parasitoids should avoid plants with aphids, but at larger scales they should not discriminate between plants with or without aphids. This study sought to address the following questions: (1) Do aphids and spatial scale affect hornworm abundance? (2) Do aphids and spatial scale affect parasitism rates? and (3) Do aphids and spatial scale affect plant female fitness?

Methods

Host Plant: *Nicotiana tabacum* L. (Solanaceae, NC95 line, ‘tobacco’ hereafter) is a cultivated annual species that supports a wide range of insects. One of the most damaging herbivores is the tobacco hornworm, whose feeding severely reduces fitness in domestic tobacco (McFadden 1968) and wild congeners (Heil and Baldwin 2002; Kessler and

Baldwin 2004). Leaf herbivory induces the production of nicotine in tobacco roots and the compound is then transported to the plant's shoots where it can serve as a defensive compound that deters herbivory (Baldwin 1997). Nicotine accounts for 95% of the total alkaloid content in tobacco and can constitute up to 14 % plant dry weight (Baldwin 1989; Sisson and Severson 1990).

Focal herbivore: *Manduca sexta* L. (Lepidoptera: Sphingidae; hereafter 'hornworm' or 'moth' as the larval and adult forms respectively), the tobacco hornworm, ranges from southern Canada to southern Brazil. Larvae feed on Solanaceous species, and tobacco is a preferred host plant (Madden and Chamberlin 1945). Hornworms are attacked by a number of natural enemies including parasitic and predaceous wasps, spiders, and birds (McFadden 1968). Adults are moderately large with a wingspan from 76 - 127 mm. Moths are strong fliers and can cover 2.0 km in a single flight (Madden and Chamberlin 1964).

Secondary herbivore: *Myzus persicae* Sulzer (Homoptera: Aphididae; 'aphid' hereafter), the green peach or tobacco aphid, feeds on tobacco phloem. Aphid infestation can cause curling or wilting of host plants and vector plant disease (Borrer et al. 1989).

Parasitoid: *Cotesia congregata* Say (Hymenoptera: Braconidae; 'parasitoid' hereafter) is a gregarious endoparasitoid of the tobacco hornworm and other sphingid larvae (Kester and Barbosa 1991). Female parasitoids attack 3rd-instar hornworms and are approximately 2 mm in length.

Experimental Design: One hundred fifty tobacco seedlings were germinated in vermiculite and transplanted 2 weeks later into 6-cell flats containing Pro-Mix BX soil

(Premier Horticulture Ltd., Red Hill, Pennsylvania, USA). Plants were transplanted into 3.78 -L pots and placed in an outdoor enclosure until they began to bolt. On July 19, 2005, elongating tobacco plants were transplanted into a 0.134 km² field plot at Kentland Farm, Montgomery County, Virginia, USA. The field plot array contained alternating strips of fescue and barley (Fig. 1). Fescue strips were 9.14 m in width and barley strips were roughly 27.43 m. Tobacco plants were planted in the barley strips in squares. Each square contained four tobacco plants, with one plant in each corner of the square. Squares were randomly assigned to spatial treatments such that the four plants in each square were positioned 5 m, 10 m, or 25 m (n = 11, 12, and 12, respectively) apart on each side. Squares were positioned so that every plant in the square was no less than 30 m from all other tobacco plants in the array, a distance greater than any of the spatial treatments. The layout was first drawn in AutoCAD (2006) for accurate distances between plants and to find the configuration that would allow the greatest number of replications while still dispersing spatial treatment squares evenly throughout the field. Tobacco plants within each square were assigned to one of two aphid treatments (presence or absence). Two plants that were side by side in the square were randomly assigned to aphid treatments by flipping a coin. The two remaining plants in the square were then assigned to aphid treatments such that there were 2 plants per treatment per square, and each plant in the square was positioned next to a plant of the same treatment on one side and a plant of the alternate treatment on the other (Fig. 2). This arrangement assured that each plant was equidistant from two other plants, one in each treatment (aphid presence or absence). Thus, an insect leaving any experimental plant would have two plants equally close, one in each treatment, to choose between. This design was used to avoid confounding

treatment choices with distance from each plant. Flags were used to map the layout in the field over a 3 day period using a Jacob's Rod mounted with a Staff Compass (8 ° declination) and a Right Angle Prism. Use of these tools ensured plants were arrayed accurately in squares despite the hilly landscape. Plant holes were dug 45.72 cm deep using an auger with a 15.24 cm bit.

Aphid treatments were maintained bi-weekly by manually removing aphids in the aphid absence treatment group with tape (Scotch®, 3M, St. Paul, MN, USA). Plants in the aphid presence treatment were established with naturally occurring aphids from the field. Tobacco plants never became heavily infested with aphids (~50 per leaf). To control for the effects of tape residue, plants in the aphid presence treatment were also taped, although to a much lesser extent. Plants were watered as needed using a tractor fitted to pull a water truck. Areas around the plants were cleared of weeds on a weekly basis and fescue strips were maintained with a bush hog twice during the experiment.

Plants were censused weekly for hornworms and parasitism. Only hornworms with cocoons were removed; these were maintained individually until wasps emerged to confirm attack by *Cotesia congregata*. To assess the impact of aphids on plant reproduction, aphid treatments were maintained until 90% of the tobacco fruits were mature. Female plant reproduction was assessed as the total number of fruits produced per plant.

