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Control of Agrilus ruficollis (Coleoptera: Buprestidae) With Insecticides and Identifying Visual Attractants for Use in a Monitoring Trap

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ABSTRACT

The rednecked cane borer, *Agrilus ruficollis* (F.), is a pest of cultivated and wild blackberries in the Midwestern and Eastern parts of the United States. Feeding, mating, egg laying and development of *A. ruficollis* from larvae to adult only occurs on primocanes, the first year vegetative growth stage of blackberries, and not on the second year fruiting stage called floricanes that die after fruiting. Damage from this pest is caused by larvae girdling primocanes and tunneling in their pith, causing formation of galls. Gall formation on the primocane increases the chance of winter injury and can also potentially reduce yields the following season. There is currently only one class of insecticide (imidacloprid) approved for use in managing the pest and no trap is available for monitoring this pest. The research presented in this dissertation was to determine if other chemical classes of insecticides would provide equal or adequate control of *A. ruficollis* as that achieved by an application of imidacloprid and determine what visual and chemical cues act as stimulants for attracting *A. ruficollis*. An efficacy study of several insecticides found that only paraffinic oil (JMS Stylet Oil) provided a level of control of *A. ruficollis* similar to that achieved by the industry standard (imidacloprid). Paints that mimicked the spectral reflectance of blackberry leaves and canes of both primocane and floricane growth stages were applied to wooden dowels or corrugated plastic (ranging from 0.3 to 2.5 cm diameter). The dowels or corrugated plastics were covered with sticky Tanglefoot® and field evaluated for attractiveness to *A. ruficollis* for three years, with modifications to the trap each year. Commercially available green or purple plastic funnel traps covered with Fluon® were evaluated for attractiveness to *A. ruficollis* in 2014. In 2011, the greatest numbers of *A. ruficollis* adults were captured on one inch prism-shaped, vertical primocane mimic traps that reflected light at a peak wavelength between 540-560 nm (green). In 2012 and 2013, field tests

demonstrated that the most *A. ruficollis* adults were captured on traps painted the same green color as traps used to monitor for emerald ash borer, *Agrilus planipennis* Fairmaire. The funnel trap testing in 2014 reinforced the previous findings that *A. ruficollis* is most attracted to the green color of emerald ash borer traps. In 2013, it was noted that colored traps usually captured significantly more *A. ruficollis* males than females (> 2.4 males: 1 female ratio). This indicated a need to determine if there was a chemical cue used by *A. ruficollis* adult females to find and select only blackberry primocanes and not floricanes. However, no differences were found in volatile compounds collected from blackberry primocanes and floricanes. Although GC/MS peaks were identified, these collected volatiles did not stimulate antennae of *A. ruficollis* adults. Overall, research for this dissertation revealed that certain highly reflective green colors attracted *A. ruficollis*. Further research should be conducted to optimize trap design and determine if there is a pheromone or primocane plant odor that enhances colored trap catch of female and/or male *A. ruficollis*. A baited colored trap will improve the monitoring for this insect and timing of insecticide applications and lead to development of a mass trapping tactic that reduces the local density of *A. ruficollis* and lessen need for insecticide application.

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DEDICATION

This dissertation is dedicated to my wonderful loving wife Sun Young Kim. You have truly made this time at the University of Arkansas worthwhile and happy for me.

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CHAPTER 1 – LITERATURE REVIEW

INTRODUCTION

Insecticides for fruit insect management have changed tremendously within the past 20 to 30 years from broad spectrum to narrow spectrum (reduced risk) chemistries. Currently, several different tactics including use of insecticides with different modes of action are recommended to successfully lower fruit pest densities. A major concern behind the continued use of one insecticide formulation is the risk of developing pest resistance. Pest resistance to older classes of insecticides has driven the industry and researchers to develop newer chemistries with different modes of action but these formulations are much more expensive to apply against insect pests (Denholm and Rowland 1992, Whalon et al. 2008).

Traps of different color and design, and semiochemicals such as pheromones and kairomones have been used for monitoring and as insect control tactics (Plimmer et al. 1982, Murlis et al. 1992, Johnson et al. 2009). Pheromones and kairomones used in traps allow growers to detect pest species from low to high densities, but do not usually correlate well to local densities or percent damage (Murlis et al. 1992). Some pheromones have been used to disrupt mating of Lepidoptera pests like codling moth, *Cydia pomonella* (L.), and Oriental fruit moth, *Grapholita molesta* (Busck) (Cardé and Minks 1995). Mating disruption uses dispensers of synthetic sex pheromones to create multiple plumes of pheromone in the treated area and prevent the insects from successfully finding their mates. Aggregation pheromones have been used to alter the behavior of insects. Aggregation pheromone at high concentrations can become repellants, as is the case in bark beetles (reviewed in Wood 1982).

Along with pheromones, developments in kairomone identification and isolation have improved the management of pest species. Fruit fermentation volatiles have been used as kairomones to attract-and-kill sap beetles, *Carpophilus* spp. (Hossain et al. 2008) or scarab

beetles (Leal 1998). Kairomones have also been used to improve monitoring systems. For example, commercially available products manuka oil (and Z-(3)-hexanol) were found to contain the same compounds as aerated ash bark samples and green leaf volatiles used to identify attractive compounds against emerald ash borer, *Agrilus planipennis* Fairmaire. These products were added to the existing colored traps to help improve trap captures for the emerald ash borer national survey conducted by the USDA-APHIS-PPQ (reviewed in Crook and Mastro 2010). To date, many semiochemicals have been isolated, identified and synthesized from insects. Currently, there is more emphasis on identifying and testing attractiveness of host plant odors (kairomones) to insects or if host plant colors and/or host leaf shapes play a role in attracting insects.

Being able to locate hosts for mating, oviposition or feeding using semiochemicals and/or visual cues is crucial for the survival of many specialist species. Most, if not all, plants emit some blend of odors to which specific insects have become responsive. Chemicals emitted by plants can act as an attractant to specialist herbivores and greatly increase the ability to find hosts (Miller and Strickler 1984, Visser 1986, Dicke 2000). The color and foliar shape of the plant can also increase the attractiveness for pest species (Roessingh and Städler 1990, Degen and Städler 1996, 1997). Herbivory can cause plants to release secondary chemicals, leading to the increased apparency of the host among non-host neighbors and attracting more herbivores (Dicke 2000) or their natural enemies (reviewed in Price et al. 1980, Vet and Dicke 1992). Research on host plant volatiles and secondary compounds has increased the potential to develop safer and more environmentally friendly control tactics. Being able to utilize naturally occurring attractant or deterrent chemicals from plants is an area that needs continued research, especially in blackberries against rednecked cane borer, *Agrilus ruficollis* (F.).

BLACKBERRIES *(RUBUS* SUBGENUS *RUBUS)*

Blackberries (*Rubus* subgenus *Rubus*), in the family Rosaceae, are native throughout most of the United States, Europe and Asia. Blackberries have a perennial root system, but the fruiting canes are biennial (Clark et al. 2007). The primocane is the first summer growth that requires a winter dormant period before becoming a floricane the following growing spring. However, recent breeding programs have identified and selected for plants in which primocanes produce fruit in the fall (Clark et al. 2005). Blackberries can be classified into three main categories; trailing, semi-erect and erect. Each category contains multiple cultivars produced by breeding programs in USDA and several universities. Flowers are found tightly clustered at the terminal end of inflorescences and are white–pink in color. The flowers are attached to a central receptacle in which the fruit is formed (Clark et al. 2007). Within the flower are many pistils each forming a drupelet consisting of a fleshy ovary and a single seed in the center. The flowers of upright blackberries are self-fertile, meaning that no pollinators are needed for development of fruit. However, pollination by insects increases the number of drupelets and the overall size of the fruit compared to self-pollinated fruit. Some of the common insect pollinators of blackberries are bees; including the honeybee *Apis mellifera* L. and bumblebees *Bombus* spp. (Fernandez and Ballington 2000).

With commercial cultivation of blackberries, the need for identification and management of pests (insects, diseases, and vertebrates) has become crucial to ensure a marketable harvest and survival of the planting. Current pests of blackberry in the eastern United States include: raspberry crown borer, *Pennisetia marginata* (Harris) (Lepidoptera: Sesiidae); rednecked cane borer, *Agrilus ruficollis* (F.) (Coleoptera: Buprestidae); strawberry clipper, *Anthonomus signatus* Say (Coleoptera: Curculionidae); stink bug complex (Johnson et al. 2005, Pyzner 2006); and a

recent pest, spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) (Bolda et al. 2010, Walsh et al. 2011).

REDNECKED CANE BORER, *AGRILUS RUFICOLLIS*

The rednecked cane borer, *Agrilus ruficollis* (F.) (Coleoptera: Buprestidae), is a pest of cultivated and wild blackberries in the Midwestern and Eastern parts of the United States. Adults emerge from late-April to mid-July (Webster 1892, Chittenden 1922, Walton 1951, Johnson 1992a). Adults are all black with the dorsal portion of the prothorax a reddish/copper color (Fabricius 1787, Hopkins 1891, Chittenden 1922). Males can be differentiated from females predominately by the presence of a groove on the ventral surface of the first two segments of the abdomen (Fig 1.1) and the short tooth on the inner margin at the apex of the mesotibiae (Fisher 1928). Eggs are oviposited onto primocanes singly or in groups of up to six eggs on the root collar or on the stem or branches (Hutchings 1923). Once the larva hatches, it enters the primocane and begins feeding just underneath the bark before entering the pith (Hopkins 1891) (Fig 1.2a,b). The first instar girdles the cane causing the formation of a gall also known as 'raspberry gouty-gall' (Walsh 1870) (Fig 1.3). The formation of a gall predisposes canes to winter injury and reduces yields the following year (Mundinger 1941, Walton 1951, Johnson and Mayes 1989, Johnson 1992a, 1992b) or can lead to cane breakage (Solomon 1995). The older larva moves into and feeds within the pith throughout the summer and then creates a pupal cavity in which it overwinters (Halderman 1846, Hopkins 1891, Chittenden 1922). The following spring, the 4th instar pupates, then ecloses to an adult that sclerotizes but remains inactive for 7-10 days before emerging from the cane (Chittenden 1922). This pest can reduce the bramble fruit yields in the eastern US by 72% (Hixson 1939, Strik et al. 2007).

Agrilus ruficollis oviposits only on canes of the lighter green primocanes that emerge in the spring and not on canes of the darker green to reddish-brown 1-yr-old floricanes. However, little is understood about this primocane host selection behavior, especially what stimuli may be involved - chemical or visual. This gap in knowledge can be viewed as one of many aspects that prevent the development of an effective trapping system and alternative management tactics. Growers typically scout for *A. ruficollis* by walking the bramble planting weekly during the day in May and June to detect the presence of *A. ruficollis* adults on primocane leaves. This scouting method is a very inefficient and labor intensive monitoring technique. The lack of an effective sampling method has made it difficult to establish an economic threshold of the number of *A. ruficollis* adults per sampling unit. As a result, growers either don't apply insecticide against *A. ruficollis* or apply one or more insecticide sprays without knowledge of presence/absence of *A. ruficollis* adults and/or if eggs are being laid.

There are also reported cultivar differences in susceptibility to *A. ruficollis* galling. Hixson (1939) listed several cultivars as comparatively resistant to attack by *A. ruficollis* including: 'Advance'; 'Austin Mayes'; 'Best of All'; 'Blower'; 'Rosborough'; 'Brazos'; 'Early Harvest'; 'Lucretia'; 'Ozark Beauty'; 'Mesereau'; and 'Youngberry'. Johnson (1992b) observed that some blackberry cultivars with two or more *A. ruficollis* galls per cane differed significantly in yield where 'Shawnee' yielded least followed by 'Comanche', 'Cheyenne' and 'Cherokee'.

Currently there are only three control tactics available to combat this pest: pruning to remove primocanes galled by *A. ruficollis* during the dormant stage and burn them (Chittenden 1922); prune primocanes that emerged prior to or during the egg laying period (Walton 1951); or apply a post bloom soil drench application of Admire Pro (imidacloprid) (Kim and Johnson 2012). It is not economical to prune galled primocanes in plantings with high densities of *A.*

ruficollis galled primocanes because this would significantly reduce the number of fruit producing floricanes the following spring and ultimately reduce the potential yield. Therefore, efficient monitoring and control measures are needed to keep this pest below economically damaging levels.

PAST RESEARCH WITH OTHER BUPRESTIDAE

Insecticidal methods for control of other buprestids in the genus *Agrilus* have generally been targeted toward trunk and limb applications, trunk injections, or soil drench applications. There have been a wide variety of active ingredients used to control various *Agrilus* sp. including; DDT, organophosphates, organochlorines, neonicotinoids (imidacloprid, dinotefuran), azadirachtin, carbamates (carbaryl), and pyrethroids (Williams and Neiswander 1959, Barter 1957, Appleby et al. 1973, Petrice and Haack 2006, McKenzie et al. 2010, Smitley et al. 2010a,b, McCullough et al. 2011, Muilenburg and Herms 2012). Along with the chemical control tactics, research into host plant odors as well as visual stimuli has been extensively studied (reviewed in Crook and Mastro 2010).

There are several reports of the attractiveness of host plant odor and visual stimuli for *Agrilus* species, but no such research has been conducted on *A. ruficollis* to date. The two-lined chestnut borer, *A. bilineatus* (Weber), was attracted to a crude steam distillate of bark from stressed oak trees used with sticky-banded trees and vane traps (Dunn et al. 1986). The emerald ash borer, *A. planipennis* responds to several plant volatiles emitted by stressed ash species (Rodriguez-Saona et al. 2006, Crook et al. 2008, de Groot et al. 2008, Marshall et al. 2010). Levels of six sesquiterpenes were elevated 24-h after girdling (stressed) ash trees. These odors that elicited antennal responses by both male and female emerald ash borer include: α-cubebene; α-copaene; 7-epi-sesquithujene; trans-β-caryophyllene; eremophilene; and α-humulene (α-

caryophyllene) (Crook and Mastro 2010). Phoebe oil, distilled from the Brazilian walnut, *Phoebe porosa* Mez., attracted emerald ash borer in the field and contains all six volatiles that stimulated emerald ash borer antennae, including 7-epi-sesquithujene, while Manuka oil, distilled from the New Zealand Manuka tree, *Leptospermum scoparium* Forst (Crook et al. 2006), contains only five out of six (Crook et al. 2008). The emerald ash borer has sensitivity to certain spectra or wavelength of light (Crook et al. 2009; Francese et al. 2010). Electroretinogram (ERG) recordings found emerald ash borer most sensitive to the visual spectrum in the UV (340 nm), violet/purple (420-430 nm), blue (460 nm), green (540-560 nm) and only eyes of female emerald ash borer responded to red (640-670 nm) regions of the spectrum (Crook et al. 2009). Based on these results, traps placed at mid (13 m) and lower (6 m) ash tree canopy height caught significantly more adult emerald ash borers on green traps than purple traps (Crook et al. 2009; Francese et al. 2010). Emerald ash borer response to traps was enhanced by incorporating specific host plant volatiles with trap colors and trap height (Francese et al. 2005, Marshall et al. 2010, Crook et al. 2012). My hypothesis is that another buprestid species, *A. ruficollis*, may be attracted to both specific color and host volatiles from its primocane blackberry host plant.

