

ABSTRACT

Title of Dissertation: ROLE OF PEACH [*PRUNUS PERSICA* (L.)
BATCSH] EXTRAFLORAL NECTARIES IN
MEDIATING NATURAL ENEMY-HERBIVORE
INTERACTIONS

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Extrafloral nectaries (EFNs) are reported to benefit some plants when ants (Hymenoptera: Formicidae) use their secretions and fend off herbivores. The significance of peach [*Prunus persica* (L.) Batsch] EFNs in mediating natural enemy-pest dynamics was studied for the ‘Lovell’ cultivar with EFNs present and absent. The first phase of the research tested the hypothesis that peach EFNs contribute indirectly to plant defense from herbivores. Trees with EFNs experienced a 6-fold increase in predators (predominantly ants), fewer herbivores, and less folivory compared to trees without EFNs. Ant exclusion techniques further revealed that trees with EFNs benefited from reduced folivory in the spring and increased vigor (trunk circumference, leaf surface area, and terminal carbon composition) only when ants were permitted in their canopies. It was concluded that the EFNs do have a defensive role with regard to foliage feeders.

The next research phase explored the impact of EFNs on biological control of a key economic pest, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), in peach orchards. Experiments revealed that trees with EFNs had higher parasitoid densities in the spring and increased parasitism of larval *G. molesta* later in the season. Ant exclusion from mature peach trees with EFNs increased *G. molesta* fruit injury by > 4-fold, indicating that EFNs have a protective role for the fruit as well.

The potential for competitive interactions between ants and other natural enemies associated with EFNs was explored in the final research phase. Studies revealed that several natural enemy groups contribute to reductions in *G. molesta* eggs, larvae, and pupae in peach orchards. Although ants antagonized the *G. molesta* egg parasitoid *Trichogramma minutum* (Riley) on trees with EFNs, the ants were crucial in reducing *G. molesta* in both the larval and pupal stages.

The implications of EFN-natural enemy-pest interactions to orchard-level biological control will likely depend on local herbivore population dynamics. However, the EFNs clearly benefit *P. persica* indirectly, through enhancement of ants and other natural enemies. Thus, EFNs are an important host-plant characteristic that should be retained in future peach cultivars in order to maximize conservation biological control.

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NECTARIES IN MEDIATING NATURAL ENEMY-HERBIVORE
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By

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Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2004

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Table of Contents

List of Tables	iv
List of Figures	v
Chapter I: The Role of <i>Prunus persica</i> [(L.) Batcsh] Extrafloral Nectaries in Plant Defense	1
Introduction	1
Materials & Methods	5
1. Do EFNs impact predator or herbivore densities in the canopy?	8
2. Is folivory reduced on trees with EFNs?	9
3. Do the EFNs enhance tree vigor?	11
4. Do ants associated with the EFNs provide protection from folivory?	12
Results	14
1. Do EFNs impact predator or herbivore densities in the canopy?	14
2. Is folivory reduced on trees with EFNs?	20
3. Do the EFNs enhance tree vigor?	22
4. Do ants associated with the EFNs provide protection from folivory?	23
Discussion	27
Chapter II: The Impact of Leaf Extrafloral Nectaries on Biological Control of a Key Economic Pest, <i>Grapholita molesta</i> (Busck) (Lepidoptera: Tortricidae), in Peach [<i>Prunus persica</i> (L.) Batcsh]	31
Introduction	31
Materials & Methods	35
1. Do EFNs or ants affect other natural enemies in the peach tree canopy?	35
2. Do EFNs or ants affect densities or parasitism of the key economic pest, <i>G. molesta</i> ?	37
3. Do ants associated with peach EFNs affect fruit injury by <i>G. molesta</i> ?	38
Results	40
1. Do EFNs or ants affect other natural enemies in the peach tree canopy?	40
2. Do EFNs or ants affect densities or parasitism of the key economic pest, <i>G. molesta</i> ?	45
3. Do ants associated with peach EFNs affect fruit injury by <i>G. molesta</i> ?	49
Discussion	49

Chapter III: Interactions Between Ants (Hymenoptera: Formicidae) and Other Natural Enemies Associated with Extrafloral Nectaries of Peach [*Prunus persica* (L.) Batsch]: Implications for a Key Pest, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae). 54

Introduction	54
Materials & Methods.....	56
1. Do ants associated with EFN-bearing peach trees disrupt biological reduction of <i>G. molesta</i> by other natural enemies?	57
2. What are the relative contributions of ants, compared to other natural enemies, to biological reduction of <i>G. molesta</i> in different life stages?	60
Results	66
1. Do ants associated with EFN-bearing peach trees disrupt biological reduction of <i>G. molesta</i> by other natural enemies?	66
2. What are the relative contributions of ants, compared to other natural enemies, to biological reduction of <i>G. molesta</i> in different life stages?	70
Discussion.....	79
Literature Cited.....	82

List of Tables

<i>Table 1. Results of mixed model ANOVAs for fixed effect of leaf EFNs on arthropod densities on ‘Lovell’ peach trees in completely randomized design study with four field replications.</i>	14
<i>Table 2. Results of mixed model ANOVAs testing for fixed effect of leaf EFNs on ant and herbivore densities on ‘Lovell’ peach trees in completely randomized design study with four field replications.</i>	17
<i>Table 3. Relative contribution of <i>M. hilleri</i> to folivory for peach trees with or without leaf extrafloral nectaries (+/-EFNs).</i>	21
<i>Table 4. Results of ANOVA testing for fixed effects of leaf EFNs, ant presence, and interaction on folivory for ‘Lovell’ peach trees in CRD split-plot study.</i>	24
<i>Table 5. Ants present on ‘Lovell’ peach trees by leaf EFN phenotype and date.</i>	27
<i>Table 6. Associations between natural enemy densities and <i>G. molesta</i> larvae in terminal shoots of peach trees with or without leaf extrafloral nectaries (+/-EFNs) or ant exclusion treatment .</i>	47
<i>Table 7. The 2 X 2 X 3 factorial treatment structure of the completely randomized split-split plot design used in 2003 field study that compared survival of sentinel <i>G. molesta</i> on ‘Lovell’ peach trees.</i>	60
<i>Table 8. Results of mixed model ANOVAs testing for effects of leaf extrafloral nectaries (EFNs), ant exclusion, and exclusion of other (non-ant) natural enemies on survival of <i>G. molesta</i> introduced on ‘Lovell’ peach trees.</i>	71
<i>Table 9. Relative impact of ants, as compared to other natural enemies, on <i>G. molesta</i> pupae introduced to ‘Lovell’ peach terminals in field experiments.</i>	79

List of Figures

Figure 1. <i>Prunus persica</i> (L.) Batsch leaves at 5X magnification illustrating three leaf EFN phenotypes produced from ‘Lovell’ X ‘Lovell’ crosses.	4
Figure 2. Field lay-out depicting two replicates used in studies comparing ‘Lovell’ peach trees with leaf EFNs present and absent.	6
Figure 3. Effect of ‘Lovell’ leaf extrafloral nectary presence (+EFN) or absence (-EFN) on predator and herbivore densities summed over 8 d in 2002 and 2003.	16
Figure 4. Ant densities on ‘Lovell’ trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN) in 2002 and 2003.	18
Figure 5. Herbivore densities on ‘Lovell’ trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN) in 2002 and 2003.	19
Figure 6. <i>P. persica</i> folivory by <i>M. hilleri</i> in the laboratory (July 2002).	20
Figure 7. Effect of ‘Lovell’ leaf extrafloral nectary presence (+EFN) or absence (-EFN) on folivory, measured as percentage of injured leaves in 5 leaf sample per tree, 2002.	21
Figure 8. Effect of leaf extrafloral nectary presence (+EFN) or absence (-EFN) on ‘Lovell’ tree vigor.	22
Figure 9. Effect of ant exclusion treatment on herbivore densities on ‘Lovell’ trees with leaf EFNs present or absent, 6 July 2003.	23
Figure 10. Monthly folivory rates, measured as percentage of injured leaves in 5 leaf sample per tree, 2003.	25
Figure 11. Average leaf surface area for ‘Lovell’ trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN), by month in 2003.	26
Figure 12. Predators other than ants on ‘Lovell’ peach trees with and without leaf extrafloral nectaries (+/-EFN).	40
Figure 13. Parasitic Hymenoptera associated with ‘Lovell’ peach trees with and without leaf extrafloral nectaries (+/-EFN).	41
Figure 14. Predator densities on ‘Lovell’ trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN), by limb jarring on 8 d in 2002 and 2003.	42
Figure 15. Effect of ant exclusion on densities of predators other than ants (by limb jarring) on ‘Lovell’ trees, August 2003.	43
Figure 16. Effect of ‘Lovell’ extrafloral nectaries (+/- EFN) on parasitic Hymenoptera densities, by sticky trap, on 3 d in 2002 and 7 d in 2003.	44
Figure 17. Interactive effect of ant exclusion treatment and leaf extrafloral nectary presence (+EFN) or absence (-EFN) for <i>G. molesta</i> larvae infesting terminal shoots of ‘Lovell’ peach trees, 29 May 2003.	46
Figure 18. Average monthly densities of larval <i>G. molesta</i> , by visual inspection, and adult <i>Braconidae</i> , by sticky trap, in plots of ‘Lovell’ peach trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN), 2003.	48
Figure 19. Monthly rates of <i>G. molesta</i> parasitism by <i>M. delicatus</i> in relation to <i>G. molesta</i> larval densities in shoots of ‘Lovell’ peach trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN), 2003.	48

<i>Figure 20. Effect of ant exclusion on percentage of fruit (20/tree harvested 13 August 2003) infested with G. molesta larvae.</i>	49
<i>Figure 21. Terminal cages (8 X 30 cm) providing partial and full natural enemy exclusion treatment in 2 X 2 X 3 level factorial study comparing survival of sentinel G. molesta eggs, larvae, and pupae.</i>	62
<i>Figure 22. Effect of ant exclusion on survival of sentinel G. molesta eggs and interactive effects of ant exclusion and leaf extrafloral nectary presence (+EFN) or absence (-EFN) on survival of sentinel G. molesta pupae on 'Lovell'.</i>	67
<i>Figure 23. Significant interactive effects of 'Lovell' leaf EFNs and ant exclusion treatment on densities of predators other than ants, by limb jarring, and parasitic Hymenoptera, by sticky trap, in September 2002.</i>	68
<i>Figure 24. Association between H. axyridis abundance (no. adults/tree) and G. molesta egg survivorship (% hatch/tree) on 'Lovell' with leaf EFNs in 2002.</i>	69
<i>Figure 25. Effect of natural enemy exclusion cages (full, partial, or none) and three-way interaction of leaf EFNs, ant exclusion, and natural enemy exclusion cages on T. minutum parasitism of G. molesta eggs introduced on 'Lovell'.</i>	72
<i>Figure 26. Effect of natural enemy exclusion cages (full, partial, or none) and three-way interaction of leaf EFNs, ant exclusion, and natural enemy exclusion cages on survival of G. molesta eggs introduced on 'Lovell'.</i>	73
<i>Figure 27. Interactive effects of natural enemy exclusion cages (full, partial, or none) and ant exclusion treatment and main effect of natural enemy exclusion cages on survival of G. molesta larvae introduced on 'Lovell'.</i>	75
<i>Figure 28. Effect of natural enemy exclusion cages (full, partial, or none) on survival of G. molesta pupae introduced on 'Lovell'.</i>	76
<i>Figure 29. Significant two-way interactions for attrition of G. molesta pupae introduced on 'Lovell': interactive effects of ant exclusion and natural enemy exclusion cages (full, partial, or none) and interactive effects of leaf EFNs and ant exclusion.</i>	78

Chapter I: The Role of *Prunus persica* [(L.) Batsch] Extrafloral Nectaries in Plant Defense

Introduction

Extrafloral nectaries (EFNs) are secretory glands commonly appearing on the petioles, stipules, and leaf margins of most peach [*Prunus persica* (L.) Batsch] cultivars (Gregory 1915, Okie 1998). Why EFNs evolved in the Rosaceae and 67 other plant families (Elias 1983) is not clear, despite considerable research on their function (see Bentley 1977a, Bentley 1983, and Rogers 1985 for reviews).

A common hypothesis is that EFNs confer plant defense by attracting ants (Hymenoptera: Formicidae) that consume the exudate and, in turn, guard the plants against herbivores (Bentley 1977a, Beattie 1985, Bronstein 1998). The “protectionist” or ant defense theory has been tested in a variety of systems, primarily in the tropics, with mixed results (Rogers 1985, Heil and McKey 2003). Numerous studies have documented ant attraction to EFN-bearing plants (e.g., Janzen 1966, Bentley 1977b, Inouye and Taylor 1979), and some authors have attributed reduced herbivory of various plant parts to ant “protection” (e.g., Janzen 1966, Bentley 1976, Fatima et al. 1992, Fonseca 1994, Oliveira et al. 1999, Rudgers 2004). However, others (e.g., O’Dowd and Catchpole 1983, Heads and Lawton 1985, Rashbrook et al. 1992) have found no benefit of ant visits to plants bearing EFNs. Their findings support an alternative hypothesis, that EFN exudate is merely a bi-product of some physiological function, e.g., excretion, which ants exploit (Wheeler 1910).

The results of various studies suggest that ants and other insect predators (e.g., Coccinellidae, Cantharidae, and Chrysopidae) regularly consume EFN secretions from *Prunus* spp. Putman (1963) found that the predators *Stethorus punctillum* (Weise), *Adalia bipunctata* (L.), *Cycloneda sanguinea* (L.), *Chrysopa carnea* (Steph.), *Camponotus pennsylvanicus* (DeGeer), and *Prenolepis imparis* (Say) regularly consumed peach EFN secretions. Using data from studies in the United States, Korea, Japan, and China, Pemberton and Vandenburg (1993) reported that 41 coccinellid species consumed EFN exudates, particularly from *Prunus* spp. Pemberton (1993) observed predacious mites (*Anystis*, Anystidae) feeding on EFNs of cherry [*Prunus sargentii* (Rehder)]. Tilman (1978) related *Formica obscuripes* (Forel) densities on black cherry [*Prunus serotina* (Ehrh.)] to timing of EFN production and reported that tent caterpillar [*Malacosoma americanum* (Fabricius)] survivorship increased with increasing distance from *F. obscuripes* colonies, suggesting the potential for facultative mutualism between *F. obscuripes* and *P. serotina*.

The timing and distribution of EFN secretion in *P. persica* suggest that the EFNs could contribute to plant defense. The EFNs are distributed on the newly-formed leaves, and their secretions are more profuse during early spring. Optimal defense theory argues that young leaves, which are rich in nitrogen (N) and are more valuable than old leaves to the plant (McKey 1974, Rhoades 1979), are more likely than old leaves to be protected chemically or behaviorally (e.g., via mutualisms with ‘body guards’). Because the young leaves lack lignin, which protects old leaves of some plants from insect herbivory (Lambers et al. 1998), and contain high amounts of N, they are especially vulnerable to insect attack (Yokoyama and Miller 1989). Both

N concentration and EFN production are higher in young terminal leaves than older stem leaves of *P. persica*. Furthermore, leaf N and EFN exudate volume peak coincidentally in early spring when leaf feeding herbivores may cause severe injury (Putman 1963, Yokoyama and Miller 1989). Heil and McKey (2003) hypothesized that EFN production in plants will coincide with the period of greatest risk of attack from herbivores. By bringing in ‘body guards’ during this critical period, the EFNs reduce deleterious effects to the plants.

P. persica has distinct advantages as a model system for the study of EFN effects, since three leaf EFN phenotypes exist: globose (small, circular EFNs producing exudate), reniform (large, kidney-shaped EFNs producing exudate), and absent (no EFNs or exudate produced) (Connors 1922, Okie 1998). The EFN phenotype displays codominant inheritance, with heterozygotes producing the globose type and homozygous alleles producing the reniform and absent types (Connors 1922, Weber et al. 1997). By studying individuals of the same cultivar, it is assumed that EFN effects can be isolated and potentially confounding host plant characteristics (e.g. canopy architecture, fruit flesh color and pubescence) held constant (Weber et al. 1997).

The current study tested the hypothesis that *P. persica* EFNs indirectly contribute to plant defense by encouraging predators that offer protection from herbivores. Experiments compared EFN-bearing and non-EFN trees of the ‘Lovell’ cultivar, which produces offspring in a ratio of 1 absent: 2 globose : 1 reniform EFN phenotypes when self-pollinated (Fig. 1) (Okie 1998). By using trees of the same cultivar that varied with respect to EFN presence, EFN-herbivore-predator interactions

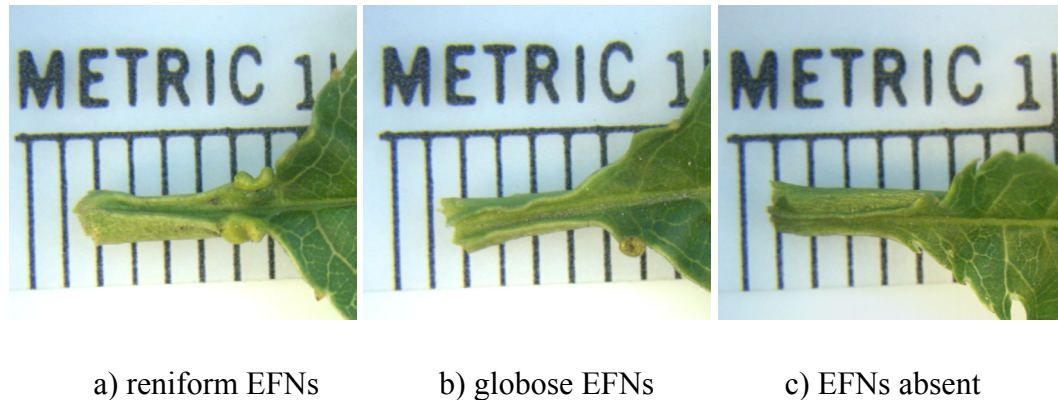


Figure 1. *Prunus persica* (L.) Batsch leaves at 5X magnification illustrating three leaf EFN phenotypes produced from ‘Lovell’ X ‘Lovell’ crosses. Gradations shown are in mm.

were explored in a system that attempted to minimize potentially confounding host plant effects. Large-scale plots with trees of the same leaf EFN type were studied in the natural setting over two years. This experimental design enabled testing of the full range of arthropods and their shifting population dynamics. Because insecticides may disrupt arthropod community structure in peach orchards (Brown and Puterka 1997), insecticide application was minimized during the studies.

Specifically, I addressed the questions: 1) Do EFNs impact predator or herbivore densities in the canopy?, 2) Is herbivory reduced on trees with EFNs?, and 3) Do the EFNs enhance tree vigor? Using a factorial design that manipulated presence of both EFNs and ants, comparing trees that had invested in EFN production and those that had not, I addressed an additional question regarding the contribution of ants to plant defense: 4) Do ants associated with the EFNs provide protection from folivory?

Materials & Methods

Field experiments comparing *P. persica* with EFNs present and absent were conducted during two years at two sites in the mid-Atlantic region: the United States Department of Agriculture Appalachian Fruit Research Station, Kearneysville, WV, and the University of Maryland Western Maryland Research and Education Center, Keedysville, MD. The two sites (situated 28 km apart) had a similar climate, topography (Hagerstown Silt-Loam soil; 3-8% slope running North-South), and potential for peach production (Pennsylvania State University 2001). Also, the landscape surrounding the experimental fields was similar at the two locations: unmanaged hedgerow c. 10 m to the west, dirt road c. 4 m to the north, and grass sod to the east and south. Treatments (EFNs present or absent) were arranged in a completely randomized design with four replicates (two per site). A replicate (0.25 ha) consisted of two plots with 40 trees of the same leaf EFN phenotype per plot. The trees were planted in 5 rows (5 X 3 m spacing) (Fig. 2). Replicates were 33 m apart and separated by a buffer hedge row of hybrid willow (*Salix* spp.) trees (Fig. 2). A buffer hedgerow also separated plots within a replicate.

One-year-old dormant ‘Lovell’ seedlings (700 total) were obtained in March 2002 (Adams County Nursery, Aspers, PA). The seedlings were produced from open-pollinated crosses of ‘Lovell’ parent material in a nursery seedling lot in 2001. Based on the predicted Mendelian segregation, it was expected that the trees would approximately follow the 1 reniform : 2 globose : 1 absent ratio for leaf EFN phenotype, enabling the selection of trees with EFNs present and absent for comparison in field studies. All seedlings were potted in March and held in the

greenhouse at 18-21°C for 4 wk to break dormancy. Trees (320 total) of a consistent size range (8-10 mm stem diameter, 60-65 cm height) were selected for field study based on leaf EFN phenotype (160 reniform type and 160 absent type) after examining leaf petioles and margins (Fig. 1) using a hand lens.