Data Analyses: Hornworm and parasitism censuses were summed over the entire season for each plant. Since each square (with 4 plants) is the unit of replication, averages were taken of plants with and without aphids to produce two values for each square. This

method was used for hornworm, parasitism, and female plant fitness. Data were analyzed in a split plot design (SAS, version 9.1) to test the whole-plot effects of spatial treatment (3 levels, 5, 10 and 25 m apart, tested using $n = 35$ as the denominator term since this is the number of experimental whole-plot units), the split plot effect of aphid treatment (2 levels, presence or absence, tested over the MS error term), and the interaction between spatial scale and aphids on hornworm abundance, parasitism, and plant female fitness. A categorical variable 'edge' was put in the model to determine if closer proximity to the woods influenced moth oviposition or parasitoid attack. Edge categories were determined by distance from the woods, beginning with the experimental units closest to the woods and ending with those furthest for a total of six edge categories, each separated by at least 30 m.

Results

Seventy-one out of 142 plants had hornworms, and there was a mean \pm s.e. of 1.31 ± 0.17 hornworms per plant. Hornworm abundance was not affected by spatial treatments (whole plot factor: $F_{2,27} = 1.55$, $p = 0.23$), aphid treatment (split plot factor: $F_{1,69} = 0.19$, $p = 0.67$), their interaction ($F_{2,69} = 0.12$, $p = 0.89$), or edge effects ($F_{5,69} = 0.72$, $p = 0.61$).

Parasitism frequency was very low with only 14 instances of parasitism on 12 of the 142 plants. Parasitism was not affected by spatial treatments (whole plot factor: $F_{2,27} = 1.79$, $p = 0.19$), aphid treatment (split plot factor: $F_{1,69} = 0.21$, $p = 0.65$), or their interaction ($F_{2,69} = 0.20$, $p = 0.82$). Marginally more parasitized hornworms were found on plants close to the woods edge ($F_{5,69} = 2.34$, $p = 0.06$). Of the 14 instances of

parasitism, 10 occurred on plants closest to the wood's edge, 1 in the group second closest to the wood's edge, and 3 in the group furthest from the edge.

Plants produced a mean \pm s.e. of 81.29 ± 3.1 fruits per plant ($n = 140$). Plant female fitness was not affected by spatial treatments (whole plot factor: $F_{2, 32} = 1.41$, $p = 0.27$), aphid treatment (split plot factor: $F_{1, 69} = 0.39$, $p = 0.54$), their interaction ($F_{2, 69} = 0.26$, $p = 0.77$), or edge effects ($F_{5, 69} = 1.57$, $p = 0.21$).

Discussion

Natural enemies and hosts often differ significantly in the scales at which they disperse or respond to spatial subdivision (e.g. Roland and Taylor 1997; Althoff and Thompson 1999; Cronin et al. 2000; Ryall and Fahrig 2005; Cronin and Reeve 2005). For example, the presence of three parasitoid species, *Microplitis tristis*, *Eurylabus tristis*, and *Bracon variator*, declined with patch size, in contrast to their host, *Hadena bicruris*, which showed no effect of patch size (Elzinga et al. 2007). In this study, spatial scale and aphids had no effect on hornworm abundance. Moths were able to colonize host plants that were spread out over 0.13 km^2 regardless of treatments, which is not surprising since hawkmoths are strong flyers (Madden and Chamberlin 1945) and can easily move between plants. However, the lack of effect of aphids on hornworm abundance, as has been found in previous research in this system (Lentz unpublished), was not expected. These results may be due to the shorter duration of aphid infestation and lower aphid densities than in previous studies. Infestation in previous experiments were such that aphid attack began early in the season (May) and plants were heavily infested (~200 per leaf) until harvest. In this study, plants were not put in the ground until mid-July due to

land use constraints and never became heavily infested (~50 per leaf). Therefore, if aphids did not change the host plant, then there is no reason why spatial scale should influence moth choice as to when to avoid aphid-infested plants.

Parasitism was not affected by aphids or spatial scale, but host plants that were closer to the woods were more likely to have parasitized hornworms. These results should be interpreted cautiously since parasitoid attack was infrequent (see Results). Low parasitoid populations could be a result of many factors. Agroecosystems have frequent and intense disturbance regimes and are recognized as particularly difficult environments for natural enemies (Landis et al. 1999). For example, tilling of annual crops can kill overwintering parasitoids (Landis et al. 2000) and destroy insect refuges that protect from environmental extremes and pesticides, and the field plot in this experiment is tilled annually. Annual harvesting and soil cultivation almost completely erase natural enemies, so that arable fields have to be recolonized from surrounding habitats yearly (Thies et al. 2005). It is also important to note that this particular field plot has never been closely associated with any of the host plants or hosts that *C. congregata* attacks.

Despite low parasitism, edge effects, as observed in this study, have been described in many cases. For example, Deans et al. (2005) examined the responses of aerial insect assemblages to a gradient of forest retention at the landscape scale. Most insect families and assemblages, including herbivores and parasitoids, were more abundant in a medium retention treatment, or subplots made of strips of harvested land that alternate with thinned forest strips and embedded in a retention harvested plot. This effect was largely due to the increased edge of the medium retention treatment in comparison to other treatments. Studies have also shown that spillover from non-crop

habitat such as forests can result in higher parasitism rates near the crop edge than the interior (Thies and Tscharrntke 1999; Tylianakis et al. 2004; Cronin and Reeve 2005), consistent with my results.

