1-1-2006

Establishment Of Blackberries And Detection And Management Of Raspberry Crown Borer

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ESTABLISHMENT OF BLACKBERRIES AND DETECTION AND MANAGEMENT OF RASPBERRY CROWN BORER

By

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A Thesis Submitted to the Faculty of Mississippi State University in Partial Fulfillment
of the Requirements for the Degree of Master of Science in Horticulture
in the Department of Plant and Soil Science

Mississippi State, Mississippi

November 2006

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Title of Study: ESTABLISHMENT OF BLACKBERRIES AND DETECTION AND MANAGEMENT OF RASPBERRY CROWN BORER

Pages in Study: 53

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Evaluations of tactics for detection and management of raspberry crown borer, RCB, *Pennisettia marginata* (Harris) on blackberries were performed in Mississippi from January 2005 - July 2006. Randomized split plots with infested blackberries and certified nursery stock of two recommended cultivars, thorny 'Chickasaw' and thornless 'Apache' were planted. Pest management tactics targeting RCB included drenches: chemical insecticide bifenthrin, recently registered for RCB management; experimental pesticide E2Y45; and entomopathogenic nematodes *Steinernema feltiae*. Insect pheromones (E,Z)3-13-octadecadien-1-ol and (E,Z)3-13-octadecadien-1-yl-acetate were evaluated as RCB lures. Effects of fungicides captan, pyraclostrobin + boscalid, lime-sulfur and insecticide acetamiprid on viability and infectivity of entomopathogenic nematode species, *S. feltiae* and *Heterorhabditis bacteriophora* were evaluated in laboratory bioassays with host greater wax moth larvae.

RCB larvae and soil pests on and around infested blackberry plants were observed. Bait traps with 5 mg of (E.Z)3-13-octadecadien-1-ol captured an adult RCB on

October 19, 2005. Laboratory bioassays indicated that lime-sulfur and pyraclostrobin + boscalid decrease nematode movement and infectivity.

DEDICATION

I dedicate this manuscript to my thoughtful and caring parents who supported me throughout my studies at Mississippi State University. Without them this research would not be possible.

ACKNOWLEDGEMENTS

I acknowledge my gratitude for the support, understanding and many challenges presented by my major advisor Dr. Frank B. Matta. He provided the opportunity and guidance necessary for accomplishment. His academic professionalism proved steadfast in coordination of our achievement.

I appreciate the contribution of knowledge and expertise extended by Dr. Blair Sampson, Dr. John Braswell, Dr. Juan Silva, Dr. David Nagel, Dr. Blake Layton, Dr. Donn Johnson, Dr. Gary Lawrence, Dr. Alan Henn, Clarissa Balbalian and Jessie Quarrels. Their brilliance illuminates the way, allowing the scientific method to proceed.

I thank Gerry Ferguson, Keith Daniels and the staff of the Oktibbeha County MAFES Experiment Station for facilitating my field research project. I am indebted to the North American Bramble Grower's Association and the USDA IR-4 Project for providing the funds necessary to undertake this work. Their continuous support has been beneficial beyond expectations.

I commend the sacrifices made by Elizabeth Montgomery, Jackie McKern, Pam Reid, Shardendu Singh, Heather Wilson and Ne Chewe. Their time and energy were valuable and their contributions were a credit to this project.

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CHAPTER I

INTRODUCTION

Blackberries, *Rubus spp.* L., are a high value crop native to and historically grown in this region (Westwood, 1993). Local production of blackberries is important for fresh market sales. The high price for fresh market blackberries is mostly due to the short postharvest life of the fruit that limits shipping from foreign production areas with lower labor costs. In 2002, US farmers harvested 56 million pounds on 8,370 acres. Most production is in Oregon and California (Rieger, 2005). In 1996, Oregon harvested 28,700,000 lbs. of blackberries grown on 5,030 acres that sold for $25,433,000 (ORBC, 2005). Average sales produced $5,056 / acre. Several years ago, there were more than 50 farms producing blackberries locally. According to Dr. Barbara Smith (2004), expert on blackberry pests and diseases at the Poplarville, MS USDA Horticultural Research Experiment Station, the primary limiting factor for blackberry production in Mississippi is the raspberry crown borer (RCB), *Pennisettia marginata* (Harris), (Lepidoptera: Sesiidae). RCB larvae bore into the crown and canes of blackberries, severely limiting fruit production, causing wilting and lodging of infested canes (Williams, 1991). While larvae are feeding inside subterranean crowns and canes, irreversible damage to plantings can occur

before the pest is detected. Dr. Smith reported that most blackberry farms in Mississippi have recently gone out of business due to this key pest.

The purpose of this research was to provide blackberry farmers with effective methods of early detection and management of the raspberry crown borer. Previous research identifies sex-attractant pheromones used to trap male RCB adults (Solomon et al., 1981). Several drench insecticides previously labeled for managing populations of RCB in crowns have been banned. The only chemical pesticide currently labeled for blackberries as a management tactic for RCB is bifenthrin. Research for new pesticide labeling continues. Entomopathogenic nematodes are a biological pest management tactic that has been proven effective against RCB (Capinera et al., 1986). The objectives of this research were to 1) trap male RCB adults using pheromone bait; 2) monitor populations of RCB living in and around infested crowns; and prevent the spread of RCB from infested blackberry plants to adjacent rows of certified nursery stock transplant blackberries in the field, using fall and spring applications of chemical insecticide and entomopathogenic nematodes, and 3) to test unknown effects of chemical pesticide applications on entomopathogenic nematodes. To accomplish the third objective, laboratory bioassays evaluating infectivity of entomopathogenic nematodes on Lepidopterous larval hosts after exposure to variable rates of chemical pesticides were conducted *in vitro*.

CHAPTER II

LITERATURE REVIEW

Establishment of Blackberries

Blackberries (*Rubus* L. subgenus *Eubatus* Focke) are a native crop in North America and Europe (Crandall, 1995). Most farms in Mississippi grow erect varieties (Braswell and Rasberry, 2000). Establishment of blackberry crops requires adequate soil fertility and amendments. Blackberries should be planted according to recommendations. Field production of blackberries requires irrigation during summer heat (Crandall, 1995). Seasonal pruning is also required. Plans should include weed, disease and pest management applications.

Thorny and thornless varieties are chosen for local production. Several varieties released by the University of Arkansas Breeding Program are recommended by the Mississippi Cooperative Extension Service (Braswell and Rasberry, 2000). Two recently released varieties include 'Apache' thornless and 'Chickasaw' thorny. 'Apache' produces large, high quality fruit, and shows excellent vigor and good disease resistance (Clark and Moore, 1999). 'Chickasaw' is a high yielding variety with improved postharvest quality, vigorous growth and moderate disease resistance (Clark and Moore, 1999). Both varieties should be successful for blackberry farming in Mississippi.

The soil at the Oktibbeha Co. North Farm Mississippi Agricultural and Forestry Experiment Station (MAFES) research plot was tested before planting, and the results were analyzed by the Mississippi State University Extension Service. The soil pH before planting was 6.2, within the optimum range 6 to 6.5 recommended by Braswell and Rasberry, (2000). Analysis of test results indicated that the soil was very fertile for blackberry production (Crouse, 2005). Fertilizer amendment recommended by Braswell and Rasberry (2000) is 11 lbs. for the 1st year and 16 lbs. for the 2nd yr. of granular 13-13-13 / 100 feet of row divided into early and late spring applications. Previously, the field research plot was cultivated for 4-5 years with soybeans and fertilized with potassium and phosphorus. Glyphosate was the only pesticide applied. Soybeans were plowed under in September 2004 to prepare for the blackberry crop. Soil type is Leeper, offering poor drainage (Brent, 1973). Brent describes that the surface organic layer is composed of 5 inches of silty clay loam, and below are several layers of silty clay, clay, silty clay loam and clay loam to 4-5 feet depth.

Planting and field preparation should provide proper growing conditions such as spacing, drainage, and irrigation. The field should be prepared without weeds (Pense, 2005). A roto-tiller is commonly used to mulch weeds into the soil and improve tilth (Shippen et al., 1980). Bare root canes require a spacing of 3-4 ft. along rows, planting slightly deeper than the previous depth indicated by the soil line on the crown (Pense, 2005). Rows should be maintained 3-4 ft. wide without weeds to provide space for canes to spread, and may be raised to improve drainage in heavy soils (Pritts and Handley, 1989). With heavy soils and a well

graded field with a gentle slope, irrigation furrows may extend beside rows from a lateral ditch at the higher end to drain gradually towards the lower end of the field (Crandall, 1995). Spacing between rows should be sufficient to accommodate tractors and implements that will be used in the field (Pritts and Handley, 1989). Between rows, mowed cover may be maintained to improve traction for equipment and for worker access (Crandall, 1995).

