SOUNDS AND SMELLS OF A SUBTERRANEAN SESIID: ACOUSTIC DETECTION AND MATING DISRUPTION OF GRAPE ROOT BORER

By
WILLIAM RYAN SANDERS

A THESIS PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE
UNIVERSITY OF FLORIDA
2010
To Patato
ACKNOWLEDGMENTS

I would like to thank Oscar Liburd for the opportunity of completing my Masters thesis in his laboratory. I would like to thank Richard Mankin for giving my idea a chance, sticking with it, and for all his input throughout the writing process. I would also like to thank Lukasz Stelinski for his open mind, ideas, patience, and input. I would also like to thank his lab, especially Wendy Meyer, and the entire Small fruit and vegetable IPM lab at the University of Florida in Gainesville. Their input and advice has been instrumental to the success of my program. I would like to thank the Florida Grape Growers Association for their financial support early on in my project and SARE for financial support throughout the rest of my program. I would like to thank all the grape growers that gave their time and allowed the use of their land for our experiments. This work could not have been done without their support, especially Bob Paulish, John Sirvent, Antonio Fiorelli, and Terry McKnight. Finally, I would like to thank Joshua Betz. He was a tireless and invaluable help with data analysis. Finally, I would like to thank Simone Harbas for her support and encouragement.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>4</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>8</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>9</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>10</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1 LITERATURE REVIEW</td>
<td>12</td>
</tr>
<tr>
<td>2 INVESTIGATION OF GRB ANTENNAE AND SENSILLA USING SCANNING ELECTRON MICROSCOPY</td>
<td>30</td>
</tr>
</tbody>
</table>

## 1 LITERATURE REVIEW

- Introduction ........................................................................................................ 12
- Literature Review .................................................................................................. 13
  - Description and Life Cycle ............................................................................. 13
    - Reproduction ................................................................................................. 15
    - Egg ............................................................................................................... 16
    - Larva ............................................................................................................. 17
    - Pupa .............................................................................................................. 18
    - Adult .............................................................................................................. 19
- Damage .................................................................................................................. 19
- Management .......................................................................................................... 20
  - Monitoring ........................................................................................................... 20
  - Weed control ....................................................................................................... 20
  - Mounding ........................................................................................................... 21
- Biological control ................................................................................................. 22
  - Chemical control: Lorsban® ............................................................................. 22
  - Chemical control: attract-and-kill .................................................................. 23
  - Mating disruption ............................................................................................... 24
- Justification ......................................................................................................... 27
- Goal and Hypotheses ............................................................................................ 28
- Specific Objectives ............................................................................................... 28

## 2 INVESTIGATION OF GRB ANTENNAE AND SENSILLA USING SCANNING ELECTRON MICROSCOPY

- Introduction ........................................................................................................... 30
- Materials and Methods ............................................................................................ 31
  - Insects ................................................................................................................. 31
- Scanning Electron Microscopy ................................................................................ 31
- Results and Discussion ............................................................................................ 32
  - Morphology of Antennae ..................................................................................... 32
    - Scape and pedicel ............................................................................................... 32
    - Trichoid rich region ......................................................................................... 33
3 ACOUSTIC DETECTION OF GRAPE ROOT BORER ........................................... 46

Introduction ............................................................................................................. 46
Materials and Methods............................................................................................ 48
   Acoustic Instruments, Signal Recording, and Soil Sampling Procedures .......... 48
   Listener Assessment of Infestation Likelihood .................................................. 49
   Digital Signal Processing and Classification ..................................................... 49
Results .................................................................................................................... 50
   Spectral Profiles ............................................................................................... 51
   Insect Sound-Impulse Bursts ............................................................................ 52
   Assessments of Infestation Likelihood ............................................................. 53
Discussion .............................................................................................................. 53

4 PHEROMONE ATTRACTIVENESS AND MATING DISRUPTION STUDIES ........ 60

Introduction ............................................................................................................. 60
   Grape Root Borer ............................................................................................. 60
   Chemical Control .............................................................................................. 61
   Mating Disruption ............................................................................................. 62
Materials and Methods............................................................................................ 65
   Chemicals and Dispensers ............................................................................... 65
   Experimental Sites ........................................................................................... 65
      Citra ........................................................................................................... 65
      Lithia .......................................................................................................... 66
      Bradenton .................................................................................................. 66
   Pheromone Attraction Experiments .................................................................. 66
      Determining the relative attractiveness of pheromone blends with trapping ................................................................. 66
      Direct observation of male GRB response to pheromone blends in the field ................................................................................................................................. 67
      Determining the relative attractiveness of SPLAT vs. septa ....................... 68
   Pheromone Disruption Experiments ................................................................ 69
      Determining the effects of pheromone blend on disruption efficacy .......... 69
      Determining the effects of dispenser density on disruption efficacy ....... 70
      Determining the effect of pheromone point source aggregation on GRB disruption ................................................................................................................................. 70
Determining load rate of pheromone in dispensers for optimal disruption ................................................................. 71
Assessing effect of orientation disruption on next season’s GRB population .............................................................................................................................. 72
Quantification of pheromone release rate from dispensers used in mating disruption investigations ......................................................................................................................... 73

Results ............................................................................................................................................................... 74
Pheromone Attraction Experiments .................................................................................................................. 74
  Determining the relative attractiveness of pheromone blends with trapping ............................................................. 74
  Direct observation of male GRB response to pheromone blends in the field .............................................................. 75
  Determining the relative attractiveness of splat vs. septa .................................................................................. 76

Pheromone Disruption Experiments ............................................................................................................... 76
  Determining the effects of pheromone blend on disruption efficacy ................................................................. 76
  Determining the effects of dispenser density on disruption efficacy ............................................................. 76
  Determining the effect of pheromone point source aggregation on GRB disruption .................................................. 77
  Determining load rate of pheromone in dispensers for optimal disruption ............................................................. 78

Assessing effect of orientation disruption on next season’s GRB population ................................................................. 78
Quantification of pheromone release rate from dispensers used in mating disruption investigations ................................................................. 79

Discussion .......................................................................................................................................................... 80

LIST OF REFERENCES ........................................................................................................................................ 95

BIOGRAPHICAL SKETCH ..................................................................................................................................... 101
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-1</td>
<td>Numbers of invertebrates recovered from root systems, listener assessments of infestation likelihood, and rates of $s_{\text{highdB}}$, $s_{\text{middB}}$, and $s_{\text{lowdB}}$ trains and bursts at sites where $s_{\text{lowdB}}$ bursts were detected, arranged in order of the rates of $s_{\text{lowdB}}$ bursts.</td>
<td>56</td>
</tr>
<tr>
<td>3-2</td>
<td>Numbers of invertebrates recovered from root systems, listener assessments of infestation likelihood, and rates of $s_{\text{highdB}}$, $s_{\text{middB}}$, and $s_{\text{lowdB}}$ trains and bursts at sites where $s_{\text{lowdB}}$ bursts were not detected, arranged in order of the summed rates of rates of $s_{\text{highdB}}$ and $s_{\text{middB}}$ bursts.</td>
<td>57</td>
</tr>
<tr>
<td>3-3</td>
<td>Listener assessments of recording sites determined by excavation to be uninfested or infested.</td>
<td>58</td>
</tr>
<tr>
<td>3-4</td>
<td>Computer assessment of recording sites determined by excavation to be uninfested or infested.</td>
<td>58</td>
</tr>
<tr>
<td>4-1</td>
<td>Effect of pheromone blend on season mean GRB catch in 2007.</td>
<td>86</td>
</tr>
<tr>
<td>4-2</td>
<td>Effect of dispenser type on mean GRB catch per trap in 2009.</td>
<td>88</td>
</tr>
<tr>
<td>4-3</td>
<td>Effect of GRB and LM pheromones on season mean catch per trap of GRB males and orientation disruption in 2008.</td>
<td>88</td>
</tr>
<tr>
<td>4-4</td>
<td>Effect of pheromone point source density on season mean catch per trap of GRB males and orientation disruption in 2008.</td>
<td>88</td>
</tr>
<tr>
<td>4-5</td>
<td>Effect of pheromone point source aggregation on season mean catch per trap of GRB male and orientation disruption in 2009.</td>
<td>89</td>
</tr>
<tr>
<td>4-6</td>
<td>Effect of load rate on season mean catch per trap and % orientation disruption in 2009.</td>
<td>90</td>
</tr>
<tr>
<td>4-7</td>
<td>Mean catch per trap for all control traps in both vineyards for both years of mating disruption experiments.</td>
<td>91</td>
</tr>
<tr>
<td>4-8</td>
<td>Results of all disruption experiments.</td>
<td>93</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Scape and pedicel of antenna of <em>Vitacea polistiformis</em></td>
<td>41</td>
</tr>
<tr>
<td>2-2</td>
<td>Trichoid rich region of antenna of <em>Vitacea polistiformis</em></td>
<td>42</td>
</tr>
<tr>
<td>2-3</td>
<td>Sensilla auricillica and type-1 basiconica of <em>Vitacea polistiformis</em></td>
<td>43</td>
</tr>
<tr>
<td>2-4</td>
<td>Types 1-3 s. basiconica &amp; type-1 s. coeloconica of <em>Vitacea polistiformis</em></td>
<td>44</td>
</tr>
<tr>
<td>2-5</td>
<td>Apical region of antenna of <em>Vitacea polistiformis</em></td>
<td>45</td>
</tr>
<tr>
<td>3-1</td>
<td>Spectral profiles of insect-produced sound impulses</td>
<td>59</td>
</tr>
<tr>
<td>4-1</td>
<td>Effect of pheromone blend on weekly mean GRB male catch in 2007</td>
<td>86</td>
</tr>
<tr>
<td>4-2</td>
<td>Effect of pheromone type on mean number of visits per observation session 2007</td>
<td>87</td>
</tr>
<tr>
<td>4-3</td>
<td>Effect of pheromone blend on mean log of visit duration per observation session in 2007</td>
<td>87</td>
</tr>
<tr>
<td>4-4</td>
<td>Effect of dispenser type on weekly mean GRB catch per trap in 2009</td>
<td>88</td>
</tr>
<tr>
<td>4-5</td>
<td>Effect of pheromone point source density on weekly mean catch per trap of GRB males in 2008</td>
<td>89</td>
</tr>
<tr>
<td>4-6</td>
<td>Effect of pheromone point source aggregation on weekly mean catch per trap of GRB male in 2009</td>
<td>90</td>
</tr>
<tr>
<td>4-8</td>
<td>Mean catch per trap of untreated plots for all experiments in 2008</td>
<td>92</td>
</tr>
<tr>
<td>4-9</td>
<td>Mean catch per trap of control plots for all experiments in 2009</td>
<td>92</td>
</tr>
<tr>
<td>4-10</td>
<td>Release rate of GRB pheromone from 1g SPLAT dispensers in 2008</td>
<td>93</td>
</tr>
<tr>
<td>4-11</td>
<td>Expected effect of increasing dispenser density on orientation disruption</td>
<td>94</td>
</tr>
<tr>
<td>4-12</td>
<td>Effect of increasing dispenser density on orientation disruption</td>
<td>94</td>
</tr>
</tbody>
</table>
The antennae of sensilla of male and female grape root borer (GRB) were investigated using scanning electron microscopy. Antennae were found to be comprised of four separate regions. Regions differed by segment shape, size, number of rami, and the types and relative numbers of sensilla found. Analogous versions of each region were found in male and female antennae. Eleven different types of sensilla were found, ten of which were found on female antennae.