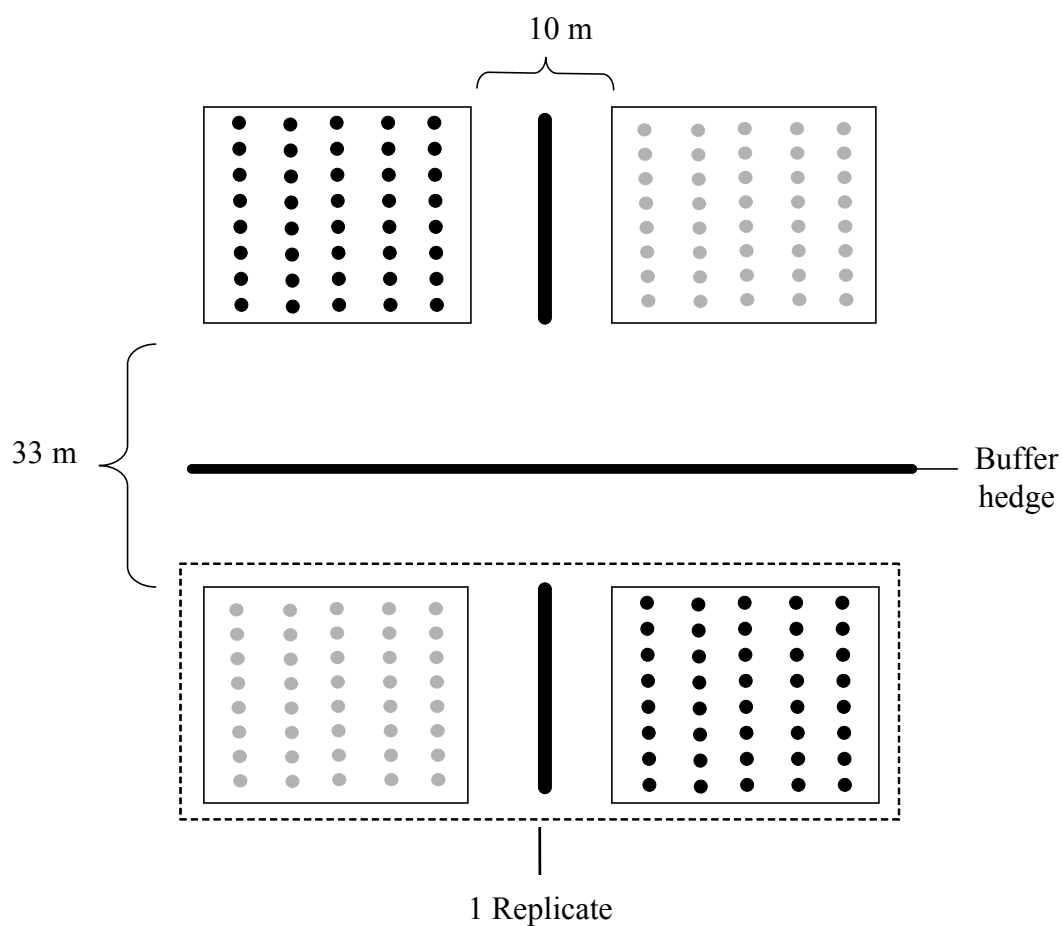


Figure 2. Field lay-out depicting two replicates used in studies comparing ‘Lovell’ peach trees with leaf EFNs present (black circles) and absent (grey circles).

Field sites were disked (19 April 2002) to break-up sod 1 wk before planting, and trees were treated (22 April) with avermectin (Agri-Mek 1.0 ml[AI]/L) to eliminate arthropods that may have infested them in the greenhouse. On 26 April trees were planted in auger dug holes (30 cm diameter X 30–35 cm deep) and covered with soil up to ~5 cm above the root system. On 17 May, 1 m tall hybrid willow trees (Greenwood Nursery, McMinnville, TN) were planted into buffer rows at each site. For rows separating replicates, 21 willow trees were planted at 2.5 m spacing (Fig. 2). For rows separating plots within a replicate, 16 willow trees were planted at 1 m spacing to form a thick buffer hedge (Fig. 2).

Mechanical cultivation to deter weed growth was performed between tree rows (11-12 June, 2-3 July, and 12-14 August 2002; 9-10 July and 4-5 August 2003), and a 0.5 m diameter area from the base of each peach tree was regularly hand-weeded from June to September (both years). An herbicide selective for grasses (Sethoxydim 0.086 kg [AI]/ha) was applied to buffer rows containing willow trees on 2 August 2002. Pre-emergent herbicides (Oryzalin 0.55 kg [AI]/ha and Oxyfluorin 0.09 kg [AI]/ha) were applied to the ground in tree rows on 15 April 2003, and Paraquat (0.12 kg [AI]/ha) was applied under tree rows on 14 May and 31 July (2003). Fungicide application was required both years to control heavy infestations of powdery mildew [*Podosphaera leucotricha* (Ell. et Ev.)] that can kill small peach trees (Rubigan 1.3 L[AI]/ha and Penncozeb 6.7 kg[AI]/ha: 29 July 2002, WV site only; Myclobutanil 0.027 kg [AI]/ha: 18-23 June and 11-14 July 2003, both sites). Chlorpyrifos (5.7 L[AI]/ha) was applied to tree trunks by handgun sprayer in all plots for control of peach tree borers (*Synanthedon* spp.) after completion of experiments in 2002 (3-7

October) and before the start of experiments in 2003 (4 April). No insecticides were applied during the field study months (May - September) of either year.

1. Do EFNs impact predator or herbivore densities in the canopy?

Predator and herbivore densities in the tree canopy were estimated twice monthly (2002: 24 and 31 May, 7 and 18 June, 3 and 16 July, 5 and 21 August; 2003: 20 and 27 May, 11 and 26 June, 7 and 21 July, 14 and 28 August). A preliminary study (M. Brown, Appalachian Fruit Research Station, Kearneysville, WV, unpublished) suggested that ants in peach orchards display temporal foraging niches within a 24 h period. Sampling was therefore done at the same time (0700-1100 hr) on a sample day to reduce variation among sample days. Sample trees were randomly selected within treatment plots each year (10 trees per plot in 2003, 4 trees per plot in 2002 due to low variability within plots). To sample a tree, a randomly selected limb was tapped three times with a rubber hose while holding a 0.58 m² canvass tray underneath to collect dislodged arthropods. Arthropods collected from the trees were identified to family or species and grouped according to function as ‘herbivores,’ ‘predators,’ or ‘others’ (e.g. detritivores, tourists). Arthropods in the ‘others’ category were not tallied for data analysis. Arthropod density data for individual sample trees within treatment plots was averaged to avoid pseudo-replication.

Arthropod density data were summed across the eight sample periods of 1 year, and analysis of variance (ANOVA) was used to test for the effect of EFNs on densities of herbivores or predators. Additional ANOVA procedures were used to test for EFN effects on the most prevalent herbivore and predator groups: *Mylocerus*

hilleri (Faust) (Coleoptera: Curculionidae) and ants (Hymenoptera: Formicidae), respectively. Separate mixed model ANOVA procedures were performed for each year (PROC MIXED, RANDOM rep rep*trt; SAS Institute 1999). A second analysis was performed for data of each sample period (8 total) each year to detect potential effects of tree phenology on predator and herbivore density. Data within sample period were log transformed to normalize distributions, and a separate mixed model ANOVA was performed for ants and herbivores (PROC MIXED, RANDOM rep rep*trt; SAS Institute 1999). When ANOVA indicated significant treatment effects, means were separated by the least-squares difference procedure using the Bonferroni adjustment to correct for multiple comparisons (LSMEANS/ADJUST=BON, alpha = 0.05; SAS Institute 1999).

2. Is folivory reduced on trees with EFNs?

Feeding studies were conducted to determine if the EFNs had direct effects on those leaf feeding herbivores that regularly appeared in the tree canopies in samples of the Section 1 study. Herbivores most commonly encountered in that study were *Mylocerus hilleri* (Faust) (Coleoptera: Curculionidae), *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae), and *Diabrotica undecimpunctata howardi* (Barber) (Coleoptera: Chrysomelidae). Ten adults per species were evaluated in the laboratory to confirm characteristic leaf injury associated with their feeding. Individuals were held alone in 710 ml paper cups (Solo Cup Co., Urbana, IL) covered with nylon mesh (20°C, 14:10 L:D photoperiod) and offered newly formed *P. persica* leaves (5 with

EFNs and 5 without EFNs) collected from the terminal tip. Leaves were examined for signs of folivory after 5 d, and herbivore injury, when present, was photographed.

In addition, the level of herbivore injury to leaves in the field was estimated on 10 randomly selected trees of each treatment plot. Because glandular activity is concentrated on new shoot growth (Yokoyama and Miller 1989), sampling was limited to newly formed leaves on the distal portion (up to 5 cm from the tip) of the terminal. The younger leaves (i.e. leaves 1-4) of a terminal are commonly folded longitudinally, and on trees with actively producing EFNs the leaf margins stick together, impeding examination of the whole leaf. Therefore, the 5th leaf distally was used as the standard position for leaf samples of all trees. Five terminals of each sample tree were randomly selected, and the 5th distal leaf of each terminal was removed (16 July and 1 August 2002). The five leaves were examined visually for the presence of folivory, and the percentage of injured leaves was recorded. Based on the results of the laboratory feeding assays, the leaf injury was classified as *M. hilleri*, *P. japonica*, or 'other herbivore' feeding. To estimate the amount of extant leaf tissue available for photosynthesis, leaf surface area (cm²) was determined for the same five leaves per tree using a leaf area meter (LI-3100, LI-COR Environmental, Lincoln, NB). For each variable (herbivory and leaf area) data for a treatment plot were averaged prior to statistical analysis. Separate ANOVAs were performed within sample dates to test for an effect of EFNs on either herbivory or leaf area (PROC MIXED, RANDOM rep rep*trt; SAS Institute 1999). When ANOVA indicated significant treatment effects, means were separated by the least-squares difference procedure (LSMEANS, alpha = 0.05; SAS Institute 1999).

3. Do the EFNs enhance tree vigor?

To assess the impact of EFNs on tree vigor, trunk growth and terminal ^{13}C composition were measured for one randomly selected peach tree per treatment plot. Trunk diameter (i.e., cross-sectional area) correlates with above-ground biomass and is used as a standard predictor of fruit yields in deciduous fruit tree production (Westwood and Roberts 1970). Because the trees in the current study had not reached fruit bearing age, trunk diameter was also an indicator of potential reproductive capacity. Trunk diameters (mm^2) were measured before (1 March 2003) and after (1 October 2003) the growing season to determine change in above-ground biomass that occurred during the active growing period (May-August). Each sample tree's trunk was measured ~5 cm above the soil level using calipers. Two perpendicular trunk diameters per tree were taken, and the two measurements were averaged.

Plant carbon assimilation during a period of seasonal growth is a useful indicator of seasonal productivity (Ehleringer 1991). On 1 March (2003) the distal 20 cm of three terminals per tree was removed for carbon analysis. Terminal samples were removed from three random locations of a similar canopy height, according to Ehleringer (1991). Following oven drying for 48 h at 55°C , terminal samples were dipped in liquid N and ground to a fine powder (passing through a 40-mesh screen) in a stainless steel mill (LM-17-732, Wiley). The mill was cleaned with pressurized air between samples. The three pulverized samples from each tree were combined, and isotopic discrimination was performed for a 5 mg subsample (^{13}C molar abundance ratio, Δ ; Isotope Services, Inc., Los Alamos, NM). To test for an EFN effect on tree vigor, separate mixed model ANOVAs were performed for carbon content and trunk

diameter (PROC MIXED, RANDOM rep rep*trt; SAS Institute 1999). When ANOVA indicated significant treatment effects, means were separated by the least-squares difference procedure (LSMEANS, alpha = 0.05; SAS Institute 1999).

4. Do ants associated with the EFNs provide protection from folivory?

To address this question, a split-plot factorial design was imposed in 2003 on the experimental plots depicted in Fig. 2. Two treatments (ants present, ants absent) were imposed on each of the EFN treatments (EFNs present, EFNs absent). Half the sample trees in each experimental plot had ants permitted; the other half had ants excluded. A completely randomized split-plot design (leaf EFN phenotype = whole plot factor; ant presence = subplot factor) was used. For each whole-plot containing 40 trees of the same EFN type (i.e., EFNs present or absent), the border trees were excluded from sampling to avoid potential edge effects; 8 trees were randomly selected for sampling.

On 3 April (2003) the 8 sample trees were each vigorously shaken to remove ants, and a 5 cm wide band of masking tape was affixed to each tree trunk ~30 cm from the ground. The ant exclusion treatment was then applied in a stratified random scheme to 1/2 of the sample trees (4 trees total). The treatment consisted of a 2 cm wide ring of tangle trap (Tanglefoot Co., Grand Rapids, MI) applied to the tape as a sticky barrier encircling the trunk. Exclusion treatments were checked weekly, and tangle trap was reapplied as necessary to ensure effectiveness of the ant barrier. The ground under the sample trees' canopies was weeded weekly to prevent ants from crawling from ground vegetation to the canopies.

Limb jarring (as described in ‘Materials & Methods,’ Section 1) was done twice monthly from May to August (20 and 27 May, 11 and 26 June, 7 and 21 July, 14 and 28 August 2003) to obtain herbivore and *M. hilleri* canopy densities on four trees of each subplot. Leaf surface area and herbivory were measured monthly (29 May, 27 June, 22 July, 19 August 2003) on four trees per subplot. Five terminals per tree were randomly selected, and the 5th distal leaf was removed. The percentage of leaves with visible herbivory presence was recorded, and the average leaf area (cm²) was determined as in the previous study (Section 2). Data for each variable (herbivory and leaf area) for each subplot’s four sample trees were averaged prior to statistical analysis. Mixed model ANOVA was used to test for the main effects of the EFNs and ants and interactive effects on all herbivores and *M. hilleri* specifically, herbivory rates, and leaf surface area (PROC MIXED, RANDOM rep rep*trt rep*trt*ants; SAS Institute 1999). Both total herbivores and *M. hilleri* density data required log transformation prior to analyses; separate ANOVAs were performed for each variable within individual sample dates. When ANOVA indicated significant treatment effects, means were separated by the least-squares difference procedure using the Bonferroni adjustment to correct for multiple comparisons (LSMEANS/ADJUST=BON, alpha = 0.05; SAS Institute 1999). To test for an association between *M. hilleri* densities and leaf injury, 2003 *M. hilleri* density data (collected 27 May, 26 June, 21 July, and 14 August by limb jarring) and herbivory estimates (from leaves collected 29 May, 27 June, 22 July, and 19 August) were combined, and correlation analysis was performed by treatment (PROC CORR Spearman’s, SAS Institute 1999).

Ant sampling was done by limb jarring described for the Section 1 study. All sampling was 0700-1100 hr. Because the actively foraging ants could not be identified without disrupting them, all ants on the canopy of one randomly selected peach tree per treatment plot were collected on 21 August (2002) and 29 August (2003) for later identification. Jeffrey Sossa (Department of Systematic Biology, Ant Laboratory, Smithsonian Institution, Washington, DC) and Sean Brady (Laboratory of Analytical Biology and Department of Entomology, Smithsonian Institution, Suitland, MD) identified the ants.

Results

1. Do EFNs impact predator or herbivore densities in the canopy?

Presence of leaf EFNs significantly affected annual total predator and herbivore densities on peach trees during both years (Table 1).

Table 1. Results of mixed model ANOVAs for fixed effect of leaf EFNs on arthropod densities on ‘Lovell’ peach trees in completely randomized design study with four field replications. The predators and herbivores were sampled by limb jarring twice monthly (May - August) in 2002 and 2003.

Year	Arthropod group	<i>ndf, ddf</i>	<i>F</i>	<i>P > F</i>
2002	Predators ^a	1, 3	28.4	<.001
	Ants	1, 3	26.7	.002
	Herbivores ^b	1, 3	13.7	.020
	<i>M. hilleri</i>	1, 3	0.7	.020
2003	Predators ^a	1, 3	247.9	.01
	Ants	1, 3	103.5	.01
	Herbivores ^b	1, 3	22.5	.03
	<i>M. hilleri</i>	1, 3	20.4	.46

^aIncludes Formicidae, Cantharidae, Coccinellidae, Asilidae, and Araneae.

^bIncludes *M. hilleri*, Aphididae, Cicadellidae, Chrysomelidae, and Scarabaeidae.

Trees with EFNs had an average of 5X greater predator density than trees without EFNs. The difference between the EFN treatments was statistically significant in both years (LSD, $P < 0.05$; Fig. 3a,b). The predators included individuals from the Cantharidae, Coccinellidae, Asilidae, Formicidae, and Araneae.

Ants consistently outranked other predator groups on trees with EFNs (97% in 2002, 78% in 2003) but were scarce on trees without EFNs (42% in 2002, 3% in 2003; Fig. 3a,b). Ants were significantly more abundant on trees with EFNs both years (LSD, $P < 0.05$, Table 1). Annual herbivore densities were significantly lower on trees with EFNs than trees without EFNs during both years. Differences were more pronounced in the second year (2003) when average annual herbivore density for trees with EFNs was half that of trees without EFNs (LSD, $P < 0.05$; Fig. 3a,b). Herbivores included individuals in the Aphididae, Cicadellidae, Chrysomelidae, and Scarabaeidae families and *M. hilleri* (Coleoptera: Curculionidae). *M. hilleri* was the most common herbivore, accounting for >50% of the total herbivore population regardless of the EFN phenotype or the year (Fig. 3a,b). EFNs significantly affected *M. hilleri* in the second year (2003; Table 1). The average annual per tree total of 9 weevils on trees with EFNs compared to 18 weevils on trees without EFNs (LSD, $P < 0.05$).

Except for the first sample date in 2003 (20 May), ant density was significantly affected by EFN presence on every sample date of both years (Table 2). After ants colonized the plots (>20 May, both years), the peach trees with EFNs had significantly larger ant populations than the trees without EFNs (LSD, $P < 0.05$; Fig. 4a,b).

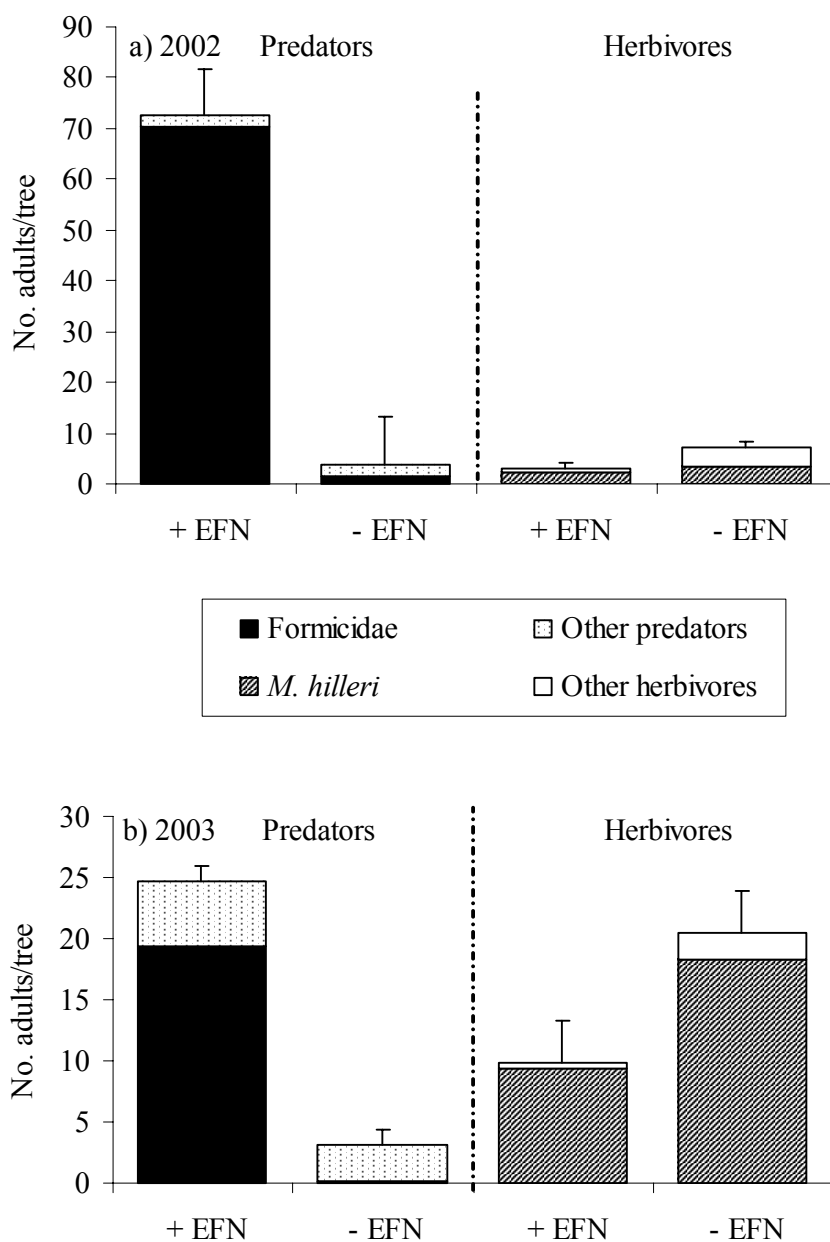


Figure 3. Effect of ‘Lovell’ leaf extrafloral nectary presence (+EFN) or absence (-EFN) on predator and herbivore densities summed over 8 d in 2002 (a) and 2003 (b). Least-squares means (+SED) are shown. ‘Other’ predators: Cantharidae, Coccinellidae, Asilidae, and Araneae; ‘Other’ herbivores: Aphididae, Cicadellidae, Chrysomelidae, and Scarabaeidae.