Summer and winter pruning are recommended. Summer pruning recommendations, beginning in June, include tipping rows to a height of 3-4 ft. and may be repeated 4 times during the growing season (Braswell and Rasberry, 2000). Winter pruning normally involves removing floricanes from the previous season (Pritts and Handley, 1989). Additionally, damaged or infested canes are removed during dormant season pruning (Crandall, 1995).

Weed management for blackberries may include both mechanical and chemical tactics. Care must be taken while removing weeds in close proximity to first-year blackberries, because hoeing and pre-emergent herbicides may be harmful to roots of young transplants (Pritts and Handley, 1989). Hand-pulling may be used around transplants, and hoeing between plants in rows, while rototillers may be used to mulch weeds between rows (Crandall, 1995). According to Mississippi Extension Service herbicide recommendations, glyphosate and diquat dibromide are both labeled for use in production of first-year brambles, and glyphosate is labeled for use during later years (2005).

According to the USDA (1978), the major crop diseases affecting blackberries in the Southeast US are as follows: anthracnose, *Elsinoe veneta,* that

damages canes and new shoots; rosette or double blossom, *Cercosporella rubi* Wint., that damages flower initiation and causes witch's broom growth of new shoots; crown and cane gall, *Agrobacterium tumefaciens* and *Agrobacterium rubi,* that cause swelling of canes and roots, and stunted vegetative growth; and gray mold, *Botrytis cinerea,* that destroys fruit before and after harvest. To treat foliar fungal disease and dieback, spread by splashing rain and prolonged moisture on leaves, the Fungicide Resistance Action Committee recommends that chemicals with different modes of action, such as captan, strobilurins, and copper hydroxide, be alternated to limit pathogen resistance to pesticides (Balbalian, 2005). *Agrobacterium radiobacter* strain K1026 is labeled for use preventing gall symptoms of caneberries at 100g product, 100 billion CFU, per gallon water in a suspension. One thousand non-infested rooted stems can be dipped in suspension before planting (DeFrancesco, 2002). For control of gray mold near harvest time, iprodione (Rovral, Bayer CropScience) is used at 2 lb. ai/acre, (DeFrancesco, 2002). Abnormal plants must be removed and destroyed.

Blackberries are hosts to several insect pests besides RCB. In Mississippi and Alabama, other major pests include aphids, mites, plant bugs and weevils (Harris, 2000 and Nesbitt, 2006). Minor blackberry pests include grasshoppers and caterpillars (Harris, 2000). Johnson (2005) lists the following additional pests in Arkansas: rednecked cane borer, weevils, beetles, tree crickets, and thrips. Aphids spread viruses to blackberries. Preventative soil fumigation and foliar insecticide are recommended by Crandall. Bifenthrin is an insecticide currently labeled for spray and drench application on blackberries (Johnson and Lewis,

2005; Nesbitt, 2006). Carbamate insecticide is also recommended by Mississippi Extension Service for application to blackberries for management of more resistant pests such as beetles, grasshoppers, plant bugs and weevils, but cannot be applied during flowering season due to potential damage to bee populations. Bt, *Bacillus thuringensis ,*biological insecticide is recommended for application to defoliating caterpillars, (Harris, 2000). The economic injury level after harvest, during seasonal rise in insect populations, is 30% defoliation (Layton, 2005).

Life Cycle of the Raspberry Crown Borer

The raspberry crown borer clearwing moth life cycle was studied by Raine in 1962. His observations are summarized here. The primary host is brambles, *Rubus spp.* Distribution covers the US and Canada. Eggs appearing reddish-brown, oval, 1.5 mm in length are laid on the underside of new leaves. Incubation period is 3-10 weeks. Hatching is influenced by moisture. Small spine-like crochets distinguish the 3.5 mm larvae, colored white with a brown head. Within a half hour of hatching, larvae crawl down to the base of the cane spinning a thread. At the crown just below soil surface, the larva forms a hibernaculum and overwinters. In the spring, first-year larvae tunnel into cambium, often causing cracks exuding frass at the base of canes. Girdling of canes may cause galls and frass to appear in autumn. Larvae grown to 29 mm, (see figure 1b) may overwinter in canes or crowns. Second year larvae girdle deeper into crowns, then in July, they tunnel to above the soil surface in canes and form reddish-brown, 26 mm long pupae with transverse rows of dorsal abdominal spines.

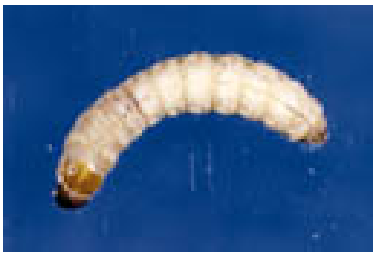
Occasionally, a pupa forms an earthen cell in the soil adjacent to the crown. Adults emerge in late summer and early fall, resembling yellow-jacket wasps, 20-25 mm long, (see figure 1a). The male's antennae are pectinate and female's are smooth. Females mate shortly after emergence and lay about 100 eggs, 2-3 per plant, mostly within 2 days, with an average life span of 6 days. Solomon reported adult RCB in west-central Mississippi from September 9 through October 11 of 1981. Wylie (1970) described RCB as a serious pest in Arkansas. A more recent study of the pest in Arkansas revealed an adult emergence period from mid-late September (McKern, 2005).

Figure 1: Raspberry crown borer (photos)

a) adult

20-25 mm length

b) larva



29 mm length

(Photos from Funt et al., 1999.)

Detection of the Raspberry Crown Borer

Pheromone traps have been successful detection methods for adult *P. marginata* (Solomon, et al., 1981) and other Sesiidae clearwing borers such as raspberry clearwing moth *Pennisettia hylaeiformis* (Priesner, 1986); currant borer moth, *Synanthedon tipuliformis* (James, 2001); peachtree borer, *Sanninoidae*

exitosa; lesser peachtree borer moths, *Synanthedon pictipes* (Gentry et al., 1984 and 1980; McLaughlin et al., 1976; Pfeiffer et al., 1991; Snow et al., 1985); and dogwood borer, *Synanthedon scitula* (Pfeiffer and Killian, 1998). Solomon et al. (1981) captured RCB adults in traps using the pheromone (E,Z)3-13-octadecadien-1-ol from September 9 – October 11 in west-central Mississippi. The research concerning *P. hylaeiformis* (Laspeyres), found in Europe, is especially valuable due to the close similarity between this species and *P. marginata* (Harris, 2000; Eichlin and Duckworth, 1988). Priesner and others found equal number of receptor sites for both (EZ)3-13-octadecadien-ol and (E,Z)3-13-octadecadien-yl acetate on the antennae of male raspberry clearwing borers. This combination did not appear in Solomon's report, and should be tested in RCB bait traps.

Management of the Raspberry Crown Borer

RCB is currently managed by preventative drench applications of bifenthrin ((Johnson and Lewis, 2005; Nesbitt, 2006). Once an infested plant is detected, it must be dug out and burned (Antonelli, 1997). McKern reported 100% control of RCB in Arkansas field trials with bifenthrin drench in late fall (2005). McKern (2005) also tested the experimental pesticide E2Y45, (Dupont®) and achieved 100% control with a fall drench. Previous successful control methods using the pesticides diazinon and permethrin (Schaefers, 1974), are now unavailable due to loss of EPA registration of these products. Schaefers was able to eliminate heavy infestation in raspberry field crops with 1 application of

diazinon or azinphos-methyl on October 12 aimed at eggs and larvae on leaves, and hibernacula just below ground level. Spring drenches on April 16 were 90% effective. The insecticide azinphos-methyl (Guthion, Bayer) lost its EPA product registration in 2005.

Entomopathogenic nematodes have been used as a successful tactic against the following clearwing borer moths: *P. marginata* (Capinera, et al., 1986); currant borer moth, *S. tipuliformis* (Miller and Bedding, 1981 and 1982); and grape root borer, *Vitacea polistiformis* (Williams et al., 2002). The most successful of these nematodes are *Heterorhabditis bacteriophora* and *Steinernema feltiae*. Williams achieved 90% control of grape root borer using 10^9 *H. bacteriophora* /A. According to Miller and Bedding in 1982, a spray application of 3.4 x 10^7 nematodes/bush using *S. feltiae,* also known as *Neoaplectana bibionis,* caused 90% control of currant borer moth in field crops. Capinera was less successful against RCB with 60,000 *S. feltiae* / plant causing 67% infected larvae. Georgis and Gaugler (1991) contribute to previous research indicating that entomopathogenic nematodes are susceptible to immobility caused by low soil-moisture. Irrigation maintaining moist soil conditions improves the populations of nematodes in the soil (Georgis and Gaugler, 1991) using insect parasitic nematodes. McKern (2005) tested 60,000 infective juvenile *S. feltiae* / blackberry plant without irrigation, but was not successful.