Along with colored traps and host odors, research into the attraction of beetles to visual decoys of the same species has been studied for various *Agrilus* sp. It has been demonstrated that decoys of adult *A. planipennis* placed on a trap surface or silhouettes of these beetles increased trap captures (Lelito et al. 2008, Domingue et al. 2013b). Similarly, decoys of *A. planipennis* adults adhered onto green plastic branch-traps also attracted *A. biguttatus* and *A. sulcicollis* (Domingue et al. 2013a). These observations of the attractiveness of trap color and the addition of visual decoys may result in more attractive traps for *Agrilus* spp.

DISSERTATION RESEARCH

The objectives of this doctoral dissertation were: 1) to compare insecticide efficacy of several classes of insecticides against *A. ruficollis*; 2) to examine the attractiveness of various designs and trap colors to *A. ruficollis*; 3) to examine whether volatiles collected from primocane and floricane blackberries are chemically unique.

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Figure 1.1 Ventral surface of one female and two male *Agrilus ruficollis*. Red circle indicates the groove that can be used to determine sex of *A. ruficollis* as described by Fisher (1928).

Figure 1.2 *Agrilus ruficollis* larval feeding in a blackberry primocane: a) early instar feeding, girdling the cane; and b) late instar feeding within the pith of primocane.

Figure 1.3 Gall formation after girdling of blackberry primocane by *Agrilus ruficollis* larva.

CHAPTER 2 – INSECTICIDAL EFFICACY OF SELECTED ACTIVE INGREDIENTS IN PREVENTING GALL FORMATION BY *AGRILUS RUFICOLLIS*

INTRODUCTION

The rednecked cane borer, *Agrilus ruficollis* (F.) (Coleoptera: Buprestidae), is a pest of cultivated (*Rubus* subgenus *Rubus*) and wild blackberries (*Rubus fruticosis* L.) in the Midwestern and Eastern parts of the United States. Adults emerge from late-April to mid-July and lay eggs on blackberry primocane leaves (Walton 1951, Johnson 1992a). The blackberry plant has a biennial stem ("canes") from the perennial root system. In its first year, a blackberry plant produces a new stem, the primocane, which is vegetative or may produce fruit in the fall ("primocane fruiting"). This cane over winters and becomes a floricane that produces flowers and fruit the following spring and summer and then the cane dies (Krewer et al. 2004). Adult feeding by *A. ruficollis* causes small, irregular holes along the edges of the leaf and often defecate black, oblong fecal pellets onto leaves. The first instar girdles the stem of a primocane causing that damaged area to swell into a gall. After girdling the cane, the larva tunnels into the pith of the primocane where in Arkansas it over winters until late April or May. In April and May galled canes can be split to find the over wintered white, legless larvae or the darkening pupae or newly eclosed adult in the discolored tunnel in the pith. This galling and tunneling damage predisposes primocanes to winter injury so the floricanes may or may not foliate and rarely ripen fruit (Mundinger 1941, Johnson and Mayes 1989, Johnson 1992a, 1992b).

There are recommended cultural control tactics for *A. ruficollis*. Growers prune and burn galled canes in the winter in plantings with less than 5% *A. ruficollis* galled canes. Elimination of nearby wild *Rubus* hosts is also recommended to lower the local population of *A. ruficollis* (Hixson 1939, Walton 1951, Pfeiffer 2011).

Chemical control is recommended only if more than 5% of canes are *A. ruficollis* galled (Johnson and Mayes 1989, Bessin 2004, Pfeiffer 2011, Studebaker 2013). Currently, the only insecticide registered against *A. ruficollis* is imidacloprid (Admire Pro). This is a synthetic neonicotinoid insecticide that is applied as a soil drench or through the irrigation system ("chemigation"). The EPA label for Admire Pro states, "DO NOT apply prebloom, during bloom or when bees are foraging."

An area of increasing concern for managing A. *ruficollis* is the interaction of insecticides with pollinators (A. *ruficollis* lay eggs during and after bloom) and the potential for this pest to develop resistance to the one registered compound, imidacloprid. Several studies investigated the lethal and sublethal effects of neonicotinoids and other compounds on pollinators. Mullins et al. (2010) examined 87 pesticides and metabolites in wax samples and 98 pesticides and metabolites in pollen samples collected from honey bee hives. Direct contact toxicity experiments conducted on bumble bee, *Bombus impatiens* (Cresson), alfalfa leafcutting bee, *Megachile rotundata* (F.), and Mason orchard bee, *Osmia lignaria* Say, demonstrated that the neonicotinoids, clothianidin and imidacloprid, were highly toxic to these bee species (Scott-Dupree et al. 2009). Oral exposure of imidacloprid concentrations greater than 50 μg/liter to honey bees induced abnormal behaviors such as bees not returning to the hive or increased time between visits to sucrose source (Yang et al. 2008).

There is a need to demonstrate efficacy of alternative classes of insecticides (different active ingredients and modes of action) to imidacloprid given the concerns noted above. Also, there are no insecticide formulations registered against *A. ruficollis* that are currently labeled as OMRI (Organic Materials Review Institute) approved for use in organic production of

blackberries or backyard production. The objective of this research was to determine the efficacy of selected synthetic and earth-derived (organic) insecticides on *A. ruficollis*.

MATERIALS AND METHODS

Chemicals. We tested both synthetic and earth-derived (organic) compounds for efficacy against *A. ruficollis* in 2010 and 2011 (Table 2.1).

The synthetic compounds tested were: acetamiprid (Assail 30SG) at rate of 0.095 kg active ingredient (AI)/hectare (United Phosphorus, Inc., King of Prussia, PA); thiamethoxam + chlorantraniliprole (Voliam Flexi) at rate of 0.061 kg (AI)/hectare (Syngenta Crop Protection, LLC, Greensboro, NC); bifenthrin (Fanfare 2EC) at rate of 0.112 kg (AI)/hectare (MANA - Makhteshim Agan of North America, Inc., Raleigh, NC); imidacloprid (Admire Pro) at rate of 0.56 kg (AI)/hectare (Bayer Environmental Science, Research Triangle Park, NC); indoxacarb (Avuant 30DG) at rate of 0.123 kg (AI)/hectare (Dupont Crop Protection, Wilmington, DE).

The organic compounds evaluated were: azadirachtin (Aza-Direct) at rate of 0.028 kg (AI)/hectare (Gowan Company, Yuma, AZ); azadirachtin and pyrethrin (Azera) at rate of 0.014 kg (AI)/hectare azadirachtin and 0.015 kg (AI)/hectare pyrethrin (MGK Company, Minneapolis, MN); pyrethrin (Pyganic 5EC) at rate of 0.112 kg (AI)/hectare (MGK Company, Minneapolis, MN); peppermint oil + rosemary oil (Ecotrol) at rate of 1.2% solution (EcoSmart Technologies, Inc., Alpharetta, GA); *Beauveria bassiana* strain GHA (Botanigard 22WP) at rate of 6.73×10^{13} spores/hectare (Mycotech Corp., Butte, MT); and paraffinic oil (Organic JMS Stylet-Oil) at rate of 1.5% solution (JMS Flower Farms Inc., Vero Beach, FL).

Efficacy Studies. We conducted the efficacy studies at the University of Arkansas Fruit Research Station in Clarksville, Arkansas (35°32'33.64"N -93°25'13.26"W). Insecticide applications were applied according to the labeled recommendation once inspections of

primocane foliage detected presence of *A. ruficollis* adults and ended when adults were no longer present. Plots were scouted for beetle presence using a direct (*in* situ) sampling method (Hutchins 1994). This was accomplished by walking the rows of blackberry plantings for 15 min to observe whether adults were present. The plants were observed from the base of the plant to the top to look for resting, feeding, or mating adults. Insecticide applications were carried out in the morning and were completed before noon.

In 2010, ten insecticide formulations were used in the efficacy study (Table 2.1). Plots were set up using a randomized complete block design with four replicates and each treatment plot had ten blackberry plants per plot, there was a four plant buffer placed between treatments (Figure 2.1). On 12 May, application of the insecticides was initiated. The imidacloprid and the thiamethoxam + chlorantraniliprole treatment were each applied once as a soil drench. All other treatments were applied as a foliar application. The two synthetic products (acetamiprid and bifenthrin) were reapplied once to the foliage on 25 May and the organic products were reapplied to the foliage weekly on 19 and 25 May. A 1 gal solution per treatment plot (equivalent to 100 gpa) was applied using a SHURflo® electric backpack sprayer (Cypress, CA) at 40 psi. On 17 and 30 September, 2010, the number of galls on each primocane per treatment plot was recorded.

Results from the 2010 efficacy study were used to remove and/or replace insecticides used in the 2011 testing. In 2011, five insecticide formulations were used for the efficacy study at the rates noted above (Table 2.1). A treatment plot was marked at ends of each blackberry row and arranged in a randomized complete block design with four replicates. Each treatment plot had 20 blackberry plants (Figure 2.2). A soil drench of Admire Pro was applied 29 April. On 2 May, the other four treatment were applied as a foliar application of 1 gal of solution per treatment plot (100 gpa) using a SHURflo® electric backpack sprayer (Cypress, CA) at 40 psi.

Avaunt and Fanfare were reapplied on 17 May. The organic formulations of Organic JMS Stylet Oil and Botanigard were reapplied weekly on 10, 17, 24 May. On 14 October, 2011 the number of galls on each primocane per treatment plot was recorded.

Statistical Analysis. Data were analyzed using analysis of variance (ANOVA) and treatment means were separated using the Waller-Duncan *k*-ratio *t*-test (SAS 2008). All data were log (x+0.5) transformed before analysis and means of untransformed data were displayed in the tables.

RESULTS

In 2010, there were no significant differences ($F = 0.37$; df = 10, 33; $P < 0.9524$) in the treatment mean numbers of *A. ruficollis* galls per plot (Table 2.2). Although the results demonstrated no significant differences, paraffinic oil (average 0 galls), *B. bassiana* (average 0.25 galls), and bifenthrin (average 0.25 galls) were carried over for another trial in 2011.

Results from 2011 demonstrated significant differences between some treatments. Although galling in plots treated with imidacloprid and paraffinic oil was similar to that in plots treated with bifenthrin and *B. bassiana*. Comparison of blackberry plots treated with imidacloprid or paraffinic oil each had significantly fewer galls per plot than either the untreated control or plots treated with indoxacarb ($F = 3.25$; df = 5, 18; $P < 0.0289$). However, the numbers of galls in plots treated with bifenthrin or *B. bassiana* were not different from plots treated with indoxacarb and the untreated control (Table 2.3).

DISCUSSION

Alternative control tactics for *A. ruficollis* were inconsistent between the two years that efficacy testing was conducted. In 2010, no significant differences were seen among any of the insecticides tested. There were two potentially confounding factors. It has been noted that *A.*

ruficollis emerge in late-April or early May (Walton 1951, Johnson 1992a), but the 2010 efficacy trial was started in the Mid May and may have been too late for effective control. There was a high visual count of RNCB adults observed in the field study planting at the start of application on 12 May 2010 using a direct sampling method (Hutchinson 1994), but these counts dropped dramatically to 25 and 1 adults per 15 minute visual inspection of the planting on 19 and 25 May, respectively. Another potential confounding factor was the actual treatment plot location (Figure 2.1). Visual observations along the planting rows showed that larger numbers of *A. ruficollis* adults were flying from plant to plant within a row, especially along the ends of rows. This potential edge effect may have been the cause of high variance leading to no significance difference among the treatments. The efficacy testing protocol was modified in 2011 so that each treatment application was applied to a different blackberry row instead of all treatments as subplots within the same blackberry row (Figure 2.2). This modification of the treatment plot layout resulted in significant differences between two of the treatments.

In 2011, the four applications of paraffinic oil (JMS Stylet Oil) were as effective as the one soil drench application of imidacloprid (Admire Pro) in preventing blackberry cane galling by *A. ruficollis*. The mode of action for paraffinic oil was through suffocation of treated insects or eggs (Bográn et al. 2006). One problem with use of paraffinic oil against *A. ruficollis* is that the EPA label states that to minimize phytotoxicity, paraffinic oil should not be applied at temperatures above 32°C. It is known that *A. ruficollis* oviposition period occurs in southern states like Arkansas during May and June when temperatures often exceed 32°C (Johnson and Mayes 1989). Therefore, it was thought that paraffinic oil could have achieved even greater effectiveness if sprays were not stopped after four applications when daily temperatures began to

exceed 32°C in May. Given this limitation, paraffinic oil still prevented enough galling of blackberry to serve as an effective alternative treatment to imidacloprid.

Although paraffinic oil was found to be effective alternative to imidacloprid for preventing gall formation, further testing should be conducted to identify additional classes of compounds to manage *A. ruficollis*. Further studies on laboratory based mortality bioassays on adult *A. ruficollis* with the different insecticide classes should also be conducted to determine whether the compound is targeting the egg, larvae, or adult life stage but this would require development of an artificial rearing method. Having alternative classes of compounds available for management of *A. ruficollis* would allow growers the option to rotate insecticides with different modes of action as part of their insecticide resistance management program. Also, the recommended timing of the application of imidacloprid after bloom may not be ideal since *A. ruficollis* lay eggs during and after bloom. Studies are needed to determine if a soil drench application of imidacloprid before or during blackberry bloom produce levels in flowers and nectar that is toxic to pollinators and significantly reduce cane galling by A. *ruficollis*.

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Table 2.1. Synthetic and earth-derived insecticides evaluated (X = year used) in blackberry field efficacy studies against *Agrilus ruficollis* in either 2010 or 2011 at the Fruit Station in Clarksville, AR.

Insecticide active ingredient	**Product name**	**Year applied** 2010	**Year applied** 2011
Bifenthrin	Fanfare 2EC	X	X
Acetamiprid	Assail 30SG	X	
Imidacloprid	Admire Pro	X	X
Indoxacarb	Avaunt		X
Thiamethoxam + Chlorantraniliprole	Voliam Flexi	X	
Peppermint oil + Rosemary oil	Ecotrol[a]	X	
Paraffinic oil	Organic JMS Stylet Oil[a]	X	X
Azadirachtin	Aza Direct[a]	X	
Pyrethrin	Pyganic 5EC[a]	X	
Azadirachtin + Pyrethrin	Azera[a]	X	
Beauveria bassiana strain GHA	Botanigard[a]	X	X

[a] Organic, earth-derived biopesticide formulation that is OMRI (Organic Materials Review Institute) approved for organic production

Table 2.2. Mean (± SE) number of *Agrilus ruficollis* induced galls per blackberry plot by insecticide treatment in Clarksville, AR (2010).

