COMPARISON OF THE EFFICACIES OF ORGANIC AND STANDARD INSECT CONTROL METHODS FOR PECAN ORCHARDS

by

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(Under the Direction of James D. Dutcher)

ABSTRACT

In contrast to conventional pecan management, there are fewer options for pest management in organic pecan, *Carya illinoinensis* (Wangenheim) K. Koch, due to the stricter regulations on the compounds that can be used. Thus, our study focused on seeking insect control methods for organic pecan. The applications of some OMRI (Organic Materials Review Institute) and non-OMRI approved biopesticides were investigated for pecan aphid complex suppression and fall webworm (*Hyphantria cunea* (Drury)) control. The effects of cool-season and warm-season intercrop planting on the enhancement of pecan aphid complex biological control were evaluated in the pecan orchard. The trail blocking and foraging disruption experiments of the secondary predators, Argentine ant (*Linepithema humile* (Mayr)) and red imported fire ant (*Solenopsis invicta* (Buren)) were conducted. The results of our study could provide organic growers with some viable insect control methods, and also offer some ideas for future organic pecan research.

INDEX WORDS: yellow pecan aphid, blackmargined aphid, black pecan aphid, fall webworm, biopesticides, intercrops, trunk sprays

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LITERATURE REVIEW

Introduction

Pecan, *Carya illinoinensis* (Wangenheim) K. Koch, is the most important nut crop that is native to North America, with the widest distribution of commercial orchards in the United States (Wood et al. 1990, Reid 2002, Thompson and Conner 2012). There are 18 major pecan producing states in the US from the Atlantic coast of Virginia and the Carolinas westward to California, and from Texas north to Illinois (Reid 2002, Thompson and Conner 2012). Fifteen US southern states and northern Mexico account for the majority (over 98%) of the world's annual pecan production (Thompson and Conner 2012). In 2014, the utilized pecan production was estimated at 132,075 tons in the US; and Georgia, with 38,000 tons (29%), lead the nation in pecan nut production (USDA 2015a). As a result, by 1950s, Georgia state has become the largest pecan producer in the nation (Jones 2006). In the US, 543,486 acres of land were used for pecan production, including a total acreage of 123,415 acres in Georgia (USDA 2014).

To secure the quantity and quality of pecan production, a thoroughly planned pecan pest management program is needed. However, there are some unique pecan characteristics that pose challenges to the implementation and development of a pest management program. (1) **Large tree size** where trees can reach more than 25 m in height, covering an area of 0.1 acre (Dutcher et al. 2012, Wells 2014). (2) **Long tree lifespan** (more than 100 years) where the long-lived profile offers pests an opportunity to adapt to the pecan production system (Harris 1991, Wood

2003). (3) **Low genetic diversity among trees planted in the improved orchards** where only a few cultivars are planted in the orchards. (4) **Long growing season** of over 7 months, where pecan leaves and nuts are exposed to an array of pests throughout the season. There are more than180 species of phytophagous insects associated with pecan in the US (Harris 1983, Ree and Knutson 1997, Dutcher et al. 2006). (5) **Wide geographic distribution of pecan production** where pecan is grown in various environmental conditions and one pest management program cannot be applied to all circumstances (Harris 1983, Reid 2002, Wood 2003). In addition to the above mentioned pecan characteristics, organic pecan pest management program has further challenges due to the more stringent regulations and standards of production that limit the use of conventional tools.

Organic agriculture provides lower potential of insect resistance to pesticides, and reduced risk of pesticides to nontarget organisms and the environment; the increase in consumer demand also gives an impetus to the emergence of organic agriculture (Heerema et al. 2007). However, relative to the rising consumer interests in organic agriculture, there is a lack of research on organic pest management program (Zehnder et al. 2007).

Pecan profile and production systems

Pecan is a deciduous tree that belongs to the walnut family, Juglandaceae. It is a monoecious plant, containing both male and female flowers developing at different parts of the pecan tree; pollen shed and stigma receptivity take place at separate times in order to avoid self-pollination which results in low nut quality or nut abortion (Conner and Wells 2007). Besides the edible nuts, pecan also provides a good source of lumber and shade (Dutcher et al. 2012, Thompson and Conner 2012). In the US, pecan grows across the Southwest, Southeast and

Midwest with a complex array of environments, but a sandy loam soil with a clay subsoil has proven the most suitable for nut production; as a result, pecan is well adapted to Georgia's soil, and the climate is also optimal for pecan production in Georgia (Jones 2006). In one growing season, pecan undergoes multiple developmental stages, including bud break, pollination, water stage, gel stage, half shell hardening, dough and shuck split; and after leaf drop, pecan enters dormancy (Ree and Knutson 1997). In spite of pecan's long growing season, insect problems usually take place after bud break stage where the bud scale splits and the leaf begins to expand, and until shuck split stage (Ree and Knutson 1997). In Georgia, bud break takes place in mid-March; pollination starts in early May while shoot and leaf continue growing; root growth increased rapidly in late May to early June; nut development starts from May through early October; defoliation occurs in mid-November (Conner and Wells 2007).

Pecan nuts are harvested from orchards and groves managed by three cultural methods based on the types of trees grown. Trees in native groves are harvested intermittently, especially during years with a heavy crop. These trees receive no to very minimal pest control. Seedling trees are full grown trees planted from seeds and cultured in orchards. These trees are harvested every season, and pest control is by integrated pest management. Improved trees are planted to known cultivars in orchards, and pest control is also by integrated pest management. Native groves and seedling orchards have much greater diversity of nuts than improved orchards (Wood 2003).

Pecan arthropods and management

While most of the 270 insect species associated with pecan in the US are beneficial or benign, multiple pest complexes have been identified in pecan (Harris 1983, Smith et al. 1996,

Reid 2002). Key insect pests that need management nearly every year are pecan nut casebearer (*Acrobasis nuxvorella* (Neunzig)), pecan weevil (*Curculio caryae* (Horn)) and hickory shuckworm (*Cydia caryana* (Fitch)). In the humid Southeastern US, yellow aphid complex ((*Monellia caryella* (Fitch) and (*Monelliopsis pecanis* (Bissell)), black pecan aphid (*Melanocallis caryaefoliae* (Davis)), phylloxera complex (*Phylloxera* spp.) and fall webworm (*Hyphantria cunea* (Drury)) were listed as major pests (Harris 1983). Pecan insect pests can be categorized into three groups: (1) Nut feeders: pecan nut casebearer, hickory shuckworm, pecan weevil, brown stink bug (*Euschistus servus* (Say)), green stink bug (*Acrosternum hilare* (Say)) and southern green stink bug (*Nezara viridula* (L.)). (2) Foliage feeders: yellow aphid complex, black pecan aphid and fall webworm. (3) Twig, stem, branch and trunk feeders: Pecan phylloxera (*Phylloxera devastatrrix* (Pergande)) and pecan spittle bug (*Clastoptera achatina* (Germar)) (Ree and Knutson 1997). The remaining insect species include predators and parasitoids associated with the major pests (Ellington et al. 2003).

Predators. The red imported fire ant (RIFA), *Solenopsis incivta* (Buren) is an important predator of pecan weevil larvae and reduces the population of weevil larva in the soil by one third (Dutcher and Sheppard 1981). However, RIFA feeds on fallen and cracked pecan kernels, and also preys on some beneficial species, such as ladybeetles (Ree and Knutson 1997). Several ladybeetle (coccinellid) and lacewing species (chrysopid) are effective biological control agents of pecan aphids (Liao et al. 1984, Liao et al. 1985). In addition, the larval stage of flower flies (Syrphidae), especially *Allograpta oblique* (Say), is also the beneficial form that feeds on pecan aphids, and syrphid fly larvae are commonly found crawling on pecan leaves when aphids are abundant (Conner and Wells 2007). Both beneficial and pest mirids (Hemipteran) can be found on pecan; the most important beneficial mirid is *Deraeocoris nebulosus* (Uhler), which preys on

multiple pecan pests, including pecan aphids, spittlebugs, caterpillars and insect eggs (Conner and Wells 2007).

Parasitoids. There are some parasitoids associated with pecan weevil, such as some tachinid flies; however, the parasitic effects are not significant from most of the pecan weevil parasitoids (Tedders 1985). Heyerdahl and Dutcher (1985) reported thirty-seven hymenopterous parasitoids of four pecan leafminers—*Stigmella juglandifoliella* (Clemens), *Cameraria caryaefliella* (Clemens), *Phyllonorycter caryaealbella* (Chambers) and *Coptodisca lucifluella* (Clemens). The egg stage of pecan nut casebearer is the host of several parasitoids; the tiny parasitic wasp of the genus *Trichogramma* lays eggs inside the pecan nut casebearer egg, and the trichogramma egg hatches into a larva which consumes the casebearer egg contents (Knutson and Ree 2005). The study from Nevárez and Rivero (2013) indicated that the releases of *Trichogramma platneri* (Nagarkatti) reduced the amount of pecan cluster damaged by pecan nut casebearer and hickory shuckworm; however, when the pest populations are high, *T. platneri* is unable to perform effective biological control to reduce the pest populations below economic threshold.

Because pecan is widely cultivated throughout diverse regions of the US, the main pest issues vary among different regions. In addition, even different orchards in the same regions could have different pests associated with economic damages; hence, the understanding of your orchard and pest is crucial to a successful pest management program.

Over the last 40 years, pecan pest management has gradually shifted from heavy reliance on broad-spectrum pesticides or tank mix pesticides to integrated approaches (Harris et al. 1998). The adverse effects caused by prophylactic and frequent sprays, included insect resistance and elimination of natural enemies (nontarget effect). For instance, research showed that the

fungicide (triphenyltin hydroxide) application in pecan diminished the biological control effects from beneficial fungi which were pathogenic to blackmargined aphid (Pickering et al. 1990); the carbaryl treatment for pecan weevil and hickory shuckworm caused the resurgence of pecan aphid population (Dutcher et al. 1985); the detection of pyrethroid efficacy loss to blackmargined aphid (Dutcher 1997). Integrated pest management (IPM) is a relatively new approach to the insect problems, utilizing multiple strategies for pest control. The pecan IPM approaches begun adoption about 40 years ago (Harris 1983). Organic agriculture is a concept newer than IPM in pecan. In fact, the increase in consumer demand gives an impetus to the growth of organic agriculture (Heerema et al. 2007). However, in comparison with the rising consumer interests, there is a lack of research on organic pest management program (Zehnder et al. 2007).

Organic pest management emphasizes the adoption of science-based and ecologically-secured strategies regulated and approved by international and national organic production standards (Zehnder et al. 2007). According to the conceptual model proposed by Wyss et al. (2005) for organic arthropod management program development, there are four different phases in the model, ranging from preventative approaches to curative methods. The first phase focuses on cultural practices that are implemented on a preventative purpose of reducing the pest infestation and damage at the initial stages of organic pest management program. Cultural preventative practices for organic pecans are selections of cultivars with tolerance toward insect pests and diseases. Pecan cultivars have shown to have tolerance toward insect pests (Arnold and Andrews 1981, Wells 2014). For example, “Pawnee” pecan shows field resistance to yellow aphid complex, on the contrary, “Stuart” is very susceptible (Thompson and Grauke 1998); some pecan cultivars have tolerance toward all three pecan aphid species (Karar et al. 2012, Conner 2014). The second phase emphasizes the ecological engineering manipulations, including

conservation biological control, intercropping and trap cropping. Evaluation of cover crops for the enhancement of aphidophagous coccinellids in pecan orchard, and the results showed that the mean densities of aphidophagous coccinellids in the mixed stands of hairy vetch (*Vicia villosa* (Roth)) and rye (*Secale cereal* (L.)) were approximately 6 times greater than in unmowed resident vegetation; and 87 times greater than in mown grasses and weeds (Bugg et al. 1990). The third phase consists of the releases of biocontrol agents, including inundation and inoculation biocontrol. In general, the third-phase strategies are used when the preventative practices are not sufficient enough to keep the pest population at low level, and other practices are required. In other words, the third-phase strategies are employed to directly cope with the pest problems. For example, LaRock and Ellington (1996) conducted pecan aphid biological control study by inoculative releases of pecan aphid predators, green lacewing (*Chrysoperla rufilabris* (Burmeister)), convergent lady beetle (*Hippodamia convergens* (Guerin-Meneville)) and multicolored Asian lady beetle (*Harmonia axyridis* (Pallas)) for seven years; the results indicated that the costs of predator releases reduced overtime, while pecan yield and quality remained high. The fourth-phase strategies are associated with curative methods, such as the application of approved organic repellents as physical barriers, pheromones for mating disruption, and insecticides derived from natural materials (plants, animal, bacteria and mineral, etc). In fact, the fourth-phase strategies are considered the last options for the management of pests, which are adopted when the control methods from the prior three phases have failed. Some potential biopesticides have been tested in pecan pest management studies. For instance, Shapiro-Ilan et al. (2013) found that the applications of eucalyptus extract combined with the fungal microbial insecticide, *Isaria fumosorosea* (Wize), were able to cause up to 82 % of black pecan aphid mortality in the field; and the nematode-derived insecticide, *Chromobacterium*

subtsugae, also had the potential to suppress pecan weevils. Overall, although there are potential or viable tactics that can be used as alternatives, the most effective methods for management of key pecan pests rely on chemical insecticides (Shapiro-Ilan et al. 2013). In the long term, further organic research is necessary in order to fulfill public demand for organic products and reduce adverse effects associated with chemical or conventional pest control strategies. Currently, pecan IPM primarily differs from organic pest management in the types of pesticides applied to control insects and diseases.

Pecan aphid management

There are three aphid species attacking pecan, including blackmargined aphid (*Monellia caryella* (Fitch)), yellow pecan aphid (*Monelliopsis pecanis* (Bissell)) and black pecan aphid (*Melanocallis caryaefoliae* (Davis)). According to Tedders (1983), in 1981, the economic losses resulting from the damages and costs of control from yellow aphid complex and black pecan aphid were ranked second and third after pecan weevil, respectively, in Georgia. The outbreaks of pecan aphids occur two times per season: one is in spring, and after that, the aphid numbers decrease to very low levels; and the other is in the late season (mid-July to October) when the aphids resurge (Dutcher et al. 2012).

In general, pecan aphids remove carbohydrates from pecan, excrete honeydew and induce the growth of sooty mold fungus, which results in the reduction of photosynthetic rate. In addition, the black pecan aphid is able to cause defoliation in the late season because while the black pecan aphid feeds on leaves, it also injects a toxin that damages the leaf tissue and creates chlorotic spots on the leaflets which ultimately lead to abscise (Ree and Knutson 1997). Moreover, the pecan aphid damages can cause detrimental effects on nut production, and reduce

nut yield (Wood et al. 1987). The yellow aphid complex and black pecan aphid have different economic thresholds where action needs to be taken to avoid economic loss. The economic thresholds for the yellow aphid complex and black pecan aphid are 20 aphids and 1 aphid per compound leaf, respectively (Harris 1983, Dutcher et al. 2012). Chemical insecticides are commonly used for pecan aphid control, such as using neonicotinoids for controlling yellow aphid complex and organophosphates for controlling black pecan aphid (Dutcher et al. 2012). However, Dutcher (1998) demonstrated that after insecticides are applied, aphid control becomes ineffective in four stages: first, aphidophagous insect populations collapse due to the application of insecticides; second, without adequate natural enemies, aphid populations increase to an unusually high level; third, more insecticides are used to control aphids, and over application not only induces resistant aphids to become the dominant genotype in the population, but also makes the insecticide an impractical control option; finally, the pecan orchard loses both natural and chemical control for aphids. As a result, it has been observed that aphid resurgence was caused by the destruction of natural enemies by multiple applications of certain pesticides; and it has also been reported that certain pesticides have lost the efficiency to control pecan aphids. For example, the pyrethroid resistance observed in blackmargined aphid (Dutcher and Htay 1985, Dutcher 1997). Other than relying mainly on chemical control for aphids, there are some alternatives. Biological control is one of the viable options, since the pecan aphids are not vectors of pecan diseases, pecan trees are able to endure some degree of aphid damage, and there are some natural and introduced aphidophagous insects that prey on and control pecan aphids (Dutcher 1998, Dutcher 2004, Dutcher et al. 2012). The inoculative releases of pecan aphid predators, green lacewing, convergent lady beetle and multicolored Asian lady beetle were found effective for pecan aphid control while the pecan yield and quality remained high (LaRock and

Ellington 1996). Other natural enemies of pecan aphids include the lady beetles—*Olla v-nigrum* (Mulsant), *Coccinella septempunctata* (L.) and *Cycloneda sanguinea* (L.); lacewings—*Chrysoperla quadripunctata* (Burmeister) and *Micromus posticus* (Walker) (Dutcher 1998). The multicolored Asian lady beetle is originated in Asia, and it preys on various aphids species in orchards and forests (Tedders and Schaefer 1994, Koch 2003). In North America, the introduction and releases of the multicolored Asian lady beetle can be traced back to 1916; yet, the establishment of the lady beetle was not documented until decades later, when Chapin and Brou (1991) reported the establishment in Louisiana and Mississippi (Gordon 1985). In Georgia, the first evidence of this lady beetle colonization was in 1990 in Buchanan after the releases of total 87,561 lady beetles from 1978 to 1981, and by 1992 the population had distributed throughout Georgia to northern Florida and eastern South Carolina (Tedders and Schaefer 1994).

Plant diversity in pecan orchards can be increased by intercrop planting. Groundcovers may provide the beneficial or innocuous insects with alternate food sources, serve as shelters for oviposition and defense against secondary predators, and offer the microclimate that favors the development of beneficial insects (Ellington et al. 1995). Besides the benefit mentioned above, other advantages of intercrops include improving soil nitrogen, promoting soil structure, suppressing harmful weeds, and reducing soil compaction (Ellington et al. 1995, Dutcher 1998).

Cool-season intercrops are planted after pecan harvest. Before the next pecan production season starts, they serve as shelters, and provide alternate prey for beneficial insects when pecan aphids are absent from pecan trees (Bugg et al. 1990). Bugg et al. (1990) found that *C. septempunctata* and *H. convergens* reproduced abundantly on the intercrops, which also supported alternate prey aphids. A diversity of different alternate prey aphid species has been found from various understory covers: hairy vetch (*Vicia villosa* (Roth)) and crimson clover

(*Trifolium incarnatum* (L.)) harbor pea aphid (*Acyrthosiphon pisum* (Harris)) and blue alfalfa aphid (*Acyrthosiphon kondoi* (Shinji)); rye (*Secale cereal* (L.)) sustains bird cherry-oat aphid (*Rhopalosiphum padi* (L.)) (Bugg et al. 1991a, Dutcher 1998). Those crops also support some thrips species as well. Lady beetles, especially *O. v-nigrum* prey on thrips after alternate aphid populations drop because of heavy rains and subsequent outbreaks of fungal pathogens (Bugg et al. 1990).

Warm-season intercrops are planted during pecan growing season in attempt to provide continuous biological control after cool-season intercrops. For example, cowpea (*Vigna unguiculata* ssp.), hairy indigo (*Indigofera hirsuta* (L.)) and sesbania (*Sesbania exaltata* [Rafinesque-Schmaltz] Cory) harbor cowpea aphid (*Aphis craccivora* (Koch)); while sorghum (*Sorghum bicolor* (L.)) supports corn leaf aphid (*Rhopalosiphum maidia* (Fitch)); and sesbania also sustains bandedwinged whitefly (*Trialeurodes abutilonea* (Haldenman)) (Bugg and Dutcher 1989, Bugg et al. 1991b). Aphidophagous insects observed in warm-season crops include coccinellid beetles (*C. septempunctata*, *H. convergens*, *O. v-nigrum* and *Cycloneda sanguinea*) and some syrphid flies (Bugg and Dutcher 1989).

In spite of all the positive impacts on biological control of pecan aphid, intercrop selection is still challenging due to some drawbacks. Understory groundcover might raise the concern of water competition between pecan and groundcover, especially for warm-season legumes; however, the concern may be diminished if the water transpired by cover crops can be replaced by irrigation system (Bugg et al. 1991b). Cool-season groundcover provides aphidophagous insects with alternate prey before pecan aphids are available on pecan trees, whereas, warm-season groundcover serves as a reservoir of aphidophaga and alternate prey while pecan aphid infestation is already underway. Thus, the alternative prey in warm-season

intercrop might keep aphidophagous insects from going up to pecan canopy (Bugg et al. 1991b). As a result, cutting the intercrops is necessary when aphidophaga are needed in pecan trees (Dutcher 1998). In contrast, some cool-season intercrops senesce, and aphidophaga move to pecan foliage while pecan is becoming infested with aphid. Cutting is also a useful tool to facilitate aphid biological control for some cool-season intercrops (Bugg et al. 1991b). Moreover, a food spray of fermented molasses study indicated that it was an effective attractant for ladybeetles and lacewings to the pecan canopy, and reductions in abundance of three pecan aphid species followed the food spray application (Dutcher et al. 2012). *C. septempunctata* and *H. convergens* are usually collected at the ground level, whereas, *O. v-nigrum* and *H. axyridi*s are commonly found from pecan tree canopy and at the ground level (Dutcher et al. 2012). The principally arboreal *O. v-nigrum* and *H. axyridi*s play a relatively important role in biological control of pecan aphids (Bugg et al. 1991a) (Mizell III 2003). In fact, different alternate prey is found from distinct intercrops, and the feeding preference for alternate prey varies among different aphidophgous species. For example, in laboratory observations, cowpea aphid does not serve as an acceptable prey for *H. axyridi*s while it is readily eaten by *H. convergens* (Dutcher et al. 1999). Aside from being a reservoir of beneficial insects, intercrops also provide resources for generalist stink bugs that also damage pecan nuts. For instance, sesbania cultures abundant alternate prey and sustains ladybeetles; however, it also has the disadvantage of harboring stink bugs (Bugg et al. 1991b). The use of trap crops might be able to address the issue by attracting kernel-feeding hemipterans away from the intercrop or pecan (Dutcher 1998, Mizell III 2003, Dutcher et al. 2006).