Third stage infective juveniles of *S. feltiae* and *H. bacteriophora* hunt through moist soil and inside openings in plant surfaces until they enter the borer larvae and penetrate to the body cavity where they release symbiotic bacteria that

infect and kill the insect while nourishing and releasing antibiotics to protect future generations of nematodes (Poinar, 2000). *H. bacteriophora* is primarily parthenogenic and *S. feltiae* is amphimictic. Although they cause mortality of young tadpoles, they have shown no adverse effects on animals from class Aves, Reptilia, Pices or Mamalia (Poinar, 2000). Since 2002, the EPA exempts the application of *S. feltiae* or *H. bacteriophora* from government regulation. Further research comparing chemical pesticides with higher concentrations of entomopathogenic nematodes applied on irrigated soil should be pursued.

Entomopathogenic Nematode Interactivity with Pesticides

Georgis and Gaugler (1991) identified chemical pesticides that were severely limiting to populations of entomopathogenic nematodes applied to field soil. Before applying pesticides that may cause negative interactions with entomopathogenic nematode treatments on blackberry plants at the research plot, laboratory bioassays are needed to evaluate effects. Previous studies tested the toxicity of crop pesticides on entomopathogenic nematodes *S. feltiae* and *H. bacteriophora* (DeNardo and Grewal 2003; Rovesti et al., 1990, 1988; Hara and Kaya, 1983, 1982). Methods used in these studies included observations of nematode posture and movement after variable time in pesticide solutions, and infectivity bioassays *in vitro* with host greater wax moth larvae, *Galleria mellonella* L. Previous pesticide bioassays used low concentrations of nematodes, but with current recommendations at 10^9 nematodes / acre (Rincon-vitova, 2005) suspended in 50 gal. soil drench, nematode concentrations of 5,000 / mL should

be tested. This study evaluated the effects of pesticides used in blackberry production on *S. feltiae* and *H. bacteriophora.*

Herbicides recommended for use during establishment of blackberry crops, according to Mississippi Cooperative Extension Service in 2005, include 2.25 lb. ai /acre glyphosate, (Roundup® Monsanto) and fluazifop-p-butyl, (Fusilade®DX Syngenta), 0.75 oz. / gal. Rovesti and Deseo in 1990 and Rovesti et al. in 1988 tested glyphosate, non-selective post-emergent herbicide, at concentrations up to 10,000 ppm, and found that it had no effect on viability or infectivity of *S. feltiae* and *H. bacteriophora* on the host *G. mellonella.* Fluazifop, post-emegent selective graminicide, was tested by Rovesti et al. in 1988, and found to have no effect on the viability and infectivity of *H. bacteriophora* at twice the recommended concentration for blackberry production.

Fungicides recommended for use on blackberries include calcium polysulfide 17,400 ppm solution sprayed to runoff at delayed bud-break (De Francesco 2002 and Pritts et al., 2005); 4 lbs./ acre Captan 50 WP in 45-100 gal. foliar spray; azoxystrobin, Abound® Syngenta, 1 oz./ gal. sprayed to runoff during flowering (Buckley and Waters 2002); and pyraclostrobin plus boscalid, 23 oz. Pristine® BASF / acre in 50-150 gal. foliar spray (Balbalian, 2005). The three foliar fungicides are sprayed during the growing season at 7-14 day intervals in rotation with a different FRAC group fungicide. The FRAC (Fungicide Resistance Action Committee) group category for captan is multi-site. The group number for azoxystrobin and pyraclostrobin is 11. The FRAC group number for boscalid is 7. These fungicides can be used in rotation to prevent survival of

resistant fungi. De Nardo and Grewal in 2003, tested azoxystrobin at slightly lower concentration than recommended rate for field application of Abound® and found no significant effect on survival or infectivity of *S. feltiae* in sand wells with host *G. mellonella.* Sulfur was tested at 10,000 ppm, and caused no significant harm to viability or infectivity of *S. feltiae* and *H. bacteriophora* (Rovesti et al., 1988 and 1990). The effects of captan, the anilide boscalid, and lime-sulfur on the entomopathogenic nematodes are not known.

The foliar insecticide acetamiprid, Assail® Cerexagri, was recommended for experimentation by Dr. Blair Sampson, Entomologist at USDA ARS Small Fruits Research Station, Poplarville, MS. Although it has not been registered for use on blackberries, it is classified as reduced risk pesticide for use in integrated pest management and is not harmful to many beneficial insects. It can be applied during flowering season as spray, unlike pesticides that kill bees. Application rate to control plant bugs and beetles on field crops is 1.1 oz. 70% WP / A. The insecticide carbaryl, recommended for foliar application on blackberries (Harris 2000), is toxic to *Heterorhabditis* and *Steinernema* entomogenous nematodes (Zimmerman and Cranshaw 1990), and cannot be applied during bloom due to bee mortality.

CHAPTER III

MATERIALS AND METHODS

Establishment of Blackberries

In February, 2005, a soil samples from the experimental plot (206 ft. by 36 ft.) at the North Farm, MSU Starkville was submitted to the Mississippi Agricultural Extension Service for nutrient availability testing. The soil was tilled along 3 longitudinal rows 4 ft. wide spaced 12 ft. apart. Twenty infested blackberry plants were collected from Jack Wilson's Farm in Wilmer, AL. Positive identification was given by putting on a pair of gloves and giving damaged canes a tug. If the larva was present, the cane would pull away at the crown (Williams, 1991). Soil samples were collected from around the infested crowns to quantify populations of larvae in the soil. Infested plants were transplanted at 10 ft. spacing along the center row of the North Farm plot, leaving 8 ft. at each end. Forty-nine 'Chickasaw' and 49 'Apache' certified blackberry plants were ordered from Bramble Berry Farms, and cultivars were separated into homogeneous split plots with 7 plants each, randomly arranged along the two outside rows. Plants were spaced 4 ft. apart along outside rows, with 49 plants on each side of the center row. Plots were irrigated as needed to maintain moist soil.

Fertilization was according to Mississippi Extension Service Information Sheet 1444 for blackberry production. One month after planting, 1/2 cup of 13-13-13 granular fertilizer was applied in a 2 ft. circle around each plant. In June of 2005, ½ cup was applied in a 2½ ft. circle. Another soil sample was submitted for analysis during dormancy after the first season, and fertilizer was applied as needed according to soil analysis recommendations. Weeds in rows were removed by hand weeding and hoeing or application of 2% glyphosate and diquat dibromide as needed the 1st year, then only glyphosate the second year (MSU Extension Service, 2004). Weeds were managed between rows with tillage or mowing. Summer and winter pruning was done according to recommendations (Braswell and Rasberry, 2000).

Detection of Raspberry Crown Borer

Sticky insect traps (Pherocon IC, Trécé Inc.) were baited and labeled with pheromone lures applied to rubber band strips. The pheromone was applied drop-wise with a micropipette onto the surface of each rubber band strip on a shielded balance. The baited rubber strip was centered along the glue surface inside the bottom of the trap. The concentrations of pheromones applied to each bait trap are as follows:

1 mg (E,Z)-3-13-octadecadien-1-ol, 1 mg (E,Z)-3-13-octadecadien-yl acetate, 1 mg (E,Z)-3-13-octadecadien-1-ol + 1 mg (E,Z)-3-13-octadecadien-1-yl acetate, and 5 mg (E,Z)-3-13-octadecadien-1-ol, (Pheromones are acquired from TRIFOLIO-M GmbH). Four bait traps were set up, each atop a 1 m stake at 66 ft.

and 134 ft. along both outside rows. Traps were monitored twice a week from August 8 through October 30, 2005. Bait traps were replaced every 15 days, alternating their positions randomly among the 4×1 m staked positions. Data of all clearwing moths with black and yellow bands were gathered as they were captured in the sticky traps.

Management of Raspberry Crown Borer

The plants in each split-plot were divided into 6 test variables and a control in randomized order as follows:

1. 0.1 lb. ai / acre bifenthrin (Capture® 2EC, FMC) fall, 1 L / plant
2. 0.1 lb. ai / acre bifenthrin (Capture® 2EC, FMC) fall and spring, 1 L / plant
3. 120,000 *Steinernema feltiae* / plant fall, 1 L / plant
4. 120,000 *S. feltiae* / plant in fall, 1 L / plant and 180,000 / plant in spring, 1.5 L / plant
5. 0.067 lb. ai / acre E2Y45-215 (Dupont®) fall, 1 L / plant
6. 0.067 lb. ai / acre E2Y45-215 (Dupont®) fall and spring, 1 L / plant
7. Control treatment with water only, 1 L / plant

Drenches were mixed in 5 gal. containers and applied by dipping and pouring on each crown using a plastic bottle marked and cut off at the desired level. Crop was irrigated as necessary to prevent desiccation of entomopathogenic nematodes for 2 weeks after each application.