In addition to showing strong edge effects, community studies suggest that parasitoids tend to have limited dispersal abilities, on the order of tens of meters, compared to their hosts (Roland 1993; Elzinga et al. 2007). For example, the abundance and diversity of parasitoids and predators are often more affected by habitat fragmentation than their herbivorous hosts (Komonen et al. 2000; Kruess and Tscharrntke, 1994). Further, some small scale studies that examine patch dispersal demonstrated that only a small portion of parasitoids dispersed up to the maximum distance measured (100 m at the most; Ellers et al. 1998; Fournier and Boivin 2000; Hagler et al. 2002; Elzinga et al. 2007). In this study, it appears that parasitoids do not disperse as well as their herbivorous hosts since hornworms were found throughout the patch but parasitoids were mostly limited to the edge closest to the woods that can serve as a parasitoid source. Habitat patchiness may lead to higher herbivory in isolated plant patches, such as those further away from the wood's edge, due to the absence of natural enemies and the ability of moths to utilize these isolated patches without pressure from parasitoids.

Several parasitoid species have shown a lack of effect of spatial scale on parasitism. Parasitism rates of the green cloverworm, *Plathypena scabra*, were examined in soybean plots that varied in the type of corridor and in location of where larvae were collected. Attack by *Cotesia marginiventris*, *Aleiodes nolophanae*, and *Campylochaeta plathypenae* did not differ with respect to the type of corridor or location of hosts (Pavuk and Barrett 1993). Dempster et al. (1995) examined the movement of 10 phytophagous

insect species and 7 of their parasitoids among host plants in patches that varied in size and distance from the source population. They found that all of the species were highly mobile regardless of distance of the patch to the source. In this study, there is little that can be concluded regarding the effects of aphids and spatial scale on *Cotesia congregata* since parasitism rates were extremely low and it is unclear if differences in spatial scale would emerge with a larger sample size or if there is truly no effect of scale.

Female plant fitness was not affected by spatial scale, which was expected since hawkmoth visitation was hypothesized to be uniform throughout the field plot. However, the lack of effect of aphids on plant fitness is contrary to previous research in this system. Previous experiments demonstrated that high density aphid attack that began in the rosette stage reduced female fitness in tobacco (Lentz unpublished). Results in this study may reflect low aphid density and late infestation (as described above).

It is difficult to draw conclusions regarding the effect of spatial scale and aphids on parasitism because very few hornworms were parasitized. Parasitism was limited to a small area of the plot closest to the woods. Yet, the data suggest that parasitoids do not disperse as well as their herbivorous hosts, and that the resulting habitat patchiness may lead to higher herbivory in isolated plant patches due to the ability of moths to utilize these isolated patches without pressure from parasitoids.

Literature Cited

- Althoff, D.M., and J.N. Thompson. 1999. Comparative geographic structures of two parasitoid-host interactions. *Evolution* 53: 818-825.
- Baldwin, I.T. 1989. Mechanism of damage-induced alkaloid production in wild tobacco. *Journal of Chemical Ecology* 15: 1661-1680.
- Bommarco, R., and J.E. Banks. 2003. Scale as modifier in vegetation diversity experiments: effects on herbivores and predators. *Oikos* 102: 440-448.
- Borror, D.J., C.A. Triplehorn, and N.F. Johnson. 1989. An introduction to the study of insects. sixth edn. Thompson Learning Inc., USA.
- Bukovinszky, T., R.P.J. Potting, Y. Clough, J.C. van Lenteren, and L.E.M. Vet. 2005. The role of pre- and post-alighting detection mechanisms in the responses to patch size by specialist herbivores. *Oikos* 109: 435-446.
- Cronin, J.T. 2003a. Matrix heterogeneity and host-parasitoid interactions in space. *Ecology* 84: 1506-1516.
- Cronin, J.T. 2003b. Movement and spatial population structure of a prairie planthopper. *Ecology* 84: 1179-1188.
- Cronin, J.T., and K.J. Haynes. 2004. An invasive plant promotes unstable host-parasitoid patch dynamics. *Ecology* 85: 2772-2782.
- Cronin, J.T., and J.D. Reeve. 2005. Host-parasitoid spatial ecology: a plea for a landscape-level synthesis. *Proceedings of the Royal Society B-Biological Sciences* 272: 2225-2235.
- Cronin J.T., J.D. Reeve, R. Wilkens, and P. Turchin. 2000. The pattern and range of movement of a checkered beetle predator relative to its bark beetle prey. *Oikos* 90: 127-138.
- Deans, A.M., J.R. Malcolm, S.M. Smith, and M.I. Bellocq. 2005. Edge effects and the responses of aerial insect assemblages to structural-retention harvesting in Canadian boreal peatland forests. *Forest Ecology and Management* 204: 249-266.
- Dempster, J.P., D.A. Atkinson, and O.D. Cheesman. 1995a. The spatial population dynamics of insects exploiting a patchy food resource. 1. Population extinctions and regulation. *Oecologia* 104: 340-353.
- Dempster, J.P., D.A. Atkinson, and M.C. French. 1995b. The spatial population - dynamics of insects exploiting a patchy food resource. 2. Movements between patches. *Oecologia* 104: 354-362.