Acoustic recordings were evaluated as potential tests for arthropod infestation as a means for improving chemical and cultural controls for GRB. Acoustic samples allowed human listeners and computer software to reliably distinguish whether a given site was infested or uninfested. More testing is needed to determine if acoustic sampling will improve control methods enough to justify the cost of sampling.

Several studies were conducted assessing the effect of pheromone blend, dispenser density, load rate, and dispenser aggregation on mating disruption of GRB. GRB and leopard moth sex pheromones were found to be attractive to GRB. Increasing dispenser density improves percent orientation disruption, but in a non-linear fashion as
predicted by the competitive attraction model. Increasing load rate was found to increase the effect of mating disruption. The reason is likely that higher load rates allow for disruption for longer periods of time as release rate from Specialized Pheromone and Lure Application Technology (SPLAT) dispensers tested was linear (~77µg/day). Dispenser aggregation decreased disruption; orientation disruption increased as area between dispensers equalized. It was found that competitive attraction is the most likely mechanism for mating disruption of GRB.
CHAPTER 1
LITERATURE REVIEW

Introduction

Grape root borer (GRB), *Vitacea polistiformis* Harris, was first reported attacking cultivated grapes in 1854 (Bambara and Neunzig 1977). Since then, GRB has become a widespread pest of grapes in the Eastern United States. Not much attention was paid to GRB until recently because it is not a problem in California, which produces 88% of all grapes in the U.S. Grape root borer is one of the most destructive pests of grapes in North (Pearson and Schal 1999) and South Carolina (Pollet 1975). It is considered the key grape pest in Georgia (Weihman 2005) and in Florida (Liburd and Seferina 2004). As the amount of grape acreage increased over the past several years in Florida (Weihman 2005), GRB has become an increasingly pressing problem. High infestations of GRB result in vine death while lower infestations weaken plants and reduce yields (Dutcher and All 1979). Grape root borer feed on grape roots, reducing vine vigor and cold tolerance, increasing susceptibility to pathogens and drought, and hastening vine death (Pearson and Meyer 1996). Grape root borer damage has resulted in enormous losses to the commercial grape industry. Entire vineyards have been destroyed in Florida and GRB was cited as the cause of total cessation of grape production in South Carolina (Pollet 1975).

Grape root borer adults resemble paper wasps which are frequently seen in vineyards. Therefore, GRB may infest a vineyard and remain unnoticed until symptoms become severe. The larval stage lives underground, inside the grape roots, making the pest difficult to detect for most of the year. Emerging adults, however, leave their pupal casings visibly above the soil surface. Monitoring for GRB can be done effectively using
multicolored bucket traps baited with female sex pheromone (Roubos and Liburd submitted).

Currently, chlorpyrifos (Lorsban®) is the only registered pesticides for GRB control (Turner et al. 2006). Lorsban® is an unacceptable method in North Carolina due to soil type, labor cost and pre-harvest interval (Pearson and Meyer 1996). Lorsban® is also not ideal for control in Florida due to its restrictions and conflicts with harvesting dates (Weihman 2005, Weihman and Liburd 2006). In addition, Lorsban® is suspected of being carcinogenic and its future use is not guaranteed (Weihman and Liburd 2006). Some alternative methods have been researched, including attract-and-kill and mating disruption, which have demonstrated potential (Weihman and Liburd 2006).

**Literature Review**

**Description and Life Cycle**

Grape root borer is a day flying clearwing moth with brown scales on the forewing. The abdomen has characteristic yellow to orange bands, which give it the appearance of a paper wasp (*Polistes*) that is commonly found in southeastern vineyards. Males are smaller than females and have four tufts of scales originating from the terminal segment of the abdomen, which may serve to dispense pheromone. Clark and Enns (1964) estimate the life cycle of GRB at two years while Sarai (1972) reported a life cycle of 3 years. Both of these estimates were based on differing sizes of larvae found in grape roots early and late in the season. Webb et al. (1992) found that GRB could fully complete their life cycle in one year in container grown vines in Florida.

These moths are native to the eastern United States. Grape root borer has been found as far north as southwestern Michigan and as far south as Miami, Florida (Webb et al. 1992). Grape root borer inhabits every eastern coastal state and has been found
as far west as Missouri (Clark and Enns 1964) and Arkansas (USDA 2003). Significant populations have been reported from areas in Ohio (Alm et al. 1989) and Pennsylvania (Jubb 1982), though generally population densities farther north are lower than in the south.

In Florida, adults typically fly, depending on geographical location, from mid June through October (Webb et al. 1992). Adults have been caught in pheromone traps as late as January in Miami, FL with significant flight activity reported to last through December (Webb et al. 1992). Peak emergence ranges from August to October throughout Florida, with peaks occurring later farther south (Weihman and Liburd 2007). Emergence periods vary drastically from 28 days (Clark and Enns 1964) to six months (Webb et al. 1992). Peak emergence also varies, and can consist of a single or bimodal peak. Latitude seems to play a role in length of emergence period with states farther north having shorter emergence periods and southern areas having longer ones (Clark and Enns 1964, Dutcher and All 1978a, Webb et al. 1992). Emergence may be affected by rainfall with dry seasons resulting in a shorter emergence period and wetter years having longer emergence periods (Clark and Enns 1964, Webb et al. 1992). In Georgia, GRB emergence has been linked to sugar concentration in the grapes (Dutcher and All 1978b). Webb et al. (1992) suggests that in Florida, the major factors influencing emergence are soil temperature, moisture, oxygen, and actively growing roots. Plant cues may also affect the emergence of GRB. For instance, Weihman and Liburd (2007) found that when grape harvest occurred two weeks later than the previous year GRB peak emergence also occurred two weeks later, though the causes are unclear.
Reproduction

Adult females attract males from long distances using a female-specific sex pheromone blend of two major components. The primary component, which comprises 99% of the blend, is \((E,Z)-2,13\text{-octadecadien-1-ol acetate}\). The secondary component is \((Z,Z)-3,13\text{-octadecadien-1-ol acetate}\). The female will perch on a branch or leaf, not necessarily the grape vine, to begin calling. The virgin female calls by lifting her abdomen and exposing her pheromone gland, which excretes the pheromone complex (Pearson and Meyer, 1996). Males detecting the pheromone plume will fly upwind sweeping the air, maintaining contact with the pheromone plume. Upon finding the calling female, males exhibit a stereotypical behavior sequence. The male approaches the female head-on, touches antennae with her, and then begins hovering over her abdomen. The male, still flying, thrusts with his genitalia until coupled. Only after coupling successfully will the male land. Mating duration varies and has been observed to last as little as 45 min or as long as 4 h and takes place between 11:30 am and 4:00 pm (Clark and Enns 1964), with female calling likely initiating at least a half an hour prior to observed mating (Dutcher and All 1978a). Mating can occur during the first calling period after eclosion and has been observed occurring with virgin females 30 minutes after call initiation (Dutcher and All 1978a). Though females typically mate once, they occasionally mate with more than one male. The second mating can take place one directly after another or after a period of oviposition. It is not known whether males mate with more than one female (Clark and Enns 1964).

There are several conflicting reports with regard to the timing of oviposition. According to Dutcher and All (1978a), oviposition begins between 8 and 9 am the day after mating and occurs only during the day for up to 8 consecutive days. This
preovipositional period is atypical of sesiids, which usually initiate oviposition immediately after mating. The typical sesiid ovipositional behavior was observed in GRB by Clark and Enns (1964). During that study, oviposition took place during the day and lasted from 3 to 14 days. Females oviposit approximately half of their eggs within 24 hours of mating (Clark and Enns 1964, Dutcher and All 1978a).

There does not appear to be a preferred oviposition site. Females will lay their eggs on the trunk of the grape vine, on low lying grape leaves and branches, on weeds near the base of the grape plant, and on the soil itself. The eggs are attached with a weak adhesive secretion, and may become dislodged by rain and wind. After laying an egg the female will fly to another plant, sometimes a considerable distance away from the initial oviposition site, before laying an additional egg. Fecundity varies, though females rarely lay more than 500 eggs (Clark and Enns 1964).