In July 2006, crowns of blackberry plants in outside rows of the North Farm plot were removed from the soil. Insects on the crowns were surveyed by brushing soil and insects from the surface onto a 2 ft. X 2 ft. screen table. RCB

larvae and tunnels in uprooted canes and crowns were quantified by visual observation of sections. Soil insects at the base of each plant in the plot were surveyed by sampling 0.5 L of soil under each crown. Data were analyzed for statistical significance using analysis of variance, (ANOVA) and Fischer's least significant difference test ($P \leq 0.05$), (LSD_{05}).

Entomopathogenic Nematode Interactivity with Pesticides

Dilution of S. feltiae from sponges

Commercial product nematodes were transported in foam coating the porous surfaces of polyether polyurethane sponge (Bedding, 1984). When sponges arrived, labeled 5 million nematodes / package for each species, they required dilution and storage. Sponges were diluted in 250 mL sterile distilled water in Erlenmeyer flask for 5 minutes, then the process was repeated by transferring the sponge to another flask. The suspensions were combined before counting.

Examination, counting and identifying commercially produced nematodes

Commercial entomopathogenic nematodes were examined and counted under a dissecting microscope to determine quality and quantity at arrival of shipment, before blackberry pesticide exposure, and at 24 and 72 hour intervals after exposure to variable treatments. Enumeration and examination of nematodes were repeated before and after exposure to variable pesticide treatment groups after 2 weeks of refrigerated storage.

Viability was noted, in terms of movement and posture, similarly to methods described by Rovesti et al. 1988, 1990. One drop of suspension containing 15-100 nematodes was transferred from flask to microscope slide using a glass capillary tube. Movement was noted for 10 nematodes in a diameter across the droplet. If a nematode did not move, it was probed with a needle. The needle was washed in 70% alcohol between treatments. Percentage of nematodes was counted for each of the following postures: J-shaped or curved; straight; or coiled, observed with tail crossing over anterior body region. Accumulation of vacuoles was noted as a sign of poor health (Lawrence, 2005).

Nematodes were quantified according to the method described by Lawrence (2005):

1. Dilute nematodes to a countable suspension in distilled water, less than 100 nematodes per 1/16 in. grid square, in a counting Petri dish.
2. Spread 10 mL homogenous nematode suspension on 90 mm diameter counting Petri dish.
3. Count nematodes in each of 18 squares in a diagonal across the dish.
4. Calculate suspension concentration using the following formula: Quantity of nematodes in 18 squares X 25.75 = quantity on the Petri dish.
5. Back calculate the dilution to find the nematode concentration of the original sample.

Exposure of S. feltiae and H. bacteriophora nematodes to pesticide solutions

Both species of nematodes were exposed to pesticide mixtures for 3 days prior to *in vitro* inoculation of wax moth larvae. Each pesticide treatment group was mixed in a 125 mL Erlenmeyer flask (pesticide treatment volume not to exceed 1 cm height) with distilled water + pesticide to make blackberry field application rate, 10% application rate, and 1% application rate concentration, leaving enough volume to add nematode suspension. Nematode suspension was added to make @5000 nematode / mL treatment concentration. Flasks were covered with parafilm and maintained at 20° C. After 24 and 72 hours, viability of nematodes was evaluated by observing drops from each flask under dissecting microscope for movement and posture using methods described above (Rovesti et al.,1990 and 1988).

In vitro pesticide bioassays

Infectivity of nematodes was evaluated after exposure to pesticides for 72 hours using *in vitro* bioassay (Rovesti et al. 1990, 1988; Woodring and Kaya, 1988). One mL nematode suspension was transferred from each pesticide exposure treatment group onto 2 Whatman #1 filter papers in a 90 mm Petri dish, using 3 Petri dishes for each treatment group. Ten conditioned *G. mellonella* larvae were arranged between the 2 filter papers in Petri dishes. Insect larvae were conditioned by submersion in 56°C water for 15 sec., then rinsed under tap water for 30 sec. Bioassay Petri dishes were stored in plastic bags and maintained near 20ºC in dark conditions for 3 weeks. Moisture in the filter paper was

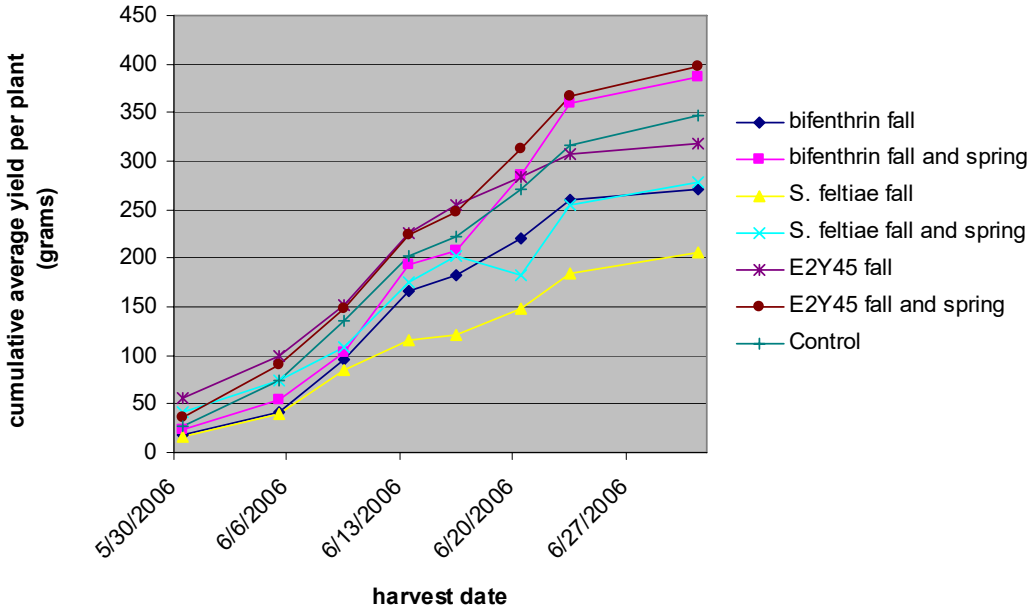
maintained by adding distilled water to prevent desiccation of the larvae. Nematodes were isolated from greater wax moth larvae to provide infectivity data. Infected wax moth larvae were transferred from the filter paper to the top of each Petri dish. Insects were cut into sections with a scalpel and the dish was flooded around the larvae under a dissecting microscope. The larval carcasses were crushed and mix until the suspension appeared homogeneous. To extract the nematodes from the suspension, the suspension was filtered between 200 mesh and 500 mesh sieves. The contents of 500 mesh sieve were rinsed into a beaker to fill 100 mL volume. Nematodes were counted using Lawrence's method. Data were analyzed using ANOVA and Fischer's LSD_{05}.

CHAPTER IV

RESULTS AND DISCUSSION

Establishment of Blackberries

The establishment of blackberries on the Oktibbeha Co. North Farm, MAFES research plot was adequate for statistical analysis of the treatment groups. Before the first insecticide treatments in late fall, 93% of the 98 experimental unit blackberry plants were showing green leaves, see figure 3. When final data was collected, 5 plants were dead along the outside rows. The average total yield per plant in 2006 was 315 g. None of the insecticides caused significant damage to plant survival. The fall + spring treatment groups of bifenthrin and E2Y45 had superior yield, see figure 2. This yield increase could be caused by lower insect populations after the spring insecticide drenches under these plants. The *S. feltiae* treatment groups had lower yields, see figure 2.



Statistical analysis using ANOVA and Fischer's $LSD_{0.05}$ indicated no significant difference between treatment yields

Figure 2: Treatment group yields

Figure 3: Research Plot 17Sept2005 with pheromone bait traps set along rows

Outer rows are nursery stock transplants with 14 alternating stretches of 7 'Apache' and 'Chickasaw' blackberries, totaling 98 plants. Middle row is infested transplants. Pheromone bait traps are set at 1/3 and 2/3 along the length of outside rows.

Detection of Raspberry Crown Borer

Results confirm previous observations by Solomon et al., (1981) indicating a pheromone lure for capturing RCB adults in traps. One adult male RCB was captured on October 19, 2005 in a folded cardboard sticky trap (Pherocon IC) using 5 mg (E,Z) 3,13-octadecadiene-1-ol as a lure. This capture date reveals a later period of adult occurrence for RCB in Mississippi compared to the mid-late September adult occurrence reported by McKern from Arkansas in 2005. The highest of the variable pheromone concentrations attracted the RCB male, indicating that the 5 mg E3Z13-18ol lure is effective for early detection, but a higher range of concentrations should be evaluated. The specimen was visually identified then verified using DNA analysis at the University of Arkansas (Johnson and McKern, 2005). Between August 18 and September 29, 2005, 75 other Sesiid moths were captured in the traps. The two other species of moths identified are the horsenettle borer, *Synanthedon rileyana* (Johnson and McKern, 2005) and the dogwood borer, *Paranthrene tabanaformis* (Brown, 2005). Both of the other moth species displayed the wasp-shaped adult body and yellow striped abdomens similar to the RCB, but their legs were black. RCB were identified by their yellow legs (Eichlin and Duckworth, 1988).