- den Boer, P.J. 1990. The survival value of dispersal in terrestrial arthropods. *Biological Conservation* 54: 175– 192.
- Eber, S. 2001. Multitrophic interactions: the population dynamics of spatially structured plant-herbivore-parasitoid systems. *Basic and Applied Ecology* 2: 27-33.
- Eber, S., and R. Brandl. 1994. Ecological and genetic spatial patterns of *Urophora cardui* (Diptera: Tephritidae) as evidence for population structure and biogeographical processes. *Journal of Animal Ecology* 63: 187-199.
- Eber, S., and R. Brandl. 1996. Metapopulation dynamics of the tephritid fly *Urophora cardui*: an evaluation of incidence-function model assumptions with field data. *Journal of Animal Ecology* 65: 621-630.
- Eber, S., and R. Brandl. 1997. Genetic differentiation of the tephritid fly *Urophora cardui* in Europe as evidence for its biogeographical history. *Molecular Ecology* 6: 651-660.
- Ellers, J., J.J.M. Van Alphen, and J.G. Sevenster. 1998. A field study of size-fitness relationships in the parasitoid *Asobara tabida*. *Journal of Animal Ecology* 67: 318-324.
- Elzinga, J.A., S. van Nouhuys, D.J. van Leeuwen, and A. Biere. 2007. Distribution and colonisation ability of three parasitoids and their herbivorous host in a fragmented landscape. *Basic and Applied Ecology* 8: 75-88.
- Fahrig, L. 2001. How much habitat is enough? *Biological Conservation* 100: 65-74.
- Fournier, F., and G. Boivin. 2000. Comparative dispersal of *Trichogramma evanescens* and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) in relation to environmental conditions. *Environmental Entomology* 29: 55-63.
- Gering, J.C., T.O. Crist, and J.A. Veech. 2003. Additive partitioning of species diversity across multiple spatial scales: Implications for regional conservation of biodiversity. *Conservation Biology* 17: 488-499.
- Hagler, J.R., C.G. Jackson, T.J. Henneberry, and J.R. Gould. 2002. Parasitoid mark-release-recapture techniques - II. Development and application of a protein marking technique for *Eretmocerus* spp., parasitoids of *Bemisia argentifolii*. *Biocontrol Science and Technology* 12: 661-675.
- Heil, M., and I.T. Baldwin. 2002. Fitness costs of induced resistance: emerging experimental support for a slippery concept. *Trends in Plant Science* 7: 61-67.

- Holt, W.V., P.M. Bennett, V. Volobouev, and P.F. Watson. 1996. Genetic resource banks in wildlife conservation. *Journal of Zoology* 238: 531-544.
- Hopper, K.R., J.E. Powell, and E.G. King. 1991. Spatial density dependence in parasitism of *Heliothis virescens* (Lepidoptera: Noctuidae) by *Microplitis croceipes* (Hymenoptera: Braconidae) in the field. *Environmental Entomology* 20: 292-302.
- Ives, A.R., P. Kareiva, and R. Perry. 1993. Response of a predator to variation in prey density at 3 hierarchical scales - lady beetles feeding on aphids. *Ecology* 74: 1929-1938.
- Jarosik, V., and L. Lapchin. 2001. An experimental investigation of patterns of parasitism at three spatial scales in an aphid parasitoid system (Hymenoptera: Aphidiidae). *European Journal of Entomology* 98: 295-299.
- Kankare, M., S. van Nouhuys, O. Gaggiotti, and I. Hanski. 2005. Metapopulation genetic structure of two coexisting parasitoids of the Glanville fritillary butterfly. *Oecologia* 143: 77-84.
- Kareiva, P., 1990. Population dynamics in spatially complex environments -theory and data. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 330: 175-190.
- Kareiva, P., and U. Wennergren. 1995. Connecting landscape patterns to ecosystem and population processes. *Nature* 373: 299-302.
- Kessler, A., and I.T. Baldwin. 2004. Herbivore-induced plant vaccination. Part I. The orchestration of plant defenses in nature and their fitness consequences in the wild tobacco *Nicotiana attenuata*. *The Plant Journal* 38 639-649.
- Kester, K.M., and P. Barbosa. 1991. Postemergence learning in the insect parasitoid, *Cotesia congregata* Say (Hymenoptera: Braconidae). *Journal of Insect Behavior* 4: 727-742.
- Komonen A., R. Penttila, M. Lindgren, and I. Hanski. 2000. Forest fragmentation truncates a food chain based on an old-growth forest bracket fungus. *Oikos* 90: 119-126.
- Kruess, A., and T. Tscharntke. 1994. Habitat fragmentation, species loss, and biological control. *Science* 264: 1581-1584.
- Landis, D.A., F.D. Menalled, J.C. Lee, D.M. Carmona, and A. Perez-Valdez. 1999. Habitat modification to enhance biological control in IPM. APS Press, St. Paul.