**Egg**

Grape root borer eggs are elliptical in shape with one truncated end and a longitudinal sulcus running along the dorsum. They are usually medium to dark brown in color and average 1.05 mm in length and 0.75 mm in width (Bambara and Neunzig 1977). Eggs kept under laboratory conditions required 13 to 22 days to hatch (Clark and Enns 1964) with an average incubation period of 18.2 days (Dutcher and All 1978a). Under laboratory conditions, 70 to 85% of eggs successfully hatch (Clark and Enns 1964), though percent hatch is probably lower under field conditions due to predation, pathogens, and precipitation. As soon as the egg hatches, the first instar larva drops to the ground and burrows into the soil to begin feeding.
Larva

The larva is the damaging stage of the pest. The majority of feeding occurs on the inner bark, though at times all tissues within the outer bark have been consumed. Borers have occasionally been found burrowed deep within the crown of the vine. Larvae appear to feed on the roots they first encounter and do not choose younger more tender roots. Larvae do not appear to move from plant to plant, or even from root to root. If their feeding kills the grape plant, the larvae die along with it (Clark and Enns 1964). Most larvae are found within the topsoil between five and 20 cm deep, though some have been found as deep as 80 cm. First instar mortality is high, with only 1.5 to 2.7% of larvae surviving to infest grape roots. Abiotic factors, including insufficient soil moisture and depth of grape roots, appear to be the main cause of mortality (Dutcher and All 1978c).

First instar larvae are whitish in color and average 2.39 mm in length and 0.42 mm in width. They have circular spiracles and relatively long setae. Head width averages 0.39 mm and the mouthparts are prognathous, but change to hypognathous orientation after feeding. Unlike the body, the head is evenly pigmented and pale brown with black ocelli (Bambara and Neunzig 1977). Late instar larvae vary from 25 to 35 mm in length (averaging 29 mm) and 4.7 to 10.5 mm in width. There is no significant change in coloration. Head width averages 3.6 mm at this stage (Barbara and Neunzig 1977).

There are differing accounts of how long it takes for a larva to reach full size. Most studies assume the two-year life cycle described by Clark and Enns (1964). It is possible that the one year life cycle found by Webb et al. (1992) is specific to Florida or certain regions within Florida. It is also possible that it is an artifact of artificial conditions of the Webb et al. (1992) study. If GRB experiences a one year life cycle, one would
expect that successful mating disruption or attract and kill experiments performed one season would decrease the emerging populations of the succeeding season. In a recent trap evaluation experiment, Weihman and Liburd (2007), placed pheromone baited traps containing an insecticide strip in 16 vineyards throughout Florida. In northern vineyards, these traps caught and killed a mean of approximately 120 males during peak emergence in 2003. In the next season, traps in the same vineyards caught a mean of about 60 males during peak emergence. It is possible that GRB have a one year life cycle in Florida and that the number of males caught and killed in the first season (over 600 per trap on average) resulted in a lower population the following year, though it is also possible that other factors may be responsible for the reduction in population density. During the winter, GRB larvae undergo obligate diapause. It has been reported, however, that GRB may become active during the winter if soil is sufficiently warm (All et al. 1987).

**Pupa**

In early summer, sufficiently grown fourth instar larvae begin pupation. Pupation requires 33 to 44 days (Clark and Enns 1964, Sarai 1972). Pupae may remain in their burrows in the crown of the vine or may move to within the top five cm of topsoil to begin pupation. Their pupal case is usually attached to a grape root and is composed of silk, frass, and/or soil. A majority of pupal casings are found within a 30 cm (1 ft.) radius of the crown (Clark and Enns 1964). According to Townsend (1980), 92% of pupal casings are found within 35 cm of the trunk. Pupae average 19 mm in length and 5.4 mm in width. They vary in color from almost yellowish to pale brown and become darker over time. Near eclosion, they are very dark brown.
**Adult**

When the pharate adult is fully formed, it wriggles \( \frac{3}{4} \) of the way out of the pupal case through the use of abdominal spikes and emerges vertically halfway above the soil. The pupal case is then split in two and the adult will fully emerge and climb onto the nearest substrate to dry its wings. Adults live an average of 7.4 days after emerging, though some females have been observed to live and oviposit up to twice that long (Clark and Enns 1964). Adults do not feed. Instead, they spend their time and energy finding mates and reproducing.

**Damage**

Grape root borer feeding is characterized by gouge-like wounds that extend longitudinally along peripheral roots and vertically on the trunk base. As larvae mature, wounds generally reach 1.3 to 1.4 mm in diameter. These wounds can cause girdling of larger roots, cutting off nutrients and water transportation from roots to the rest of the plant. Smaller roots can be completely destroyed by feeding. A single larva is capable of causing 6% girdling of the vine trunk resulting in 47% reduction in yield. Two or three larvae are capable of killing the entire vine (Dutcher and All 1979). Outward signs of damage are not evident until the season after the damage occurs. Damaged vines require several seasons to completely repair themselves. Damage caused by GRB results in reduction of vine vigor. Specifically, average leaf area, berry and cane yields are significantly decreased. In addition, wounds in the roots make the plant more susceptible to freeze damage, drought, and pathogens (Dutcher and All 1979).

Grape root borer attacks European grapes, *V. vinifera*, muscadine grapes, *Vitis rotundifolia*, bunch grapes, *V. labrusca*, and hybrid bunch grapes, *Euvitis* spp. In addition, GRB are capable of completing their life cycle on wild grapes. When
contemplating control methods, it is important to consider areas with wild grapes as a reservoir for the pest (Weihman 2005).

Management

Monitoring

Digging up grape roots to look for GRB larvae can damage vines. Therefore, monitoring for adults is the only viable way to determine if a vineyard has a GRB population. Monitoring for adults is done primarily through pheromone trapping. It is possible to assess populations by counting pupal casings, but this is time consuming and labor intensive. According to Townsend and Micinski (1981), the sex ratio over the season is approximately 1:1. Therefore, even though traps baited with the female sex pheromone will only capture males, the method may still give an idea of population size and potential for reproduction. In the past, both wing traps and bucket traps have been baited with female pheromone to monitor GRB populations, but a recent study showed that green bucket traps are superior in many respects, including long term cost effectiveness, to sticky traps for this purpose (Weihman and Liburd 2007). More recently, Roubos and Liburd (submitted) showed that a multicolored bucket trap is more effective in capturing male GRB than green bucket traps.

Weed control

Weeds provide a substrate for oviposition and are preferred as mating sites (Sarai 1972). Weeds also create a humid microclimate for first instar larvae, and provide cover for newly hatched larvae as well as newly eclosed adults. By providing humidity and cover from predators, weeds help to counter the two main causes of pre-feeding mortality. Vineyards should therefore be kept weed free.
Mounding

When GRB larvae are ready to pupate, they usually migrate to within the top 5 cm of topsoil to form their pupal case. From this depth, pharate adults are able to ascend out of the soil. By building a layer of soil around the base of the grape plant after larvae have already migrated, the distance the pharate adult must cover to eclose is much greater. As this length increases, mortality increases. Sarai (1969) found 100% mortality when the mounds were 19 cm high. Any larvae that begin migrating after the mound has been built are not affected. They are able to burrow to within the top 5 cm of the mound and pupate successfully. Mounding must be performed just before peak emergence to maximize mortality. Since 92% of pupal casings are found within 35 cm of the trunk, the mound should extend to a 50 cm radius around the plant (Dutcher and All 1979). All et al. (1985) reported 83% control with mounding. They also report that in addition to preventing emerging adults, mounding can increase first instar mortality by effectively increasing root depth. However, to maximize pupal mortality, the mound must be placed after pupation, not before hatching. Mounding has also been shown to decrease the need for herbicide application (Kennedy et al. 1979).

Mounding may not be a useful control method in Florida. Grape roots would grow into the mound quickly because muscadine varieties, the most common in Florida, grow rapidly and have shallow root systems. Additionally, emergence occurs bimodally in some regions. In these regions, mounding would need to be done twice or only part of the population would be controlled. In such areas, mounding can become cost prohibitive.
**Biological control**

There have been several studies assessing different methods of biological control. The most promising research includes nematodes and fungal pathogens, as these methods have the potential to be developed into a product for commercial use. Clark and Enns (1964) found that *Beauveria bassiana* (Balsamo) Vuillemen and *Metarrhizium anisopliae* (Metchnikoff) Sorokin were occasionally responsible for larval and pupal mortality, resulting in desiccated chalky-white larvae and pupae.

Williams et al. (2002) found that several nematode species are effective against grape root borer. The two species that showed the most promise were *Heterorhabditis bacteriophora* (GPS11 strain) and *H. zealandica*. They reported that *H. zealandica* caused 93% mortality at high concentrations (60,000 per plant) under greenhouse conditions. Only a small area, within 50 cm of the vine trunk, needs to be treated at such high doses; therefore, the treatment is economically viable. These nematodes are applied topically, but are able to move through the soil, find GRB larvae, and infect them.

**Chemical control: Lorsban®**

According to Dutcher and All (1979), the economic injury level of GRB is extremely low (0.074 larvae per vine) meaning that insecticide control measures should be used as soon as GRB are detected. Currently, only one chemical, chlorpyrifos, is registered for GRB control (Turner et al. 2006). Lorsban®, the commercially available form of chlorpyrifos, is an acetylcholinesterase inhibitor and is applied as a soil drench to prevent newly hatched first instar larvae from reaching grape roots. Adlerz (1984) reported that Lorsban® is effective against GRB in muscadines but not as effective in bunch grapes. The root structures of these grape genera differ, with muscadine roots
being shallower. This may allow some roots to be within the thin layer of soil that retains Lorsban®, protecting them from infestation. In Florida, most grapes grown are muscadine, so Lorsban® has some control potential.