Insecticide active ingredient	Amount (AI)/hectare	Mean number of galls per plot in September [a]
Acetamiprid	0.095 kg	0.5 ± 0.3a
Imidacloprid	0.560 kg	0.5 ± 0.4a
Bifenthrin	0.112 kg	0.3 ± 0.2a
Thiamethoxam + Chlorantraniliprole	0.061 kg	0.8 ± 0.6a
Peppermint oil + Rosemary oil	1.2% solution	0.5 ± 0.4a
Paraffinic oil	1.5% solution	0.0 ± 0.0a
Azadirachtin	0.028 kg	0.5 ± 0.3a
Pyrethrin	0.112 kg	0.0 ± 0.0a
Azadirachtin + Pyrethrin	0.014 kg	0.8 ± 0.6a
Beauveria bassiana strain GHA	6.73×10^{13} spores	0.3 ± 0.2a
Untreated Control		0.5 ± 0.3a

[a] Means followed by the same letter are not significantly different (Waller-Duncan *k*-ratio *t*-test, $P < 0.05$)

Table 2.3. Mean (± SE) number of *Agrilus ruficollis* induced galls per blackberry plot by insecticide treatment in Clarksville, AR (2011).

Insecticide active ingredient	Amount (AI)/hectare	Mean number of galls per plot in October [a]
Bifenthrin (Fanfare 2EC)	0.112 kg	12.9 ± 6.43ab
Imidacloprid (Admire Pro)	0.56 kg	2.5 ± 1.19b
Indoxacarb (Avaunt)	0.123 kg	15.0 ± 3.94a
Paraffinic oil (Organic JMS Stylet Oil)	1.5% solution	4.0 ± 2.12b
Beauveria bassiana (Botanigard)	6.73 x 10^{13} spores	10.0 ± 3.81ab
Untreated control		16.0 ± 1.83a

[a] Means followed by the same letter(s) are not significantly different (Waller-Duncan *k*-ratio *t*-test, $P < 0.05$)

1	2	3	4	5	6	7	8	9	10	11	Rep 1
9	10	4	11	5	2	8	7	1	6	9	Rep 2
3	7	11	4	1	2	5	8	7	1	6	Rep 3
7	2	3	4	1	6	11	10	8	5	9	Rep 4

Figure 2.1. Insecticide efficacy plot design for 2010. Each row indicates a row of blackberry plants where Labels 1-12 indicate an insecticide treatment as follows: 1) untreated control, 2) bifenthrin, 3) acetamiprid, 4) imidacloprid, 5) thiamethoxam + chlorantraniliprole, 6) peppermint oil + rosemary oil, 7) paraffinic oil, 8) azadirachtin, 9) pyrethrin, 10) azadirachtin + pyrethrin, and 11) *Beauveria bassiana*.

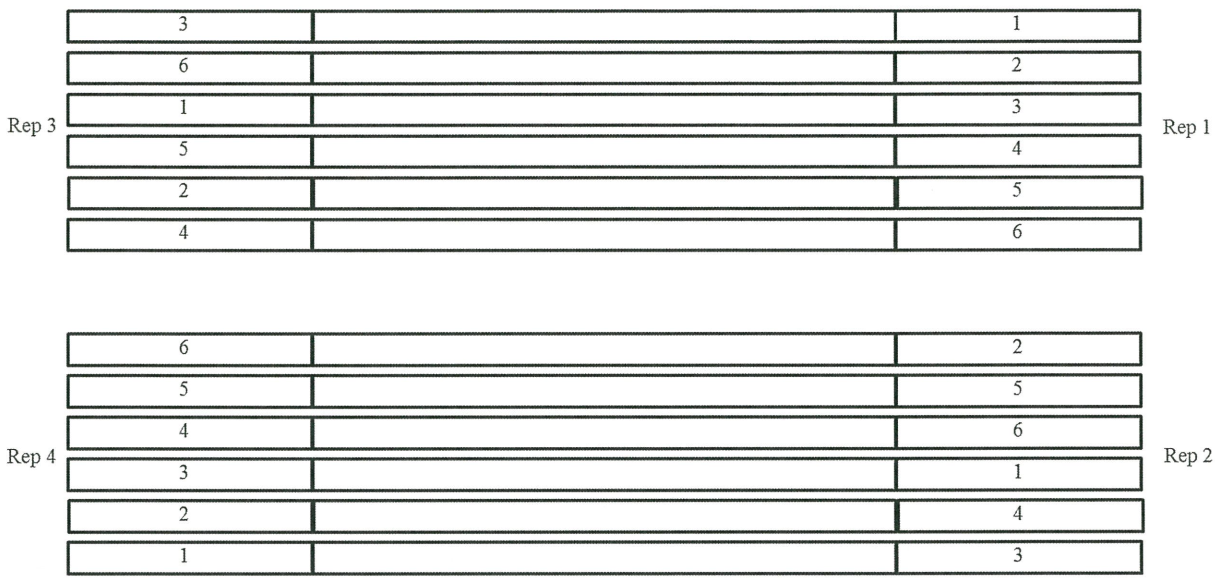

Figure 2.2. Insecticide efficacy plot layout for 2011. Each row indicates a row of blackberry plants with a treatment plot on each end where Labels 1-6 indicate an insecticide treatment as follows: 1) bifenthrin, 2) imidacloprid, 3) indoxacarb, 4) paraffinic oil, 5) *Beauveria bassiana*, 6) untreated control.

CHAPTER 3 – ATTRACTION OF *AGRILUS RUFICOLLIS* TO VARIOUS COLORS AND IDENTIFICATION OF ATTRACTIVE ODORS FROM BLACKBERRY PLANTS

INTRODUCTION

Rednecked cane borer, *Agrilus ruficollis* (F.) (Coleoptera: Buprestidae), is a pest of cultivated (*Rubus* subgenus *Rubus*) and wild blackberries (*Rubus fruticosis* L.) in the Midwestern and Eastern parts of the United States and emerges from late-April to mid-July (Walton 1951, Johnson 1992a). This pest can potentially reduce the fruit harvest by 72% (Hixson 1939), this can affect the nearly 7,100 tons of bramble fruit (Strik et al. 2007) produced annually in the eastern US. *Agrilus ruficollis* oviposits only on the lighter green primocanes that emerge in the spring and not on the darker green to reddish-brown 1-yr-old floricanes. The early *A. ruficollis* instar girdles the cane causing galling which predisposes canes to winter injury and reduces yields the following year (Mundinger 1941, Walton 1951, Johnson and Mayes 1989, Johnson 1992a, 1992b). Johnson (1992b) also noted that blackberry plants with two or more *A. ruficollis* galls per cane also significantly reduced yields on certain varieties.

There are reported cultivar differences in susceptibility to *A. ruficollis* galling. Hixson (1939) listed several cultivars as comparatively resistance to attack by *A. ruficollis* including: 'Advance'; 'Austin Mayes'; 'Best of All'; 'Blower'; 'Rosborough'; 'Brazos'; 'Early Harvest'; 'Lucretia'; 'Ozark Beauty'; 'Mesereau'; and 'Youngberry'. However, the mechanism behind these host resistance are still unknown.

Currently there are only three control tactics available to combat this pest: 1) removing galled primocanes during the dormant stage and burn them; 2) prune primocanes that emerged prior to or during the egg laying period; 3) or apply a post-bloom soil drench of Admire Pro (imidacloprid). A fourth tactic can be implemented by using resistant plants as mentioned above,

but most are older varieties that have been replaced with higher yielding varieties. Pruning off galled primocanes in plantings with high *A. ruficollis* infestations during the dormant season significantly reduces the number of fruit producing floricanes the following spring and ultimately reduces the potential yield. Therefore, efficient monitoring and control measures are needed to keep this pest below economic damaging levels.

Efficient trapping or monitoring can prevent unwarranted insecticide sprays; however, no trap has been developed for monitoring this pest. Growers can walk weekly through a blackberry planting in May and June searching for presence of *A. ruficollis* adults on primocane leaves. There are several reports of the attractiveness of host plant visual and odor stimuli for insect species in the genus *Agrilus*, but no such research has been conducted on *A. ruficollis* to date.

The two-lined chestnut borer, *A. bilineatus* (Weber), was attracted to a crude bark steam distillate from stressed oak trees used with sticky-banded trees and vane traps (Dunn et al. 1986). The emerald ash borer (EAB), *A. planipennis* Fairmaire (Coleoptera: Buprestidae), exhibited antennal responses to several plant volatiles emitted by ash species (Rodriguez-Saona et al. 2006, Crook et al. 2008, de Groot et al. 2008, Lelito et al. 2008, Marshall et al. 2010). Six identified sesquiterpenes that were elevated 24-h after girdling (stressed) ash trees and noted to elicit antennal responses by both male and female *A. planipennis* included: a-cubebene; a-copaene; 7-epi-sesquithujene; trans-b-caryophyllene; eremophilene; and a-humulene (a-caryophyllene). Phoebe oil attracted EAB in the field and contained all six of the antennally active *A. planipennis* volatiles, including 7-epi-sesquithujene (Crook et al. 2008). Along with the attraction to the bark volatiles, a green leaf volatile (3Z)-hexanol was shown to be attractive for males (de Groot et al. 2008, Grant et al. 2010). A female pheromone, (3Z)-dodecen-12-olide (3Z-lactone), has been shown to have antennal activity for male *A. planipennis* (Bartelt et al. 2007, Silk et al. 2011,

Ryall et al. 2012). The identification of both stressed tree volatiles and green leaf volatiles have been combined with advancements in trap color for this pest.

Electroretinogram (ERG) recordings found *A. planipennis* most sensitive to the visual spectrum in the UV (340 nm), violet/purple (420-430 nm), blue (460 nm), green (540-560 nm) but only female EAB respond to red (640-670 nm) regions of the spectrum (Crook et al. 2009). Traps placed at mid (13 m) and lower (6 m) ash tree canopy heights caught significantly more *A. planipennis* on green traps than purple traps (Crook et al. 2009; Francese et al. 2010). The response by *A. planipennis* to sticky traps has been enhanced by incorporating specific host plant volatiles with trap colors and trap height (Francese et al. 2005, Lelito et al. 2008, Marshall et al. 2010, Crook et al. 2012). Recent identification of host volatiles from ash trees and visual stimuli that are attractive to *A. planipennis* brings up the question as to whether *A. ruficollis* may be attracted to host volatiles and visual stimuli from its blackberry host plant. Although the identification of volatiles from blackberry fruit and bound aromas in leaves has been investigated (Humpf and Schreier 1991, Qian and Wang 2005, Du et al. 2010a, 2010b), the identification of volatiles from the actual plant has yet to be determined.

Little is understood about the host selection behavior for *A. ruficollis*, especially what stimuli may be involved, chemical or visual. This prevents the development of an effective trapping system, leaving us with the current inefficient and labor intensive monitoring technique of walking bramble plantings to detect presence of *A. ruficollis* adults. This has made it nearly impossible to establish an economic threshold of the number of *A. ruficollis* adults per sampling unit, which has led to unjustified and/or untimely use of insecticides or no control tactic being used. The objectives of this study were 1) to determine attractiveness of colored traps to *A.*

ruficollis and 2) to determine if the volatile bouquet emitted from blackberry primocanes differs from floricanes.

MATERIALS AND METHODS

Trap Color

Samples of blackberry primocanes and floricanes (leaves and stems) were taken to a local hardware store to be characterized for color and purchase color matching paints. These paints along with the following colors: yellow (John Deere yellow, Krylon Products Group, Cleveland, OH); purple spray paint (Rich Plum, Krylon Products Group, Cleveland, OH); emerald ash borer traps light purple, dark purple and green (Crook et al. 2008), were analyzed using a Jazz spectrometer (Ocean Optics, Dunedin, FL) to determine the color spectra reflected peak wavelength and the change in percent reflectance across the visible wavelengths. For 2013, color matched paints were used for all traps besides the emerald ash borer purple, which used the commercially available emerald ash borer trap. Traps were painted with paint that matched the peak wavelength and percent reflectance of blackberry primocane (leaf and cane) and floricane (leaf and cane) and color matched to emerald ash borer green traps (Table 3.1).

Trap Field test

2011

Gray sheet metal (3.8 cm X 8.9 cm) that mimicked a blackberry leaf shape had paints applied that mimicked blackberry primocane and floricane (leaf and cane), or John Deere yellow or purple spray paint, and unpainted control. For each color, four leaf orientations were made: vertical, horizontal, 45° tilted up, and 45° tilted down. Four painted metal strips of each orientation were alternately attached onto 0.6 m long x 1.0 cm diameter dowel rods (Figure 3.1). To test the color and effect of cane diameter on attractiveness to *A. ruficollis*, 61.0 cm long

wooden dowel rods of different diameters (2.5 cm, 1.3 cm, 1.0 cm, 0.3 cm) were painted with the colors listed above (Figure 3.2). The painted rods were attached vertically to a piece of rebar exposed 15.2 cm above soil next to a blackberry plant, coated with Tanglefoot® (Tanglefoot Co., Grand Rapids, MI) using a paint brush. Tanglefoot® was cleaned off and reapplied as needed. Traps were spaced 3 m apart in a randomized complete block design with eight replicates. Four blocks were set out at the University of Arkansas Fruit Station in Clarksville, AR (35°32'33.64"N 93°25'13.26"W) on 19 May and four at a grower planting in Tontitown, AR (36° 9'36.77"N 94°17'24.95"W) on 17 May. Traps were checked weekly and all captured *A. ruficollis* were counted and removed. Traps were deployed in the field until two weeks of zero captures were recorded.

2012

Trap design was modified in 2012 with the addition of emerald ash borer traps (dark purple, light purple, and green). These traps replaced the purple spray paint used in 2011 and were used to determine if commercially available traps would attract *A. ruficollis*.

Testing of the attractiveness of leaf colors was conducted by painting primed sheet metal (3.8 cm X 8.9 cm) with leaf mimic colors (primocane and floricane), yellow, and the unpainted (primed) control. While the corrugated plastic of the three emerald ash borer trap colors of green, or light or dark purple were cut into rectangles (3.8 cm X 8.9 cm) (Table 3.1). During the flight period of *A. ruficollis* in May and June, traps with each test color, four painted metal strips or corrugated plastics were alternately attached onto 61.0 cm long x 1.3 cm diameter dowel rods was positioned 45° tilted downwards (Figure 3.3a). These colored leaf traps on rods were positioned 3 m apart in blackberry rows and anchored in a vertical orientation to a piece of rebar exposed 15.2 cm above soil.

To test the color attractiveness of the canes, 0.6 m long wooden dowel rod 1.3 cm inch in diameter (Figure 3.3b) was either painted a cane mimic color, yellow, or one of the three emerald ash borer trap colors (Figure 3.3c) of green, or light or dark purple (Table 3.1) (2.5 cm x 61 cm prism shaped) and unpainted control. At similar spacing (stated previously) in the blackberry row, painted rods were anchored vertically to a piece of rebar exposed 15.2 cm above the soil. All traps were coated with Tanglefoot® using a paint brush after deployment into the field. Just before the initiation of flight of *A. ruficollis* adults traps were placed in a randomized complete block design with seven replicates. Five replicates were installed at the Fruit Research Station in Clarksville, AR on 17 April and the remaining two replicates were installed at a local grower planting in Tontitown, AR on 27 April. Treatment traps were rearranged after each count period to eliminate trap location effect. Clean sticky traps were set out every two weeks. Traps were checked weekly and all captured *A. ruficollis* adults were counted and removed. Later these adults were sexed under a stereomicroscope looking for a groove (male) or no grove (female) along the ventral surface of the abdomen (Fisher 1928). Traps were deployed in the field until two weeks of zero captures were recorded.