- Landis, D.A., S.D. Wratten, and G.M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45: 175-201.
- Legaspi, B.C., and J.C. Legaspi. 2005. Foraging behavior of field populations of *Diadegma* spp. (Hymenoptera: Ichneumonidae): Testing for density-dependence at two spatial scales. *Journal of Entomological Science* 40: 295-306.
- Lei, G.C., and I. Hanski. 1997. Metapopulation structure of *Cotesia melitaearum*, a specialist parasitoid of the butterfly *Melitaea cinxia*. *Oikos* 78: 91-100.
- Levin, S.A. 1992. The problem of pattern and scale in ecology. *Ecology* 73: 1943-1967.
- Madden, A.H., and F.S. Chamberlin. 1945. Biology of the tobacco hornworm in the southern cigar-tobacco district. *Technical Bulletin* 896: 1-51.
- Norowi, H.M., J.N. Perry, W. Powell, and K. Rennolls. 2000. The effect of spatial scale on interactions between two weevils and their parasitoid. *Ecological Entomology* 25: 188-196.
- Pavuk, D.M., and G.W. Barrett. 1993. Influence of successional and grassy corridors on parasitism of *Plathypena scabra* F. (Lepidoptera: Noctuidae) larvae in soybean agroecosystems. *Environmental Entomology* 22: 540-546.
- Ray, C., and A. Hastings. 1996. Density dependence: Are we searching at the wrong spatial scale? *Journal of Animal Ecology* 65: 556-566.
- Roland, J. 1993. Large scale forest fragmentation increases the duration of tent caterpillar outbreak. *Oecologia* 93: 25-30.
- Roland, J., and P.D. Taylor. 1997. Insect parasitoid species respond to forest structure at different spatial scales. *Nature* 386: 710-713.
- Rothman, L.D., and D.C. Darling. 1991. Spatial density dependence - effects of scale, host spatial pattern and parasitoid reproductive strategy. *Oikos* 62: 221-230.
- Ryall, K.L., and L. Fahrig. 2005. Habitat loss decreases predator-prey ratios in a pine-bark beetle system. *Oikos* 110: 265-270.
- Schneider, D. 1994. *Quantitative ecology: spatial and temporal scaling*. Academic Press, San Diego, CA.
- Schooler, S.S., A.R. Ives, and J. Harmon. 1996. Hyperparasitoid aggregation in response to variation in *Aphidius ervi* host density at three spatial scales. *Ecological Entomology* 21: 249-258.

- Sisson, V.A., and R.F. Severson. 1990. Alkaloid composition of the *Nicotiana* species. *Beitrage Zur Tabakforschung International* 14: 327-339.
- Taylor, P.D., L. Fahrig, K. Henein, and G. Merriam. 1993. Connectivity is a vital element of landscape structure. *Oikos* 68: 571-573.
- Tenhumberg, B., M.A. Keller, A.J. Tyre, and H.P. Possingham. 2001. The effect of resource aggregation at different scales: Optimal foraging behavior of *Cotesia rubecula*. *American Naturalist* 158: 505-518.
- Thies, C., I. Roschewitz, and T. Tschardtke. 2005. The landscape context of cereal aphid- parasitoid interactions. *Proceedings of the Royal Society B-Biological Sciences* 272: 203-210.
- Thies, C., I. Steffan-Dewenter, and T. Tschardtke. 2003. Effects of landscape context on herbivory and parasitism at different spatial scales. *Oikos* 101: 18-25.
- Thies, C., and T. Tschardtke. 1999. Landscape structure and biological control in agroecosystems. *Science* 285: 893-895.
- Tschardtke, T., and R. Brandl. 2004. Plant-insect interactions in fragmented landscapes. *Annual Review of Entomology* 49: 405-430.
- Tylianakis, J.M., R.K. Didham, and S.D. Wratten. 2004. Improved fitness of aphid parasitoids receiving resource subsidies. *Ecology* 85: 658-666.
- Umbanhowar J., J. Maron, and S. Harrison. 2003. Density-dependent foraging behaviors in a parasitoid lead to density-dependent parasitism of its host. *Oecologia* 137: 123-130.
- van der Meijden. E., and C. van der Veen-van Wijk. 1997. Tritrophic metapopulation dynamics: a case study of ragwort, the cinnabar moth, and the parasitoid *Cotesia popularis* Academic Press, San Diego.
- van Nouhuys, S., and J. Ehrnsten. 2004. Wasp behavior leads to uniform parasitism of a host available only a few hours per year. *Behavioral Ecology* 15: 661-665.
- Van Nouhuys, S., and I. Hanski. 1999. Host diet affects extinctions and colonizations in a parasitoid metapopulation. *Journal of Animal Ecology* 68: 1248-1258.
- van Nouhuys, S., and I. Hanski. 2000. Apparent competition between parasitoids mediated by a shared hyperparasitoid. *Ecology Letters* 3: 82-84.
- Weisser, W.W. 2000. Metapopulation dynamics in an aphid-parasitoid system. *Entomologia Experimentalis et Applicata* 97: 83-92.

- Wiens, J.A. 1989. Spatial scaling in ecology. *Functional Ecology* 3: 385-397.
- Wiens, J.A., R.L. Schooley, and R.D. Weeks. 1997. Patchy landscapes and animal movements: Do beetles percolate? *Oikos* 78: 257-264.
- Williams, D.W., and A.M. Liebhold. 2000. Spatial scale and the detection of density dependence in spruce budworm outbreaks in eastern North America. *Oecologia* 124: 544-552.
- With, K.A., S.J. Cadaret, and C. Davis. 1999. Movement responses to patch structure in experimental fractal landscapes. *Ecology* 80: 1340-1353.
- With, K.A., D.M. Pavuk, J.L. Worchuck, R.K. Oates, and J.L. Fisher. 2002. Threshold effects of landscape structure on biological control in agroecosystems. *Ecological Applications* 12: 52-65.