There are problems with Lorsban® use, however. Lorsban® is only permitted to be used once per season and only maintains toxicity in the soil for four weeks. The emergence period in Florida is four to six months and often has bimodal peaks; therefore application of Lorsban® in Florida can only control part of the population. In addition, Lorsban® is limited to use 35 days pre-harvest. In many parts of Florida, peak emergence coincides with harvest; therefore Lorsban® cannot be used when it would be most effective. Weihman and Liburd (2007) recommend applying Lorsban® after harvest to maximize mortality in both bunch and muscadine grapes in Florida.

Finally, as an organophosphate, Lorsban®'s future use is not guaranteed. The Food Quality Protection Act (FQPA) of 1996 has resulted in the elimination or restriction of some broad-spectrum pesticides. It is unclear how the FQPA will affect Lorsban® use in the future, but it is necessary to find alternatives to Lorsban® in the mean time.

**Chemical control: attract-and-kill**

Attract-and-kill involves the placement droplets that contain female sex pheromone (attract) and insecticide (kill). Weihman and Liburd (2006) evaluated Attract-and-kill, but the results were inconclusive in part because the vineyards tested had small populations. In addition, the supplier, IPM Tech, provided them with the incorrect pheromone during the second year. Attract-and-kill remains an attractive alternative to Lorsban® application and merits further study.
Mating disruption

Mating disruption is a technique used to suppress the ability of male moths to find females. Inability to find mates results in low reproductive success rates and a potentially drastic reduction in population the succeeding season. Generally, the female sex pheromone is placed in dispensers which are then distributed throughout the vineyard. It is unclear which mechanism is mainly responsible for the success of mating disruption, though several possibilities have been offered. The main competing hypotheses are competitive attraction, desensitization, camouflage, and sensory imbalance. According to competitive attraction, males follow the pheromone plume produced by dispensers and when they locate the source are unable to mate and must search again. The desensitization hypothesis states that males become less sensitive or less responsive to female sex pheromone than they normally would be through habituation or adaptation. The camouflage hypothesis states that males are unable to find calling females because the guiding boundaries, which are necessary for anemotaxis, of their natural pheromone plumes are obscured by synthetic pheromone plumes. The sensory imbalance theory is defined as “disrupting mate-finding not via adaptation or habituation but by interfering with the male’s ability to perceive (as opposed to receive) the normal sensory inputs associated with their species’ sex pheromone” (Miller et al. 2006). Preliminary behavioral studies conducted in the field have suggested that the main mechanism in mating disruption for grape root borer is competitive attraction (Sanders unpublished data).

Mating disruption has been used with success in multiple crops with various lepidopteran pests. Codling moth, *Cydia pomonella* L., is the key pest in western U.S. pear and apple orchards and mating disruption has proved successful with the number
of hectares treated by mating disruption increasing from near zero in 1990 to nearly 40,000 in 2000 (Brunner et al. 2002). Mating disruption has also proven successful in vineyards with the vine moth, *Eupoecilia ambivella*, and the grape berry moth, *Lobesia botrana* in Germany. In more than 98% of vineyards implementing mating disruption in the Wuerttemberg region, attack damage did not exceed the economic threshold (Kast 2001, Louis and Schirra 2001).

Mating disruption has also proven successful in controlling several Sesiid pests; including peach tree borer, *Synanthedon exitiosa* (Say), lesser peach tree borer, *S. pictipes* (Grote and Robinson) and currant clearwing moth, *S. tipuliformis* (Clerk). In a field study testing mating disruption on lesser peach tree borer, Pfeiffer et al. (1991) reported complete trap shutdown in pheromone treated plots and a 19 to 97% reduction in population compared with control over three years as measured by pupal case counts. In this study, mating disruption outperformed pesticides recommended for lesser peach tree borer control and is recommended by the authors to replace pesticide treatments.

Johnson et al. (1991) report that mating disruption using either of the two main components of the GRB female sex pheromone reduced GRB population levels, as assessed by exposed pupal casings, the following season. They also report that mating disruption using the minor component, *(Z,Z)*-3,13-octadecadien-1-ol, was more effective than using the major component, *(E,Z)*-2-13-octadecadien-1-ol. Commonly, trap shutdown is used to assess the success of mating disruption treatments. It is presumed that if males cannot find traps (trap shut down) baited with the synthetic blend of the female sex pheromone they are unable to successfully follow a pheromone plume to a
female and consequently cannot mate with the same frequency. It is conceded that mating is still possible under these conditions, but it is unlikely and probably depends on population density (Weihman and Liburd 2006). Weihman and Liburd (2006) report successful trap shutdown using the sex pheromone of the Leopard moth, Zeuzera pyrina L., to control GRB. In behavioral studies conducted under field conditions, I have seen GRB males following a pheromone plume produced by dispensers releasing Leopard moth pheromone. It has also been shown that the synthetic form of the natural GRB sex pheromone has great potential for the control of GRB (Webb and Mortensen 1990). Currently, GRB sex pheromone is more expensive than the Leopard moth pheromone and commercially unavailable for use as mating disruptant.

There are several advantages to mating disruption. One of the major advantages of mating disruption is its’ specificity. Sex pheromones of one species may influence the behavior of closely related species but will not affect natural enemies that may help in controlling the pest population. Mating disruption can also be long lasting, depending on the dispenser type chosen, with one treatment lasting the entire season. It is also difficult for insects to develop resistance to mating disruption. Finally, the pheromones and dispensers used are non-toxic and some dispensers are naturally biodegradable.

Mating disruption also has disadvantages. It is only effective for low to moderate pest populations because as population increases, the chance of a random encounter and subsequent mating increases. Mating disruption can be quite costly, especially if the sex pheromone is difficult to synthesize. Another problem with mating disruption is that it does not kill the pest. Therefore, it is unable to control pest immigration. If females are mating outside of the treatment area and flying in to lay eggs, mating disruption can
not mitigate that. Mating disruption is not a stand alone method of control. If it is to be successful, it must be used in combination with other IPM strategies.

**Justification**

Grapes grown in Florida are used for fresh fruit, U-pick, jam, juice, and especially wine. Most grapes grown in Florida are destined to become wine. In 2003, the 13 wineries registered with Florida Grape Growers Association (FGGA) produced over $8,000,000 in wine sales (WineAmerica, 2009). The number of registered Florida wineries has increased from 13 to 17 since then (FGGA 2009), and since Florida is this country’s third largest wine consuming state (UGA 1997), demand will not be a limiting factor for further growth. In 2007, Florida was the 6th largest producer of wine; 1,667,618 gallons (WineAmerica, 2009). There are now 17 wineries registered with FGGA. In addition, as the green movement gains momentum and more consumers choose locally produced goods to minimize their carbon footprints, Florida grape growers will be able to satisfy consumers’ ecological concerns as well as their palettes. In Florida, the amount of land devoted to grape cultivation has steadily increased over the past several years and is now over 1,000 acres (Weihman 2005).

As the amount of grape acreage increased over the past several years in Florida (Weihman 2005), GRB has become an increasingly pressing problem. High infestations of GRB result in vine death while lower infestations weaken plants and reduce yields (Dutcher and All 1979). Grape root borer feed on grape roots, reducing vine vigor and cold tolerance, increasing susceptibility to pathogens and drought, and hastening vine death (Pearson and Meyer 1996). Grape root borer damage has resulted in enormous losses to the commercial grape industry. Entire vineyards have been destroyed in
Florida and GRB was cited as the cause of total cessation of grape production in South Carolina (Pollet 1975).

GRB represents the single largest obstacle to sustaining the rapid growth of Florida’s blossoming grape industry. Current control methods are too expensive, ineffective, and unreliable. Therefore, it is imperative that alternatives be explored.

Goal and Hypotheses

The overall goals of my research were to investigate alternative control methods for Grape root borer in Florida vineyards and to gain a better understanding of GRB as well as the mechanisms responsible for mating disruption. I hypothesized that researchers would be able to predict the presence or absence of arthropods in the root systems of grape vines using acoustic samples. I also hypothesized that Leopard moth pheromone would prove attractive to GRB and that competitive attraction was the mechanism responsible for mating disruption in our system. Following the competitive attraction model, I hypothesized that: 1) GRB pheromone would be more effective than LM pheromone as a mating disruptant and 2) that % orientation disruption would increased as pheromone point source density increased, point source aggregation decreased, and pheromone load rate increased.

Specific Objectives

- To investigate antennae and sensilla of GRB using scanning electron microscopy
- To evaluate acoustic detection as a tool for improving mounding
- To determine the relative attractiveness of GRB and Leopard moth pheromones through the use of trapping and behavioral observation studies
- To determine the most efficient approach to orientation disruption of GRB and investigate the mechanism(s) responsible:
- Determine the pheromone release rate from SPLAT dispensers
- Compare the orientation disruption efficacy of two pheromone blends
- Determine the most efficient dispenser density
- Determine lowest effective load rate
- Assess the effect of dispenser aggregation
CHAPTER 2
INVESTIGATION OF GRB ANTENNAE AND SENSILLA USING SCANNING ELECTRON MICROSCOPY

Introduction

An insect's ability to survive and reproduce is heavily dependent on gathering large amounts of information from its environment. Relevant information is received by many sense organs; mechanosensitive, chemosensitive, thermosensitive, and thigmosensitive. Chemical signals are detected by sensory neurons housed within sensilla found mainly on insect antennae and maxillary palpalae. Since the function of a sensillum can often be deduced from its structure (Keil 1997), cataloging and examining an insect's sensilla is an important first step towards a comprehensive understanding of an insect’s behavior. Moths, especially those with crepuscular or nocturnal habits, rely heavily on olfaction for finding food, mates and oviposition sites. The ultrastructure of pheromone sensitive sensilla has been studied numerous times, though the studies generally focus on the males of large moths like Bombyx mori (Steinbrecht and Müller 1991), Manduca sexta (Sanes and Hildebrand 1976), Antheraea polyphemus (Keil 1987) and A. pernyi (Keil 1987, Zimmerman 1991). While there have been some exceptions, like Shields and Hildebrand (1999 a&b), the olfactory systems of female moths have been largely ignored. This study is an attempt to catalog and describe all types of sensilla appearing on the antennae of male and female grape root borer, Vitacea politiformes Harris (Sesiidae), using light and scanning electron microscopy. The current results lay the foundation for future electrophysiological investigation to confirm the functions of individual antennal sensilla of V. politiformis.
Materials and Methods

Insects

Two female specimens of *Vitacea polistiformis* were received from the McGuire Center for Lepidoptera & Biodiversity of the Florida Museum of Natural History in Gainesville, Florida. The two females loaned were collected in 1985. One was in good condition with fully intact antennae, the second was in poor condition with only one antenna, which was missing the apical 3 segments. The males used (n=3) were caught at a pheromone baited trap from a vineyard in Citra, FL and preserved in 70% ethanol until they could be prepared for scanning electron microscopy (SEM).

