THE DISTRIBUTION OF THE BLACK PECAN APHID *MELANOCALLIS CARYAEFOLIAE*

(HOMOPTERA: APHIDIDAE) BETWEEN THE UPPER AND LOWER SURFACES OF

PECAN FOLIAGE

by

CHRISTIAN M. PAULSEN, JR.

(Under the Direction of John Ruberson)

ABSTRACT

An orchard survey determined that nymphs of *M. caryaefoliae* regularly feed on both surfaces of pecan foliage, while other pecan aphids feed predominately on the lower leaf surface. Aphid populations on laboratory pecan seedlings were similarly distributed. Comparison of *M. caryaefoliae* nymphs reared on each leaf surface found the upper surface offers no fitness advantage. Observations of aphid density found evidence that crowding by heterospecific aphids contributes to *M. caryaefoliae* movement to the upper surface, while conspecific crowding has no effect. Field observations and experiments on laboratory seedlings found that some aphidophagous lady beetle and lacewing larvae predominately search the lower leaf surface for prey. *M. caryaefoliae* may settle on the upper leaf surface because it is a habitat with reduced probability of enemy encounters.

INDEX WORDS: *Melanocallis caryaefoliae,* Aphididae, Homoptera, enemy-free space,
                 foliage-feeding, crowding, *Monellia caryella*, *Monelliopsis pecanis,*
                 Coccinellidae, Chrysopidae.

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CHAPTER 1

INTRODUCTION

Pecan is a major horticultural crop for the state of Georgia, and Georgia produces the most pecans of any state in the US. The black pecan aphid *Melanocallis caryaefoliae* is among the most important pecan pests, costing the Georgia pecan industry millions of dollars per year in damage and control costs. *M. caryaefoliae*'s unique mechanism of feeding on foliage led me to suspect that its distribution on pecan foliage differs from other pecan feeding aphids. The manuscripts which comprise this thesis describe the orchard surveys and laboratory experiments I conducted to ascertain the distribution of *M. caryaefoliae*, as well as the experiments I conducted to determine the ecological causes of this distribution.

It is hoped that the results of this research may help to improve the control of *M. caryaefoliae*.

CHAPTER 2

LITERATURE REVIEW

Pecan

The pecan, *Carya illinoinensis* (Wangenh.) K. Koch, is native to North America. As a species, it is believed to have originated in the Cretaceous period in southern Illinois and spread to Iowa, Kansas, Missouri, Tennessee, Mississippi, Arkansas, Texas, and Mexico as the Gulf of Mexico receded south from the mouth of the Ohio River to its current location (Edelson 1982, McEachern 2010). Native Americans fed on the nuts—and planted them, contributing to the spread of the trees—long before Europeans arrived on the continent. "Pecanes" was the word used by several Indian nations along the Mississippi River to describe three different shelled nut varieties (Brison 1974). As early as 1528, Spanish explorers documented the pecan trees and the Indians' use of the nuts (McEachern 2010). "Pecane" was adopted to refer specifically to *C. illinoinensis* by French settlers in Louisiana (Brison 1974).

Settlers from Europe soon acquired a taste for pecans and began planting more trees, spreading them further. Both George Washington and Thomas Jefferson planted pecan trees (McEachern 2010). The first successful pecan graft was performed in 1846 or 1847 by Antoine, a slave gardener at the Oak Alley plantation, north of New Orleans. In 1857, the first pecan plantings in Georgia occurred by accident, when a storm and shipwreck washed a barrel of nuts ashore near the Georgia-Florida state line. In 1874, William Nelson opened the world's first commercial pecan orchard in New Orleans, while in Pascagoula, Mississippi, John Lassabe planted the seed that would eventually become the 'Stuart' cultivar. In 1876, Edmond E. Risien

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began his work in pecan cultivation that would lead to the introduction (in 1924) of 'Western', the most-planted cultivar in the world. The first named cultivar, 'Van Deman', was released in 1877, and others were released in the years after. By 1900, commercial pecan rearing had grown popular enough that speculators entered the market purely to make quick profits by buying land and selling orchards. The money lost or swindled in this speculation gave the pecan industry a negative reputation for some years, but this speculation also led to a large number of trees being planted in Georgia and Texas. The first pecan conference, the Nut Growers Convention, was held in Macon, GA in 1902.

Since then, pecan has become the United States' most important native horticultural crop (Brison 1974), and Georgia produces the largest volume of pecans in the US (Agricultural Statistics Board 2010). In 2007, pecan farms covered over 114,000 acres of Georgia, mainly in the middle and southern regions (USDA National Agriculture Statistics Service 2008).

As commercial pecan growing led to the creation of monocultures, existing herbivores became economically significant pests. The first reports of insect damage were outbreaks of the pecan weevil *Cucurlio caryae* (Horn) (Coleoptera: Curculionidae) in 1903 and 1904, reducing the pecan crop in Texas and Georgia (McEachern 2010). Today, economically significant arthropod pests of pecan include *C. caryae*, hickory shuckworm *Cydia caryana* (Fitch) (Lepidoptera: Tortricidae), pecan leaf scorch mite *Eotetranychus hicoriae* (McGregor) (Acari: Tetranychidae), kernel-feeding Hemiptera, pecan nut casebearer *Acrobasis nuxvorella* Neunzig (Lepidoptera: Pyralidae), pecan bud moth *Gretchena bolliana* (Slingerland) (Lepidoptera: Tortricidae), fall webworm *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae), longhorn beetles *Prionus* spp. (Coleoptera: Cerambycidae), hickory nut curculio *Conotrachelus hicoriae* Schoof (Coleoptera: Curculionidae), and hickory shoot curculio *Conotrachelus aratus* (Germar)

(Coleoptera: Curculionidae) (Hudson and Dutcher 2007), stink bugs (Hemiptera: Pentatomidae) (Mizell 2005), and three species of foliage-feeding aphids.

Aphids.

The black pecan aphid *Melanocallis caryaefoliae* (Davis) (Hemiptera: Aphididae) is a common sight on pecan foliage in Georgia and other states. While pecan is by far the aphid's most common host, *M. caryaefoliae* is sometimes observed on other species of *Carya*, but not on other genera (Rogers 1960, Tedders 1978). In fact, the first description of the species simply noted they occurred on hickory (Davis 1910). Moznette (1929) noted that the aphid had become an economically important pest of pecan "only in the last few years". Two other aphids, the yellow pecan aphid *Monelliopsis pecanis* (Davis) and the blackmargined aphid *Monellia caryella* (Fitch), also commonly feed on Georgia pecans. All three species often co-occur on the same individual trees.

All three pecan aphids are monoecious—they complete their entire life cycles on pecan and do not require a secondary host plant. The fundatrices (the first generation of parthenogenetic females) appear in mid-spring: from late March to early April for *M. caryaefoliae* and *M. pecanis* (correlating well with the beginning of pecan tree development), early to late April for *M. caryella*. These are followed by several generations of parthenogenetic females, called viviparae (a maximum of 30 generations in *M. caryaefoliae*, 32 in *M. pecanis*, and 33 in *M. caryella*). Sexuales (oviparae and males) appear in October and persist through November (for *M. caryaefoliae* and *M. pecanis*) or early December (for *M. caryella*). These deposit eggs under the bark of pecan limbs. The eggs overwinter and hatch into fundatrices the following spring. With the exception of the oviparae, all adults of all three species are alates (Tedders 1978).

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All three aphids also follow a similar pattern of seasonal distribution (Tedders 1978, Tedders et al. 1992). After the fundatrices appear, aphid density increases, then quickly declines in June and may remain low through July. The cause of this decline is unclear. Increasing summer temperatures would be the most obvious hypothesis: during these months, air temperatures can reach 35 to 40° C for several hours every day, even within the pecan canopies. Brief exposure to these temperatures can increase mortality of all three aphids (Kaakeh and Dutcher 1993), and prolonged exposure can halt development (Tedders et al. 1992). Of the three pecan aphids, *M. caryaefoliae* is the most heat-tolerant (Kaakeh and Dutcher 1993, Tedders et al. 1992). However, Tedders (1978) notes that large aphid populations can build up on pecan foliage in sleeve cages during these same months. He also notes that natural enemy populations are too low during this period, and the effects of heavy rainfall are too temporary, for either to explain the population declines during these months. All three aphid populations begin increasing again in late July and the following months (Tedders 1978, Tedders et al. 1992). Of the three, *M. caryella* has the greatest capacity to build up large populations rapidly (higher fecundity rate and intrinsic rate of increase, and shorter generation time relative to the other pecan aphids) (Kaakeh and Dutcher 1992). None of the aphids regularly build up to damaging population levels prior to July, when the first damage from aphid feeding appears on pecan foliage (Tedders 1978).

All three pecan aphids feed on the phloem cells of leaf veins. This causes injury to the plant and the pecan crop through several mechanisms. First, consumption of phloem removes carbohydrates from the leaves, and is a direct drain on the energy reserves of the tree, which would normally be used to produce the pecan crop (Wood et al. 1987). During their lifetimes, an individual *M. caryella*, *M. pecanis*, and *M. caryaefoliae* consumes 301, 36.1, and 44.8 joules, respectively (Wood et al. 1987). All three aphids were found to reduce the starch content of roots

and stems of greenhouse pecan seedlings, with *M. caryella* and *M. pecanis* causing a greater loss

of starch than *M. caryaefoliae* does (Tedders et al. 1982). It is calculated that a season-long

standing population of one *M. pecanis* per compound leaf on a 70-year-old 'Stuart' cultivar tree

can reduce the nut yield by 2.41 kg (Wood et al. 1987), a loss of $7.66 at 2009 values

(Agricultural Statistics Board 2010); a similar population of *M. caryella* can cause an 18.13 kg

reduction (Wood et al. 1987), a loss of $57.61.

Of the energy extracted, very little is used for aphid growth. Growth efficiency (calories

in growth divided by calories consumed) is highest in *M. caryaefoliae* (17–25%), followed by *M.*

pecanis (9–16%), then *M. caryella* (1–5%) (Wood and Tedders 1985, Wood et al. 1987). Even

less of the energy consumed (0.5–5.4%) is used for aphid respiration (Wood and Tedders 1985).

Most (76.3–98.4%) of the energy extracted from pecan phloem is not used at all by the aphids,

but excreted in the form of honeydew (Wood et al. 1987). The carbohydrate content of

honeydew varies between the pecan aphids. As *M. caryella* utilized the least energy from the

phloem, its honeydew contains the highest energy content in the form of carbohydrates (Wood et

al. 1987). Its honeydew contains 9 and 13 times more glucose equivalents than *M. pecanis* and

M. caryaefoliae, respectively (Tedders and Wood 1987).

When the aphids' honeydew is deposited on pecan leaves, it serves as a substrate for the

growth of sooty molds, which block sunlight and reduce leaf photosynthesis (Tedders and Smith

1976). The pecan aphids also directly reduce photosynthesis via their feeding. All three aphids

destroy leaf cells with their stylets (Tedders and Thompson 1981). Chlorophyll content of leaves

is reduced after feeding by the pecan aphids, with *M. pecanis* and *M. caryaefoliae* causing the

greatest loss (Wood et al. 1987). Overall, leaf net photosynthesis decreases as aphid density

increases. For *M. caryella* and *M. pecanis*, photosynthesis declines exponentially with aphid

density; for *M. caryaefoliae*, photosynthesis declines linearly with aphid density (Wood and Tedders 1986). However, *M. caryaefoliae* at any given density have a more detrimental effect on photosynthesis than either of the other two aphids (Wood and Tedders 1985, 1986). Damage to the leaves from multiple aphids feeding can result in premature leaf abscission (Wood et al. 1985).

M. caryaefoliae possesses an additional, unique mechanism for damaging pecan foliage. Feeding by *M. caryaefoliae* causes premature senescence-like chlorosis, beginning on the foliage at the point of stylet insertion and spreading outward. The affected region begins turning yellow within two days of the aphid's feeding and eventually dies. The presence of these chlorotic lesions further reduces leaf photosynthesis and speeds up leaf abscission (Lakin 1972, Cottrell et al. 2009). Perhaps surprisingly, the ability to cause this damage decreases with the aphid's instar; chlorotic lesions appeared fastest in response to first instar feeding and slowest in response to adult feeding (Lakin 1972).

It appears that these chlorotic lesions increase the nutritional value of the leaf phloem. Feeding by *M. caryaefoliae* increases the concentration of free amino acids in the leaf phloem (Petersen and Sandström 2001). *M. caryaefoliae* is not the only aphid known to alter its host in this manner: the senescence-like feeding damage of the greenbug *Schizaphis graminum* (Rondani) increases the amino acid content of wheat (*Triticum aestivum* L.) phloem (Dorschner et al. 1987, Petersen and Sandström 2001). *M. caryaefoliae* are known to prefer chlorotic over healthy leaf tissue (Lakin 1972, Cottrell et al. 2009). The presence of these lesions also has effects on the aphid's fitness: aphids allowed to mature from first instar to adulthood on chlorotic leaf tissue were more fit (they developed faster, into larger adults) than aphids forced to feed on healthy leaf tissue during development (Cottrell et al. 2009). *M. caryaefoliae* also excretes a

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lower quantity of carbohydrates than the other pecan aphids (thirteen times less glucose than *M. caryella* excretes [Tedders and Wood 1987]), suggesting that its phloem consumption is lower (Petersen and Sandström 2001). While the chlorotic lesions induced by *M. caryaefoliae* feeding appear to make the phloem nutritionally advantageous for some days, the severe leaf damage eventually resulting from these alterations is disadvantageous (Petersen and Sandström 2001). The mobility of the alate adults might circumvent this disadvantage.

In some manner because of its ability to induce these chlorotic lesions, *M. caryaefoliae* is the most damaging of the pecan aphids. The damage and pest control costs associated with this aphid can outweigh those of *M. caryella* and *M. pecanis* combined (Ellis and Dutcher 1999, Hudson and Dutcher 2004, 2006, 2008). Pest management strategies reflect *M. caryaefoliae*'s capacity to cause damage. Action thresholds for controlling *M. caryella* and *M. pecanis* are an average of 20 combined aphids per compound leaf prior to August 1, or an average of 10 per compound leaf after August 1. The action threshold for controlling *M. caryaefoliae* is an average of one aphid (or aphid-damaged area) per compound leaf (McVey and Ellis 1979).

M. caryaefoliae does not affect all pecan cultivars equally. It favors some cultivars over others, as demonstrated by probing longer and building up larger populations on these leaves. *M. caryaefoliae*'s preferred cultivars are 'Oconee', 'Gloria Grande', 'Shoshoni', and 'Cheyenne'; its least preferred are 'Pawnee' and 'Cape Fear'. The other pecan aphids also preferred the latter two less, and their most preferred were 'Shoshoni', 'Cheyenne', and 'Tejas' (Kaakeh and Dutcher 1994). Alate viviparae of *M. caryaefoliae* also have a general preference for whichever cultivar they fed on as nymphs; this preference is apparently mediated by some water-soluble factor(s) on the leaf surface (Wood and Reilly 1998). When examining damage rather than aphid populations, 'Sioux', 'Cape Fear', 'Farley', 'Cowley', 'Grabhols', 'Barton', 'Gloria Grande' and

'Money Maker' are the least susceptible to *M. caryaefoliae* (i.e. exhibited the least damage when experimentally exposed to the aphids) and 'Choctaw', 'Oconee', 'Sumner', 'Schley', and 'Desirable' are the most susceptible among the commonly-grown cultivars (Wood and Reilly 1998, Chen et al. 2009). Some varieties' foliage also demonstrate an antixenosis-like effect and suppress the populations of *M. caryaefoliae*, but there is little correlation between this effect and the damage inflicted on pecan. For example, 'Choctaw' and 'Schley' both suppress *M. caryaefoliae* populations, but small aphid populations still inflict severe damage on these varieties (Wood and Reilly 1998, Chen et al. 2009).

Within a pecan tree canopy, aphids do not show a consistent preference for any side (north, south, east, or west) of the tree (Edelson 1982). At the beginning of the season, *M. caryaefoliae* prefers the shade (i.e. interior) leaves, and they move to the sun (i.e. peripheral) leaves as the season progresses (Moznette 1929). The three aphids tend to favor different locations on each compound leaf: *M. caryaefoliae* prefer the basal halves of basal leaflets, *M. caryella* prefer the basal halves of middle leaflets (particularly the areas adjacent to the primary leaf veins), and *M. pecanis* prefer the apical halves of apical leaflets (Tedders, 1978).

M. caryella and *M. pecanis* feed predominantly on the lower (i.e. abaxial) surfaces of leaves, largely ignoring the upper (i.e. adaxial) surface (Tedders 1978). This behavior is very common among foliage-feeding tree aphids (Blackman and Eastop 1994, Hopkins and Dixon 1997), as it enables the aphids to take advantage of the lower surface's protection from rain, solar radiation, honeydew dropped by aphids on leaves above, and protection by raised leaf venation against dislodgement through the brushing action of other leaves during wind (Hopkins and Dixon 1997, Dixon 2005).

The literature is unclear about the leaf-side preferences of *M. caryaefoliae*. The first description of the species (Davis 1910) noted them "mainly on the upper sides of leaves, but also on the lower sides" on hickory trees. Richards (1960) and Petersen and Sandström (2001) corroborated this. In contrast, Tedders (1978) and Kaakeh and Dutcher (1994) observed *M. caryaefoliae* settling mainly on the lower leaf surface on orchard trees and on isolated leaves in a lab, respectively. And Walker (1932) stated that they have no leaf-surface preference per se, but a negative phototaxis, and will settle on either surface if the leaf is shaded.

Behavioral and morphological data about the pecan aphids are consistent with observations of *M. caryaefoliae* on the upper leaf surfaces. *M. caryaefoliae* feed on the phloem cells of quaternary leaf veins, which are small but available to be fed upon from either leaf surface (Tedders and Thompson 1981, Kaakeh and Dutcher 1994). *M. caryella* and *M. pecanis* feed on the primary, secondary, and tertiary veins, and their proboscises are rarely long enough to reach the phloem cells of these larger veins from the upper leaf surface (Tedders and Thompson 1981, Kaakeh and Dutcher 1994). *M. caryaefoliae* is therefore the only pecan aphid capable of regularly feeding on the upper leaf surface, but this does not explain why it would face the aforementioned environmental hazards of the upper surface—solar radiation, rain, honeydew, and dislodgement—to do so.

Natural enemies.

The pecan aphid complex is preyed upon by many generalist predators and a few specialist parasitoids. Many of these natural enemies preferentially target *M. caryella* and *M. pecanis* rather than *M. caryaefoliae*.

The major predators on pecan foliage are the lady beetles (Coleoptera: Coccinellidae) (Tedders 1978). Prior to the introduction of *Harmonia axyridis* (Pallas), the most common lady

beetles observed on pecan were *Olla v-nigrum* (Say) and *Hippodamia convergens* (Guérin-Meneville) (Tedders 1978, Mizell 2007). These were occasionally observed feeding on pecan aphids in large numbers, but not often enough to make them useful biological control agents (Tedders and Angalet 1981). Other, less common ladybeetles observed were *Coleomegilla maculata* (DeGeer), *Cycloneda sanguinea* L., and *Cycloneda munda* (Say) (Mizell 2007). The exotic lady beetle *Coccinella septempunctata* L. was observed to complete development when fed on pecan aphids in the laboratory; when given a choice, *M. caryella* was its preferred prey and *M. caryaefoliae* its least preferred. In 1978, *C. septempunctata* was introduced to several Georgia pecan orchards in hopes of controlling the pecan aphids; it successfully established, but remained on lower branches when observed on pecan foliage at all (Tedders and Angalet 1981). Mizell (2007) reports these species (in addition to many non-lady beetle predators of aphid) being displaced by another introduced species, *Harmonia axyridis*, in Florida pecan orchards and some Georgia orchards. Numbers of *M. caryella* and *M. pecanis* are also greatly reduced since the establishment of *H. axyridis* (Mizell 2007).

Lacewings (Neuroptera) also contribute to the control of pecan aphids. The lacewing population of Georgia pecan trees includes four Chrysopidae (*Chrysoperla rufilabris* [Burmeister], *Chrysoperla plorabunda* [Fitch], *Chrysopa quadripunctata* Burmeister, and *Chrysopa nigricornis* Burmeister), three Hemerobiidae (*Micromus posticus* [Walsh], *Hemerobius humilinus* L., and *Micromus substanticus* Walker) and one Coniopterygidae, *Coniopteryx westwoodi* Melander. Of these, *C. rufilabris* is the most numerous (Dinkins et al. 1994). Chrysopids preferentially oviposit on aphid-infested pecan leaves rather than aphid-free ones (Petersen and Hunter 2002, Kunkel and Cottrell 2007). When given a choice between species, the adults preferred infestations of *M. caryella* over *M. caryaefoliae*, but their larvae will

readily accept either species as food (Petersen and Hunter 2002), and develop equally well on either (Kunkel and Cottrell 2007).

Pecan aphid mortality from lady beetles and lacewings is high enough that some species have been recommended for use in integrated pest management on pecan (LaRock & Ellington, 1996). These aphid predators search the upper leaf surfaces less than the lower surfaces (Dixon, 1970; Hopkins & Dixon, 1997).

Other predators observed on pecan include *Zelus exsanguis* (Stål), *Sinea spinipes* (Herrich-Schaeffer) (both Hemiptera: Reduviidae), *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), and *Allograpta obligua* (Say) (Diptera: Syrphidae) (Tedders 1978).

The native parasitoids of the pecan aphid complex are *Aphelinus perpallidus* (Gahan) (Hymenoptera: Aphilinidae) and an unidentified species of *Trioxys* (Hymenoptera: Braconidae). (Tedders 1978). *A. perpallidus* attacked all species of pecan aphid (Tedders 1978). Two other braconids, *Trioxyx pallidus* Halliday and *Trioxys complanatus* (Quilis), were introduced to the orchards at the USDA Agricultural Research Service, Southeastern Fruit and Tree Nut Research Lab in 1974, but it is doubtful whether they established. In the lab, these braconids are capable of developing on any of the pecan aphids, but overwhelmingly preferred *M. caryella* (Tedders 1977). There is no known parasitoid in Georgia specifically targeting *M. caryaefoliae*.