Management of Raspberry Crown Borer

Experimental data in this study do not provide conclusive results for the effects of the pesticide treatments on RCB. Tunneling was observed in cross sections of crowns as follows: Two out of 14 fall nematode treatments, 1/14 fall

and spring nematode treatments, and 1/13 control treatments. One RCB larva, identified by Dr. Sampson (2006) was located in a tunnel in one of the fall nematode treatment blackberries. The breeding patterns of emerging adults could have been disrupted by flooding and high winds that swept through the research plot in 2005, caused by hurricanes Katrina on August 30 and Rita on September 23. The high distribution rate of RCB egg laying, 2-3 eggs per plant and 100 eggs per female (Raine, 1962), should have provided a more uniform infestation rate. The 3-10 week incubation period for the eggs laid on blackberry leaves was interrupted by the extreme weather conditions. This could have been the cause for the low rate of infestation of the blackberries.

The soil drench insecticide treatments also affected other insect populations. Several species of beetles and weevils were uncovered when the plants were removed from the soil in July, 2006. Flea beetles, carambid beetles, click beetles, june beetles, and billbugs are common agricultural pests found in the samples that could damage blackberries (Sampson, 2006). The fall treatment of bifenthrin and E2Y45 both resulted in a significant decrease in the population of beetles and weevils removed from the outer surface of the crowns when the plants were removed from the soil. The fall treatment of E2Y45 resulted in a significant decrease in the beetle and weevil populations from the 0.5 L soil samples beneath crowns. Fire ants were also a nuisance in the plot. Ant populations were observed on or beneath all experimental treatments except the fall + spring bifenthrin drenched plants. Fall treatment of bifenthrin and the fall + spring treatment of E2Y45 both had very low ant populations, mean counts 0.08

and 0.23 per plant, respectively, compared to the other treatments, ranging from 2.92 – 23.08 mean ants per plant.

Entomopathogenic Nematode Interactivity with Pesticides

Data indicate that exposure to blackberry pesticides lime sulfur and pyraclostrobin + boscalid caused a decrease in movement and infectivity of entomopathogenic nematodes. Negative effects of pesticides were observed during exposure of the nematodes to pesticides in Erlenmeyer flasks prior to inoculation of bioassays, *H. bacteriophora (* figure 4) and *S. feltiae* (figure 5). For both species of nematodes, lime-sulfur in full spray concentration or 10% treatment groups stopped movement. Pyraclostrobin + boscalid fungicide at full concentration stopped movement at 24 hours, but both species recovered a low rate of movement after 72 hours. Due to the poor survival of *H. bacteriophora* during storage, data for movement of this species is not conclusive, but data for *S. feltiae* is sufficient for statistical analysis (Table 1). All concentrations of lime-sulfur caused significant decrease in movement of *S. feltiae*. Exposure to pyraclostrobin + boscalid caused significant decrease in movement after 24 and 72 hours.

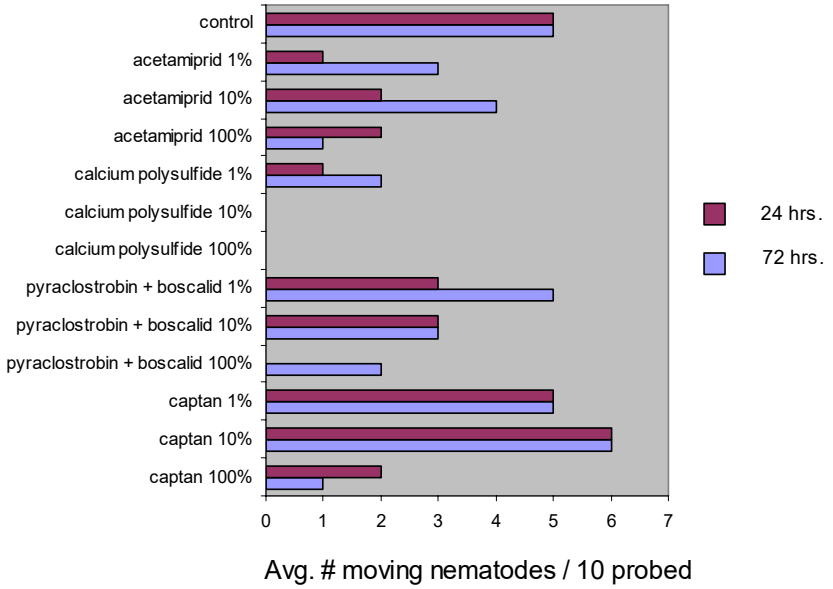
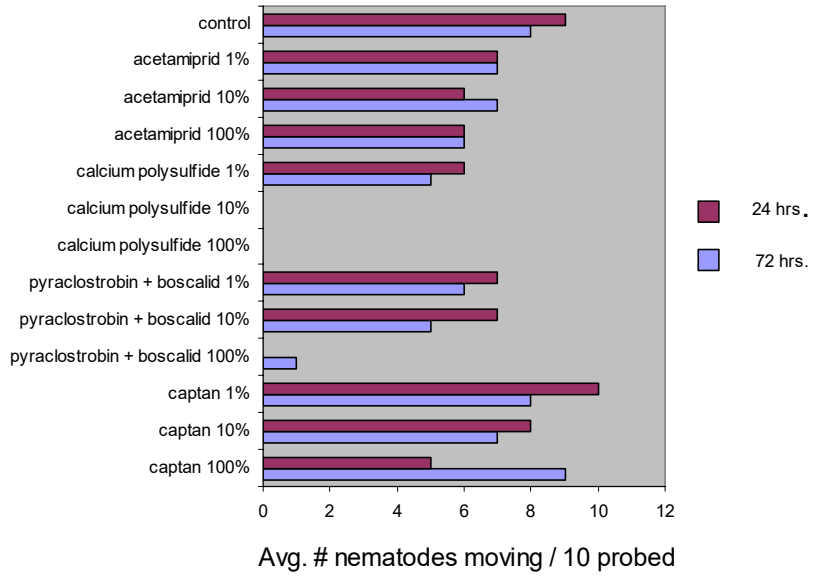


Figure 4: Movement of *H. bacteriophora* after 24 and 72 hour pesticide exposure



At 24 hr. and 72 hr. intervals after nematodes were added to pesticide treatment flasks, movement in response to probing was observed. Pesticide solutions were prepared at 100%, 10%, and 1% labeled application rate to test the effects of variable levels of pesticides on both species of entomopathogenic nematodes.

Figure 5: Movement of *S. feltiae* after 24 and 72 hour pesticide exposure

Table 1: Effect of pesticides on movement of *S. feltiae* after 24 and 72 hours of exposure

Pesticide concentration	avg. decreased % movement after 24 hours*	avg. decreased % movement after 72 hours*
control	1.5 c**	6.5 d
lime sulfur 100%	86.5 a	86.5 a
lime sulfur 10%	86.5 a	86.5 a
lime sulfur 1%	51.5 a,b	61.5 a,b
captan 100%	21.5 a,b	6.5 d
captan 10%	6.5 b,c	16.5 d
captan 1%	1.5 c	5 d
pyraclostrobin + boscalid 100%	51.5 a,b	56.5 a,b,c
pyraclostrobin + boscalid 10%	11.5 b,c	31.5 b,c,d
pyraclostrobin + boscalid 1%	10 b,c	26.5 c,d
acetamiprid 100%	16.5 b,c	26.5 c,d
acetamiprid 10%	16.5 b,c	10 d
acetamiprid 1%	10 b,c	10 d

*Percent movement is observed by counting moving and non-moving nematodes along a diameter across the sample on a slide

**Column numbers followed by the same letter are not significantly different as determined by Fischer's least significant difference test ($P \leq 0.05$)

After 2 weeks of refrigerated storage in 0.1% formalin suspension, significant changes in movement and posture were observed. Average movement counts of *S. feltiae* stimulated by needle remained above 8/10 before and after storage. However, movement of *H. bacteriophora* decreased from 7/10 to 1/10 average counts. According to Woodring and Kaya (1988), *Steinernema spp.* survive storage conditions for longer periods than *Heterorhabditis.* After storage, mean count of *S. feltiae* with vacuoles was below 2%, while mean of 14% vacuolated *H. bacteriophora* was observed. Appearance of vacuoles in stored nematodes shows negative correlation with observed movement, r value -0.84. Coil posture in *H. bacteriophora* remained low after storage, but in *S. feltiae* average curling nearly doubled from 6.7% before to 12.1% after storage. This

may indicate a reaction to environmental toxins as indicated by Hara and Kaya (1983).