Table 2. Results of mixed model ANOVAs^{a,b} testing for fixed effect of leaf EFNs on ant and herbivore densities on ‘Lovell’ peach trees in completely randomized design study with four field replications. The ants and herbivores were sampled by limb jarring.

Arthropod group tested	2002							
	24-May	31-May	7-Jun	18-Jun	3-Jul	16-Jul	5-Aug	21-Aug
Ants								
<i>ndf, ddf</i>	1, 3	1, 3	1, 3	1, 3	1, 3	1, 3	1, 3	1, 3
<i>F</i>	59.2	92.2	157.5	163.9	33.5	29.7	353.2	279.0
<i>P</i>	0.01	0.01	<0.001	<0.001	0.01	0.01	<0.001	<0.001
Herbivores ^c								
<i>ndf, ddf</i>	1,3	1,3	1,3	1,3	1,3	1,3	1,3	1,3
<i>F</i>	2.3	21.6	6.2	6.9	11.1	9.3	0.1	1.5
<i>P</i>	0.12	0.02	0.13	0.04	0.02	0.05	0.52	0.3
Arthropod group tested	2003							
	20-May	27-May	11-Jun	26-Jun	7-Jul	21-Jul	14-Aug	28-Aug
Ants								
<i>ndf, ddf</i>	1,3	1,3	1,3	1,3	1,3	1,3	1,3	1,3
<i>F</i>	2.0	22.4	40.2	19.2	145.4	5.4	110.7	2.3
<i>P</i>	0.26	0.02	0.01	0.02	0.001	0.10	0.002	0.04
Herbivores ^c								
<i>ndf, ddf</i>	1,3	1,3	1,3	1,3	1,3	1,3	1,3	1,3
<i>F</i>	1	27.3	1	27.5	10.3	13.2	2.5	3.3
<i>P</i>	0.39	0.01	0.39	0.01	0.05	0.04	0.21	0.17

^a Mixed model ANOVA tested for EFN effect.

^b Mixed model ANOVA tested for main effect of EFNs, ants, and interaction; only EFN main effect results shown here.

^c Includes *M. hilleri*, Aphididae, Cicadellidae, Chrysomelidae, and Scarabaeidae.

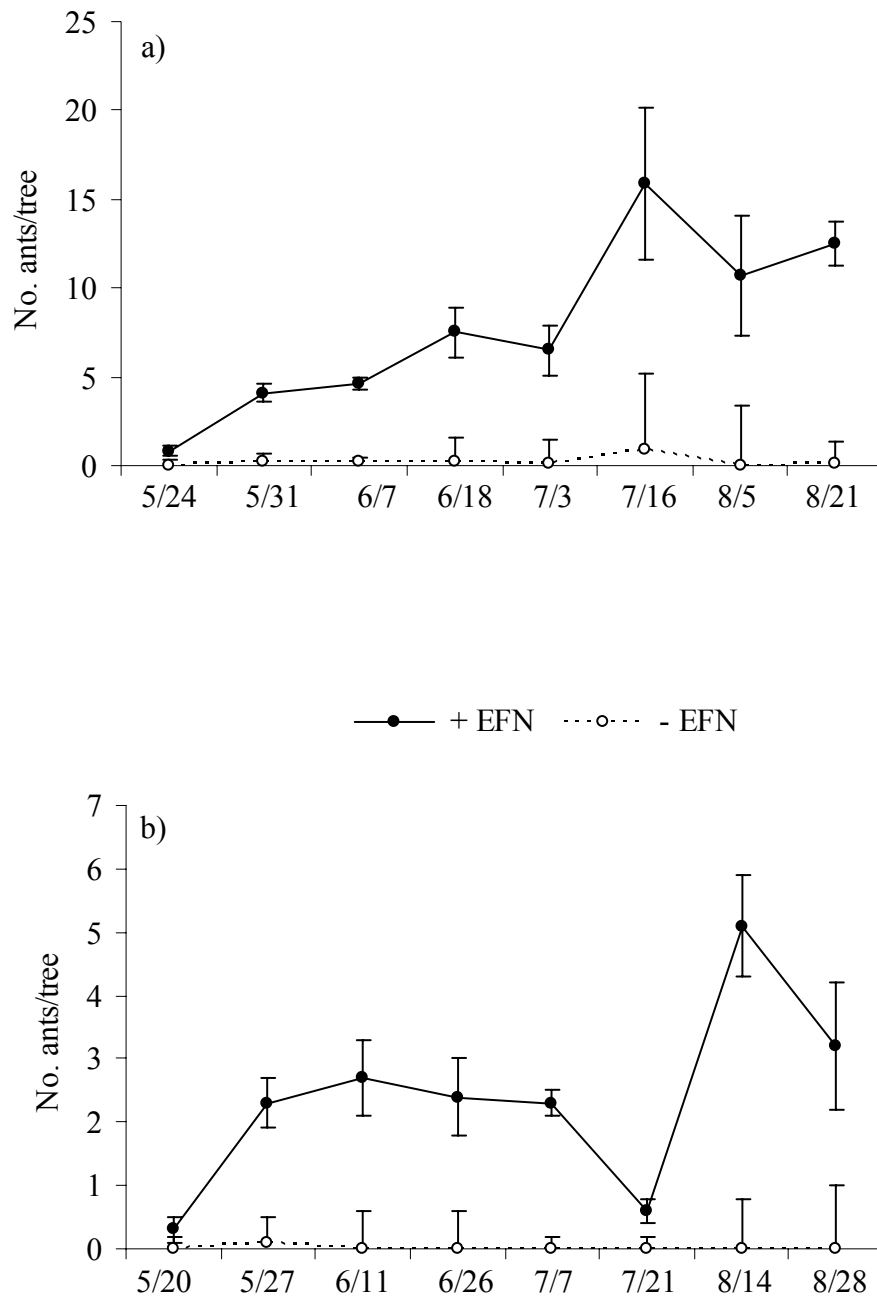


Figure 4. Ant densities on 'Lovell' trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN) in 2002 (a) and 2003 (b). Geometric means are plotted with 95% CI.

By contrast, the trees with EFNs had smaller herbivore populations than the trees without EFNs (Fig. 5). However, differences in herbivore densities due to EFNs were statistically significant on less than half of the sample periods (Fig. 5).

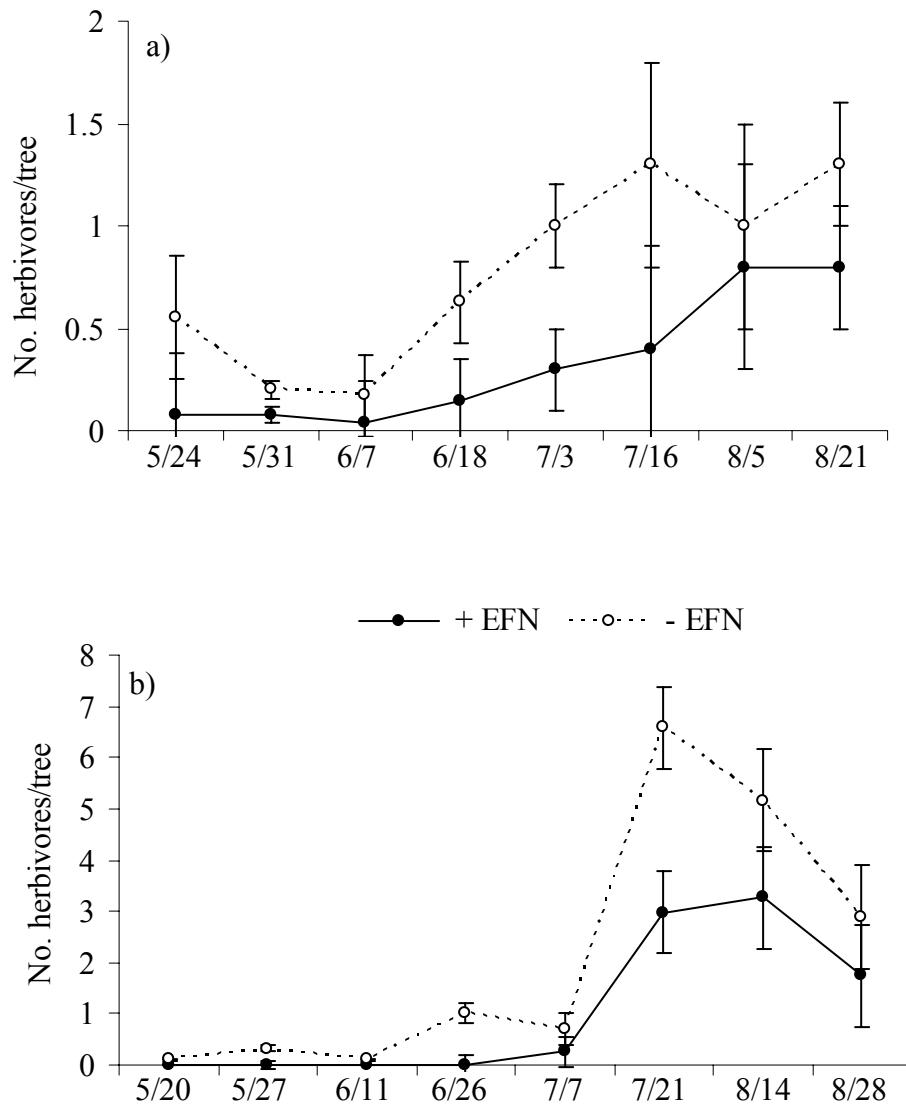


Figure 5. Herbivore densities on 'Lovell' trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN) in 2002 (a) and 2003 (b). Geometric means are plotted with 95% CI.

2. Is folivory reduced on trees with EFNs?

Laboratory evaluations confirmed folivory by *P. japonica* and *M. hilleri* on both peach leaf types (+ EFN and - EFN). *P. japonica* feeding caused "lace-like" injury (removal of leaf tissue between the veins) (Hogmire 1995). *M. hilleri* feeding caused leaf injury that was easily distinguished from that of *P. japonica* (Fig. 6). The weevil fed on the leaf margins and then chewed inwardly in a winding pattern, consuming leaf veins along the way. *Diabrotica* spp. did not feed on *P. persica* leaves of either leaf type (+ EFN or - EFN) in the laboratory.

M. hilleri feeding accounted for >98% of folivory for *P. persica* leaves with or without EFNs in the field on both sample dates in 2002 (Table 3).



Figure 6. *P. persica* folivory by *M. hilleri* in the laboratory (July 2002).

Table 3. Relative contribution of *M. hilleri* to folivory for peach trees with or without leaf extrafloral nectaries (+/-EFNs), measured as percentage injured leaves in 5 leaf sample per tree, 2002.

Herbivore species	16 July		1 August	
	+ EFNs	- EFNs	+ EFNs	- EFNs
<i>M. hilleri</i>	98.3%	99.7%	98.6%	98.2%
<i>P. japonica</i>	1.7%	0.3%	1.4%	1.8%

A significant EFN effect on folivory was detected on both sample dates in 2002 (16 July: $ndf = 1$, $ddf = 3$, $F = 69.6$, $P = 0.004$; 1 August: $ndf = 1$, $ddf = 3$, $F = 43.2$, $P = 0.007$). Trees with EFNs had less than half the level of folivory observed for trees without EFNs (Fig. 7). Folivory in the field was consistent with feeding injury caused by *M. hilleri* in laboratory assays. The leaf surface area did not differ significantly between trees with and without EFNs.

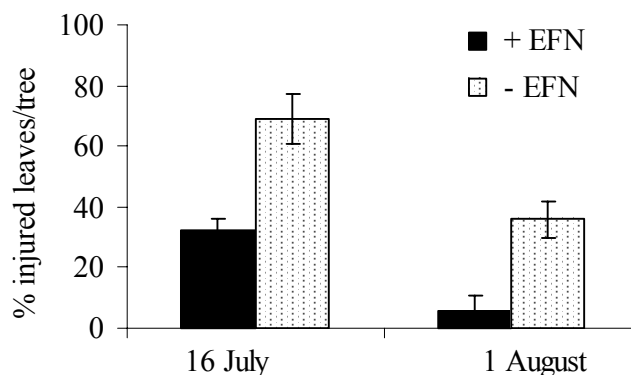


Figure 7. Effect of ‘Lovell’ leaf extrafloral nectary presence (+EFN) or absence (-EFN) on folivory, measured as percentage of injured leaves in 5 leaf sample per tree, 2002. Least-squares means (\pm SEM) are shown.

3. Do the EFNs enhance tree vigor?

Carbon isotopic composition of tree terminals collected after the first year of growth differed significantly between trees of the two EFN types ($ndf = 1$, $ddf = 3$, $F = 18.7$, $P = 0.02$). On average, trees with EFNs had significantly more ^{13}C than trees without EFNs (1 March 2003; LSD, $P < 0.05$; Fig. 8). However, difference in tree trunk growth was not detected until the second year (October 2003: $ndf = 1$, $ddf = 3$, $F = 15.5$, $P = 0.03$). Trunks of trees with EFNs were significantly thicker than trunks of trees without EFNs (LSD, $P < 0.05$; Fig. 8).

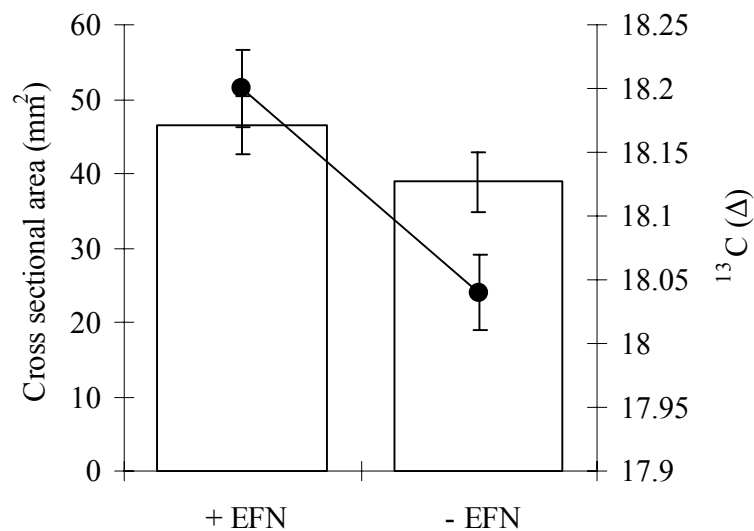


Figure 8. Effect of leaf extrafloral nectary presence (+EFN) or absence (-EFN) on 'Lovell' tree vigor: trunk cross sectional area >2 y field growth (primary Y axis, open bars) and terminal C >1 y field growth (secondary Y axis, solid circles). Least-squares means (\pm SEM) are shown.

4. Do ants associated with the EFNs provide protection from folivory?

The ant exclusion barriers effectively excluded ants from the peach tree canopies. Ants were not detected in limb jar samples of trees with ant exclusion treatment. The ant exclusion treatment significantly affected herbivore densities ($ndf = 1$, $ddf = 6$, $F = 8.58$, $P = 0.026$). The overall herbivore load increased significantly when ants were excluded (LSD, $P < 0.05$; Fig. 9). However, the exclusion of ants had no impact on the density of *M. hilleri*.

A significant EFN by ant exclusion interaction was detected for folivory in May 2003 (Table 4). When ants were present, a significant ~15-fold increase in folivory was observed for leaves without EFNs as compared to leaves with EFNs (LSD, $P < 0.05$; Fig. 10a).

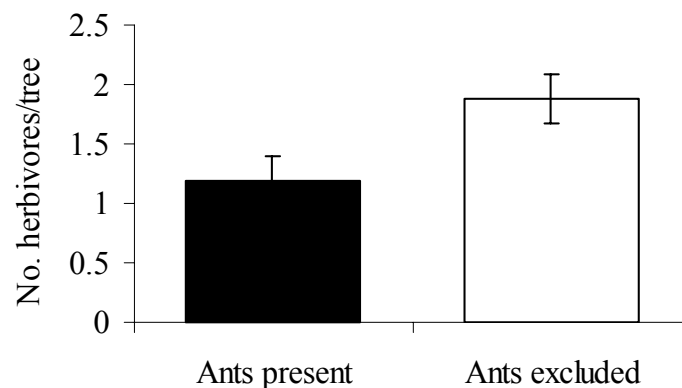


Figure 9. Effect of ant exclusion treatment on herbivore densities on ‘Lovell’ trees with leaf EFNs present or absent, 6 July 2003. Geometric means are plotted with 95% CI.

Table 4. Results of ANOVA^a testing for fixed effects of leaf EFNs, ant presence, and interaction on folivory for ‘Lovell’ peach trees in CRD split-plot study with 4 field replications; sampling was performed monthly (May - August) 2003.

Sample date	<i>ndf, ddf</i>	<i>F</i>	<i>P > F</i>
29 May			
EFNs	1, 3	29.5	0.02
Ants	1, 6	0.4	0.53
Interaction	1, 6	6.5	0.04
27 June			
EFNs	1, 3	139.8	0.001
Ants	1, 6	0.1	0.89
Interaction	1, 6	0.2	0.69
22 July			
EFNs	1, 3	25.0	0.02
Ants	1, 6	12.9	0.01
Interaction	1, 6	2.2	0.19
19 August			
EFNs	1, 3	101.1	0.002
Ants	1, 6	2.3	0.18
Interaction	1, 6	0.5	0.51

^aSeparate mixed model ANOVAs were performed for each sample date; PROC MIXED, RANDOM rep rep*trt rep*trt*ants; SAS Institute 1999.

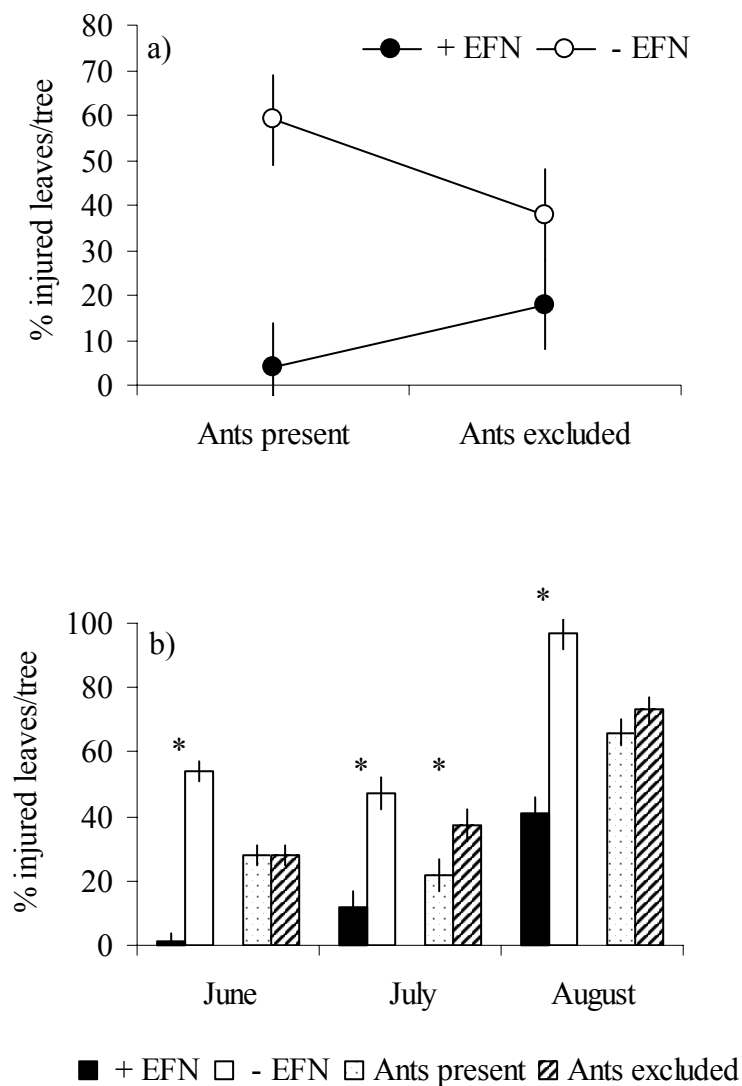


Figure 10. Monthly folivory rates, measured as percentage of injured leaves in 5 leaf sample per tree, 2003: interactive effect of ant exclusion treatment and leaf extrafloral nectary presence (+EFN) or absence (-EFN) in May (a) and main effects of EFNs and ant exclusion (b). *Indicates significant main effect determined by ANOVA for that month. Least-squares means (\pm SEM) are shown.