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CHAPTER 3

DISTRIBUTION OF THE BLACK PECAN APHID (HOMOPTERA: APHIDIDAE) ON

UPPER AND LOWER LEAF SURFACES

Abstract

Three aphid species regularly feed on the foliage of pecan: the black pecan aphid

Melanocallis caryaefoliae (Davis), the yellow pecan aphid *Monelliopsis pecanis* (Davis), and the

blackmargined aphid *Monellia caryella* (Fitch). *M. caryaefoliae* appears unique among these for

frequently being observed feeding on the upper surface of pecan leaves. This is risky behavior,

given the environmental hazards associated with the upper surface. This study was undertaken to

confirm the distribution of *M. caryaefoliae*, and to investigate density and predation as potential

causes. A pecan orchard survey found all three aphid species and stages predominantly on the

lower leaf surface, except for the *M. caryaefoliae* nymphs, which were evenly distributed

between upper and lower leaf surfaces. This survey also found aphidophagous lacewing larvae

predominately on the lower leaf surface, while lady beetle larvae were evenly distributed

between upper and lower surfaces. These distributions are consistent with the hypothesis that *M.

caryaefoliae* nymphs' settling on the upper leaf surface may be a strategy of reducing encounter

rates with natural enemies. Observations of manipulated aphid nymphs on laboratory pecan

seedlings revealed nymph distributions consistent with field observations. *M. caryaefoliae*

nymph movement to the upper leaf surface correlated with the density of other aphid species, but

with other aphids absent *M. caryaefoliae* still fed on the upper surface, regardless of conspecific

density. Crowding-induced dispersal may influence nymph distribution, but it is not the primary cause of nymph movement to the upper leaf surface.

Introduction

Three aphid species (Hemiptera: Aphididae) are regularly found on pecan [*Carya illinoinensis* (Wangenh.) K. Koch] foliage: the black pecan aphid *Melanocallis caryaefoliae* (Davis), the yellow pecan aphid *Monelliopsis pecanis* (Davis), and the blackmargined aphid *Monellia caryella* (Fitch) (Tedders 1978). These aphid species' feeding on pecan foliage can result in economic injury exhibited through decreased yield and quality of the pecan crop (Dutcher 1984). Typically, feeding by these aphids results in depletion of chlorophyll and carbohydrates, decreased leaf area and leaf photosynthesis (Wood et al 1985), and defoliation. In addition, excessive honeydew accumulation on pecan foliage serves as a substrate for black sooty mold growth and inhibits photosynthesis (Tedders and Smith 1976). *M. caryaefoliae* causes further damage by inducing premature senescence-like conditions in the leaf tissue surrounding its feeding site (Cottrell et al 2009), so it is of particular economic importance (Hudson and Dutcher 2004, 2006, 2008).

All three aphid species have similar life cycles. They are holocyclic and monoecious, completing their entire life cycle on pecan trees. The fundatrices appear from late March to late April and are followed by more than 20 generations of viviparae (Tedders 1978). In all three species, all viviparae are alates. Sexuales (apterous oviparae and alate males) appear from mid-October to early December (Tedders 1978).

Aphids generally demonstrate positive geotaxis and negative phototaxis (Pettersson et al 2007), and most foliage-feeding tree aphids feed on the lower (i.e., abaxial) surface of leaves, ignoring the upper (i.e., adaxial) surface (Blackman and Eastop 1994, Hopkins and Dixon 1997).

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Settling on the lower leaf surface offers protection from rain, solar radiation, honeydew dropped by aphids from leaves above (Hopkins and Dixon 1997), and the protruding leaf veins of the lower leaf surface give a degree of protection from being brushed off by the movement of other leaves (Dixon 2005). *M. pecanis* and *M. caryella* fit this trend, and predominantly settle on the lower surface of pecan leaves. Tedders (1978) noted that on mature leaves, more than 80% of *M. caryella* and more than 97% of *M. pecanis* were found on the lower leaf surface, and that all feeding damage from these aphids was observed on that surface. However, the literature is ambiguous regarding the distribution of *M. caryaefoliae* on the lower and upper leaf surfaces. Davis (1910) noted the aphids settling "mainly on the upper sides of leaves, but also on the lower sides" on hickory trees, and Richards (1960) noted similar behavior on pecan. However, Tedders (1978) surveyed pecan orchards weekly for the duration of the 1973, 1974, and 1975 growing seasons and observed first instars on the lower surface of developing pecan leaves in spring, and about 74% of *M. caryaefoliae* on the lower leaf surface during the late summer and fall. Kaakeh and Dutcher (1994) noted that fourth-instar *M. caryaefoliae* settled on the lower surface of excised pecan leaves from various cultivars. Walker (1932) stated that negative phototaxis was more important than leaf surface preference, so *M. caryaefoliae* may be found on either surface provided the leaf is shaded. Our own casual observations, both of aphid infestations in pecan orchards and of the *M. caryaefoliae* colony in the lab, did not find an apparent preference for either leaf surface.

Behavioral and morphological data can partially explain these distributions. All three aphid species feed on the phloem cells of the leaf veins. *M. caryaefoliae* feed on the quaternary veins (Kaakeh and Dutcher 1994), which are small but available to be fed upon from either leaf surface (Tedders and Thompson 1981). *M. pecanis* feed mainly on the tertiary veins, and *M.*

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caryella feed mainly on the primary and secondary veins (Kaakeh and Dutcher 1994); even though these aphids have longer proboscises than *M. caryaefoliae*, they usually can only reach the phloem cells of these larger veins by feeding from the lower leaf surface (Tedders and Thompson 1981). Thus, only *M. caryaefoliae* is able to feed on both the upper and lower leaf surfaces, but this does not explain why the nymphs would face the environmental hazards of the upper leaf surface—solar radiation, rain, honeydew, and dislodgement—to do so.

It is possible that increasing aphid density may foster dispersal. *M. caryaefoliae* nymphs typically settle at a feeding site and remain there until they complete development (Cottrell et al. 2009), possibly eliciting dispersal (to other locations on the same leaf surface or to the other leaf surface) of first instars exposed to higher densities. Additionally, *M. caryaefoliae* in crowded populations are more prone to jumping when disturbed (Tedders 1978), a response that would likely facilitate dispersal. Crowded populations also result in smaller adult aphids (Tedders 1978) and fewer offspring (Kaakeh and Dutcher 1992), so dispersal could increase fitness. Thus, *M. caryaefoliae* may disperse to the upper leaf surface in response to increasing aphid density such that the negative fitness effects of crowding on the lower surface may outweigh the risks of mortality from the environmental hazards of the upper surface.

Alternately, *M. caryaefoliae* may move to the upper leaf surface to escape predators. The tree-feeding aphid *Monaphis antennata* (Kaltenbach) has adopted a predator avoidance strategy (Berdegue et al. 1996) of regularly feeding on upper leaf surfaces (Hopkins and Dixon 1997). This strategy exploits the fact that generalist aphid predators such as larval lady beetles (Coleoptera: Coccinellidae) and lacewings (Neuroptera) exhibit negative geotaxis (Hodek and Honĕk 1996) and search the upper leaf surfaces more rarely than the lower surfaces (Dixon 1970, Hopkins and Dixon 1997). Lady beetles and lacewings are also known to prey on pecan aphids,

including *M. caryaefoliae* (Tedders and Angelet 1981; Kunkel and Cottrell 2007), in sufficient quantities that some species have been recommended for use in integrated pest management approaches for controlling all three aphid species on pecan (LaRock and Ellington 1996). Mortality from these predators may be high enough to make feeding on the upper leaf surface a viable predator avoidance strategy for *M. caryaefoliae*.

The purpose of this study was two-fold. The first was to determine whether the leaf-side distribution of *M. caryaefoliae* in both orchard and laboratory environments differed significantly from that of *M. caryella* and *M. pecanis*. The second was to examine whether aphid density or the distribution of their natural enemies potentially explains leaf-side preference by *M. caryaefoliae*. To these ends, aphids and natural enemies were sampled from a pecan orchard to determine the leaf side distribution of all three pecan-feeding aphids, as well as the distribution of their lady beetle and lacewing natural enemies. Additionally, the distribution and density of manipulated aphids on potted pecan seedlings were examined in the laboratory.

Materials and Methods

Aphid and predator field observations. Ten pecan trees ('Schley' cultivar) were selected from an orchard at the USDA, ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA. Trees in this commercial-like orchard were approximately 100 yrs old and, during 2009, no insecticides were applied to these trees. From the middle and lower two-thirds of each tree's foliage, 15 compound leaves were sampled—ten sun leaves (leaves from the canopy periphery) and five shade leaves (leaves from the canopy interior)—on a weekly basis. Leaves were selected at random, such that the north, south, east and west quadrants of the canopy were represented, as pecan aphids do not exhibit a consistent preference for any quadrant (Edelson 1982, Edelson and Estes 1987). The survey ran for nine weeks, from mid-June to early

August 2009. The numbers of aphids and predators on each leaf surface (upper and lower), were visually determined and recorded in the field. Adults of each aphid species were counted separately; *M. caryeafoliae* nymphs were counted separately, while the visual similarity of *M. pecanis* nymphs and *M. caryella* nymphs made it necessary to pool them. All species of lacewing were pooled, but each life stage was counted separately; the same was done for lady beetles. While the species of observed predators was not recorded, prior surveys have found the neuropteran population of Georgia pecan trees to include four chrysopids (*Chrysoperla rufilabris* [Burmeister], *Chrysoperla plorabunda* [Fitch], *Chrysopa quadripunctata* Burmeister, and *Chrysopa nigricornis* Burmeister, with *C. rufilabris* the most numerous), three hemerobiids (*Micromus posticus* [Walsh], *Hemerobius humilinus* L., and *Micromus substanticus* Walker) and one coniopterygid, *Coniopteryx westwoodi* Melander (Dinkins et al. 1994). Lady beetles associated with pecan include *Harmonia axyridis* (Pallas), *Olla v-nigrum* (Mulsant), *Coccinella septempunctata* (L.), *Hippodamia convergens* Guerin-Meneville, *Cycloneda sanguinea* L., and *Cycloneda munda* (Say), with *H. axyridis* being the most numerous (Bugg et al. 1991, Mizell 2007). Analysis of variance (ANOVA) was performed for each species or group, treating each leaf as an experimental unit and the trees as blocks. Initially, a full factorial was performed, with block (trees), leaf surface (upper or lower surface), shade (sun leaves vs. shade leaves), and sampling date as factors. Then, the interactions which were not significant at $P < 0.05$ (specifically block × shade, block × date, and all three-way and four-way interactions) were pooled with the error (Analytical Software, 2008). For interactions where a significant difference was detected ($P < 0.05$), Tukey's Honestly Significant Difference (HSD) test was used to separate the means and evaluate simple main effects (Roberts and Russo 1999, Analytical Software 2008).

Pecan seedlings. All pecan seedlings used in aphid rearing and laboratory experiments were greenhouse-grown from open-pollinated seeds ('Desirable' cultivar) collected from pecan orchards at the USDA, ARS, SEFTNRL, Byron, GA. Leaf discs were taken from fully expanded simple leaves using a 1.8 cm-diameter Birkenstrand leaf punch sampler (Rabbit Tool USA, Rock Island, IL) (Cottrell et al. 2009).

Insects. A colony of *M. caryaefoliae* was established from individuals collected from pecan orchards at the USDA, ARS, SEFTNRL, Byron, GA with periodic introduction of new aphids from the field. Alate viviparae were maintained on potted pecan seedlings in 60 × 120 × 70 cm wooden frame cages with mesh sides and a glass top. The cages were lit with two 40-watt fluorescent plant growth lamps placed above the glass, operating at L:D 14:10 h photoperiod, and the cage room was kept at 26 °C ± 1 °C. Separate colonies for alate viviparae of *M. caryella* and *M. pecanis* were maintained under the same conditions.

Aphid distribution on upper and lower leaf surfaces in the laboratory: Effect of single or multiple aphid species. Aphid distribution on pecan foliage was examined using laboratory trials where single or multiple aphid species were released onto foliage and their distribution examined over time. For each trial, 15 potted seedlings were used. Each seedling was trimmed so only three simple leaves remained, thus making all plants structurally uniform. In single species assays, done for each of the three aphid species, four adult aphids were placed on the upper surface of each leaf. In assays using multiple species (i.e., *M. caryaefoliae*, *M. pecanis*, and *M. caryella* together on the same seedling), five adults of each species were placed on the upper surface of each leaf. Each individual plant was placed in a clear, plastic, cylindrical cage, 28 × 41 cm (h × d), under the same photoperiod and temperature conditions as the aphid colonies. The plants were kept in these cages with the adult aphids for 24 h to allow time to

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deposit nymphs. The adult aphids were then removed and the 15 plants, with only nymphs, placed in an empty 60 × 120 × 70 cm cage, as previously described. Immediately following the removal of the adults (i.e., 0 d), three plants were destructively sampled and the number of nymphs on the upper and lower surface of each leaf was recorded. Every two days afterwards (i.e., 2, 4, 6, and 8 d) three more plants were sampled and the location of nymphs was recorded.

Experiments were performed with *M. caryaefoliae* alone (three trials), *M. pecanis* alone (two trials) and *M. caryella* alone (two trials). Three trials of the mixed aphid species experiment, using all three species, were performed. For the mixed species experiment, numbers of *M. pecanis* and *M. caryella* were pooled when counted because of difficulties in differentiating nymphs. In some instances, nymphs completed development and began depositing nymphs on some leaves by day 6 or 8; thus, data from these leaves were excluded from statistical analysis. Within each experiment (single and multiple aphid species), the heterogeneity chi-square statistic was calculated to determine whether the data from multiple trials of the same experiment could be pooled (Zar 1999). Analysis of variance (ANOVA) was used to test whether leaf surface affected aphid distribution (Analytical Software 2008).

Aphid distribution on upper and lower leaf surfaces in the laboratory: Effect of nymphal density. In addition to collection of aphid distribution data on leaves, the surface area of the leaves was measured with a LI-3100C Area Meter (Li-Cor Biosciences, Lincoln NE). Leaf areas were recorded from two of the three trials of the *M. caryaefoliae*-only experiment, and from all three trials of the mixed aphid species experiment. From this, nymph density was calculated, both for the lower leaf surface and for the entire leaf. For each leaf, the proportion of nymphs on the lower surface was calculated; because many of these proportions were very high or very low, the data were normalized using Zar's (1999) modification of the Freeman and

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Tukey arcsine transformation. Pearson's correlation coefficient (r) was used to test for correlation between the transformed proportions and nymphal density (at $P < 0.05$ significance) against the null hypothesis that $\rho = 0$.

Results

Field observations: Aphids. Comparing the two leaf surfaces, the *M. caryaefoliae* nymphs were the only aphid group for which there was no significant difference in numbers between the upper and lower leaf surfaces (Table 1.1, Figure 1.1A). All others, including adult *M. caryaefoliae*, had significantly higher numbers on the lower leaf surface (Table 1.1, Figure 1.1A). The percentages of *M. caryaefoliae* nymphs and adults (respectively) on the lower leaf surface were 49.98 and 86.66% (Figure 1.1A). The percentages of *M. caryella* adults, *M. pecanis* adults, and their pooled nymphs (respectively) on the lower leaf surface were 98.57, 98.56, and 99.17% (Figure 1.1A).

Comparing the effects of shade on aphid abundance, the *M. caryella* adults were the only group for which there was no significant difference in numbers between the sun and shade leaves (Table 1.1, Figure 1.1B). Significantly more adult *M. pecanis* were observed on sun than shade leaves, as were pooled nymphs of *M. pecanis* and *M. caryella* (Table 1.1, Figure 1.1B). In contrast, more adults and nymphs of *M. caryefoliae* were observed on shade than sun leaves (Table 1.1, Figure 1.1B).

The abundance of *M. caryefoliae* nymphs did vary significantly between trees, but this did not affect the distribution between leaf surfaces (there was no significant tree × leaf surface interaction) (Table 1.1). They were affected by the interaction of shade × leaf surface (Table 1.1). There was no significant difference in means between the upper and lower surfaces within either sun or shade leaves; but there was a significant difference between the lower surface

means on shade (9.30 ± 0.60) versus sun foliage (7.18 ± 0.43) (Figure 1.2). The interaction of date × shade was also significant (Table 1.1): when nymph numbers rose in late July, the increase was greater on shade foliage (Figure 1.3). Although mean numbers of nymphs varied significantly by date, there was never a significant difference between leaf surfaces within any sampling date (Table 1.1, Figure 1.4A).

Unlike the nymphs, the *M. caryefoliae* adults did not vary in numbers between trees (Table 1.1). Like the nymphs, the interaction of tree × leaf surface was not significant, while the interaction of shade × leaf surface was significant (Table 1.1). The mean on the lower surface was significantly greater on shade (2.40 ± 0.23) than sun foliage (1.90 ± 0.15). However, there was no significant difference between the upper surface means for the shade (0.30 ± 0.05) or sun leaves (0.33 ± 0.03). The adult means also varied over time with a significant interaction of date × leaf surface (Table 1.1). During the last three sample dates, the number of adults on the lower side was significantly higher than the number on the upper side (Figure 1.4B).

The *M. caryella* adults (Fig. 1.5A), *M. pecanis* adults (Fig. 1.5B), and their pooled nymphs (Fig. 1.5C) all displayed trends over time similar to the *M. caryaefoliae* adults: their numbers on the lower surface varied over time, while the numbers on the upper surface remained near zero. For the yellow pecan aphid adults, neither the tree nor the tree × leaf surface interaction had a significant effect on abundance (Table 1.1). For *M. caryella* adults and the pooled nymphs, the effects of both tree and the tree × leaf surface interaction were significant (Table 1.1). For *M. caryella* adults, abundance on the lower leaf surface varied between trees from 0.76 ± 0.13 to 0.31 ± 0.11 aphids per leaf, while no tree's upper leaf surface abundance differed significantly from zero. For the pooled nymphs of *M. caryella* and *M. pecanis*,

abundance on the lower leaf surface varied between trees from 12.85 ± 1.82 to 7.09 ± 0.98, while abundance on the upper leaf surface varied from 0.14 ± 0.06 to 0.02 ± 0.01 aphids per leaf.

Field observations: Lacewings and lady beetles. No lacewing pupae or adults were observed during the survey, and only one lady beetle adult was observed. Those three groups were excluded from statistical analysis.

Comparing predator abundance between the two leaf surfaces, the numbers of lacewing eggs and larvae varied significantly between the upper and lower surfaces, while no lady beetle stage varied significantly with leaf surface (Table 2, Figure 1.6A). For lacewing eggs and larvae, the date × leaf surface interaction was significant as well (Table 2): the lower-surface mean of larvae was 0.05 ± 5.8E-3 overall (with an increase to 0.11 ± 0.03 on July 30); while the overall mean on the upper surface was 8.9E-3 ± 2.6E-3, and on no date was the upper-surface mean significantly different from zero.

Comparing effect of shade on predator abundance, only lady beetle larval abundance varied significantly between sun and shade leaves (Table 2, Figure 1.6B). The date × shade interaction was significant as well: in the interior, the mean per leaf side was 4.4E-3 ± 2.2E-3, and on no date was the mean significantly different from zero (Figure 1.7). Predator abundance did not vary significantly between trees, nor was there a significant tree × leaf surface interaction for any of the predators (Table 1.2).

Aphid distribution on upper and lower leaf surfaces in the laboratory: Effects of single or multiple aphid species. For the experiment with *M. caryaefoliae* alone on the potted pecan seedlings, there was significant heterogeneity between the three trials ($\chi^2_2 = 21.25$, $P < 0.0001$) so these data could not be pooled. However, in all three trials, more nymphs were observed on the upper leaf surfaces than the lower ones (Fig. 8a); these differences were

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significant in trial one ($F_{1,84} = 6.44$, $P = 0.0130$) and trial three ($F_{1,84} = 7.98$, $P = 0.0062$), but not in trial two ($F_{1,68} = 1.07$, $P = 0.3048$). The percentages of *M. caryaefoliae* on the lower leaf surface in the three trials were 34.4, 45.9, and 38.3%, respectively (Figure 1.8A).

There was no significant heterogeneity between the trials for *M. pecanis* ($\chi^2_1 = 0.06$, $P = 0.8065$) or for *M. caryella* ($\chi^2_1 = 0.12$, $P = 0.7290$), so both trials for each species were pooled. The leaf surface had a significant effect on average nymph count for both *M. pecanis* ($F_{2,140} = 98.08$, $P < 0.0001$) and *M. caryella* ($F_{2,126} = 45.73$, $P < 0.0001$). The mean percentages of *M. caryella* and *M. pecanis*, respectively, on the lower leaf surface were 95.5 and 92.5% (Figure 1.8A).

In the experiment using all three aphid species mixed, there was significant heterogeneity of *M. caryaefoliae* distributions between trials ($\chi^2_2 = 15.45$, $P = 0.0004$). However, for *M. caryaefoliae*, neither the leaf side ($F_{1,186} = 2.25$, $P = 0.1353$) nor the trial ($F_{2,186} = 1.25$, $P = 0.2889$) alone had a significant effect on the number of nymphs. While the leaf side × trial interaction was significant ($F_{2,186} = 3.45$, $P = 0.0338$), the HSD test did not find any difference between any leaf side in any trial; as ANOVA is more powerful than the HSD test, this disagreement between results probably indicates that the more conservative HSD would have detected differences at a larger sample size. The percentage of *M. caryaefoliae* nymphs observed on the lower leaf surface in trials 1, 2, and 3 was 55.38, 23.44, and 50.00%, respectively (Fig. 1.8B). Among the pooled *M. pecanis* and *M. caryella* nymphs, there was no significant heterogeneity in distributions between trials ($\chi^2_2 = 4.43$, $P = 0.1092$). However, leaf side ($F_{1,186} = 191.96$, $P < 0.0001$) and trial ($F_{2,186} = 30.53$, $P < 0.0001$) both had significant effects on nymph numbers, and their interaction was significant ($F_{2,186} = 27.94$, $P < 0.0001$). The percentage of

yellow nymphs observed on the lower leaf surface in trial 1, 2, and 3 was 84.8, 93.9, and 95.9%, respectively (Figure 1.8B).