Although red imported fire ant is an important predator of pecan pests, such as the southern green stink bug and pecan weevil, it also disrupts pecan biological control by feeding

on beneficial insects (Ree and Knutson 1997, Dutcher 1998). Red imported fire ants feed on aphid honeydew and aphidophaga (Ree and Knutson 1997, Dutcher 1998). Alternate prey aphids and pecan aphids attract fire ants to intercrops and pecan trees. The application of an insecticide on the tree trunks is used to hinder fire ants from foraging up to pecan canopy, where the ants interfere with beneficial insects. Sesbania naturally repels red imported fire ant (Kaakeh and Dutcher 1992, Bugg and Dutcher 1993, Dutcher and Beaver 2005).

In pecan orchards, the best candidates of the intercrop vary from orchard to orchard because of different climate conditions, soil nutrition requirements, and alternate species needed, etc. (Bugg et al. 1991b, Dutcher 1998). Although there are some tradeoffs and side effects of adopting intercrop, planting intercrop still plays an important role in biological control in sustainable or organic pecan production, as long as the drawbacks are diminished by proper intercrop selection and management (Bugg et al. 1991b, Diver and Ames 2000).

Organic agriculture and organic pecan production

The USDA organic regulations describe organic agriculture as the application of a set of cultural, biological, and mechanical practices that support the cycling of on-farm resources, promote ecological balance, and conserve biodiversity. These include maintaining or enhancing soil and water quality; conserving wetlands, woodlands, and wildlife; and avoiding use of synthetic fertilizers, sewage sludge, irradiation, and genetic engineering (USDA 2015b).

In general, there are two reasons for wanting to develop organic pecan production (Bock et al. 2012). First, there is an increased margin of profit for selling organic pecan due to higher sale prices; however, the dollar value varies according to the location, nut quality and market (Flores 2008, Bock et al. 2012). Second, the adverse effects of agricultural activities on the

environment are reduced, and the products are free of synthetic chemicals (Diver and Ames 2000, Bock et al. 2012). Organic pecans generally have higher per-pound cost of production than conventionally grown ones, yet the yields are often lower due to nutrient deficiencies and pest damages (Heerema et al. 2007). However, there are some exceptions. The average yield produced by the well-managed organic pecan system is 44 pounds per tree which surpasses the yield from conventional management system (25 pounds per tree) (Flores 2008). In addition, lower chemical costs and reduced inputs also benefit the dollar value of organic pecan, if nut quality is comparable to conventional pecan (Heerema et al. 2007, Bock et al. 2012).

Typically, organic pecan orchards are established either at planting or by converting from conventional to organic (Bock et al. 2012). A three-year period is required for the converted orchard or the land that is not already certified as organic to obtain organic certification for selling pecans as organic (Heerema et al. 2007). In contrast to conventional pecan management, there are fewer options for pest management in organic pecan due to the stricter regulations on the compounds that can be used; furthermore, research focusing on organic pecans is relatively scarce (Heerema et al. 2007). In spite of the disadvantageous facts, some research still show promising results of organic-approved compounds on pecan pests, and some organic pecan orchards also develop successful organic pecan production (Flores 2008, Allen 2015).

Scope of research

The goal of my study is to search for potential organic pest management tactics for pests in pecan. My study consists of three projects.

In the first project, biopesticides were evaluated for efficacy against two pecan insect pests in two separate trials: (1) two OMRI (Organic Materials Review Institute) listed

biopesticides, 70% Neem Oil (70% clarified hydrophobic extract of neem oil) and Entrust® (22.5 % spinosyn A&D), and one non-OMRI approved biopesticide, Abba Ultra® (3.74 % abamectin) were evaluated for control of fall webworm in a laboratory bioassay; (2) Abba Ultra®, 70% Neem Oil and OII-YS™ (8% yucca extract, chitosans) were evaluated for efficacy against pecan aphids in the field. I also monitored the nontarget effects of the test materials on the abundance of lacewing, a predator of pecan aphids (Neuroptera: Chrysopidae), in pecan canopies. The hypotheses were: (1) is the survival of fall webworm larvae reduced after feeding on pecan foliage treated with biopesticides? (2) are the abundances of pecan aphids and predatory lacewings decreased after the application of biopesticides to the pecan foliage?

Second, I evaluated the effects of different intercrops on the enhancement of alternate prey aphids and beneficial insects (lady beetles) in the intercrops, and the suppression of pecan aphid in pecan canopies. The hypothesis was: in pecan orchards, do intercrops enhance biological control by maintaining higher abundance of lady beetles and lower abundance of pecan aphid in the pecan trees relative to a mowed sod check?

Third, I developed a method to prevent ants from foraging in the pecan tree canopies. (1) I compared RIFA trail blocking effects of different concentrations of methyl anthranilate with the nontreated control in greenhouse experiments; (2) determined if trunk applications of Tangle-Trap® Sticky Coating and Bird Stop™ (26.4% of methyl anthranilate) would prevent Argentine ant and RIFA from foraging in the pecan tree canopy in a controlled replicated field trial. The hypotheses were (1) does methyl anthranilate block the foraging trail of RIFA? (2) does trunk applications of Bird Stop™ (a commercial formulation of methyl anthranilate) disrupt the foraging of the Argentine ant and RIFA in a pecan orchard? Is the trail blocking effect of Bird Stop™ better than the Tangle-Trap® Sticky Coating? Is the combination of Bird Stop™ and

Tangle-Trap® Sticky Coating better than either material alone for blocking the foraging trail of the ants?

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CHAPTER 2

EVALUATION OF THE EFFICACY OF BIOPESTICIDES FOR CONTROL OF FALL WEBWORM, YELLOW PECAN APHID, BLACKMARGINED APHID, AND BLACK PECAN APHID

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Abstract

Two separate laboratory tests were conducted to evaluate the efficacy of selected biopesticides for fall webworm (*Hyphantria cunea* (Drury)) control. The biopesticides evaluated were Abba Ultra® (3.74 % abamectin) at 31.25, 62.5 and 78.13 ml of formulation/100 liter of water; 70 % Neem oil (70% clarified hydrophobic extract of neem oil) at 1% and 1.5 % formulation v:v in water; and Entrust® (22.5 % spinosyn A&D) at 31.25 and 78.13 ml of formulation/100 liter of water. All the test concentrations of Abba Ultra® and Entrust® caused significantly higher mortality of fall webworm larvae than the nontreated control at 10 and 13 days post-treatment in the first and second laboratory tests, respectively; and no fall webworm larvae were able to successfully undergo pupation and reach adulthood. Compared with the nontreated control, the two test rates of 70 % Neem oil did not show significant higher fall webworm mortality, or significantly lower pupation and adult emergence rates in the first webworm bioassay; while they showed slightly higher mortality and lower pupation and adult emergence rates in the second test, but the efficacy was very low compared to Abba Ultra® and Entrust®.

Field trials were carried out to test the effects of Abba Ultra® at 62.5 and 78.13 ml of formulation/100 liter of water; 70 % Neem oil at 1% and 1.5 % formulation v:v in water; and OII-YS™ (8% yucca extract (chitosans)) at 500 ml of formulation/100 liter of water on suppression of pecan aphids (*Monellia caryella* (Fitch), *Monelliopsis pecanis* (Bissell), and *Melanocallis caryaefoliae* (Davis)) and nontarget effects on the densities of lacewing (predator of pecan aphids; Neuroptera: Chrysopidae) eggs in pecan canopies. The results indicated that only Abba Ultra® resulted in reduction of pecan aphid abundances in pecan canopies at 1 day post-treatment. Compared with the nontreated control, no treatments show significantly lower

lacewing egg densities in pecan canopies on all the assessment dates (1, 6 and 13 days post-treatment).

Key words: feeding bioassay, neem oil, abamectin, spinosad

Introduction

The fall webworm, *Hyphantria cunea* (Drury), is a foliar-feeding pest of pecan, *Carya illinoinensis* (Wangenheim) K. Koch. It is native to North America, and has a wide distribution in other countries, including China, Japan, Korea and several countries in Europe (Li et al. 2013). Although it occasionally causes economic injury to pecan, the fall webworm larva is voracious, and is able to consume large amount of pecan foliage by its gregarious feeding behavior (Polles and Payne 1975). Damage may reduce nut production and quality (Ree and Knutson 1997). In the southern states, the fall webworm usually has two generations every year, and there are two different forms of the fall webworm—red-headed and black-headed (Hyche 1999, Tyson 2011). The two forms vary in seasonal activity and behavior, but they share similar life cycles. For example, red-headed form larvae tend to stay closely related to the web until fully grown, whereas black-headed form larvae have a tendency to leave the original web and split into smaller webs when they are one-half grown (Hyche 1999). The larvae are covered with long white and black hairs, and they feed on pecan leaves within silk webs covering pecan shoots and leaves. In general, more than 100 larvae can be found in a silk web, and webs can be seen from late spring to fall (Gill 1924, Ree and Knutson 1997). The larvae stop feeding at full growth of last instar, and they leave the webs and crawl to the ground debris or bark cracks where they prepare to pupate. The last generation overwinters as pupae, and the adults are unable to emerge until next year in late spring or summer (Gill 1924, Li et al. 2013).

Chemical pesticides, such as carbamate (carbaryl), organophosphate and diamide are used for fall webworm management (Polles and Payne 1975, Hudson et al. 2006, Li et al. 2013). In comparison with chemical pesticides, biopesticides usually provide lower risk of insect resistance development, and are often safer to nontarget organisms and the environment (EPA 2016). For example, *Bacillus thuringiensis* Berliner formulated as Dipel® HG, was efficacious against fall webworm in field trials, and *Bacillus thuringiensis* Berliner formulated as Thuricide® 90 TS even caused similar larval mortality to DDT in laboratory condition (Buffam et al. 1969, Polles and Payne 1975). Other potential biological pesticides have been tested against fall webworm, including spinosyn, azadirachtin, *Metarhizium anisopliae* (Met.) (entomopathogenic fungus) and essential oils derived from eucalyptus species (Brudea et al. 2012, Tserodze and Meskhi 2012, Ebadollahi et al. 2013, Akca et al. 2014, Saruhan et al. 2014). In organic farming systems, pesticides options are limited, and pest management is generally more challenging; furthermore, in all pecan orchards, the recommended material for control of fall webworm is Dipel, an OMRI approved insecticide (Hudson et al. 2006).

The blackmargined aphid (*Monellia caryella* (Fitch)), yellow pecan aphid (*Monelliopsis pecanis* (Bissell)), and black pecan aphid (*Melanocallis caryaefoliae* (Davis)) are considered key pests of pecan in Georgia. According to Tedders (1983), in 1981, the economic losses resulting from the damages and costs of control from yellow aphid complex (blackmargined aphid and yellow pecan aphid) and black pecan aphid were ranked second and third after pecan weevil, respectively, in Georgia. The outbreaks of pecan aphids usually occur in spring and in the late season (mid-July to October); between the two outbreaks, the aphid populations remain at very low levels, and the aphids resurge at the second outbreak (Dutcher et al. 2012). Three aphid species can coexist on pecan leaves at the same time. The three aphids remove carbohydrates

from pecan and excrete honeydew which induces the growth of sooty mold fungus; in return, feeding damage by the aphids reduces the photosynthetic rate of the leaf. Black pecan aphid causes defoliation in severe cases in the late season. While the black pecan aphid is feeding on leaves, it also injects a toxin that damages the leaf tissue and creates chlorotic spots on the leaflets which ultimately lead to abscise (Ree and Knutson 1997).

The pecan aphid feeding injury can cause detrimental effects on nut production, and reduce nut yield (Wood et al. 1987). Chemical insecticides are commonly used for pecan aphid control, such as using neonicotinoids for controlling yellow aphid complex and organophosphates for controlling black pecan aphid (Dutcher et al. 2012, Brenneman et al. 2016). However, Dutcher (1998) demonstrated that after insecticides are applied, aphid control becomes ineffective by continual application. As a result, it has been observed that aphid resurgence was caused by the destruction of natural enemies by multiple applications of certain pesticides; and it has also been reported that certain pesticides have lost the efficiency to pecan aphids; for example, the pyrethroid resistance in blackmargined aphid (Dutcher and Htay 1985, Dutcher 1997). Besides these negative impacts of chemical insecticides, the environmental concerns and safety to the operators also need to be considered; and seeking an alternative approach is important. In organically managed pecan, the pest management strategies adopted should be approved by international and national organic production standards (Zehnder et al. 2007).

The objectives of the study were to (1) evaluate two OMRI (Organic Materials Review Institute) listed biopesticides, 70% Neem Oil and Entrust®, and one non-OMRI approved biopesticide, Abba Ultra® for fall webworm management in pecan; (2) to determine the potential of Abba Ultra®, 70% Neem Oil and OII-YS™ for suppressing pecan aphids in the field. We also

monitored the nontarget effects of the test materials on the densities of lacewing (predator of pecan aphids; Neuroptera: Chrysopidae) eggs in pecan canopies. Neem oil was selected for the study because it is an OMRI recommended insecticide that has repellent, anti-feedant and direct contact toxicity to insects. Entrust® and Abba Ultra® were selected for this trial because both have contact and ingestion toxicity; previous research revealed that spinosad product performed excellent control against fall webworm larvae (Brudea et al. 2012, Saruhan et al. 2014); abamectin was also found effective against some aphid species (Nada and Gaffar 2012, Gaikwad et al. 2014). OII-YS™ is a chitinase inhibitor that is derived from natural products that has shown efficacy against plant pathogens and insects. Our hypothesis was that biopesticides would significantly control pest species without decreasing lacewing eggs relative to the nontreated control.

Materials and Methods

Fall Webworm Laboratory Bioassay. The fall webworm larvae were collected from the nontreated pecan trees near Coastal Plain Experiment Station, University of Georgia Tifton campus. An extended reach pruner was used to collect the pecan shoots and leaves with fall webworms feeding on. The fall webworm larvae were picked from the collected pecan shoots and leaves by forceps, and maintained in petri dishes (10 cm×1.5 cm) under laboratory conditions (22 °C and 14 L: 10 D).

Two separate tests were conducted. In the first test, two different insecticides were used at two different rates to test the efficacy for control of fall webworm under laboratory conditions—Abba Ultra® (3.74 % abamectin, Makhteshim-Agan of North America, Inc. d/b/d ADAMA, Raleigh, NC) at 31.25, 62.5 and 78.13 ml of formulation/100 liter of water.; 70 %

Neem oil (70% clarified hydrophobic extract of neem oil, Monterey Lawn and Garden Products, Inc., Fresno, CA) at 1% and 1.5 %. In the second test, two insecticides at two rates were evaluated for efficiency—Entrust® (22.5 % spinosyn A&D, Dow agrosciences LLC, Indianapolis, IN) at 31.25 and 78.13 ml of formulation/100 liter of water, and 70 % Neem oil at 1% and 1.5 % formulation v:v in water. Abba Ultra® was evaluated at three rates—31.25, 62.5 and 78.13 ml of formulation/100 liter of water. Deionized water was use to dilute the insecticides and also served as the control treatment in both tests.

Nontreated pecan leaves were collected from the field, and rinsed and washed by deionized water. After the water on the leaves dried out, pecan leaves were dipped into the test insecticides (treatments). Control leaves were dipped in deionized water. Dipped leaves were left to dry and fed to the fall webworm larvae. Five larvae and two treated leaves were placed on the filter paper (90 mm in diameter, Waterman International Ltd., Maidstone, UK) in a petri dish. Each petri dish was covered with lid, and also sealed with Parafilm M® (Pechiney Plastic Packaging, Chicago, IL) to reduce water loss, but few small openings were made on the Parafilm M® to allow slightly air flow. For each replicate, if the treated leaves were all consumed, untreated leaves were provided until pupation. Each treatment was replicated five times. The same methods were applied to two separate experiments conducted on June 16th and 23rd in 2015.

Fall webworm larvae were checked 3 and 10 days post-treatment for the first fall webworm test; and 3 and 13 days post-treatment for the second test. The numbers of the dead larvae were recorded, and the mortality was calculated. The larva was considered dead if not moving after being probed with a paint brush. The pupation and adult emergence rates were

recorded about 6 weeks post-treatment. The efficacy of different test materials (treatments) was calculated by Abbott's formula: $\frac{\%\ \text{mortality in the treated sample} - \%\ \text{mortality in the control}}{100 - \%\ \text{mortality in the control}} \times 100\%$.

To compare the mortality, pupation and adult emergence rates obtained from different treatments, the data were analyzed using GLIMMIX procedure with Beta distribution in SAS (SAS Software 2013), and type III tests were used to determine whether the treatment effect is significant or not at $\alpha = 0.05$. Then *t*-test ($\alpha=0.05$) was used to specify the significant differences between treatments.

Field Trials of Pesticide Applications for Pecan Aphids. The field trials were carried out in 2015 at the Ponder Farm of the University of Georgia Tifton Campus located in Tift County, GA, USA. The 4.6 ha research site was planted to the pecan cultivar "Desirable" on sandy loam soil on squares at a tree density of 61 trees/ha in1986. The trees at the time of the study were 10 m in height.

Three different pesticides were applied at different rates to test the ability to suppress pecan aphid complex under field conditions—Abba Ultra® at 62.5 and 78.13 ml of formulation/100 liter of water, 70 % Neem oil at 1% and 1.5 % formulation v:v in water, and OII-YS™ (8% yucca extract (chitosans), O2YS™ corporation, Carrollton, GA) at 500 ml of formulation/100 liter of water. Water was use to dilute the pesticides and also served as the control treatment in the study. The treatments were applied to the pecan foliage on June 11th 2015 by an airblast sprayer (Streamline 823, 300 gallon air sprayer, Durand-Wayland, Inc., LaGrange, GA). Twenty-four pecan trees were selected for the trial, and in between each chosen tree, there was at least one nontreated tree that served as a buffer zone. The six treatments were randomly assigned to the selected trees in a completely randomized design with 4 replicates.

The abundances of pooled lacewing eggs and pecan aphids in pecan canopies were examined at 1 (June 12nd), 6 (June 17th) and 13 (June 24th) days after treatment. For each treated trees, five pecan terminals (shoots) were randomly collected from 4 m above the ground by extended reach pruner (STIHL® PP 100 Extended Reach Pruner, STIHL Inc., Virginia Beach, VA) for lacewing egg inspection; and one compound leaf was randomly selected from each collected shoot for pecan aphid inspection (five compound leaves in total per sample). The lacewing eggs and pecan aphid complex (yellow pecan aphid, blackmargined aphid and black pecan aphid) were enumerated.

To compare the mean numbers of lacewing eggs and pecan aphids obtained from different treatments on the indicated dates, the count data were analyzed using GLIMMIX procedure with negative binominal distribution in SAS (SAS Software 2013), and type III tests were used to determine whether the treatment effect is significant or not at $\alpha = 0.05$. Then *t*-test ($\alpha=0.05$) was used to specify the significant differences between treatments.

Results

Fall Webworm Laboratory Bioassay. In the first experiment, at 3 days post-treatment, significantly higher mortality was found in Abba Ultra® (mortality: 20%) than other treatments (both rates of Abba Ultra® vs nontreated control: $t_{20}= 2.99$, $P< 0.01$; vs both rates of 70 % Neem Oil: $t_{20}= 2.25$, $P< 0.05$), but no differences were found between the two different Abba Ultra® concentrations; very low mortality was observed in nontreated control (mortality: 0) and both the concentrations of 70 % Neem Oil, mortality rates were both 4%, and not significantly different between nontreated control (Table 2.1). At 10 days post-treatment, the mortality observed in both Abba Ultra® concentrations reached 100%, and was significantly higher than other treatments

(both rates of Abba Ultra® vs nontreated control: t_{20}= 6.43, P< 0.0001; vs 1% of 70 % Neem Oil: t_{20}= 6.31, P< 0.0001; vs 1.5% of 70 % Neem Oil: t_{20}= 6.17, P< 0.0001). Again, no significant differences were shown between the 70% Neem Oil and the nontreated control.