Scanning Electron Microscopy

The heads of the insects were removed and the antennae were excised under 40 × magnification (Leica, Wild MC3 stereomicroscope, Heerbrugg, Switzerland) and subsequently agitated in distilled water for 2 min in an attempt to remove the scales. After 2 min, the distilled water was removed and replaced and agitation was repeated. Antennae were rinsed in this manner until the rinsing dish contained very few scales and subsequently kept in 70% ethanol for approximately 24 h. Antennae were then dehydrated in a graded series of 75, 80, 85, 90, and 99.9% ethanol:water (Onagbola et al. 2008); antennae were maintained for 1 h at each gradation. Thereafter, antennae were mounted on aluminum stubs with double-sided copper sticky tape and kept in a drying chamber (25 ± 1 °C, 10 ± 1% RH) for approximately 5 days. The antennae were sputter coated with gold/palladium (40:60) in a LADD SC-502 (Vermont, USA) high resolution sputter coater and subsequently examined with a Kevex® S-530 (Hitachi, Tokyo, Japan) SEM operated at 10, 15 or 20 kV.
Results and Discussion

Morphology of Antennae

Antennae of both sexes have four regions that vary drastically from one another in appearance and distribution of sensilla and are given here in order of occurrence from most proximal to most distal; the scape and pedicel, a region densely populated by large type-1 sensilla trichoidea (subsequently referred to as the trichoid rich region), a 'club-like' region with much broader antennomeres than other regions and devoid of type-1 s. trichoidea, and the apical three segments. Light microscopy revealed striking sexual dimorphism in the antennal structure, but this dimorphism was largely restricted to the trichoid rich region.

Male antennae were bipectinate (Figure 2B) while female antennae were mostly filiform (Figure 2A); both having the previously mentioned “club-like” region. All three female antennae measured, 9.8 to 10 mm long, were longer than male antennae, 8.8 to 9.1 mm. Female antennae also contained more antennomeres, 62, than male antennae, 55 to 58. As found in Manduca sexta (Shields and Hildebrand 1999a), the leading edge of the antennae of both sexes were completely devoid of scales and heavily packed with sensilla while the trailing edge was densely packed with scales and contained very few sensilla. Eleven types of sensilla were found; Bohm’s bristles, two different s. trichoidea, three types of s. basiconica, one type of s. auricillica, one type of sensilla chaetica, two types of s. coeloconica (one of which was found only on male antennae), and one type of squamiform sensilla.

Scape and pedicel

Scales remained over a majority of the surface area of the scape and pedicel in all antennae investigated despite our attempt at scale removal. Three patches of Bohm’s
bristles were visible (Figure 1A) on the antenna of both sexes in approximately the same area. Two patches occur dorsally on the lateral (the side corresponding with the trailing edge of the flagellum) side of the pedicel; one occurs on the proximal edge while the other occurs on the distal edge of this antennomere. The third patch of bristles is on the medial face of the pedicel, the face corresponding to the leading edge of the flagellum. A thin line of bristles follows the edge of the pedicel between the proximal patch of bristles and this third patch. In addition, four s. squamiforme (Figure 1B) form a line from the leading to the trailing edge across the dorsal surface (Figure 1A). On the pedicel, these structures are normally almost completely covered by scales.

**Trichoid rich region**

The trichoid rich region (Figure 2-2) of the antenna was of similar length in both sexes; between 31 and 34 antennomeres. This region was marked by an overwhelming presence of large type-1 s. trichoidea. In females, the s. trichoidea projected from the leading surface in two rows. The rows joined at the dorsal and ventral edges of the leading edge, loosely forming a crescent shape. In males this region is bipectinate, marked by two horn-like inflexible projections of the cuticle. These projections were heavily laden with type-1 s. trichoidea on the leading surfaces. The projections were smallest on the edges of the region and largest in the center of the region. Larger “horns” contained more and larger sensilla than smaller projections. On the dorsal and ventral surfaces of these projections, other sensilla were sparsely scattered, the numbers and locations of which were similar to the two nearby proximal and distal projections, but which changed with size. Up to one type-2 s. coeloconica, three s. auricillica, one s. chaetica, and up to five scales can be found on the outer surface (i.e.: most dorsal or ventral) of each projection. In addition, up to five type-2 s. trichoidea and
no scales were found on the outer surface of smaller projections while no type-2 s. trichoidea and up to five scales were found on the outer surface of larger projections. The trailing edge of male antennomeres was densely scaled and contained only squamiform sensilla. However, in female antennae this heavily scaled region populated only with squamiform sensilla was narrower and covered less of the dorsal and ventral surfaces. Therefore, the dorsal and ventral surfaces of female antennae had more type-2 s. trichoidea in this region as compared with male antennae. All female antennomeres in this region carried 6 to 8 type-2 s. trichoidea and 16 to 24 s. auricillica while male antennomeres sometimes had neither of these sensilla.

**Club-like region**

Karalius (1994) described a region of antennae in *Synathedon tipuliformis* as a “slightly expressed bulb,” however no pictures accompanied the description so what is meant is unclear. In *V. polistiformis*, the club-like region was sexually dimorphic only in the number of antennomeres composing the region. This region consisted of 17 segments in males and 24 segments in females. Each antennomere in this region is broader than it is long. This region was found to be devoid of type-1 trichoid s. and the leading surface was covered with type-2 s. trichoidea, with over 100 present on most antennomeres. The antennomeres nearest the distal edge of this region were smaller than others in this region and therefore did not have as many sensilla. “Club-like” antennomeres also contained between two and four type-1 s. basiconica, 2 to 6 type-2 s. basiconica, up to ten s. auricula, and at most a single type-1 s. coeloconica distributed over the leading surface.
Apical region

The apical three segments were much smaller than those of every other region and were inset into the edge of the ‘club-like’ region of the antenna in both sexes. This region contained up to 33 deeply socketed, rigid s. chaetica (Figure 2-5) found which were absent on all other antennal regions of both sexes. No other sensilla were found in this region. While examining hydrated specimens preserved in 70% ethanol, this region was readily flexible and fairly elastic. When applying gentle pressure to one sensillum chaetica on the apical antennomere, the entire apical region was depressed into the cavity of the apical segment of the “club-like region” before the s. chaetica was deformed.

Morphology of Sensilla

Bohm’s bristles

These sensilla were found in several patches only on the pedicel. Their occurrence on insect antennae is well documented in Coleoptera (Merivee et al. 1999), Hymenoptera (Crook et al. 2008), Psocoptera (Hu et al. 2009), and Lepidoptera. Those photographed in this study (Figure 2-1A) are typical of Bohm’s bristles and closely resemble bristles photographed by Cuperus (1982) in Yponomeuta viginipunctatus and those photographed by Gomez and Carrasco (2008) in Talponia batesi. These structures are aporous and function as mechanoreceptors (Karalius 1994, Hallberg et al. 2003), encoding changes in antennal position (as reviewed in Sane et al. 2007).

Sensilla trichoidea

We found two types of s. trichoidea. Both are filiform in shape but type-1 (Figs. 2) were several times longer and thicker than type-2 (Figures 2-2A, 2-4A, and 2-4B) and their cuticular shafts differed in ultrastructure. The circumferential ridges of shafts of
type-1 trichoid sensilla were diagonal and form what Shields and Hildebrand (1999a) termed a herringbone pattern whereas those of type-2 form a helical pattern. Both s. trichoidea were porous and likely function as olfactory receptors. The abundance of s. trichoidea varied drastically from region to region. Type-1 s. trichoidea were restricted to the trichoid rich region of the antennae in both sexes and were found in much higher numbers on male antennae than female antennae (Figure 2-2). Type-2 s. trichoidea were more common on female antennae; they were present on all antennomeres except for those composing the apical region and the scape and pedicel in females, but were absent in the trichoid region of male antennae on antennomeres with large cuticular protrusions (Figure 2-2B). It has been reported that grape root borer females respond to plumes of conspecific pheromone and have the same response threshold as males (Pearson and Schal 1999). Pearson and Schal (1999) also report that males respond more strongly than females when presented with only the minor component (1% of the natural blend). Since males have more type-1 s. trichoidea than females, it is probable that these sensilla are sensitive to pheromone, especially the minor component.

**Sensilla basiconica**

We found three types of s. basiconica. The three categories were distinguished based on shape and location. Type-1 s. basiconica (Figures 2-3D and 2-4C) were found on the cuticular protrusions of male antennae, one per cuticular extension, and on the leading edge of female antennae in the trichoid rich region, as well as on the leading edge of the “club-like” region of both sexes. In the latter two instances, two to four type-1 s. basiconica were present and equidistant from one another on each antennomere forming a band across the surface. These sensilla were “spine-like,” rigid in
appearance, and were always observed as perpendicular to their socket. Images depicting the ultrastructure of these sensilla were ambiguous and we were unable to determine whether this type of sensillum was porous or aperous. Of the three subtypes of s. basiconica, these were the least common.