EFN presence significantly affected folivory during June, July, and August (Table 4); leaves without EFNs had significantly higher folivory than leaves with EFNs during these months (LSD, $P < 0.05$; Fig. 10b). In July, a significant ant exclusion effect was also detected for folivory (Table 4). Average leaf injury per tree was significantly higher on trees with ants excluded in July (LSD, $P < 0.05$; Fig. 10b).

Trees with EFNs had a greater leaf surface area than trees without EFNs (Fig. 11). This trend was apparent throughout the 2003 season, but the EFN effect was statistically significant only in June and August (June: $ndf = 1$, $ddf = 3$, $F = 29.7$, $P = 0.01$; August: $ndf = 1$, $ddf = 3$, $F = 20.7$, $P = 0.02$). The ant exclusion treatment had no effect on leaf surface area.

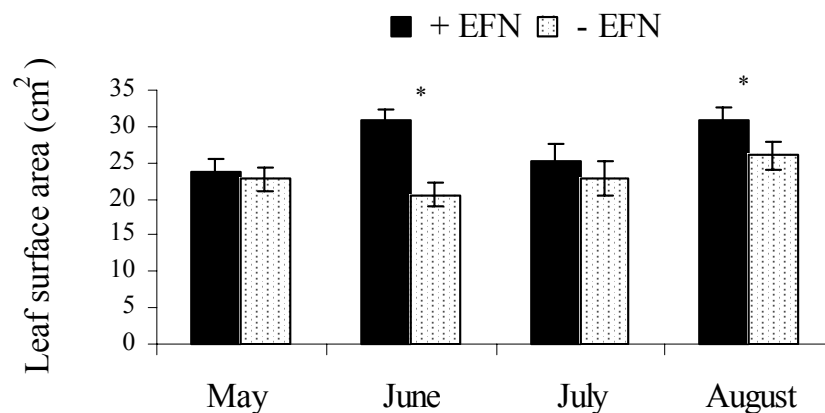


Figure 11. Average leaf surface area for ‘Lovell’ trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN), by month in 2003. *Indicates significant effect of leaf EFNs determined by ANOVA for that month. Least-squares means (\pm SEM) are shown.

M. hilleri density and leaf herbivory were significantly correlated on both tree types when ants were excluded (+ EFN: $\rho = 0.73$, $P = 0.001$; - EFN: $\rho = 0.51$, $P = 0.039$; Spearman's correlation coefficients, $N = 16$). Ants collected from the tree canopies appear in Table 5.

Discussion

This study clearly showed that EFNs increase predator populations and decrease herbivores in 'Lovell' *P. persica* trees (Figs. 3 & 5). The response of predacious ants (Fig. 4) to peach trees with EFNs is especially noteworthy. Ants are voracious generalists and may significantly impact a wide range of herbivores (Way and Khoo 1992, Stradling 1987). Overall herbivory was significantly less on trees with EFNs than without EFNs (Fig. 7), although densities of the principal herbivore, *M. hilleri*, were not impacted by either the ants or EFNs (Fig. 3). Ants apparently did not kill or remove sufficient numbers of *M. hilleri* to affect abundance of this insect.

Table 5. Ants present on 'Lovell' peach trees by leaf EFN phenotype and date; sampling was by limb jarring between 7 and 11 am ($N = 4$ trees per leaf phenotype per year).

Tree leaf phenotype	Ant species collected	
	21 August 2002	29 August 2003
+ EFN	<i>Formica nitidiventris</i> Emery <i>Lasius neoniger</i> Emery	<i>Formica nitidiventris</i> Emery <i>Lasius neoniger</i> Emery
- EFN	<i>Lasius neoniger</i> Emery	<i>Formica nitidiventris</i> Emery <i>Lasius neoniger</i> Emery

Folivory for trees with EFNs was nearly eliminated in May if ants were permitted in the tree canopy (Fig. 10a). In contrast, when ants were excluded >15% of the leaves were injured by herbivores (Fig. 10a). *M. hilleri* densities were positively correlated with leaf injury when ants were excluded from both trees with and without EFNs. This suggests that ants successfully deterred or disrupted *M. hilleri* feeding, although the exact mechanism is not known.

Trees with reduced folivory would theoretically have the advantage of greater vigor or reproductive fitness because their capacity for photosynthesis would increase. Trees of the study were too young to reproduce. However, the measures of vigor on the young trees confirmed a positive effect of the EFNs. Trees with EFNs, which experienced less herbivory than trees without EFNs during the first field season, had significantly higher carbon levels than trees without EFNs (Figs. 7 & 8) and therefore greater growth capacity. By the second growing season, significant differences in tree vigor were apparent. The trees with EFNs had a greater trunk diameter (Fig. 8) and a greater leaf surface consistently from May to August (Fig. 11). These growth enhancements are expected to carry over to subsequent years when the trees have reached a fruiting age and could potentially impact reproductive fitness (Westwood and Roberts 1970).

Data of the study supports the argument that trees with EFNs depend on ants for protection in spring (May 2003; Fig 10a). The link between EFN resources and the ants' protective function is crucial to the theory of indirect plant defense, as increases in ant densities alone do not prove defense. Furthermore the possibility that other natural enemies contributed to the protection must be eliminated.

The significant interaction between ants and EFNs in May (Fig. 10a) revealed that if ants were present, the trees with EFNs had substantially less folivory (an average of 4%, as compared to 60% for trees without EFNs; Fig. 10a). If ants were excluded, trees with EFNs did not benefit from the EFNs; they were as vulnerable to herbivory as trees without EFNs (Fig. 10a). Trees with the ant exclusion treatment still had arthropod predators, such as coccinellids, cantharids, and asilids, in the canopies. However, the other predators did not effectively provide protection from herbivores during May, because herbivory rates were comparable for trees with and without EFNs under the ant exclusion treatment in May (Fig. 10a). Therefore, when leaves first emerge in the spring, investment in leaf EFNs as a defense strategy appears to be highly effective but dependent on ants.

As the growing season progressed, protection from herbivory was no longer restricted to ants. After May, the effect of EFNs was independent of ants, and the degree of protection decreased (Fig. 10b). By August, 41% of the sample leaves on trees with EFNs had some folivory, compared to 1% in June (Fig. 10b), despite higher ant densities observed for trees with EFNs (Fig. 4b). However, the August injury level was still significantly lower for trees with EFNs than for trees without EFNs (Fig. 10b).

EFN production continued through August. Nitrogen concentration in peach leaves changes seasonally, peaking in early spring and then declining, and seasonal changes in EFN exudate chemistry might be expected (Yokoyama and Miller 1989). Change in EFN chemistry through the season may affect the seasonal dynamics of natural enemy-herbivore interactions. For example, ant aggression toward herbivores

such as *M. hilleri* could change depending on the ants' dietary constraints and EFN exudate sugar content. Josens et al. (1998) showed that the feeding habits of ants in the *Camponotus* genus are affected by both the concentration of sucrose and the viscosity of nectar. Various sugar-feeding ants become more protective of sugar resources and aggressive toward herbivores when food is scarce (Way 1963). Natural enemies (other than ants) using the EFN secretions also could have disrupted *M. hilleri* feeding and contributed to host plant protection after May. For example, the coccinellid *Harmonia axyridis* (Pallas) readily consumes peach EFN exudates in the laboratory (C.R. Mathews, Appalachian Fruit Research Station, Kearneysville, WV, unpublished). *H. axyridis* has displaced several coccinellid species in mid-Atlantic region orchards (Brown and Miller 1998) and could possibly affect other beetles, such as *M. hilleri*.

In summary, peach tree EFNs contribute indirectly, via ant protection, to early season defense from a principal herbivore, *M. hilleri*. While protection from folivory could involve a complex of arthropod natural enemies, the ants are clearly a dominant force in the peach natural enemy community. Because ants are highly competitive and may disrupt other natural enemies (DeBach 1974, Perfecto and Castineiras 1998), the potential for negative as well as positive impacts should be further explored.

Chapter II: The Impact of Leaf Extrafloral Nectaries on Biological Control of a Key Economic Pest, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), in Peach [*Prunus persica* (L.) Batsch]

Introduction

Located on the petiole, stipules, and leaf margins of most peach [*Prunus persica* (L.) Batsch] cultivars (Gregory 1915, Okie 1998), extrafloral nectaries (EFNs) are glandular structures that exude nectar. Numerous studies have documented ant associations with EFN-bearing plants, particularly in the tropics (Janzen 1966, Bentley 1977a, Beattie 1985, Rogers 1985, Heil and McKey 2003). My research in Chapter I revealed that EFN-bearing ‘Lovell’ peach trees attract ants and benefit from the ants’ presence. The EFNs may encourage potential mutualisms with additional natural enemies, such as parasitic wasps (Hymenoptera) that feed on nectar (Leius 1960, Powell 1986, Vinson and Barbosa 1987, Pemberton and Lee 1996, Quicke 1997, Lewis et al. 1998). The extrafloral nectar from a variety of plants is recognized as a valuable resource for adult ichneumonid and braconid parasitoids (Bugg et al. 1989, Stapel et al. 1997, Baggen et al. 1999) and may be important in efforts to conserve natural enemies (Barbosa 1998, Bugg and Pickett 1998, Gurr et al. 1998, Baggen et al. 1999, Landis et al. 2000). However, except for work on cotton (*Gossypium* spp.), the influence of EFNs on natural enemy effectiveness has been largely overlooked (Beattie 1985, Rogers 1985).

Information on multi-trophic level interactions in which plants mediate natural enemy attack of herbivores, e.g. through provision of resources or chemical

communication (Barbosa 1998, DeMoraes et al. 2000), suggests that plant traits such as EFNs may affect natural enemies and herbivores variably. Cotton varieties without EFNs are less attractive to some lepidopteran pests (Lukefahr et al. 1965). However, removal of EFNs from cotton plants decreased a wide range of natural enemies (Adjei-Mafo and Wilson 1983) and reduced biological control by parasitoids (Lingren and Lukefahr 1977, Treacy et al. 1987). Therefore, plant breeding efforts that select for varieties of cotton without EFNs may reduce opportunities for biological control. Larvae of the lacewing *Chrysoperla plorabunda* (Fitch) frequently use extrafloral nectar of cotton plants. The cotton nectar provides nutrition that enhances larval longevity of the lacewing (Limburg and Rosenheim 2001). Stapel et al. (1997) reported that the extrafloral nectar of cotton increased the level of parasitism in *Helicoverpa zea* (Boddie) by the braconid parasitoid *Microplitis croceipes*. Clearly, plant breeding may significantly alter traits such as EFNs and therefore influence the effectiveness of natural enemies impacted by them (Bottrell et al. 1998).

Fruit breeding programs have developed many modern peach cultivars with the EFNs removed (Okie 1998) without regard to effects on insect pests or natural enemies (Scorza and Sherman 1996). Before the present research, little was known about EFN-natural enemy relations in peach, although others had observed that natural enemies (ants and others) forage around peach EFNs and consume the extrafloral nectar. Putman (1958) suggested that the EFNs may help protect 'Elberta' peach from European red mites [*Panonychus ulmi* (Koch)]. Later, Putman (1963) showed that common predators in peach orchards [*Stethorus punctillum* (Weise), *Adalia bipunctata*

(L.), *Cycloneda sanguinea* (L.), *Chrysopa carnea* (Steph.), *Camponotus pennsylvanicus* (DeGeer), and *Prenolepis imparis* (Say)] consumed the EFN exudate.

Chemical analysis of EFN secretions of the peach cultivar ‘Earli-Glo’ revealed carbohydrates and amino acids (Caldwell and Gerhardt 1986). Parasitoid adults require these components for energy and egg formation (Vinson and Barbosa 1987, Lewis et al. 1998). EFN production for *Prunus* spp. is higher in young terminal leaves than in older stem leaves, and EFN volume peaks in early spring (Putman 1963, Yokoyama and Miller 1989). The EFN secretions could potentially sustain natural enemy populations in early spring before host/prey are available and therefore enhance biological control of pest herbivores, especially if EFN feeding increased fitness or effectiveness of the natural enemies (Hodek 1962, Price et al. 1980, Van Emden 1990, Barbosa 1998, Barbosa and Benrey 1998, Bugg and Pickett 1998, Landis et al. 2000). The limited information on the effects of EFNs on natural enemy fitness indicates that natural enemies benefit from EFN feeding. *Macrocentrus ancylicivorus* (Roh.), an important parasitoid of the oriental fruit moth [*Grapholita molesta* (Busck)], lived longer when presented young peach leaves that produced greater amounts of EF nectar than on older leaves (Putman 1963). In the laboratory, peach extrafloral nectar increased longevity of and egg parasitism by the oriental fruit moth egg parasitoid *Trichogramma minutum* (Shearer and Atanassov 2004). However, no one has determined how natural enemies other than ants or key pests respond to peach EFNs in the field.

It is especially important to understand the potential for competitive interactions among different natural enemies that feed on EFN secretions. The

predominance of ants on EFN-bearing peach trees (Chapter I) could reduce the effectiveness of other natural enemies. Ant exclusion from citrus canopies in Australia resulted in a 2-fold increase in beneficial arthropod abundance (James et al. 1999). Ants excluded from pineapple in Hawaii led to increased mealybug [*Dysmicoccus brevipes* (Cockerell)] parasitism by *Anagyrus ananatis* (Gahan) (Gonzalez-Hernandez et al. 1999). Likewise, when ants were excluded from grapefruit [*Citrus paradisi* (MacFayden)] trees, parasitism of the scale *Aonidiella aurantii* (Maskell) by the parasitoid *Aphytis melinus* (DeBach) increased (Murdoch et al. 1995).

The current study examined the relationship between EFNs, ants, other natural enemies, and the oriental fruit moth (*G. molesta*). The oriental fruit moth is a key pest of peaches in the mid-Atlantic region (Allen 1962, Hogmire 1995). *G. molesta* attacks the peach tree (fruits and young terminals) in the larval stage (Rothschild and Vickers 1991). Insecticides are regularly applied to control the pest in the mid-Atlantic region (Hogmire 1995). Parasitoids attacking *G. molesta* (larval stage) in the mid-Atlantic include several ichneumonids and braconids (*Macrocentrus* spp.) (Allen 1962).

Field experiments compared peaches with two leaf gland types (reniform EFNs and no EFNs) and attempted to hold other host plant characteristics (e.g. canopy architecture and fruit attributes) constant by using the same peach cultivar, 'Lovell.' Studies addressed the following specific questions: 1) Do EFNs or ants affect other natural enemies in the peach tree canopy?, 2) Do EFNs or ants affect densities or

parasitism of the key economic pest, *G. molesta*?, and 3) Do ants associated with peach EFNs affect fruit injury by *G. molesta*?

Materials & Methods

The study, conducted in 2002 and 2003, used the orchard plots (0.1 ha per plot) described in Chapter I (Fig. 2). The treatments (peach trees with and without EFNs) were in a completely randomized design and replicated four times. In 2003, plots were split (completely randomized split-plot design, Chapter I), to measure the whole plot effects of leaf EFNs and the subplot effects of ants (ants excluded or not).

1. Do EFNs or ants affect other natural enemies in the peach tree canopy?

Tree canopy densities of predators were estimated in 2002 and 2003 by limb jarring. Densities of parasitic Hymenoptera were estimated by sticky traps. Limb jarring, as described in Chapter 1, was performed twice monthly (2002: 24 and 31 May, 7 and 18 June, 3 and 16 July, 5 and 21 August; 2003: 20 and 27 May, 11 and 26 June, 7 and 21 July, 14 and 28 August). Ten trees per plot were sampled in 2002. In 2003, under the split-plot design, sampling was reduced to 8 trees (4 trees per subplot). Dislodged predators were counted and identified to family or species and later grouped as total predators, Araneae, Asilidae, Coccinellidae, Formicidae, or 'other.' Sticky trap sampling for parasitic Hymenoptera was conducted monthly during 2002 (18 June, 3 July, and 22 August). In 2003 the interval between sticky trap samples was shortened to two weeks (29 May, 10 and 26 June, 7 and 29 July, and 14 and 28 August), in order to assess parasitoid population dynamics. The sticky traps were clear 18 cm diameter plastic dinner plates with their inner 15 cm diameter coated in a

thin layer of Tangletrap (Tanglefoot Co., Grand Rapids, MI). Four traps per plot were suspended by string from the terminals of four randomly selected trees. After 48 h, traps were covered with saran wrap, transported to the laboratory, and frozen until the captured insects could be examined by stereo microscope. In 2002, the parasitic Hymenoptera were not separated below the order level. In 2003, they were identified to at least superfamily. For each sample period, natural enemy density data for the four sample trees of a sub-plot were averaged to avoid pseudo-replication.

Data for sample periods of each year required log transformation before statistical analysis. Separate analyses were performed for the total predator (non-ant) collection, total parasitoid collection, and for specific taxa known to attack *G. molesta* (Asilidae, Araneae, Braconidae, and *T. minutum*). Analyses were conducted for each year's sample period (8 total), to capture seasonal dynamics that potentially could have varied with either tree or arthropod phenology. For the 2002 data, a mixed model ANOVA tested for the EFN effects on natural enemy densities (PROC MIXED, RANDOM rep rep*trt; SAS Institute 1999). For the 2003 data, mixed model ANOVA tested for the main effects of EFNs and ants on natural enemy densities and the interactions (PROC MIXED, RANDOM rep rep*trt rep*trt*ants; SAS Institute 1999). When ANOVA indicated a significant treatment effect, means were separated by the least-squares difference procedure using the Bonferroni adjustment to correct for multiple comparisons (LSMEANS/ADJUST=BON, alpha = 0.05; SAS Institute 1999).

2. Do EFNs or ants affect densities or parasitism of the key economic pest, *G. molesta*?

G. molesta populations were monitored 4 times in 2002 (24 May, 23 June, 23 July, 19 August) and 2003 (29 May, 26 June, 22 July, 14 August). Eight randomly selected trees per plot were sampled both years (2003: 4 trees with ant exclusion, 4 trees without ant exclusion). The sampling spanned the period when *G. molesta* normally infests peaches in the experimental sites (H. Hogmire, West Virginia University Kearneysville Tree Fruit Education Center, pers. commun., 2002). An entire tree was visually inspected for new shoot flagging injury and frass characteristic of *G. molesta* larval feeding in stems (Rothschild and Vickers 1991). The total number of flagged shoots and larvae (one flagged shoot = one larva) per tree was recorded. To avoid recounting injured terminals in subsequent samples, flagged shoots (~12 cm long) were cut off and transported to the laboratory.

Shoots collected in 2003 were held for emerging *G. molesta* moths and adult parasitoids. Each shoot was held in a 710 ml paper cup (Solo Cup Co., Urbana, IL). A store-bought red apple (variety 'Red Delicious'), washed with dish soap (Ivory) to remove wax coating and rinsed with deionized water, was added to each cup as food for larvae emerging from the shoot (Bobb 1939). Cups were covered with nylon mesh and held in a growth chamber (22°C, 16:8 L:D photoperiod) and checked weekly for *G. molesta* adults and parasitoid adults. Percentage parasitism was calculated as the percent of available hosts (i.e., shoots from which either an adult moth or adult wasp emerged) from which an adult parasitoid emerged.

Graphical examinations and univariate analyses suggested that data within sample periods of a year were appropriate for ANOVA. For 2002 data collected in the CRD study, mixed model ANOVAs (within sample periods) tested for the effects of EFNs on *G. molesta* densities (PROC MIXED, RANDOM rep rep*trt; SAS Institute 1999). For 2003 data collected in the split-plot study, mixed model ANOVAs tested for the main effects of EFNs and ants and the EFN X ant interaction on *G. molesta* densities and percentage parasitism of *G. molesta* within sample periods (PROC MIXED, RANDOM rep rep*trt rep*trt*ants; SAS Institute 1999). Percentage parasitism data were arcsine ($\sqrt{}$) transformed prior to ANOVA. When ANOVA indicated significant treatment effects, means were separated by the least-squares difference procedure using the Bonferroni adjustment to correct for multiple comparisons (LSMEANS/ADJUST=BON, alpha = 0.05; SAS Institute 1999).

3. Do ants associated with peach EFNs affect fruit injury by *G. molesta*?

Because trees of the previous studies were too young to fruit, this study evaluated *G. molesta* injury to fruit in an orchard of mature peach trees at the USDA Appalachian Fruit Research Station (Kearneysville, WV). The study used two 0.25 ha blocks (140 trees each) of the ‘Loring’ cultivar, which has the globose leaf EFN phenotype (Fig. 1). Trees were planted in 1997 and used previously in orchard management studies. Each block was flanked by apple [*Malus domestica* (Borkh)] trees on one side and woody hedgerows on the other sides.