Aphid distribution on upper and lower leaf surfaces in the laboratory: Effects of nymphal density. In observations with *M. caryaefoliae* alone, there was no significant correlation between the density on the entire leaf (both upper and lower leaf surfaces) and the proportion of nymphs on the lower leaf surface ($r = 0.01$, d.f. = 52, $P = 0.9709$) (Figure 1.9A), but there was a significant positive correlation between the proportion of nymphs on the lower leaf surface and the density of nymphs on the lower leaf surface ($r = 0.50$, d.f. = 52, $P = 0.0001$) (Figure 1.9B).

On the plants with all three species mixed, the density of the *M. caryaefoliae* alone followed the same pattern as on the plants without the other aphid species. There was no significant correlation between the proportion of *M. caryaefoliae* nymphs on the lower leaf surface and the density of *M. caryaefoliae* nymphs on the whole leaf ($r = -0.02$, d.f. = 64, $P = 0.8917$). There was a significant positive correlation between the proportion on the lower leaf surface and the density of *M. caryaefoliae* nymphs on the lower leaf surface ($r = 0.61$, d.f. = 64, $P < 0.0001$) (Fig 1.10A). There was a was significant negative correlation between the proportion of *M. caryaefoliae* on the lower leaf surface and the combined density of *M. caryella* and *M. pecanis* on the lower leaf surface ($r = -.33$; d.f. = 64; $P = 0.0076$) (Fig. 1.10B). There was no significant correlation between the proportion of *M. caryaefoliae* on the lower leaf surface and the total density on the lower surface ($r = -0.13$; d.f. = 64; $P = 0.3020$) (Figure 1.10C).

Discussion

The distribution of *M. caryaefoliae* nymphs on the leaves was unique among the pecan-feeding aphids. Both in the orchard and in the laboratory, they were observed about equally

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distributed between the upper and lower surfaces of leaves, while the adults and nymphs of *M. caryella* and *M. pecanis* were predominantly observed on the lower surface. Surprisingly, *M. caryaefoliae* adults were also predominantly observed on the lower leaf surfaces.

The difference between the distributions of the nymphs and the adults of *M. caryaefoliae* has a few implications. First, it suggests that the nymphs are actively dispersing before finding a feeding site, not simply feeding where they are born, and that the nymphs on the upper surface move to the lower surface shortly before or after reaching maturity. Second, the difference also suggests that different pressures are operating on the different stages, causing nymphs to feed on upper leaf surfaces but not the adults. Further, the factor(s) causing these nymphs to move to the upper leaf surface is not strong enough to make them altogether abandon the lower leaf surface, suggesting that the selective pressures are intermittent.

Explanations for *M. caryaefoliae* distribution on leaf surfaces should account for these facts. As such, hypotheses include: (1) nymphs disperse when the lower leaf surface becomes too crowded, (2) morphological or nutritional differences occur between the two pecan leaf surfaces, or (3) the nymphs are moving to the upper leaf surface to escape natural enemies.

Dispersal is a common response to high density of conspecifics in many species of aphids. Among other aphid species with both apterous and alate generations, tactile stimuli from conspecifics can induce a greater proportion of dispersal-adapted, alate offspring (Johnson 1965). Poor nutritional quality in the host plant, which can be induced by higher aphid densities, has also been implicated in production of more alates (Wadley 1923), though a metadata analysis suggests this correlation only exists above a given nutritional quality threshold (Müller et al. 2001). However, *M. caryaefoliae* differ from many aphids in two relevant ways. First, their viviparae are exclusively alates. This could explain the difference between adult and nymph

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distributions: dispersing adults can fly to new leaves or new plants and settle on the lower surfaces, while nymphs must walk to a new location, so the hazardous upper leaf surface may be the only location accessible to them. Second, *M. caryaefoliae* produce chlorotic lesions in association with feeding. The presence of these lesions during nymphal development has a positive effect on aphid fitness, and a foraging nymph will preferentially feed on leaves which are already chlorotic (Cottrell et al. 2009). Thus, conspecific crowding (below a particular density threshold) could result in faster creation of larger chlorotic lesions and thus benefit *M. caryaefoliae*. In this case, feeding on the upper leaf surface could also benefit these aphids, by allowing them to share chlorotic lesions from both leaf sides. However, crowding from other pecan aphids might cause *M. caryaefoliae* to disperse, as *M. caryella* and *M. pecanis* also damage leaves by their feeding, negatively affecting the growth of any aphids feeding on these leaves later (Leser 1981, Edelson 1982). In particular, damage from *M. caryella* feeding inhibits *M. caryaefoliae*'s ability to induce chlorotic lesions (Petersen and Hunter 2001, Petersen and Sandström 2001).

It is not surprising that the results of these experiments were ambiguous regarding the effect of density on *M. caryaefoliae* nymph distribution. If nymphs are moving to the upper leaf surface in response to overcrowding on the lower surface, then a significant negative correlation should be observed between the density of nymphs and the proportion of nymphs on the lower leaf surface. This negative correlation was not observed (nymphs were found in significant numbers on both leaf surfaces at all aphid densities) when examining the density of conspecifics, whether *M. caryaefoliae* were alone or all three aphids were present on the seedling. On the other hand, the expected negative correlation was observed between the proportion of *M. caryaefoliae* on the lower surface and the density of *M. caryella* and *M. pecanis*. Although this correlation

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was statistically significant, it only explained 10.8% of the observed variation, indicating that other factors influenced *M. caryaefoliae*'s distribution—and *M. caryaefoliae* nymphs still fed on the upper surface when *M. caryella* and *M. pecanis* were completely absent. In addition, interactions between the pecan aphid species may be less common under orchard conditions, as each aphid prefers a different region of the compound leaf: *M. caryaefoliae* prefer the basal halves of basal leaflets, *M. caryella* prefer the basal halves of middle leaflets, and *M. pecanis* prefer the apical halves of apical leaflets (Tedders 1978). Thus, overcrowding by other aphids on the lower leaf surface may be a cause for *M. caryaefoliae* movement to the upper leaf surface, but it is not the only one, nor the most important.

Morphological or nutritional differences between the leaf surfaces may be responsible for the distribution of *M. caryaefoliae* nymphs. Tissues within the same plant are known to vary in their nutritional quality for herbivorous insects. Pecan leaves within the same terminal vary with age, and age affects the nutritional quality of the leaf (Kennedy et al. 1950). Even within the same compound leaf, the three pecan-feeding aphids' preferences for different regions (Tedders 1978) may reflect differing distributions of nutrients. On the other hand, it is less likely that there is a nutritional difference between the two leaf surfaces, because *M. caryaefoliae* feeds on the same phloem from either surface. However, morphological differences between the two surfaces could affect aphid fitness by making it easier or harder to feed. If this fitness difference favors aphids on the upper leaf surface, and is of similar magnitude to the risks of mortality from environmental hazards on the upper surface, then a population equally distributed between both surfaces would be expected. Stomata are one major difference between the two surfaces: stomata only occur on the lower surface of pecan leaves. The greater density of stomata on peripheral than interior leaves (Lombardini et al. 2009), and the greater lower-leaf-surface abundance of *M.*

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caryaefoliae nymphs on interior than peripheral leaves, may indicate that stomatal density on the lower leaf surface influences nymphs. Since *M. caryaefoliae* feed on quaternary leaf veins (Tedders 1978), a greater density of stomata on a leaf may correspond with a smaller leaf area suitable for feeding. However, before examining any potential mechanisms of leaf-surface fitness effects in-depth, it would be preferable to compare the effect of each leaf surface on nymphal development (Cottrell et al. 2009), to determine for certain whether leaf surface affects *M. caryaefoliae* fitness.

Natural enemies may also explain the distribution of *M. caryaefoliae*. Fitness tradeoffs between differing mortality factors can result in a species, or a specific life stage of a species, distributing about evenly between two niches. The winter cherry bug *Acanthocoris sordidus* Thunberg deposits 15–67% of its eggs on non-host plants; the higher nymphal mortality on the non-host is offset by lower egg mortality (Nakajima and Fujisaki 2010). Pecan leaves may be a similar system: if lady beetles and lacewings mainly forage on the lower surface of pecan leaves, as they do in other systems (Dixon 1970, Hopkins and Dixon 1997), and if mortality from these predators is comparable to that from the environmental hazards of feeding on the upper leaf surface, both leaf surfaces could be equally advantageous to *M. caryaefoliae* nymphs, and they could be expected to distribute about evenly between the two surfaces. Further, because the adults can avoid predators and fly to new feeding sites, adults are under less pressure from predation and can be expected to preferentially settle on the lower leaf surface. Field observations of predators reveal that the upper leaf surface is not completely devoid of natural enemies, but nevertheless has reduced predator abundance compared to the lower surface. Lacewing larvae were predominantly observed on the lower leaf surfaces, while the lady beetle larvae were observed on both leaf sides. The late-July increase in *M. caryaefoliae* in the canopy

interior, just prior to the increase of lady beetle larvae on the canopy periphery, suggests a relationship between the foraging of the lady beetles and the distribution of the aphids. It also bears noting that predator numbers were low through most of the season. While populations of one lady beetle for every 70 aphids (or occasionally as low as 1:200) can prevent outbreaks (Hagen and Van den Bosch 1968), the predator population in this orchard did not prevent any outbreaks; it is possible the selection pressure from predation was intermittent. Further studies are needed on these predators' searching patterns on pecan foliage and their predation on black pecan aphids before it can be determined whether *M. caryaefoliae* nymphal distribution on leaf surfaces is a predator avoidance strategy.

The pecan aphid parasitoid literature suggests they are unlikely to exert a significant, direct influence on *M. caryaefoliae* distribution, because no parasitoids specializing in black pecan aphids are known from Georgia. Two parasitoids—*Aphelinus perpallidus* Gahan (Hymenoptera: Aphelinidae), and *Trioxys monelliopsis* Starý and Marsh (Hymenoptera: Braconidae)—are known to be native to Georgia (Mizell and Schiffhauer 1990), and two more—*Tryoxis pallidus* (Haliday) and *Trioxys complanatus* Quilis—were introduced as biological control agents, but their establishment is uncertain (Tedders 1977, Starý and Marsh 1982). *A. perpallidus* will parasitize *M. caryaefoliae*, but prefers *M. caryella* or *M. pecanis* (Mizell and Schiffhauer 1990); the two introduced species almost exclusively targeted *M. caryella* in laboratory trials (Tedders 1977). A field survey comparing rates of parasitism between all three pecan aphids could still be very informative.

In conclusion, the distribution of *M. caryaefoliae* nymphs between the upper and lower leaf surfaces is unique among pecan-feeding aphids. Movement by these nymphs to the upper leaf surface is not correlated with conspecific density and is correlated with density of other

pecan-feeding aphids, but crowding from other aphids cannot be the sole reason black pecan aphid nymphs feed on the upper surface. The distribution of nymphs is consistent with the hypothesis that some morphological or nutritional difference between the two leaf surfaces favors the black pecan aphid nymphs feeding on the upper leaf surface. The distribution is also consistent with the hypothesis that the upper leaf surface is a relatively enemy-free space, and the observed distribution of lacewing predators is consistent with this hypothesis. Further experiments on the relative fitness of aphids feeding on each leaf surface, and on the predation patterns of lacewings and lady beetles, could determine whether either of these hypotheses are correct.

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Tables

Table 1.1.

F-statistics and numerator degrees of freedom from ANOVA for the pecan aphids observed in

the orchard from June through August 2009.

Source[a]	df[b]	F				
		Black pecan aphid adults	Black pecan aphid nymphs	Blackmargined aphid adults	Yellow pecan aphid adults	Yellow aphid nymphs
Tree	9	1.73	4.03 ***	4.36 ***	1.61	2.67 **
Leaf surface	1	293.19 ***	0.77	206.45 ***	419.55 ***	466.07 ***
Shade	1	5.04 *	10.62 **	0.07	5.04 *	21.05 ***
Date	8	143.78 ***	276.51 ***	65.17 ***	61.41 ***	76.57 ***
Tree × leaf surface	9	1.22	0.93	3.49 **	1.53	2.59 **
Shade × leaf surface	1	6.07 *	7.11 **	0.14	5.04 *	20.37 ***
Date × leaf surface	8	78.44 ***	1.06	68.38 ***	76.01 ***	91.03 ***
Date × shade	8	1.73	10.14 ***	0.43	2.97 **	6.91 ***

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[a]The interaction effects not listed here were pooled with the error.

[b]For all terms, the denominator degrees of freedom = 2654.

Table 1.2.

F-statistics and numerator degrees of freedom from ANOVA for the aphid natural enemies

observed in the orchard from June through August 2009.

Source[a]	df[b]	F				
		Lacewing eggs	Lacewing larvae	Lady beetle eggs	Lady beetle larvae	Lady beetle pupae
Tree	9	0.82	1.37	0.78	0.74	0.82
Leaf surface	1	46.48 ***	31.54 ***	1.49	0.84	0.76
Shade	1	0.38	0.98	1.49	7.54 **	0.76
Date	8	1.91	3.22 **	0.66	5.04 ***	2.98 **
Tree × leaf surface	9	1.08	0.92	0.78	1.15	1.42
Shade × leaf surface	1	0.00	0.00	1.49	0.03	0.08
Date × leaf surface	8	3.29 **	2.18 *	1.32	0.70	0.29
Date × shade	8	0.42	0.91	0.66	2.70 **	0.38

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[a]The interaction effects not listed here were pooled with the error.

[b]For all terms, the denominator degrees of freedom = 2654.
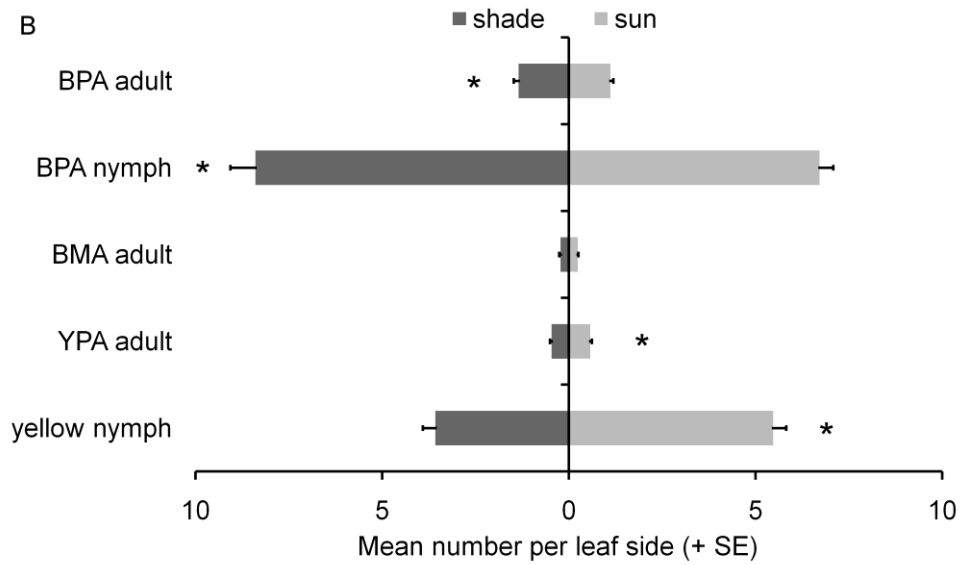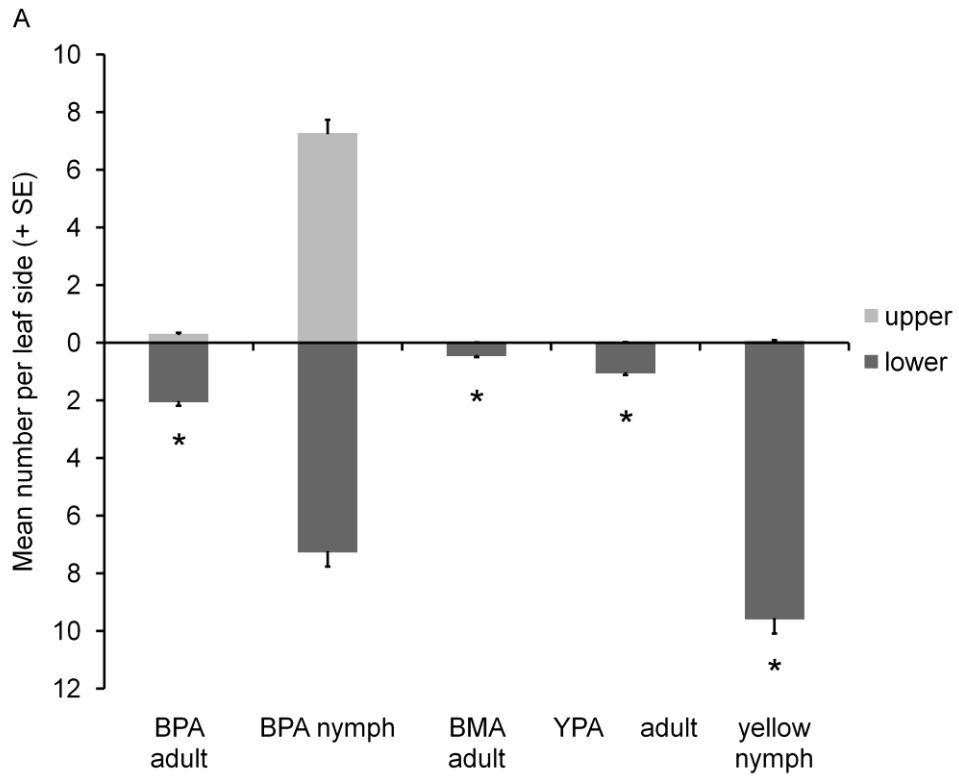
Figures

Aphid abundance from the field survey, pooled across sampling dates and trees. "BPA", black pecan aphid *M. caryaefoliae*; "YPA", yellow pecan aphid *M. pecanis*; "BMA", blackmargined aphid *M. caryella*; "yellow nymph", nymphs of *M. pecanis* and *M. caryella*, pooled due to visual similarity. An asterisk indicates significant differences ($P < 0.05$) between paired bars. (A) Comparison of upper and lower leaf surfaces ($n = 1350$). (B) Comparison of shade ($n = 450$) and sun foliage ($n = 900$).

Figure 1.2.

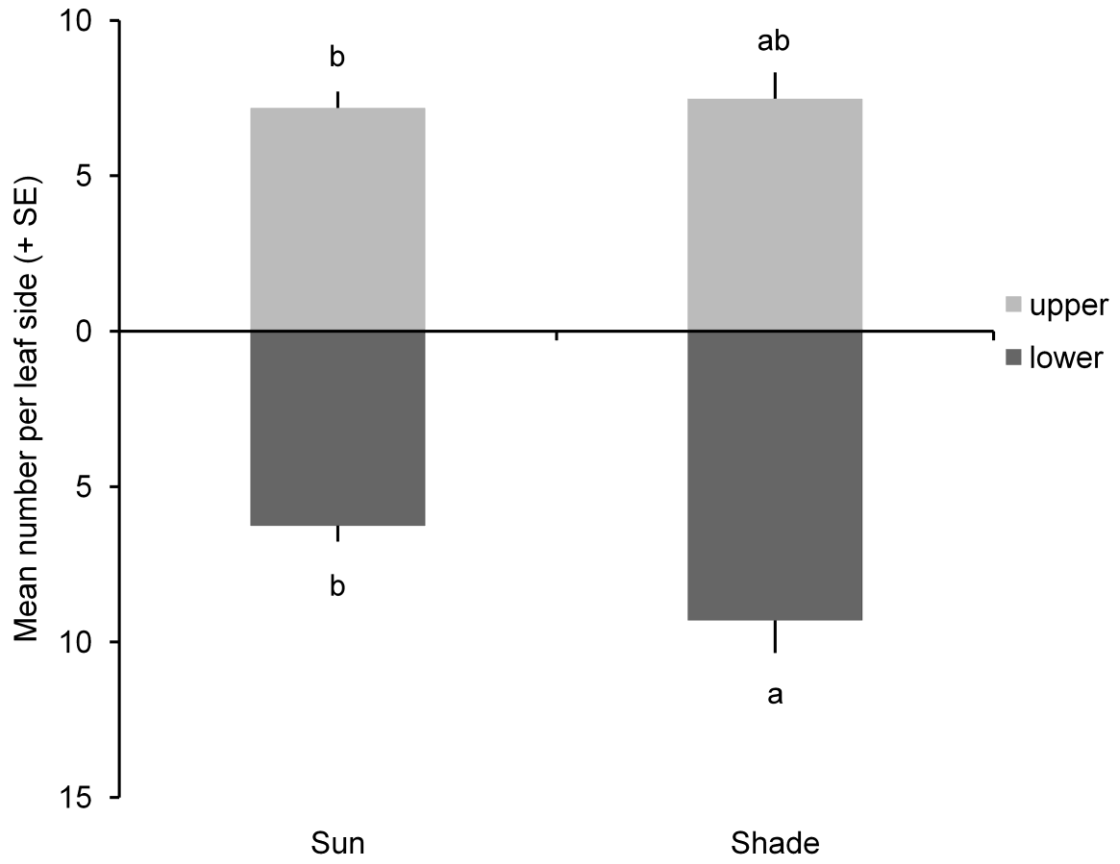Comparison of the effects of leaf surface and shade on *M. caryaefoliae* abundance. For shade leaves, $n = 450$; for sun leaves, $n = 900$. Within the same column, the same letters indicate no significant difference ($P < 0.05$) between aphids on upper and lower leaf surfaces. Comparing between columns, the same letters indicate no significant difference ($P < 0.05$) between aphids on shade and sun foliage.