2013

Traps in 2013 were modified to a single design; vertical prism shaped, using clear corrugated plastic sheets (2.5 cm wide each side) with a length of 61.0 cm that was glued onto a wooden dowel rod 1.3 cm in diameter (Figure 3.4a). Traps in this configuration used in the 2012 study were observed to be easier to clean and reapply Tanglefoot® once deployed. Therefore, all traps were made into this vertical prism shape.

Each prism trap was coated with a white primer (White Multipurpose Zero VOC* Latex Primer, B51 W450, Sherwin Williams Ltd., Richardson, TX) then coated with two to three coats

of one of these paints: a blackberry leaf or cane mimic color, emerald ash borer green or white primer (control), emerald ash borer light purple plastic traps were modified into prism shaped traps as mentioned above (Table 3.1). On 8 May, traps were placed in a blackberry planting in a randomized complete block design with nine replicates at the University of Arkansas Fruit Station in Clarksville, AR. Traps were spaced 4.6 m apart within the blackberry row and anchored vertically to a piece of rebar exposed 15.2 cm above the soil. All traps were coated with Tanglefoot® after deployment into the field. Traps were checked weekly and captured *A. ruficollis* adults were counted and removed. The number of beetles per trap of each sex (described above) was counted and the sex ratio was calculated. Traps were deployed in the field until two weeks of zero captures were recorded.

2014

A field trial was conducted in 2014 to determine whether commercially available colored multifunnel traps would be effective in capturing *A. ruficollis*. The multifunnel traps were either a green or purple 12-unit Lindgren funnel trap that was coated with Fluon® (ChemTica USA, Durant, OK) (Fig. 3.4 c-d). Traps were placed in blackberry plantings at the University of Arkansas Fruit Station in Clarksville, AR, the grower planting in Tontitown and a new location of wild blackberries in Springdale (36° 9'45.84"N 94° 5'23.46"W), AR. Traps were spaced 20 m apart along the planting with a total of 10 replicates. Traps were filled with a 50% anti-freeze to water solution to drown captured insects and insects were strained from drowning solution weekly. Traps were deployed in the field until two weeks of zero captures were recorded.

Degree Day Modeling

Data from the 2011-2014 field trials along with data from Johnson and Mayes (1989) was used to determine whether *A. ruficollis* adult activity can be predicted using a degree day model. Weather data for Fayetteville, AR from 1985-1987 was gathered from weatherunderground.com while weather data from Clarksville was collected from the weather station at the experiment station. The maximum and minimum air temperature was used to calculate degree day accumulation numbers using the following formula (average daily temperature – degree day base). These degree day numbers were combined to determine cumulative degree days at time of first activity, peak activity, and last activity. Base temperatures ranging from 40°F to 57°F were tested to determine if a common base temperature is available to use for predicting *A. ruficollis* activity.

Volatile Collection

Volatiles from primocanes and floricanes were collected by cutting four 0.6 m long sections of the cane and placing them into a clear Teflon bag (American Durafilm, Holliston, MA) (61 cm x 61 cm). Canes were cut throughout May 2014 between 8:00 – 10:00 am. One bag was left empty to serve as the control for each rep. Each bag was customized with an inlet and outlet port along the sides of the bag. A 4-chamber air delivery system (Analytical Research Systems, Inc., Gainesville, FL) with a carbon filter was used to push odor-free, filtered air through Teflon® tubing (odor-free, filtered air) and activated charcoal filter at a flow rate of 1.5 liter/min into the bag while volatile ladened air exits through a volatile collection trap (0.6 cm diameter x 10.2 cm long) packed with 50 mg Porapak Q absorbent powder (Southern Scientific Inc., Micanopy, FL) connected to a vacuum pump pulling at a rate of 0.5 liter/min. The collection of volatiles (eight replicates) was allowed to run for four hrs and trap eluted with 3 ml of dichloromethane and concentrated to 0.5ml with nitrogen. All solvent extracts were kept at -

80°C before injecting samples into gas chromatograph – mass spectrometry (GC-MS) for analysis (modified from Crook et al. 2008).

Porapak Q extracted samples were injected into a Varian 3900 GC in the laboratory for initial identification of volatiles and also to determine if the collection method was conducted properly using a modified gas chromatograph (GC) method originally described by Crook et al. (2008). A 1µl sample of the volatile was injected into the GC in a splitless mode with the temperature for the injector at 220°C and FID at 250°C. The carrier gas of Helium was flowed through the capillary column (Varian Factor Four, VF-3ms 30m) at 40cm/sec. The GC oven was programmed to increase in temperature at a rate of 8°C/min from 40°C to 300°C with an initial and final hold time of 1 and 5 min, respectively. Volatile collections from primocane and floricane along with a control (empty Teflon bag) were analyzed on GC. Samples that showed peaks on the GC were later injected into a Varian 450 GC coupled with Varian 320 Triple Quadruple EI/CI GC/MS (University of Arkansas State Wide Mass Spectrometry Facility, Fayetteville, AR) to identify and quantify peaks. The GC output into the MS was operated in full-scan (M/Z 50-350) and selected ion monitoring modes (TIC at m/z 68, 120, and 93) with electron-impact ionization. To identify each volatile peak, its retention time (RT) was compared to a computerized mass spectral data library (National Institute of Standards and Technology - Version 2008, Gaithersburg, Maryland).

Statistical Analysis

Trap count data collected from the trap experiment were log (x +.05) transformed before analysis. Difference between cane and leaf traps was analyzed by a paired t-test while analysis of the different colors within cane and leaf was analyzed using PROC GLM with main effects for treatment and block (SAS 2008). Waller-Duncan *k*-ratio *t* test ($\alpha = 0.05$) was used to make

pairwise comparisons between treatments. Comparison of sex ratio was analyzed using two-way ANOVA to test the main effects and then a *t* test was conducted on individual colors. Waller-Duncan *k*-ratio *t* test ($\alpha = 0.05$) was used to make pairwise comparisons between treatments. The gender count data for each color was log (x+.05) transformed before analysis. Data for the 2014 field trial of two funnel trap colors were log (x+.05) transformed before analysis by a paired t-test.

RESULTS

Trap color

A yearly comparison of color analyses of blackberry plant parts and colored traps identified differences in the mean reflected wavelength and percentage reflectance (Table 3.1). Peak wavelengths of light emitted by blackberry plant parts varied from light green at 546 nm for primocane canes to 554 nm for primocane leaves and 550 nm for floricane leaves to a reddish-brown floricane cane at 630 nm.

Yearly color matching from the local hardware store produced very similar paint colors mimicking those reflected by the actual plant. For colors used in the 2011 trials, the paint color matched primocane leaf and cane peak had wavelengths at 545 nm and 556 nm, respectively (Table 3.1, Figure 3.5). These wavelengths were slightly greater, by 5-6 nm, than the actual plant readings. For the 2012 trials the same colors were used as in 2011, but the purple was replaced with actual emerald ash borer (dark purple, light purple, and green) corrugated plastic traps (Table 3.1, Figure 3.6). In comparison to the different green colored traps, the emerald ash borer green trap had peak reflection at 544 nm and 50% reflectance compared to < 25% reflectance from all natural blackberry plant parts. For the 2013 field trials, paint was used to color all traps except emerald ash borer light purple, which used the actual emerald ash borer light purple

plastic trap. Paint colors for primocane mimicking traps were more closely matched to the actual plant color reading (Table 3.1, Figure 3.7). All the traps painted to mimic a blackberry plant part had used a flat or high gloss paint that reflected from 7 to 23% of the light compared to 49-54% light reflectance by the emerald ash borer green trap.

Trap field test

2011

Results from field testing the attractiveness to *A. ruficollis* of the different colors and orientations of the leaf mimics demonstrated that there was no significant difference in beetles captured for the orientation of the leaf for any of the colors. This was also true when comparing the different diameter sizes as well as colors. When data for different orientations for colors were pooled together for analysis, there were no significant differences seen when comparing total *A. ruficollis* capture amongst the different leaf colors. However, there were significantly more *A. ruficollis* captured per trap painted primocane cane and floricane cane colors compared to the unpainted control and yellow ($F = 4.59$; $df = 4, 35$; $P = 0.0044$) (Table 3.2). When comparing total capture between cane to leaf, there were significantly more *A. ruficollis* captured on cane mimic traps than on leaf mimic traps ($t = 2.70$, $df = 78$, $P = 0.0086$).

2012

Field testing of colored traps resulted in significantly more *A. ruficollis* captured on traps mimicking a blackberry cane (2.24 beetles per trap/192.02 cm^2) than a blackberry leaf (0.90 beetles per trap/106.68 cm^2) (t-value = 3.38, $df = 96$, $P = 0.001$). The emerald ash borer green traps shaped as a vertical cane captured significantly more beetles than any other colored cane trap ($F = 6.09$, $df = 6, 42$, $P = 0.0001$) (Table 3.3). The emerald ash borer green trap shaped as a blackberry leaf captured significantly more beetles than any other colored leaf trap (F = 14.87,

df= 6, 42, $P < 0.0001$). Similar *A. ruficollis* adult catches were obtained by cane traps colored yellow, emerald ash borer light purple, and mimics of a primocane or floricane. When comparing total capture between cane to leaf, there were significantly more *A. ruficollis* adults captured on cane mimic traps compared to the leaf mimic ($t = 3.38$, df = 96, $P = 0.001$). When data were pooled together for all colors, no differences were seen in the sex ratio of beetles caught for each color.

2013

Field testing of color traps attractiveness demonstrated similar results as the previous year (Table 3.1). Vertical prism-shaped cane traps colored primocane leaf or emerald ash borer trap green captured significantly more beetles per trap than any other colors, followed by primocane cane and floricane leaf colored traps ($F = 8.81$, df = 6, 56, $P < 0.0001$) (Figure 3.8). The traps colored to mimic floricane cane, emerald ash borer purple trap, and the white control had the fewest beetles per trap.

Analysis of the sex ratio in 2013 demonstrated that there were significantly more males (40.0 beetles) than females (7.9 beetles) captured among the colored traps ($F = 64.60$, df = 1, 118, $P < 0.0001$). When comparing the sex ratio for each individual color, there were significantly more males than females captured except on traps mimicking floricane leaf color and emerald ash borer trap purple (Table 3.4).

2014

The commercially available *A. planipennis* traps captured significantly more *A. ruficollis* adults in the green funnel traps than the purple funnel traps (t-value = 4.45, df=18, $P = 0.0003$) There was a 139 fold difference in the number of *A. ruficollis* captured per green trap (41.7 beetles) than per purple trap (0.3 beetles) (Figure 3.9).

Degree Day Modeling

Data from field tests in 2011-2014 along with data from Johnson and Mayes (1989) was used to calculate cumulative degree days at first activity, peak activity, and last activity at various degree day base temperatures. Results from this observation demonstrated that there were no similar degree days that can be used to predict first activity, peak activity, and last activity for *A. ruficollis* (Table 3.6).

Volatile Collection

The GC/MS analysis of volatile collections from primocanes and floricanes produced no detectable differences between the compounds. Collected volatiles were either contaminated or results from the GC/MS showed similar peaks for both volatile samples. The compounds that were identifiable in a floricane sample were (Figure 3.10); 3, 4-pentadienal (RT 4.4), cyclopentane, 2 propenyl (RT 4.6), 2-butynal (RT 7.0), 1H pyrrole, 3-methyl (RT 8.2 min), 5-trizaborane (RT 8.8), and 1,2 cyclobutane dicarbonitrile (RT 9.7). Compounds identified from a primocane sample were (Figure 3.11); 3, 4 pentyl-1-ol (RT 4.4), 3, 4 pentadienal (RT 7.6), 1H pyrrole, 3-methyl (RT 8.2 min), 5-trizaborane (RT 8.7), and 1, 2 cyclobutane dicarbonitrile (RT 9.7).

DISCUSSION

Investigation into the visual and chemical cues attracting *A. ruficollis* adults to blackberries demonstrated varying results. In terms of replicating the actual color of the plant, we were able to closely match each paint to the specific plant reflectance spectrum except floricane cane presumably due to color variability of mature canes. Percent reflectance was doubled for primocane mimics, but this increase was not achieved for floricane colors. The percent reflectance of all the paints only ranged from 7% - 23% for the plant mimic colors, the yellow

spray paint was at 83%, and the reflectance from the emerald ash borer traps ranged from 14% - 54%. From 2010 to 2013 the paints were adjusted to better match the actual plant color and were most closely matched for the 2013 testing. With adjustments to the paint made every year, increases in trap captures were also seen.

The original test conducted in 2011 investigated whether the color of the trap and leaf orientation or cane diameter size would increase beetle captures. Since no differences were observed in *A. ruficollis* response to leaf orientation or cane diameter, the following year all leaf traps were positioned in the downward orientation and all cane traps were made using one diameter size (1.3 cm). The results from 2011 demonstrated that the colors mimicking primocane and floricane canes captured the most beetles. However, for the tests conducted in 2012 emerald ash borer green traps captured significantly more *A. ruficollis* adults for both cane and leaf traps, followed by traps mimicking the color of a primocane, floricane, and emerald ash borer light purple trap. When comparing the percent reflectance of the colors to the respective trap data, the plant color mimicked traps with high percent reflectance had greater numbers of beetles captured than plant mimicked colors with low reflectance. This increase capture of beetles on higher reflectance traps was seen in all three years of the study. The trap with the highest percent reflectance, emerald ash borer green trap (49-54%), had the highest number of beetles captured which ranged from a 2.5 – 14 fold increase. This increase in trap capture from traps with higher percent reflectance was also seen by Crook et al. (2009) when various colors were tested on *A. plannipenis*. These findings indicate the importance of having traps with high reflectance compared to low reflectance from natural blackberry plant parts. Although there was a greater number of *A. ruficollis* adults captured on the vertical cane traps than the leaf mimic trap, this may be attributed to trap area differences: cane mimic of EAB colors measured 179.07 cm^2 and

plant colors measured 192.02 cm^2 compared to leaf mimic of EAB colors measured 105.66 cm^2 and plant leaf colors measured 106.68 cm^2. There was a 2.1 fold increase in the average beetles per trap on cane mimic traps (2.5 beetles) versus leaf mimic traps (1.2 beetles) in 2012. Investigation into the use of commercially available green multifunnel traps supports the findings that the emerald ash borer trap green color is effective in capturing *A. ruficollis*. These green multifunnel traps also had much better capture of *A. planipennis* than purple multifunnel traps (Francese et al. 2011, 2013). These findings have application that will allow growers to use a green multifunnel fluon coated trap or the green prism-shaped sticky trap for monitoring *A. ruficollis*.