The mortality caused by Abba Ultra® (62.5 and 78.13 ml of formulation/100 liter of water) increased from 20 to 100% from 3 to 10 days post-treatment, while the mortality detected in the 1.5% of the 70% Neem Oil treatment only increased slightly from 4% to 12%. And the mortality of the rest two treatments (Nontreated control and 1% of the 70% Neem Oil) remained the same during both post-treatment dates.

The pupation rates in two Abba Ultra® concentrations were both 0, and were significantly lower than other treatments (both rates of Abba Ultra® vs nontreated control: t_{20}= -7.09, P< 0.0001; vs 1% of 70 % Neem Oil: t_{20}= -7.09, P< 0.0001; vs 1.5% of 70 % Neem Oil: t_{20}= -6.90, P< 0.0001); the pupation rates in nontreated control and 1% of the 70% Neem Oil treatments were both 96%, and it was 88% in the 1.5% of the 70% Neem Oil treatment, whereas there were no significant differences among these three treatments. The adult emergence rates were nearly the same as the pupation rates among all treatments; the only difference was found in the nontreated control treatment with 88% adult emergence rate caused by the failure to turn into adults from pupae.

In the second experiment, at 3 days post-treatment, both Entrust® treatments showed significantly higher mortality (40% and 52%) than other treatments (Entrust® 31.25 ml of formulation/100 liter of water vs all other treatments: t_{32}= 6.82, P< 0.0001; Entrust® 78.13 ml of formulation/100 liter of water vs all other treatments: t_{32}= 7.77, P< 0.0001), and the mortality rates in other treatments were all 0, but the mortality was not significantly different between the two Entrust® concentrations (Table 2.2). Except for the nontreated control treatment which

remained 0 mortality, the mortality in all other treatments rose from 3 to 13 days post-treatment. As a result, the mortality in Abba Ultra®— 31.25, 62.5 and 78.13 ml of formulation/100 liter of water increased from 0 to 92, 88 and 92%; 70% Neem Oil— 1 and 1.5% rose from 0 to 60 and 28%; and Entrust®— 31.25 and 78.13 ml of formulation/100 liter of water increased from 40 and 52% to 100%, respectively. At 13 days post-treatment, all treatments showed significantly higher mortality than nontreated control (Abba Ultra® at 31.25 and 78.13 ml of formulation/100 liter of water: t_{32}= 6.82, P< 0.0001; Abba Ultra® at 62.5 ml of formulation/100 liter of water: t_{32}= 6.51, P< 0.0001; 1% of 70 % Neem Oil: t_{32}= 5.10, P< 0.0001; 1.5% of 70 % Neem Oil: t_{32}= 2.89, P< 0.01; both rates of Entrust®: t_{32}= 7.13, P< 0.0001); Entrust® caused the highest mortality, and all the three concentrations of Abba Ultra® were slightly lower than Entrust®, but the mortality observed from the two pesticides was not significantly different, and no significant differences were detected within different concentrations from both two pesticides. The 70% Neem Oil treatments caused higher mortality than nontreated control, and the mortality was significantly different between the two concentrations; the low concentration caused higher mortality relatively to the high concentration (1% vs 1.5% of 70 % Neem Oil: t_{32}= 2.57, P< 0.05).

The pupation rates in the three Abba Ultra® concentrations and two Entrust® concentrations were 0, and were significantly lower than other treatments (Table 2.2) (Abba Ultra® and Entrust® vs nontreated control: t_{32}= -6.41, P< 0.0001; vs 1% of 70 % Neem Oil: t_{32}= -2.56, P< 0.05; vs 1.5% of 70 % Neem Oil: t_{32}= -4.45, P< 0.0001). The pupation rates in 70% Neem Oil treatments were significantly lower than nontreated control (1% 70% Neem Oil: t_{32}= -4.67, P< 0.0001; 1.5% 70% Neem Oil: t_{32}= -2.85, P< 0.01), and the low concentration (1% of 70% Neem Oil) had significantly lower pupation rate relatively to the high concentration (1% vs 1.5% of 70 % Neem Oil: t_{32}= -2.21, P< 0.05). The nontreated control reached 100% pupation.

The adult emergence rates were similar to the pupation rates among all treatments. However, the adult emergence rates found in the 70% Neem Oil concentrations and nontreated control treatment were slightly lower, because of the failure of turning into adults from pupae.

Field Trials of Pesticide Applications for Pecan Aphids. Lacewing egg densities in pecan canopies are given in Table 2.3. At 1 and 6 days post-treatment, no significant differences with the lacewing egg densities were found due to different treatment applications. At 13 days post-treatment, the lacewing egg densities in both Abba Ultra® at 62.5 and 78.13 ml of formulation/100 liter of water, 1.5% of 70 % Neem oil, and OII-YS™ were significantly greater than nontreated control (Abba Ultra® at 62.5 ml of formulation/100 liter of water: t_{18}= 3.36, P< 0.01, at 78.13 ml of formulation/100 liter of water: t_{18}= 3.04, P< 0.01; 1.5% of 70 % Neem oil: t_{18}= 2.21, P= 0.0405; and OII-YS™: t_{18}= 2.72, P= 0.0141) and 1% of 70 % Neem oil (Abba Ultra® at 62.5 ml of formulation/100 liter of water: t_{18}= 3.55, P< 0.01, at 78.13 ml of formulation/100 liter of water: t_{18}= 3.18, P< 0.01; 1.5% of 70 % Neem oil: t_{18}= 2.57, P< 0.05; and OII-YS™: t_{18}= 2.95, P= 0.00086).

Pecan aphid densities in pecan canopies are given in Table 2.4. At one day post-treatment, the pecan aphid densities in both Abba Ultra® concentrations were 1.25 aphids/sample, which were significantly lower than other treatments, and no significant differences were observed between two Abba Ultra® concentrations; the pecan aphid densities were 8.25 aphids/sample in nontreated control (Abba Ultra® at 62.5 and 78.13 ml of formulation/100 liter of water: t_{18}= -2.95, P= 0.0085), 7.75 aphids/sample in OII-YS™ (Abba Ultra® at 62.5 and 78.13 ml of formulation/100 liter of water: t_{18}= -2.85, P= 0.0107), 7.25 aphids/sample in 1.5% of 70 % Neem oil (Abba Ultra® at 62.5 and 78.13 ml of formulation/100 liter of water: t_{18}= -2.74, P= 0.0136), and 7.00 aphids/sample in 1% of 70 % Neem oil (Abba

Ultra® at 62.5 and 78.13 ml of formulation/100 liter of water: t_{18}= -2.68, P= 0.0154). At 6 days post-treatment, from the majority of the samples, the pecan aphid densities were 0s (17 out of 24 samples); pecan aphids were only observed from 2 out of 4 samples from nontreated control, 2 out of 4 samples from Abba Ultra® at 62.5 ml of formulation/100 liter of water, 2 out of 4 samples from 1.5% of 70 % Neem oil, and 1 out of 4 samples from OII-YS™. At 13 days post-treatment, no significant differences were found among all the treatments.

Discussion

Our two fall webworm tests indicated that both Entrust® and Abba Ultra® showed higher efficacy of fall webworm control relatively to all other treatments in both two tests. Entrust® insect-killing action was faster, as indicated by the higher mortality observed in Entrust® treatment than any other treatments at 3 days post-treatment in the second test. Previous research also revealed that spinosad product, Laser™ performed excellent control against fall webworm larvae (Brudea et al. 2012, Saruhan et al. 2014). The U.S. Environmental Protection Agency (EPA) has classified spinosad as a reduced-risk material, and Entrust® is an OMRI certified pesticide, thus it could offer one more option for fall webworm control to the organic growers (Ruiu 2015).

The efficacy of Abba Ultra® at 3 days post-treatment in both tests was 20% or even 0, but all the webworms moved slowly and stopped feeding on the leaves, and the larvae ended up having high mortality on the second assessment date. Given the voracious feeding behavior of the fall webworm, pesticides with faster action or anti-feedant effect would be better choices for fall webworm control (Polles and Payne 1975). However, contrary to Entrust®, Abba Ultra® is not an OMRI certified product approved for organic use.

Although in the second test, higher larval mortality of fall webworm was observed in 70% Neem Oil than the control on the second assessment date, the 70% Neem Oil concentrations did not show higher mortality than Entrust® and Abba Ultra® on all the assessment dates in both two tests; in addition, the pupation rate and adult emergence rate caused by 70% Neem Oil were also always higher than Entrust® and Abba Ultra®. On the contrary, according to Saruhan et al. (2014), NeemAzal T/S (1% Azadirachtin) was found effective against fall webworm larvae, and the mortality of the fall webworm was dose dependent. In our two tests, all the neem oil treated leaves were consumed during experiments; however, Akca et al. (2014) detected the anti-feedant effect of NeemAzal T/S against the fall webworm larvae from the laboratory bioassay. Some previous research indicates that the neem tree genome, the growing environment and the geographic origin of neem trees can cause the various effects of neem-derived pesticides (Isman 2006, Kraiss and Cullen 2008). This may explain the different effects of neem-derived pesticides discovered from this study and others (Liu and Liu 2005, Akca et al. 2014, Saruhan et al. 2014).

Our pecan aphid field trials indicated that Abba Ultra® provided better control of pecan aphids than 70% Neem Oil and nontreated control at 1 day post-treatment. Besides pecan aphids, abamectin was also found effective against other aphid species; for example, Nada and Gaffar (2012) indicated that the abamectin product, Vertemic® showed excellent control against cowpea aphid (*Aphis craccivora* (Koch)) under laboratory conditions; Gaikwad et al. (2014) observed that abamectin offered better control of okra aphid (*Aphis gossypii* (Glover)) relatively to nontreated control in field trials. However, in our field trials, the suppression of pecan aphid did not last to 6 and 13 days post-treatment. By contrast, according to Tedders et al. (1984), under laboratory conditions, avermectin B_1 (≥80% avermectin B_1a and ≤20% avermectin B_1b) provided significant suppression of yellow pecan aphid, and offered 2 weeks residual protection of pecan

seedlings against all three pecan aphid species. The possible explanation to the short period of pecan aphid suppression could be the nature of abamectin, which is photodegradable in sunlight (Peterson et al. 1996, Escalada et al. 2008). As a result, in Tift County, GA, the daylight hours in June 2015 were approximately 14 hours per day, thus the long daylight hours might present more chances of the exposure of the pecan leaves to sunlight (The United States Naval Observatory).

The 70% Neem Oil treatments did not show better pecan aphid control than the nontreated control treatment in our field trials. However, some previous studies demonstrated that neem-derived products were effective against different aphid species; Azatrol®, Triple Action Neem Oil (70% Neem Oil) and Pure Neem Oil (100 % Neem Oil) were found highly effective against green peach aphid (*Myzus persicae* (Sulzer)) after sprayed on the foliage of sweet pepper plants under greenhouse conditions (Shannag et al. 2014); aqueous extract of neem seeds caused nymphal mortality, and reduced the survival period and fecundity of cotton aphid (*A. gossypii*) (Santos et al. 2004); Neemix® and neem seed oil caused high nymphal mortality of soybean aphid (*Aphis glycines* (Matsumura)) and increased its development time to adulthood (Kraiss and Cullen 2008). The different results between previous research and our study could also be attributed to the neem tree genome, the growing environment and the geographic origin of neem trees, which lead to the various effects of neem-derived pesticides (Liu and Liu 2005, Isman 2006, Kraiss and Cullen 2008).

OII-YS™ is a natural adjuvant that contains Chitosan, and the product is said to increase plant's innate defenses and enhance plant health, vitality and vigor (O2YS Corporation 2016). In our study, OII-YS™ had no significant effects on pecan aphid control and the densities of lacewing eggs in pecan canopies. The effect on pecan aphid suppression is unknown when OII-

YS™ is mixed with other materials, but according to our results, OII-YS™ did not show non-target effects, which in our study would be observed as a reduction of the lacewing egg density.

Compared with nontreated control, the Abba Ultra® and 70% Neem Oil treatments did not cause significant reduction of the lacewing egg densities in pecan canopies. Although in comparison with chemical pesticides, biopesticides are usually safer to nontarget organisms and the environment, further research is needed to demonstrate whether these test biopesticides have any nontarget effects on other beneficial insects of pecan (EPA 2016). Besides lacewings, some lady beetle species are also important pecan aphid predators. Previous research indicated that the neem-derived product, Neemix® increased the development time of the first and third instar *Harmonia axyridi*s (Pallas) larvae to adult, but had no effects on adult *H. axyridis*; Neemix® caused the delay of pupation in *Coccinella septempunctata* (L.) and deformation of wings and elytra in adults, but the LC_{50} values for first and fourth instars were much higher than the recommended rate for pea aphid (*Acyrthosiphon pisum* (Harris)) control (Banken and Stark 1997, Kraiss and Cullen 2008).

Overall, in our study, we have observed some biopesticides (including Entrust®, an OMRI approved pesticide) that were effective for fall webworm control, but further field trials need to be carried out to test the efficacy of the products in the field conditions. In spite of the fact that 70% Neem Oil did not show better pecan aphid control than nontreated control in our field trials, based on previous research on other aphid species, other neem-derived products might have the potential for pecan aphid control, but further research is needed to test the effects. Abba Ultra® only showed short period of pecan aphid suppression, thus future research could focus on how to extend the pecan aphids suppression period. To employ the biopesticides into

practical use in the pecan field, the nontarget effects on more varieties of beneficial species in the pecan field need to be monitored.

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Table 2.1 Mortality, pupation rate and adult emergence rate of fall webworm larvae, and efficacy with Abba Ultra® (62.5 and 78.13 ml of formulation/100 liter of water), 70 % Neem oil (1 and 1.5 %) and nontreated control[1].

Test Materials	Concentration	Mortality		Efficacy[2]	Pupation Rate	Adult Emergence Rate
		Day 3	Day 10			
Nontreated Control	-	0 b	0 b	-	96% a	88% a
Abba Ultra®	62.5 ml /100 l	20% a	100% a	100%	N/A	N/A
	78.13 ml /100 l	20% a	100% a	100%	N/A	N/A
70% Neem Oil	1%	4% b	4% b	4%	96% a	96% a
	1.50%	4% b	12% b	12%	88% a	88% a

[1]Different letters in the same column indicate statistically significant differences among different treatments. (*t*-test; α=0.05)

[2]The efficacy was calculated by Abbott's formula: $\frac{\%\ \text{mortality in the treated sample} - \%\ \text{mortality in the control}}{100 - \%\ \text{mortality in the control}} \times 100\%$.

Table 2.2 Mortality, pupation rate and adult emergence rate of fall webworm larvae, and efficacy with Abba Ultra® (31.25, 62.5 and 78.13 ml of formulation/100 liter of water), 70 % Neem oil (1 and 1.5 %), Entrust® (31.25 and 78.13 ml of formulation/100 liter of water) and nontreated control[1].

Test Materials	Concentration	Mortality		Efficacy[2]	Pupation Rate	Adult Emergence Rate
		Day 3	Day 13			
Nontreated Control	-	0 b	0 d	-	100% a	92% a
Abba Ultra®	31.25 ml /100 l	0 b	92% a	92%	0 d	0 c
	62.5 ml /100 l	0 b	88% ab	88%	0 d	0 c
	78.13 ml /100 l	0 b	92% a	92%	0 d	0 c
70% Neem Oil	1%	0 b	60% b	60%	40% c	36% b
	1.5%	0 b	28% c	28%	52% b	44% b
Entrust®	31.25 ml /100 l	40% a	100% a	100%	N/A	N/A
	78.13 ml /100 l	52% a	100% a	100%	N/A	N/A

[1]Different letters in the same column indicate statistically significant differences among different treatments. (*t*-test; α=0.05)

[2]The efficacy was calculated by Abbott's formula: $\frac{\%\ \text{mortality in the treated sample} - \%\ \text{mortality in the control}}{100 - \%\ \text{mortality in the control}} \times 100\%$.

Table 2.3 Mean numbers of lacewing eggs per 5 shoots in different pesticide treatments[1].

Test Materials	Concentration	June 12, 2015	June 17, 2015	June 24, 2015
Nontreated Control	-	5.50 a	6.50 a	1.00 b
Abba Ultra®	62.5 ml /100 l	5.50 a	5.25 a	6.75 a
	78.13 ml /100 l	5.00 a	8.00 a	5.25 a
70% Neem Oil	1%	7.75 a	6.75 a	0.50 b
	1.50%	7.25 a	7.25 a	3.50 a
OII-YS™	500 ml /100 l	8.75 a	6.00 a	4.50 a

[1]Means for treatments in the same sampling date that are labeled with different letters are significantly different. (GLIMMIX procedure; *t*-test, α=0.05)

Table 2.4 Mean numbers of pecan aphids per 5 leaves in different pesticide treatments[1].

Test Materials	Concentration	June 12, 2015	June 17, 2015	June 24, 2015
Nontreated Control	-	8.25 a	5.00 a	3.00 a
Abba Ultra®	62.5 ml /100 l	1.25 b	3.50 a	2.00 a
	78.13 ml /100 l	1.25 b	0 a	3.50 a
70% Neem Oil	1%	7.00 a	0 a	3.00 a
	1.50%	7.25 a	14.00 a	2.50 a
OII-YS™	500 ml /100 l	7.75 a	9.75 a	3. 00 a

[1]Means for treatments in the same sampling date that are labeled with different letters are significantly different. (GLIMMIX procedure; *t*-test, $\alpha=0.05$)

CHAPTER 3

EVALUATION OF INTERCROPS FOR BIOLOGICAL CONTROL OF YELLOW PECAN APHID, BLACKMARGINED APHID AND BLACK PECAN APHID IN A "DESIRABLE" CV. PECAN ORCHARD

[1]Liu, T. and J.D. Dutcher. 2016. To be submitted to *Biological Agriculture & Horticulture*.

Abstract

To test cultural practices that have been suggested to improve biological control of the pecan aphid complex, intercrop trials were conducted from 2014 to 2016 in a 'Desirable' pecan orchard in Tift County, GA. Comparison of cool-season intercrops of white Dutch clover (*Trifolium repens* L.) plus hairy vetch (*Vicia villosa* Roth), joint vetch (*Aeschynomene americana* L.) alone, white Dutch clover alone, hairy vetch alone, and mowed sod were evaluated, and intercrops of hairy vetch alone and white Dutch clover plus hairy vetch harbored more alternate prey aphids and lady beetles than mowed sod. Only white Dutch clover plus hairy vetch had a lower abundance of pecan aphids in the canopy than mowed sod. In the cool-season intercrop trial in 2016, white Dutch clover produced more abundant alternate prey aphids than mowed sod, and hairy vetch and white Dutch clover plus hairy vetch had higher abundances of both alternate prey aphids and predatory lady beetles; however, only hairy vetch intercrop was associated with lower abundances of pecan aphids in the canopy. Joint vetch was not evaluated in 2016. In the warm-season intercrop trial 2015, mowed sod, hairy indigo (*Indigofera hirsuta* (L.)) alone and a mixed planting of hairy indigo plus buckwheat (*Fagopyrum esculentum* Moench, variety: Mancan) plus alyce clover (*Alysicarpus vaginalis* L. DC.) were compared, and cowpea aphid (*Aphis craccivora* (Koch)) was observed in all intercrop treatments. Multicolored Asian lady beetle (*Harmonia axyridi*s (Pallas)) was observed in hairy indigo; both *H. axyridi*s and convergent lady beetle (*Hippodamia convergens* (Guerin-Meneville)) were observed in hairy indigo plus buckwheat plus alyce clover and mowed sod; *Coleomegilla maculata* (DeGeer) was found in hairy indigo plus buckwheat plus alyce clover. Lower pecan aphid density was observed in hairy indigo plus buckwheat plus alyce clover. Although the intercrop studies indicated that some intercrops have potential to sustain abundant lady beetles and alternate prey aphids, the

evidence of pecan aphid biological control enhancement is not compelling, because on most of the sampling dates, no significant differences with pecan aphid density were found between mowed sod and other intercrop treatments.

Key words: white Dutch clover, hairy vetch, joint vetch, hairy indigo, buckwheat, alyce clover

Introduction

The blackmargined aphid (*Monellia caryella* (Fitch)), yellow pecan aphid (*Monelliopsis pecanis* (Bissell)), and black pecan aphid (*Melanocallis caryaefoliae* (Davis)) are considered key pests of pecan (*Carya illinoinensis* (Wangenheim) K. Koch) in Georgia. The three aphids remove carbohydrates from pecan, excrete honeydew and induce the growth of sooty mold fungus, which leads to the reduction of photosynthetic rate. In consequence, the pecan aphid damages can cause detrimental effects on nut production, and reduce nut yield (Wood et al. 1987).