On 3 April 2003, 20 trees were randomly selected from each of the two 0.25 ha blocks. The trees were pruned so their canopies did not contact the ground or

adjacent trees. Ten of the 20 trees of each block were randomly assigned to the ant exclusion treatment. The treatments (ants excluded, ants present) were arranged in a randomized complete block. Ant exclusion bands were applied, as described in Chapter I. The bands were inspected weekly, and Tangletrap was reapplied as necessary to maintain the ant barrier. Tree canopies were inspected weekly to ensure that no branches or foliage contacted the ground or adjacent trees, which could lead to ants using them as bridges. When any tree needed pruning, all 40 trees in the study were pruned in the same manner. In this way, potential physiological responses to pruning (e.g. plant release of volatile chemicals) were held constant across blocks and treatments. Mating disruption pheromones (Isomate-LPTB and Isomate-P, 140 lures/0.5 ha, Biocontrol Limited, Vancouver, WA) were used to disrupt peach tree borers (*Synanthedon* spp.) in both blocks. Both blocks received fungicide sprays (Rubigan 1.3 L[AI]/ha and Penncozeb 6.7 kg[AI]/ha) at monthly intervals, and the grass aisles were mowed regularly from May to August.

On 13 August, 20 mature peaches per tree were randomly selected and harvested. The fruits were examined visually for external insect injury and then cut into quarters to examine internal flesh. Larvae inside the fruits were examined under a stereo microscope to confirm the presence of the anal comb, the taxonomic feature that is indicative of *G. molesta*. The percentage of fruit (per 20 fruit sample) infested with *G. molesta* was recorded. Data were arcsine ($\sqrt{\quad}$) transformed, and a mixed model ANOVA was performed to determine if ant exclusion significantly affected the percentage *G. molesta* injured fruit (PROC MIXED, RANDOM block block*trt; SAS Institute 1999).

Results

1. Do EFNs or ants affect other natural enemies in the peach tree canopy?

The ant exclusion treatment effectively precluded ant foraging in the canopies of both tree types. Coccinellidae accounted for 30-38% of predators (other than ants) on trees with EFNs and 14-23% of predators (other than ants) on trees without EFNs (Fig. 12). Aside from ants, Araneae were the predominant natural enemy group found on trees lacking EFNs during 2002 and 2003 and comprised 31-44% of natural enemies (other than ants) found on trees with EFNs (Fig. 12). Asilidae and Cantharidae were present on both tree types (with and without EFNs) but accounted for <26% of predators sampled annually (Fig. 12: ‘Other predators’).

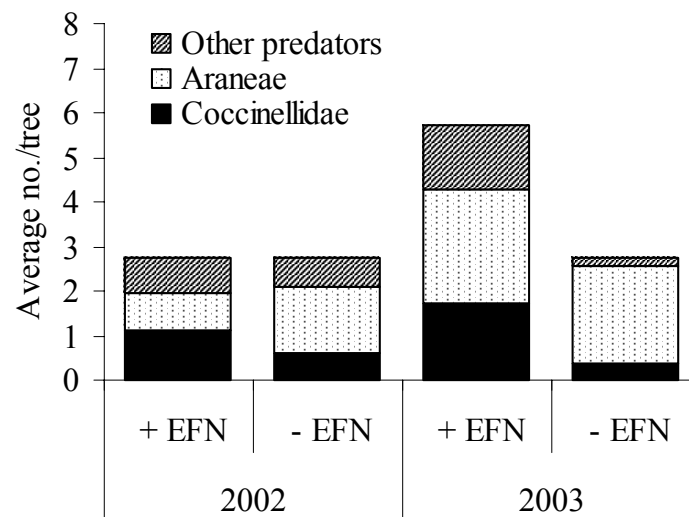


Figure 12. Predators other than ants on ‘Lovell’ peach trees with and without leaf extrafloral nectaries (+/-EFN). Average adult densities, by limb jarring, were summed over 8 sample periods per year. ‘Other’ predators: Cantharidae, Asilidae.

The Chalcidoidea were the dominant parasitic Hymenoptera group in 2003. They accounted for >52% of the parasitic wasps collected on sticky traps of trees in both EFN treatments (Fig. 13). Ichneumonoidea comprised 30% of parasitoids collected annually from trees with EFNs but only 10% of the parasitoids from trees without EFNs (Fig. 13). The oriental fruit moth egg parasitoid *T. minutum* accounted for ~5% of parasitoids collected from either tree type (+EFN or -EFN; Fig. 13). Individuals in the superfamilies Platygastroidea, Proctotrupoidea, and Ceraphronoidea were also collected during 2003 (Fig. 13: ‘Other parasitoids’).

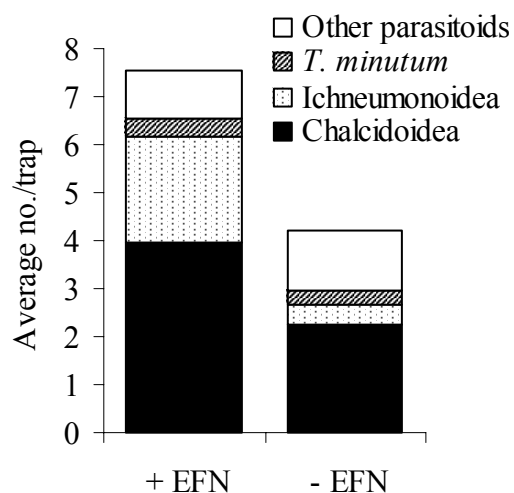


Figure 13. Parasitic Hymenoptera associated with ‘Lovell’ peach trees with and without leaf extrafloral nectaries (+/-EFN). Average adult densities, by sticky trap, were summed over 8 sample periods in 2003. ‘Other’ parasitoids: Platygastroidea, Proctotrupoidea, and Ceraphronoidea.

Predator densities were higher on trees with EFNs than on trees without EFNs (Fig. 14). This trend was apparent from May through July of both years, but the EFN effect was significant only on 27 May 2003 ($ndf=1$, $ddf=3$, $F=22.1$, $P=0.018$).

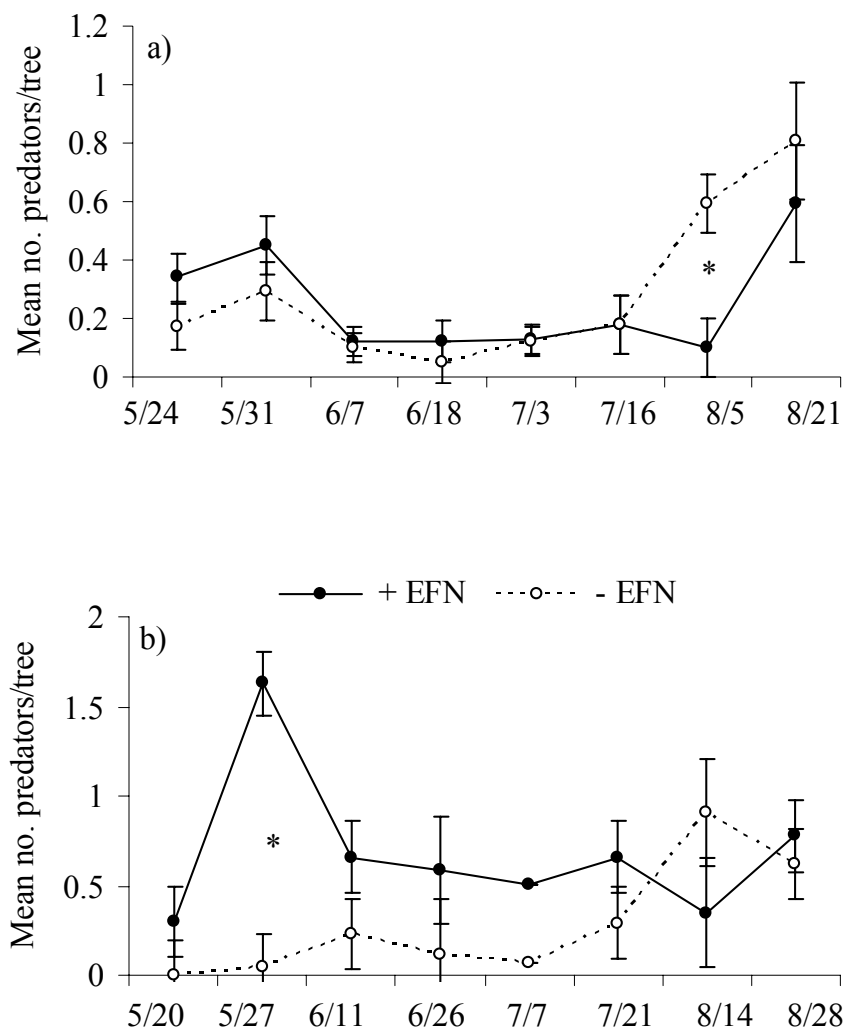


Figure 14. Predator densities on ‘Lovell’ trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN), by limb jarring on 8 d in 2002 (a) and 2003 (b). Geometric means are plotted with 95% CI. *Significant EFN effect detected by ANOVA.

Trees with EFNs had significantly higher average predator densities than trees without EFNs on 27 May 2003 (LSD, $P < 0.05$; Fig. 14b). The predator response varied during the August sample periods of both years. In 2002, there was a highly significant EFN effect for the 5 August sample period, with greater predator densities on trees lacking EFNs ($ndf = 1$, $ddf = 3$, $F = 106.5$, $P = 0.002$; Fig. 14a; LSD, $P < 0.05$). In 2003, ant exclusion significantly affected predators in August ($ndf = 1$, $ddf = 6$, $F = 8.1$, $P = 0.029$). Significantly more predators inhabited trees with ant exclusion than trees with ants permitted to forage in the canopy during this period (14 August: LSD, $P < 0.05$; Fig. 15). Ant exclusion also significantly affected spiders on 14 August 2003 ($ndf = 1$, $ddf = 3$; $F = 0.03$, $P = 8.7$), with higher spider densities on trees with ants excluded regardless of leaf EFN type (LSD, $P < 0.05$). Asilid densities were not significantly affected by EFNs or ants in any sample period of either year.

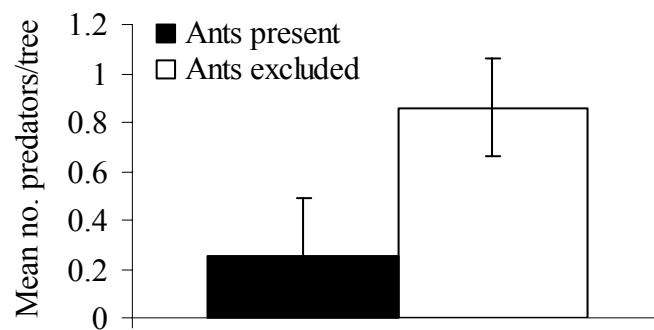


Figure 15. Effect of ant exclusion on densities of predators other than ants (by limb jarring) on ‘Lovell’ trees, August 2003. The graph combined predator data from both trees with and without EFNs. Geometric means with 95% CI are plotted.

The data of Figure 16 show a consistent trend of higher parasitic Hymenoptera abundance (by sticky trap) for trees with EFNs than trees without EFNs in both years.

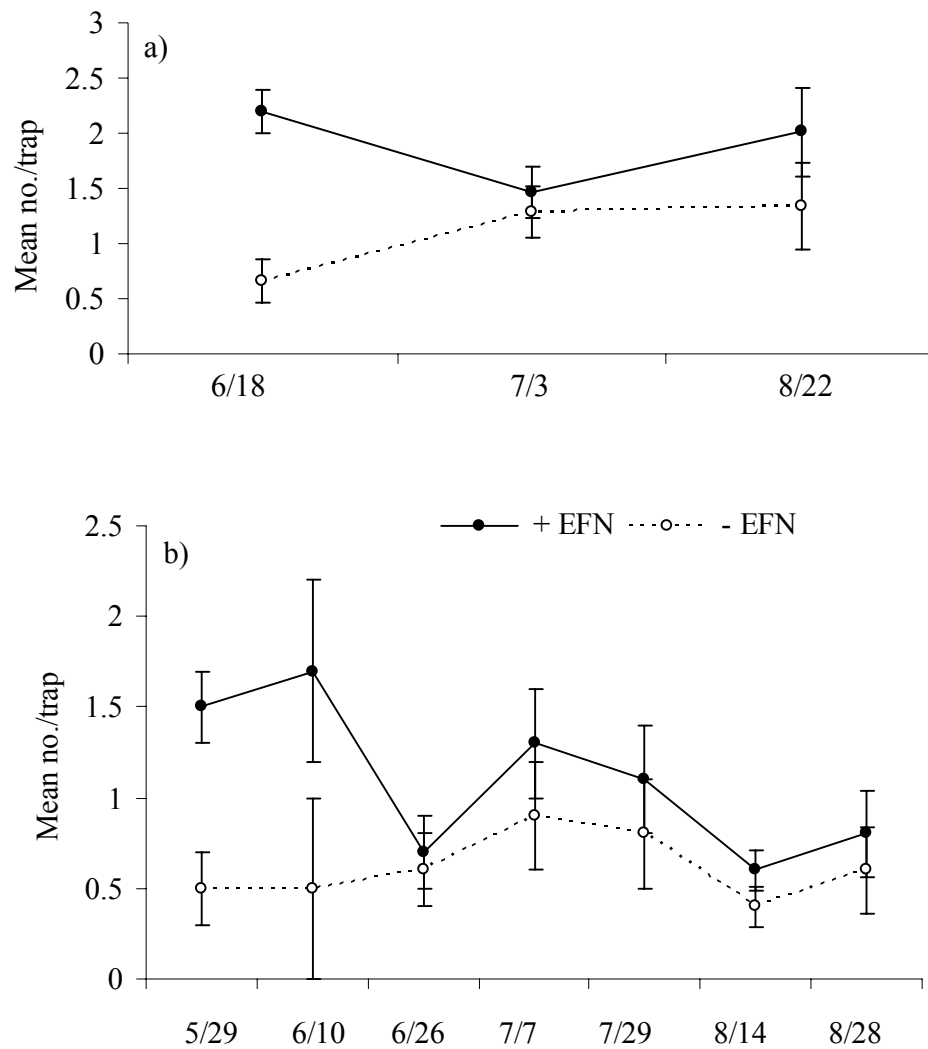


Figure 16. Effect of ‘Lovell’ extrafloral nectaries (+/- EFN) on parasitic Hymenoptera densities, by sticky trap, on 3 d in 2002 (a) and 7 d in 2003 (b). Parasitoid density data from both trees with and without EFNs were combined. Geometric means are plotted with 95% CI.

The EFN effect was significant for parasitoids (species combined) collected in May 2003 and in June both years (all dates: $ndf = 1$, $ddf = 3$; 18 June 2002: $F = 100.6$, $P = 0.002$; 29 May 2003: $F = 9.7$, $P = 0.02$; 10 June 2003: $F = 4.89$, $P = 0.04$).

Significantly more parasitoids were found on trees with EFNs than trees without EFNs in May and June each year (LSD, $P < 0.05$; Fig. 16a,b). Separate analyses for Braconidae and *T. minutum* indicated no significant effects of EFNs or ant exclusion on densities of any sample period.

2. Do EFNs or ants affect densities or parasitism of the key economic pest, *G. molesta*?

A significant interactive effect of EFNs and the ant exclusion treatment was found for *G. molesta* larvae in shoots during the first sample period of 2003 (29 May: $ndf = 1$, $ddf = 6$, $F = 65.2$, $P = 0.02$). When ants were not excluded from the tree canopies, trees with EFNs had significantly fewer flagged shoots than trees without EFNs (LSD, $P < 0.05$; Fig. 17). When ants were excluded, *G. molesta* injury did not differ between trees with or without the EFNs. Neither EFNs nor ants significantly impacted the pest's larval densities in terminal shoots during the June-August sample periods in 2002 or 2003.

No significant correlations between *G. molesta* shoot densities and natural enemy groups were detected in any month of 2002. In the first sample period of 2003, asilid densities were negatively correlated with larval flagging on trees with EFNs on which ants were not excluded. However, no significant correlation was detected

between *G. molesta* and any of the natural enemy groups on trees without EFNs (May; Table 6). Braconid abundance was positively correlated with *G. molesta* injury on trees with EFNs on which ants were not excluded (June 2003; Table 6). Spiders were negatively correlated with *G. molesta* injury on trees without EFNs and on which ants had not been excluded (August 2003; Table 6).

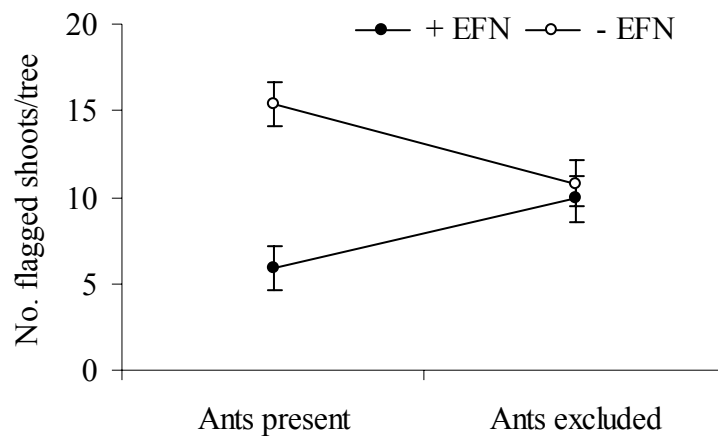


Figure 17. Interactive effect of ant exclusion treatment and leaf extrafloral nectary presence (+EFN) or absence (-EFN) for *G. molesta* larvae infesting terminal shoots of 'Lovell' peach trees, 29 May 2003. Least-squares means (\pm SEM) are shown.

Table 6. Associations between natural enemy densities^a and oriental fruit moth larvae in terminal shoots^b of peach trees with or without leaf extrafloral nectaries (+/-EFNs) or ant exclusion treatment. Results are from Spearman's rank correlation analyses performed by treatment within four sample periods in 2003 (N=16 per period).

Sample period	Natural enemy group
	ρ , P
May	Asilidae
+ EFNs, ants present	-0.53, 0.03
June	Braconidae
+ EFNs, ants present	0.59, 0.02
August	Araneae
- EFNs, ants present	-0.65, 0.002

^a Total number per tree by limb jarring (27 May, 26 June, 29 July, and 14 August) and 24 h sticky trap sampling (29 May, 26 June, 22 July, and 14 August).

^b Total number of flagged terminal shoots, by visual inspection (29 May, 26 June, 29 July, and 14 August).

The abundance of *G. molesta* larvae peaked on peach trees with EFNs in July (per tree mean = 8.7, SEM = 2.6) (Fig. 18). By contrast, the abundance of larvae peaked in May (per tree mean = 13.1, SEM = 0.9) on trees without EFNs and then gradually declined (Fig. 18). Braconids reached a maximum density in May on trees with EFNs (per tree mean = 0.75, SEM = 0.2), contrasted with a peak density in June (per tree mean = 0.13, SEM = 0.1; Fig. 18) on trees without EFNs. The EFNs significantly affected *G. molesta* parasitism by *M. delicatus* (Hymenoptera: Braconidae) in July 2003 ($ndf = 1$, $ddf = 3$, $F = 6.8$, $P = 0.04$) (Fig. 19). Percentage parasitism was significantly higher on trees with EFNs in July 2003 (LSD, $P < 0.05$; Fig. 19).

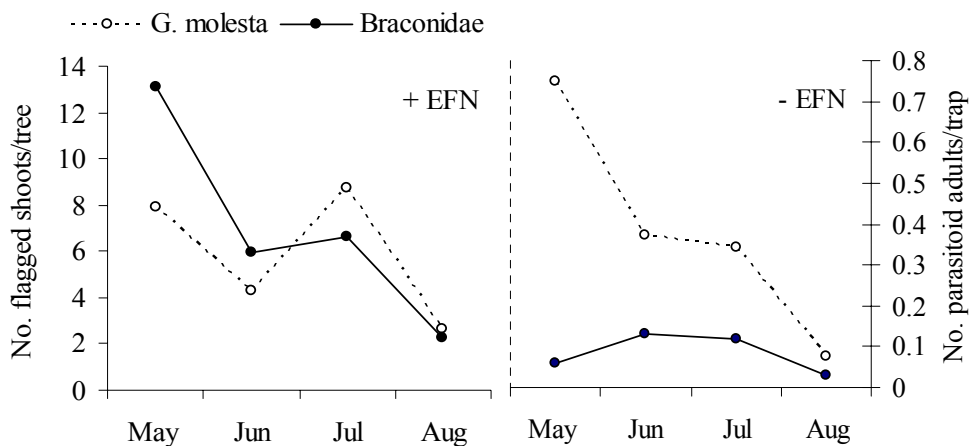


Figure 18. Average monthly densities of larval *G. molesta* (Y_1 axis), by visual inspection, and adult Braconidae (Y_2 axis), by sticky trap, in plots of 'Lovell' peach trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN), 2003.