Since more beetles were captured onto cane mimics than leaf mimics, all traps in 2013 were made to have a surface area of 179.07cm^2. When the traps were made with the same surface area, results were very similar to the previous years, i.e., cane traps had significantly higher counts than did leaf traps. Testing in 2013 showed that traps colored emerald ash borer green trap and primocane leaf captured the greatest number of beetles followed by primocane cane and floricane leaf. Although it appears that the primocane leaf color performed better than previous years, the colors were rearranged for 2013 to better match the actual plant color. Therefore, for 2013 the color that was designated as primocane cane for 2011-2012 was changed to primocane leaf for 2013 and the same was done for primocane leaf for 2011-2012. Another reason for the increase capture for traps in the color range of 540-550 nm is the close proximity to the plant reflectance and that it blends well into the canopy decreasing the chance of avoidance behavior which was also seen in a study with *A. planipennis* (Francese et al. 2010).

Although there were no significant differences observed in 2012 when comparing male (11.8 beetles) and female (13.4 beetles) capture on cane or leaf mimics or based on color, this

was not the case in 2013. The average number of beetles captured in 2013 was heavily skewed towards males (40.0 beetles) versus females (7.9 females). The sex ratio results from 2012 show similarities to colored trap studies conducted on *A. planipennis* that more females were captured on purple colored traps while more males were captured on green colored traps (Crook et al. 2009, Francese et al. 2010). However, in 2013 even the purple colored trap was skewed towards more male captures. The increase of male capture on green colored traps can be associated with male presence in the plant canopy looking for mates, which has been associated with *A. planipennis* behavioral observations in the field (Rodriguez-Saona et al. 2006).

Based on trap captures from 2011 to 2014, the seasonal flight of *A. ruficollis* occurred from late-April until mid-July with peak adult capture in the traps from mid-June to late-June (Figure 3.10). In comparison, Johnson and Mayes (1989) found that adult emergence from galled canes held in an insectary occurred from late-April into June. An attempt was made to formulate a degree day (DD) model (lower developmental temperature estimated to be 50°F) that predicts first, peak and last *A. ruficollis* adult emergence from adult emergence data from caged galled blackberry canes in an insectary (Johnson and Mayes 1989) and first, peak and last *A. ruficollis* adult flight using trap data from this study (Table 3.6). However, based on these findings there seems to be no similarities in the first, peak, and last adult activity among all years observed. Once a rearing technique is developed for *A. ruficollis*, a lower developmental temperature can be derived. Then the DD model can be improved so that a grower can predict the *A. ruficollis* flight and egg laying periods in order to time insecticide sprays or time use of other control tactics like mass trapping.

Investigations into the chemical cues of attraction for *A. ruficollis* yielded no positive results. This could have been due to errors in volatile collections or that there are no differences

in the volatile profile between primocane and floricane plant parts (leafs and stems). Although, there have been studies that identified volatiles collected from blackberry fruit (Du et al. 2010a, 2010b, Qian and Wang 2005). Although Humpf and Schreier (1991) demonstrated that blackberry leaves contained 2-methyl-2-hepten-6-ol (sulcatol), the aggregation pheromone for the ambrosia beetles *Gnathotrichus sulcatus* (LeConte) and *G. retusus* (LeConte), this compound was not found in any of our blackberry samples. Preliminary analysis of volatile collections from primocane and floricanes only had identifiable peaks on floricane samples. Some of these peaks were observed to elicit activity in other insects; 2-cyclohexen-1-one which is related to an antiaggregation pheromone for *Dendroctonus pseudotsugae* Hopkins (Reviewed in Byers 1989), calamenene which was found to be an attractive compound for *Xyleborus glabratus* Eichhoff (Hanula and Sullivan 2008), and acetophenone which is an antiaggregation compound for several *Dendroctonus* sp. (Pureswaran and Borden 2004, Erbilgin et al. 2007). However, none of these compounds were found in the present study. Further investigations into identifying the volatile components from primocanes and floricanes is necessary to get a better understanding on whether there is a chemical stimulus attracting *A. ruficollis* to oviposit only on primocanes or that odor has no role in oviposition.

We demonstrated that a trap reflecting in the 540 nm range with a percent reflectance above 23% has the greatest capture of *A. ruficollis*, which was similar to experiments with *A. planipennis* (Crook et al. 2009, Francese et al. 2010). However, these colored traps captured mostly male *A. ruficolls*. A commercially available multifunnel green trap demonstrated its efficiency in capturing *A. ruficollis* and looks promising as a monitoring tool. Now a grower could monitor for *A. ruficollis* adults. They can buy the clear sheets of plastic, cut out and shape into a prism, apply primer and green paint and Tanglefoot® and attach colored prism trap to a

dowel for support. Many green prism-shaped sticky traps could be made for the price of one green, fluon coated multifunnel trap ($35 per trap; ChemTica USA, Durant, OK).

We still need to identify the blackberry attractant volatile in order to develop a kairomone or aggregation pheromone lure to bait a colored trap for monitoring both sexes of *A. ruficollis*. Having a baited, colored monitoring trap will allow blackberry growers to effectively monitor *A. ruficollis* adult flight. This can ultimately lead to more effective pest control, decreased insecticidal applications, and possibly a mass trapping tactic. Further research is needed to determine the optimal percent reflectance and also determine if the addition of blackberry volatile bouquet will significantly increase capture of *A. ruficollis* adults.

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Table 3.1. Jazz spectrometer reading of peak wavelength and percent reflectance of blackberry primocane and floricane plant parts, paints mimicking these parts, and yellow and purple spray paint used in traps for emerald ash borer (EAB), *Agrilus plannipenis*.

	2011		2012		2013	
Color	**Wavelength (nm)**	**Reflectance (%)**	**Wavelength (nm)**	**Reflectance (%)**	**Wavelength (nm)**	**Reflectance (%)**
Primocane Leaf (plant)	554	10	-	-	-	-
Primocane Cane (plant)	546	13	-	-	-	-
Floricane Leaf (plant)	550	10	-	-	-	-
Floricane Cane (plant)	630	15	-	-	-	-
Primocane Leaf	545	18	545	18	556	23
Primocane Cane	556	23	556	23	545	18
Floricane Leaf	520	7	520	7	550	8
Floricane Cane	619	13	619	13	619	13
Yellow Spray Paint	579	83	579	83	NA	NA
Purple Spray Paint	420, 670, 790	17, 25, 45	NA	NA	NA	NA
EAB Green Trap	NA	NA	542	49	545	54
EAB Dark Purple Trap	NA	NA	440, 600	19, 14	NA	NA
EAB Light Purple Trap	NA	NA	432, 605, 700	23, 16,19	432, 605, 700	23, 16,19
Unpainted Control (Metal)	448, 616	10	-	-	NA	NA
Unpainted Control (Wood)	622, 673	56	-	-	NA	NA
Unpainted Control (Primer)	NA	NA	NA	NA	431, 574	94

Table 3.2. Mean (±SE) number of *Agrilus ruficollis* adults captured per colored sticky trap mimicking blackberry canes and leaves of primocanes and floricanes from 2011 field test in Clarksville and Tontitown, AR.

	Mean number of beetles captured per trap mimicking	
Trap Color	**Cane**[a]	**Leaf**[a]
Primocane	3.8 ± 0.8a	0.9 ± 0.48a
Floricane	2.9 ± 1.06a	0.9 ± 0.35a
Purple	1.8 ± 0.59ab	0.5 ± 0.38a
Yellow	0.5 ± 0.19b	1.1 ± 0.40a
Control	0.6 ± 0.32b	0.5 ± 0.27a

[a] Mean values in the same column with the same letter are not significantly different (Waller-Duncan *k-ratio* t-test, $p < 0.05$).

Table 3.3. Mean (±SE) number of *Agrilus ruficollis* adults captured per colored sticky trap mimicking blackberry canes and leaves of primocanes and floricanes or emerald ash borer colored traps from 2012 field test in Clarksville and Tontitown, AR.

	Mean number of beetles captured per trap mimicking	
Trap Color	**Cane**[a]	**Leaf**[a]
Primocane	2.1 ± 0.70b	1.3 ± 0.47b
Floricane	1.7 ± 0.75bc	0.6 ± 0.37c
Emerald Ash Dark Purple	0.7 ± 0.47c	0.3 ± 0.18c
Emerald Ash Light Purple	1.1 ± 0.59bc	0.0c
Emerald Ash Green	7.4 ± 0.95a	4.1 ± 1.16a
Yellow	2.0 ± 0.69bc	0.0c
Control	0.6 ± 0.30c	0.0c

[a] Mean values in the same column with the same letter are not significantly different (Waller-Duncan *k*-ratio *t*-test, $P < 0.05$).

Table 3.4. Sex ratio of *Agrilus ruficollis* adults captured per colored sticky traps from 2012 field test in Clarksville and Tontitown, AR.

Trap Color	**Cane M:F Ratio** [a]	**Leaf M:F Ratio** [a]	**Total M:F Ratio** [a]
Primocane	4.50a	0.40a	1.57a
Floricane	1.75a	2.00a	1.80a
Emerald Ash Borer Dark Purple	0.67a	1.00a	0.75a
Emerald Ash Borer Light Purple	0.33a	0.00a	0.33a
Emerald Ash Borer Green	0.76a	0.86a	0.80a
Yellow	0.44a	0.00a	0.44a
Unpainted Control	2.00a	0.00a	2.00a

[a] Mean values in the same column with the same letter are not significantly different (Pairwise chi-square analyses performed using a Bonferroni-adjusted α value = 0.0024).

Table 3.5. Mean (±SE) number of *Agrilus ruficollis* adult males and females captured per colored sticky trap in blackberry fields in Clarksville and Tontitown, AR (2013).

Trap Color	Male[a]	Female[a]	df	*t*-value	*P*[b]	Total M:F Ratio[c]
Primocane Leaf	12.4 ± 1.91a	1.6 ± 0.50a	16	-4.43	0.0004*	8.00a
Primocane Cane	5.6 ± 1.02ab	0.8 ± 0.28a	16	-4.35	0.0005*	7.14a
Floricane Leaf	4.9 ± 1.23b	1.2 ± 0.36a	16	-1.98	0.0650	4.00a
Floricane Cane	2.1 ± 0.59bc	0.7 ± 0.33a	16	-3.27	0.0049*	3.17a
Emerald Ash Green	9.9 ± 1.81a	2.4 ± 0.80a	16	-3.19	0.0057*	4.05a
Emerald Ash Purple	1. 9 ± 0.59c	0.8 ± 0.36a	16	-1.58	0.1342	2.43a
Control (Primer only)	3.2 ± 1.10bc	0.6 ± 0.34a	16	-3.15	0.0062*	5.80a

[a] Mean values in same column with the same letters are not significantly different (Waller-Duncan *k-ratio* t-test, $p < 0.05$).

[b] *P* values indicate a significant difference between males and females (*t*-test, $P < 0.05$)

[c] Mean values in the same column with the same letter are not significantly different (Pairwise Chi-square analyses performed using a Bonferroni-adjusted α value = 0.0024).

Table 3.6. Degree days (base 50°F) accumulated from January 1 to first, peak and last *Agrilus ruficollis* adult emergence from caged galled canes in an insectary in Fayetteville, AR from 1985 to 1987 (Johnson and Mayes 1989) and adult flight activity derived from traps monitored from 2011 to 2014 in Tontitown and Clarksville, Arkansas.

	Adult Emergence (Johnson and Mayes 1989)			**Adult Flight (Capture in Traps)**			
Activity period	1985	1986	1987	2011	2012	2013	2014
First	652	487	1033	895	661	523	359
Peak	739	593	1436	1002	796	943	715
Last	1127	746	1771	1428	1823	2316	1973

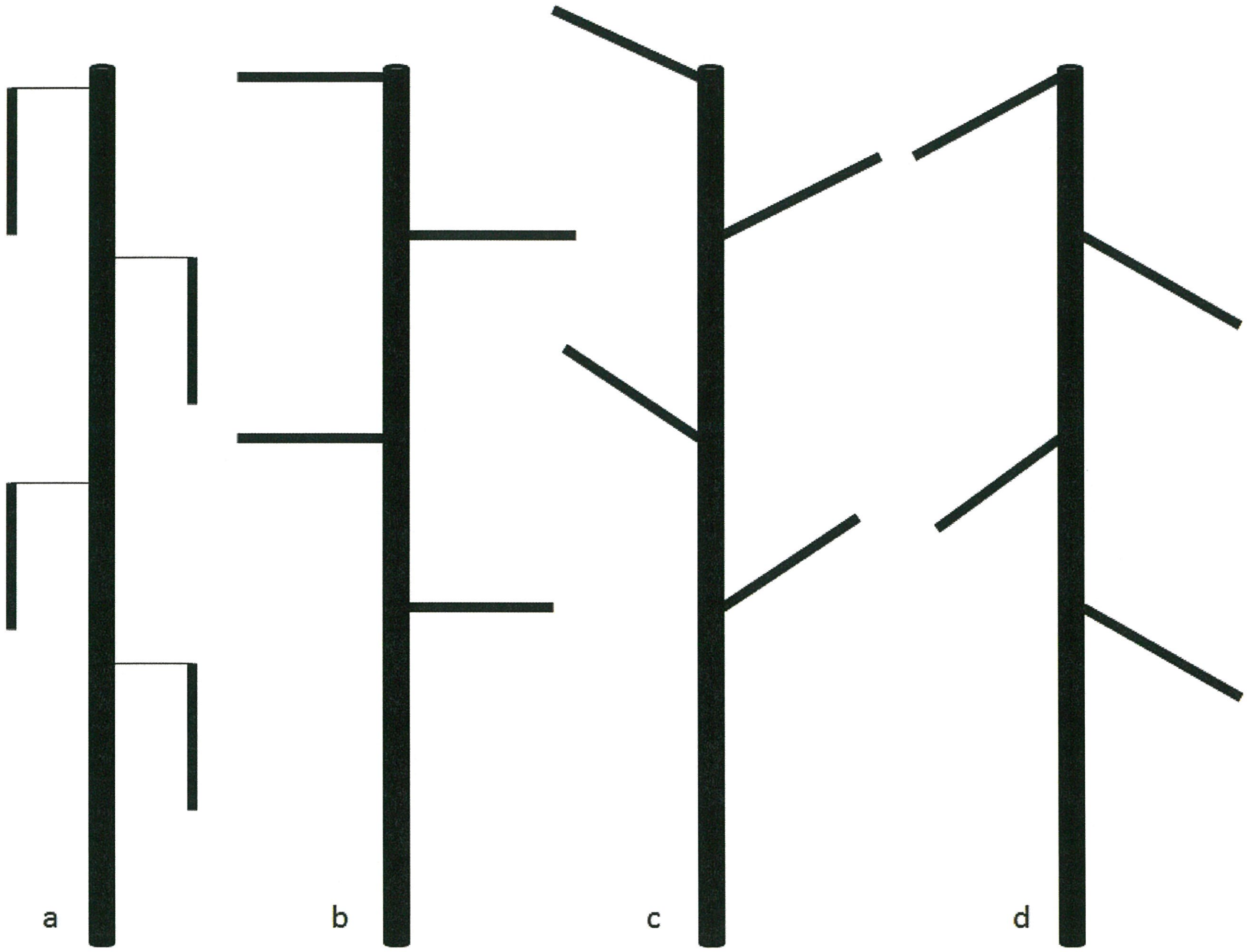

Figure 3.1. Diagram of blackberry leaf mimic traps for 2011 field testing. Orientation of leaves: a) vertical, b) horizontal, c) 45° upward, d) 45° downward.