Type-2 s. basiconica (Figure 2-4A) were filiform in appearance and multi-porous. They were observed on both male and female antennae only on the leading edge of the “club-like” region. The presence of dozens of pores on the surface of these sensilla implies an olfactory purpose.

Type-3 s. basiconica (Figure 2-4B) were multi-porous, broad, flattened and peg-like in appearance and found in meager numbers on the leading surface of the “club-like” region both male and female antennae and on the leading edge of the trichoid rich region of female antennae. Of the three subtypes of s. basiconica, these were the most common on both male and female antennae. The presence of pores in the ultrastructure of these sensilla implies they play a role in olfaction.

Sensilla coeloconica

S. coeloconica are typified as cuticular pegs in sunken pits. We tentatively report two s. coeloconica in grape root borer. Type-1 s. coeloconica (Figure 2-4D) fits the established description for these sensilla well. We were unable to determine the porosity of the peg surface of s.coeloconica. Therefore, an olfactory role for these sensilla can not be ruled out. However, it has been reported that neurons involved in hygroreception are stimulated by distortions in the sensillum lymph caused by changes in humidity (Steinbrecht and Müller 1991). It seems possible that sufficient mechanical disturbance of the cuticle could displace sensillum lymph, simulating changes in humidity. Since type-1 s. coeloconica are deeply recessed in a pit and the pits are
surrounded by microtrichia that overlap it, the peg seems to be relatively well protected from mechanical deformation. We postulate that type-1 s. coeloconica are involved in hygroreception.

While type-1 was found in multiple places on both male and female antennae, type-2 (Figure 2-4C) was found only incidentally in two out of over 200 SEM images taken. As this sensilla was only observed in two images (both on male antennae); neither of which show clearly that the two pegs are in a pit and neither of which allow a view of the peg sockets or the ultrastructure of the two pegs, we can only speculate that this type of sensilla may be involved in detecting chemicals (Keil 1987), temperature, or humidity (McIver and Hutcinson, 1972). It is possible that these sensilla only occur on males, but it seems unlikely given the documentation of every other sensilla type on both male and female antennae and the necessity of both male and female GRB to be sensitive to changes in humidity and temperature. In addition, given the ability of female GRB to detect conspecific sex pheromone (Pearson and Schal 1999) and the need for females to locate oviposition sites, it seems unlikely that this sensillum would exhibit sexual dimorphism.

**Sensilla squamiforme**

These sensilla (Figure 2-1B) always occurred in regions normally covered by scales and thus were found on all antennomeres of both sexes except for those occurring in the apical region (of both sexes). The ultrastructure of these sensilla was remarkably similar to scales; aporous and with multiple grooves along the entire length of the sensillum. Those described here were very similar to those documented by Cuperus (1983) in *Yponomeuta vigintipunctatus*. Due to the lack of pores in the surface, an olfactory role can be ruled out. Their slender scale-like appearance is similar to other
reported mechanoreceptors (Hallberg et al. 2003) and this is most likely their role in grape root borer.

**Sensilla chaetica**

These sensilla were found only in the apical region, with a vast majority of them occurring on the apical segment (Figure 2-5). The ultrastructure of these sensilla is remarkably similar to the squamiform sensilla and to antennal scales. These sensilla were rigid and the longest and thickest structures on the antennae. The sockets of these sensilla are drastically different from those of all other sensilla documented in this study. They extended beyond the cuticle and up the base of the sensilla like a sleeve. It is unclear what role they serve as the sockets seem to make the sensilla inflexible and no pores were observed in the surface or on the tips.

**Conclusions**

Eleven types of sensilla were found of which ten were found on the antennae of both male and female grape root borer. The only type of sensillum, type-2 s. coeloconica, not found on female antennae was discovered on images of male antennae, however our small sample size does not rule out the possibility of its presence on female antennae. The main differences between male and female antennae were observed in the trichoid rich region. Male antennae were bipectinate, having two rami, while female antennae lacked these structures. Male rami were densely fringed on both sides by type-1 s. trichoidea (for a total >160) while female antennomeres in this region were limited to approximately 60 type-1 s. trichoidea. Female antennae had more type-2 s. trichoidea in this region than males which may indicate that these sensilla are involved in detecting chemicals involved in host plant or oviposition site location and not detection of sex pheromones. The club-like region of
female antennae contained more segments than male antennae, causing female antennae to be longer than male antennae, though the size, shape, and sensilla populations of antennomeres in this region were similar in both sexes.
Figure 2-1. Scape and pedicel of antenna of *Vitacea polistiformis*. A) The scape and pedicel of a female antenna. Present are scales, sc, Bohm’s bristles, B, and s. squamiforme, S. B) A closer look at s. squamiforme, S. At this magnification the similarity to scale structure is discernible.
Figure 2-2. Trichoid rich region of antenna of *Vitacea polistiformis*. A) 3 segments of the trichoid rich region of the female antenna. Type-1 s. trichoidea, **T1**, form two rows and form the majority of sensilla on the leading surface. The trailing surface has been stripped of most of its scales but a few still remain. A s. squamiforme, **S**, that would have been hidden by scales is visible s. auricillica, **A**, are present on the outer surfaces of each antennomere as well as in clusters of three on the leading surface. Type-2 s. trichoidea, **T2**, are present on the outer surfaces of each antennomere of this region on female antennae. B) 3 segments of the trichoid rich region of male antennae. As in the female, the trailing surface was covered with scales, **sc**, but most have been removed revealing s. squamiforme, **S**. Visible are the cuticular extensions that drastically increase the surface area of the leading surface. These extensions are populated by dozens of type-1 s. trichoidea, **T1**.
Figure 2-3. Sensilla auricillica and type-1 basiconica of *Vitacea polistiformis*. A-C) Images are of s. auricillica. The size and shape of these sensilla varied. More varieties exist than are depicted here. D) Image depicts type-1 s. basiconica and socket.
Types 1-3 s. basiconica & type-1 s. coeloconica of *Vitacea polistiformis*. A) Shown is a type-2 s. basiconica, B2, in the context of its location. The ultrastructure was photographed separately. Also shown are type-2 s. trichoidea, T2, and non-innervated microtrichia, mt. B) Shown is a type-3 s. basiconica, B3. The surface is covered with groups of pores which appear to form ridges in the sensillar surface. One can also see the helical ridges of the type-2 s. trichoidea, T2, and the microtrichia, mt. C) This image was taken in an attempt to capture the type-1 s. basiconica, B1. The putative type-2 s. coeloconica, C2. Two pegs are visible and may be recessed in a pit. D) Shown is type-1 s. coeloconica surrounded by microtrichia.
Figure 2-5. Apical region of antenna of *Vitacea polistiformis*. A) Close up showing the scale-like ultrastructure of the s. chaetica found on the apical segment of the antennae of grape root borer. B) Pictured are the first two of the three apical antennomeres of the apical region. In this image, the s. chaetica, C, present nowhere else on the antennae can be seen. The sockets of these sensilla are the only found that extend beyond the cuticular surface. Even the empty scale sockets shown here are less obtrusive. Type-2 trichoid sensilla, T2, can be seen on the edge of the first segment of the “club-like” region found on the antennae of both sexes. Some scales, sc, are also present that were not removed by the preparation.
CHAPTER 3
ACOUSTIC DETECTION OF GRAPE ROOT BORER

Introduction

The number of registered Florida wineries has increased from 13 to 17 in the past 4 years (Florida Grape Growers Association 2009), and since Florida is this country’s third largest wine consuming state (University of Georgia 1997), demand will not be a limiting factor for further growth. In addition, as the green movement gains momentum and more consumers choose locally produced goods to minimize their carbon footprints, Florida grape growers will be able to satisfy consumers’ ecological concerns as well as their palettes. In Florida, the amount of land devoted to grape cultivation has steadily increased over the past several years and is now over 1,000 acres (Weihman 2005). As the grape industry has grown, Grape root borer (GRB), *Vitacea polistiformis* Harris, has become an increasingly pressing problem. GRB is a widespread pest of grapes in the Eastern United States. It is the key pest of grapes in Florida (Liburd and Seferina 2004) and Georgia (Weihman 2005) and an important pest in North (Pearson and Schal 1999) and South Carolina (Pollet 1975). GRB larvae burrow into the soil immediately after hatching until they reach grape roots. Once there, larvae feed on roots reducing vine vigor and cold tolerance, increasing susceptibility to pathogens and drought, and hastening vine death (Pearson and Meyer 1996). High infestations of GRB result in vine death while lower infestations weaken plants and reduce yields and a low economic injury level has been established in Georgia for GRB, 0.074 larvae per vine (Dutcher and All 1979). One GRB larva feeding at the root crown can cause as much as 47% decrease in yield. Entire vineyards have been destroyed in Florida at least in part due to
GRB infestation and GRB was cited as the cause of total cessation of grape production in South Carolina (Pollet 1975).

Currently, the organophosphate chlorpyrifos is the only chemical registered in Florida for GRB control (Turner et al. 2006). Lorsban® is the commercially available formulation of the chemical and is applied to the root area as a soil drench. Lorsban® is not an ideal control tool for GRB because it is highly toxic to birds, fish, aquatic invertebrates, and honeybees. It is also moderately toxic to pets and livestock and is suspected of being carcinogenic to humans (Food Quality Protection Act, 1996).

Florida vineyards are relatively small, usually between three and ten acres, and are typically family owned and operated. Most grape growers live on site with family and many are reluctant to use Lorsban®, despite the possible financial consequences of avoiding it, because of the potential safety hazard to themselves, family, employees and the environment. They also realize that consumers are becoming increasingly wary of pesticide use and want to provide goods produced using a safer alternative. The lack of a safe, economically viable management plan for GRB is the biggest barrier to the stability, profitability, and growth of the Florida grape industry.