Variations in infectivity of entomopathogenic nematodes on greater wax moth larvae resulting from 72 hour exposure to variable rates of blackberry pesticides is indicated by data, *S. feltiae* in figure 6 and *H. bacteriophora* in figure 7. Acetamiprid insecticide had little negative effect on the rate of juvenile nematodes found in host larvae with *S. feltiae*. Wax worms were highly infected by *H. bacteriophora* following actetamiprid treatment groups. The 10% acetamiprid application rate treatment caused a significant increase above the control group *H. bacteriophora* population. Possibly, the insecticide decreased the resistance of greater wax moth larvae to nematode infestation. Lime sulfur was toxic to nematodes at the 100% and 10% application rates, suppressing infectivity for both nematode species. The 1% lime sulfur treatments caused a significant decrease in infectivity of *H. bacteriophora*, but no significant decrease of *S. feltiae.* Full application rate of captan caused no significant reduction in the rate of infectivity for both nematode species. Pyraclostrobin + boscalid reduced the rate of infectivity of *S. feltiae* at 100% and 10% application rates, but not significantly at 1% rate. The 100% pyraclostrobin + boscalid treatment caused 0 infectivity of *H. bacteriophora,* but nematode population was not decreased significantly by lower concentrations. Pristine® fungicide contains 12.8% pyraclostrobin and 25.2% boscalid as active ingredients. The active ingredient azoxystrobin is shown to have no effect on *S. feltiae* (De Nardo and Grewal, 2003). Although azoxystrobin and pyraclostrobin are both FRAC group

11 fungicides, their toxicity to nematodes may vary. Further research could isolate boscalid and pyraclostrobin as separate treatments.

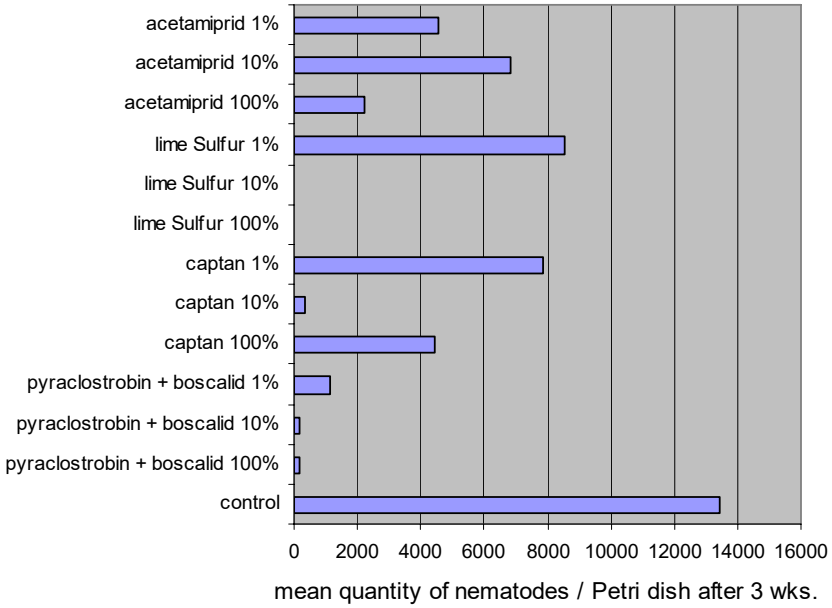
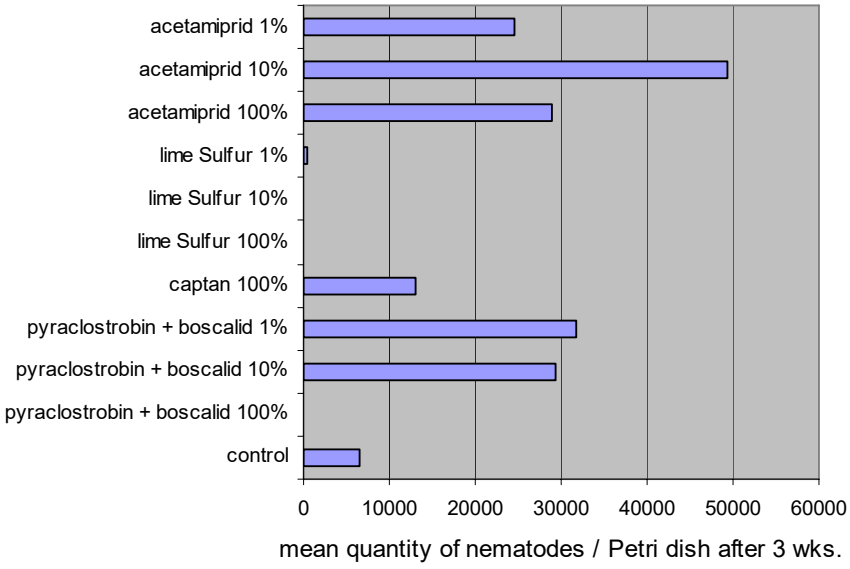


Figure 6: Effect of variable pesticide rates on infectivity of *S. feltiae*



1 mL of suspension from pesticide treatment flasks after 72 hrs. exposure were pipetted into Petri dishes with heat conditioned *Galleria mellonella* greater wax moth larvae to test effects of pesticide exposure on infectivity of entomopathogenic nematode species.

Figure 7: Effect of variable pesticide rates on infectivity of *H. bacteriophora*

Table 2: Effects of pesticide exposure on infectivity of entomopathogenic nematodes

	H. bacteriophora				*S. feltiae*			
treatment concentration	pyraclostrobin + boscalid	captan	lime-sulfur	acetamiprid	pyraclostrobin + boscalid	captan	lime-sulfur	acetamiprid
Control	6,495 a,b*	6,495 a	6,495 A	6,495 b	13418 a	13418 a	13418 a	13418 a
100%	0 b	13,075 a	0 B	1,121 a,b	200 a,b	4,462 a,b	0 B	28,869 b
10%	29,297 a	-	0 B	1,912 a	172 b	319 b	0 B	49,240 a,b
1%	31,816 a	-	8526 B	954 a,b	1,116 a,b	7,840 a,b	8,526 a,b	24,577 a,b

* treatments in each column indicated by the same letter are not significantly different as determined by Fischer's least significant difference test ($P \leq 0.05$)

CHAPTER V

SUMMARY AND CONCLUSIONS

Blackberry crops, historically common in Mississippi are severely limited by the raspberry crown borer, *Pennisettia marginata* (Harris). Larval populations grow undetected underground until permanent damage caused by tunneling in crowns and canes decrease yields and destroy crops. The adult moths disperse eggs to large numbers of plants, spreading infestations from other plots or wild brambles. Early detection and prevention of this major pest is necessary for re-establishment of this native high value crop for regional fresh market production.

Detection of RCB can be facilitated by implementing pheromone lures and sticky traps set along crop rows. Previous research in Mississippi with RCB and the insect sex-attractant pheromone (E,Z)3-13-octadecadien-1-ol (Solomon et al., 1981) is confirmed by the results of this study. The lure concentration 5 mg / trap is sufficient to capture the adult male. The period of adult appearance is now extended beyond the results of previous studies into late October in Mississippi. Traps should be set to capture adults throughout the reproductive stage of the RCB life cycle, from August through October. Farmers should be able to identify the pests visually by their yellow and black banded abdomens and yellow legs to distinguish them from other insects captured in the traps. There is an opportunity

for mating disruption research with pheromone lures distracting RCB males away from their mates, as described by McLaughlin et al. (1976) concerning peachtree borers. Although unlike peaches, brambles often grow wild and mating will still occur outside the range of pheromone distraction, thereby allowing gravid females from distant sites to lay eggs on crops within a few days flying range.

Current pest management of RCB in blackberries includes pesticide labeling for chemical insecticide drench with bifenthrin at the rate 0.1 lb. ai / acre (Capture® 2EC, FMC). This application has proven effective in Arkansas with spring treatments (McKern, 2005). Experimental pesticide E2Y45 (Dupont®) 0.067 lb. ai / acre has also shown excellent control against RCB in Arkansas with fall drenches (McKern, 2005). The data in this thesis does not show any damage from RCB on crowns treated with these rates of bifenthrin or E2Y45. Unfortunately, severe weather conditions caused by hurricanes Katrina and Rita in late summer and early fall of 2005 disrupted the concurrent RCB mating, egg laying and incubation periods. The population of RCB did not reproduce enough to provide sufficient data for statistical analysis. Tunnels were observed in a few crowns from control and entomopathogenic nematode treatments, suggesting the need for chemical drenches to manage this pest. Further research with entomopathogenic nematodes should involve timing of applications and application rate of nematode suspensions.