Chemical insecticides are commonly used for pecan aphid control, such as using neonicotinoids for controlling yellow aphid complex and organophosphates for controlling black pecan aphid (Dutcher et al. 2012). However, Dutcher (1998) demonstrated that after insecticides are applied, aphid control becomes ineffective by continual applications. As a result, it has been observed that aphid resurgence was caused by the destruction of natural enemies by multiple applications of certain pesticides; and it has also been reported that certain pesticides have lost the efficiency to control pecan aphids; for example, the pyrethroid resistance in blackmargined aphid (Dutcher and Htay 1985, Dutcher 1997). Other than relying mainly on chemical control for aphids, there are some alternatives. Biological control with natural and introduced aphidophagous insects is a viable option (Dutcher 1998, Dutcher 2004, Dutcher et al. 2012).

Plant diversity in pecan orchards can be increased by intercrop planting. Groundcovers may provide the beneficial species with alternate food sources, serve as shelters for oviposition and defense against secondary predators, and offer the microclimate that favors the development of beneficial insects (Ellington et al. 1995).

Cool-season intercrops are planted after pecan harvest. Before the next pecan production season starts, they serve as shelters, and provide alternate prey for beneficial insects when pecan aphids are absent from pecan trees (Bugg et al. 1990). Bugg et al. (1990) found that *Coccinella septempunctata* (L.) and *Hippodamia convergens* (Guerin-Meneville) reproduced and were more abundant on the intercrops that also supported alternate prey aphids. Furthermore, a diversity of different alternate prey aphid species has been found from various understory covers: hairy vetch (*Vicia villosa* (Roth)) and crimson clover (*Trifolium incarnatum* (L.)) harbor pea aphid (*Acyrthosiphon pisum* (Harris)) and blue alfalfa aphid (*Acyrthosiphon kondoi* (Shinji)); rye (*Secale cereal* (L.)) sustains bird cherry-oat aphid (*Rhopalosiphum padi* (L.)) (Bugg et al. 1991a, Dutcher 1998). Those crops also support some thrips species. Lady beetles, especially *Olla v-nigrum* (Mulsant) prey on thrips after alternate aphid populations drop because of heavy rains and subsequent outbreaks of fungal pathogens (Bugg et al. 1990).

Warm-season intercrops are planted during pecan growing season in attempt to provide continuous biological control after cool-season intercrops. For example, cowpea (*Vigna unguiculata* ssp.), hairy indigo (*Indigofera hirsuta* (L.)) and sesbania (*Sesbania exaltata* [Rafinesque-Schmaltz] Cory) harbor cowpea aphid (*Aphis craccivora* (Koch)); while sorghum (*Sorghum bicolor* (L.)) supports corn leaf aphid (*Rhopalosiphum maidia* (Fitch)); and sesbania also sustains bandedwinged whitefly (*Trialeurodes abutilonea* (Haldenman)) (Bugg and Dutcher 1989, Bugg et al. 1991b). Aphidophagous insects observed in warm-season crops include

coccinellid beetles (*C. septempunctata*, *H. convergens*, *O. v-nigrum* and *Cycloneda sanguinea*) and some syrphid flies (Bugg and Dutcher 1989).

In spite of all the positive impacts on biological control of pecan aphid, intercrop selection is still challenging due to some drawbacks. Understory groundcover might raise the concern of water competition between pecan and groundcover, especially for warm-season legumes. Aside from being a reservoir of beneficial insects, intercrops also provide resources for generalist stink bugs that also damage pecan nuts. For instance, sesbania cultures abundant alternate prey and sustains ladybeetles; however, it also has the disadvantage of harboring stink bugs that injure pecan kernels (Bugg et al. 1991b, Dutcher 1998, Mizell III 2003, Dutcher et al. 2006).

In pecan orchards, the best selections of the intercrop vary from orchard to orchard because of different climate conditions, soil nutrition requirements, and alternate species needed, etc. (Bugg et al. 1991b, Dutcher 1998). Although there are some tradeoffs and side effects of adopting intercrop, planting intercrop still plays an important role in biological control in sustainable or organic pecan production, as long as the drawbacks are diminished by proper intercrop selection and management (Bugg et al. 1991b, Diver and Ames 2000, Zehnder et al. 2007).

The objectives of this study were (1) to determine the effects of different intercrops on the enhancement of alternate prey aphids and beneficial insects (lady beetles) in the intercrops; (2) to evaluate different intercrops for pecan aphid suppression in pecan canopies. The hypothesis was that intercrops would harbor higher numbers of lady beetles and alternate prey aphids, and also maintain higher abundances of lady beetles and lower densities of pecan aphids in the pecan trees relative to a mowed sod check.

Materials and Methods

All the intercrop studies were conducted from 2014 to 2016 at the Ponder Farm of the University of Georgia Tifton Campus located in Tift County, GA, USA. About 12.3 acre of the farm was used for the intercrop studies, and it consists of approximately 300 thirty-year-old pecan trees planted to the cultivar "Desirable" on sandy loam soil. The pecan trees in the research farm were planted in rows with approximately 12-m tree intervals, and in between each tree row, there is an approximately 7-m-wide vegetation area (aisle) that received the intercrop seeds in our studies.

Cool-season intercrop studies. Two separate intercrop trials were conducted in 2014/2015 and 2016.

Cool-season intercrop trial 2014/2015: The intercrops planted were (1) white Dutch clover (*Trifolium repens* L.) plus hairy vetch (*Vicia villosa* Roth) mixture, (2) joint vetch (*Aeschynomene americana* L.), (3) white Dutch clover alone and (4) hairy vetch alone. The mowed sod which received no seeds served as the control treatment. Except for white Dutch clover which was already per-inoculated, other legumes were mixed with seed inoculant (Guard-N inoculant, INTX Microbials, LLC, Kentland, IN) prior to seeding. The intercrops were broadcasted by the handheld seed spreader (EarthWay® 2750 nylon bag handheld spreader, 25 lb., Earthway Product Inc., Bristol, IN) in December 2014. Each intercrop was planted in plots (3×3 trees, with 9 trees per plot), containing 2 vegetation aisles where the intercrops were seeded. The experiment was carried out in a completely randomized design with three replicates (plots) for each treatment.

Insects in the intercrops were assessed by sweep sampling. On May 12th, 19th and 29th in 2015, fifty back and forth sweeps were taken from each plot (25 sweeps per aisle) by sweep net

(15 in. dia., BioQuip, Rancho Dominguez, CA). The alternate prey aphids and pooled lady beetles (larvae, pupae and adults) obtained from sweep samples were enumerated, and the alternate prey aphids and adult lady beetles were identified to species. The densities of pooled lady beetles and pecan aphids—blackmargined aphid, yellow pecan aphid and black pecan aphid, in pecan canopies were examined on June 5th, 12th, 19th, 25th and July 2nd in 2015 after the intercrops were mown. On the first sampling date (June 5th), except for hairy vetch, two plots were randomly selected from each treatment, and two trees were randomly chosen from each selected plot for pecan aphid and lady beetle sampling; for the hairy vetch, two trees were randomly selected from each plot for sampling. On the rest 4 sampling dates (June 12th, 19th, 25th and July 2nd), one tree was randomly chosen form each plot for sampling. Five terminals (shoots) were randomly collected from 4 m above the ground by extended reach pruner (STIHL® PP 100 Extended Reach Pruner, STIHL Inc., Virginia Beach, VA) for lady beetle inspection; and one compound leaf was randomly selected from each collected terminal for pecan aphid inspection (five compound leaves in total per sample).

Cool-season intercrop trial 2016: The intercrops planted were (1) white Dutch clover plus hairy vetch mixture, (2) white Dutch clover alone, (3) hairy vetch alone, and (4) mowed sod. Except for white Dutch clover which was already per-inoculated, hairy vetch were mixed with seed inoculant (Guard-N inovulanT, Verdesian Life Sciences LLC, Cary, NC) prior to seeding. The intercrops were broadcasted by tow broadcast spreader (EarthWay® 2050TP estate tow broadcast spreader, Earthway Product Inc., Bristol, IN) in late January 2016. Each intercrop was planted in plots (3×3 trees, with 9 trees per plot), containing 2 vegetation aisles where the intercrops were seeded. The experiment was carried out in a completely randomized design with five replicates (plots) for each treatment.

Insect abundances in the intercrops were assessed by sweep sampling. On March 9th, 24th and 31st; April 7th, 19th and 28th; and May 9th, 23rd and 26th in 2016, fifty back and forth sweeps were taken from each plot (25 sweeps per aisle) by sweep net. The alternate prey aphids and pooled lady beetles (larvae, pupae and adults) obtained from sweep samples were enumerated, and the alternate prey aphids and adult lady beetles were identified to species. The densities of pooled ladybeetles and pecan aphids—blackmargined aphid, yellow pecan aphid and black pecan aphid, in pecan canopies were examined on May 2nd, 9th, 23rd and 26th; June 2nd, 9th, 16th, 23rd and 30th; and July 7th, 14th, 21st and 28th in 2016. Intercrops were mown on May 27th. On each indicated sampling date, the central tree was chosen from each plot for pecan aphid and lady beetle sampling. Five terminals (shoots) were randomly collected from 4 m above the ground by extended reach pruner for lady beetle inspection; and one compound leaf was randomly selected from each collected shoot for pecan aphid inspection (five compound leaves in total per sample).

Warm-season intercrop study. The warm-season intercrop trial was conducted in 2015. Intercrops sown in late July were (1) hairy indigo and (2) hairy indigo plus buckwheat (*Fagopyrum esculentum* Moench, variety: Mancan) plus alyce clover (*Alysicarpus vaginalis* L. DC.) mixture, and (3) mowed sod (which received no seeds). Hairy indigo and alyce clover were mixed with seed inoculant (Guard-N inoculant, INTX Microbials, LCC, Kentland, IN) prior to seeding. The intercrops were broadcasted by tow broadcast spreader. Each intercrop was planted in the adjacent two rows (vegetation aisles) with one tree row containing approximately 11 pecan trees in between the intercrops. There was a non-seeded vegetation aisle that separated two different types of intercrop treatments.

Insects in the intercrops were assessed by sweep sampling on September 17th; October 7th, 21st and 28th; and November 4th in 2015. Fifty back and forth sweeps were taken from each

intercropped row by sweep net. The species of alternate aphids and adult lady beetles obtained from sweep samples were identified. The pecan aphids and lady beetles in pecan canopy were examined on September 17th and 25th; and October 2nd and 7th in 2015. On each indicated sampling date, four trees were randomly chosen from pecan trees planted in between the same type of intercrop (treatment) rows for pecan aphid and lady beetle sampling. Five terminals were randomly collected from 4 m above the ground by extended reach pruner for lady beetle inspection; and one compound leaf was randomly selected from each collected terminal for pecan aphid inspection (five compound leaves in total per sample).

Statistical Analyses. To compare the insect counts obtained from different intercrops and pecan canopies on the indicated dates, the count data were analyzed using GLIMMIX procedure with negative binominal distribution in SAS (SAS Software 2013), and type III tests were used to determine whether the treatment effect is significant or not at $\alpha = 0.05$. Then *t*-test ($\alpha=0.05$) was used to specify the significant differences between treatments.

Results

Cool-season intercrop trial 2014/2015. Five species of lady beetles were observed in the sweep samples, including *H. convergens*, *C. septempunctata*, *Cycloneda munda* (Say), *Coleomegilla maculata* (DeGeer) and *Harmonia axyridi*s (Pallas). All the intercrops supported *H. convergens* and *C. septempunctata*. However, only hairy vetch and white Dutch clover harbored *C. munda*; white Dutch clover plus hairy vetch and white Dutch clover alone sustained *C. maculata*; and white Dutch clover plus hairy vetch and hairy vetch alone supported *H. axyridi*s.

Pooled lady beetle counts in intercrops (Figure 3.1 and Table 3.1). On May 12th, the mean numbers of pooled lady beetles (per sweep sample) observed in white Dutch clover plus hairy vetch and hairy vetch alone were significantly higher than mowed sod (t_{10}= 2.66, P< 0.05; t_{10}= 3.14, P< 0.05); while joint vetch and white Dutch clover alone showed no significant differences with mowed sod. Pooled ladybeetle abundance in joint vetch was significantly higher than white Dutch clover alone (t_{10}= 2.67, P< 0.05). On May 19th, none of the intercrops showed significantly greater mean abundances of pooled lady beetles than mowed sod; white Dutch clover plus hairy vetch and hairy vetch alone were significantly higher than white Dutch clover alone (t_{10}= 2.49, P< 0.05; t_{10}= 2.42, P< 0.05). On May 29th, no significant differences were shown among all treatments.

Pea aphid and green peach aphid (*Myzus persicae* (Sulzer)) were identified as alternate prey aphids in the sweep samples, but green peach aphids were only found from joint vetch, white Dutch clover alone and mowed sod on May 19th, and the number was very low compared to pea aphid. Mean abundances on May 19th were 0.33 green peach aphids/sample and 2.67 pea aphids/sample in joint vetch; 3.67 green peach aphids/sample and 11.33 pea aphids/sample in white Dutch clover alone; and, 2.67 green peach aphids/sample and 30.67 pea aphids/sample in mowed sod.

Alternate prey aphid counts in intercrops (Figure 3.2 and Table 3.2). On May 12th, the mean abundances of alternate prey aphids (per sweep sample) found in white Dutch clover plus hairy vetch and hairy vetch alone were significantly higher than mowed sod (t_{10}= 3.13, P< 0.05; t_{10}= 3.31, P< 0.01); but joint vetch and white Dutch clover alone were not significantly different from mowed sod; and joint vetch also showed no significant differences with white Dutch clover plus hairy vetch and hairy vetch alone. On May 19th, no significant differences were found

between any treatments; and on May 29th, very low numbers of alternate prey aphids were observed (only found in one of the white Dutch clover plus hairy vetch samples).

Pooled lady beetle and pecan aphid densities in trees in different intercrop plots (Figure 3.3 and Table 3.3). The mean densities of pooled lady beetles (mean numbers of pooled lady beetles per 5 shoots) that we observed were very low; the highest density was 1 in hairy vetch on June 25th. And, in the majority of the samples, no lady beetles were observed; there were 82 samples in total, but lady beetles were only found in 10 samples. No significant differences in the mean densities of lady beetles were detected due to the different types of intercrop treatments in all sampling dates.

On June 5th and 12th, the mean numbers of pecan aphids per 5 leaves were significantly lower in white Dutch clover plus hairy vetch than in mowed sod (t_{17}= -2.15, P= 0.0462 on June 5th; t_{10}= -2.43, P< 0.05 on June 12th); whereas there were no significant differences among joint vetch, white Dutch clover, hairy vetch and mowed sod treatments; and also no significant differences were detected among white Dutch clover plus hairy vetch, joint vetch, white Dutch clover alone and hairy vetch alone. On the rest of the sampling dates (June 19th, 25th and July 2nd), no significant differences in the mean densities of pecan aphids in the tree canopy were found due to different types of intercrop treatments.

Cool-season intercrop trial 2016. Five species of lady beetles were observed in the sweep samples, including *H. convergens*, *C. septempunctata*, *O. v-nigrum*, *C. maculata* and *H. axyridi*s. All the intercrops harbored *H.* convergens, *C. septempunctata* and *H. axyridi*s. However, only hairy vetch alone and white Dutch clover alone supported *C. maculata*; white Dutch clover plus hairy vetch and white Dutch clover alone sustained *O. v-nigrum*.

Pooled lady beetle counts in intercrops (Figure 3.4 and Table 3.4). On March 24th, 31st and May 9th, no lady beetles were observed in mowed sod; since the lady beetles counts in the mowed sod were all 0s, and there was no variation, the GLIMMIX procedure in SAS failed to interpret the data and compare the data from different treatments. Thus, the data from these three sampling dates were analyzed by SAS after dropping the mowed sod treatment. After dropping mowed sod, on March 31st and May 9th, no significant differences were found among the three treatments (white Dutch clover plus hairy vetch, white Dutch clover alone and hairy vetch alone); but on March 24th, hairy vetch alone showed significantly higher mean abundances of pooled lady beetles than white Dutch clover alone and white Dutch clover plus hairy vetch treatments (t_{12}= 2.60, P< 0.05; t_{12}= 2.76, P< 0.05).

For the rest sampling dates after March 31st, significant differences were only detected on April 28th; both hairy vetch alone and white Dutch clover plus hairy vetch showed significantly higher mean abundances of pooled lady beetles than mowed sod (t_{16}= 2.79, P< 0.05; t_{16}= 2.22, P= 0.0415), but there were no significant differences among hairy vetch alone, white Dutch clover plus hairy vetch and white Dutch clover alone.

Alternate prey aphid counts in intercrops (Figure 3.5 and Table 3.5). On March 9th, no intercrop treatments showed significantly higher mean abundances of alternate prey aphids (per sweep sample) than mowed sod; hairy vetch alone was significantly higher than white Dutch clover plus hairy vetch (t_{16}= 3.13, P< 0.01); mowed sod and white Dutch clover alone were not significantly different from hairy vetch alone and white Dutch clover plus hairy vetch. On March 24th, hairy vetch alone and white Dutch clover plus hairy vetch showed significantly higher mean abundances of alternate prey aphids than mowed sod (t_{16}= 4.02, P= 0.001; t_{16}= 3.23, P< 0.01), and white Dutch clover alone showed no significant differences with white Dutch clover plus

hairy vetch and mowed sod. On March 31st and April 7th, all intercrop treatments showed significantly higher mean numbers of alternate prey aphids than mowed sod treatment (on March 31st, white Dutch clover/hairy vetch: t_{16}= 3.89, P< 0.01; white Dutch clover alone: t_{16}= 5.73, P< 0.0001; hairy vetch alone: t_{16}= 6.20, P< 0.0001; on April 7th, white Dutch clover plus hairy vetch: t_{16}= 2.81, P< 0.05; white Dutch clover alone: t_{16}= 3.21, P< 0.01; hairy vetch alone: t_{16}= 3.63, P< 0.01); on March 31st, hairy vetch alone was significantly higher than white Dutch clover plus hairy vetch (t_{16}= 3.21, P< 0.01), while white Dutch clover alone was not significantly different from hairy vetch alone and white Dutch clover plus hairy vetch; on April 7th, no significant differences were found among the intercrop treatments (excluding mowed sod). On April 19th, the mean numbers of alternate prey aphids in hairy vetch were significantly higher than all other treatments (white Dutch clover plus hairy vetch: t_{16}= 2.33, P< 0.05; white Dutch clover alone: t_{16}= 3.69, P< 0.01; mowed sod: t_{16}= 3.79, P< 0.01); and no significant differences were observed among the rest treatments. On April 28th, only white Dutch clover alone showed significantly higher mean abundances of alternate prey aphids than mowed sod (t_{16}= 2.35, P< 0.05); white Dutch clover plus hairy vetch and hairy vetch alone showed no significant differences with white Dutch clover alone and mowed sod. On May 9th, no significant differences were detected between any treatments. On the last two sampling dates (May 23rd and 26th), no alternate prey aphids were observed in all the intercrop treatments.

Pooled lady beetle and pecan aphid densities in trees in different intercrop plots (Figure 3.6 and Table 3.6). The mean densities of pooled lady beetles (mean numbers of pooled lady beetles per 5 shoots) that we observed were very low; the highest density was 0.6 in white Dutch clover alone and hairy vetch alone on June 23th. And in the majority of the samples, no lady beetles were found; there were 260 samples in total, but lady beetles were only observed in 21

samples. In all the sampling dates, no significant differences in the mean densities of lady beetles were detected because of different intercrop treatments.

From the total 13 sampling dates, significant differences with the mean densities of pecan aphids (mean numbers of pecan aphids per 5 leaves) were only found on two sampling dates—May 26th and June 30th. On May 26th, the pecan aphid density in hairy vetch was significantly lower than mowed sod (t_{16}= -3.10, P< 0.01); however, white Dutch clover alone and white Dutch clover plus hairy vetch showed no significant differences with mowed sod and hairy vetch alone. On June 30th, hairy vetch alone had significantly lower pecan aphid abundances than white Dutch clover plus hairy vetch (t_{16}= -2.32, P< 0.05); however, no intercrops showed significantly lower pecan aphid abundances than mowed sod.

Warm-season intercrop trial 2015. Lady beetle species (Table 3.7). Three species of adult lady beetles were found in sweep samples, including *H. axyridi*s, *H. convergens* and *C. maculata*. The majority of the adult lady beetles collected were *H. axyridi*s (11 *H. axyridi*s vs 17 pooled adult lady beetles), and only one *C. maculata* was observed (on Oct. 28th) in this experiment. Most of the lady beetles observed in this experiment were obtained from the first two sampling dates (Sep. 17th and Oct. 7th); on the rest sampling dates, only one *H. axyridi*s and one *C. maculata* were found in hairy indigo plus buckwheat plus alyce clover treatment on Oct. 28th, and one *H. axyridi*s was observed from hairy indigo on Nov. 4th.