The practice of mounding has shown some promise as an effective control alternative to pesticides. When GRB larvae are ready to pupate, they usually migrate to within the top 5 cm of topsoil to form their pupal case. From this depth, pharate adults are able to ascend from the soil. Creating a circular soil mound around the base of the vine after larvae have already migrated and begun pupation increases the distance they must travel as pharate adults to reach the soil surface before they eclose. Mortality increases as this distance increases. Sarai (1969) found 100% mortality when mounds
were 19 cm high. Once GRB emergence begins to decline for the year, mounds must be removed so that mounding may be done the next year. Currently, mounding is so labor intensive that it is unattractive to most growers. The cost of mounding would be greatly decreased if growers were able to easily determine whether or not a given plant is infested with GRB, eliminating the cost of treating uninfested vines. This study evaluated the potential of acoustic detection methods as a means of determining the presence or absence of GRB larvae in an individual grapevine’s root system. If successful, this detection system should decrease the amount of Lorsban® used for control of GRB in vineyards and make the sustainable practice of mounding much more attractive to growers, encouraging good stewardship and a healthier environment. The acoustic detection method could be implemented wherever GRB is a problem beyond Florida viticulture.

**Materials and Methods**

**Acoustic Instruments, Signal Recording, and Soil Sampling Procedures**

Acoustic records were collected from 28 root systems at Blue Heron Vineyard near Lithia, FL, and 8 root systems at Sirvent’s Farm and Vineyards near Florahome, FL between April 28 and June 9, 2009. Air temperatures ranged between 29 and 35 °C during the recordings. Two accelerometer amplifiers and a recorder (details of the instruments are described in Mankin et al. 2009) were set in the storage bed of an electric cart for transport through the vineyard rows to sites with vines exhibiting symptoms of GRB infestation: wilting, yellowed or dead leaves, and reduced leaf area as compared with neighboring plants of the same variety. A 30 cm nail was inserted into the root system of the selected vine. The accelerometer was attached to the nail head by a magnet. One or more listeners took notes and monitored the signals during a
recording period of 3 min or longer. Within 1 to 2 h after recording, the vine was excavated and the contents of the root system were examined to obtain an independent verification of whether a site was uninfested or contained insects.

**Listener Assessment of Infestation Likelihood**

Subterranean larvae typically produce spectrally distinctive, 3 to 10 ms sound impulses during movement and feeding activities (Mankin et al. 2000, 2009). These sound impulses can be identified and recognized as insect-produced sounds by most listeners after 10 to 20 min practice with the accelerometer and headphones. In this experiment, the listeners were primarily Richard Mankin and Will Sanders. Occasionally, Everett Foreman, Betty Weaver, Mackenzie Egan, John Sirvent, and Bob Paulish also served as listeners.

Assessments were performed as in Mankin et al. (2007), where low indicates detection of no valid, insect-produced sounds or only a few faint sounds during a recording period, medium indicates detection of sporadic or faint groups of valid sounds, and high indicates detection of frequent, easily detectable groups of valid sounds. No attempt was made to distinguish between pest and non-pest species in the assessment. Comparisons between the distributions of assessed infestation likelihoods at infested and uninfested recording sites were performed using the NPAR1WAY procedure in SAS (SAS Institute 2004).

**Digital Signal Processing and Classification**

Recorded signals were band-pass filtered between 0.2 and 5 kHz to facilitate subsequent analysis, and visualized with audio playback using Raven 1.3 software (Charif et al. 2008). In initial screenings, we confirmed the presence of groups (trains) of discrete, 3 to 10 ms impulses separated by intervals < 250 ms that had occurred
frequently where insects were recovered in previous studies (Mankin et al. 2009). Trains containing 6 or more impulses were a focus of analysis because they often were identified as insect sounds in playbacks of recordings from infested sites.

The impulses and impulse trains detected in the recordings were analyzed with customized software, DAVIS (Digitize, Analyze, and Visualize Insect Sounds, Mankin et al. 2000), which discarded long-duration, low frequency background noise (Mankin et al. 2007) and then compared the spectrum of a 512-point time-slice centered around the peak of each impulse against averaged spectra (spectral profiles) constructed as described in Results.

The impulse sequences were screened to identify and characterize trains of impulses that listeners typically classify as separate, individual sounds. Each train was labeled according to the spectral profile matched by a plurality of its impulses. The beginning and ending times of impulse trains, their labels, and the number of impulses per train were stored in separate train-sequence spreadsheets for each recording.

Results

The root systems at 25 of 36 recording sites exhibited *V. polistiformis* larval damage, although only one live larva was recovered. Altogether, 27 root systems contained one or more invertebrates of various species (Tables 3-1 and 3-2). Among these were 41 Coleoptera (including four *Mycotrupes*, three Tenebrionids, one Cerambycid, four *Phyllophaga* larvae and one *Anomala* larva) one Cetoniid larva, six *Lepisma saccharina* (L), and three burrowing roaches. Six sites contained *Solenopsis invicta* Buren workers, and three had termite workers. Other organisms found in the root systems included five unidentified worms, three Diplopoda, three large spiders, and an earthworm. Only the *V. polistiformis* was to be targeted as a pest (see Discussion), but
for purposes of categorizing sites, we considered a site to be infested if the excavated root system contained one or more invertebrates capable of producing sounds.

**Spectral Profiles**

Two types of impulses that are typical of insect-produced sounds (Mankin et al. 2000, 2009) appeared frequently in initial screenings of signals detected at recording sites where excavations verified infestation. A third type of impulse with a highly distinctive spectral pattern appeared less frequently. Spectral profiles of these impulses (Mankin et al. 2000) were calculated to assist in discriminating insect sounds from background noise (Figure 3-1). A profile of one of the two most frequently occurring insect sound impulses, $s_{\text{highdB}}$, was constructed from a series of 128 consecutive impulses in a relatively noise-free recording that contained several insect burrowing sounds. The second profile, $s_{\text{middkB}}$, was constructed from a series of 94 consecutive impulses in a recording that contained several larval scraping sounds of slightly lower frequency. The third, less frequently occurring profile, $s_{\text{lowdB}}$, was constructed from a 0.1 s period containing 13 consecutive impulses of this distinctive type. The three types of impulses had similar temporal patterns but their spectral patterns diverged at frequencies above 2.6 kHz.

Various types of background noise also occurred frequently in all recordings, comprising about 80% of all sounds detected. Continuous noise could be discounted easily because insect sounds usually occur as brief impulse bursts (Mankin et al. 2009), but some low-frequency impulsive noise was discarded by matching it with one of two noise profiles. To exclude higher-frequency noise impulses, we constructed a noise profile, $n_{\text{highdB}}$ (Figure 3-1), as an average spectrum of impulses produced during a gust of light wind. A second noise profile, $n_{\text{lowdB}}$ (Figure 3-1), was constructed as an average
spectrum of a 5 s period where impacts of water droplets from an irrigation hose were detected.

**Insect Sound-Impulse Bursts**

Although isolated $s_{\text{highdB}}$, $s_{\text{middB}}$, and $s_{\text{lowdB}}$ impulses occurred frequently in the recordings, most of the signals that listeners interpreted as insect sounds appeared in bursts of more than 6 but less than 50 impulses of a given type, similar to bursts that have been detected in other insect acoustic detection studies (Mankin et al. 2008a,b, 2009). In analogy with such studies, we defined trains of type $s_{\text{highdB}}$, $s_{\text{middB}}$, and $s_{\text{lowdB}}$ impulses to be a series of impulses of each type, separated by durations $< 0.25$ s. Bursts of type $s_{\text{highdB}}$, $s_{\text{middB}}$, and $s_{\text{lowdB}}$ were trains of each impulse type that contained at least 7 but less than 50 impulses. We analyzed recordings from each of the 36 root systems using the DAVIS signal analysis system (Mankin et al. 2000) matching each detected impulse against the three signal and two noise profiles (Figure 3-1). The rates of detection of trains and bursts in the nine root systems that contained bursts of type $s_{\text{lowdB}}$ are listed in Table 3-1. One site, assessed by listeners at high likelihood of infestation, contained a $V. \text{polistiformis}$ larva as well as two $\text{Phyllophaga}$ larvae. Bursts of type $s_{\text{highdB}}$ also were detected at this site. The rates of detection of trains and bursts in the other root systems are listed in Table 3-2. As in previous studies (Mankin et al. 2009), the rate of trains was correlated with, but not necessarily proportional to, the rate of bursts at each recording site. There were 14 root systems in which no bursts of any insect-sound profile type were detected. Five of these did contain insects but nine were found to be uninfested when they were excavated.
Assessments of Infestation Likelihood

The listener assessments of infestation likelihood matched significantly with the presence or absence of insects in the root systems at the recording sites (Table 3-3). Only one infested site was ranked at low likelihood of infestation, and all of the sites ranked at high likelihood of infestation were infested.

To develop a computer assessment of infestation likelihood, we examined the rates of bursts of different types detected at different infested and uninfested sites, and constructed indicators of infestation likelihood as described in Mankin et al. (2007). Sites with rates of bursts of all three insect-sound profile types < 0.5 / min were considered to have low likelihood of infestation, while sites with rates of bursts of any insect-sound profile type > 1.5 / min were assessed at high likelihood of infestation. Sites with intermediate rates were assessed at medium likelihood. Assessments of the results in Tables 3-1 and 3-2 based on these criteria are listed in Table 3-4. The computer assessments, like the listener assessments in Table 3-3, matched significantly with the presence or absence of insects in the root systems at the recording sites.

Discussion

One of the goals of the acoustic detection study was to develop a method for targeting *V. polistiformis* infestations to decrease the cost and effort involved in treatments such as mounding. Although it would be helpful to obtain more recordings from *V. polistiformis* larvae, the results of the study nevertheless provide some insight into how targeting might be accomplished. An important finding was that the vineyard contains a large variety of nontarget, sound-producing insects. The signals produced by such insects could easily confound the identification of a targeted pest unless the pest
produced a distinctive, easily identifiable sound that distinguished it from nontarget insects.