Biological pest management of RCB in blackberry crops can be achieved with moderate success by applying entomopathogenic nematodes (Capinera et al., 1986). Interactions between nematodes and other pesticides used for regular

cropping of blackberries are important factors for optimizing pest management strategies. Results of this study should be considered when timing pesticide applications. Soil residue of lime-sulfur, applied in early spring, could decrease viability and infectivity of *S. feltiae* and *H. bacteriophora.* Pyraclostrobin + boscalid exposure caused a decrease in movement and infectivity of both nematode species at full concentration, but at lower levels no significant decrease in infectivity was observed. These results indicate a need to avoid simultaneous application with nematode species, but spray applications would be diluted before contacting an existing nematode population in the soil. Biological control nematodes could be applied between bud-break and bloom to avoid negative pesticide interactions. Captan and acetamiprid were not shown to be harmful to the beneficial nematodes. Integrated pest management strategies are improved by decreasing use of pesticides that harm non-target organisms.

Blackberry farmers should be able to re-establish crops in Mississippi based on this research. Establishment of blackberry crops can be achieved with the materials and methods provided here. Early detection pheromone traps and preventative pest management options from previous research are tested, showing many positive results in Mississippi. Soil interactions with chemical pesticides limiting viability of beneficial entomopathogenic nematode applications are identified and should be avoided to improve the efficacy of a safer biological alternative to chemical pesticides.

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APPENDIX A

WORK LOG FOR BLACKBERRY RESEARCH PLOT AT OKTIBBEHA COUNTY MISSISSIPPI AGRICULTURE AND FORESTRY EXPERIMENT STATION (MAFES) NORTH FARM

Date	Activity
30Dec2004	Collected 19 transplants showing symptoms of borer damage from infested farm and transported to tilled MAFES Oktibbeha Co. North Farm research plot Sampled infested canes and soil insects
31Dec2004	Collected plot soil sample for MSU Soil Analysis Lab Heeled in transplants
5Jan2005	Planted 19 transplants along center row Staked off 3 rows 4ft. wide × 196 ft. long with 12 ft. between row centers
26Feb2005	Planted 49 ‘Chickasaw’ and 49 ‘Apache’ certified nursery stock bare-root blackberry plants along two outside rows according to treatment plan
25Mar2005	Graded plot after heavy rains indicated low areas Fertilized all blackberry plants with ½ cup 13-13-13 / plant
4Apr2005	Submitted necrotic plant samples from plot to MSU Plant Pathology Lab
7,9Apr2005	Hand-weeded 2 ft. circle around each blackberry plant
15Apr2005	Sprayed 4 oz./gal 18% glyphosate + 0.73 % diquat, 2-3 gal. per row outside 2 ft. circles
20Apr2005	Sampled diseased plants from plot and observed root damage
29Apr2005	Installed irrigation system composed of furrows along each row, 20 in. poly-pipe in ditch along the west side of plot emptying into furrows, and metal pipes connecting poly-pipe to irrigation riser head
6May2005	Replaced dead plants due to 18Mar05 freeze damage Irrigated furrows and watered plants with a bucket
7-9May2005	Irrigated furrows and watered plants with a bucket

10May2005	Irrigated plants with a bucket potted bare-root 'Chickasaw' and 'Apache' nursery stock with 70% peat and 30% perlite media and began watering every day
11May2005	Irrigated furrows and watered plants with a bucket
12May2005	Irrigated plants with a bucket Pruned dead stems from canes in pots and field plot
13,16-17May2005	Irrigated plants with a bucket
18May2005	Irrigated furrows and watered plants with a bucket
19May2005	Irrigated plants with a bucket Hand-weeded 2 ft. diameter circle around each plant
20May2005	Irrigated furrows and watered plants with a bucket
21May2005	Irrigated furrows and watered plants with a bucket
22May2005	Irrigated plants with a bucket
23May2005	Irrigated furrows and watered plants with a bucket
24-25May2005	Irrigated plants with a bucket
26May2005	Irrigated furrows and watered plants with a bucket
27-28May2005	Irrigated plants with a bucket
2Jun2005	Fertilized pot plants with 50 ppm chelated iron, 0.133 L for 1 gal. pots and 0.233 L for 2 gal pots to correct deficiency symptoms Observed 3/19 defoliated center row transplants and apparent plot deer damage
3Jun2005	Hoed 2½ ft. circle around plot plants
4-5Jun2005	Irrigated plants with a bucket
7Jun2005	Pruned damaged stems on plot Submitted samples of plot plant brown leaf-spot to MSU Plant Pathology Lab

8Jun2005	Applied 300 ppm 20-20-20 fertilizer drench to pot plants, 1 pt. to 1 gal. pots and 1.5 pt. to 2 gal. pot
9Jun2005	Replanted 5 ‘Chickasaw’ and 6 ‘Apache’ dead or severely diseased plot plants Irrigated plants with a pail
10Jun2005	Sprayed 2-3 gal./row glyphosate solution, 2.5 oz. 41% ai. concentrate/gal.
14Jun2005	Hoed 2½ ft. circle around plot plants
15Jun2005	Fertilized all blackberry plants with ½ cup 13-13-13 / plant Replaced 1 ‘Chickasaw’ and 1 ‘Apache’ with dieback in plot Irrigated plants with a bucket
16Jun2005	Rototiller cultivated between and outside rows Irrigated furrows and watered plants with a bucket
17Jun2005	Irrigated furrows and watered plants with a bucket
18Jun2005	Irrigated plants with a bucket
19Jun2005	Irrigated furrows and watered plants with a bucket
20Jun2005	Irrigated plants with a bucket
21Jun2005	Irrigated furrows and watered plants with a bucket
22Jun2005	Irrigated plants with a bucket
23Jun2005	Irrigated furrows and watered plants with a bucket
24Jun2005	Irrigated plants with a bucket Replaced 1 ‘Chickasaw’ and 3 ‘Apache’ with dieback in plot Samples were analyzed by Mississippi State University Plant Pathology Lab as leaf blotch, *Mycosphaerella confusa* and cane blight Fungicide was recommended

25Jun2005	Irrigated furrows and watered plants with a bucket
28Jun2005	Irrigated plants with a bucket
3Jul2005	Irrigated furrows and watered plants with a bucket
13Jul2005	Hand-weeded and hoed 2 ft. circle around each plant
20Jul2005	Sprayed glyphosate along rows, outside 2 ft. circles
27Jul2005	Replaced 4 'Apache' showing dieback symptoms with pot plants
1Aug2005	Sprayed glyphosate between rows with tractor and box sprayer
3Aug2005	Broadcast sprayed Pyraclostrobin + boscalid (Pristine®, BASF) 23 oz./acre using a tractor and highboy
9Aug2005	Set pheromone traps atop 4 1-m stakes with variable pheromone concentrations Began monitoring traps for clearwing moths with yellow/black rings on abdomen
10Aug2005	Hoed weeds in 2 ft. circle around each plant Broadcast fungicide captan 50WP (4lb./acre) using tractor and highboy
20Aug2005	Hand-pulled weeds growing through plants Irrigated furrows and watered plants with a bucket
26-27Aug2005	Hoed 2 ft. circle around each plant
29Aug2005	Removed pheromone traps to prepare for hurricane Katrina
31Aug2005	Replaced pheromone traps after hurricane Repaired irrigation poly-pipe
3Sep2005	Mowed between rows and outside plot along perimeter
4Sep2005	Spread pine bark mulch 2 in. depth in a 2 ft. circle around each plant
5Sep2005	Irrigated furrows and watered plants with a bucket

7Sep2005	Irrigated furrows and watered plants with a bucket
9Sep2005	Sprayed glyphosate along rows outside 2 ft. circles
14Sep2005	Irrigated furrows and watered plants with a bucket
16-17Sep2005	Shoveled out furrows, realigned and rebuilt rows damaged by hurricane Katrina
21Sep2005	Irrigated furrows and watered plants with a bucket
23Sep2005	Mowed between rows and outside plot along perimeter
28Sep2005	Took samples of floral dieback to Plant Pathology Lab Pathogens identified were *Alternaria sp., Curvilaria sp., and Exserohilum sp.*
3Oct2005	Took 6 in. depth soil samples within 2 ft. circles around plants Analysis indicated very high levels of Phosphorus and recommendations for reduced fertilizer application in the spring Broadcast sprayed 100 ml Azoxystrobin 22.9% ai / acre (Abound®, Syngenta) using tractor and highboy
4Oct2005	Identified dogwood borers captured in pheromone traps with Dr. Brown at Insect Taxonomy Museum, Mississippi State University
5Oct2005	Irrigated furrows and watered plants with a bucket
9Oct2005	Irrigated furrows and watered plants with a bucket
12Oct2005	Broadcast sprayed captan 50WP (4lbs./acre) using tractor and highboy
15Oct2005	Irrigated furrows and watered plants with a bucket Deer tracks noted near defoliated plants
22Oct2005	Irrigated furrows and watered plants with a bucket
24Oct2005	Observed 93/98 blackberry plants showing leaves at first freeze
6Nov2005	Irrigated furrows and watered plants with a bucket