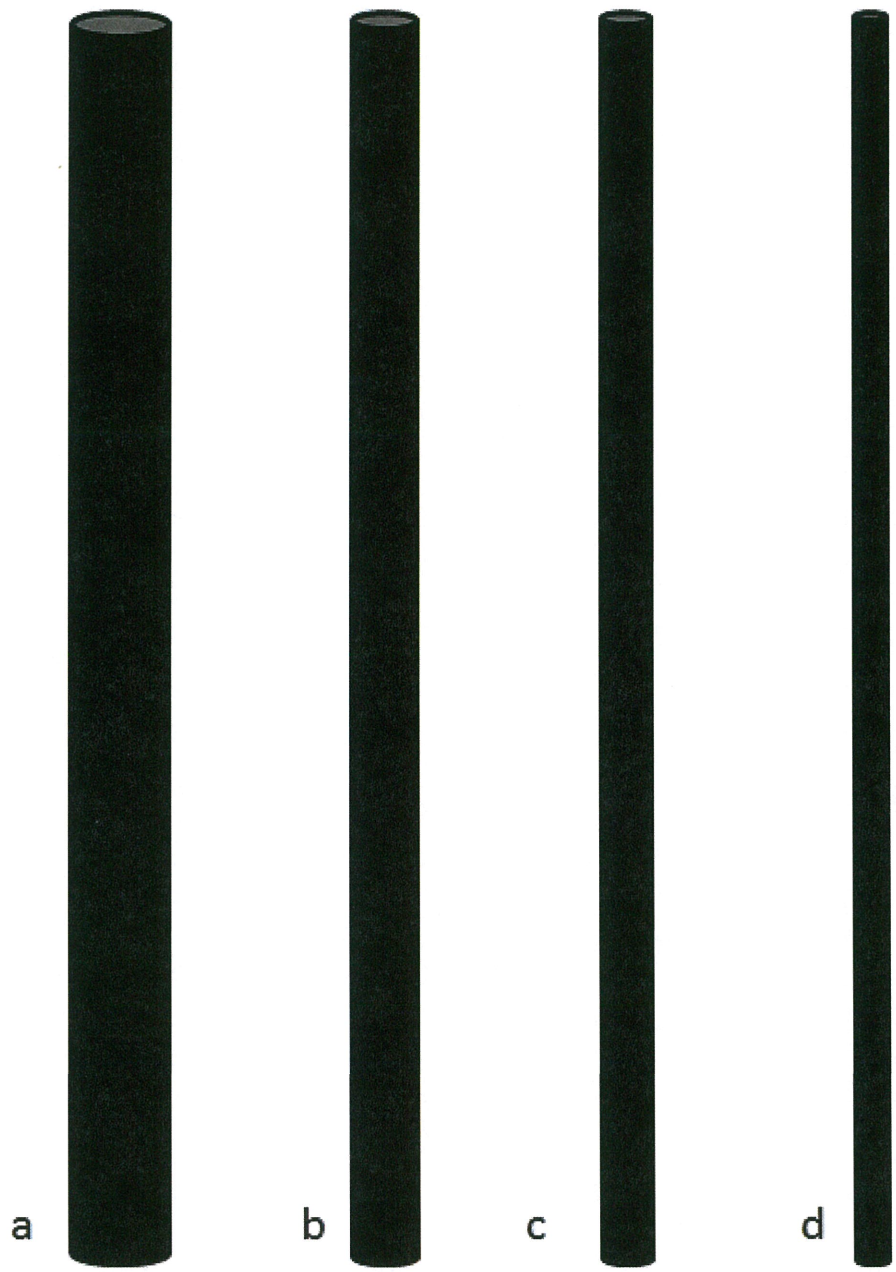

Figure 3.2. Diagram of wooden dowels of blackberry cane mimic traps for 2011 field testing. a) 2.5 cm diameter, b) 1.3 cm diameter, c) 1.0 cm diameter, d) 0.3 cm diameter.

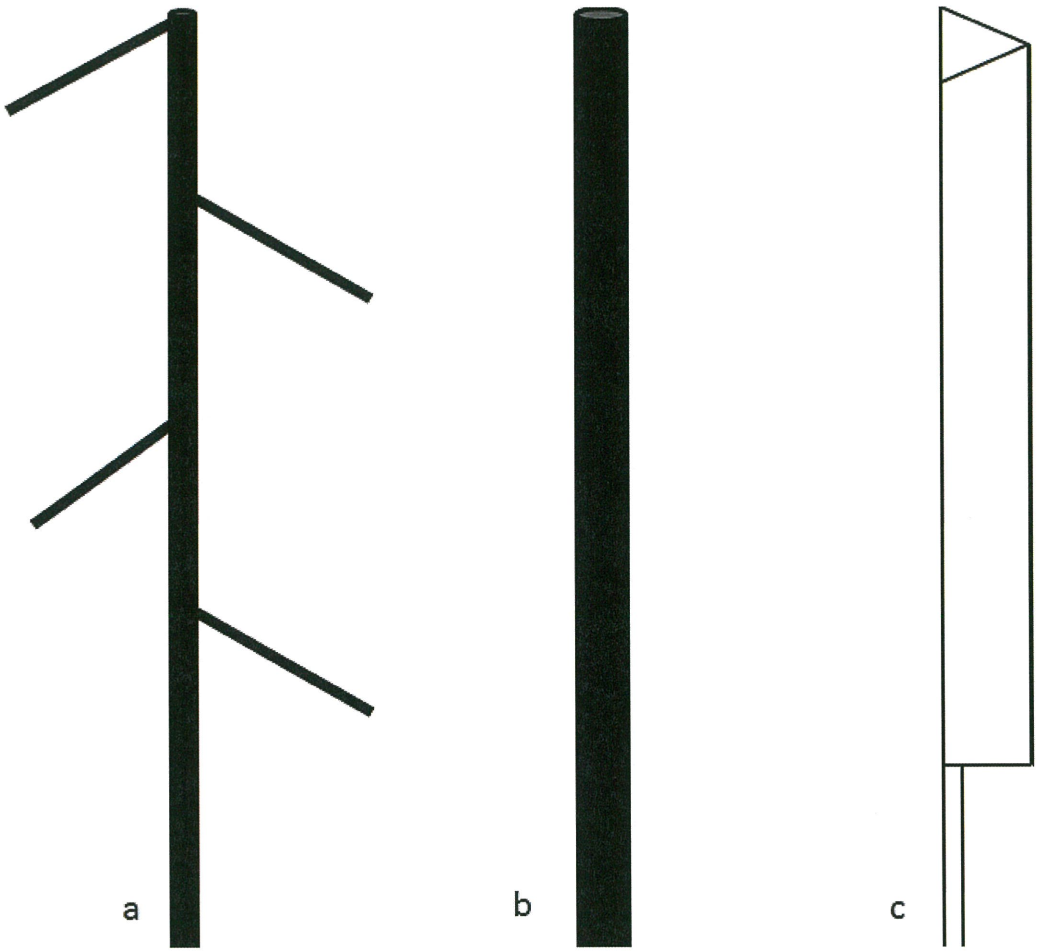

Figure 3.3. Diagram of blackberry plant (a) leaf mimic and cane mimic using either (b) painted wooden dowels (1.3 cm diameter) or-c) green or purple corrugated plastic sheets folded into a prism shape (see Fig. 3.4). All these traps were field evaluated for attractiveness to *Agrilus ruficollis* adults in 2012.

Figure 3.4. a) Diagram of prism-shaped vertical trap used in 2013 field testing. b) Green sticky trap noted as most attractive to *Agrilus ruficollis* adults in blackberry plantings in 2013. c) Green and d) purple multifunnel traps coated with fluon. These traps were evaluated for attractiveness to *A. ruficollis* adults in 2014.

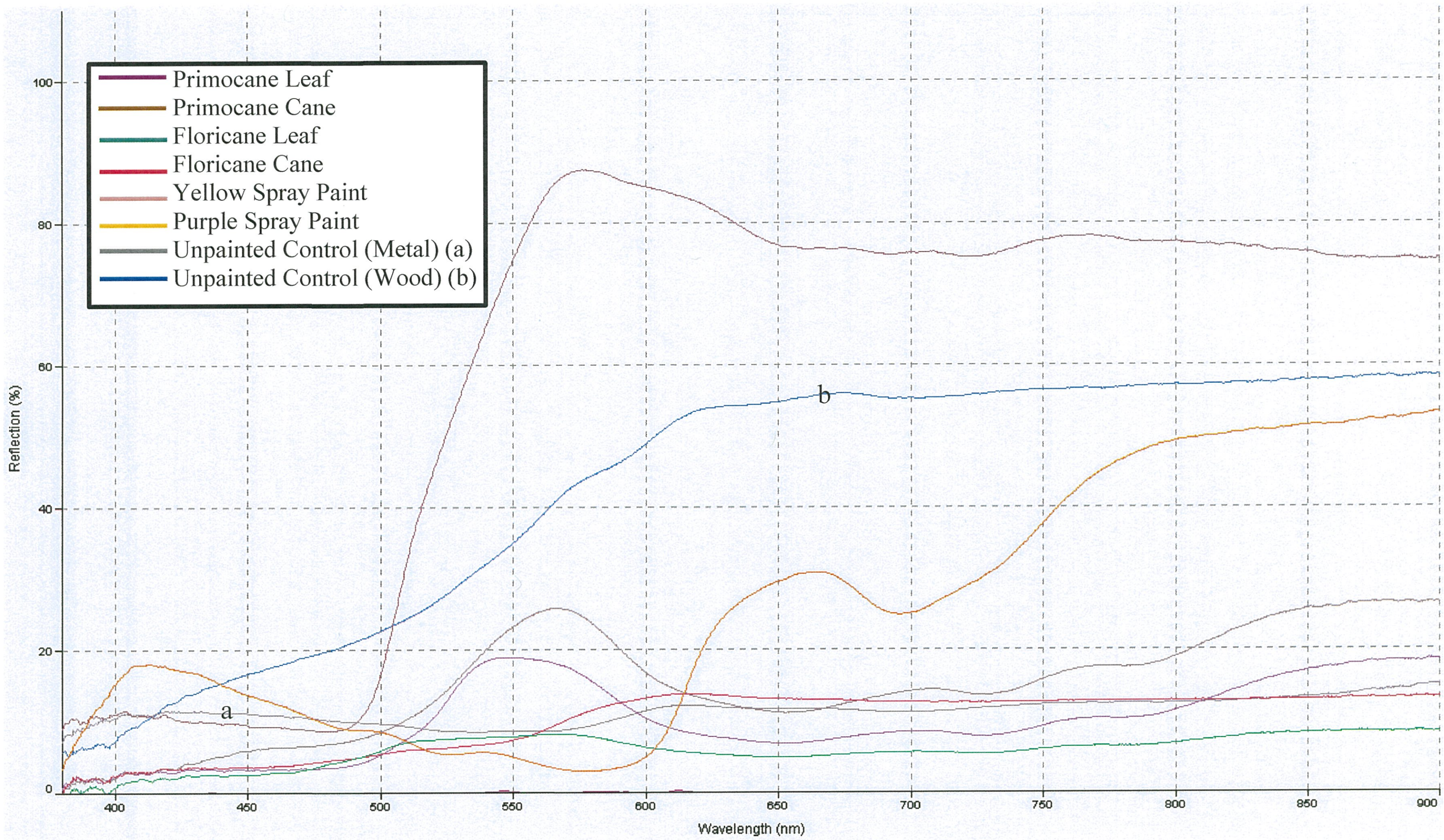

Figure 3.5. Color spectra of traps used in 2011 field trial.

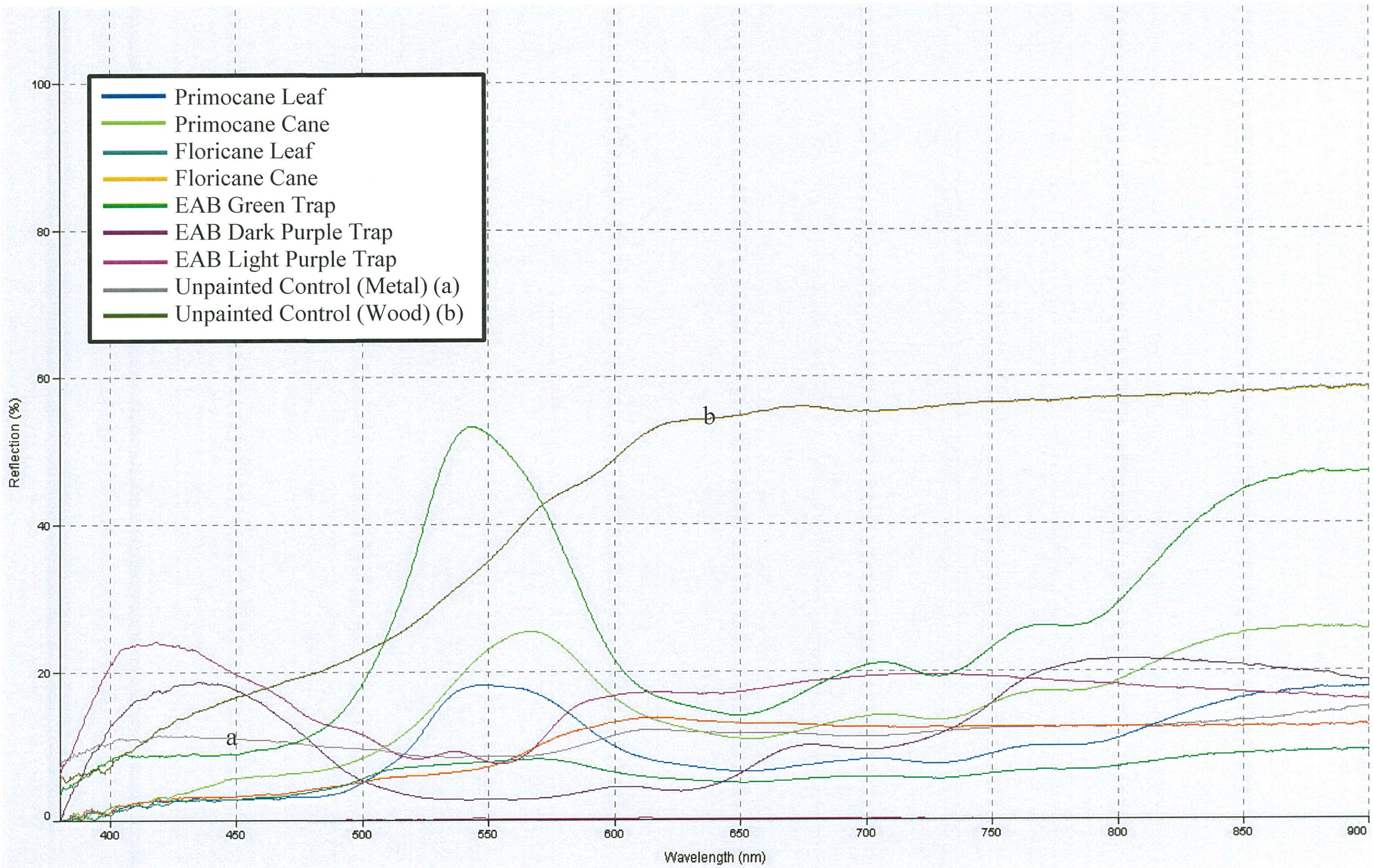

Figure 3.6. Color spectra of traps used in 2012 field trial.