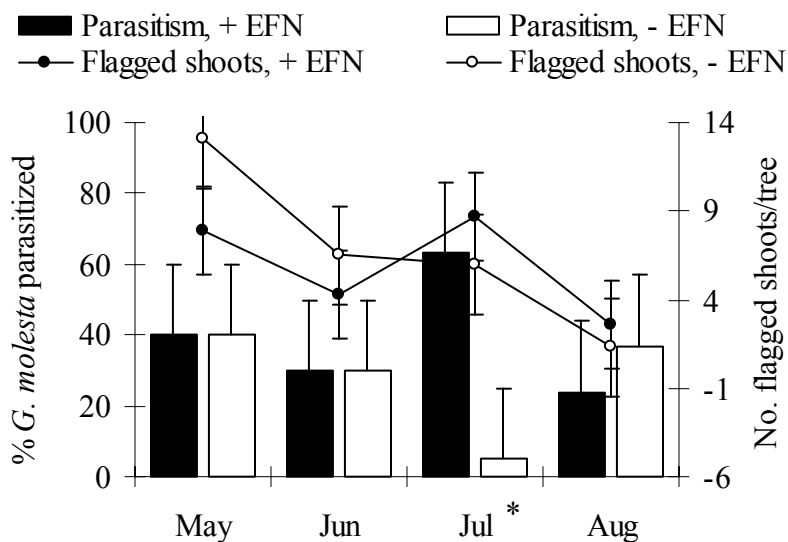


Figure 19. Monthly rates of *G. molesta* parasitism by *M. delicatus* (Y_1 axis) in relation to *G. molesta* larval densities (Y_2 axis) in shoots of 'Lovell' peach trees with leaf extrafloral nectaries present (+EFN) or absent (-EFN), 2003. Back-transformed means are plotted with 95% CI. *Significant EFN effect within month detected by ANOVA.

3. Do ants associated with peach EFNs affect fruit injury by *G. molesta*?

The ant exclusion treatment significantly increased the level of fruit injury by *G. molesta* on the mature peach trees ($ndf = 1$, $ddf = 19$, $F = 7.3$, $P = 0.02$). The percentage of fruit injured was ~5 times greater on trees on which ants were excluded (LSD, $P < 0.05$; Fig 20).

Discussion

The results of these studies suggest that the leaf EFNs generally have a positive impact on natural enemies in peach orchards. The increase in ants due to the EFNs did not result in any major disruptions of other natural enemies. Only one negative effect was detected, and that was late (5 August 2002) in the growing season (Fig. 15).

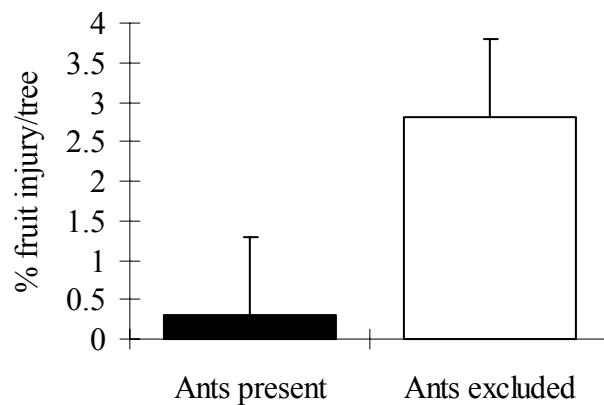


Figure 20. Effect of ant exclusion on percentage of fruit (20/tree harvested 13 August 2003) infested with *G. molesta* larvae. The study was conducted on 8 y old 'Loring' peach trees with leaf EFNs. Back-transformed means and 95% CI are shown.

Trees with EFNs generally had greater numbers of natural enemies associated with them than trees without EFNs (Figs. 12, 13 & 14). The statistically significant increases in non-ant natural enemies in May and June (Figs. 14 & 16) on trees with EFNs could be important in reducing pests later in the season. Sugar resources in the spring are particularly important for adult parasitic wasps that rely exclusively on nectars for food sources (Leius 1960, Quicke 1997). Sugar resources can enhance wasp fecundity, longevity and attack rates (Powell 1986, Vinson and Barbosa 1987, Olson et al. 2000, Fadamiro and Heimpel 2001) and lead to increased time spent searching for hosts (Lewis et al. 1998). Stearns (1928) reported that sugar feeding increased the longevity of *M. ancylivorus*, a predominant parasitoid of *G. molesta* in the mid-Atlantic region. Fadamiro and Heimpel (2001) showed that the longevity of *M. grandii* (Goidanich), which parasitizes lepidopteran larvae, increased by 2 d if exposed to sugar for 24 h early in life.

The significant EFN X ant interaction detected for *G. molesta* densities in May 2003 (Fig. 17) suggests that ants exerted pressure on 1st and 2nd generation *G. molesta* on EFN trees. About 30% fewer terminal shoots were infested by *G. molesta* on trees with EFNs when ants were not excluded (Fig. 17). However, the negative correlation detected for asilids and *G. molesta* on trees with both EFNs and ants present suggests that asilids could have contributed to reductions in *G. molesta* as well.

The EFNs had an especially dramatic impact on parasitism of *G. molesta* larvae by the braconid *M. delicatus* in July 2003 (Fig. 19). Neither braconid nor *G. molesta* densities differed on trees with and without EFNs in July. Therefore, the

increased parasitism in the EFN treatment was apparently not because of a numerical response to food resources or hosts. However, the EFNs could have enhanced wasp tenure time or effectiveness via increased host-finding or attack rates (Powell 1986, Lewis et al. 1998). The drastic increase in *M. delicatus* parasitism on trees with EFNs in July has potential economic significance, as oriental fruit moth larvae at that time can cause heavy losses to peach fruits. In the mid-Atlantic region, larvae of the first three generations of *G. molesta* feed almost exclusively on peach terminal shoots. Larvae of later generations attack the fruits (Hogmire 1995, L. Hull, Pennsylvania State University Fruit Research and Education Center, Bigglerville, PA, pers. commun., 2002). Thus, the parasitoid's action on the July infestation (4th or 5th generation) may lessen economic injury to the fruits.

While the impact of ants on *G. molesta* was not clearly demonstrated in the studies using young peach trees, the results of the study using mature trees (Fig. 20) provided striking evidence of ants' ability to reduce fruit injury. The Chinese have relied on ants to suppress fruit injury in citrus orchards for centuries, and anecdotal evidence suggests that fruit production is not possible in some regions of China without the actions of ants (Groff and Howard 1925, Olkowski and Zhang 1998). The results of my earlier work (Chapter I) suggested that ants associated with EFN-bearing peach trees deterred feeding habits of the key herbivore, *M. hilleri*. Ants could have removed or eaten *G. molesta* eggs or larvae. Tilman (1978) observed that *Formica obscuripes* (Forel) associated with EFNs of *Prunus serotina* (Ehrh.) would remove *Malacosoma americanum* (Fabricius) larvae and that a variety of beetles dropped from the canopy when they encountered ants. Way and Cammell (1989) reported that

removal of the coconut caterpillar (*Opisina arenosella* Walker) eggs by several ant species contributed significantly to control of this pest. Although the underlying mechanism was not elucidated in this study, the results clearly indicate that ants were important in reducing *G. molesta* fruit injury.

Much research has focused on the provision of non-crop resources as a means of conserving natural enemies (i.e., diversification: Risch et al. 1983, Van Emden 1990, Andow 1991, Landis et al. 2000). Peach EFNs represent a durable (i.e. present during entire growing season) resource with the advantage of requiring no additional production modifications. Several mechanisms potentially associated with peach EFNs could impact arthropods, including leaf surface chemistry and semiochemicals associated with EFN secretions (Barbosa and Benrey 1998). For instance, Elzen et al. (1986) found that glanded cotton produces a synomone that attracts the parasitoid *Camponotus sonorensis* (Cameron). Furthermore, within glanded peach cultivars there may be variation with respect to EFN attractiveness to both natural enemies and herbivores (e.g. due to differences in nutritional composition, synomones, etc.). Thus, further research on specific aspects of peach EFN-natural enemy-pest interactions could lead to a conservation biological control strategy with optimal effectiveness. Lewis et al. (1997) have called for pest management tactics based on knowledge of multitrophic level interactions. Such strategies become more important as the availability of effective chemical pest controls diminishes due to lost registration and increased insect resistance. *G. molesta* is already resistant to organophosphorus and carbamate insecticides formerly used on a wide scale in peach production (Pree et al. 1998, Shearer and Atanassov 2004). As Scorza and Sherman (1996) pointed out, a

vast void exists in terms of definitive research on the genetics of peach resistance to insects and disease. Results of the current study indicate that removal of the EFN trait could seriously erode the contributions of natural enemies to *G. molesta* biological control.

Chapter III: Interactions Between Ants (Hymenoptera: Formicidae) and Other Natural Enemies Associated with Extrafloral Nectaries of Peach [*Prunus persica* (L.) Batsch]: Implications for a Key Pest, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae)

Introduction

EFN-ant interactions have been documented extensively for a variety of systems, but potential EFN interactions with other (non-ant) natural enemies have largely been overlooked (Beattie 1985). My research of Chapter I revealed that peach trees with EFNs encourage ants and that the trees benefit from reduced leaf herbivory and increased vigor, as compared to trees without EFNs. Furthermore, research of Chapter II indicated that spring densities of the oriental fruit moth [*Grapholita molesta* (Busck)], a major pest of peach, are reduced for EFN-bearing trees with ants in their canopies. However, because natural enemies other than ants were not excluded in the study, the contributions of ants, versus other natural enemy groups, were not discernable.

My research of Chapter II showed that in addition to ants, peach trees with EFNs supported higher densities of Asilidae, Cantharidae, Coccinellidae, Araneae, and parasitic Hymenoptera. Coccinellids (Putman 1963, Pemberton and Vandenberg 1993), chrysopids (Putman 1963), and cantharids (C.R. Mathews, Appalachian Fruit Research Station, Kearneysville, WV, unpublished) consume the extrafloral nectar of

peach. Two important parasitoids of *G. molesta*, *Macrocentrus ancylivorus* (Roh.) and *Trichogramma minutum* (Riley), also use peach EFN and benefit from increased longevity when the resource is available to them (Putman 1963, Shearer and Atanassov 2004).

The potential for ants to interfere with the actions of other natural enemies, via competitive interactions or intraguild predation (Rosenheim et al. 1995), has been largely disregarded. Ants exhibit aggressive and territorial behavior, especially in the presence of sugar resources (Way 1963). The ants consume a variety of arthropods and often physically remove or cause an avoidance response (e.g., dropping) of arthropods from plants (Way 1963, Sudd 1965). Ant interference may reduce parasitism levels, particularly when tending honey-dew producing homopterans (e.g., Aphididae and Coccoidea, DeBach 1974, Perfecto and Castineiras 1998). Thus, EFN-pest-natural enemy interactions may be complex and require detailed examination to determine the overall effect on the pest of interest.

The current study examined interactions between peach EFNs, natural enemies, and biological control (in the sense of Stern et al. 1959) of *G. molesta*, a key economic pest of peach in the mid-Atlantic region (Hogmire 1995, Allen 1962). *G. molesta* females deposit eggs of the first three generations on the underside of leaves of newly emerged shoots, and neonate larvae bore into the shoots and feed. Fourth or fifth instars exit the shoots and usually pupate in leaf axils of the tree canopy or under bark (Rothschild and Vickers 1991). The eggs of later generations (i.e., 4th or 5th) are deposited directly on the fruit surface (Hogmire 1995, L. Hull, Pennsylvania State University Fruit Research and Education Center, Bigglerville, PA, pers. commun.,

2002). Several parasitoids attack *G. molesta* in the mid-Atlantic region, including *Macrocentrus* spp. (larval stage) and *Trichogramma minutum* Riley (egg stage) (Allen 1962).

In the first phase of this study (experiments performed in 2002), the effects of EFNs and ants were examined using a completely randomized split-plot design (whole plot factor= EFNs; subplot factor = ant exclusion). Natural enemy densities and biological reductions of sentinel pests were compared for trees with or without leaf EFNs and with or without ant exclusion. In the second phase (experiments performed in 2003), natural enemy exclusion was added as a third factor to selected trees of the split-plot field study, creating a split-split plot design (whole plot factor = EFNs; subplot factor = ant exclusion; sub-subplot factor = natural enemy exclusion). The exclusion of all other natural enemies with limb cages (Debach 1974) enabled separation of the relative impacts of ants versus other natural enemies on survival of set pest densities. The study addressed the following specific questions: 1) Do ants associated with EFN-bearing peach trees disrupt biological reduction of *G. molesta* by other natural enemies?, and 2) What are the relative contributions of ants, compared to other natural enemies, to biological reduction of *G. molesta* in different life stages?

Materials & Methods

The study, conducted in 2003, used the orchard plots (each plot 0.1 ha) of ‘Lovell’ peach trees planted in a completely randomized design, as described in Chapter I (Fig. 2).

*I. Do ants associated with EFN-bearing peach trees disrupt biological reduction of *G. molesta* by other natural enemies?*

This phase of the study used 10 randomly selected trees from each 0.1 ha plot. On 22 August 2002, the ant exclusion treatment was applied to 5 (randomly selected) trees of each plot, as described in Chapter I. The experiment was arranged in a split-plot design (whole plot: +/- leaf EFNs; subplot: +/- ant exclusion treatment).

*Ia. *G. molesta* eggs*

To determine the impacts of EFNs and ants on survival of *G. molesta* in the egg stage, sentinel eggs were introduced on 6 September 2002. Eggs laid on sheets of wax paper were obtained from a laboratory colony (Pennsylvania State University Fruit Research and Education Center, Bigglerville, PA). The wax paper sheets were cut into 10 cm x 10 cm squares with 10-20 eggs per square. This egg density was chosen based on the average number of oriental fruit moth eggs per tree observed in established peach orchards (L. Hull, Pennsylvania State University Fruit Research and Education Center, Bigglerville, PA, pers. commun., 2002). The squares (1 square per tree) were pinned to newly formed terminal shoots on the peach tree, on the underside of either the 3rd or 4th distal leaf where eggs are naturally oviposited by females (Allen 1962). Five trees per subplot (5 with ant exclusion, 5 without) received sentinel eggs. Eggs were retrieved after 48 h and held in the laboratory in individual petri dishes in a growth chamber (20°C, 16:8 L:D photoperiod). Percentage eggs parasitized was calculated after 20 d. In addition, percentage eggs completing development (hatched) was calculated, because mortality also occurs when an adult *T. minutum* female feeds

upon a host egg but does not oviposit in it (Allen 1962, Vasquez et al. 1997). Data for the 5 trees per subplot were averaged prior to statistical analysis. Data were arcsine ($\sqrt{}$) transformed, and ANOVA was used to test for the main effects of EFNs, ants and the interactive effect on percentage survival (hatch) and percentage parasitism (PROC MIXED; SAS Institute 1999). Means were separated by the least-squares difference procedure (LSMEANS; alpha = 0.05; SAS Institute 1999).

Ib. G. molesta pupae

To determine the impacts of EFNs and ants on survival of *G. molesta* in the pupal stage, sentinel pupae were introduced on 27 August 2002. *G. molesta* commonly pupate in leaf axils or bark openings within the tree canopy (Rothschild and Vickers 1991). Pupae were attached to 5 trees per subplot (5 with ant exclusion, 5 without) using a fine insect pin inserted through the posterior end of the pupa. One pupa per tree was affixed at the leaf axil angle on the lowest terminal extending from the trunk. For one tree per subplot, the pupa was covered with a fine polyester mesh sleeve cage, as a check to verify that pupae were not dislodged during the exposure period. Pupae on the other 5 trees per subplot were exposed to predation. After 24 h, pupae were examined for signs of predation, and the percentage of intact pupae surviving per plot was recorded. Data for the 5 trees per subplot were averaged prior to statistical analysis. Data were arcsine ($\sqrt{}$) transformed, and ANOVA was used to test for the main effects of EFNs and ants and the EFN-ant interactive effects on percentage survival (PROC MIXED; SAS Institute 1999). Means were separated by the least-squares difference procedure (LSMEANS; alpha = 0.05; SAS Institute 1999).

Ic. Natural enemy densities

Limb jarring (for predators) and sticky trap sampling (for parasitic Hymenoptera) were performed in the tree canopies (5 with ant exclusion, 5 without), as described in Chapter I, to estimate densities of non-ant natural enemies that could have potentially contributed to biological reductions of *G. molesta* introduced in field experiments (5 September 2002). Data for the 5 trees per subplot were averaged prior to statistical analysis. Data were log transformed, and separate ANOVAs within dates were used to test for the main effects of EFNs and ants and the EFN-ant interactive effects on predator and parasitoid density (PROC MIXED; SAS Institute 1999). A separate ANOVA was performed for *Harmonia axyridis* (Pallas), which was commonly observed feeding upon *G. molesta* eggs in the field. When ANOVA indicated significant fixed effects, means were separated by the least-squares difference procedure (LSMEANS; alpha = 0.05; SAS Institute 1999). To determine if there was an association between natural enemies found in the tree canopies and mortality rates for sentinel *G. molesta*, natural enemy density data (limb jarring and sticky traps) were combined with pest survivorship data (sentinel introductions). Correlation analysis was performed by treatment (+/- EFN) within dates for the following variables: adult *H. axyridis*, Coccinellidae, Cantharidae, Araneae, Formicidae, Asilidae, parasitic Hymenoptera, percentage *G. molesta* egg survival, and percentage *G. molesta* pupal survival (Spearman's CORR; SAS Institute 1999).

2. *What are the relative contributions of ants, compared to other natural enemies, to biological reduction of *G. molesta* in different life stages?*

This phase of the study, performed in 2003, used four randomly chosen trees per plot, two with and two without the ant exclusion treatment. To avoid potential interference with the natural arthropod population dynamics, sample trees used in previous studies (Chapters I,II and Chapter III, Section 1) were not used in the current study. Three levels of natural enemy exclusion were imposed on the four trees per plot to create a 2 x 2 x 3 factorial design (EFN presence X ant exclusion X natural enemy exclusion, Table 7). Survival of different life stages of *G. molesta*, obtained from a laboratory colony (Pennsylvania State University Fruit Research and Education Center, Biglerville, PA) and introduced into the field, was measured in each of the treatment combinations.

Table 7. The 2 X 2 X 3 factorial treatment structure of the completely randomized split-split plot design used in 2003 field study that compared survival of sentinel *G. molesta*^a on ‘Lovell’ peach trees.

Factor	Levels
Leaf extrafloral nectaries (EFNs)	present absent
Ant exclusion	Tangletrap trunk barrier no barrier
Natural enemy exclusion	partial terminal cage full terminal cage no cage

^a Introduced in egg, larval, and pupal stages from laboratory colony.

The natural enemy exclusion treatment was achieved via use of cylindrical terminal cages (20 cm diameter) constructed of aphid-resistant polyester netting (32 x 32 mesh per 2.5 cm) and double seamed lengthwise with polyester thread (132 mg mercerized cotton, Coats) (Debach 1974). For each tree, three individual terminals received either no cage (control), a partial exclusion cage, or a total exclusion cage. Both exclusion cage types (partial and full) were buttressed by an inner support (30 cm long X 18 cm diameter) constructed of 0.04 mm polycarbonate sheet (AIN Plastics, Virginia Beach, VA) and a 4 mm diameter dowel rod (60 cm long) (Fig. 21). Partial exclusion cages were unseamed (open) on both ends (Fig. 21a), and full exclusion cages were double seamed (closed) at the distal end (Fig. 21b). On 28 May 2003, three terminals of each plot's four trees were selected at random and shaken vigorously to remove all arthropods. Exclusion cages were established on 2 of the 3 terminals. A support was fastened to each limb with two twist ties, and a cage was slid over the support and secured with four metal butterfly clips. The open (unseamed) end of the full exclusion cage was tied shut with a twist tie. The partial exclusion cage remained open on both ends to mimic the microhabitat of the full exclusion cage while permitting natural enemy entry. The control terminal (no exclusion cage) was identified with two permanently placed twist ties. Throughout the season, the three designated terminals per tree were pruned lightly to maintain a comparable length (~60-70 cm), and caged terminals were checked regularly to insure cage effectiveness. Exclusion cages were opened only to introduce *G. molesta* from the laboratory colony and to check progress of the insects' development.