<u>Figure 1.3.</u>

Comparison of *M. caryaefoliae* nymph abundance between sun and shade foliage over time. An asterisk indicates significant differences ($P < 0.05$) between numbers on a given date.
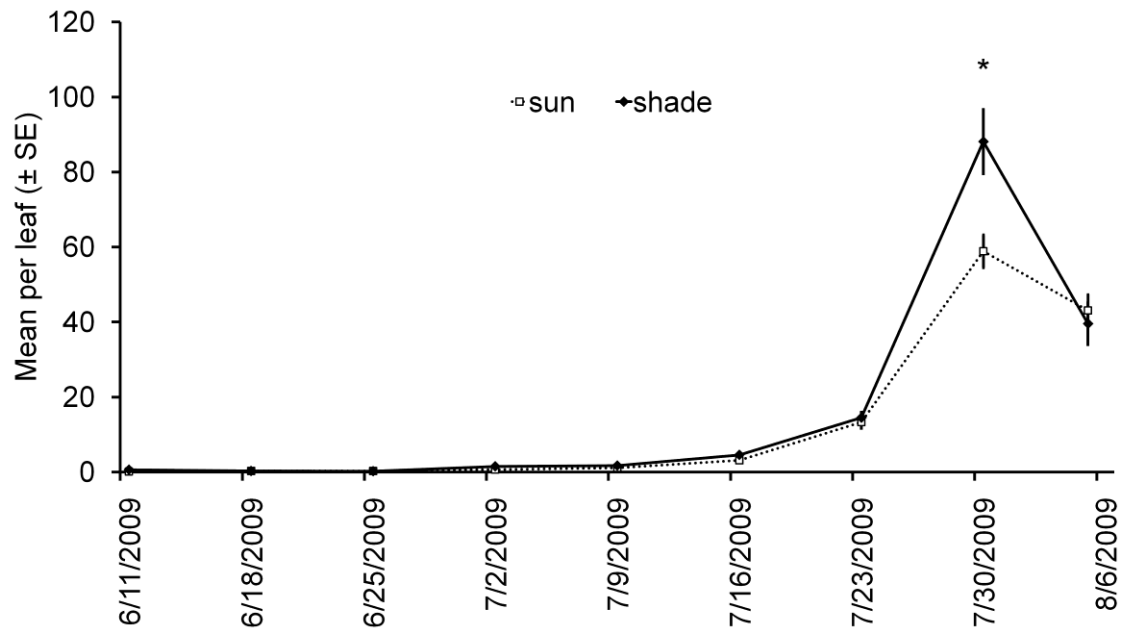
Figure 1.4.

Comparison of *M. caryaefoliae* nymphs and adult abundance between leaf surfaces over time.

An asterisk indicates significant differences (*P* < 0.05) between the two surfaces on a given date.

(a) Black pecan aphid nymphs. (b) Black pecan aphid adults.
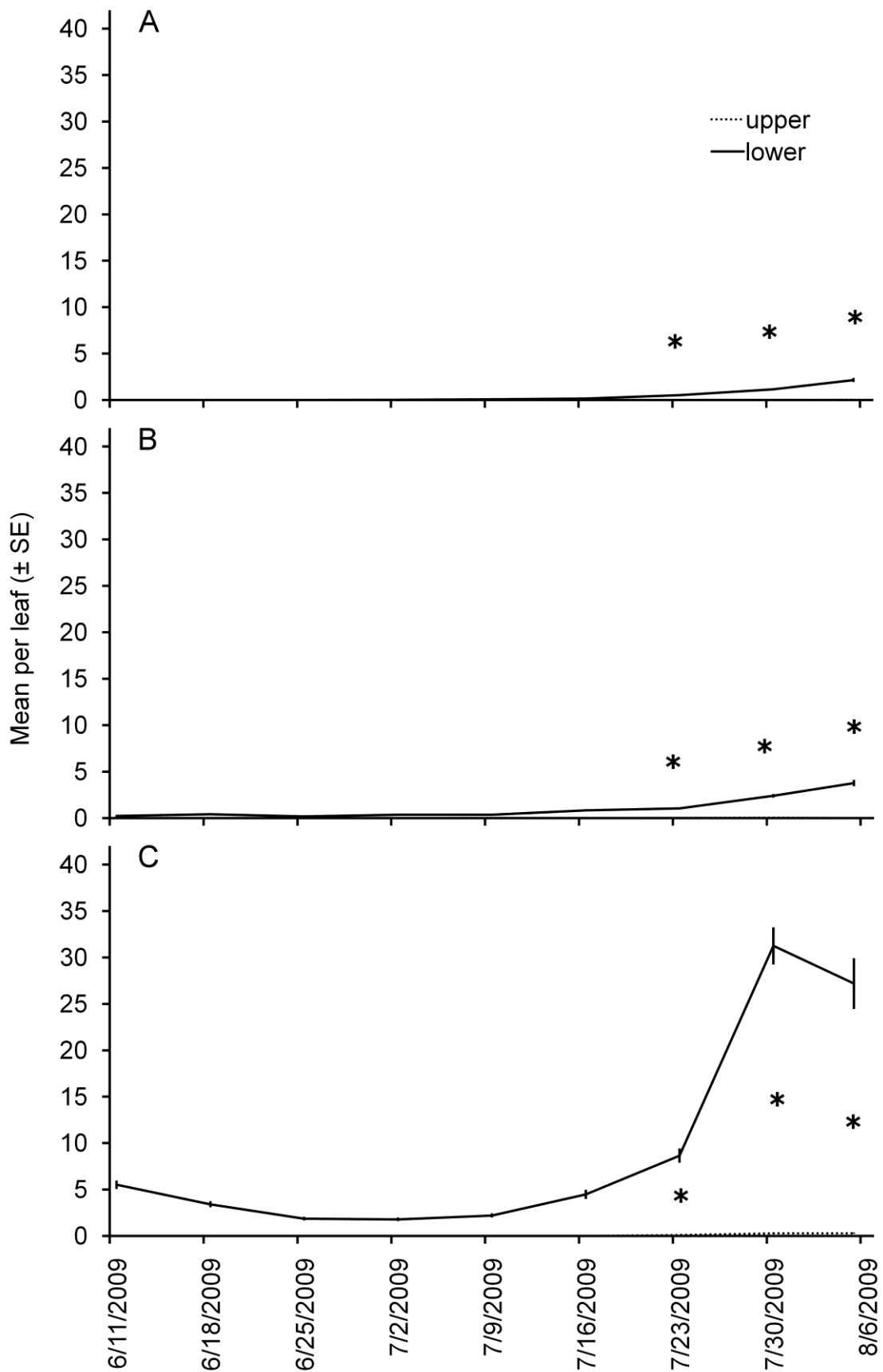
Figure 1.5.

Comparison of *M. pecanis* and *M. caryella* nymph and adult abundance between leaf surfaces

over time. An asterisk indicates significant differences ($P < 0.05$) between the two surfaces on a

given date. (a) Blackmargined aphid adults. (b) Yellow pecan aphid adults. (c) Nymphs of

blackmargined aphids and yellow pecan aphids, pooled due to their visual similarity.
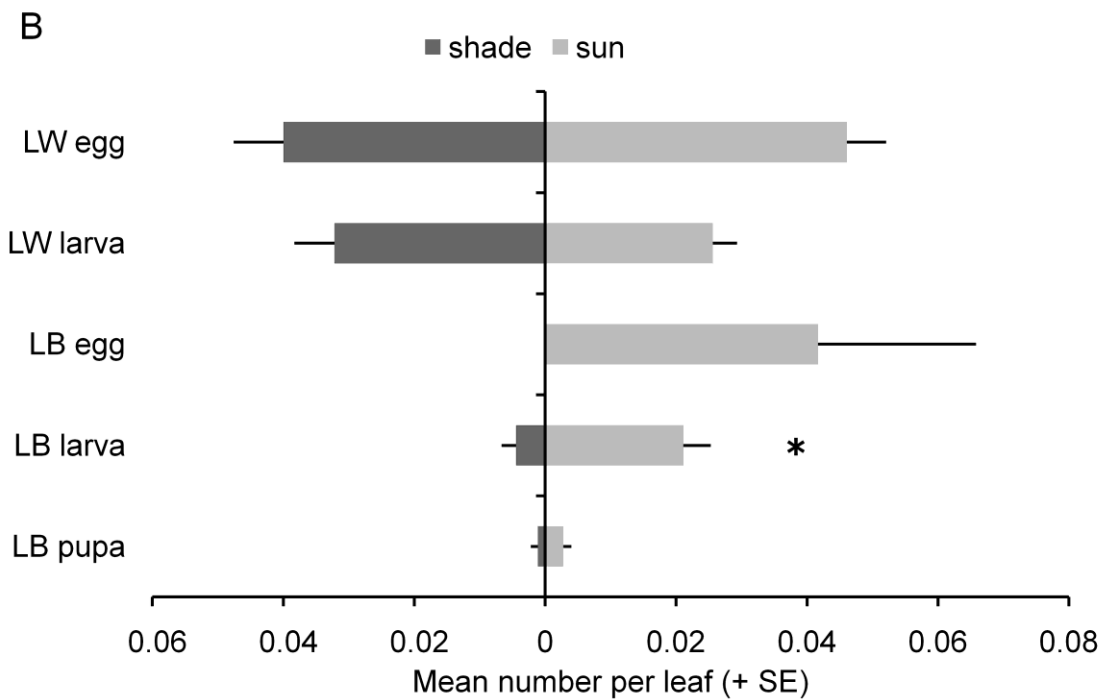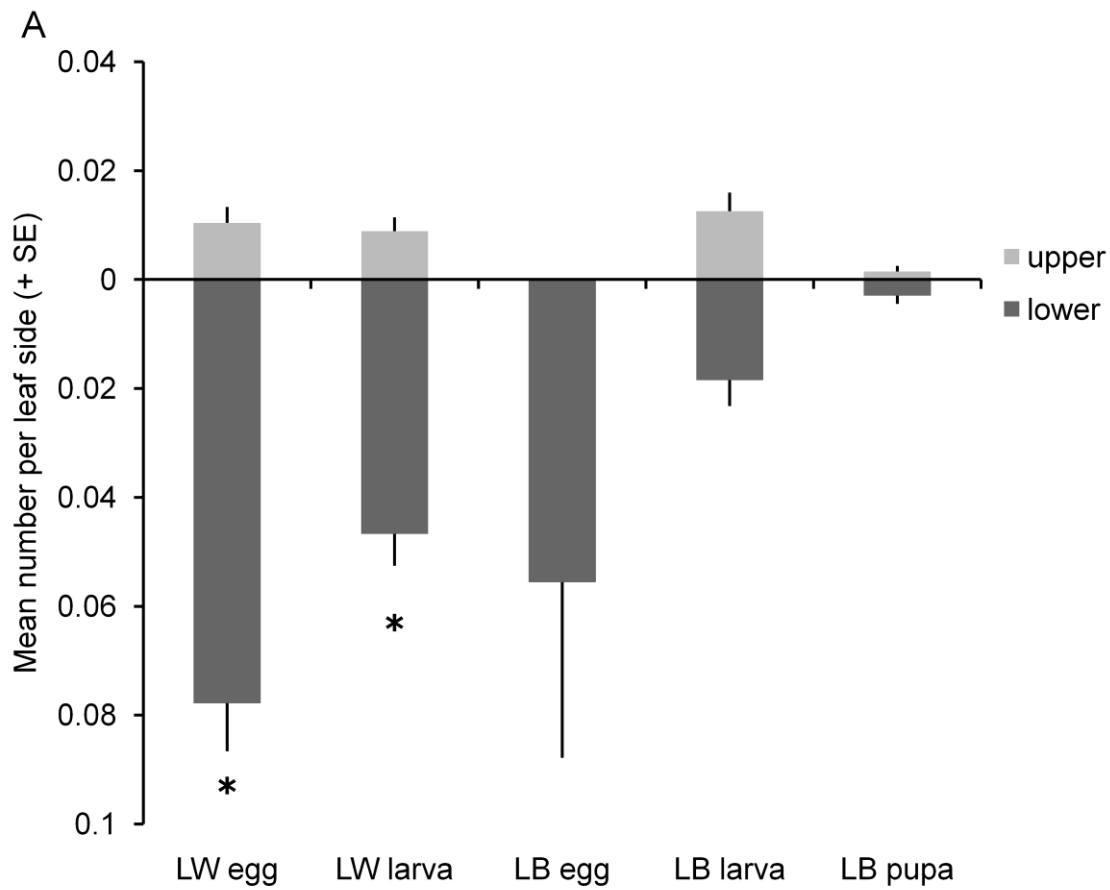
<u>Figure 1.6.</u>

Natural enemy abundance from the field survey, pooled across sampling dates and trees. "LW", lacewing; "LB", lady beetle. An asterisk indicates significant differences ($P < 0.05$) between paired bars. (a) Comparison of upper and lower leaf surfaces ($n = 1350$). (b) Comparison of shade ($n = 450$) and sun foliage ($n = 900$).
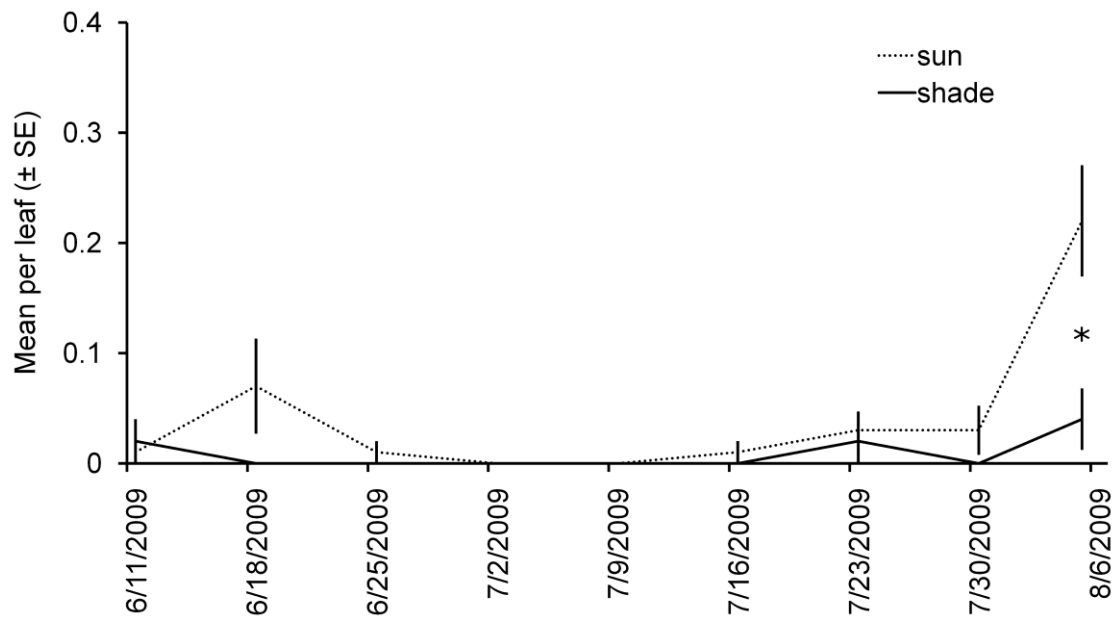
Figure 1.7.

Comparison of lady beetle larva abundance between sun and shade foliage over time. An asterisk indicates significant differences ($P < 0.05$) between numbers on a given date.
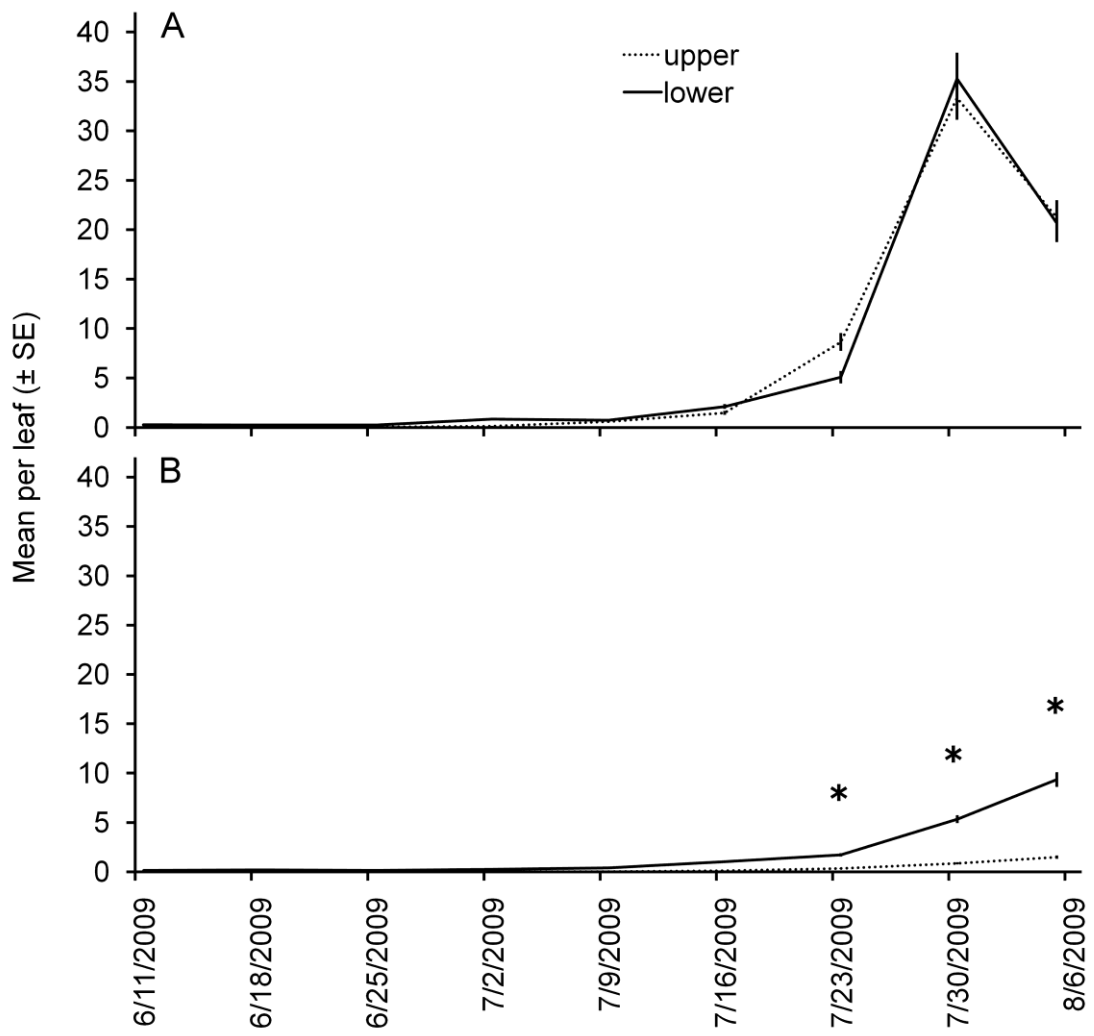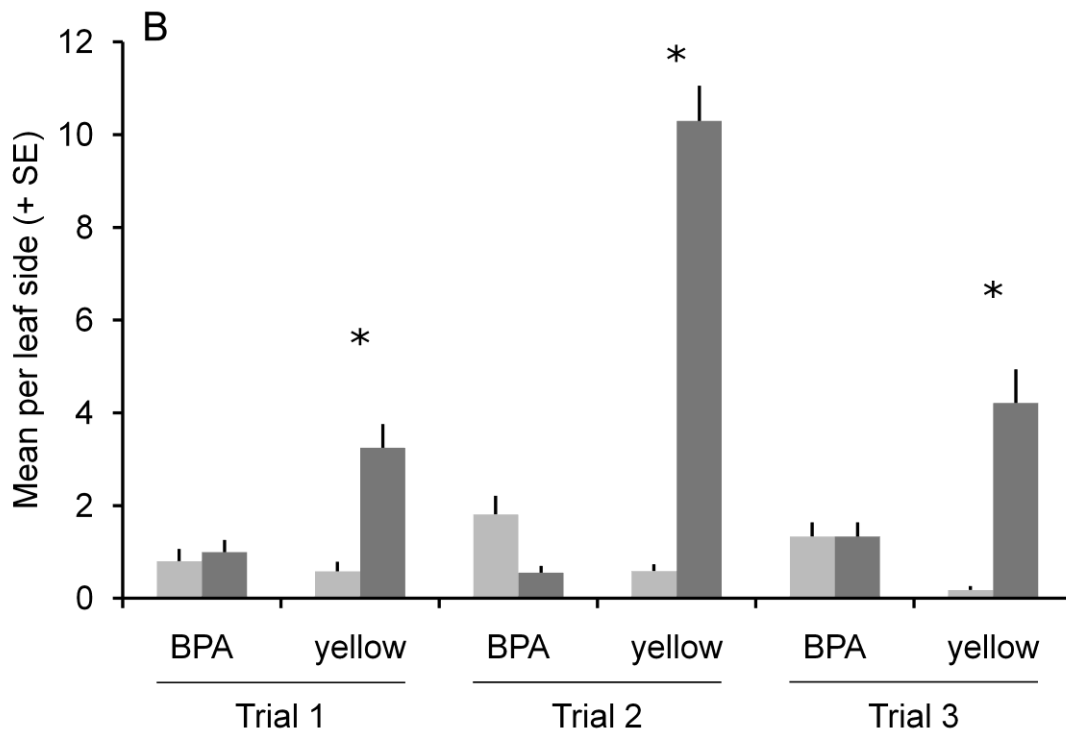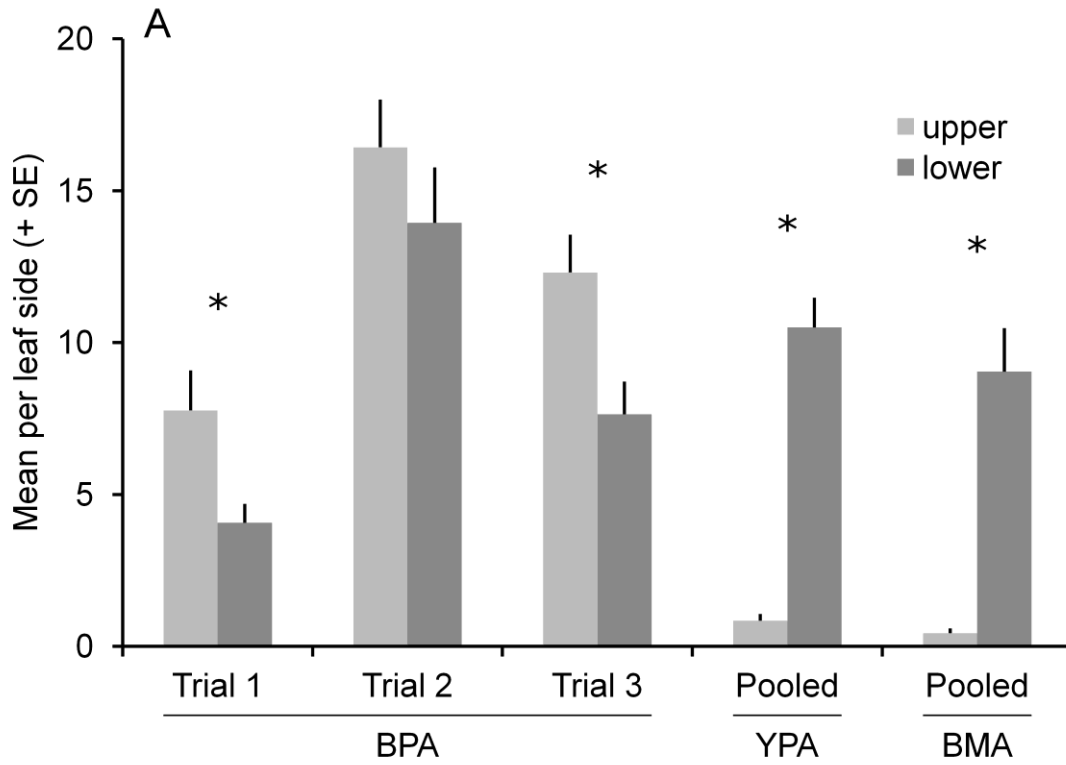
Figure 1.8.