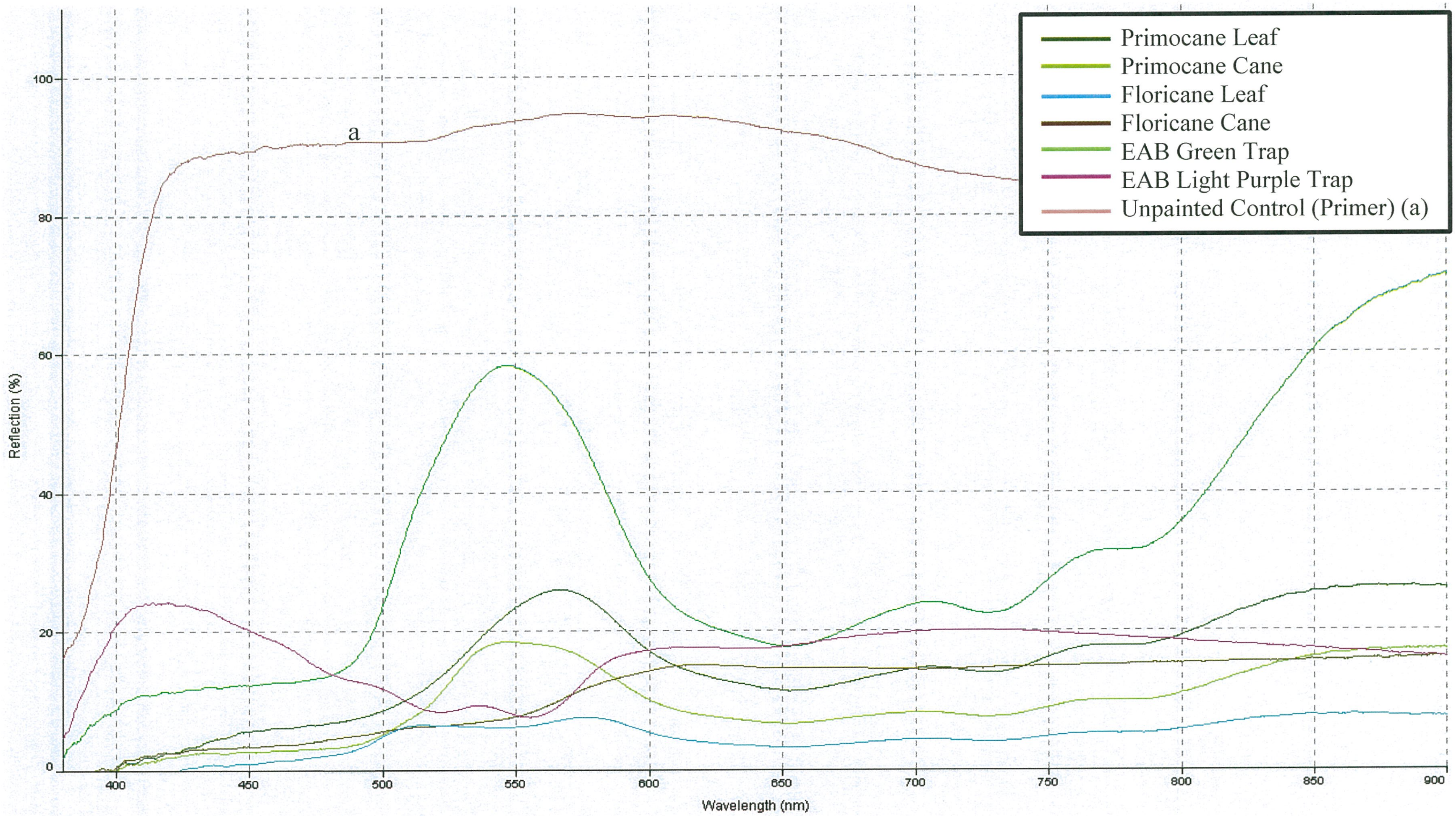

Figure 3.7. Color spectra of traps used in 2013 field trial.

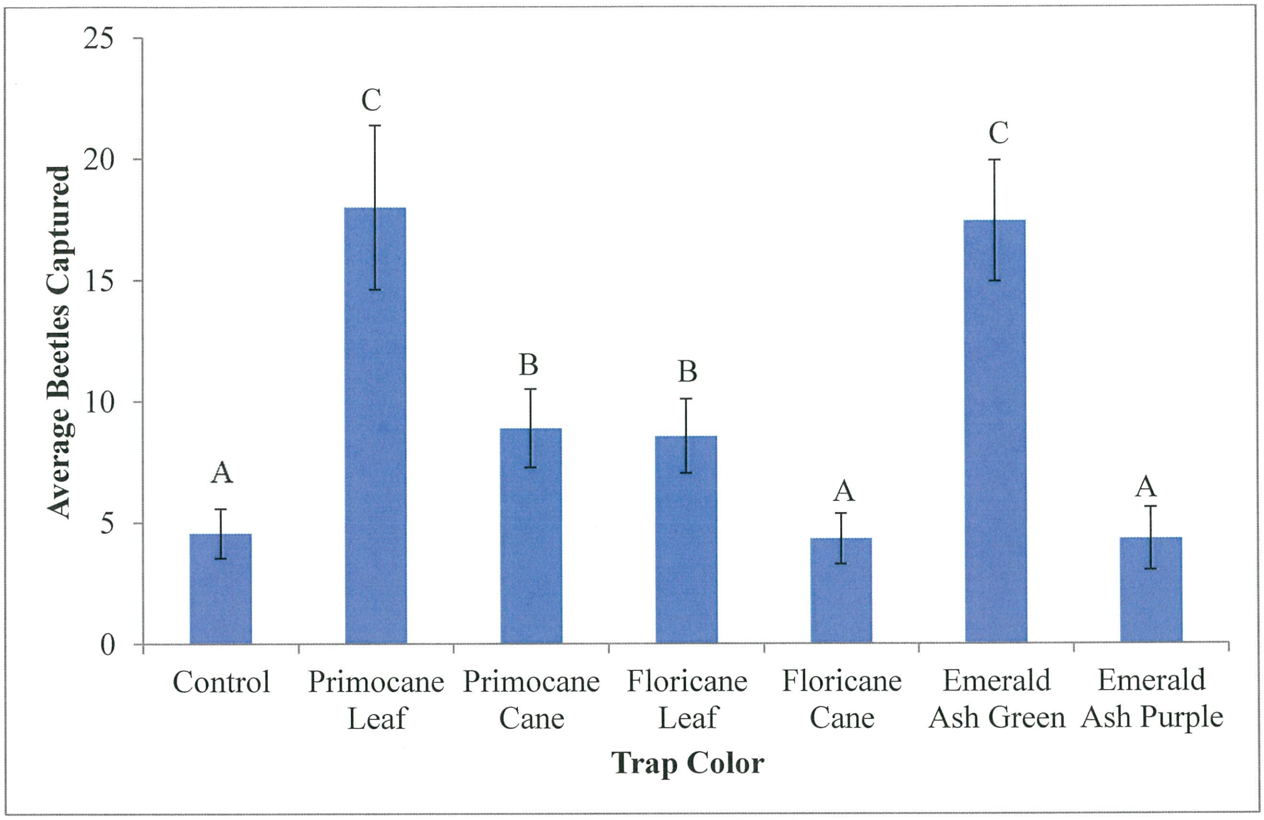

Figure 3.8. Mean (± SE) number of *Agrilus ruficollis* adults captured per colored trap in blackberry plantings in Tontitown and at the Fruit Station in Clarksville, AR (2013). Mean bars followed by the same letter are not significantly different (Waller-Duncan *k*-ratio *t*-test, $P < 0.05$).

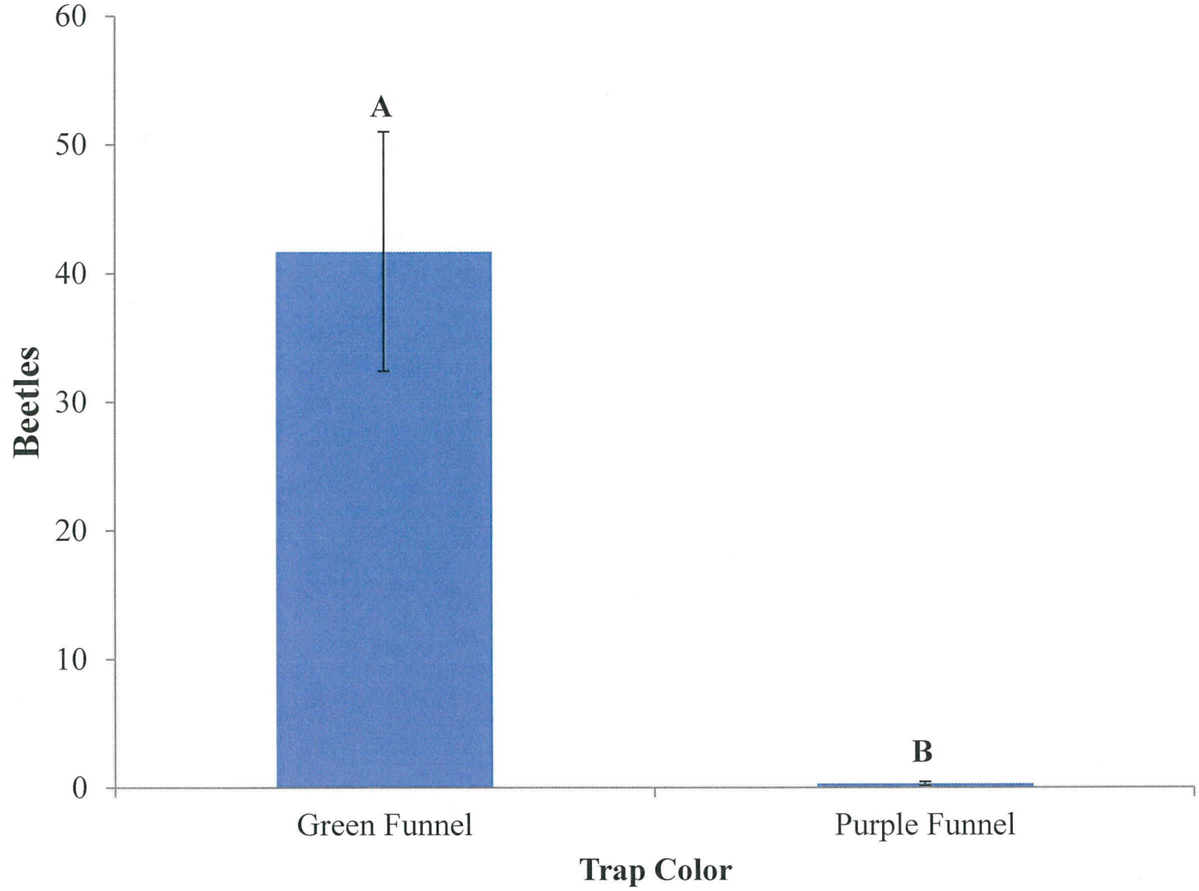

Figure 3.9. Mean (± SE) number of *Agrilus ruficollis* adults captured per funnel trap color from three blackberry sites in Arkansas: wild blackberries in Springdale, commercial blackberries in Tontitown and blackberry selections at the Fruit Station in Clarksville, AR (2014). Mean bars followed by the same letter are not significantly different (*t*-test, $P < 0.05$).

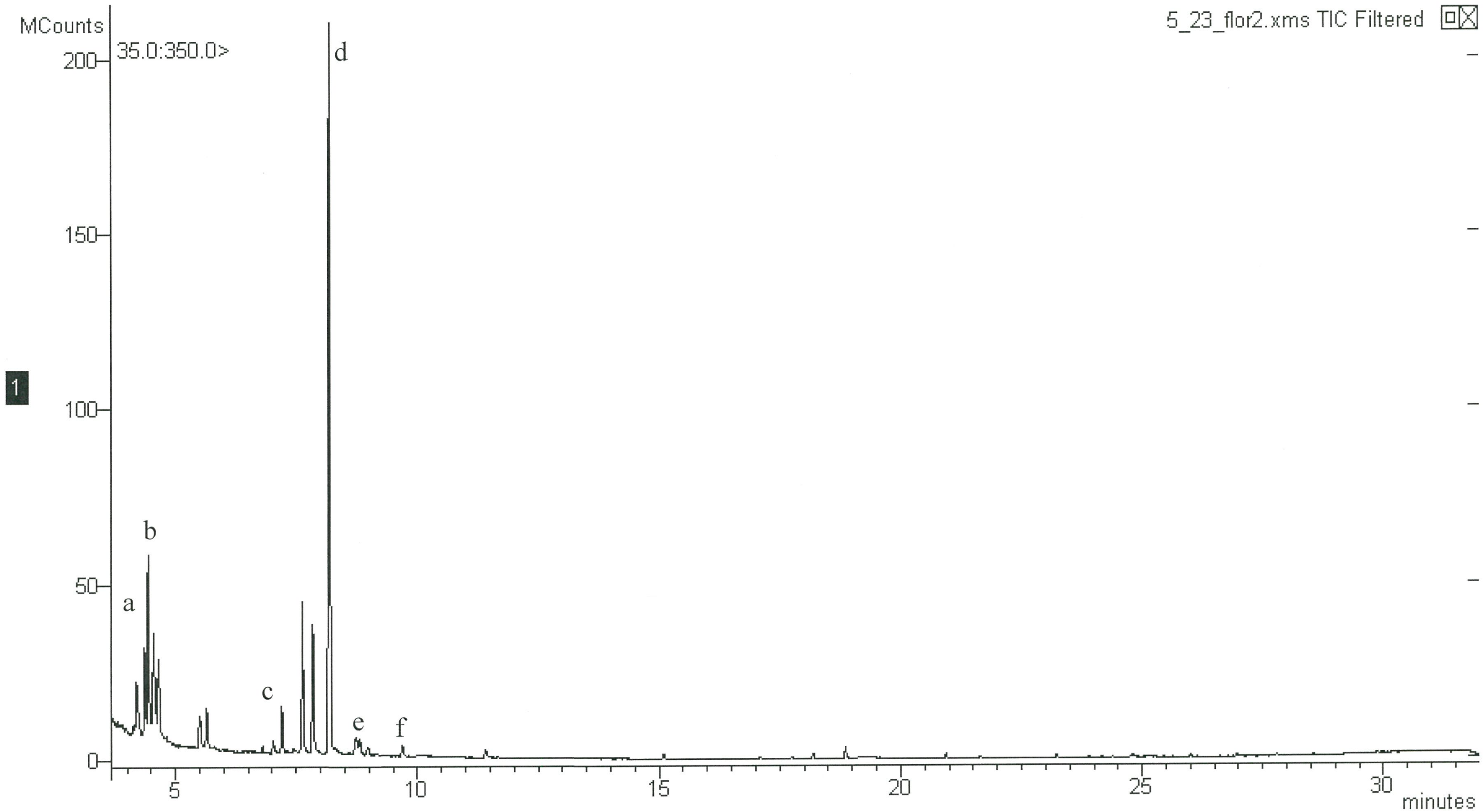

Figure 3.10. GC/MS graph of floricane volatile collection. Letters on graph correspond to the following compounds: a) 3, 4-pentadienal (RT 4.4), b) cyclopentane, 2 propenyl (RT 4.6), c) 2-butynal (RT 7.0), d) 1H pyrrole, 3-methyl (RT 8.2 min), e) 5-trizaborane (RT 8.8), and f) 1,2 cyclobutane dicarbonitrile (RT 9.7).