Figures

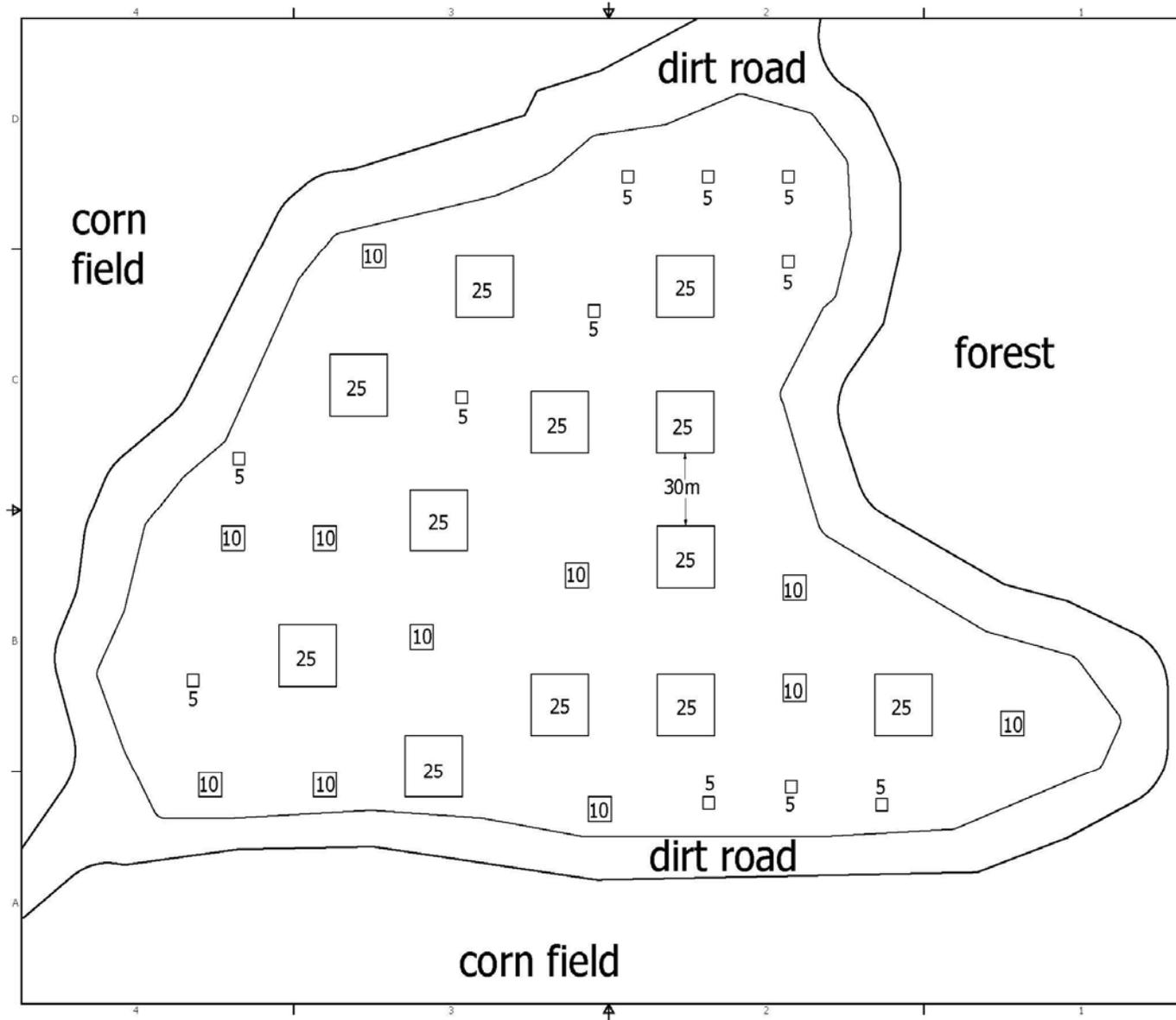


Figure 1. Spatial configuration of experimental units. Larger squares (25) = 25 m, medium squares (10) = 10 m, and small squares (5) = 5 m.

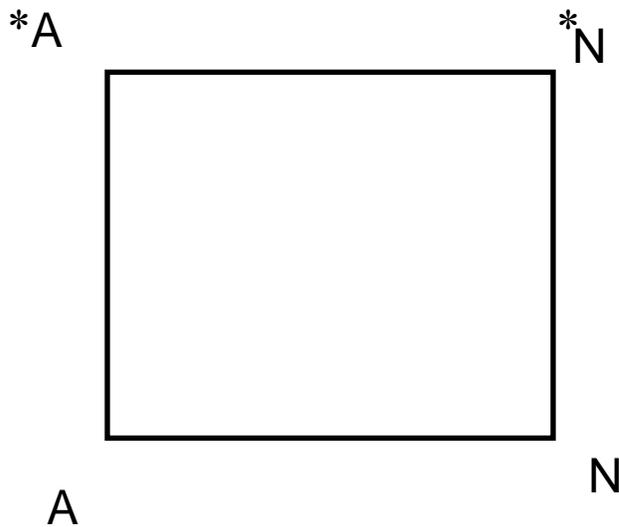


Figure 2. Arrangement of aphid treatments within experimental units. Aphid treatments of the first two tobacco plants were decided at random (as indicated by *) while the remaining plant treatments were determined so that there were 2 plants per treatment per square, and each plant in the square was positioned next to a plant of the same treatment on one side and a plant of the alternate treatment on the other. This arrangement assured that each plant was equidistant to two other plants, one in each treatment (aphid presence or absence). A = aphid presence, N = aphid absence.