Mean pecan aphid nymph numbers from lab experiments. "BPA", black pecan aphid *M. caryaefoliae*; "YPA", yellow pecan aphid *M. pecanis*; "BMA", blackmargined aphid *M. caryella*; "yellow", nymphs of *M. pecanis* and *M. caryella*, pooled due to visual similarity. An asterisk indicates significant differences ($P < 0.05$) between paired bars. (a) Trials with each aphid species on separate plants. The heterogeneity between trials with black pecan aphids was significant, so the trials could not be pooled. Both trials with yellow pecan aphid could be pooled, as could blackmargined aphid. (b) Trials with all three aphid species on the same plants. Heterogeneity between the numbers of *M. caryaefoliae* was significant, so the trials could not be pooled.
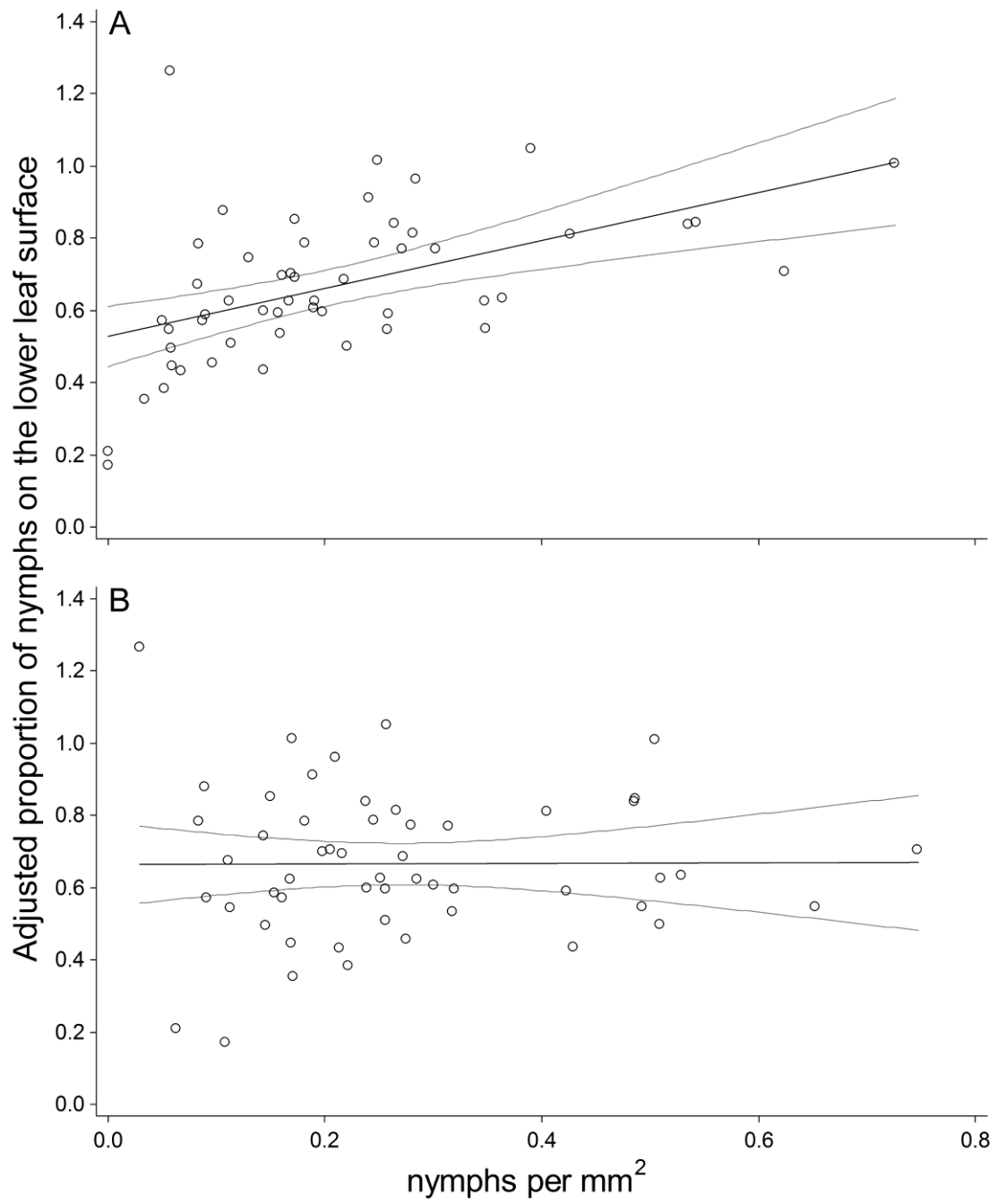
M. caryaefoliae distribution between leaf surfaces vs. density from solo lab experiments. The

proportions of *M. caryaefoliae* nymphs on the lower leaf surface were arcsine transformed to

normalize their distribution. The curves represent ± 95 % C.I. for regression. (A) Distribution vs.

density of nymphs on the entire leaf. The formula of the regression line is:

$\sin^{-1}\sqrt{proportion} = 0.5281 + (0.6650)density$; $r^2 = 0.00$. (B) Distribution vs. density of

nymphs on the lower leaf surface. The formula of the regression line is:

$\sin^{-1}\sqrt{proportion} = 0.6636 + (6.82\text{E}-03)density$; $r^2 = 0.25$.
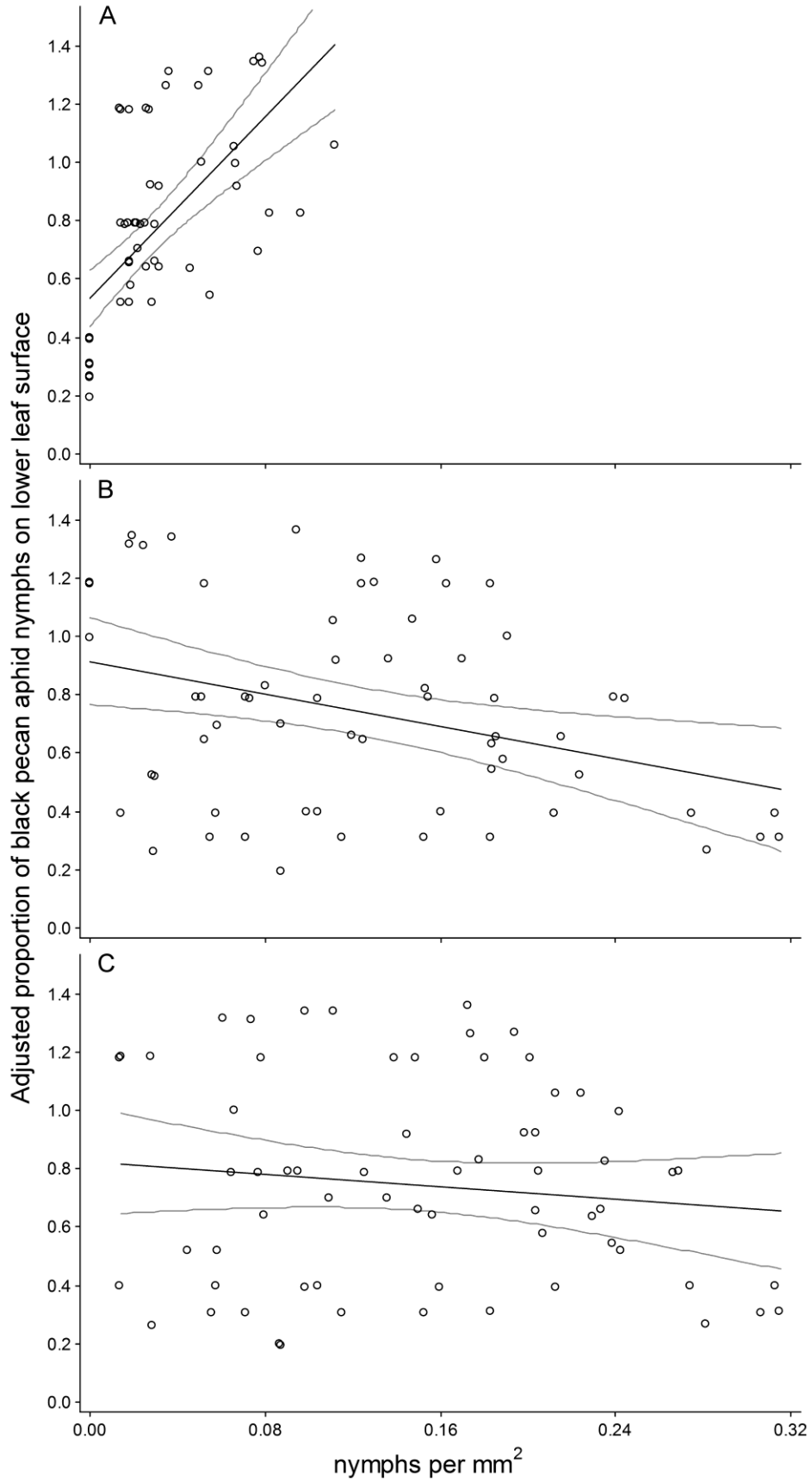
Figure 1.10.

M. caryaefoliae distribution between leaf surfaces vs. density from mixed lab experiments. The proportions of *M. caryaefoliae* nymphs on the lower leaf surface were arcsine transformed to normalize their distribution. The curves represent $\pm$ 95 % C.I. for regression. (A) *M. caryaefoliae* distribution vs. *M. caryaefoliae* density on the lower leaf surface. The formula of the regression line is: $\sin^{-1}\sqrt{proportion} = 0.5322 + (7.7831)density$; $r^2 = 0.38$. (B) *M. caryaefoliae* distribution vs. density of "yellow aphids" (i.e. the pooled nymphs of blackmargined and yellow pecan aphids) on the lower leaf surface. The formula of the regression line is: $\sin^{-1}\sqrt{proportion} = 0.9149 + (-1.3915)density$; $r^2 = 0.11$. (C) *M. caryaefoliae* distribution vs. density of all three aphid species on the lower leaf surface. The formula of the regression line is: $\sin^{-1}\sqrt{proportion} = 0.8251 + (-0.5435)density$; $r^2 = 0.02$.

CHAPTER 4

TOP OR BOTTOM: EFFECTS OF LEAF SURFACE AND PREDATION ON BLACK PECAN

APHID *MELANOCALLIS CARYAEFOLIAE* DISTRIBUTION ON PECAN FOLIAGE

Abstract

The black pecan aphid *Melanocallis caryaefoliae* (Davis) (Hemiptera: Aphididae) is

unique among pecan [*Carya illinoinensis* (Wangenh.) K. Koch] foliage-feeding aphids for its

distribution on individual leaves: adults feed predominately on the lower leaf surface, while

nymphs have no preference for either surface. To test whether the nymphs feed on the upper

surface due to some inherent property of the pecan foliage offering a fitness advantage on the

upper surface, first instar *M. caryaefoliae* were reared to adulthood on the upper and lower

surfaces of leaves on orchard trees, and on excised leaf discs in the laboratory. No differences in

development were observed between the two surfaces in the orchard; in the laboratory, the

aphids on the lower surface developed faster, into larger adults. A few explanations for the

difference of results between the two experiments are considered, but neither result explains why

the nymphs feed on the upper leaf surface. To test the hypothesis that the upper surface

represents an enemy-free space, the foraging behavior of aphidophagous larvae of *Harmonia

axyridis*, *Olla v-nigrum* (both Coleoptera: Coccinellidae), and *Chrysoperla rufilabris*

(Neuroptera: Chrysopidae) on laboratory pecan seedlings was observed, and their impact on

populations of aphids on orchard foliage and on laboratory pecan seedlings was observed.

Evidence was observed of *H. axyridis* and *C. rufilabris* searching the lower surface more often,

while *O. v-nigrum* appears to search both sides equally. This suggests the *M. caryaefoliae* nymphs would often encounter fewer predators on the upper leaf surface.

Introduction

Among the three aphid (Hemiptera: Aphididae) species which feed on pecan [*Carya illinoinensis* (Wangenh.) K. Koch] foliage, the black pecan aphid *Melanocallis caryaefoliae* (Davis) is of particular interest. First, it is uniquely damaging to pecan foliage. Feeding by the other two pecan aphids [the yellow pecan aphid *Monelliopsis pecanis* (Davis) and the blackmargined aphid *Monellia caryella* (Fitch)] depletes leaf chlorophyll and carbohydrates, leading to reduced leaf area, reduced photosynthesis, and defoliation (Wood et al. 1985). Excessive honeydew accumulation further inhibits photosynthesis by serving as a substrate for black sooty mold (Tedders and Smith 1976). All of which result in decreased yield and quality of the pecan crop (Dutcher 1984). Not only can feeding by *M. caryaefoliae* cause all of the above, it also induces premature, senescence-like conditions in the leaf tissue surrounding the feeding site (Cottrell et al. 2009). This damage can cause premature leaf abscission, thus making *M. caryaefoliae* of particular economic importance (Hudson and Dutcher 2004, 2006, 2008).

M. caryaefoliae is also distinguished from *M. pecanis* and *M. caryella* by its distribution on pecan leaves. The latter feed predominantly on the lower (i.e. abaxial) surfaces of leaves, ignoring the upper (i.e. adaxial) surface (Tedders 1978, Chapter 3). This behavior is very common among foliage-feeding tree aphids (Blackman and Eastop 1994, Hopkins and Dixon 1997), as it enables the aphids to take advantage of the lower surface's protection from rain, solar radiation, honeydew dropped by aphids on leaves above (Hopkins and Dixon 1997), and dislodgement by contact with other leaves (Dixon 2005). In contrast, *M. caryaefoliae* distributions appear to vary over time. Davis (1910) and Richards (1960) noted this aphid

settling preferentially on the upper surface of hickory or pecan leaves. Tedders (1978) sampled an orchard on a weekly basis for an entire growing season, noting the earliest first instars settled primarily on the underside of developing pecan leaves, and that in late summer and fall ~74% (presumably of pooled nymphs and adults) were on the lower leaf surface. Kaakeh and Dutcher (1994) noted that, in the laboratory, fourth-instar *M. caryaefoliae* settled on the lower surface of excised pecan leaves from various cultivars. Other than Tedders (1978) none of the authors specified the duration of observations, or specified whether the stated distributions applied to nymphs, adults, or both. The authors (Chapter 3) conducted a nine-week field survey in summer 2010 and observed adult *M. caryaefoliae* preferentially settled on the lower pecan leaf surface, while the nymphs were distributed about equally between both surfaces throughout the survey.

Behavioral and morphological data partially explain these distributions because each species feeds on the phloem cells of leaf veins. *Melanocallis caryaefoliae* feed on the quaternary veins, which are small but available to be fed upon from either leaf surface (Tedders and Thompson 1981, Kaakeh and Dutcher 1994). *Monellia caryella* and *M. pecanis* feed on the primary, secondary, and tertiary veins, and the aphid proboscises are rarely long enough to reach the phloem cells of these larger veins from the upper leaf surface (Tedders and Thompson 1981, Kaakeh and Dutcher 1994). Thus, only *M. caryaefoliae* is able to feed on the upper leaf surface, but this does not explain why the nymphs (and almost exclusively the nymphs) would face the environmental hazards of the upper surface—solar radiation, rain, honeydew, and dislodgement—to do so.

It was theorized that increased aphid density could induce first instar *M. caryaefoliae* to move to the upper leaf surface. While experiments with aphids on pecan seedlings in the laboratory found that movement to the upper surface was correlated with the density of *M.*

caryella and *M. pecanis*, the *M. caryaefoliae* nymphs would still move to the upper surface when they were the only species present, regardless of their density (Chapter 3). Density-induced dispersal cannot be the only cause for these aphids feeding on the upper surface.

Morphological or nutritional differences between the leaf surfaces may be responsible for the distribution of *M. caryaefoliae* nymphs. Tissues within the same plant are known to vary in their nutritional quality for herbivorous insects. Pecan leaves within the same terminal vary with age, and age affects the nutritional quality of the leaf (Kennedy et al. 1950). Even on the same compound leaf, the three pecan-feeding aphids prefer different areas, possibly reflecting differing distributions of nutrients: *M. caryaefoliae* prefer the basal halves of basal leaflets, *M. caryella* prefer the basal halves of middle leaflets, and *M. pecanis* prefer the apical halves of apical leaflets (Tedders 1978). There is a remote possibility of a similar difference between the upper and lower leaf surfaces. A difference in nutritional quality is unlikely, as *M. caryaefoliae* are feeding on the same leaf veins regardless of which surface they feed from, but the two surfaces are morphologically different. For example, stomata only occur on the lower leaf surface. If any morphological differences make feeding from the upper surface significantly easier for *M. caryaefoliae* nymphs, this may offset the risks of mortality associated with that surface.

Alternately, *M. caryaefoliae* may move to the upper leaf surface to escape predators. The tree-feeding aphid *Monaphis antennata* (Kaltenbach) has adopted a predator avoidance strategy of regularly feeding on leaf tops (Hopkins and Dixon 1997), exploiting the fact that generalist aphid predators such as larval Coccinellidae (Coleoptera) and Chrysopidae (Neuroptera) search the upper leaf surfaces less frequently than the lower surfaces (Dixon 1970, Hopkins and Dixon 1997). Coccinellidae and Chrysopidae are also known to prey on pecan aphids, including *M.*

caryaefoliae (Tedders and Angelet 1981, Kunkel and Cottrell 2007), in sufficient quantities that some species have been recommended for use in integrated pest management on pecan (LaRock and Ellington 1996). Mortality from these predators may be high enough to make feeding on the upper leaf surface a viable predator avoidance strategy for *M. caryaefoliae*.

The purpose of this study was to examine two potential causes for the observed *M. caryaefoliae* distribution—leaf nutrition and predation. The effect of pecan leaf surface on aphid fitness was examined by experimentally rearing *M. caryaefoliae* from first instar to adulthood on both the upper and lower leaf surfaces, both on orchard trees and on detached leaf discs in the lab. The predation behavior of larval *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae), *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), and *Olla v-nigrum* (Say) (Coleoptera: Coccinellidae) was examined via direct observation of their movements on laboratory pecan seedlings, and by observation of aphid populations from leaves (both in the laboratory and the orchard) on which the larvae had been feeding.

Materials and Methods

Pecan seedlings. All pecan seedlings used in aphid rearing and laboratory experiments were greenhouse-grown from open-pollinated seeds ('Desirable' cultivar) collected from pecan orchards at the USDA, ARS Southeastern Fruit and Tree Nut Research Lab, Byron, GA. Leaf discs were taken from fully expanded simple leaves using a 1.8-cm-diameter Birkenstrand leaf punch sampler (Rabbit Tool USA, Rock Island, IL) (Cottrell et al. 2009).

Insects. In the field, *M. caryaefoliae*, *M. pecanis*, and *M. caryella* are all holocyclic and monoecious, capable of completing their entire life cycle on a single pecan tree. The fundatrices appear from late March to late April and are followed by more than 20 generations of viviparae

(Tedders 1978). In all three species, all viviparae are alates. Sexuales (apterous oviparae and alate males) appear from mid-October to early December (Tedders 1978).

Separate colonies of *M. caryaefoliae*, *M. pecanis*, and *M. caryella* were maintained from individuals collected from pecan orchards at the USDA-ARS SEFTNRL, Byron, GA with periodic introduction of new aphids from the field. By maintaining the colonies on potted pecan seedlings, at a temperature of 26 ± 1° C and a L14:D10 photoperiod, the life cycle was held in stasis, so the nymphs matured to alate viviparae year-round. Colonies were kept in 60 × 120 × 70 cm wooden frame cages with mesh sides and a glass top, lit by two 40-watt fluorescent plant growth lamps placed above the glass.

Some experiments required a discrete group of first-instar *M. caryaefoliae* or *M. pecanis*; these were obtained by preparing 9 cm petri dishes containing 1% water agar and ten pecan leaf discs per dish (Reilly and Tedders 1990). Twenty adult aphids were placed in each dish and allowed to nymphoposit for 24 h (Cottrell et al. 2009); between 20 and 40 first instars were produced in each dish in that interval.

Harmonia axyridis and *O. v-nigrum* were from established laboratory colonies periodically supplemented with field-collected specimens at the USDA-ARS SEFTNRL, Byron, GA. Two to three larvae were kept per 9 cm Petri dishes; adults were pooled, ~100 per 18 cm × 13.5 cm × 9.5 cm clear plastic boxes for mating, then moved back to petri dishes for egg-laying. All were kept in an environmental chamber (Percival Scientific 36L1X, Perry, IA, USA) at 25 ± 1° C and a L14:D10 photoperiod. They were fed a diet of frozen *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs, supplemented with a meat-based protein diet (Beneficial Insectary, Redding CA, USA) and live aphids from the colonies. Moisture was provided by wetted cotton dental wicks (Cottrell and Shapiro-Ilan 2008).