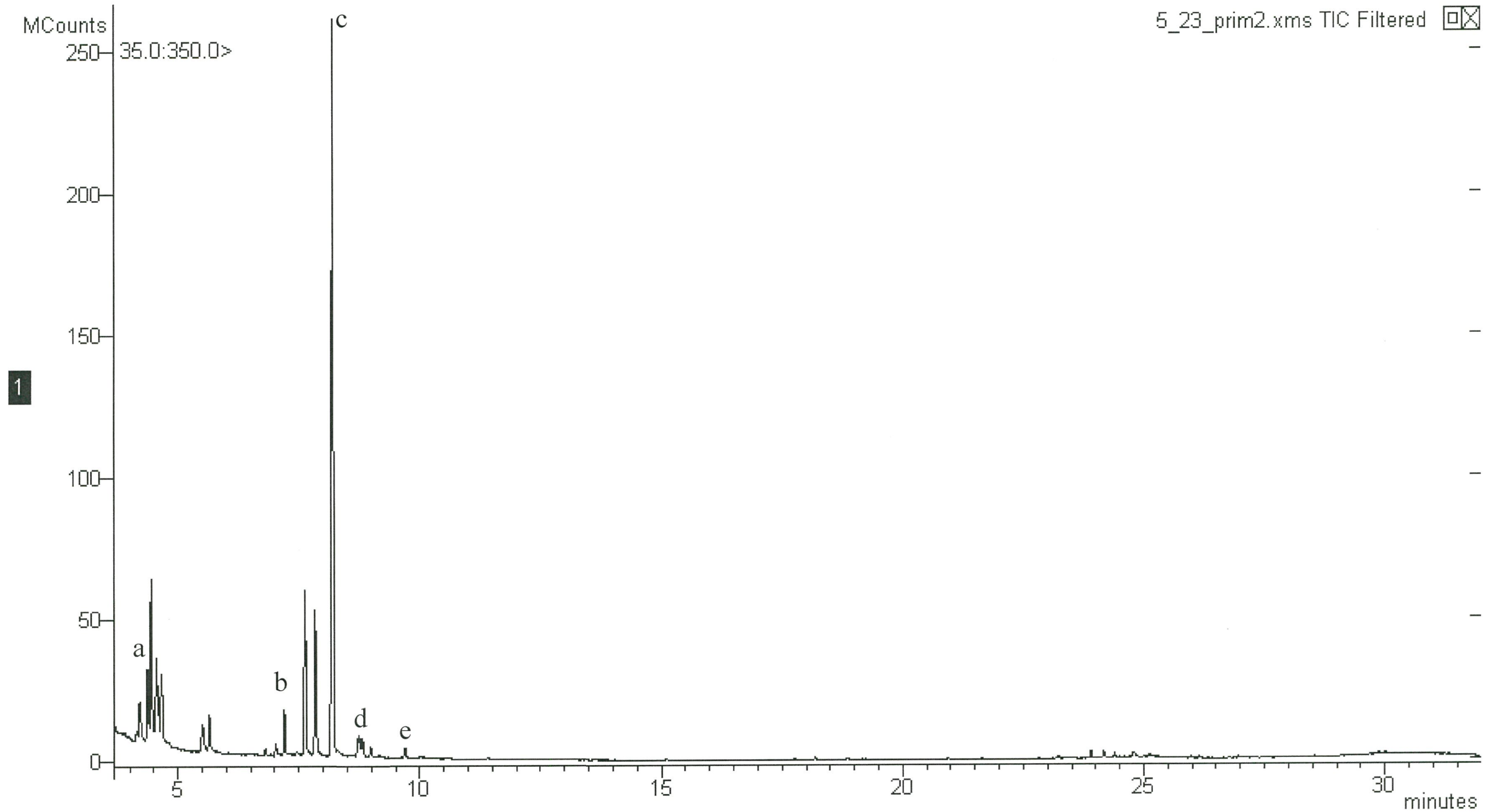

Figure 3.11. GC/MS graph of primocane volatile collection. Letters on graph correspond to the following compounds: a) 3, 4 pentyl-1-ol (RT 4.4), b) 3, 4 pentadienal (RT 7.6), c) 1H pyrrole, 3-methyl (RT 8.2 min), d) 5-trizaborane (RT 8.7), and e) 1, 2 cyclobutane dicarbonitrile (RT 9.7).

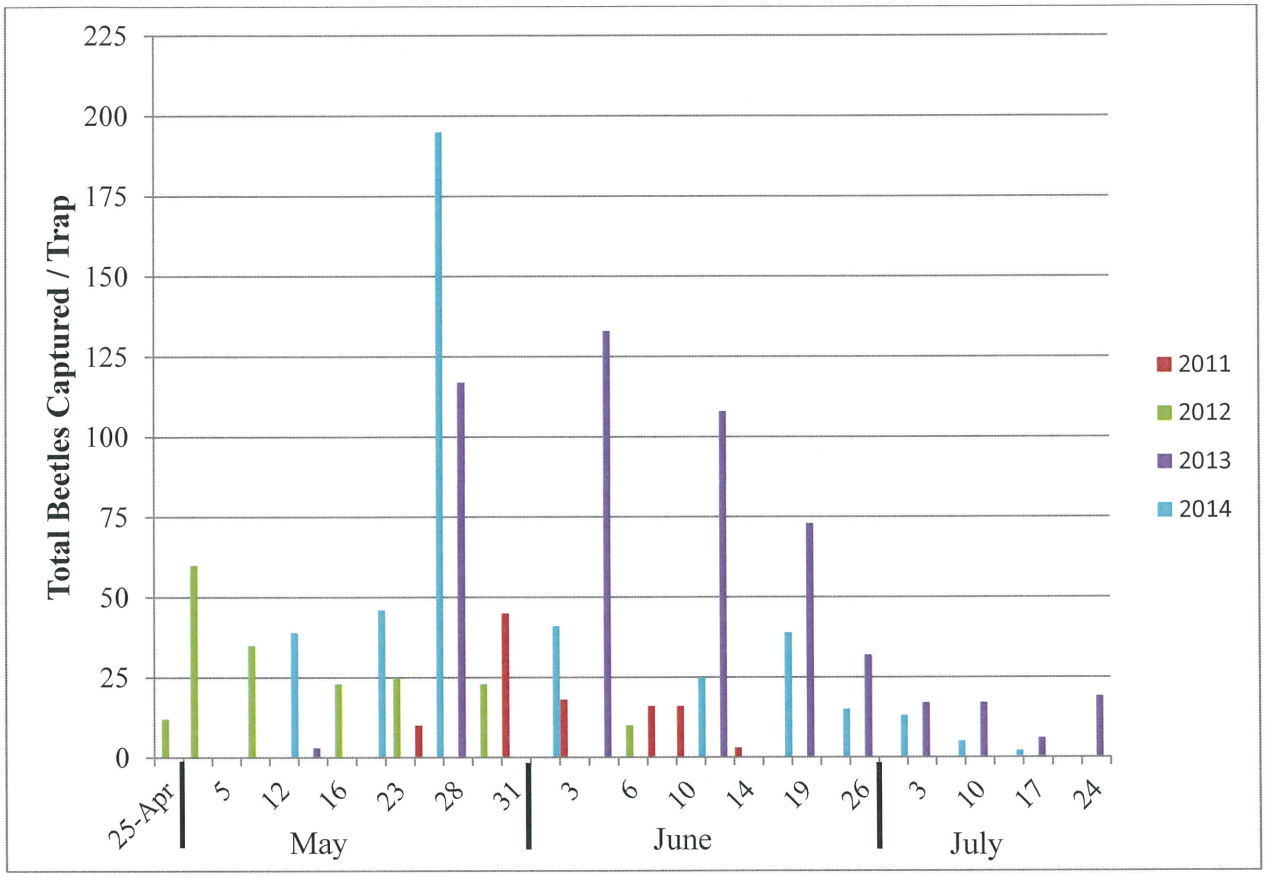

Figure 3.12. Seasonal changes in the number of *Agrilus ruficollis* captured in traps from 2011 - 2014.

CHAPTER 4 – CONCLUSION

The rednecked cane borer, *Agrilus ruficollis* (L.), is considered a pest of cultivated blackberries in the eastern United States. Harvested acreage of blackberry production has increased from 2009-2011 in the eastern United States from 7,100 to 7,300 (USDA 2012). With the increase in planted acreage, the need to effectively monitor and control blackberry pests is crucial. Primocane galling caused by the developing rednecked cane borer larvae can weaken the cane and predispose it to winter injury or cane breakage and reduce yield the following year (Mundinger 1941, Walton 1951, Johnson and Mayes 1989, Johnson 1992a, 1992b, Solomon 1995). If left uncontrolled, this pest has the potential to reduce yields by 72% (Hixson 1939, Strik et al. 2007). The current management methods for this pest include a soil drench application of imidacloprid (neonicotinoid) post bloom (Bessin 2004, Pfeiffer 2011, Kim and Johnson 2012), pruning off galled canes during the dormant season (Chittenden 1922), or pruning off canes that emerged prior to or during the egg laying period (Walton 1951).

There may be negative effects of imidacloprid on pollinators through residue exposure in pollen, nectar, and other plant materials. A study conducted on the pesticide residues found inside bee hives demonstrated that 87 and 98 different pesticides and metabolites, including neonicotinoids, were found in wax samples and pollen samples respectively (Mullins et al. 2010). Along with the exposure through hive materials, direct contact exposure of neonicotinoids were shown to be highly toxic to bumble bees, *Bombus impatiens* Cresson, alfalfa leafcutter bees, *Megachile rotundata* (F.), and orchard mason bees, *Osmia lignaria* Say (Scott-Dupree et al. 2009).

There was a need to evaluate other compounds against the rednecked cane borer. Results from this dissertation have demonstrated that the horticultural oil JMS Style Oil can be used as

either a conventional or an OMRI (Organic Materials Review Institute) approved alternative to imidacloprid. However, treatments with the horticultural oil are recommended to be halted if temperatures exceed 32°C to prevent phytotoxic effects. Further research is still needed to determine other insecticide classes that are effective in controlling this pest in order to prevent the development of insecticide resistance, especially if there are only one or two effective compounds. Aside from investigating alternative classes of insecticides for the management of this pest, further research into the attraction of *A. ruficollis* for trap development was researched.

There has been extensive research into the visual and chemical attractants of pests in the *Agrilus* genus, with emerald ash borer, *A. planipennis*, (Rodriguez-Saona et al. 2006, Crook et al. 2008, de Groot et al. 2008, Lelito et al. 2008, Crook et al. 2009; Francese et al. 2010, Marshall et al. 2010, Crook et al. 2012) and the goldspotted oak borer, *A. auroguttatus* Schaeffer, being the most recent heavily studied (Coleman and Seybold 2010, Coleman and Seybold 2011, Coleman et al. 2012, Haavik et al. 2013, Jones et al. 2013). In terms of visual attractants for *A. ruficollis*, the current trap color study captured more *A. ruficollis* males than females on all the trap colors that reflected light in the 540-560nm range. This was similar to the attractiveness of male and female *A. planipennis* (Crook et al. 2009). However, the dark purple *A. planipennis* traps tested did not attract significantly more *A. ruficollis* females than males. In contrast, a study conducted using electroretinograms revealed traps that reflected in the red spectrum range (640-650nm) attracted only female *A. planipennis* (Crook et al. 2009). Along with a spectral range of attractiveness, results from this dissertation have found that traps with higher percent reflectance of colored traps coincided with higher trap captures. This increase in beetles captured on higher percent reflectance colors was also seen on *A. planipennis* traps (Crook et al. 2009, Francese et al. 2010).

Initial investigations into the various trap designs demonstrated that a vertical trap mimicking a blackberry cane captured the most *A. ruficollis* adults. This can be in part attributed to the larger surface area presented by the cane mimics (179.07 - 192.02 cm^2) over the leaf mimics (105.66 – 106.68 cm^2). Changing all the traps to the prism-shaped type (179.07 cm^2) allowed for significantly more differences in trap captures between the different colors and percent reflectance compared to the previous years, there was a 2.9 fold increase in the total number of beetles captured from 2012 (188 beetles) to 2013 (532 beetles). An interesting observation made on the beetles captured on the traps for two years is that the majority of the adults captured were males. Results from the 2013 study demonstrated a significantly higher number of males captured for all traps tested. With more males than females being captured in traps, there is a need to determine the attractant color and possibly chemical cues that improve trap capture of *A. ruficollis* females. Recording electroretinograms could determine the spectral sensitivity regions for male and female *A. ruficollis*. Then traps of specific reflectance ranges, especially red (640-650nm), and surface area could be optimized from field evaluations of attractiveness. The presence of one or more decoys of *A. ruficollis* on traps may increase capture as seen with other buprestid species (Lelito et al. 2007, 2008, Domingue et al. 2011, 2012).

A gas chromatograph/mass spectrometer (GC/MS) analysis of blackberry primocanes and floricanes did not produce any measureable results. Additional samples of aromatic compounds of blackberry need to be collected and analyzed before testing it through a GC-EAD to identify the biologically active peaks and then test attractiveness to *A. ruficollis* adults of a lure containing one or more aromatic compounds.

Although we still know little about the chemical attractants of *A. ruficollis*, we could recommend that growers set out vertical, emerald ash borer green traps reported here as most

attractive to *A. ruficollis* adults. Monitoring these traps will aid growers in detecting presence/absence of *A. ruficollis* adults. Being able to improve timing of insecticides against this pest will allow growers to maintain productivity of blackberries with minimal detrimental effects of overuse of insecticides on the environment. Continuing the research on the behavior of this insect to the aromatic host stimuli will be beneficial.

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