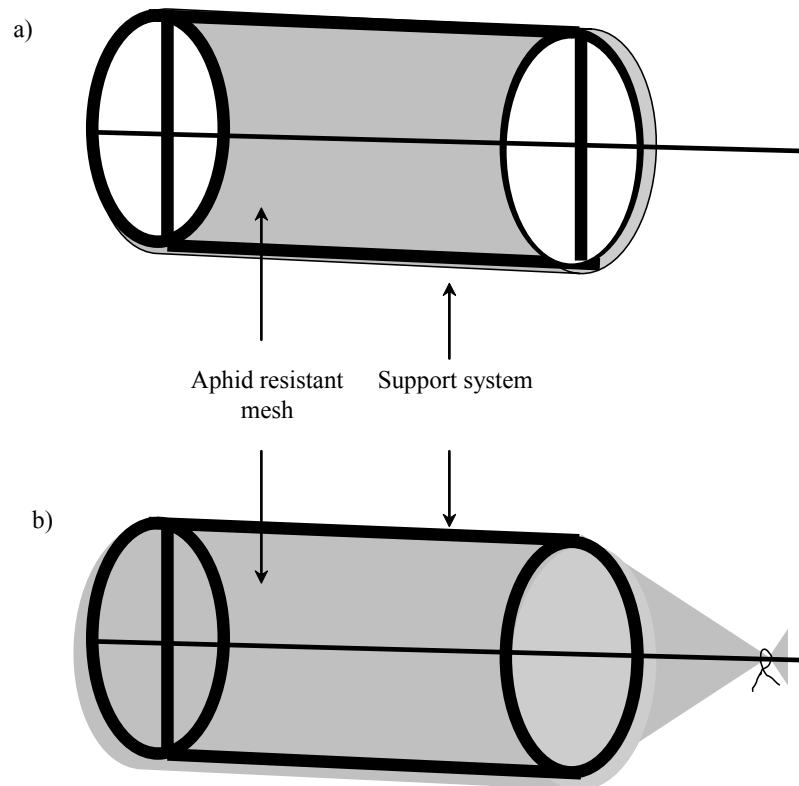


Figure 21. Terminal cages (8 X 30 cm) providing partial (a) and full (b) natural enemy exclusion treatment in 2 X 2 X 3 level factorial study comparing survival of sentinel *G. molesta* eggs, larvae, and pupae. One cage of each type (a and b) was established on an individual terminal of the same ‘Lovell’ peach tree; a third terminal (control) was not caged.

A greenhouse experiment (C.R. Mathews, Appalachian Fruit Research Station, Kearneysville, WV, unpublished) indicated that survival of *G. molesta* eggs (deposited on wax paper sheets that were pinned to the underside of the leaf) and neonate larvae (<12 h old, transferred to the terminal shoot with a camel hair brush)

was equal on the two leaf phenotypes (reniform EFNs and no EFNS) of ‘Lovell’ trees. Therefore, it was concluded that *G. molesta* eggs and larvae introduced in the field experiments would have an equal chance of surviving when placed on trees with and without EFNs.

2a. *G. molesta* eggs

This portion of the study determined if ants associated with EFN-bearing trees interfered with the parasitoid *T. minutum* that attacks *G. molesta* eggs and also assessed the relative contribution of ants (compared to other natural enemies) in causing egg mortality. *G. molesta* eggs (15-20, on small pieces of wax paper) from the laboratory colony were attached (by pinning the wax paper) to the underside of one peach tree leaf of the caged (partial cage, full cage) and uncaged (open) terminals, as described in Section 2. After 48 h, the number of eggs that remained was recorded and transported to a growth chamber (22°C, 16:8 L:D photoperiod) to determine hatch and parasitism rates. The experiment was repeated three times in 2003 (beginning 29 May, 16 July, and 2 September).

Data for the two trees per subplot were averaged within dates prior to statistical analysis. Following arcsine ($\sqrt{\quad}$) transformation, a mixed model ANOVA was performed within experiment dates (PROC MIXED, Y=EFN | ants | cage; RANDOM rep rep*trt rep*trt*ants rep*trt*ants*cage; SAS Institute, 1999). The dependent variables of percentage hatch and percentage parasitism were tested separately for the effects of EFNs, ant exclusion, and caging. When ANOVA indicated significant treatment effects, means were separated by the least-squares

difference procedure using the Bonferroni adjustment to correct for multiple comparisons (LSMEANS/ADJUST=BON, alpha = 0.05; SAS Institute 1999).

2b. *G. molesta* larvae

This part of the study determined if ants associated with EFN-bearing trees interfered with larval parasitoids of *G. molesta* and also assessed the relative contribution of ants (compared to other natural enemies) in reducing *G. molesta* larvae. Using a camel hair brush, three neonate *G. molesta* larvae from the laboratory colony were placed individually on three randomly selected shoots (1 larva per shoot) of each of the caged (partial cage, full cage) and uncaged (open) terminals. After 3 d, the terminals were examined to verify that the larvae had established successfully. The presence of gummosis (produced by the tree) at the larval entry site indicated successful shoot infestation. At the start of the experiment, the number of infested shoots was standardized to one per terminal (by removing the additional 1 or 2 shoots, depending on infestation success). After 5-7 d, the single infested shoot per terminal was removed and held in a laboratory growth chamber (22°C, 16:8 L:D photoperiod) to determine parasitism and survival of the larvae. The experiment was repeated three times in 2003 (beginning 23 July, 20 August, and 4 September).

Following arcsine ($\sqrt{\quad}$) transformation of the data, a mixed model ANOVA was performed within experiment dates (PROC MIXED, Y=EFN | ants | cage; RANDOM rep rep*trt rep*trt*ants rep*trt*ants*cage; SAS Institute, 1999). The dependent variables of percentage larval survival and percentage parasitism were tested separately for the effects of EFNs, ant exclusion, and caging. When ANOVA indicated significant treatment effects, means were separated by the least-squares

difference procedure using the Bonferroni adjustment to correct for multiple comparisons (LSMEANS/ADJUST=BON, alpha = 0.05; SAS Institute 1999).

2c. *G. molesta* pupae

This aspect of the study determined if ants associated with EFN-bearing trees interfered with parasitoids that attack *G. molesta* pupae. Pupae used in the study were produced in the laboratory. Late instar *G. molesta* larvae were put on rearing medium (Bioserv #F9649B, Frenchtown, NJ) and provided strips of corrugated cardboard (0.5 cm X 1 cm), in which the larvae pupated. Three pupae (in the cardboard strips) were pinned to a stem of each of the caged (partial cage, full cage) and uncaged (open) terminals. After 4 d, the pupae were retrieved and held in petri dishes in a growth chamber (22°C, 16:8 L:D photoperiod) to determine percentage parasitism and survival. The experiment was repeated three times in 2003 (beginning 26 June, 4 August, and 4 September).

A second experiment determined the relative importance of ants (compared to other natural enemies) as predators of *G. molesta* pupae. *G. molesta* pupae used in this experiment were removed from the cardboard strips by gently separating the corrugated layers. Three pupae were attached (pinned at the posterior end of the pupa) to each of the caged (partial cage, full cage) and uncaged (open) terminals. The pupae were examined after 24 h for predation or removal by natural enemies, and the percentage attrition per tree was recorded. The experiment was repeated three times in 2003 (beginning 3 June, 26 July, and 19 August). Following arcsine ($\sqrt{\quad}$) transformation, a mixed model ANOVA was performed within experiment dates for

the percentage attrition (PROC MIXED, Y=EFN | ants | cage; RANDOM rep rep*trt rep*trt*ants rep*trt*ants*cage; SAS Institute 1999). When ANOVA indicated significant treatment effects, means were separated by the least-squares difference procedure using the Bonferroni adjustment to correct for multiple comparisons (LSMEANS/ADJUST=BON, alpha = 0.05; SAS Institute 1999). When the analysis indicated significant differences among the ant exclusion treatments, the relative impact of ants (compared to other natural enemies), was calculated as follows: The mean percentage attrition occurring when ants were excluded was subtracted from the mean percentage attrition when ants were not excluded.

Results

1. Do ants associated with EFN-bearing peach trees disrupt biological reduction of G. molesta by other natural enemies?

1a. G. molesta eggs

Ants significantly affected both parasitism and hatch rates for *G. molesta* eggs (parasitism: $ndf = 1$, $ddf = 6$; $F = 8.2$, $P = 0.03$; hatch: $ndf = 1$, $ddf = 6$; $F = 9.2$, $P = 0.02$). When ants were excluded from the trees, egg parasitism was significantly higher, and egg hatch was significantly lower, independent of the presence of EFNs (LSD, $P < 0.05$; Fig. 22a). Differences in *G. molesta* egg parasitism and hatch rates for trees with and without EFNs were not detected ($P > 0.05$).

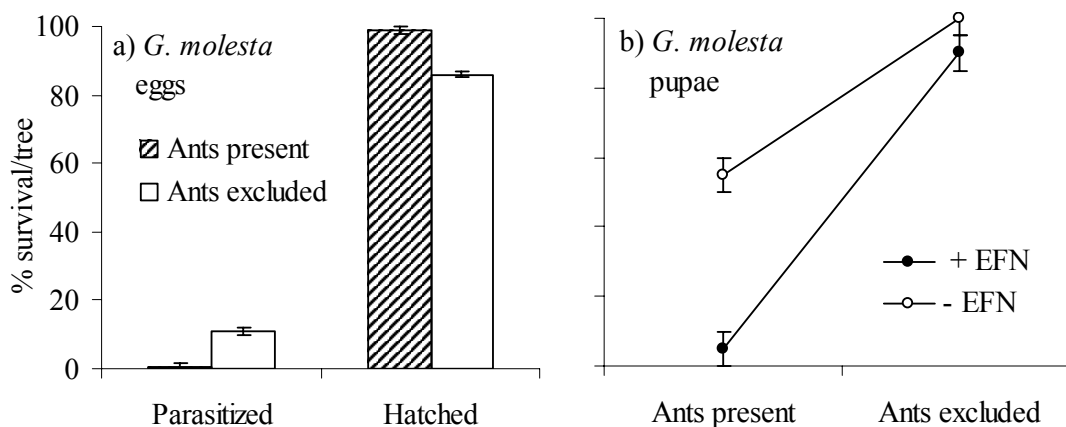


Figure 22. Effect of ant exclusion on survival of sentinel *G. molesta* eggs (a) and interactive effects of ant exclusion and leaf extrafloral nectary presence (+EFN) or absence (-EFN) on survival of sentinel *G. molesta* pupae (b) on 'Lovell' peach trees. Back-transformed means and 95% CI are shown.

1b. *G. molesta* pupae

A significant EFN X ant interaction was detected for *G. molesta* pupal survival ($ndf = 1$, $ddf = 6$; $F = 9.9$, $P = 0.02$). Pupal survival was significantly lower on trees with EFNs and ants (Fig. 22b). Pupal survival in full exclusion cages was 100% regardless of treatment, indicating that abiotic conditions did not contribute to pupal attrition in the other treatments.

1c. Natural enemy densities

The natural enemy groups (parasitic wasps and predators other than ants) responded similarly to treatments. A significant interaction of EFN treatment and ant exclusion treatment was detected for natural enemy densities in the tree canopies (parasitic wasps by sticky trap: $ndf = 1$, $ddf = 6$, $F = 10.6$, $P = 0.02$; non-ant predators by limb jarring: $ndf = 1$, $ddf = 6$, $F = 18.5$, $P = 0.005$). Ant exclusion lead to

significantly higher average densities of both predators and parasitoids on trees with EFNs than trees without EFNs (LSD, $P < 0.05$; Fig. 23a,b). When ants were not excluded, the densities of other predators did not differ between trees with and without EFNs. Predators included Cantharidae, Coccinellidae, Asilidae, and Araneae. The parasitic Hymenoptera included Chalcidoidea, Ichneumonoidea, Platygastroidea, Proctotrupoidea, and Ceraphronoidea.

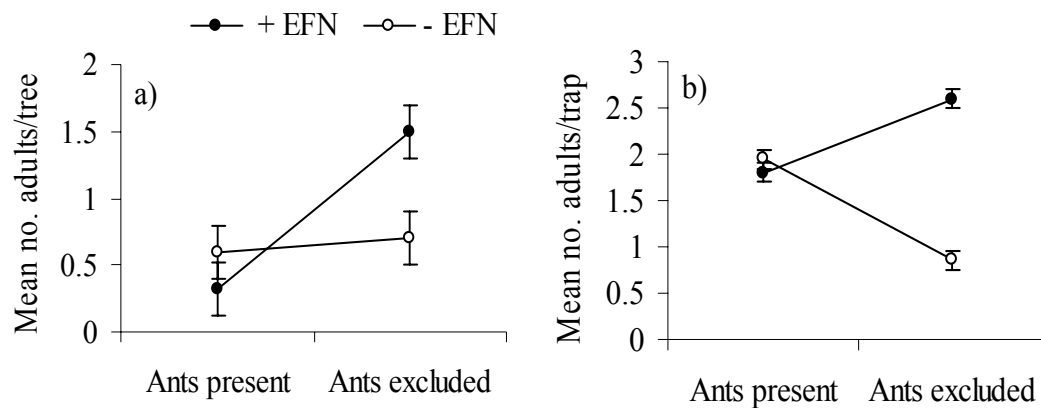


Figure 23. Significant interactive effects of 'Lovell' leaf extrafloral nectaries (EFNs) and ant exclusion treatment on densities of predators other than ants, by limb jarring (a), and parasitic Hymenoptera, by sticky trap (b), in September 2002. Geometric means are plotted with 95% CI.

A separate analysis for *Harmonia axyridis* revealed a significant EFN X ant interaction ($ndf = 1$, $ddf = 6$, $F = 7.4$, $P = 0.03$). *H. axyridis* adult densities were significantly higher on trees with EFNs when ants were excluded (mean log density = 0.26 ± 0.05) versus not excluded (mean log density = 0.07 ± 0.05 ; LSD, $P < 0.05$). The correlation analysis indicated a highly significant negative association between adult *H. axyridis* and *G. molesta* egg survivorship on trees with EFNs ($N=8$, $\rho = -0.85$, $P = 0.008$; Fig. 24). Ants also were negatively correlated with *G. molesta* pupal survivorship on those trees ($N=8$, $\rho = -0.88$, $P = 0.005$).

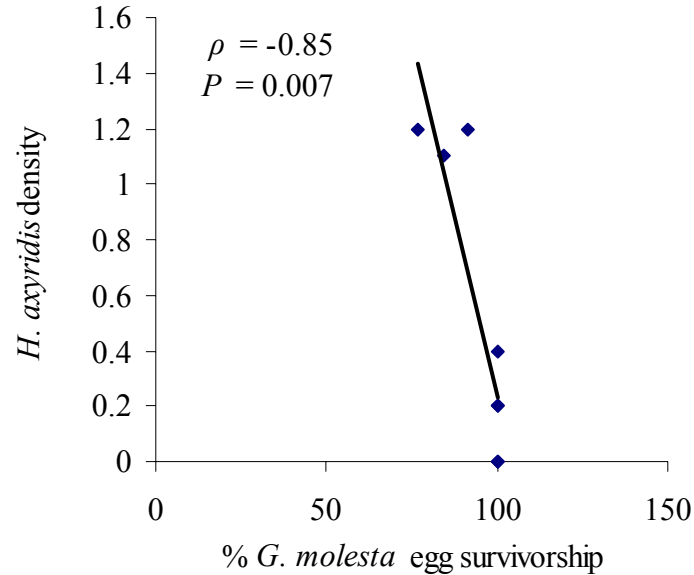


Figure 24. Association between *H. axyridis* abundance (no. adults/tree) and *G. molesta* egg survivorship (% hatch/tree) on ‘Lovell’ peach trees with leaf EFNs, 2002. Based on Spearman’s rank correlation analysis ($N=8$).

2. *What are the relative contributions of ants, compared to other natural enemies, to biological reduction of G. molesta in different life stages?*

ANOVA results for the split-split plot experiments that introduced different stages of laboratory reared *G. molesta* to the treatments of Table 7 appear in Table 8. The cage effect was significant for every *G. molesta* life stage during every experiment date (Table 8). *G. molesta* mortality was consistently lowest in the full exclusion cage, as compared to the partial and no cage treatments, indicating that abiotic factors were not significant sources of mortality.

2a. *G. molesta* eggs

T. minutum was the only parasitoid emerging from *G. molesta* eggs. No egg parasitism was detected in either the partial or full exclusion cages of any experiment (Fig 25a,b). The analysis showed a significant EFN X ant X cage interaction for egg parasitism on 16 July (Table 8). Parasitism was significantly higher on trees with EFNs when ants were excluded and significantly lower on trees with EFNs when ants were not excluded (LSD, $P < 0.05$; Fig. 25b). Average egg parasitism increased by 70% on EFN-bearing trees when ants were excluded and eggs were completely exposed (i.e., no cage) (Fig. 25b). A significant EFN X ant X cage interaction was also found, on 16 July, for the percentage of eggs that hatched after being removed from the field (Table 8). Egg survival was significantly lower on trees with EFNs when ants were excluded (LSD, $P < 0.05$; Fig. 26b). An 8-fold decrease in mean survival rate was found for uncaged eggs, as compared to eggs in full exclusion cages (Fig. 26b).

Table 8. Results of mixed model ANOVAs^a testing for effects of leaf extrafloral nectaries (EFNs), ant exclusion, and exclusion of other (non-ant) natural enemies on survival of *G. molesta* introduced on ‘Lovell’ peach trees. Completely randomized split-split plot experiments (+/-EFNs, +/-ants, exclusion cages: full, partial, or none) were conducted on three dates in 2003.

Dependent variable	Fixed effect ^b	ndf, ddf	Experiment date		
			F value ^c		
			29-May	16-Jul	2-Sept
% egg parasitism	cage	2, 24	8.4*	33.4***	6.3*
	EFN*ant*cage	2, 24	0.6	9.1*	1.4
% egg survival (hatch)	cage	2, 24	6.3**	12.8**	3.6*
	EFN*ant*cage	2, 24	0.8	3.9*	0.4
			23-Jul	12-Aug	20-Aug
% larval survival (to adult stage)	cage	2, 24	4.1*	39.2***	12.8**
	ant*cage	2, 24	4.9*	3.0	0.4
% pupal survival (to adult stage)	cage	2, 24	16.1***	11.0**	19.6***
			26-Jun	4-Aug	4-Sept
% pupal attrition from tree canopy	ant	1, 6	13.7*	13.9**	21.2**
	cage	2, 24	13.4***	18.9***	29.9***
	ant*cage	2, 24	5.6**	4.1*	12.9***
	EFN*cage	2, 24	2.0	7.9**	4.5**

^a A separate mixed model ANOVA was performed for each dependent variable (Y) listed and for each experiment date; (MODEL: Y=EFN|ant|cage; RANDOM rep rep*EFN rep*EFN*ant rep*EFN*ant*cage).

^b Effects for which no significance was found on any date are excluded.

^c * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

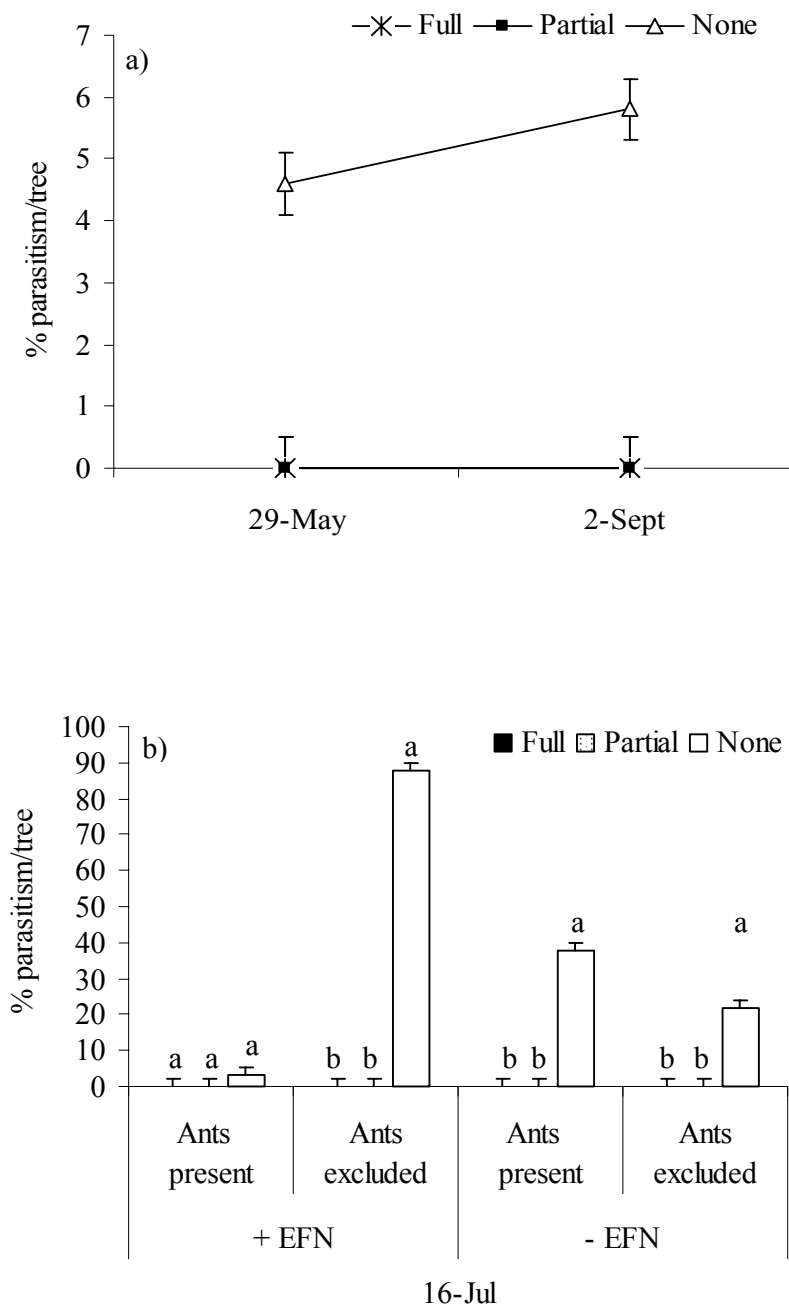


Figure 25. Effect of natural enemy exclusion cages (full, partial, or none) (a) and three-way interaction of leaf extrafloral nectaries (EFNs), ant exclusion, and natural enemy exclusion cages (b) on *T. minutum* parasitism of *G. molesta* eggs introduced on ‘Lovell’ peach trees. Split-split-plot field experiments were conducted in 3 m, 2003. Back-transformed means are plotted with 95% CI. Bars within EFN and ant exclusion treatment sharing lower case letters were not significantly different by LSD ($P=0.05$).