2Dec2005	Applied pesticide as @ 1 L drench mixture per plant to crowns for each of the following treatment groups: 1. 0.1 lb. ai / acre Capture® 2EC (FMC) fall 2. 0.1 lb. ai / acre Capture® 2EC (FMC) fall and spring 3. 120,000 *Steinernema feltiae* / plant fall 4. 120,000 *S. feltiae / plant* fall and spring 5. 0.067 lb. ai / acre E2Y45-215 (Dupont®) fall 6. 0.067 lb. ai / acre E2Y45-215 (Dupont®) fall and spring 7. Control treatment with water only Method: Drenches were mixed in 5 gal. containers and applied by dipping and pouring on the crown using a plastic bottle marked and cut off at the 1 L level.
23Feb2006	Shoveled to clear drainage along outside row furrows and drainage ditch along eastern side of plot
28Feb2006	Straightened furrows and hipped outside rows to original 4 ft. width
13Mar2006	Pruned diseased and damaged canes Hand-weeded and hoed 2 ft. circle around each plant
25Mar2006	Fertilized each plant with 9 lbs./acre 34-0-0 (10 g/plant) according to recommendations from soil analysis at Mississippi State Soil Testing Lab Hoed weeds along outside rows and hoed tall weeds along plot perimeter
29Mar2006	Applied pesticide as drench mixture per plant to crowns for each of the following treatment groups labeled same as the fall application: 1. 1 L water 2. 1 L 0.1 lb. ai / acre Capture® 2EC (FMC) fall and spring 3. 1 L water 4. 180,000 *S. feltiae* in 1.5 L water / plant spring application 5. 1 L water 6. 1 L 0.067 lb. ai / acre E2Y45-215 (Dupont®) fall and spring 7. control treatment with 1 L water Drenches were mixed in 5 gal. containers and applied by dipping and pouring on the crown using a plastic bottle marked and cut off at the specified level.

4Apr2006	Due to red marginal leaf discoloration symptomatic of nutrient deficiencies, 1/3 cup 13-13-13 was applied to the base of each plant
5Apr2006	Irrigated furrows and watered plants with a bucket
15-16Apr2006	Mowed between rows and along plot perimeter
18Apr2006	Irrigated furrows and watered plants with a bucket Built 6 ft. 7-wire electric fence around plot with 4V solar generator
20Apr2006	Sprayed glyphosate along rows outside 2 ft. circles
21Apr2006	Irrigated furrows and watered plants with a bucket
25Apr2006	Irrigated furrows and watered plants with a bucket
28Apr2006	Irrigated furrows and watered plants with a bucket
30Apr2006	Harvested
3Jun2006	Harvested Irrigated furrows and watered plants with a bucket
4Jun2006	Mowed between rows and along plot perimeter
5Jun2006	Harvested Irrigated furrows and watered plants with a bucket
6-7Jun2006	Hand-weeded and hoed 2 ft. circles around plants
7Jun2006	Irrigated furrows and watered plants with a bucket
8Jun2006	Irrigated furrows and watered plants with a bucket
9Jun2006	Harvested
11Jun2006	Harvested Irrigated furrows and watered plants with a bucket
13Jun2006	Harvested Irrigated furrows and watered plants with a bucket
14Jun2006	Harvested

15Jun2006	Irrigated furrows and watered plants with a bucket
16Jun2006	Harvested Irrigated furrows and watered plants with a bucket
18Jun2006	Pruned canes to 3 ft. height Began mowing plot weeds
19Jun2006	Raked plot and discarded prunings
20Jun2006	Harvested
21Jun2006	Sprayed glyphosate along rows outside 2 ft. circles, under fence wires and along irrigation pipe
22Jun2006	Pruned diseased and damaged canes Harvested
23Jun2006	Harvested
25Jun2006	Irrigated furrows and watered plants with a bucket
26Jun2006	Mowed weeds along outside rows and in furrows
27Jun2006	Mowed between rows and along plot perimeter Irrigated furrows and watered plants with a bucket
29Jun2006	Irrigated furrows and watered plants with a bucket
1Jul2006	Harvested
3Jul2006	Irrigated plants with a bucket
9Jul2006	Irrigated plants with a bucket
10Jul2006	Cut canes at the base with long-handled loppers
11Jul2006	Removed primary crowns (largest crown from each planting with 2nd year growth) from plot soil along south row, brushed the soil from crowns individually onto 2ft. X 2ft. screen table, then labeled and enclosed them in black plastic bags Crushed all soil clumps larger than 1 in. diameter with brush handle, and brushed small particles through mesh

	Recorded all insects on screen Repeated screening procedure for 0.5 L soil sample from beneath each crown
12Jul2006	Repeated crown removal and soil screening procedures for plants along the plot north row
13Jul2006	Cut each crown into 2-3 in. cross sections with a reciprocating saw Observed tunneling and collected a larva within a crown tunnel Observed 3 crowns with crown gall symptoms Sample submitted to Plant Pathology Lab was identified as *Agrobacterium tumefaciens*

APPENDIX B

CALCULATIONS

Field pesticide applications

15 ft. between rows X 4 ft. along rows = 60
43,560 ft.^{2} / acre ÷ 60 = 726 plants / acre
98 experimental treatment plants / 726 plants per acre =
0.1349 acres treated
0.1349 acres treated / 98 plants = 0.0013765 acres / plant

0.0013765 acres X 200 gal. pesticide solution = 0.275 gal. ≈
1 L pesticide solution / plant

28 plants for each treatment in the fall =
28 L pesticide solution for fall treatments =
2 X 14 L / 5 gal. bucket
14 plants for each treatment in the spring =
14 L pesticide solution for spring treatments

E2Y45 35% ai formulation
0.067 lbs. ai / acre E2Y45 X 454 g / lb. = 30.418 g ai / acre
30.418 g ai / acre X 0.0013765 acres / plant = 0.04187 g ai. / plant
0.04187 g ai ÷ 0.35 ai formulation = 0.11963 g formulation / plant
0.11963 g formulation / plant X 14 = 1.675 g formulation / bucket

Bifenthrin 25.1% ai formulation
0.1 lbs. ai / acre bifenthrin X 454 g / lb. = 45.4 g ai. / acre
45.4 g ai / acre X 0.0013765 acres / plant = 0.062493 g ai. / plant
0.062493 g ai / plant ÷ 0.251 ai formulation =
0.24898 g formulation / plant
0.24898 g formulation / plant X 14 = 3.486 g formulation / bucket

S. feltiae nematode concentrations are counted using method
from Dr. Lawrence, (p. 24 above)
120,000 / plant X 14 treatments / bucket = 1,680,000 nematodes / bucket

Fertilizer

98 nursery stock + 24 infested transplants = 122 new plantings
(122 × ½ cup) + (122 × ½ cup) + (122 × 1 cup) = 244 cups
1 cup 13-13-13 granular slow release fertilizer weighs 195 g or 0.429 lbs.
244 × 0.429 lbs = 64.676 lbs.

Nematode pesticide exposure treatment groups

Captan

4 lbs. / acre Captan 50 WP dissolved in 50 gal. spray solution
4 lbs. / 417 lbs. water X 0.489 = 0.00469 or 4,690 ppm captan
Make 2 flasks for each treatment group, for 2 nematode species
4,690 ppm captan
469 ppm captan
46.9 ppm captan

Pyraclostrobin + boscalid

23 oz. 12.8% pyraclostrobin + 25.2% boscalid Pristine® / @50 gal. spray / A
23/6400 X 0.128 = 0.000460 or 460 ppm pyraclostrobin
23/6400 X 0.252 = 0.000906 or 906 ppm boscalid
Make 2 flasks for each treatment group, for 2 nematode species
460 ppm pyraclostrobin + 906 ppm boscalid
46 ppm pyraclostrobin + 90.6 ppm boscalid
4.6 ppm pyraclostrobin + 9.06 ppm boscalid

Calcium polysulfide

6 gal. 29% Calcium polysulfide / 100 gal. spray, according to product label
0.06 X 0.29 = 0.0174 or 17,400 ppm recommended spray
Make 2 flasks for each treatment group, for 2 nematode species
17,400 ppm calcium polysulfide
1,740 ppm calcium polysulfide
174 ppm calcium polysulfide

Acetamiprid

1.1 oz. Assail® 70% acetamiprid/ @50 gal. spray / A
1.1/6400 X 0.7 = 0.000120 or 120 ppm acetamiprid
Make 2 flasks for each treatment group, for 2 nematode species
120 ppm acetamiprid
12 ppm acetamiprid
1.2 ppm acetamiprid