A colony of *Chrysoperla rufilabris* was established in summer 2010 with eggs purchased from Beneficial Insectary (Redding CA, USA) and supplemented with adults and larvae collected from pecan orchards at the USDA-ARS SEFTNRL, Byron, GA. Two to three larvae and pupae were kept per 9 cm petri dish in environmental chambers (Percival Scientific 36L1X, Perry, IA, USA) at $25 \pm 1°$ C and a L14:D10 photoperiod. They were fed frozen *E. kuehniella* eggs and supplemented with live aphids from the colonies. Moisture was provided by wetted cotton dental wicks. Adults were pooled for both mating and egg collection in open-ended, 17×17 cm (h × d) 1-gallon ice cream cartons (Neptune Paper Products Inc, Newark, NJ, USA) with cheesecloth covering both ends. The sides were lined with paper, for easy removal of eggs. The cylinders were kept at $26 \pm 1°$ C and a L14:D10 photoperiod. They were fed on wheast (Beneficial Insectary, Redding CA, USA) and water was provided by wetted sheets of rolled cotton.

Fitness effects of upper or lower leaf-side preference: laboratory experiment.
Individual pecan leaf discs were placed on 6 cm Petri dishes containing 1% water agar. In two of the four treatments, the discs were placed with the upper leaf surface against the agar and a single first-instar *M. caryaefoliae* was placed on the exposed lower surface (Cottrell et al. 2009); half of these dishes were maintained in an upright position (unnatural leaf orientation) and the other half were inverted (natural leaf orientation). For the remaining two treatments, the lower leaf surface was placed against the agar and the first instar was placed on the exposed upper surface; half of these dishes were maintained in an upright position (natural leaf orientation) and the other half were inverted (unnatural leaf orientation). The dishes were placed in an environmental chamber (Percival Scientific 36L1X, Perry, IA, USA) at $27 \pm 1°$ C and a L14:D10 photoperiod. Aphids were observed daily until they reached adulthood, taking note of mortality,

the instar of the survivors, and the presence of chlorosis (characterized as a visibly-discolored yellow spot around the aphid's feeding site) (Cottrell et al. 2009). Body length of aphids surviving to adulthood was measured under a dissecting microscope (Illharko and van Harten 1987).

There were 30 agar plates per treatment and all four treatments were replicated across three blocks. Analysis of variance was used to determine whether leaf surface, orientation, or the interaction of leaf surface × orientation had a significant effect on nymph survival. Additionally, we used ANOVA to determine if there was a treatment effect regarding the occurrence of visible chlorosis, development time to adulthood, and the body length of resulting adults (Analytical Software 2008). For binomial variables—nymph survival and occurrence of visible chlorosis— the counts were summed within each treatment × block combination before subjecting them to ANOVA. Where interaction between factors was significant, simple effects were examined by Scheffe's F method for simultaneous contrasts (Meyer 1991, Analytical Software 2008).

Fitness effects of leaf-side preference: field experiment. This experiment was conducted using three trials with each trial done in a separate orchard on a different pecan cultivar ('Stuart', 'Desirable' and 'Caddo', respectively) at the USDA, ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA, USA. Trial 1 was conducted using three compound leaves from ten trees whereas trials 2 and 3 used five compound leaves from six trees. Leaves were randomly selected from around the lower periphery of trees. Starting dates for the trials were August 19, September 2 and September 24, 2010. Using clip cages, pairs of first-instar *M. caryaefoliae* were confined to separate, adjacent leaflets on each compound leaf—one on an upper leaf surface and one on a lower surface. Only leaflets with no visible damage were selected. Often, healthy basal leaflets (*M. caryaefoliae*'s preferred feeding sites) were not

available, so median or apical leaflets were used; the paired-leaflet design of this test insured that the effects of leaflet location were consistent between treatments. Nymphs were observed daily until they reached adulthood, taking note of mortality, instar, and the occurrence of visible chlorosis. Measurements of adult body size could not be taken in the field.

As in the lab experiment, ANOVA was used to determine whether the leaf surface had a significant effect on nymph survival, occurrence of visible chlorosis, or development time to adulthood (Analytical Software 2008). For the binomial variables (nymph survival and chlorosis) the counts were summed within each tree before subjecting them to ANOVA.

Predation effects on aphid distribution: laboratory experiment. Three trials were conducted. In each, ten pecan seedlings were trimmed down to three simple leaves, and high vacuum grease (Dow Corning, Midland, MI, USA) was applied around the petioles to prevent aphids or predators from exiting a leaf. First-instar aphids were placed on the leaves, in ratios based on field observations (Chapter 3). In the first trial, 9 *M. caryaefoliae* were placed on both the upper and lower leaf surfaces and 12 *M. pecanis* were placed on the lower leaf surface. For the second and third trials, the same ratio was used but the numbers were increased to 12 *M. caryaefoliae* on both leaf surfaces and 16 *M. pecanis* on the lower surface. Transferred aphids were allowed to settle for 24 h. During this time, second or third instar *C. rufilabris*, *H. axyridis* and *O. v-nigrum* were selected, set aside from their colonies, and provided only water. In trials 1 and 2, these larvae were starved for 2 h prior to the experiment; in trial 3, they were starved for 2 h 40 min. For the experiment, the starved larvae were placed on the leaf petiole, distal to the applied grease, allowed to forage (2 h in the first two trials, 3 h in the third trial), and then removed. After predator removal, the number of aphids remaining on each leaf surface was recorded. The number of aphids missing from the seedling (whether due to predation or simply

falling off) was calculated and the chi-square test was used to determine whether the distribution of these disappearances differed between the predator treatments and the control.

Predation effects on aphid distribution: field experiment. Four trials were conducted, on August 30, September 2, 10, and 17, 2010. In each, 'Schley' cultivar pecan trees were selected from orchards at the USDA, ARS, SEFTNRL, Byron, GA, USA. Trials 1–3 were conducted in the same orchard and used two different trees per trial whereas trial 4 was conducted in a different orchard and used three trees. In trials 1–3, nine terminals (three replicates of three terminals each) were selected from each tree, and in trial 4, six terminals (two replicates of three terminals each) were selected from each, so within each trial there were 6 replicates (n = 18 terminals). Each terminal was trimmed down to two compound leaves. Any leaves that could brush against these leaves were trimmed, and high vacuum grease was placed around the base of each terminal, to prevent any insects from crawling off the terminal, but movement between the two compound leaves was possible. Each terminal within a replicate received one of three treatments: third-instar *H. axyridis* or *O. v-nigrum* (starved as in the laboratory experiments) were placed on petioles within the terminals, while the control terminals received no larvae. In trial 1, two larvae were placed per terminal and allowed to forage for 4 h before removal. Due to concerns that 4 h was not enough time to give significant results, foraging time was lengthened to 18 h in trial 2. However, this caused concern that the larvae would exhaust the aphid populations on the terminals, so for trials 3 and 4, only one larva was placed per terminal and allowed to forage for 18 h before removal.

After the larvae were removed, numbers of nymphs and adults of *M. caryaefoliae*, *M. caryella*, and *M. pecanis* on both leaf surfaces were recorded. Accurate counts of aphids prior to the test could not be taken without affecting their subsequent retention on leaves, especially

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adults, so the effects of the treatments were determined by using the chi-square test to compare distribution (upper vs. lower leaf surface) between treatments. This was done for the numbers of *M. caryaefoliae* nymphs individually, and for the pooled numbers of all aphids and life stages. Where a significant change was detected, the proportions were separated using a modified form of Zar's (1999) multiple comparisons for proportions (Analytical Software 2008).

Foraging behavior of larval Chrysopidae and Coccinellidae on pecan seedlings. Pecan seedlings were trimmed down to three or four simple leaves, so that upper and lower leaf surfaces of remaining leaves were visually unobstructed. High vacuum grease was applied around the base of each leaf petiole to prevent specimens from exiting the leaf. One leaf was used at a time in each trial. For recording, a plant was placed under an overhead closed-circuit camera, propping up the pot at an angle as necessary so the leaf of interest was angled between 20 and 45 degrees from the horizontal. A mirror placed parallel to the leaf was used to simultaneously make the lower leaf surface visible to the camera.

Trials were either conducted with or without aphids on the leaves. When aphids were used, 20 first instar *M. caryaefoliae* and 20 first instar *M. pecanis* were placed near the leaf midrib, half on the upper surface and half on the lower surface; this was done 24 h beforehand, to give the aphids time to disperse and settle.

Separate trials were performed with second and third instars of *C. rufilabris* (7 trials with aphids, 9 without aphids), *H. axyridis* (8 trials with aphids, 8 without aphids) and *Olla v-nigrum* (9 trials with aphids, 9 without aphids). All predator larvae were starved for 20 to 24 h before the observations were made.

For each observation, one predator larva was deposited on a leaf petiole and observed for 15 minutes. For this duration, The Observer® XT 9.0 software package (Noldus Information

Technology, Wageningen, The Netherlands) was used to record larval behavior for later review. Either during the initial observation or during later review, the software was also used to document the duration of a larva's location (upper leaf surface, lower leaf surface, or petiole), certain behaviors (searching, food handling, or resting) and the total combined duration of each location × behavior combination. ANOVA was used to determine whether there was a significant difference in durations spent on different plant locations or performing different behaviors, and whether the presence of aphids affected these factors (i.e., ANOVA tested the effect of location, behavior, location × behavior, location × aphid, behavior × aphids, and location × behavior × aphids). Tukey's HSD was used to separate the means (Analytical Software 2008).

Results

Fitness effects of leaf-side preference: laboratory experiment. There were significant effects on the time required for *M. caryaefoliae* to mature from the first instar to adult for both leaf surface ($F_{1,253}$ = 18.23, P < 0.0001) and leaf orientation ($F_{1,253}$ = 5.95, P = 0.0154) in addition to a significant interaction of surface × orientation ($F_{1,253}$ = 8.54, P = 0.0038). *Melanocallis caryaefoliae* nymphs reared on the lower leaf surface with the leaf disc inverted (i.e., natural leaf orientation) or upright required a mean (± SE) of 7.97 ± 0.06 or 8.01 ± 0.09 d to mature to adulthood, respectively. Nymphs reared on the upper surface with the disc inverted (unnatural leaf oriention) or upright required 8.60 ± 0.09 or 8.13 ± 0.10 d to mature to adulthood, respectively. In spite of the significant leaf surface × orientation interaction, there was a trend of aphids maturing more quickly on the lower surface, regardless of leaf orientation, and this difference was statistically significant on inverted leaf discs (i.e., natural leaf orientation). Leaf orientation did not have a consistent effect because on the lower surface, aphids on inverted leaf

discs matured more quickly, while on the upper surface, aphids on inverted discs tended to mature more slowly (though not significantly so).

Adult body length was significantly affected by leaf surface ($F_{1,250} = 13.96$, $P = 0.0002$) but not by orientation ($F_{1,250} = 2.19$, $P = 0.1404$). In addition, there was a significant interaction of surface × orientation ($F_{1,250} = 8.45$, $P = 0.0040$). *M. caryaefoliae* reared on the lower leaf surface with dishes inverted (i.e., natural orientiation) or upright measured 1.63 ± 0.02 (mean ± SEM) or 1.60 ± 0.01 mm, respectively. Those reared on the upper surface with dishes inverted or upright (i.e., natural position) measured 1.49 ± 0.03 or 1.58 ± 0.02 mm, respectively. In spite of the significant leaf surface × orientation interaction, there was a trend that aphids on the lower surface grew to be larger than aphids on the upper surface, regardless of orientation, and this difference was statistically significant on the inverted leaf discs. There was no significant difference between orientations for aphids on the lower leaf surface, but for aphids on the upper leaf surface, upright dishes (i.e., natural orientation) resulted in longer adults.

The proportion of aphids which survived to adulthood was significantly affected by leaf surface ($F_{1,6} = 15.12$, $P = 0.0046$) but not by leaf orientation ($F_{1,6} = 4.46$, $P = 0.0678$), nor by their interaction ($F_{1,6} = 0.11$, $P = 0.7475$). Whether a leaf disc was upright or inverted had no significant effect on survival with regard to either surface (Fig. 2.1A). However, survival on the lower leaf surface of inverted discs was significantly greater than on the upper surface of upright discs (Figure 2.1A).

Leaf surface had a significant effect on visible chlorosis ($F_{1,6} = 9.68$, $P = 0.0144$), but not leaf orientation ($F_{1,6} = 0.04$, $P = 0.8409$) or their interaction ($F_{1,6} = 0.69$, $P = 0.4308$). In spite of the significant ANOVA results for leaf surface, the conservative mean separation procedure did not separate these means (Figure 2.1B).

Fitness effects of leaf-side preference: field experiment. The time to complete trials 1, 2 and 3 was 12, 11, and 21 d, respectively. During each trial, the mean (± SE) daily high and low temperature was 32.4 ± 0.5 and 22.3 ± 0.4, 33.2 ± 0.5 and 19.2 ± 0.8, and 27.1 ± 0.8 and 13.1 ± 0.8 °C, respectively. Total precipitation during trials 1, 2, and 3 was 7.5, 0.4 and 13.4 cm, respectively.

Survival to adulthood was not significantly affected by leaf surface in trial 1 ($F_{1,18} = 0.00$, $P = 1.0000$), trial 2 ($F_{1,10} = 0.36$, $P = 0.5634$), or trial 3 ($F_{1,10} = 1.79$, $P = 0.2108$). Survival in the first 24 h was not significantly affected by leaf surface in trial 1 ($F_{1,18} = 1.27$, $P = 0.2743$), trial 2 ($F_{1,10} = 0.06$, $P = 0.8174$), or trial 3 ($F_{1,10} = 0.03$, $P = 0.8636$). For nymphs confined to the lower leaf surface, the mean proportion (± SEM) to survive in trials 1, 2, and 3 was 0.27 ± 0.08, 0.44 ± 0.10, and 0.22 ± 0.08, respectively. The proportion of nymphs that died within 24 h of being placed on the lower surface in trials 1, 2, and 3 was 0.57 ± 0.09, 0.34 ± 0.09, and 0.28 ± 0.08, respectively; the first-day mortality represented 77 ± 9, 59 ± 12, and 35 ± 10%, respectively, of overall nymph mortality. For nymphs confined to the upper leaf surface, the mean proportion to survive in trials 1, 2, and 3 was 0.27 ± 0.08, 0.36 ± 0.09, and 0.42 ± 0.09, respectively. The proportion of nymphs that died within 24 h of being placed on the upper surface in trials 1, 2, and 3 was 0.40 ± 0.09, 0.40 ± 0.09, and 0.35 ± 0.09, respectively; the first-day mortality represented 55 ± 11, 60 ± 11, and 61 ± 12%, respectively, of overall nymph mortality. Overall survival did not vary significantly between trials ($F_{2,38} = 3.76$, $P = 0.0323$), but survival in the first 24 h did ($F_{2,38} = 10.30$, $P = 0.0003$); significantly more nymphs died in the first 24 h in trial 1 than in trials 2 or 3.

Occurrence of visible chlorosis was not affected by leaf surface in trial 1 ($F_{1,18} = 0.87$, $P = 0.3630$), trial 2 ($F_{1,10} = 0.43$, $P = 0.5245$), or trial 3 ($F_{1,10} = 0.04$, $P = 0.8501$). On leaves with

nymphs confined to the lower surface, the mean proportion (± SEM) to display visible chlorosis in trials 1, 2, and 3 was 0.30 ± 0.09, 0.45 ± 0.10, and 0.48 ± 0.09, respectively. On leaves with nymphs confined to the upper surface, the mean proportion to display visible chlorosis in trials 1, 2, and 3 was 0.20 ± 0.07, 0.37 ± 0.09, and 0.42 ± 0.09, respectively.

Predation effects on aphid distribution: laboratory experiment. In trial 1, there was no significant correlation between treatment and distribution of missing *M. caryaefoliae* nymphs ($\chi^2_2 = 2.28$, $P = 0.3191$), nor one with total missing aphids ($\chi^2_2 = 2.57$, $P = 0.2771$) (Figure 2.2A). In the trial 2, there was a significant correlation between treatment and distribution of missing *M. caryaefoliae* nymphs ($\chi^2_2 = 8.99$, $P = 0.0112$): the distribution of missing aphids differed significantly between the *C. rufilabris* and *O v-nigrum* treatments, but neither treatment differed from the control (Figure 2.2B). There was no significant correlation with pooled percentages ($\chi^2_2 = 2.94$, $P = 0.2300$). In trial 3, there was no significant correlation between treatment and missing *M. caryaefoliae* nymphs ($\chi^2_2 = 4.44$, $P = 0.1088$), nor missing pooled aphids and treatment ($\chi^2_2 = 5.09$, $P = 0.0783$) (Figure 2.2C).

Predation effects on aphid distribution: field experiment. In trial 1 (August 30) there was a significant correlation between *M. caryaefoliae* nymph distribution and treatment ($\chi^2_2 = 25.19$, $P < 0.0001$). In both the *H. axyridis* and *O. v-nigrum* treatments, there was a smaller proportion of *M. caryaefoliae* nymphs on the lower leaf surface than for the control (Figure 2.3A). In trial 2 (September 2), there was a significant correlation between *M. caryaefoliae* nymph distribution and treatment ($\chi^2_2 = 35.27$, $P < 0.0001$). In the *O. v-nigrum* treatment, the proportion of *M. caryaefoliae* nymphs on the lower surface was significantly smaller than the control or *H. axyridis* proportions. The *H. axyridis* treatment was not significantly different from the control (Figure 2.3B). In trial 3 (September 10), there was a significant correlation between

M. caryaefoliae nymph distribution and treatment ($\chi^2_2 = 22.36$, $P < 0.0001$). In the *O. v-nigrum* treatment, the proportion of *M. caryaefoliae* nymphs on the lower surface is significantly larger than the control or *H. axyridis* proportion. The *H. axyridis* treatment had a smaller proportion of *M. caryaefoliae* nymphs on the lower leaf surface, but was not significantly different from the control (Figure 2.3C). In trial 4 (September 17), there was a significant correlation between *M. caryaefoliae* nymph distribution and treatment ($\chi^2_2 = 12.44$, $P = 0.0020$). In the *O. v-nigrum* treatment, the proportion of *M. caryaefoliae* nymphs on the lower surface is significantly larger than the control proportion whereas *H. axyridis* did not have a significant effect (Figure 2.3D).

Foraging behavior of chrysopid and coccinellid larvae on pecan seedlings.

Chrysoperla rufilabris larvae spent significantly more total time on the lower leaf surface than on either the upper leaf surface or off the leaf (Table 1, Figure 2.4A). In the absence of aphids, *H. axyridis* larvae spent significantly more total time on the lower leaf surface than anywhere else; when aphids were present, there was not a significant difference in time between the two leaf surfaces (Table 1, Figure 2.4B).

For *O. v-nigrum* larvae, there was no significant difference in time spent on either leaf surface, regardless of whether or not aphids were present (Table 1, Figure 4C). When aphids were present, the larvae spent significantly more time consuming honeydew than they did consuming aphids on either leaf surface (Figure 2.4C).

Larvae of all three predator species showed significant behavior × aphid presence/absence interactions (Table 1). For obvious reasons, larvae did not feed on aphids or honeydew when aphids were absent. While coccinellids, including *H. axyridis*, will augment their diets with plant foliage (Moser et al. 2008, Lundgren 2009), no plant feeding by any larvae was noted.

When focusing exclusively on the time when predators were either searching for aphids or feeding on them, *C. rufilabris* spent significantly more time on the lower leaf surface than anywhere else when aphids were present ($F_{2,42} = 8.64$, $P = 0.0007$), but the difference was not significant when aphids were absent ($F_{2,42} = 0.83$, $P = 0.4422$). Similarly, *H. axyridis* spent significantly more time searching or feeding on the lower leaf surface than the upper surface when aphids were present ($F_{2,42} = 10.31$, $P = 0.0002$), but not when aphids were absent ($F_{2,42} = 2.96$, $P = 0.0628$). *Olla v-nigrum* did not spend significantly more time searching or feeding on aphids on the lower surface, either with the aphids present ($F_{2,42} = 3.01$, $P = 0.0586$) or absent ($F_{2,42} = 0.57$, $P = 0.5703$) (Figure 2.5).

Discussion

According to Berdegue et al. (1996), three facts must be established in order to conclude that a particular niche is an enemy-free space. First, one needs to demonstrate that natural enemies have a negative effect on prey fitness in the original habitat. Second, to rule out the possibility that the alternative is an inherently better habitat, one needs to demonstrate that the prey have greater fitness in the original habitat when natural enemies are absent. Third, one needs to demonstrate that the prey have greater fitness in the alternative habitat when natural enemies are present.

Generalist aphid predators, most significantly larval lady beetles and lacewings, have been observed on pecan foliage and feeding on *M. caryaefoliae* (Tedders and Angelet 1981, LaRock and Ellington 1996, Kunkel and Cottrell 2007), so Berdegue et al.'s (1996) first criterion is met.

We tested Berdegue et al.'s (1996) second criterion by comparing the fitness of *M. caryaefoliae* nymphs reared on each leaf surface. In the laboratory experiment, nymphs reared on

the lower surface of detached pecan leaf discs matured faster, into larger adults, than did nymphs reared on the upper surface. However, no significant difference was detected between aphids reared on the two surfaces of leaflets still attached to trees in the orchard. A number of possible explanations exist for this discrepancy. It is possible that the fitness effect observed in the lab was an artifact of the study design. Pecan leaves lack stomata on the upper leaf surface (Lombardi et al. 2009), so for the treatments with that surface exposed, the stomata on the lower surface were against the agar. This would likely inhibit leaf respiration or potentially affect the ability of *M. caryaefoliae* to induce senescence-like chlorosis important to normal development.