Conclusions

Indirect interactions play a key role in structuring communities and can have strong effects on food web dynamics (Abrams et al. 1996; Polis and Strong 1996; Berlow 1999; Dicke et al. 2003). However, species interactions have traditionally been examined in a pairwise manner (Levins et al. 1990) even though the effects of introducing other species into a community are not additive. Slight changes in community context can quantitatively and qualitatively alter the outcomes of associations between pairs of species through indirect interactions (Worthen and Moore 1991; Bacher and Friedli 2002; Bronstein and Barbosa 2002; Bronstein et al. 2003).

The majority of plant species support complex faunas of herbivores and their natural enemies that interact as a complex system, but the relationships among parasitoids, single herbivore species, and plants are usually studied without considering additional herbivores. In this dissertation I conducted field and laboratory experiments to examine how a second herbivore on a shared host plant affects the dynamics of a plant-herbivore-parasitoid system. I examined how the aphid herbivore (*Myzus persicae*) changed interactions between domestic tobacco (*Nicotiana tabacum*), the tobacco hornworm (*Manduca sexta*), and a specialist parasitoid (*Cotesia congregata*). Aphid-feeding on tobacco reduced both the abundance of hornworms and the incidence of parasitism (Chapter 2). Subsequent research examined the specific mechanisms underlying these altered interactions. Chapter 2 examined the role of density-dependence and demonstrated that moth oviposition was not influenced hornworm egg density, but the proportion of caterpillars attacked by parasitoids was inversely density dependent with the number of caterpillars on a host plant. The mechanism that may best explain

changes in hornworm abundance and parasitism is host plant quality, as is demonstrated in Chapter 3. Aphid attack of host plants increased hornworm mortality, did not affect parasitoid performance, and increased parasitoid search time. These results suggest that aphids can mediate parasitoid-caterpillar interactions through changes in host plants that reduce hornworm survival and alter parasitoid behavior. Lastly, I examined how the outcomes of interactions that are altered by aphids change with spatial scale (Chapter 4). I found no effect of either on hornworm abundance and parasitism.

In this system, aphids alter parasitoid-caterpillar interactions through changes in plant quality that reduce hornworm performance and abundance, and in turn, influence parasitoid attack. This work presents a novel approach in which to consider herbivores and their impacts on communities, and provides insight about parasitoid foraging strategies and host acceptance decisions in a multispecies context. Further, this work demonstrates that the outcome of multispecies interactions may not be predictable from pairwise interactions. Dissecting these interactions in a relatively simple agricultural system has provided a starting point in which to examine these effects before expanding into more complex natural systems and broadens our understanding of how community context alters the outcomes of tritrophic interactions.

Literature Cited

- Abrams, P.A., B.A. Menge, and G.G. Mittelbach. 1996. The role of indirect effects in food webs. In Polis, G.A. and Winemiller, K.O. (eds.), Food webs: integration of patterns and dynamics. Chapman and Hall, p.371-395.
- Bacher, S., and J. Friedli. 2002. Dynamics of a mutualism in a multi-species context. Proceedings of the Royal Society of London B, Biological Sciences 269: 1517-1522.
- Berlow, E.L. 1999. Strong effects of weak interactions in ecological communities. Nature 398: 330-334.
- Bronstein, J.L. and P.Barbosa. 2002. Multitrophic/multispecies mutualistic interactions: the role of non-mutualists in shaping and mediating mutualisms. p.44-65 in B. Hawkins and T. Tscharntke, eds. Multitrophic level interactions. Cambridge University Press, Cambridge.
- Bronstein, J.L., W.G. Wilson, and W.F. Morris. 2003. Ecological dynamics of mutualist/antagonist communities. The American Naturalist, supplement 162: S24-S39.
- Dicke, M., J.G. den Boer, M. Hofte, and M.C. Rocha-Granados. 2003. Mixed blends of herbivore-induced plant volatiles and foraging success of carnivorous arthropods. Oikos 101: 38-48.
- Polis, G.A., and D.R. Strong. 1996. Food web complexity and community dynamics. American Naturalist 147: 814-846.
- Worthen, W.B. and J.L. Moore. 1991. Higher-order interactions and indirect effects: a resolution using laboratory *Drosophila* communities. American Naturalist 138: 1092-1104.

A m a n d a J . L e n t z

E d u c a t i o n

Doctorate of Philosophy, Virginia Tech, anticipated completion 8/07
advisor: Dr. Lynn S. Adler GPA: 3.93

Master of Science, Virginia Commonwealth University, 12/01
advisor: Dr. Karen M. Kester GPA: 3.61

Bachelor of Science, Virginia Tech, 5/96, Double major, Biology and Art History

P r o f e s s i o n a l E x p e r i e n c e

Laboratory Instructor, Virginia Tech: 2002 – 2007

Overall rating, 11 labs: 3.82 on a 4.0 scale.