Cowpea aphid was the only alternate prey aphid species observed in this experiment. However, the cowpea aphid counts obtained from the sweep samples were very low; the highest count was 9 aphids/sample, found in one of the sweep samples in the hairy indigo plots on Oct. 21st, and the counts from other samples ranged from 0 to 4. The first cowpea aphid observed was in the hairy indigo plus buckwheat plus alyce clover treatment on Oct. 7th.

Pooled lady beetle and pecan aphid densities in trees in different intercrop treatments (Figure 3.7 and Table 3.8; Figure 3.8 and Table 3.9). On Sep. 17th, the hairy indigo plus buckwheat plus alyce clover mixed treatment showed significantly lower mean abundances of pecan aphids than the hairy indigo and mowed sod (t_9= -3.30, P< 0.0092; t_9= -4.27, P< 0.01), and no significant differences were observed between these two treatments; whereas, the hairy indigo plus buckwheat plus alyce clover treatment revealed significantly lower mean abundances of pooled lady beetles than mowed sod treatment (t_9= -2.90, P< 0.05), and hairy indigo was not significantly different from mowed sod or hairy indigo plus buckwheat plus alyce clover. On the contrary, on Sep. 25th, the pecan aphid abundances obtained in the mowed sod treatment was significantly lower than the hairy indigo and hairy indigo plus buckwheat plus alyce clover (t_9= -3.49, P< 0.01; t_9= -2.59, P< 0.05), and no significant differences were observed between these two treatments. Except for the first sampling date (Sep. 17th), no significant differences with the mean densities of pooled lady beetles were observed among all treatments on the rest sampling dates (Sep. 25th, Oct. 2nd and 7th). On Oct. 2nd, the pecan aphid density showed no significant differences among all treatments. On the last sampling date (Oct. 7th), the hairy indigo plus buckwheat plus alyce clover treatment revealed significantly lower pecan aphid density than mowed sod treatment (t_9= -2.50, P< 0.05), whereas, hairy indigo was not significantly different from mowed sod and hairy indigo plus buckwheat plus alyce clover.

Discussion

In the cool-season intercrop trial 2014/2015, both the mean abundances of pooled lady beetles and alternate prey aphids observed in hairy vetch alone and white Dutch clover plus hairy vetch on May 12th were significantly higher than mowed sod; the mean abundances of pooled

lady beetles in hairy vetch (30.00 beetles/sample) and white Dutch clover plus hairy vetch (23.00 beetles/sample) were 6 and 4.6 times greater than mowed sod (5.00 beetles/sample), respectively; and the mean number of alternate prey aphids in hairy vetch (169.67 aphids/sample) and white Dutch clover plus hairy vetch (136.00 aphids/sample) were 56.56 and 45.33 greater than mowed sod (3.00 aphids/sample), respectively. However, on the rest sampling dates (May 19th and 29th), no intercrops showed significantly higher mean abundances of pooled lady beetles and alternate prey aphids than mowed sod. On June 5th and 12th, the mean abundances of pecan aphids in pecan canopies in white Dutch clover plus hairy vetch were significantly lower than mowed sod. In the cool-season intercrop trial 2014/2015, white Dutch clover plus hairy vetch showed the ability to sustain higher abundances of pooled lady beetles and alternate prey aphids, and also keep the pecan aphid density at lower levels on certain assessment dates. By contrast, hairy vetch alone also showed the ability to sustain higher numbers of pooled lady beetles and alternate prey aphids, but it did not improve pecan aphid biological control.

In the cool-season intercrop trial 2016, on March 24th, 31st and May 9th, we are not able to compare the mean numbers of pooled lady beetles observed in mowed sod with other treatments, because SAS failed to interpret the results, and on March 31st and May 9th, no significant differences were observed among other three treatments; however, on these three sampling dates, all other treatments had higher mean numbers of pooled lady beetles than mowed sod, which remained 0 on all three sampling dates. The mean abundances of pooled lady beetles observed in hairy vetch were significantly higher than mowed sod on 1 out of 9 sampling dates, and the mean abundances of alternate prey aphids were always the highest among all other treatments on the first 5 sampling dates (with 4 out of 9 sampling dates significantly higher than mowed sod). In

white Dutch clover plus hairy vetch, the mean abundances of pooled lady beetles and alternate prey aphids were significantly higher than mowed sod on 1 out of 9, and 3 out of 9 sampling dates, respectively. In white Dutch clover alone, the mean abundances of alternate prey aphids were significantly higher than mowed sod on 3 out of 9 sampling dates; but no significant differences with the mean abundances of pooled lady beetles were observed between white Dutch clover and mowed sod. However, on the last four sampling dates (April 28th, May 9th, 23rd and 26th) only very low numbers of alternate prey aphids were observed (the highest is 8.2 in white Dutch clover). This phenomenon could be attributed to the senescence of the intercrops. In spite of the better ability of sustaining lady beetles and alternate prey aphids in some intercropped plots than mowed sod on some sampling dates, the pecan aphid abundances observed on 12 out of 13 sampling dates did not show significant differences among all treatments; only on May 26th, the density in hairy vetch alone were found significantly lower than mowed sod. In the cool-season intercrop trial 2016, in comparison to other intercrop treatments, hairy vetch alone was the only treatment that showed the ability to harbor higher numbers of pooled lady beetles and alternate prey aphids, and also keep the pecan aphid density at lower levels on certain assessment dates.

Based on the results of the two cool-season intercrop trials, the effects of the cool-season intercrops on the enhancement of pecan aphid biological control were not consistent in both two seasons, and on most of the sampling dates, no significant differences with the pecan aphid densities were discovered among all treatments. The pecan aphid densities were found significantly lower than mowed sod only on two sampling dates in white Dutch clover plus hairy vetch in the cool-season intercrop trial 2014/2015, and one sampling date in hairy vetch in the

cool-season intercrop trial 2016. In both two intercrop trials, the mean numbers of pooled lady beetles in intercrops were not analogous to the pooled lady beetle density in pecan canopies.

On all the sampling dates in both two cool-season intercrop trials, none of the pecan aphid density exceeded the economic thresholds, of 20 yellow aphids, and 1 black pecan aphid per compound leaf (Harris 1983, Dutcher et al. 2012). The highest density, 28.4 pecan aphids per 5 leaves, was found on June 16th in 2016 in hairy vetch alone. One of the reasons why the intercrops that harbored significantly higher lady beetles and alternate prey aphids did not perform better pecan aphid biological control could be attributed to the low levels of pecan aphid infestation that may not have been sufficient to attract and sustain the lady beetles. Another reason could be that the intercropped plots were too small or closely spaced, so the lady beetles harbored by the original intercrops could fly to pecan canopies in different intercropped plots to search for food; especially when the food sources were scarce or almost consumed in the original pecan canopy. Furthermore, the different crop fields in the vicinity with aphid infestations or any lady beetle food sources might also attract the lady beetles to migrate from the research farm to other fields. To solve this problem, a food spray of fermented molasses and baker's yeast might be helpful; previous study revealed that it was an effective attractant for ladybeetles and lacewings in pecan canopies, and the decreases of three pecan aphid species were associated with the food spray application (Dutcher 2004, Dutcher et al. 2012).

In the warm-season intercrop trial, 1 out of 4 sampling date showed significant difference with the mean densities of lady beetles in pecan canopy; but the highest one was found in mowed sod. However, significantly lower mean numbers of pecan aphids were observed in hairy indigo plus buckwheat plus alyce clover and mowed sod on two and one sampling dates, respectively. Due to the lack of replicates of intercrop planting, we are not able to compare the insects (lady

beetles and alternate prey aphids) obtained in different intercrops with the lady beetle densities in pecan canopies. Therefore, the significant higher lady beetle density in pecan canopies in mowed sod could result from the high pecan aphid density (512.50 aphids/sample on Sep. 17th) that attracted lady beetles originating from any other intercrops because of the closely spaced intercropped rows, or the ability that mowed sod had to enhance the lady beetles density and reduce the pecan aphid density in pecan canopies. In pecan canopies in hairy indigo plus buckwheat plus alyce clover, the lower mean densities of pecan aphids did not correspond with the (higher) mean densities of lady beetles. One of the possible explanations of this phenomenon is that the lady beetle sampling methods we used might not be able to precisely scout highly dispersive insects; as a matter of fact, in both two cool-season and the warm-season intercrop trials, very low lady beetle densities were observed in pecan canopies. The other is that the intercrops might also be able to sustain other aphidophaga besides lady beetles, thus, the pecan aphid density might also be affected by other species (Smith et al. 1996a). For example, Bugg and Dutcher (1989) found that buckwheat and sesbania were attractive to aphidophagous syrphid flies; Bugg et al. (1990) observed some aphidophagous syrphid flies on narrow-leafed lupin, arrowleaf clover, crimson clover, 'Cahaba White' vetch and hairy vetch; Smith et al. (1996b) demonstrated that the increased density of green lacewing, *Chrysoperla rufilabris* (Burmeister), a pecan aphid predator in pecan canopies was associated with the legume ground cover (red clover, crimson clover and hairy vetch) in the pecan orchard.

Different aphidophagous insects and alternate prey afforded by various intercrops might also have different impacts on pecan aphid biological control. For instance, *C. septempunctata* and *H. convergens* are usually collected at the ground level, whereas, *O. v-nigrum* and *H. axyridi*s are commonly found from pecan tree canopy and at the ground level (Dutcher et al.

2012). The principally arboreal *O. v-nigrum* and *H. axyridi*s play a relatively important role in biological control of pecan aphids (Bugg et al. 1991a) (Mizell III 2003). In consequence, different alternate prey is harbored by distinct intercrops, and the feeding preference for alternate prey varies among different aphidophgous species. For example, in laboratory observations, cowpea aphid does not serve as an acceptable prey for *H. axyridi*s, while it is eaten by *H. convergens* (Dutcher et al. 1999).

Overall, in our study, although we have found some intercrops having potential to sustain lady beetles and alternate prey aphids, the evidence of pecan aphid biological control enhancement is not compelling, because on most of the sampling dates, no significant differences with pecan aphid density were found between mowed sod and other intercrop treatments. Larger plot size, greater intercrop plot separation, better isolation from other aphid infested crop fields, and more aphidophagous species scouting varieties are necessary to prove the effects of intercrop planting on the enhancement of pecan aphid biological control. To adopt intercrops for pecan aphid biological control, further research needs to address the intercrop effects on the soil fertility, the risk of culturing pecan pests, and water competition between pecan and intercrops, etc.

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Figure 3.1 Mean numbers of pooled lady beetles per sweep sample in different intercrop treatments in the cool-season intercrop trial 2014/2015. * indicates where significant differences were found between treatments (GLIMMIX procedure; *t*-test, α=0.05).

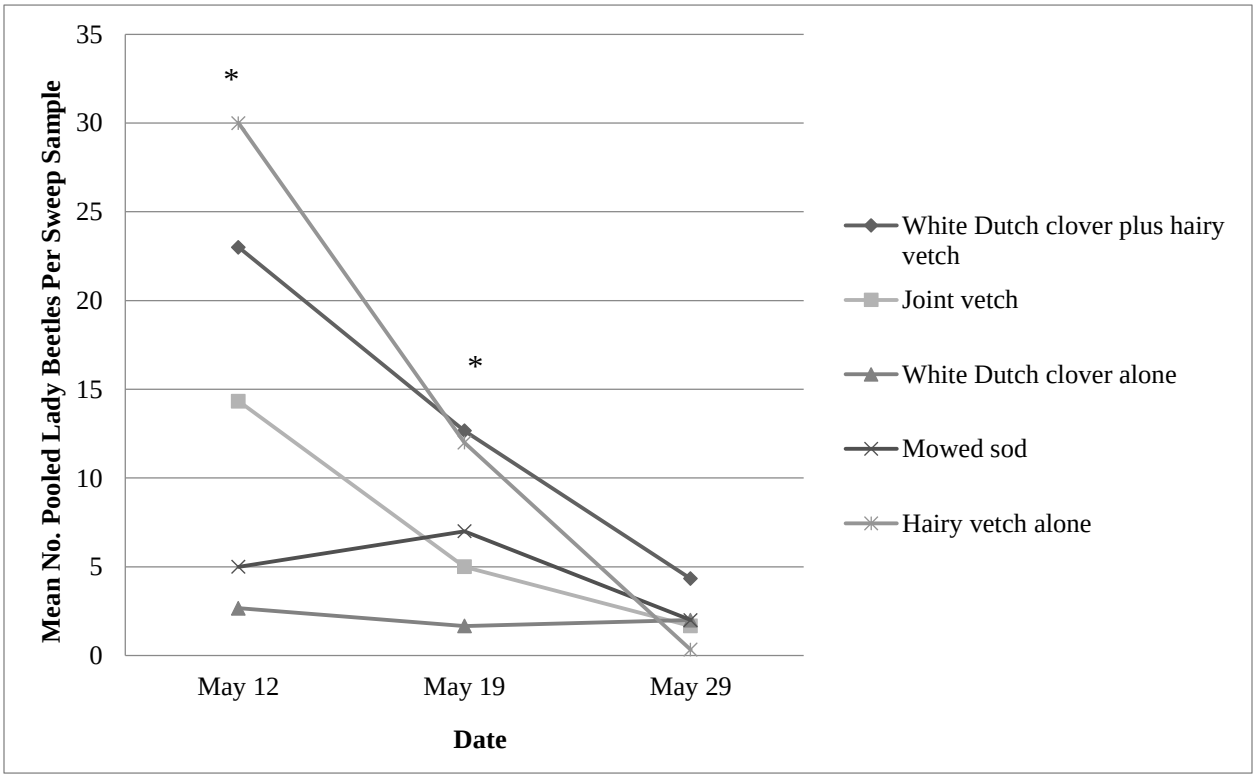

Figure 3.2 Mean numbers of alternate prey aphids per sweep sample in different intercrop treatments in the cool-season intercrop trial 2014/2015. * indicates where significant differences were found between treatments (GLIMMIX procedure; *t*-test, α=0.05).

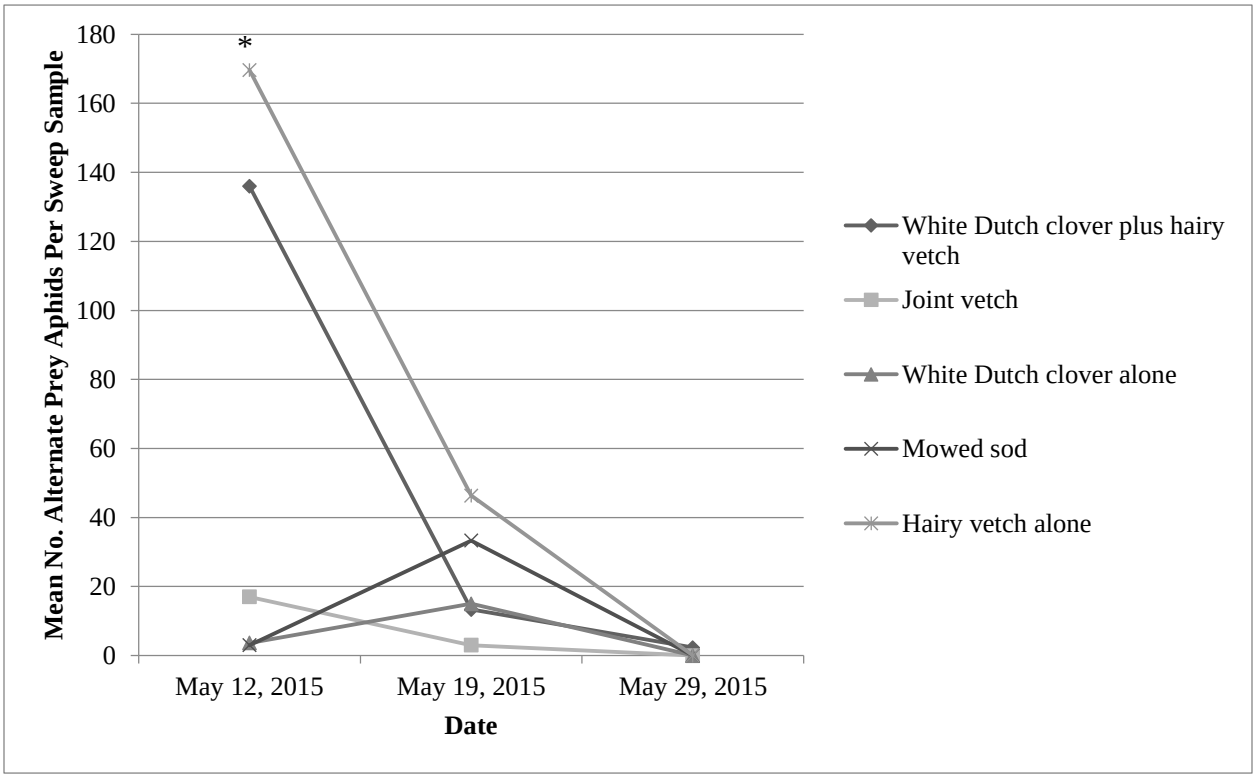

Figure 3.3 Mean numbers of pecan aphids per 5 leaves in trees in different intercrop plots in the cool-season intercrop trial 2014/2015. * indicates where significant differences were found between treatments (GLIMMIX procedure; *t*-test, α=0.05).

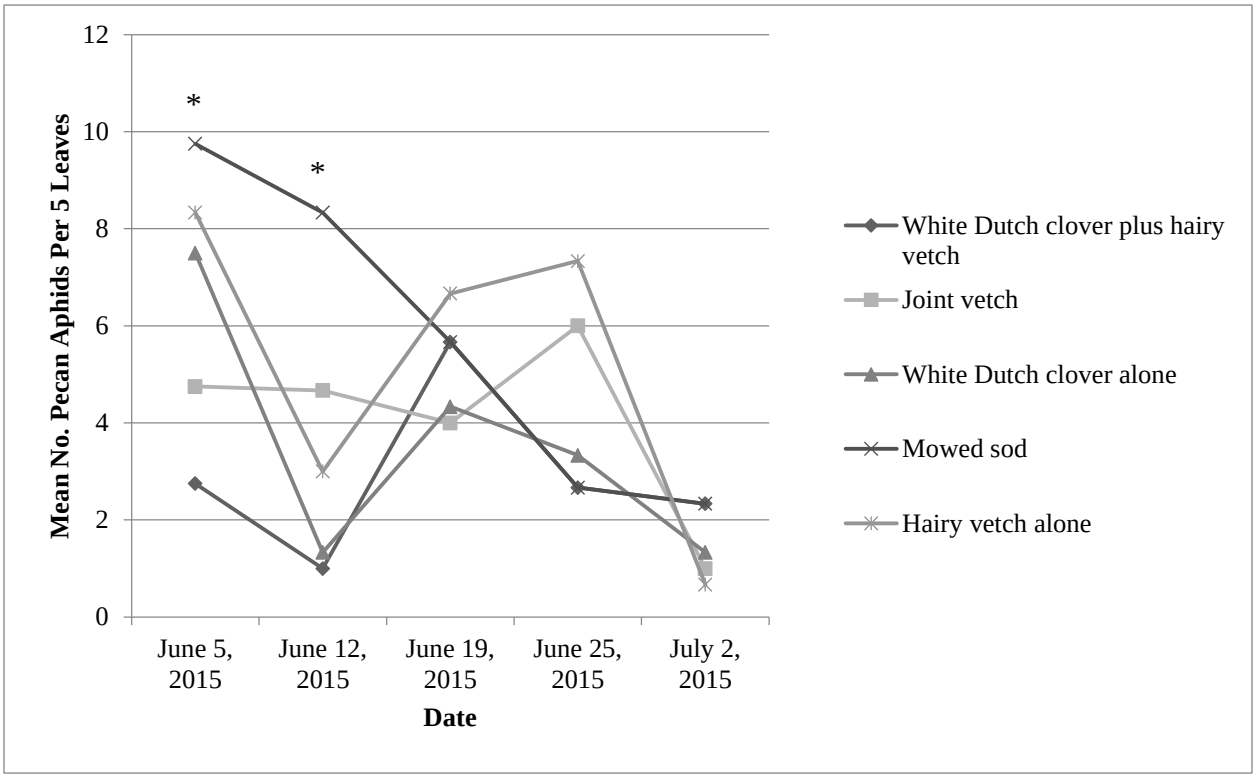

Figure 3.4 Mean numbers of pooled lady beetles per sweep sample in different intercrop treatments in the cool-season intercrop trial 2016. * indicates where significant differences were found between treatments (GLIMMIX procedure; *t*-test, α=0.05).