However, if the fitness effect observed in the lab is genuine, then several factors could account for the effect not being observed in the orchard. Fewer aphids were used in the field experiment, and the numbers surviving to adulthood were much lower, perhaps too low to detect a difference in aphid fitness. Alternately, it is possible that the extra mortality factor(s) in the orchard counteract the fitness benefit offered by the lower leaf surface. Predation and dislodgement can be ruled out as causes of mortality, because the clip cages used to secure the nymphs to the leaves should have protected them. All pecan cultivars are susceptible to *M. caryaefoliae* but two of the cultivars used in the field experiments ('Stuart' in trial 1 and 'Caddo' in trial 2) are less susceptible to *M. caryaefoliae* than 'Desirable', the cultivar used in the lab experiment (Wood and Reilly 1998). However, trial 2 of the field experiment also used 'Desirable' and still yielded mortality comparable to the other field trials. Mortality within the first 24 h after aphid transfer is generally indicative of nymphs failing to establish on a leaf. Thus, significantly higher mortality in trial 1 ('Stuart') than trial 2 ('Desirable'), suggests that cultivar may have affected survival. However, overall mortality did not differ significantly between any of the trials, so other factors must be at work as well. Because the field trials were

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conducted in late August, September, and October, much foliage on the selected trees already had damage from aphid feeding. Only leaflets with no visible damage were selected, but it is possible that a number of leaves had damage from *M. caryella* or *M. pecanis*, which was not readily apparent yet significantly reduced the nutritional value of the leaves (Tedders 1978, Tedders and Thompson 1981). In addition, some nymphs had to be placed on median or apical leaflets; this may have increased mortality as basal leaflets are their preferred feeding site (Tedders 1978, Kaakeh and Dutcher 1992). At temperatures below 30 °C, *M. caryaefoliae* development rates decrease with temperature, and mortality increases as temperatures fall below 20 °C (Tedders et al. 1992, Kaakeh and Dutcher 1993). It is possible the low night temperatures, particularly in the final trial, led to increased mortality.

While the evidence for the lower leaf surface providing an inherent fitness benefit to developing *M. caryaefoliae* is ambiguous, it is unambiguous that the upper leaf surface is, at best, equally advantageous to the nymphs as a feeding site. As there is no fitness benefit to counteract the environmental hazards of the upper surface, we can conclude that Berdegue et al.'s (1996) second criterion is met: in the absence of natural enemies, *M. caryaefoliae* fitness is decreased on the upper leaf surface.

To test Berdegue et al.'s (1996) third criterion, we examined the distribution of aphids between the upper and lower leaf surfaces following predator foraging, to determine whether leaf surface made a difference in the likelihood of an *M. caryaefoliae* nymph being dislodged or consumed. In the field experiments, all trials saw foraging by larval *O. v-nigrum* result in *M. caryaefoliae* nymph distribution shifting significantly from the control distribution—but the direction of the shift varied, suggesting in two trials that *O. v-nigrum* were foraging preferentially on the lower surface, and in the other two trials, that they were foraging on the

upper surface. Foraging by larval *H. axyridis* resulted in *M. caryaefoliae* nymph distribution shifting (relative to the control) significantly toward the upper leaf surface, but only in one trial. In the laboratory trials, however, there were no significant differences in number of missing aphids between the predator treatments and the control.

We also observed the searching behavior of predaceous larvae, to determine that they searched the lower leaf surface more commonly than the upper surface. *Chrysoperla rufilabris* was observed to spend more total time on the lower leaf surface, both with aphids present and absent, and to search more on the lower surface when aphids were absent. *Harmonia axyridis* was observed to spend more time on the lower surface, searching and total, when aphids were absent. *Olla v-nigrum* did not spend a significantly different length of time on either leaf surface.

In short, we observed instances where predators consumed more aphids on the lower leaf surface, but these were not consistent enough for us to state with confidence that this regularly occurs in the natural system. Berdegue et al.'s (1996) third criterion was sometimes met. This fits with Sheirs and De Bruyn's (2002) observation that predator-prey interactions—and thus the existence of enemy-free space—are not static. A given niche can serve as an enemy-free space in one season, and not be enemy-free space the next season (Sheirs and De Bruyn 2002). This may explain the variations within this study, and the discrepancy between our own observations from the prior summer (Chapter 3) and the distributions of control treatments of the field trials in this study. Dynamic enemy-free spaces could also explain variation within the literature, with some authors noting a preference for the upper surface (Davis 1910, Tedders 1978), some a preference for the lower surface (Richards 1960), and some noting no preference (Walker 1932).

It is also worth noting that, comparing the two coccinellids, it was the exotic *H. axyridis* that was observed searching the lower leaf surface more often, while the native *O. v-nigrum*

spent equal time on both leaf surfaces. As *M. caryaefoliae* is itself a native species, the searching behavior of its native predator could represent co-evolution: the aphids moved to the upper leaf surface, evaded predation, and flourished, so *O. v-nigrum* began foraging on the upper surface to exploit the aphid population there. Mizell (2007) has noted *H. axyridis* displacing other aphidophagous arthropods on some pecan habitats. Perhaps exploiting upper-surface *M. caryaefoliae* populations, which *H. axyridis* has left alone, helps *O. v-nigrum* avoid being completely displaced by the invasive competitor. However, the native *C. rufilabris* also concentrates on the lower leaf surface, indicating that factors besides the native/exotic dichotomy are involved in determining where the predators search.

The predaceous larvae's feeding on honeydew was also interesting. Coccinellidae in general are known to supplement their diet with honeydew. Honeydew is not an adequate dietary substitute for aphids (Wäckers et al. 2008), but can prolong the coccinellid larvae's life when high-quality prey are absent (Lundgren 2009). The length of time that *O. v-nigrum* was observed feeding on honeydew—more time than they spent feeding on aphids on either surface—was an unexpected observation. If these preferences are also shown by larvae in the field, and are not merely an artifact of the laboratory test, then the presence of honeydew increases the upper leaf surface's value as an enemy-free space by distracting some predators that do search there. Further, since *M. pecanis* and *M. caryella* nymphs excrete honeydew in greater quantities, and with a higher concentration of sugars, than *M. caryaefoliae* nymphs do (Wood & Tedders 1986; Wood et al., 1987; Petersen & Sandström, 2001), it is possible that *M. caryaefoliae* nymphs benefit indirectly from the proximity of the other two pecan aphids.

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Tables

Table 2.1.

F-statistics and degrees of freedom from ANOVA from predator behavior observations. One asterisk indicates significance at $0.01 < P < 0.05$; **, significance at $0.001 < P < 0.01$; ***, significance at $P < 0.001$.

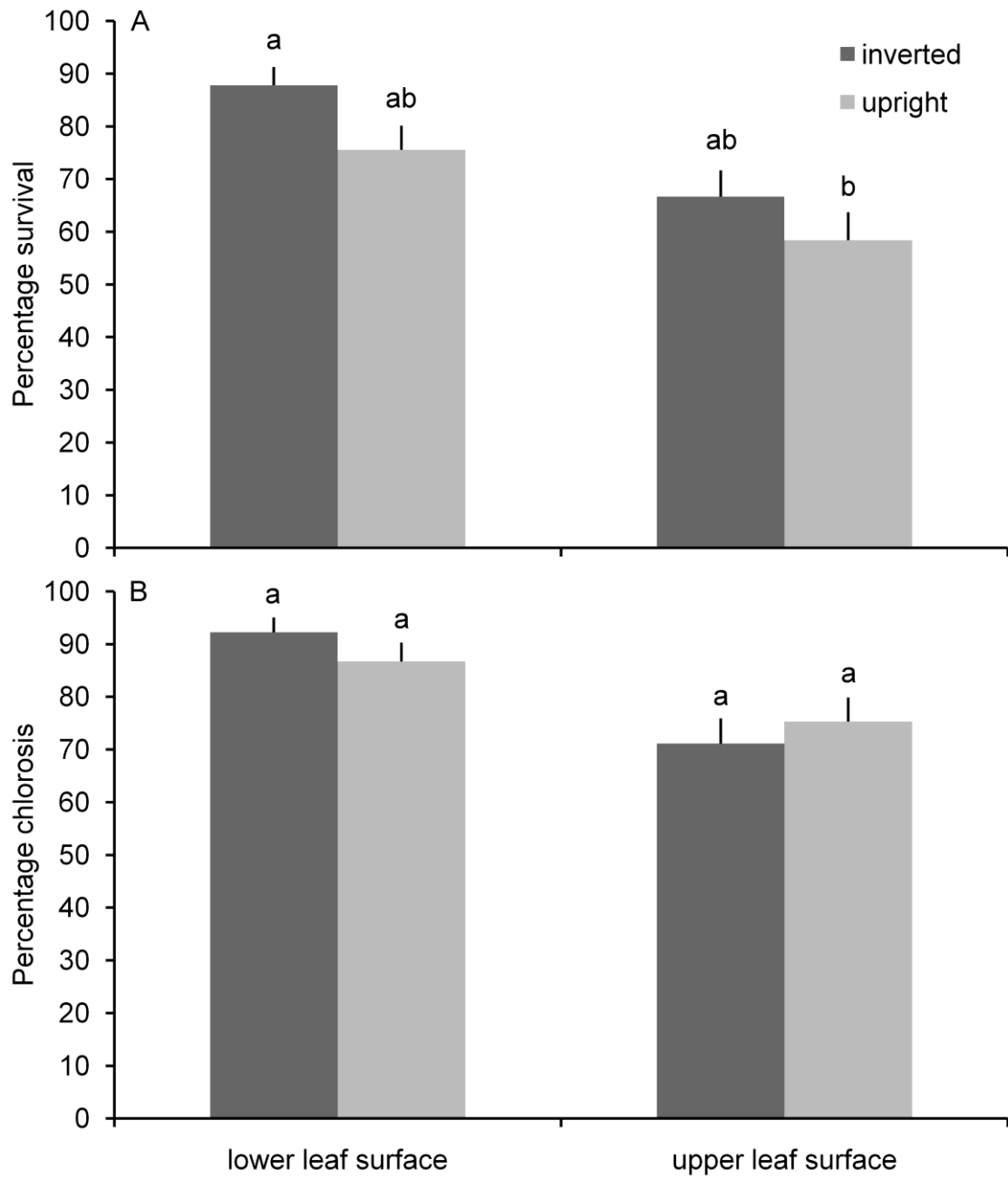|	Numerator d.f.	C. rufilabris	H. axyridis	O. v-nigrum
Behavior	3	4.98**	2.42	7.42***
Location	2	15.88***	9.09***	4.99**
Loc × aphids	3	0.24	0.61	2.47
Behav × aphids	3	3.58*	4.53**	4.56**
Behav × loc	6	3.47**	2.21*	4.51**
Behav × loc × aphids	6	1.92	2.44*	1.26
Denominator d.f.		168	168	192

Figures

Figure 2.1.

Effects of leaf surface and orientation on *M. caryaefoliae* survival and occurrence of chlorosis.

First instar *M. caryaefolieae* were reared on pecan leaf discs on 1% water agar plates under one

of four treatments: lower leaf surface exposed, leaf disc inverted; lower surface exposed, disc

upright; upper leaf surface exposed, disc inverted; upper leaf surface exposed, disc upright. The

experiment consisted of three blocks, each with 30 nymphs per treatment. Same letters indicate

means which did not differ significantly (at $P < 0.05$). (A) Comparison of survival to adulthood

(mean ± SE) between treatments. (B) Comparison of the occurrence of visible chlorotic lesions
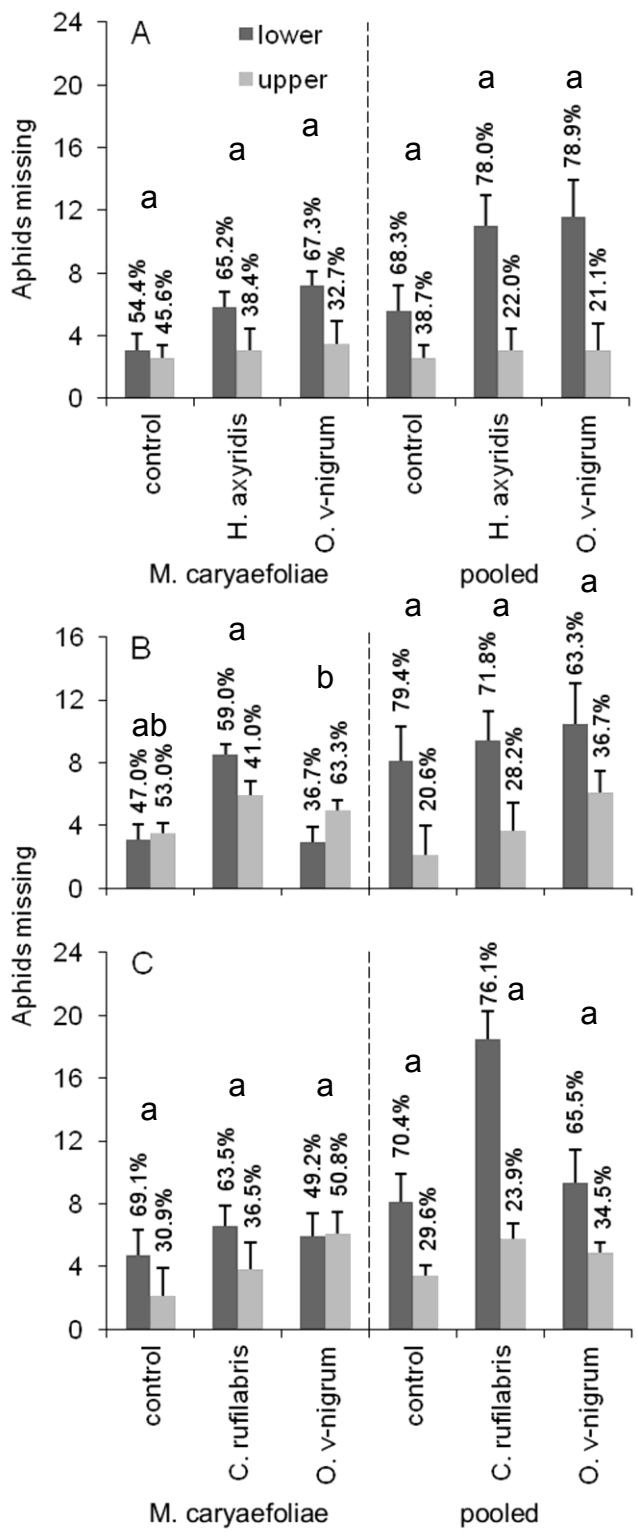
(mean ± SE) on the leaves between treatments.

Figure 2.2.

Effects of predator foraging on *M. caryaefoliae* and *M. pecanis* populations on laboratory pecan seedlings. Mean (± SE) number of aphids missing from laboratory pecan seedlings after allowing one predaceous larva (starved for ~2 h beforehand) to forage. Numbers above the columns indicate that leaf surface's percentage of the total missing aphids for that treatment. Same letters above paired columns indicate the distribution of missing aphids did not differ significantly (at $P < 0.05$) between the treatments. (A) 18 September, 2010 trial. Larvae were allowed to forage for 2 h. Initial populations: 9 *M. caryaefoliae* nymphs on the upper and lower surface each, and 12 *M. pecanis* nymphs on the lower surface. (B) 29 September, 2010 trial. Larvae were allowed to forage for 2 h. Initial populations: 12 *M. caryaefoliae* nymphs on the upper and lower surface each, and 16 *M. pecanis* nymphs on the lower surface. (C) 2 October, 2010 trial. Predaceous larvae were allowed to forage for 3 h. Initial populations: 12 *M. caryaefoliae* nymphs on the upper and lower surface each, and 16 *M. pecanis* nymphs on the lower surface.

A
66.8%
71.7%
76.0%
79.7%
71.8%
76.3%

B
59.0%
64.0%
56.4%
72.2%
66.5%
50.3%

C
51.1%
54.5%
67.0%
72.5%
70.2%
77.5%
upper
lower

D
87.5%
91.3%
93.5%
92.8%
93.0%
94.2%

mean (+ S.E.) aphids per terminal

control   Harmonia   Olla   control   Harmonia   Olla
           axyridis   v-nigrum   axyridis   v-nigrum

M. caryefoliae nymphs   all aphids pooled

control   Harmonia   Olla   control   Harmonia   Olla
           axyridis   v-nigrum   axyridis   v-nigrum

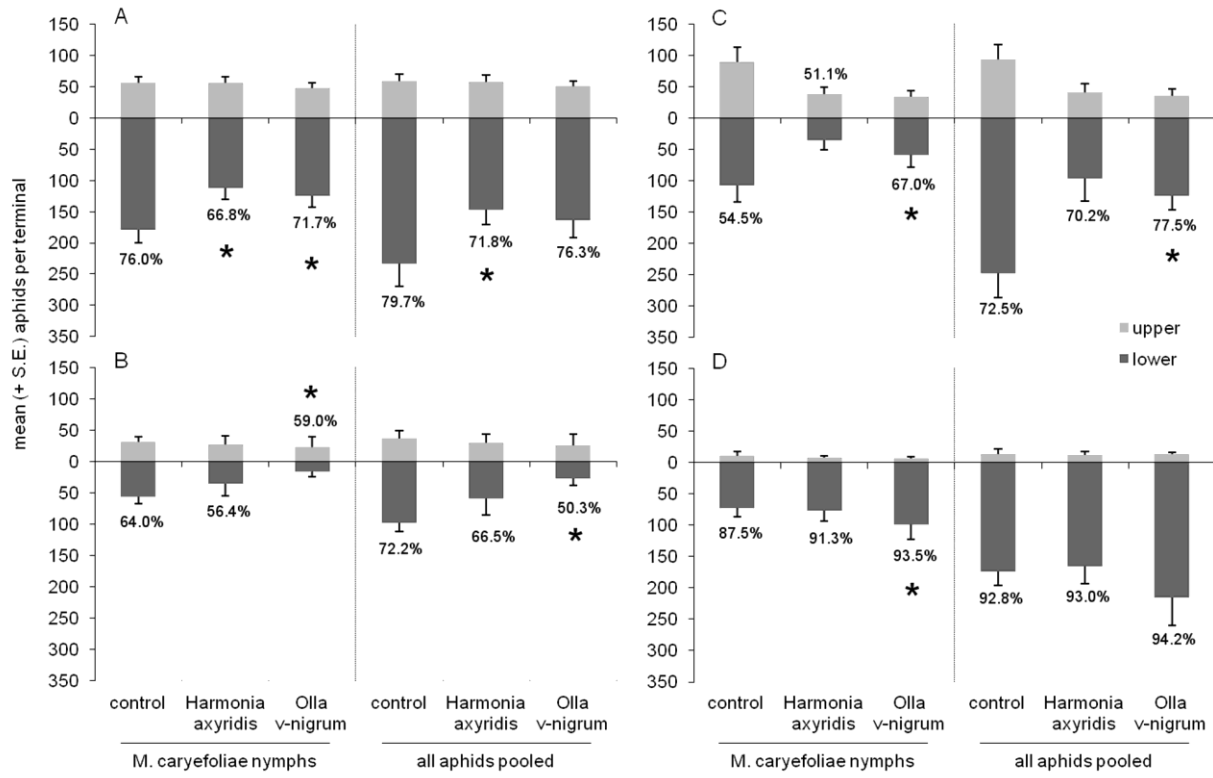M. caryefoliae nymphs   all aphids pooled

Figure 2.3.

Effects of predator foraging on pecan aphid populations on orchard pecan leaves. Coccinellid larvae were introduced to terminals (two leaves) on 'Schley' cultivar foliage. Counts of aphids were taken after allowing the larvae to forage. Within each treatment, the leaf surface that had the greater number of aphids is indicated, along with its percentage of the total aphids. An asterisk indicates that the distribution differed significantly ($P < 0.05$) from the control. "All aphids pooled" includes adults and nymphs of *M. caryaefoliae*, *M. caryella*, and *M. pecanis*. (A) Trial 1 (August 30) with two larvae per terminal, foraging on foliage in the Stuart Weevil Block for 4 h. (B) Trial 2 (September 2) with two larvae per terminal, foraging on foliage in the Stuart Weevil block for 18 h. (C) Trial 2 (September 10) with one larva per terminal, foraging on foliage in the Stuart Weevil block for 18 h. (D) Trial 3 (September 17) with one larva per terminal foraging on foliage in the Biocontrol Block for 18 h.
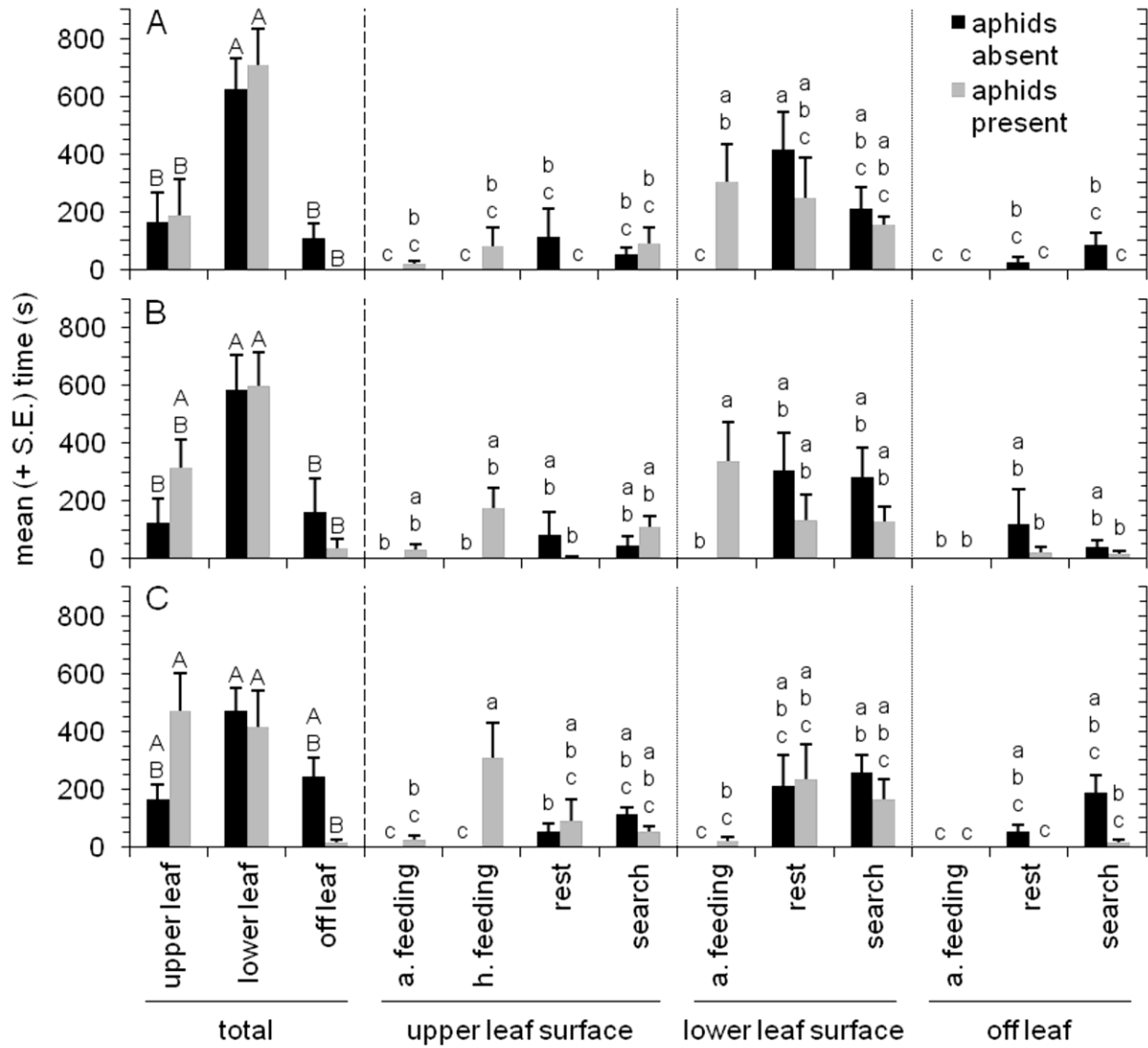
Figure 2.4.