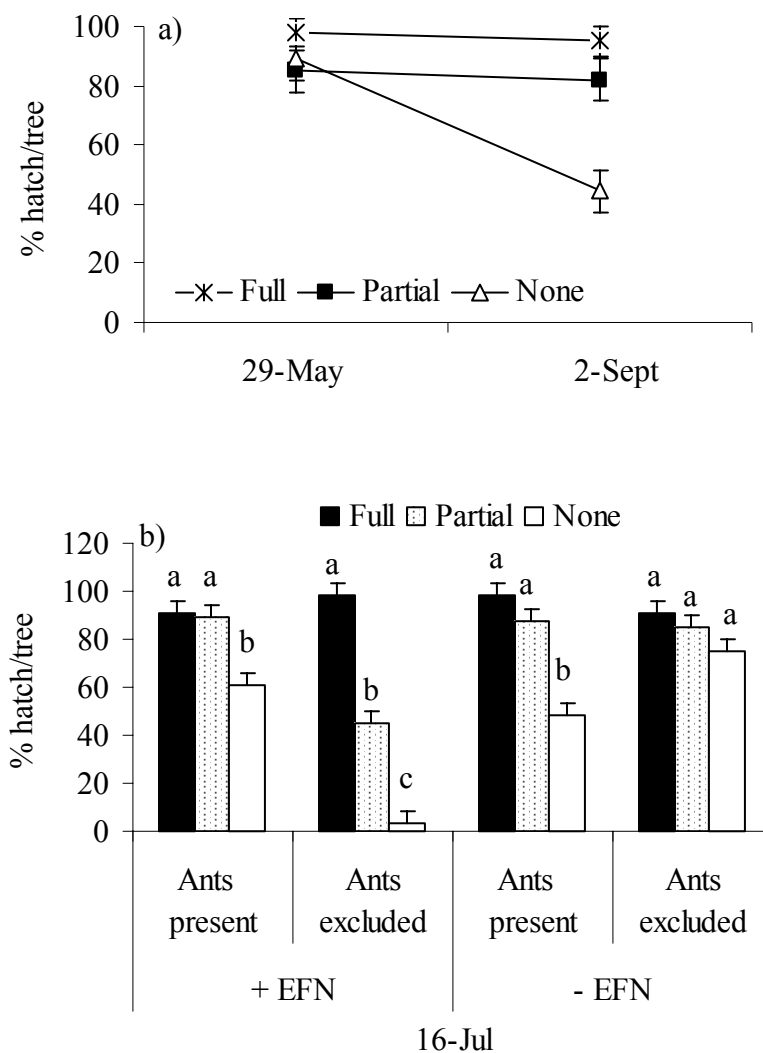


Figure 26. Effect of natural enemy exclusion cages (full, partial, or none) (a) and three-way interaction of leaf extrafloral nectaries (EFNs), ant exclusion, and natural enemy exclusion cages (b) on survival of *G. molesta* eggs introduced on ‘Lovell’ peach trees. Split-split-plot field experiments were conducted in 3 m, 2003. Back-transformed means are plotted with 95% CI. Bars within EFN and ant exclusion treatment sharing lower case letters were not significantly different by LSD ($P=0.05$).

Egg survival rates of every experiment were significantly lower for eggs exposed to natural enemies (i.e., no cages or partial cages) than eggs placed in the full exclusion cages, regardless of the EFN or ant exclusion treatment (Fig. 26a,b). Adults of *H. axyridis* were commonly observed to prey on the *G. molesta* eggs in the field during the experiments.

2b. *G. molesta* larvae

Parasitism of the *G. molesta* larvae was too low to permit statistical analysis. Parasitoids (all *M. delicatus*) emerged from only 6 of all the sentinel larvae (N= 288 total over four experiments) introduced in the field.

A significant ant X cage interaction was found for percentage larvae surviving field exposure (% reaching adult stage) on 23 July (Table 8). For trees with ant exclusion, larval survival did not differ among cage treatments (full, partial, or none) (Fig. 27a). However, when ants were present (no ant exclusion), significantly fewer *G. molesta* larvae (mean = $0.0 \pm 9.7\%$) survived on terminals exposed to natural enemies (no exclusion cage) as compared to terminals with either exclusion cage type (partial or full) (LSD, $P < 0.05$; Fig. 27a). Caging was the only significant factor impacting larval survival during the other two experiments (12 and 20 August, Table 8). On both dates, larval survival was significantly higher in the full exclusion cages (LSD, $P < 0.05$; Fig. 27b). On average, a four-fold decrease in survival was recorded for larvae exposed to natural enemies (Fig. 27b).

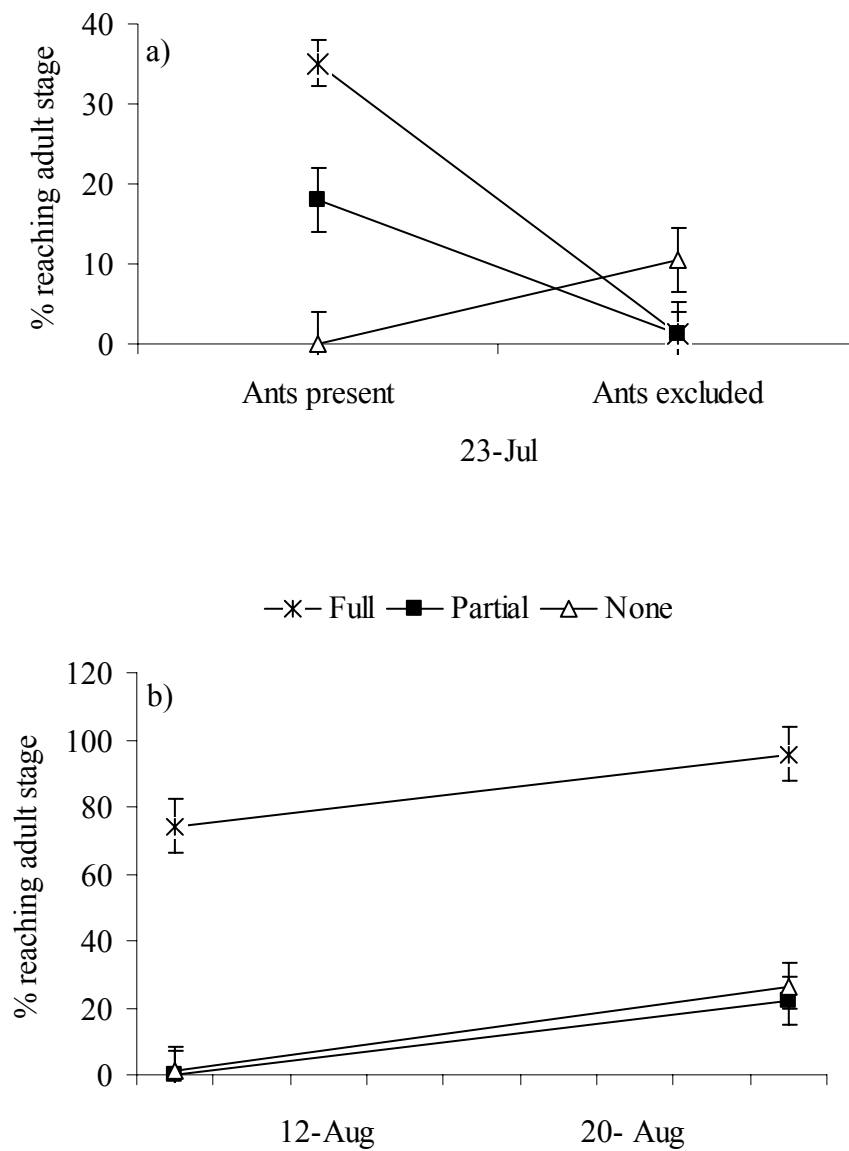


Figure 27. Interactive effects of natural enemy exclusion cages (full, partial, or none) and ant exclusion treatment (a) and main effect of natural enemy exclusion cages (b) on survival of *G. molesta* larvae introduced on 'Lovell' peach trees, 2003. Back-transformed means are plotted with 95% CI.

2c. *G. molesta* pupae

Parasitoids emerged from only two of all the sentinel pupae (N= 864 total over four experiments) exposed to field natural enemies. Both of the parasitoids were *Itopectis* sp. that is known to attack *G. molesta* pupae in the eastern U.S. (Allen 1962). Cage treatment (full, partial, and none) was the only significant factor impacting pupal survival (% reaching adult stage) in the field (Table 8). Pupal survival was significantly higher in the full exclusion cages of every experiment (LSD, $P < 0.05$; Fig. 28). Average survival rates ranged from 90-100% for pupae in the full exclusion cages (Fig. 28). By comparison, survival dropped as low as 41% (26-June; Fig. 28) on the uncaged (open) terminals.

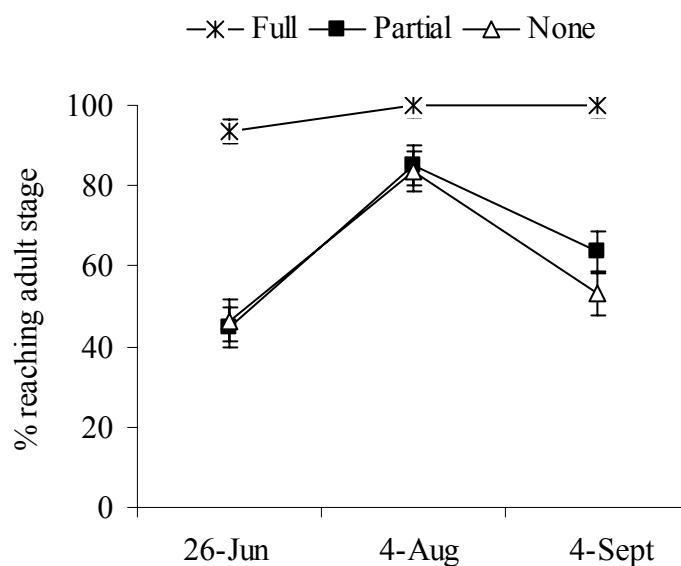


Figure 28. Effect of natural enemy exclusion cages (full, partial, or none) on survival of *G. molesta* pupae introduced on 'Lovell' peach trees, 2003. Back-transformed means are plotted with 95% CI.

A significant interactive effect of ant exclusion and natural enemy exclusion (caging) was detected for removal of *G. molesta* pupae (% attrition) during every month (Table 8). No pupae disappeared from the exclusion cages of any experiment. When ants were not excluded, pupal attrition was consistently and significantly higher in the partial or no cage treatments (compared to pupal attrition in full exclusion cages) (LSD, $P < 0.05$; Fig. 29a). Pupal attrition increased, on average, by 45% when pupae were exposed to both ants and other natural enemies (compared to pupal attrition in the full exclusion cages) (Ants present, Fig. 29a). When ants were excluded, the response was variable, and attrition rates were not always higher for pupae exposed to non-ant natural enemies (compared to pupal attrition in full exclusion cages) (Fig. 29a).

The relative impact of ants versus other natural enemies on *G. molesta* pupal attrition is shown in Table 9. The average increase in pupal attrition due to ants ranged from 19-60% (Table 9). A significant EFN X cage interaction was detected for pupal attrition during July and August (Table 8). On these dates, pupal attrition on trees with EFNs was consistently higher when natural enemies were not excluded (partial or no cage treatments) compared to the full exclusion cage treatments (LSD, $P < 0.05$; Fig. 29b). Ants were commonly observed to feed on *G. molesta* pupae put on the trees. Larvae of *H. axyridis* were also observed feeding on the pupae. Attrition rates were highest for pupae placed in partial exclusion cages of trees with EFNs during both July and August and were, on average, 5X higher in partial exclusion cages than in full exclusion cages (LSD, $P < 0.05$; Fig. 29b). For trees without EFNs, pupal attrition was not affected by natural enemy treatment (Fig. 29b).

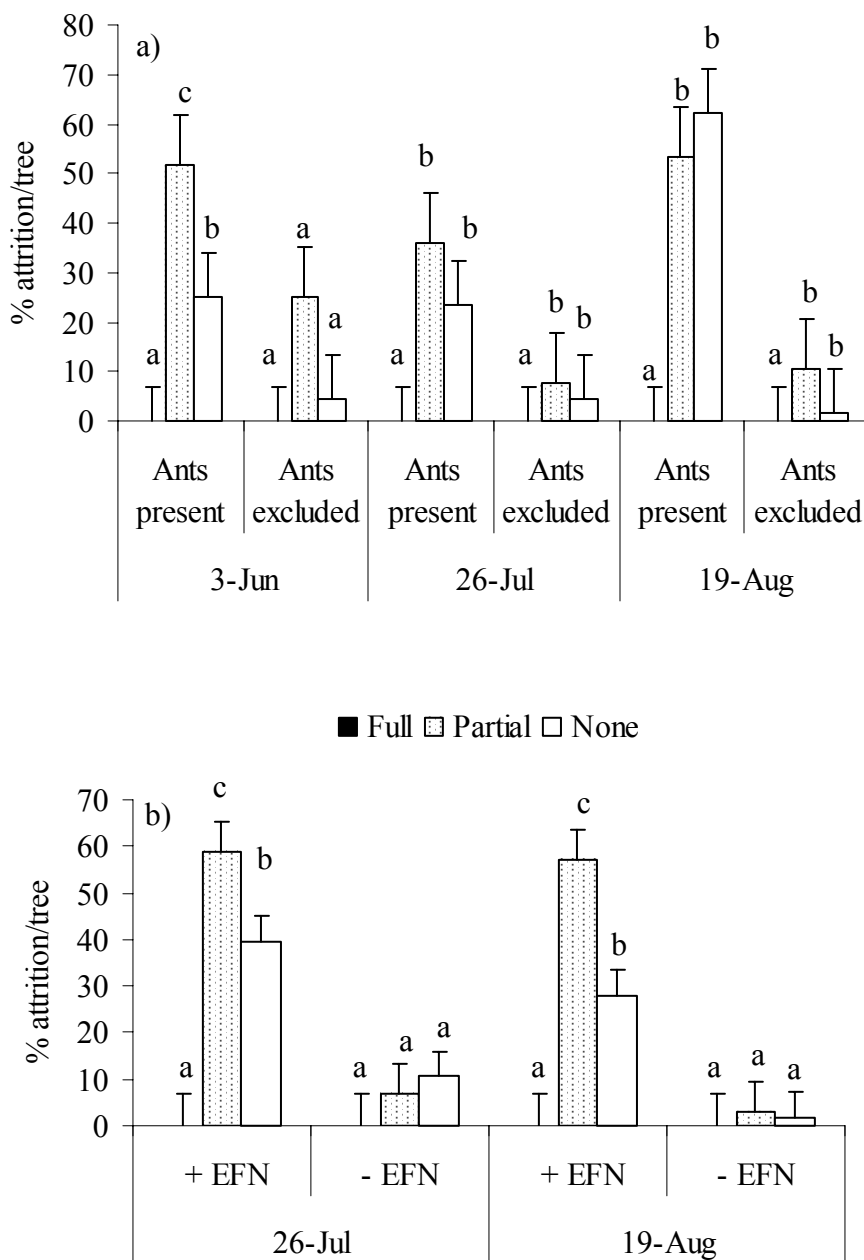


Figure 29. Significant two-way interactions for attrition of *G. molesta* pupae introduced on ‘Lovell’ peach trees in 2003: interactive effects of ant exclusion and natural enemy exclusion cages (full, partial, or none) (a) and interactive effects of leaf extrafloral nectaries (EFNs) and ant exclusion (b). Back-transformed means are plotted with 95% CI. Columns within ant exclusion treatment (a) or EFN treatment (b) sharing lower case letters were not significantly different by LSD ($P=0.05$).

Table 9. Relative impact^a of ants, as compared to other natural enemies, on *G. molesta* pupae introduced to ‘Lovell’ peach terminals in field experiments performed in 3 m, 2003; three pupae per cage type were pinned directly to the peach terminal and exposed to natural enemies for 48 h.

Date	Magnitude of increase in average percentage attrition when ants were not excluded	
	<i>Terminal cage type</i>	
	<i>Partial exclusion</i>	<i>No exclusion</i>
3-June	26.7	20.7
26-July	28.0	19.2
19-August	42.9	60.6

^aFor each date, relative impact was calculated within cage type by subtracting the least-squares mean of percentage attrition under conditions of ant exclusion from the least-squares mean percentage attrition when ants were not excluded. Least-squares means were generated from ANOVA with least-squares mean separation (LSD; SAS Institute, 1999).

Discussion

The results indicate that ants disrupt the actions of certain natural enemies of *G. molesta* eggs. The ants reduced egg parasitism by *T. minutum* and increased egg survival (Fig. 22a). The highly significant negative association between *G. molesta* egg survival and *H. axyridis* densities (Fig. 24) suggests that the coccinellid was likely the key predator reducing *G. molesta* eggs on EFN trees. Coccinellids consume a variety of lepidopteran eggs in orchards (Hogmire 1995), including those of *G. molesta* (Rothschild and Vickers 1991). That the densities of non-ant natural enemies (Fig. 23), and *H. axyridis* specifically, were reduced only on EFN-bearing trees with

ants present suggests competition between ants and other natural enemies for the extrafloral nectar. All of the natural enemy groups encountered in the studies -- coccinellids (Hagen 1962, Putman 1963, Pemberton and Vandenburg 1993), parasitic Hymenoptera (Putman 1963, Bugg et al. 1989, Shearer and Atanassov 2004), and Araneae (Taylor and Foster 1996) -- are known to use EFN secretions. While ants diminished the effects of other natural enemies on *G. molesta* eggs in trees with EFNs, their presence resulted in a decrease in pupae on these trees (Fig. 22b). The results clearly showed a negative association between ants and pupal survival on trees with EFNs and suggest a protective effect of ants associated with EFN resources.

G. molesta egg parasitism on trees with EFNs was reduced by >90% when ants were not excluded (Fig. 25b). This finding is not surprising, as nectar availability is a key factor contributing to the success of *Trichogramma* wasps in the field (Smith 1996), and only the trees with EFNs provided extrafloral resources.

The results of these studies clearly showed that natural enemies are more important than abiotic factors in reducing survival of eggs (Fig. 26a), larvae (Fig. 27), and pupae (Fig. 28) of *G. molesta*. The drastic reduction (~90%) in egg survival when ants were excluded from trees with EFNs (Fig. 26b) suggests that other natural enemies were responsible for most egg mortality. However, larval survival was lowest in the presence of ants in July (Fig. 27a). Ants, which attack many species of lepidopteran larvae (Jaynes and Marucci 1947, Sudd 1965, Tilman 1978, Way and Khoo 1992, Daane and Dlott 1998), probably consumed (or removed from the trees) the late-instar *G. molesta* larvae as they emerged from terminal shoots in search of pupation sites. Although species other than ants were important natural enemies of the

pupae, ants were apparently more important (Fig. 29a). Ants were responsible for 19-60% of pest reduction on terminals that were partially or fully caged (Table 9). Other natural enemies removed 1.5-25% of the sentinel pupae (Fig. 29a).

Overall, this study revealed that several natural enemy groups may contribute to reductions in *G. molesta* in peach orchards. While ants antagonized other natural enemies on trees with EFN resources, they also provided considerable reduction of *G. molesta* in both the larval and pupal stages. At the individual tree scale, trees with EFNs generally fared better than trees without EFNs, due to higher ant and natural enemy densities and subsequent pest reductions. However, the implications of EFN-natural enemy-pest interactions to orchard-level biological control will likely depend on local *G. molesta* population dynamics. From an economic standpoint, targeting eggs and larvae of later *G. molesta* generations (i.e., 4th and 5th) would be crucial for reducing fruit injury as eggs of these generations are deposited directly on fruit and develop into fruit-feeding larvae. Reduction of later generation eggs and larvae could be achieved by direct mortality in those life stages (i.e., parasitism or predation) or by reduction of any life stage of previous generations. Thus, enhanced mortality of pupae in the earlier generations, as shown in the current study for trees with EFNs and their ant associates, could be significant in reducing the damage-causing latter generations. The results demonstrate the complexities involved in assessing the outcomes of multi-trophic level interactions.

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