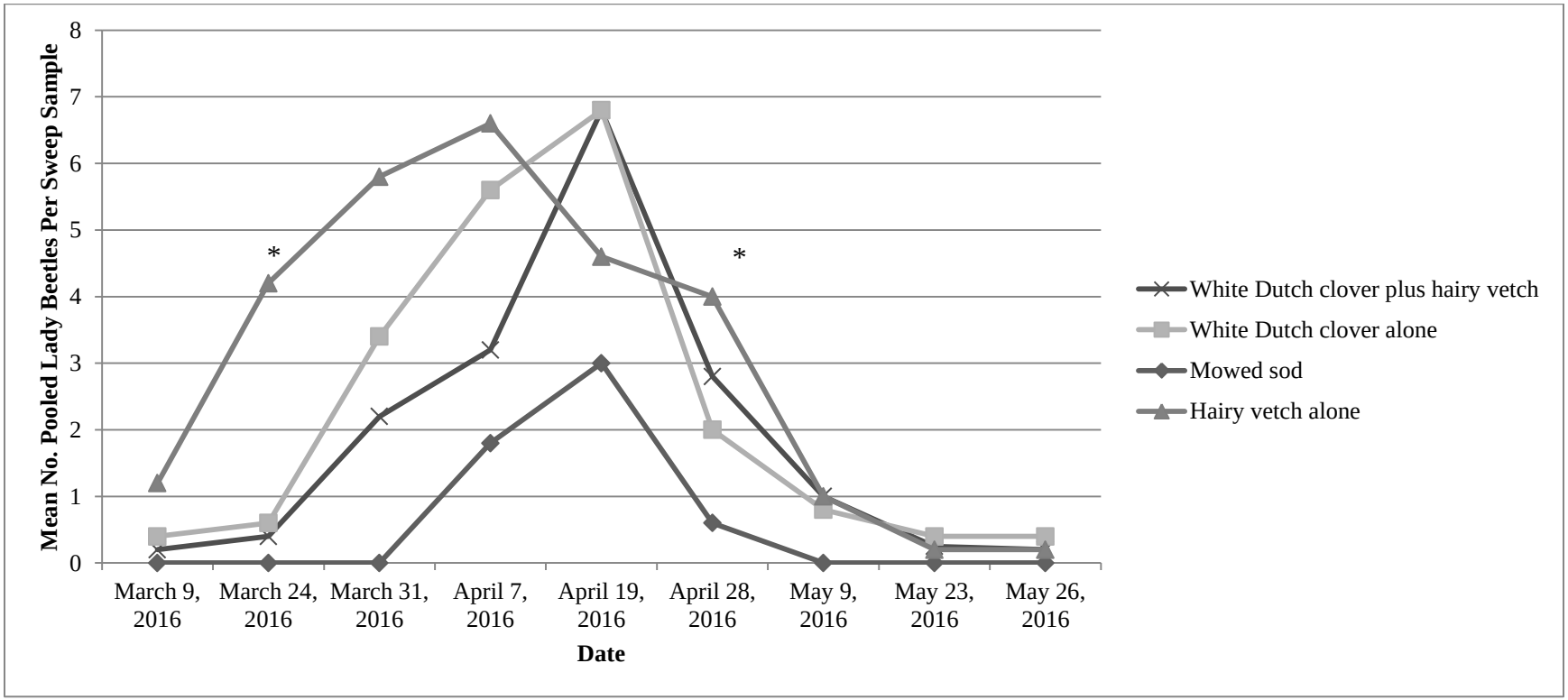

Figure 3.5 Mean numbers of alternate prey aphids per sweep sample in different intercrop treatments in the cool-season intercrop trial 2016. * indicates where significant differences were found between treatments (GLIMMIX procedure; *t*-test, α=0.05).

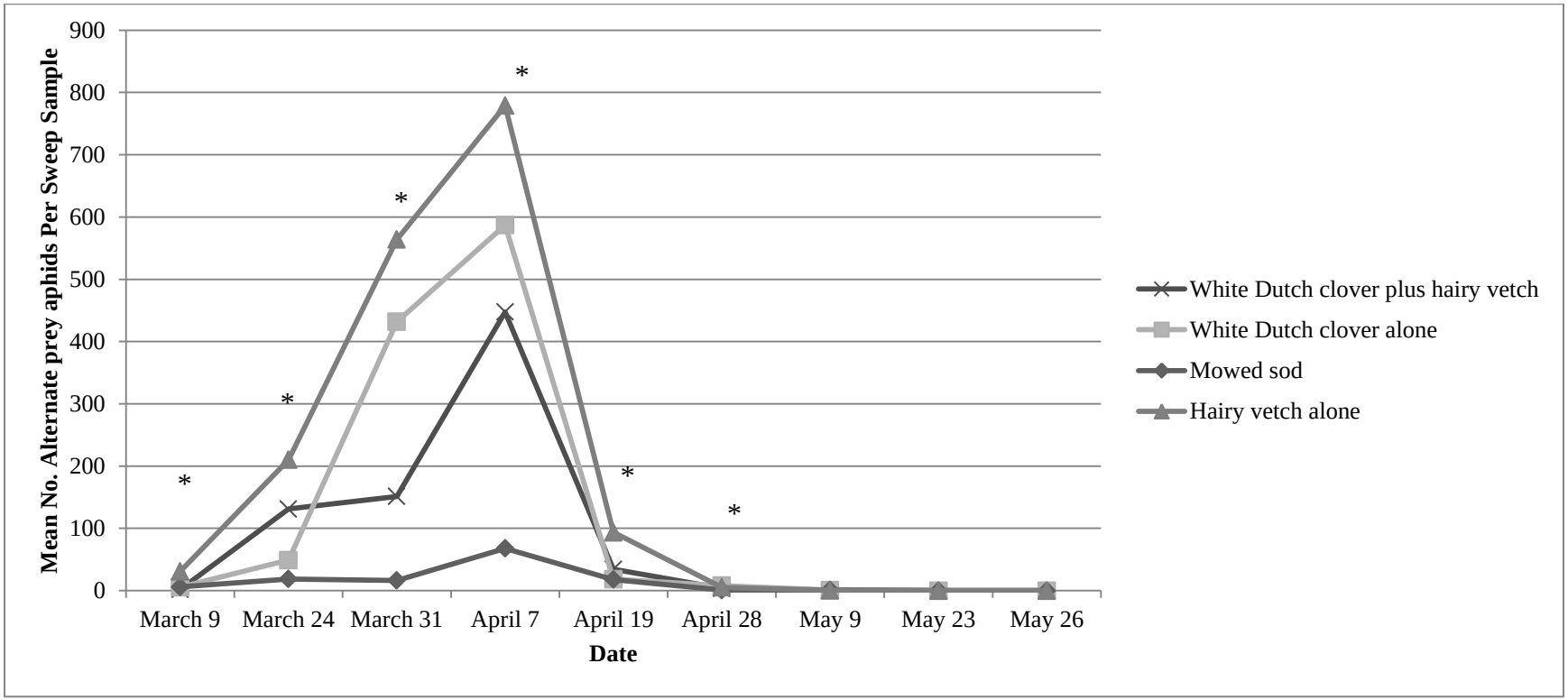

Figure 3.6 Mean numbers of pecan aphids per 5 leaves in trees in different intercrop plots in the cool-season intercrop trial 2016. * indicates where significant differences were found between treatments (GLIMMIX procedure; *t*-test, α=0.05).

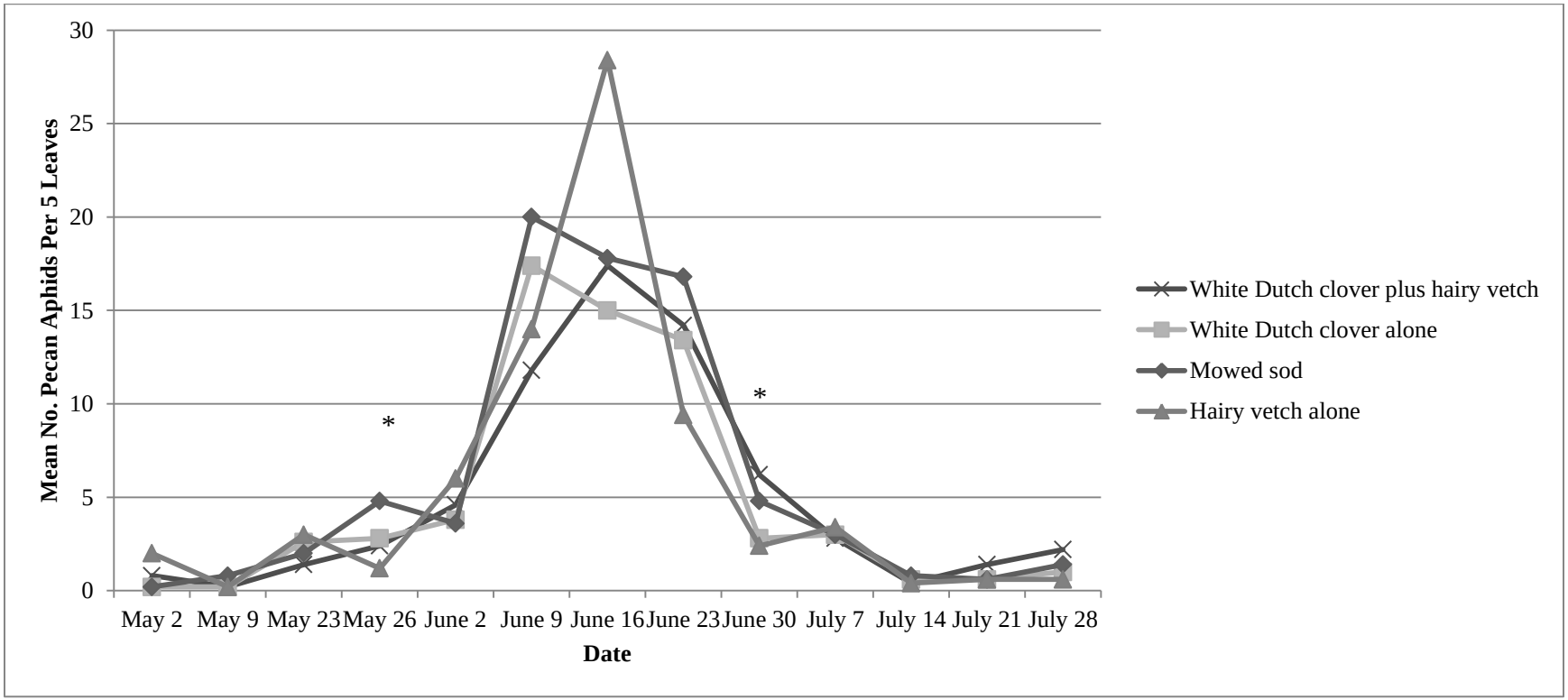

Figure 3.7 Mean numbers of lady beetle per 5 shoots in trees in different intercrop treatments in the warm-season intercrop trial 2015. * indicates where significant differences were found between treatments (GLIMMIX procedure; *t*-test, α=0.05).

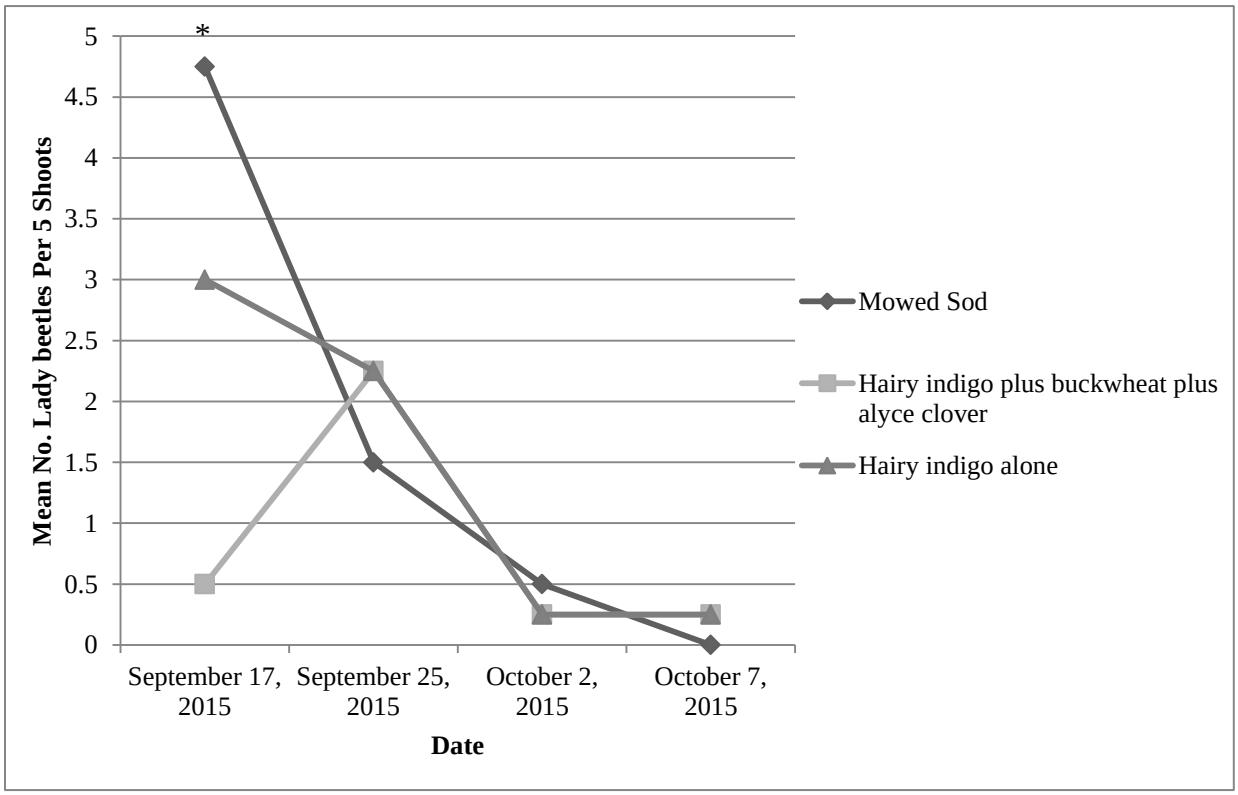

Figure 3.8 Mean numbers of pecan aphids per 5 leaves in trees in different intercrop treatments in the warm-season intercrop trial 2015. * indicates where significant differences were found between treatments (GLIMMIX procedure; *t*-test, α=0.05).

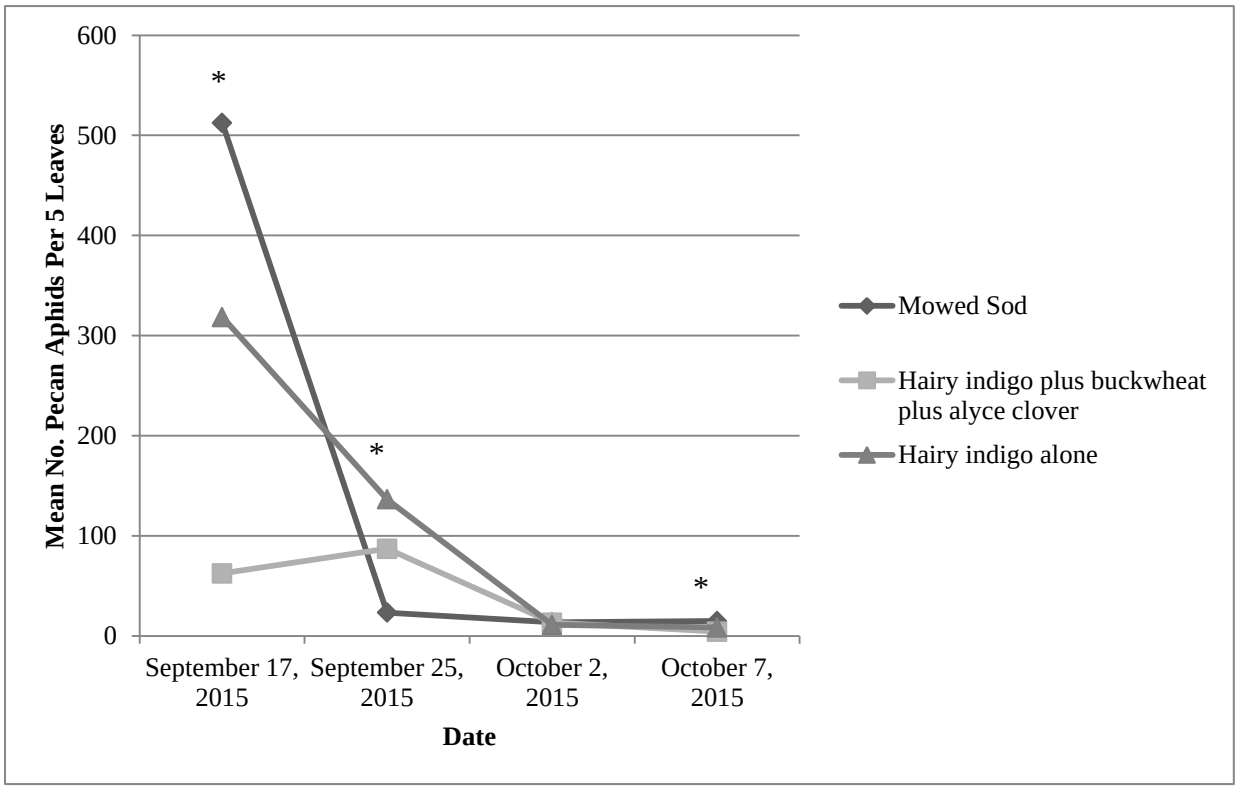

Table 3.1 Mean numbers of pooled lady beetles per sweep sample in different intercrop treatments in the cool-season intercrop trial 2014/2015[1].

Date	Intercrop Treatments				
	White Dutch clover plus hairy vetch	Joint vetch	White Dutch clover alone	Mowed sod	Hairy vetch alone
May 12, 2015	23.00 a	14.33 ab	2.67 c	5.00 bc	30.00 a
May 19, 2015	12.67 a	5.00 ab	1.67 b	7.00 ab	12.00 a
May 29, 2015	4.33 a	1.67 a	2.00 a	2.00 a	0.33a

[1]Means for treatments in the same sampling date (row) that are labeled with different letters are significantly different. (GLIMMIX procedure; *t*-test, α=0.05)

Table 3.2 Mean numbers of alternate prey aphids per sweep sample in different intercrop treatments in the cool-season intercrop trial 2014/2015[1].

Date	Intercrop Treatments				
	White Dutch clover plus hairy vetch	Joint vetch	White Dutch clover alone	Mowed sod	Hairy vetch alone
May 12, 2015	136.00 a	17.00 ab	3.67 b	3.00 b	169.67 a
May 19, 2015	13.33 a	3.00 a	15.00 a	33.33 a	46.33 a
May 29, 2015	2.33	0.00	0.00	0.00	0.00

[1]Means for treatments in the same sampling date (row) that are labeled with different letters are significantly different. (GLIMMIX procedure; *t*-test, α=0.05)

Table 3.3 Mean numbers of pecan aphids per 5 leaves in trees in different intercrop plots in the cool-season intercrop trial 2014/2015[1].

Date	Intercrop Treatments				
	White Dutch clover plus hairy vetch	Joint vetch	White Dutch clover alone	Mowed sod	Hairy vetch alone
June 5, 2015	2.75 b	4.75 ab	7.5 ab	9.75 a	8.33 ab
June 12, 2015	1.00 b	4.67 ab	1.33 ab	8.33 a	3.00 ab
June 19, 2015	5.67 a	4.00 a	4.33 a	5.67 a	6.67 a
June 25, 2015	2.67 a	6.00 a	3.33 a	2.67 a	7.33 a
July 2, 2015	2.33a	1.00 a	1.33 a	2.33 a	0.67 a

[1]Means for treatments in the same sampling date (row) that are labeled with different letters are significantly different. (GLIMMIX procedure; *t*-test, α=0.05)

Table 3.4 Mean numbers of pooled lady beetles per sweep sample in different intercrop treatments in the cool-season intercrop trial 2016[1].

Date	Intercrop Treatments			
	White Dutch clover plus hairy vetch	White Dutch clover alone	Mowed sod	Hairy vetch alone
March 9, 2016	0.20 a	0.40 a	0.00 a	1.20 a
March 24, 2016	0.40 b	0.60 b	0.00	4.20 a
March 31, 2016	2.20 a	3.40 a	0.00	5.80 a
April 7, 2016	3.20 a	5.60 a	1.80 a	6.60 a
April 19, 2016	6.80 a	6.80 a	3.00 a	4.60 a
April 28, 2016	2.80 a	2.00 ab	0.60 b	4.00 a
May 9, 2016	1.00 a	0.80 a	0.00	1.00 a
May 23, 2016	0.25 a	0.40 a	0.00 a	0.20 a
May 26, 2016	0.20 a	0.40 a	0.00 a	0.20 a

[1]Means for treatments in the same sampling date (row) that are labeled with different letters are significantly different. (GLIMMIX procedure; *t*-test, α=0.05)

Table 3.5 Mean numbers of alternate prey aphids per sweep sample in different intercrop treatments in the cool-season intercrop trial 2016[1].