Comparison of time spent by chrysopid or coccinellid larvae searching, resting, and feeding on a pecan seedling during lab observations. Individual larvae were starved for 20 to 24 h and then allowed to forage on an individual leaf for 15 min. For the trials with aphids present, 20 first instar *M. caryaefoliae* and 20 first instar *M. pecanis* were placed on the leaves 24 h beforehand. Same letters above columns indicate means which are not significantly different ($P < 0.05$); mean separation of total time on each leaf location was performed separately from mean separation of behavior by leaf location. "A. feeding" indicates feeding on aphids; "h. feeding" indicates feeding on honeydew. (A) *Chrysoperla rufilabris*; for trials with aphids n = 7; for trials without aphids n = 9. (B) *Harmonia axyridis*; for trials with aphids n = 8; for trials without aphids n = 8. (C) *Olla v-nigrum*; for trials with aphids n = 9; for trials without aphids n = 9.
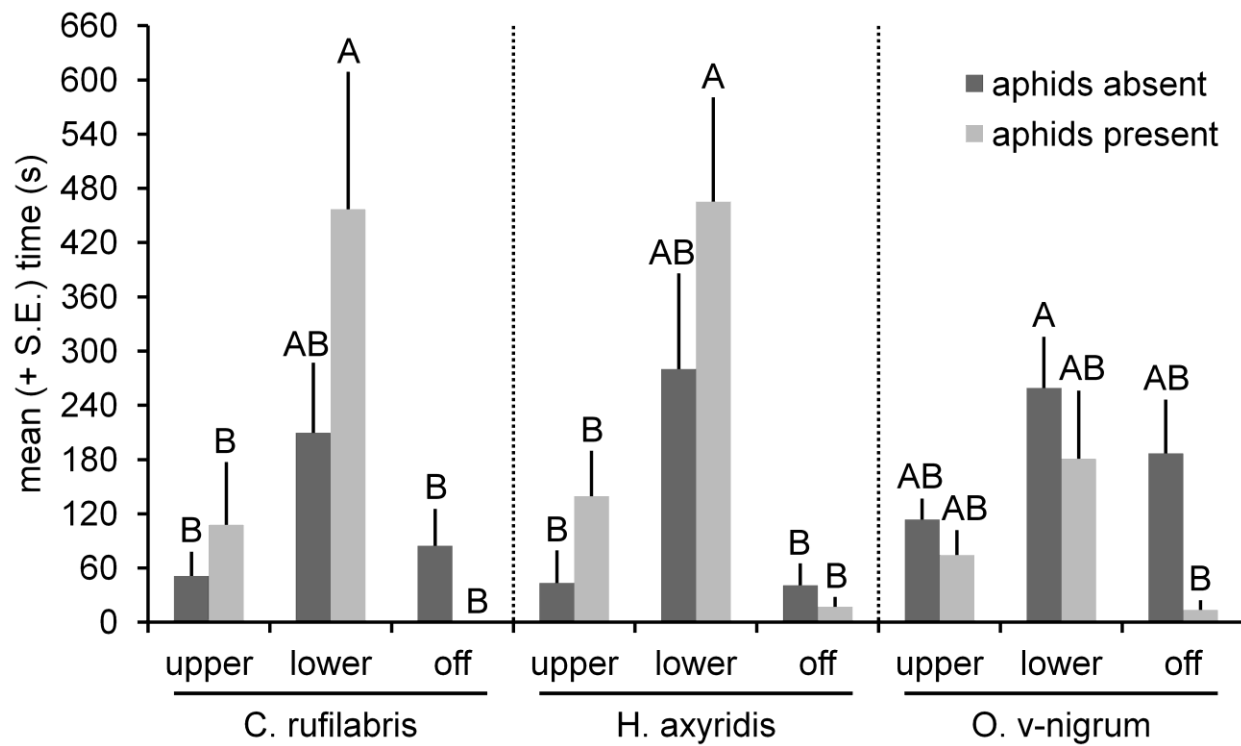
Figure 2.5.

Time spent by predaceous larvae on plant locations (upper leaf surface, lower leaf surface, and off the leaf) while either searching for or consuming prey. Individual larvae were starved for 20 to 24 h and then allowed to forage on an individual leaf for 15 min. For the trials with aphids present, 20 first instar *M. caryaefoliae* and 20 first instar *M. pecanis* were placed on the leaves 24 h beforehand. Same letters above columns indicate means which did not differ significantly (at $P < 0.05$). For *Chrysoperla rufilabris* n = 7 trials with aphids and n = 9 trials without aphids. For *Harmonia axyridis*, n = 8 trials with aphids and n = 8 trials without aphids. For *Olla v-nigrum* n = 9 trials with aphids and n = 9 trials without aphids.

CHAPTER 5

DISCUSSION

The distribution of *M. caryaefoliae* nymphs on pecan foliage observed in 2009 was unique among the pecan-feeding aphids. Both in the orchard and in the laboratory, they were observed about equally distributed between the upper and lower surfaces of leaves, while *M. caryella* and *M. pecanis* nymphs and adults were predominantly observed on the lower surface. Surprisingly, the adults of the black pecan aphid were also predominantly observed on the lower leaf surfaces. This most closely matched Walker's (1932) description that *M. caryaefoliae* had no leaf surface preference, provided the leaves were shaded; however, we are not aware of references in the prior literature to *M. caryaefoliae* adults and nymphs having independent distributions. Another tree foliage aphid, *Monaphis antennata* (Kaltenbach), has a similar distribution, with nymphs feeding almost exclusively on the upper leaf surfaces and the adults moving to the lower surface (Hopkins and Dixon 1997). However, it is interesting to note that, during the 2010 experiment testing predator larvae foraging on orchard pecan trees, in the control treatments, more *M. caryaefoliae* nymphs were observed on the lower leaf surface than on the upper surface.

These observations have several implications. First, the fact that adult *M. caryaefoliae* are found on the lower surface while nymphs are found both on the lower and upper surfaces suggests that the nymphs are actively dispersing before finding a feeding site, and not simply feeding where they are born, and that those on the upper leaf surface return to the lower surface shortly before or after maturing to adults. Second, the fact that adults and nymphs have different

preferences suggests that different selective pressures are operating on the different stages. Third, the factor(s) causing nymphs to move to the upper leaf surface is intermittent: on some occasions the nymphs are predominantly observed on the lower surface, and at no point did the nymphs abandon the lower leaf surface altogether. The occurrence of nymphs about equally on both the upper and lower surface appears to be similar to other food webs involving fitness tradeoffs between multiple, counteracting mortality factors. For example, the winter cherry bug *Acanthocoris sordidus* Thunberg deposits 15–67% of its eggs on non-host plants, and these nymphs return to the host plants after hatching (Nakajima and Fujisaki 2010). The higher nymph mortality on the non-host is offset by lower egg mortality, making both habitats viable ovipositing sites (Nakajima and Fujisaki 2010).

Three hypotheses to explain the *M. caryaefoliae* distributions were considered and tested: (1) Morphological or nutritional differences between the two leaf surfaces make the upper surface preferable. (2) Nymphs disperse when the lower leaf surface becomes too crowded. (3) Nymphs move to the upper leaf surface to escape natural enemies.

The first hypothesis is that differences between the leaf surfaces affect *M. caryaefoliae*— that, in the absence of the associated environmental hazards, the upper leaf surface is preferable for nymphs. By this hypothesis, the inherent fitness advantages offered by the upper surface mitigate the disadvantage posed by the environmental hazards. Since *M. caryaefoliae* feed on the same quaternary leaf veins, regardless of which surface they feed from, nutritional differences between the two surfaces seem unlikely. Morphological differences, such as the complete absence of stomata on the upper leaf surface, could facilitate or inhibit nymph feeding and affect nymph fitness. However, no evidence was observed suggesting that the upper surface offered an advantage. No significant difference was detected between aphids reared on the two surfaces of

leaves on orchard trees. Meanwhile, in the laboratory, nymphs reared on detached pecan leaf discs were more fit (i.e. matured faster, into larger adults) on the lower surface than on the upper surface.

A number of possible explanations exist for the discrepancy between orchard and laboratory results. It is possible that the fitness effect observed in the lab was an artifact of the study design. Due to the lack of stomata on the upper leaf surface, exposing that surface entailed pressing all the stomata on the lower surface into the agar. This may have inhibited respiration and decreased the nutritional value of these leaves.

However, if the fitness effect observed in the lab is genuine, then several factors could account for the effect not being observed in the orchard. Fewer aphids were used in the field experiment, and the numbers surviving to adulthood were much lower, perhaps too low to detect a difference in aphid fitness. Alternately, it is possible that the extra mortality factor(s) in the orchard counteract the fitness benefit offered by the lower leaf surface. Predation and dislodgement can be ruled out as causes of mortality, because the clip cages used to secure the nymphs to the leaves should have protected them. All pecan cultivars are susceptible to *M. caryaefoliae* but two of the cultivars used in the field experiments ('Stuart' in trial 1 and 'Caddo' in trial 2) are less susceptible to *M. caryaefoliae* than 'Desirable', the cultivar used in the lab experiment (Wood and Reilly 1998). However, trial 2 of the field experiment also used 'Desirable' and still saw overall mortality comparable to the other field trials. Mortality within the first 24 h after aphid transfer is generally indicative of nymphs failing to establish on a leaf. Thus, significantly higher early mortality in trial 1 ('Stuart') than trial 2 ('Desirable'), suggests that cultivar may have affected survival. However, overall mortality did not differ significantly between any of the trials, so other factors must be at work as well. Because the field trials were

conducted in late August, September, and October, much foliage on the selected trees already had damage from aphid feeding. Only leaflets with no visible damage were selected, but it is possible that a number of leaves had damage from *M. caryella* or *M. pecanis*, which was not readily apparent yet significantly reduced the nutritional value of the leaves (Tedders 1978, Tedders and Thompson 1981). In addition, some nymphs had to be placed on median or apical leaflets; this may have increased mortality as basal leaflets are their preferred feeding site (Tedders 1978, Kaakeh and Dutcher 1992). At temperatures below 30 °C, *M. caryaefoliae* development rates decrease with temperature, and mortality increases as temperatures fall below 20 °C (Tedders et al. 1992, Kaakeh and Dutcher 1993). It is possible the low night temperatures, particularly in the final trial, led to increased mortality.

Regardless of which experiment's results more closely resemble the dynamics present in the orchard, neither experiment showed the upper surface of pecan leaves offering a fitness advantage to *M. caryaefoliae* nymphs. These nymphs are not feeding on the upper surface because the habitat is inherently preferable.

Because the leaves themselves are not inducing the nymphs to feed on the upper surface, some other factor in the environment must be doing so. Our second hypothesis was that *M. caryaefoliae* nymphs disperse in response to high aphid densities, and this dispersal results in nymphs on the upper surface. Dispersal is a common response to high density of conspecifics in many species of aphids. In species possessing both apterous and alate generations, tactile stimuli from conspecifics can induce a greater proportion of dispersal-adapted, alate offspring (Johnson 1965). Poor nutritional quality in the host plant, which can be induced by higher aphid densities, has also been implicated in production of a greater proportion of alates (Wadley 1923, Müller et al. 2001).

Movement by *M. caryaefoliae* nymphs in response to crowding would be indicated by a significant negative correlation between density and the proportion of nymphs on the lower leaf surface. When examining the density of conspecifics, this negative correlation was not observed, indicating that *M. caryaefoliae* nymphs were found on both leaf surfaces regardless of their density on the leaf. The aphid's unique feeding method may account for this. The chlorotic lesions created by *M. caryaefoliae* feeding increase the nutritional quality of the phloem (Petersen and Hunter 2001, Petersen and Sandström 2001). The presence of these lesions during nymphal development has a positive effect on aphid fitness, and a foraging black pecan aphid will preferentially feed on leaves which are already chlorotic (Lakin 1972, Cottrell et al. 2009). Thus, conspecific crowding (below a particular density threshold) could result in faster creation of larger chlorotic lesions and benefit the nymphs. In this case, feeding on the upper leaf surface could also benefit these aphids, by allowing them to share chlorotic lesions from both leaf sides. However, prolonged chlorosis leads to dead leaf tissue and premature leaf abscission, which are disadvantageous for *M. caryaefoliae*. Thus, crowding may be an advantage in the short term and a disadvantage in the long term, explaining why *M. caryaefoliae* are not gregarious (Walker 1932).

When examining the density of *M. caryella* and *M. pecanis*, there was a significant negative correlation between their density and the proportion of *M. caryaefoliae* nymphs on the lower leaf surface—as density of heterospecifics increased on the lower surface, proportionally fewer *M. caryaefoliae* nymphs were found there. This distribution suggests that *M. caryaefoliae* nymphs are dispersing in response to the presence of other pecan aphids, and some of these settle on the upper leaf surface. Heterospecific-induced dispersal is consistent with prior observations that competition between *M. caryaefoliae* and *M. caryella* is asymmetrical, and that *M. caryella*

feeding damage lowers the leaf tissue's nutritional quality and inhibits the ability of *M. caryaefoliae* to induce chlorosis (Leser 1981, Edelson 1982, Petersen and Hunter 2001, Petersen and Sandström 2001). However, the observed correlation between heterospecific density and *M. caryaefoliae* distribution only explained 11% of the distribution variation observed in the mixed-aphid experiments, and for obvious reasons it does not explain why *M. caryaefoliae* nymphs moved to the upper leaf surface in the single-aphid experiments.

A third hypothesis is that *M. caryaefoliae* nymphs move to the upper leaf surface to escape natural enemies, predominantly predators which search the lower leaf surface. In other systems, aphidophagous lady beetles and lacewings mainly forage on the lower surface of foliage (Dixon 1970, Hopkins and Dixon 1997). The summer 2010 field survey supported this hypothesis: while lady beetle larvae were observed about equally on both leaf surfaces, a significantly larger number of lacewing larvae were observed on the lower leaf surface.

To further test this hypothesis, experiments were carried out to determine if three common aphid predators—*Harmonia axyridis*, *Olla v-nigrum*, and *Chrysoperla rufilabris*—favor the lower leaf surface when foraging, and whether they consume more aphids on the lower surface. The results were mixed. When larvae were experimentally allowed to forage on orchard foliage for four trials, there was evidence of *H. axyridis* feeding preferentially on the lower surface in one trial, and of *O. v-nigrum* feeding preferentially on the lower surface in two trials—but *O. v-nigrum* appeared to feed preferentially on the upper surface in the two remaining trials. When a similar experiment was conducted in the laboratory on potted pecan seedlings, no evidence for larval leaf surface preference was observed, as aphid mortality on the treated leaves did not differ significantly from the controls. Direct observations of the foraging predators found

that *C. rufilabris* and *H. axyridis* larvae spent more time on the lower surface when aphids were absent, while *O. v-nigrum* larvae spent equal time on each leaf surface.

According to Berdegue et al. (1996), three things must be established in order to conclude that a particular habitat is an enemy-free space. First, it must be established that natural enemies have a negative effect on prey fitness in the original habitat. This requirement has been fulfilled by prior observations of larval lady beetles, lacewings, and other generalist aphid predators on pecan foliage, feeding on *M. caryaefoliae* (Tedders and Angelet 1981, LaRock and Ellington 1996, Kunkel and Cottrell 2007). Second, to rule out the possibility that the alternative is an inherently better habitat, it must be established that the prey have greater fitness in the original habitat when natural enemies are absent (Berdegue et al. 1996). Our comparison of *M. caryaefoliae* nymphal development on the two leaf surfaces found that the upper surface offered no fitness advantage in isolation, and the environmental hazards associated with the upper surface make the lower surface more advantageous, so the second criterion is fulfilled. Third, it must be established that the prey have greater fitness in the alternative habitat when natural enemies are present (Berdegue et al. 1996). This could be established by demonstrating that the natural enemies of *M. caryaefoliae* search the lower leaf surface more frequently than the upper leaf surface. From our results, it appears that the upper surface of pecan foliage is not completely devoid of aphid predators, but nevertheless carries a reduced risk of predator encounters at least sometimes.

This variation in enemy encounters over time fits with Sheirs and De Bruyn's (2002) observation that predator-prey interactions—and thus the existence of enemy-free space—are dynamic and can vary between or within seasons. This may explain why *O. v-nigrum* foraging varied between trials, as well as why the *M. caryaefoliae* distributions on control leaves in the

orchard trials did not match the observed distributions from the prior summer. Dynamic enemy-free spaces could also explain variation within the literature, with some authors noting a preference for the upper surface (Davis 1910, Tedders 1978), some a preference for the lower surface (Richards 1960), and some noting no preference (Walker 1932).

It is also worth noting that, comparing the two coccinellids, it was the exotic *H. axyridis* that was observed searching the lower leaf surface more often, while the native *O. v-nigrum* spent equal time on both leaf surfaces. As pecan and *M. caryaefoliae* are also native species, the searching behavior of *O. v-nigrum* could represent co-evolution: the aphids moved to the upper leaf surface, evaded predation, and flourished, so *O. v-nigrum* began foraging on the upper surface to exploit the aphid population there. Perhaps exploiting that niche, which *H. axyridis* has left alone, helps *O. v-nigrum* avoid being completely displaced by the invasive competitor.

The predaceous larvae feeding on honeydew was also interesting. Coccinellidae in general are known to supplement their diet with honeydew. Honeydew is not an adequate substitute for aphids (Wäckers et al. 2008), but can prolong the coccinellid larva's life when high-quality prey are absent (Lundgren 2009). The length of time that *O. v-nigrum* was observed feeding on honeydew—more time than they spent feeding on aphids on either surface—is surprising. If these preferences are also shown by larvae in the field, and are not merely an artifact of the laboratory test, then the presence of honeydew increases the upper leaf surface's value as an enemy-free space by distracting some predators that do search there.

The effect of pecan aphid parasitoids on *M. caryaefoliae* populations is unknown, but the literature suggests they are unlikely to exert a significant, direct influence, because no parasitoids specializing in *M. caryaefoliae* are known from Georgia. The native *Aphelinus perpallidus* Gahan (Hymenoptera: Aphelinidae) will parasitize black pecan aphids, but prefers

blackmargined or yellow pecan aphids (Mizell and Schiffhauer 1990). Two exotic species, *Trioxys pallidus* (Haliday) and *Tryoxis complanatus* Quilis (Hymenoptera: Braconidae)—introduced in Georgia as biological control agents, but their establishment is uncertain (Tedders 1977, Starý and Marsh 1982)—almost exclusively targeted black-margined aphids in laboratory trials (Tedders 1977). A field survey comparing rates of parasitism between all three pecan aphids could still be very informative.

The interactions suggested by our data and prior research can be summarized as follows. *M. caryaefoliae*, *M. caryella*, and *M. pecanis* all feed on pecan foliage. *M. caryella* and *M. pecanis* feed almost exclusively on the lower leaf surface, because their proboscises are not long enough to feed on the phloem cells of the primary, secondary, and tertiary leaf veins from the upper surface (Tedders and Thompson 1981). The large numbers of these aphids exclusively on the lower leaf surface makes it advantageous for some generalist aphid predators, such as lacewing larvae and the exotic ladybeetle *H. axyridis*, to predominately forage for food on the lower leaf surface. In contrast with the other pecan aphids, *M. caryaefoliae* feeds on the phloem cells of quaternary leaf veins, which are accessible from either leaf surface (Tedders and Thompson 1981). Feeding by *M. caryaefoliae* induces chlorosis, which increases the nutritional value of leaf tissue in the short term but leads to tissue death and leaflet abscission in the long term (Lakin 1972, Petersen and Sandström 2001, Cottrell et al. 2009), thus their dispersal is independent of conspecific density. Because feeding damage from *M. caryella* (and possibly *M. pecanis*) inhibits the ability of *M. caryaefoliae* to induce chlorosis (Petersen and Hunter 2001, Petersen and Sandström 2001), crowding by heterospecific aphids does induce *M. caryaefoliae* to disperse. Both leaf surfaces carry unique mortality risks for *M. caryaefoliae*: the upper surface is exposed to environmental hazards—solar radiation, precipitation, honeydew dropped by

aphids on leaves overhead, and dislodgement due to leaves brushing in the wind (Hopkins and Dixon 1997, Dixon 2005)—while the lower surface is carries a greater risk of predator encounters. Thus, neither surface consistently offers a significant fitness advantage to *M. caryaefoliae* nymphs. The nymph distribution between the two surfaces varies with the relative severity of the mortality factors. However, all adult *M. caryaefoliae* are alates, thus they are more mobile than the nymphs and under less selection pressure from predators. They remain predominantly on the lower leaf surface. Complicating this distribution, at least one native predator, the lady beetle *O. v-nigrum*, has adapted to exploit *M. caryaefoliae* by foraging on the upper leaf surface as often as it does on the lower surface. Thus, the upper leaf surface is only intermittently an enemy-free space. Since aphid predators augment their diets with honeydew (Lundgren 2009), the presence of honeydew on the upper leaf surface may distract predators foraging on the upper surface and mitigate the mortality from this predator. As the *M. caryaefoliae* nymphs prefer to remain sedentary once they begin feeding (Cottrell et al. 2009), honeydew on the upper leaf surface does not impede them as it does their predators.

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