Honors Biology Laboratory (Fall 2006; designed and implemented course)

Majors Biology Laboratory (Fall of 2002 – 2005)

Plant Taxonomy (Spring 2005, 2006, 2007; created photographic atlas for student reference and web page with materials)

Advisor and Supervisor, Virginia Tech: 2003 – 2005

Mentor (David Sharp), undergraduate independent research project. Fall 2004 - Spring 2005. Project included submitted publication of original research.

Supervisor (Fall 2003 - 2006), volunteer undergraduates and work study students. Provide research experience to prepare students for graduate school and the job market.

Mentor Assistant (La Tron Brown), Virginia Tech 5/03 - 8/03

Research Assistant, Virginia Tech (Lynn Adler) and University of Georgia (Rebecca Irwin): 1/03 - 5/03 and 1/04 - 5/04

Assisted in research to examine the role of toxic nectar in Carolina Gessamine. Duties included pollinator observations, catching and swabbing bees, making pollen stains, emasculating flowers, censuses for nectar robbing etc...

**Laboratory Manager, Virginia Commonwealth University (Karen Kester):
1/02 - 8/02**

Examined the role of ovipositional experience on parasitoid preference. Oversaw the rearing of laboratory insect colonies, *Manduca sexta* and *Cotesia congregata*. Maintained pest-free greenhouse plants. Ordered necessary supplies and collected field insects.

**Laboratory Researcher, Virginia Commonwealth University (Karen Kester):
5/98 - 5/00**

Conducted experimental research to examine the effect of learning on the ovipositional decisions of parasitic wasps. Maintained laboratory colonies and greenhouse and collected field insects.

**Adjunct Laboratory Instructor, Virginia Commonwealth University (Don
Fristch): 1997 - 2002, General Biology**

Instructed non-major biology students as a separate class from lecture. During the last year I taught 13 labs in one semester and 6 labs the following semester.

P u b l i c a t i o n s

Adler, Lynn S., Michael Wink, Melanie Distl, and Amanda J. Lentz. 2006. Leaf herbivory and nutrients increase nectar alkaloids. *Ecology Letters*, 9: 960-967.

In review, *Ecological Entomology*. Lentz, Amanda J. and Lynn S. Adler. Nectar amino acids attract antagonists.

In review, *Journal of Insect Behavior*. Lentz, Amanda J. and Karen M. Kester. Postemergence learning affects sex ratio allocations in a gregarious insect parasitoid.

In review, *Journal of Insect Behavior*, by David Sharp (independent study student mentored by me). Sharp, David, Amanda J. Lentz, Johanna Barron, and Lynn S. Adler. Floral nicotine does not affect egg-laying and nectar consumption in *Manduca sexta*.

Four manuscripts from M.S. and Ph.D. work in preparation

Grants

National Science Foundation, Doctoral Dissertation Improvement Grant, 2005 - The Effect of Secondary Herbivores on Tritrophic Interactions. PI's: Robert Jones, Amanda Lentz, and Lynn Adler. \$10,782.00.

American Museum of Natural History 2004 - The Effects of Auxiliary Herbivores on Tritrophic Interactions. PI: Amanda Lentz. \$1500.00.

Graduate Research Development Program 2004 - Do Aphids Affect Nicotine Concentration in Tobacco Plants? PI: Amanda Lentz. \$375.00.

Graduate Research Development Program 2003 - Do Female Hawkmoths Prefer Amino Acid Enhanced Nectar? PI: Amanda Lentz. \$500.00.

Presentations

Oral Presentations:

"The Role of Aphids in Parasitoid-Host Interactions"

2006 Virginia Tech Research Day, Blacksburg, VA. Invited.

2006 National Meeting: Ecological Society of America, Memphis, TN.

"Postemergence Experience Affects Sex Ratio Allocation of *Cotesia congregata* (Say)"

2003 National Meeting: Ecological Society of America, Savannah, GA.

2003 National Meeting: Entomological Society of America, Ft. Lauderdale, FL.

Poster Presentations:

"Nectar Amino Acids Attract Antagonists"

2006 Virginia Tech, College of Science Round Table Meeting. Invited.

2005 Virginia Tech, Virginia Tech Research Day, Blacksburg VA.

2005 National Meeting: Ecological Society of America, Montreal, Canada.

"Effects of sequential learning experiences on the foraging behavior of the parasitic wasp, *Cotesia congregata* Say (Hymenoptera: Braconidae)" 1999 - 2001.

National Meeting: American Institute of Biological Sciences, 51st Annual Meeting, Washington D.C., 2001.

National Meeting: Society for Integrative and Comparative Biology, Annual Meeting, Atlanta GA, 2000.

Regional Meeting: Eastern Branch of the Entomological Society of America, 70th Annual Meeting, Virginia Beach Virginia, 1999.

University Meeting: Daniel T. Watts Day Research Symposium, Richmond VA, 1999.

A w a r d s

Awarded 2007, Outstanding Graduate Teaching Instructor, Department of Biological Sciences

First Place Poster Award 2005, Virginia Tech Research Day: "Nectar Amino Acids Attract Antagonists".

Nominated for Graduate Student Teaching Excellence Award, College of Science.

O u t r e a c h

The Scientific Method: Experimental Design and Applications, June 7, 2007, Oak Knoll Middle School 8th grade for Alison Dossick, Hanover, VA.

M e m b e r s h i p s

Ecological Society of America
Entomological Society of America
Sigma Chi