	Intercrop Treatments			
Date	White Dutch clover plus hairy vetch	White Dutch clover alone	Mowed sod	Hairy vetch alone
March 9, 2016	2.00 b	5.60 ab	6.00 ab	31.00 a
March 24, 2016	131.20 ab	49.20 bc	18.80 c	210.20 a
March 31, 2016	151.40 b	431.80 ab	16.40 c	564.00 a
April 7, 2016	447.60 a	587.00 a	67.80 b	779.00 a
April 19, 2016	34.20 b	18.60 b	17.80 b	93.60 a
April 28, 2016	3.60 ab	8.20 a	1.00 b	5.20 ab
May 9, 2016	0.80 a	1.00 a	1.20 a	0.40 a
May 23, 2016	0.00	0.00	0.00	0.00
May 26, 2016	0.00	0.00	0.00	0.00

[1]Means for treatments in the same sampling date (row) that are labeled with different letters are significantly different. (GLIMMIX procedure; *t*-test, α=0.05)

Table 3.6 Mean numbers of pecan aphids per 5 leaves in trees in different intercrop plots in the cool-season intercrop trial 2016[1].

Date	Intercrop Treatments			
	White Dutch clover plus hairy vetch	White Dutch clover alone	Mowed sod	Hairy vetch alone
May 2, 2016	0.80 a	0.20 a	0.20 a	2.00 a
May 9, 2016	0.20 a	0.20 a	0.80 a	0.20 a
May 23, 2016	1.40 a	2.60 a	2.00 a	3.00 a
May 26, 2016	2.40 ab	2.80 ab	4.80 a	1.20 b
June 2, 2016	4.60 a	3.80 a	3.60 a	6.00 a
June 9, 2016	11.80 a	17.40 a	20.00 a	14.00 a
June 16, 2016	17.40 a	15.00 a	17.80 a	28.40 a
June 23, 2016	14.20 a	13.40 a	16.80 a	9.40 a
June 30, 2016	6.20 a	2.80 ab	4.80 ab	2.40 b
July 7, 2016	2.80 a	3.00 a	3.00 a	3.40 a
July 14, 2016	0.40 a	0.60 a	0.80 a	0.40 a
July 21, 2016	1.40 a	0.60 a	0.60 a	0.60 a
July 28, 2016	2.20 a	1.00 a	1.40 a	0.60 a

[1]Means for treatments in the same sampling date (row) that are labeled with different letters are significantly different. (GLIMMIX procedure; *t*-test, α=0.05)

Table 3.7 Total numbers of pooled immature and adult lady beetle species obtained from different intercrop treatments in the warm-season intercrop trial 2014/2015.

Date	Intercrop Treatments	Lady beetle			
		Pooled immature	Adult		
			H. axyridis	*H. convergens*	*C. maculata*
September 17, 2015	Mowed sod	21	1	1	0
	Hairy indigo plus buckwheat plus alyce clover	24	2	1	0
	Hairy indigo alone	17	1	0	0
October 7, 2015	Mowed sod	0	3	0	0
	Hairy indigo plus buckwheat plus alyce clover	1	0	3	0
	Hairy indigo alone	2	2	0	0
October 21, 2015	Mowed sod	0	0	0	0
	Hairy indigo plus buckwheat plus alyce clover	0	0	0	0
	Hairy indigo alone	0	0	0	0
October 28, 2015	Mowed sod	0	0	0	0
	Hairy indigo plus buckwheat plus alyce clover	0	1	0	1
	Hairy indigo alone	0	0	0	0
November 4, 2015	Mowed sod	0	0	0	0
	Hairy indigo plus buckwheat plus alyce clover	0	0	0	0
	Hairy indigo alone	0	1	0	0
All sampling dates (Total)	Mowed sod	21	4	1	0
	Hairy indigo plus buckwheat plus alyce clover	25	3	4	1
	Hairy indigo alone	19	4	0	0

Table 3.8 Mean numbers of lady beetles per 5 shoots in trees in different intercrop treatments in the warm-season intercrop trial 2015[1].

Date	Intercrop Treatments		
	Mowed Sod	Hairy indigo plus buckwheat plus alyce clover	Hairy Indigo alone
September 17, 2015	4.75 a	0.50 b	3.00 ab
September 25, 2015	1.50 a	2.25 a	2.25 a
October 2, 2015	0.05 a	0.25 a	0.25 a
October 7, 2015	0 a	0.25 a	0.25 a

[1]Means for treatments in the same sampling date that are labeled with different letters are significantly different. (GLIMMIX procedure; *t*-test, α=0.05)

Table 3.9 Mean numbers of pecan aphids per 5 leaves in trees in different intercrop treatments in the warm-season intercrop trial 2015[1].

Date	Intercrop Treatments		
	Mowed Sod	Hairy indigo plus buckwheat plus alyce clover	Hairy Indigo alone
September 17, 2015	512.50 a	62.50 b	318.50 a
September 25, 2015	23.25 b	87.00 a	136.50 a
October 2, 2015	13.50 a	13.25 a	11.00 a
October 7, 2015	14.75 a	4.25 b	8.50 ab

[1]Means for treatments in the same sampling date that are labeled with different letters are significantly different. (GLIMMIX procedure; *t*-test, α=0.05)

CHAPTER 4

EVALUATION OF ANT FORAGING DISRUPTIVE TECHNIQUES FOR ANT CONTROL IN THE PECAN ORCHARD

[1]Liu, T. and J.D. Dutcher. 2016. To be submitted to *Journal of Economic Entomology*.

Abstract

The Argentine ant, *Linepithema humile* (Mayr) and red imported fire ant (RIFA), *Solenopsis invicta* (Buren) trail up pecan tree trunks to feed on pecan aphid honeydew, and interfere with the biological control agents of pecan aphids in pecan. A series of ant trail blocking experiments was carried out at a greenhouse to determine the trail blocking effects of 1, 5 and 10% of the methyl anthranilate against the red imported fire ant (RIFA), *Solenopsis invicta* (Buren). The effects were investigated for 120 min after treatment. Based on the eleven identical experiments conducted at different dates, compared with nontreated control, 1 % of the methyl anthranilate offered significantly better trail blocking effects until 60 min post-treatment; 5 and 10% of the methyl anthranilate showed significantly better trail blocking effects throughout the whole experiment. Field trials were carried out in a pecan orchard to test the foraging disruption effects of Tangle-Trap® Sticky Coating (Paste formula, The Tanglefoot Company, Grand Rapids, MI), Bird Stop™ (containing 26.4% of methyl antranilate and 73.6% of other ingredients, BIRD-X formulated for Corvus Repellent Inc., Greeley, CO) and the combination of the two treatments (Tangle-Trap® Sticky Coating and Bird Stop™) against RIFA and Argentine ant, *Linepithema humile* (Mayr). Seven days after the treatments were applied to the pecan tree trunks, the efficiency of the disruption of ant foraging was evaluated weekly (4 weeks in total). The results revealed that Bird Stop™ and Tangle-Trap® Sticky Coating plus Bird Stop™ effectively prevented ants from trailing up the pecan tree trunks for 4 weeks; while Tangle-Trap® Sticky Coating was effective for 2 weeks.

Key words: invasive ants, methyl anthranilate, Tangle-Trap® Sticky Coating, Bird Stop™

Introduction

Argentine ant, *Linepithema humile* (Mayr), has been considered an important pest in natural, urban and agricultural environments in the United States since its introduction into New Orleans, LA in 1891 (Foster 1908, Vega and Rust 2001, Silverman and Brightwell 2008). Argentine ants are omnivores with preference for sugar (honeydew), thus they are often found in homes in urban areas and in agricultural fields with infestations of honeydew-producing pests. In agricultural settings, Argentine ant damages are usually associated with the interference with the biological control agents of honeydew-producing hemipteran pests or even plant pathogen vectors (Shorey et al. 1992, Sisk et al. 1996, Silverman and Brightwell 2008, Westermann et al. 2016). For example, the Argentine ant is a secondary pest in California citrus orchards due to its association with increased densities of aphids, mealybugs and unarmored scales (Woglum and Borden 1921, Moreno et al. 1987). In previous studies, the repellency and efficacy of different materials, including conventional insecticides, essential oils and semiochemicals, had been evaluated for control of Argentine ant (Knight and Rust 1990, Sisk et al. 1996, Wiltz et al. 2007, Scocco et al. 2012). In agricultural habitats, field trials have been carried out; organophosphate insecticides, synthetic pheromones, ant-repellent semiochemicals, etc. have been tested in grapevines and citrus orchards by different application methods (Shorey et al. 1996, Sisk et al. 1996, Westermann et al. 2016). However, there is a lack of research focusing on the Argentine ant management in pecan orchards.

The red imported fire ant (RIFA), *Solenopsis invicta* (Buren), is an invasive species widely spread throughout the world, including the southeastern United States (Kaakeh and Dutcher 1992, Ascunce et al. 2011). In agricultural environments, it has been reported that RIFA damages the farming equipment, irrigation lines, electrical switch boxes, and even disrupts the

farming activities by biting or stinging farm operators (Tedders et al. 1990, Harris et al. 2003). RIFA feeds on aphid honeydew, alternate prey aphids and aphidophaga in pecan; and aphids attract fire ants to intercrops and pecan trees (Ree and Knutson 1997, Dutcher 1998). For instance, RIFA tends alternate prey aphids (cowpea aphids, *Aphid craccivore* Koch) in the cowpea (*Vigna unguiculata* ssp. *unguiculata* (L.) Walper) intercrops, and preys on the eggs, larvae, and pupae of green lacewing, *Chrysoperla rufilabris* (Burmeister), which is a predator of pecan aphids (Bugg and Dutcher 1989, Tedders et al. 1990, Dutcher et al. 1999). To improve pecan aphid biological control, the application of an insecticide (chlorpyrifos) on the tree trunks was used to hinder RIFA from foraging up to pecan canopies and interfering with beneficial insects. Sesbania (*Sesbania exaltata* [Rafinesque-Schmaltz] Cory) naturally repels RIFA; an animal repellent (butyl carbitol acetate) was also found a prominent repellent of RIFA (Kaakeh and Dutcher 1992, Bugg and Dutcher 1993, Dutcher 2004, Dutcher and Beaver 2005).

Two studies were conducted. First, RIFA trail blocking effects were measured in greenhouse bioassays that compared different concentrations of methyl anthranilate to the nontreated control. Second, the applications of Tangle-Trap® Sticky Coating and Bird Stop™ (a formulation of methyl anthranilate) to the tree trunk were compared for effectiveness for prevention ants from foraging in the pecan tree canopy in a controlled replicated field trial. Our hypothesis was that methyl anthranilate would provide better RIFA trail blocking effects than the nontreated control; and trunk applications of Tangle-Trap® Sticky Coating and Bird Stop™ would disrupt the foraging of the Argentine ant and RIFA in a pecan orchard relative to the nontreated control.

Materials and Methods

Trail blocking of the RIFA.

Ant colonies. RIFA colonies were collected at the Ponder Farm of the University of Georgia Tifton Campus located in Tift County, GA, USA. Each collected colony with original soil was maintained in a 5-gallon plastic bucket coated with four inches of "Insect a Slip" (Fluoropolymer dispension, BioQuip Products, Inc., Rancho Dominguez, CA) on the top of the inner bucket to prevent the ants from escaping. The colonies were kept in a greenhouse (6×8×6 ft. 6 in., ShelterLogic®, Watertown, CT) to avoid direct sunlight, rain or other rough weather conditions. The ant diet consisted of dried mealworms (Chubby Mealworms, Las Vegas, NV) and organic golden light blue agave (Madhava Natural Sweeteners, Longmont, CO). Water was always provided. There were 11 fire ant colonies in total in the greenhouse, and if any colonies were found inactive, the colonies were replaced by new colonies collected from the same field location (the Ponder Farm).

Test materials. The potential ant repellents tested were 1, 5 and 10% of the methyl anthranilate (99% purity, Aldrich Chemical company, Inc., Milwaukee, WI). A 70% ethanol solution was used to dilute the methyl anthranilate, and it also served as the control treatment.

Ant trail blocking bioassay. A series of trail blocking experiments was carried out at the greenhouse. To starve the ants, all the ants were food limited 36 to 72 hours before the bioassay. A wood stick (3.8×1.9×48.5 cm) was set up in the middle of the bucket with one end sticking into the ant colony in the soil and the other connecting to a hexagonal weigh tray (1.8 in diameter inner base, 2.6 in diameter inner top). About 12 cm below the hexagonal weigh tray, 8 cm of the wood stick was covered by Parafilm M® (5×10 cm, Pechiney Plastic Packaging, Chicago, IL) to prevent the wood stick from absorbing the test chemicals; and the chromatography paper (2×38

cm, Waterman Ltd., Maidstone, UK) was used to wrap around the Parafilm M® band. After that, the ant food (dried mealworms and organic golden light agave) was provided to the ants at the hexagonal weigh tray. After the foraging ants had built the trail up to the food, the wood stick with the hexagonal weigh tray was taken out from the bucket. And a paint brush (2 in, the Wooster Brush Company, Wooster, Ohio) was used to brush down all the ants on the wood stick back to the bucket. Later on, the ant-free wood stick was applied with 1.2 ml of the test chemical concentration on the chromatography paper, and the used hexagonal weigh tray was replaced with the clean one with ant food. The same arrangement was set up with the wood stick and the hexagonal tray as previously described.

The number of ants crossing the treated area (including the ones staying on the chromatography paper) during a 5-min period was recorded for the first hour; after that, it was recorded every 10 min until 120 min post-treatment. At any assessment points, if the ant counts exceeded 200, the ant counts were recorded as 200. In each experiment, 8 ant colonies were randomly chosen from the 11 colonies for the test with each treatment containing 2 replicates. Eleven identical experiments were conducted.

Statistical Analyses. The ant counts observed from the 2 replicates of the same treatment at each indicated assessment time in the same experiment were calculated as averaged ant counts before statistical analysis. To compare the ant counts obtained from different treatments at the indicated assessment time, we treated the dates as the random effect since we have conducted 11 identical experiments at 11 different dates. Thus, the mixed procedure with repeated measures in SAS was used to analyze the data, and type III tests were used to determine whether the treatment effect is significant or not at $\alpha = 0.05$ (SAS Software 2013). The significant differences

between treatments at the indicated assessment time were elucidated through Tukey-Kramer test (α=0.05).

Foraging disruption of the Argentine ant and RIFA.

The Site. The field trials were conducted at the Ponder Farm of the University of Georgia Tifton Campus located in Tift County, GA, USA in July 2016. About 12.3 acre of the farm was used for the field trials, and it consists of approximately 300 thirty-year-old pecan trees planted to the cultivar "Desirable" on sandy loam soil. The pecan trees in the research farm were planted in rows with approximately 12-m tree intervals, and in between each tree row, there is an approximately 7-m-wide ground vegetation area.

Test materials. The materials tested in the field experiment included Tangle-Trap® Sticky Coating (Paste formula, The Tanglefoot Company, Grand Rapids, MI), and Bird Stop™ (containing 26.4% of methyl anthranilate and 73.6% of other ingredients, BIRD-X formulated for Corvus Repellent Inc., Greeley, CO) at the highest suggested rate for hand sprayer, where 7.7 oz. of the product was mixed with 1 gal. of water. 1% of the NU FILM P (Miller Chemical and Fertilizer, LLC., Hanover, PA) was added to the Bird Stop™ formulation as a spreader/sticker while mixed. The four treatments were Tangle-Trap® Sticky Coating, Bird Stop™, the combination of the two treatments (Tangle-Trap® Sticky Coating and Bird Stop™), and nontreated control.

Field experiment. Pecan trees were randomly chosen for the field trials; however, if the chosen trees lacked the forage of ants on the trees, the trees were re-picked until ants were found. The old and dead barks of the chosen trees were scraped off at about 115-130 cm above ground, creating a 15 cm wide scraped ring around the trunk. Treatments were applied by the following methods: The Tangle-Trap® Sticky Coating was directly applied to the scraped area around the

trunk by putty knife; the Bird Stop™ formulation was thoroughly sprayed, covering around the tree trunk up from the ground to about 70 cm above the soil surface by a pressure sprayer (430-3G-101, Solo®, Newport News, VA); the combined treatment included the application of both Tangle-Trap® Sticky Coating and Bird Stop™; and the scraped untreated trees were served as the control treatment.

Seven days after the application of the treatments, the efficiency of the disruption of ant foraging was evaluated weekly (4 weeks in total). On each assessment day, the efficiency of the foraging disruption was assessed by the following steps. A petri dish (without lid) containing 4 g of clover honey (Great Value™, Wal-Mart Stores, Inc., Bentonville, AR) was placed at 2 cm above the scraped area at the tree trunk, and fixed on the tree by a bent metal nail plate (3-1/8×5 in, NP35, USP®, MiTek Holdings, Inc., Chesterfield, MO) which was connected to the tree trunk by two nails. The numbers of ants (including RIFA and Argentine ant) passing the treated area and reaching the petri dish were recorded at 15, 40 and 65 min after the honey was provided. At 15 and 40 min, the ant counts were obtained directly by counting the ants in the petri dishes in the field, and if the ant counts exceeded 200 per petri dish, the ant counts were recorded as 200; while at 65 min, the petri dishes were collected and brought back to the laboratory to record the precisely final ant counts. The 4 treatments were randomly assigned to the selected trees in a completely randomized design with 4 replicates.

Statistical Analyses. To compare the mean numbers of ants obtained from different treatments at the indicated assessment time, we treated the 4 sampling dates as the random effect since the efficiency of the disruption of ant foraging was evaluated for four weeks. Thus, the mixed procedure with repeated measures in SAS was used to analyze the data, and type III tests were used to determine whether the treatment effect is significant or not at $\alpha = 0.05$ (SAS

Software 2013). The significant differences between treatments at the indicated assessment time were elucidated through Tukey-Kramer test (α=0.05).

Results

Trail blocking of the RIFA (Figure 4.1 and Table 4.1).

Significant differences with the mean numbers of RIFA were detected due to different treatments from 10 min to 120 min post-treatments; at 5 min post-treatment, no significant differences with the mean numbers of RIFA were detected among the 4 treatments (Figure 4.1). At all the assessment time during 10 to 35 min post-treatment, compared to the nontreated control, the three methyl anthranilate treatments showed significantly lower mean numbers of RIFA, while no significant differences were observed among the three methyl anthranilate concentrations (Table 4.1). At 40 min post-treatment, the mean numbers of RIFA obtained in all the methyl anthranilate treatments were significantly lower than nontreated control; and 10% methyl anthranilate showed significantly lower mean numbers of RIFA than 1% methyl anthranilate, while no significant differences were detected between the 1 and 5%, and 5 and 10% methyl anthranilate treatments. At 45, 50 and 55 min post-treatment, compared with the nontreated control, all the methyl anthranilate treatments showed significantly lower mean numbers of RIFA; 5 and 10% methyl anthranilate concentrations had significantly lower mean numbers of RIFA than the 1% methyl anthranilate, but there were no significant differences between the 5% and 10% methyl anthranilate treatments. During 45 to 55 min post-treatment, although 1% methyl anthranilate showed significantly lower mean numbers of RIFA than the nontreated control, the mean ant counts increased from 1.05 to 54.05 from 5 to 55 min post-treatment; on the contrast, the mean ant counts obtained from the 5 and 10% methyl anthranilate

treatments remained low from 5 min to 55 min post-treatment (the highest mean ant counts obtained during 5 to 55 min post-treatment were 5.32 and 1.36 in 5 and 10 % methyl anthranilate treatments, respectively). From 60 to 120 min post-treatment, no significant differences were observed between the nontreated control and 1% methyl anthranilate treatment, and the mean ant counts ranged from 84.86-95.41 and 61.45-79.14 in the nontreated control and 1% methyl anthranilate treatments, respectively; whereas the 5 and 10% methyl anthranilate treatments still showed significantly lower mean numbers of RIFA than the nontreated control, but actually the mean ant counts slightly increased from 6.68 to 19.18 and 2.09 to 9.05 in the 5 and 10% methyl anthranilate treatments, respectively; no significant differences between the 5% and 10% methyl anthranilate treatments were detected.

Foraging disruption of the Argentine ant and RIFA (Figure 4.2 and Table 4.2).

At 1 week post-treatment, 15 min after the honey was provided, no ants were observed in the petri dishes from the Tangle-Trap® Sticky Coating, Bird Stop™ and Tangle-Trap® Sticky Coating plus Bird Stop™ treatments, and the mean numbers of ants found in the nontreated control were significantly higher than the three treatments (Table 4.2). Forty min after the honey was provided, no ants were observed in Tangle-Trap® Sticky Coating and Tangle-Trap® Sticky Coating plus Bird Stop™ treatments, and the mean numbers of ants found in the nontreated control were significantly higher than the rest three treatments; while no significant differences were found among these three treatments. Sixty-five min after the honey was provided, the mean numbers of ants collected in Tangle-Trap® Sticky Coating remained zero, and the mean numbers of ants found in the nontreated control were significantly higher than the rest three treatments.

At 2 weeks post-treatment, 15 and 40 min after the honey was provided, no significant differences with the mean numbers of ants were detected among all treatments (Table 4.2).

Sixty-five min after the honey was provided, the mean numbers of ants collected in the nontreated control were significantly higher than the rest three treatments.

At 3 weeks post-treatment, 15 min after the honey was provided, no significant differences with the mean numbers of ants were observed among all treatments (Table 4.2). Forty min after the honey was provided, the mean numbers of ants found in the nontreated control were significantly higher than the rest three treatments, and these three treatments were not significantly different. Sixty-five min after the honey was provided, Bird Stop™ and Tangle-Trap® Sticky Coating plus Bird Stop™ showed significantly lower mean numbers of ants than the nontreated control; whereas no significant differences were observed between Tangle-Trap® Sticky Coating and the nontreated control, and among Tangle-Trap® Sticky Coating, Bird Stop™ and Tangle-Trap® Sticky Coating plus Bird Stop™.

At 4 weeks post-treatment, 15 and 40 min after the honey was provided, no significant differences with the mean numbers of ants were detected among all treatments (Table 4.2). Sixty-five min after the honey was provided, Bird Stop™ and Tangle-Trap® Sticky Coating plus Bird Stop™ revealed significantly lower mean numbers of ants than the nontreated control; while no significant differences were observed between Tangle-Trap® Sticky Coating and the nontreated control, and among Tangle-Trap® Sticky Coating, Bird Stop™ and Tangle-Trap® Sticky Coating plus Bird Stop™.

In the ant foraging disruption field trials, the majority of the ant counts obtained from the petri dishes in Bird Stop™ and Tangle-Trap® Sticky Coating plus Bird Stop™ was zero (43 out of 48 samples in both treatments); the highest ant counts obtained in Tangle-Trap® Sticky Coating plus Bird Stop™ were 12 ants/ per petri dish (at 65 min at 4 weeks post-treatment), and in Bird Stop™ were 25 ants/ per petri dish (at 65 min at 1 week post-treatment). In the Tangle-

Trap® Sticky Coating treatment, 30 out of 48 samples showed zero ant count, and 12 out of the 18 samples which showed more than zero ant count never exceeded 12 ants/ per petri dish; in fact, the ant counts obtained from the rest 6 samples ranged from 38 to 149 ants/ per petri dish, and they were all observed from the same replicate of the Tangle-Trap® Sticky Coating treatment at 3 and 4 weeks post-treatment.

At 2 weeks post-treatment, at the assessment points 15 and 40 min, the SAS results indicated that no significant differences were detected among all treatments; however, the mean numbers of ant counts observed in the nontreated control were 86.50 and 84.50 ants/ per petri dish at 15 and 40 min, respectively, and the mean numbers of ant counts obtained in other three treatments never exceeded 1.75 ants/ per petri dish. The explanation to the SAS interpretation might be that one of the replicates of the nontreated control treatment showed zero ant counts at 15 and 40 min, and the highest ant counts obtained from other replicates were 200 ants/ per petri dish for both assessment points, thus, the variation was very large; by contrast, at the same assessment points 15 and 40 min, the majority of the ant counts found in the rest 3 treatments was zero with the highest ant counts, 5 ants/ per petri dish in Tangle-Trap® Sticky Coating, thus, the variation was smaller. The similar phenomenon was also observed at 3 weeks post-treatment at the assessment point 15 min. To prove our inference, we analyzed the data again by using the same SAS program after removing the replicate of nontreated control, which zero ant counts were obtained at 2 weeks post-treatment at 15 and 40 min and 3 weeks post-treatment at 15 min. The results revealed that the mean numbers of ants in the nontreated control were significantly higher than the rest three treatments at 2 weeks post-treatment at 15 and 40 min, and no significant differences were observed among these three treatments; at 3 weeks post-treatment at 15 min, Bird Stop™ and Tangle-Trap® Sticky Coating plus Bird Stop™ showed significantly

lower mean numbers of ants than the nontreated control, but no significant differences were observed between Tangle-Trap® Sticky Coating and the nontreated control, and among Tangle-Trap® Sticky Coating, Bird Stop™ and Tangle-Trap® Sticky Coating plus Bird Stop™.

By looking at the mean numbers of ants per petri dish obtained in different treatments at 65 min after honey was provided at 1 to 4 weeks post-treatment, we found that Bird Stop™ and Tangle-Trap® Sticky Coating plus Bird Stop™ effectively prevented ants from trailing up the pecan tree trunks for 4 weeks; while Tangle-Trap® Sticky Coating was effective for 2 weeks (Figure 4.2 and Table 4.2).

Discussion

The greenhouse ant trials revealed that at 5 min post-treatment no significant differences with the mean numbers of RIFA were observed among all treatments; this scenario could be attributed to the length of time the RIFA needs to reach the food. In spite of the fact that the trail blocking effects were only evaluated for 120 min, and it is uncertain how long the trail blocking effects of the 5 and 10% methyl anthranilate treatments would last after 120 min, based on the results of the trail blocking effect offered by 1% methyl anthranilate, the loss of trail blocking effect after 55 min post-treatment could be referred to the aging of the chemical. As a result, the similar phenomenon was observed in the study conducted by Scocco et al. (2012). In that study, fresh essential oils (spearmint, wintergreen, peppermint, cinnamon and clove oils) at 0.10, 1 and 10% concentrations were all repellent to Argentine ants; while after the essential oils were aged for one week, the 0.1% wintergreen, peppermint, cinnamon and clove oils were no longer repellent, but all the aged essential oils at 1 and 10% still remained the repellency. Although our research indicated that the 5 and 10 performed excellent trail blocking effects, to apply methyl

anthranilate as ant trail blockers, further research is needed to slow down the aging process and seek for long lasting formulation.

According to the ant foraging disruption field trials, at all the assessment time at 3 and 4 weeks post-treatment, one of the replicates of the Tangle-Trap® Sticky Coating treatment (6 samples in total) showed 38 to 149 ants/ per petri dish while other replicates of Tangle-Trap® Sticky Coating (18 samples in total) never exceeded than 12 ants/ per petri dish. This scenario might result from the ground debris, dust, insects and other substances that covered the Tangle-Trap® Sticky Coating on the tree trunk. In addition, the high ant counts found from that replicate of the Tangle-Trap® Sticky Coating might pointed out why at 65 min at 3 weeks post-treatment and at 65 min at 4 weeks post-treatment, the mean numbers of ant counts obtained from the nontreated control were 3.45 and 1.95 times higher than Tangle-Trap® Sticky Coating, but SAS specified that the two treatments were not significantly different. The reason why one of the replicates of the nontreated control treatment showed zero ant counts at 2 weeks post-treatment at 15 and 40 min and 3 weeks post-treatment at 15 min is uncertain; the reason could be associated with more attractive food sources at ground level than the honey at the tree trunk, or could be the microclimate conditions near that nontreated tree, etc.

Overall, in the ant foraging disruption field trials, we found that Bird Stop™ and Tangle-Trap® Sticky Coating plus Bird Stop™ effectively prevented ants from trailing up the pecan tree trunks for 4 weeks; while Tangle-Trap® Sticky Coating was effective for 2 weeks. Since the active ingredient of Bird Stop™ is methyl anthranilate, the results of the greenhouse and field trials were corresponding.

Dutcher and Beaver (2005) reported that butyl carbitol acetate effectively prevented RIFA from foraging up the pecan tree trunk for one week, and the reduced foraging effect lasted

two weeks after application; moreover, the trunk barrier treated with butyl carbitol acetate were more effective than methyl anthranilate and Tanglefoot® in hindering RIFA from crossing. Wiltz et al. (2007) found that some essential oils (basil, citronella, lemon, peppermint and tea tree oil) reduced the frequency of the Argentine ant and RIFA crossing treated barriers in laboratory conditions. The repellency and efficacy of some insecticides have also been investigated. The application of chlorpyrifos (organophosphate) on the pecan tree trunk prevented RIFA from trailing up to pecan canopies; in laboratory studies, cypermethrin (pyrethroid) and heptachlor (organochlorine) showed high repellency and efficacy against Argentine ant (Knight and Rust 1990, Dutcher 2004).

RIFA is an important predator of pecan weevil (*Curculio caryae* (Horn)) larvae at the ground level, thus, in order to keep RIFA at the ground level, trunk application is probably a better choice than other control methods conducted on the orchard floor (Dutcher and Sheppard 1981). However, pecan tree trunk is a refuge for overwintering aphidophagous insects, therefore applying insecticides to tree trunks can pose a concern for the conservation of aphidophaga (Mizell and Schiffhauer 1987). In our study, both Bird Stop™ and Tangle-Trap® Sticky Coating effectively prevented ants from foraging up the pecan trunk; furthermore, Tangle-Trap® Sticky Coating is an OMRI certified material, thus it could be a potential option to organic growers. However, we did not know if Bird Stop™ causes any detrimental effects on aphidophaga, and how much Tangle-Trap® Sticky Coating affects non-target insects. In fact, we found different insects trapped by Tangle-Trap® Sticky Coating, including lacewing larvae and adult lady beetles. As a result, before adopting these two materials into practical use, further research is needed to investigate the non-target effects of Tangle-Trap® Sticky Coating and Bird Stop™ on aphidophaga in pecan orchards.

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Figure 4.1 Mean numbers of RIFA crossing treated area in different treatments from 5 to 120 min after treatment. * indicates where significant differences were found between treatments (The mixed procedure; Tukey-Kramer test, α=0.05).

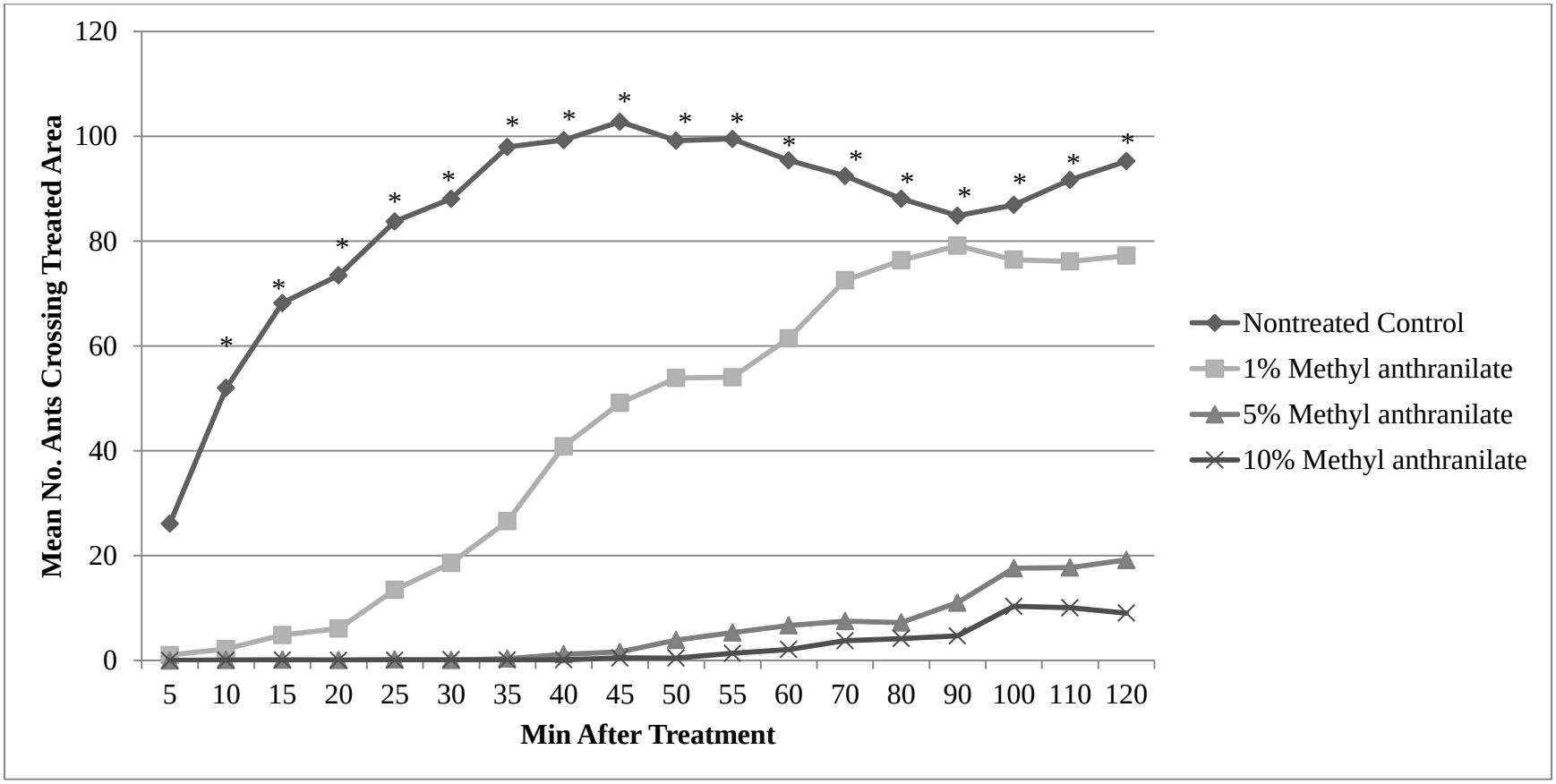

Figure 4.2 Mean numbers of ants per petri dish obtained in different treatments at 65 min after honey was provided at 1 to 4 weeks post-treatment. * indicates where significant differences were found between treatments (The mixed procedure; Tukey-Kramer test, α=0.05).

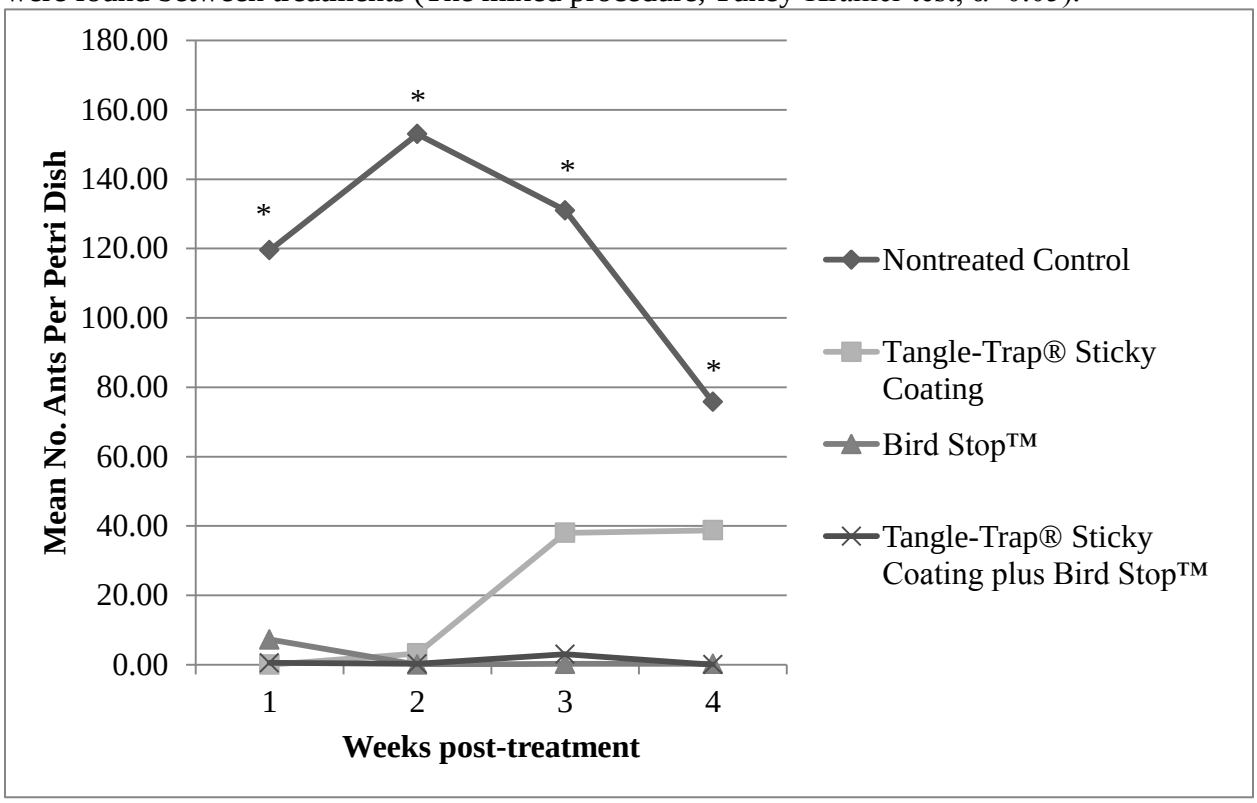

Table 4.1 Mean (±SE) numbers of RIFA crossing treated area in different treatments from 5 to 120 min after treatment[1].

Min after treatment	Treatment			
	Nontreated Control	1% Methyl anthranilate	5% Methyl anthranilate	10% Methyl anthranilate
5	26.09 (±10.26) a	1.05 (±0.58) a	0 a	0 a
10	52.00 (±11.89) a	2.18 (±1.03) b	0.09 (±0.06) b	0.05 (±0.05) b
15	68.18 (±15.45) a	4.86 (±2.42) b	0.14 (±0.10) b	0.09 (±0.09) b
20	73.50 (±15.36) a	6.09 (±2.95) b	0.09 (±0.09) b	0.05 (±0.05) b
25	83.77 (±17.07) a	13.50 (±5.09) b	0.18 (±0.12) b	0.05 (±0.05) b
30	88.09 (±18.30) a	18.59 (±6.29) b	0.09 (±0.06) b	0.14 (±0.14) b
35	97.95 (±20.74) a	26.59 (±6.69) b	0.36 (±0.22) b	0.09 (±0.09) b
40	99.27 (±21.54) a	40.86 (±10.27) b	1.18 (±0.58) bc	0.14 (±0.10) c
45	102.77 (±20.96) a	49.14 (±12.74) b	1.64 (±0.97) c	0.50 (±0.45) c
50	99.18 (±21.24) a	53.91 (±15.16) b	3.91 (±2.33) c	0.45 (±0.41) c
55	99.50 (±20.01) a	54.05 (±14.90) b	5.32 (±2.63) c	1.36 (±1.22) c
60	95.41 (±19.91) a	61.45 (±15.90) a	6.68 (±3.86) b	2.09 (±1.81) b
70	92.45 (±20.45) a	72.55 (±20.83) a	7.50 (±4.02) b	3.77 (±3.35) b
80	88.09 (±19.41) a	76.36 (±22.74) a	7.23 (±4.07) b	4.18 (±3.98) b
90	84.86 (±20.21) a	79.14 (±24.71) a	11.05 (±5.14) b	4.68 (±4.34) b
100	86.91 (±21.56) a	76.45 (±24.78) a	17.59 (±9.30) b	10.32 (±9.38) b
110	91.68 (±22.74) a	76.14 (±24.83) a	17.73 (±9.86) b	10.05 (±9.30) b
120	95.27 (±22.99) a	77.23 (±24.72) a	19.18 (±10.22) b	9.05 (±8.15) b

[1]Different letters in the same row indicate statistically significant differences among different treatments. (Tukey-Kramer test; α=0.05)

Table 4.2 Mean (±SE) numbers of ants per petri dish obtained in different treatments at 15, 40 and 65 min after honey was provided at 1 to 4 weeks post-treatment[1].

		Treatment			
		Nontreated Control	Tangle-Trap® Sticky Coating	Bird Stop™	Tangle-Trap® Sticky Coating plus Bird Stop™
1 week post-treatment	At 15 min	70.50 (±27.04) a	0 b	0 b	0 b
	At 40 min	87.25 (±43.23) a	0 b	5.25 (±5.25) b	0 b
	At 65 min	119.50 (±28.90) a	0 b	7.25 (±5.99) b	0.50 (±0.50) b
2 weeks post-treatment	At 15 min	86.50 (±42.52) a	0 a	0 a	0.25 (±0.25) a
	At 40 min	84.50 (±42.30) a	1.75 (±1.18) a	0 a	0.25 (±0.25) a
	At 65 min	153.00 (±63.09) a	3.25 (±2.93) b	0 b	0.25 (±0.25) b
3 weeks post-treatment	At 15 min	78.75 (±46.83) a	20.00 (±20.00) a	0 a	0 a
	At 40 min	116.50 (±48.25) a	19.00 (±17.67) b	0 b	0 b
	At 65 min	131.00 (±28.95) a	38.00 (±35.37) ab	0.25 (±0.25) b	3.00 (±3.00) b
4 weeks post-treatment	At 15 min	19.00 (±7.91) a	11.75 (±8.93) a	0 a	0 a
	At 40 min	24.00 (±7.08) a	27.75 (±27.42) a	0 a	0 a
	At 65 min	75.75 (±25.26) a	38.75 (±36.78) ab	0.25 (±0.25) b	0 b

[1]Different letters in the same row indicate statistically significant differences among different treatments. (Tukey-Kramer test; α=0.05)