A partial, temporary solution to this problem is to include ambiguous signals as evidence of the targeted pest, i.e., to err on the side of inclusion. Considering the invertebrates and the burst rates in Table 3-1, for example, targeting all the sites that contained $s_{\text{highdB}}$, $s_{\text{middB}}$, and $s_{\text{slowdB}}$ bursts would result in treatment of only nine of 36 sites, one of which contained a $V. \textit{polistiformis}$. Treating $\frac{1}{4}$ of all sites will be much less costly than treating all sites.

It is common for a vineyard to have approximately 735 vines per hectare. Assuming that the average price for unskilled farm labor is $20, fill dirt and delivery is free, that it takes ten minutes to build a mound around a vine and ten more minutes to remove the soil at the end of the season, it would cost approximately $4900 to treat one hectare. If we assume that our findings of 25% infestation level apply to any vineyard, a farmer would save $\frac{3}{4}$ of $4234 on mounding. It would therefore need to cost $3675 to acoustically sample all vines for the farmer to break even.

Both human listeners and computer software were able to predict the presence or absence of infestation fairly well based upon spectral profiles. However, human listeners were more likely to commit type I error while the computer was more likely to commit type II error. Unfortunately, it is more dangerous to commit type II error in this circumstance. A type I error will cause the treatment of a vine when it is unnecessary, slightly raising the cost of treatment. A type II error will leave an infested site untreated, allowing GRB to emerge and reproduce. Without further refining of the spectral profiles
or improvement of the software’s analysis algorithm, it is recommended for a human listener to assess likelihood of infestation for pest management decisions.
Table 3-1. Numbers of invertebrates recovered from root systems, listener assessments of infestation likelihood, and rates of $s_{\text{highdB}}$, $s_{\text{middB}}$, and $s_{\text{lowdB}}$ trains and bursts at sites where $s_{\text{lowdB}}$ bursts were detected, arranged in order of the rates of $s_{\text{lowdB}}$ bursts

<table>
<thead>
<tr>
<th>Number recovered</th>
<th>Ass’d infest.</th>
<th>Rate (No./min) of $s_{\text{highdB}}$</th>
<th>Rate (No./min) of $s_{\text{middB}}$</th>
<th>Rate (No./min) of $s_{\text{lowdB}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>train</td>
<td>burst</td>
<td>train</td>
</tr>
<tr>
<td>0 0 1 4</td>
<td>$\text{med}m$</td>
<td>6.7</td>
<td>0</td>
<td>4.7</td>
</tr>
<tr>
<td>≥1 0 2 3</td>
<td>$\text{high}$</td>
<td>8.3</td>
<td>2.7</td>
<td>2.8</td>
</tr>
<tr>
<td>0 0 2 0</td>
<td>$\text{high}$</td>
<td>23.4</td>
<td>3.2</td>
<td>8.5</td>
</tr>
<tr>
<td>≥1 0 0 0</td>
<td>$\text{med}m$</td>
<td>10.6</td>
<td>0</td>
<td>14.9</td>
</tr>
<tr>
<td>0 3 2 1</td>
<td>$\text{high}$</td>
<td>3.6</td>
<td>0</td>
<td>2.1</td>
</tr>
<tr>
<td>0 2 0 1$^d$</td>
<td>$\text{high}$</td>
<td>8.0</td>
<td>0.6</td>
<td>9.2</td>
</tr>
<tr>
<td>0 0 2 1</td>
<td>$\text{high}$</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0 1 3 8</td>
<td>$\text{med}m$</td>
<td>1.5</td>
<td>0</td>
<td>13.4</td>
</tr>
<tr>
<td>0 0 1 0</td>
<td>$\text{med}m$</td>
<td>2.7</td>
<td>0</td>
<td>4.3</td>
</tr>
</tbody>
</table>

$^a$Including, *Phyllophaga* sp., *Anomala* sp., Tenebrionid sp. $^b$Including *Mycotrupes* sp. $^c$Other invertebrates included Lumbricid sp., Diplopoda sp., *Blatella* sp., *Lepisma saccharina* (L), *Nerthra stygica* Say, and large spider. $^d$One *V. polistiformis* larva was found in the root system at this recording site.
Table 3-2. Numbers of invertebrates recovered from root systems, listener assessments of infestation likelihood, and rates of $s_{\text{highdB}}$, $s_{\text{middB}}$, and $s_{\text{lowdB}}$ trains and bursts at sites where $s_{\text{lowdB}}$ bursts were not detected, arranged in order of the summed rates of rates of $s_{\text{highdB}}$ and $s_{\text{middB}}$ bursts.

<table>
<thead>
<tr>
<th>No. recovered</th>
<th>Ants or Beetle larvae$^a$</th>
<th>Beetle larvae$^b$</th>
<th>Other invert.$^c$</th>
<th>Infestation likelihood</th>
<th>Rate (No./min) of $s_{\text{highdB}}$ trains</th>
<th>Rate (No./min) of $s_{\text{middB}}$ trains</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\geq 1$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 $h_{\text{high}}$</td>
<td>61.8</td>
<td>28.2</td>
</tr>
<tr>
<td></td>
<td>$\geq 1$</td>
<td>0</td>
<td>0</td>
<td>1 $h_{\text{high}}$</td>
<td>37.6</td>
<td>14.4</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0 $h_{\text{high}}$</td>
<td>13.4</td>
<td>10.0</td>
</tr>
<tr>
<td>$\geq 1$</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1 $h_{\text{high}}$</td>
<td>17.4</td>
<td>2.3</td>
</tr>
<tr>
<td>$\geq 1$</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0 $h_{\text{high}}$</td>
<td>3.3</td>
<td>0</td>
</tr>
<tr>
<td>$\geq 1$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 $m_{\text{medium}}$</td>
<td>26.3</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>$\geq 1$</td>
<td>0</td>
<td>1</td>
<td>2 $m_{\text{medium}}$</td>
<td>3.2</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0 $h_{\text{high}}$</td>
<td>15.8</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>$\geq 1$</td>
<td>1</td>
<td>0</td>
<td>1 $h_{\text{high}}$</td>
<td>9.7</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>$\geq 1$</td>
<td>0</td>
<td>1</td>
<td>0 $l_{\text{low}}$</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>$\geq 1$</td>
<td>0</td>
<td>2</td>
<td>0 $m_{\text{medium}}$</td>
<td>7.6</td>
<td>0.7</td>
</tr>
<tr>
<td>$\geq 1$</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0 $m_{\text{medium}}$</td>
<td>15.8</td>
<td>0.7</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1 $m_{\text{medium}}$</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$\geq 1$</td>
<td>1</td>
<td>0</td>
<td>0 $m_{\text{medium}}$</td>
<td>14.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$\geq 1$</td>
<td>0</td>
<td>0</td>
<td>0 $m_{\text{medium}}$</td>
<td>3.6</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 $l_{\text{low}}$</td>
<td>1.3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$\geq 1$</td>
<td>0</td>
<td>0</td>
<td>0 $l_{\text{low}}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 $l_{\text{low}}$</td>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$\geq 1$</td>
<td>0</td>
<td>0</td>
<td>0 $m_{\text{medium}}$</td>
<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0 $m_{\text{medium}}$</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$\geq 1$</td>
<td>0</td>
<td>0</td>
<td>0 $l_{\text{low}}$</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0 $m_{\text{medium}}$</td>
<td>1.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$\geq 1$</td>
<td>0</td>
<td>0</td>
<td>0 $l_{\text{low}}$</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 $l_{\text{low}}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$\geq 1$</td>
<td>0</td>
<td>1</td>
<td>0 $m_{\text{medium}}$</td>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td>$\geq 1$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 $m_{\text{medium}}$</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$Including, *Phyllophaga* sp., *Anomala* sp., Tenebrionid sp., Cetoniid sp, Cerambycid sp.

$^b$Including *Mycotrupes* sp.

$^c$Including Lumbricid sp., Diplopoda sp., Mutilid sp. *Blatella* sp., *Lepisma saccharina* (L), *Nerthra stygica* Say, and large spider.
Table 3-3. Listener assessments of recording sites determined by excavation to be uninfested or infested

<table>
<thead>
<tr>
<th>Assessed infestation likelihood</th>
<th>Number of sites uninfested</th>
<th>infested</th>
</tr>
</thead>
<tbody>
<tr>
<td>low</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>medium</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>high</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>

Note: P = 0.0002 that listener assessment is independent of the absence or presence of infestation in the excavated roots (Wilcoxon two-sample exact test, S = 61.5, Z = -4.09)

Table 3-4. Computer assessment of recording sites determined by excavation to be uninfested or infested

<table>
<thead>
<tr>
<th>Assessed infestation likelihood</th>
<th>Number of sites uninfested</th>
<th>infested</th>
</tr>
</thead>
<tbody>
<tr>
<td>low</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>medium</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>high</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>

Note: P = 0.0005 that computer assessment is independent of the absence or presence of infestation in the excavated roots (Wilcoxon two-sample exact test, S = 67.5, Z = -3.83)
Figure 3-1. Spectral profiles of insect-produced sound impulses, $s_{\text{highdB}}, s_{\text{middB}},$ and $s_{\text{lowdB}}$, compared with spectral profiles of wind-gust noise, $n_{\text{highdB}}$, and low frequency background noise, $n_{\text{lowdB}}$, used in analyses to distinguish insect produced sounds from background noise. The subscripts, highdB, middB, and lowdB, refer to the magnitudes of the relative spectrum levels of these profiles near 2.6 kHz, the midpoint of the 0.2-5 kHz range of frequencies analyzed. Spectrum level is relative to the maximum acceleration measured in the 0.2-5 kHz reference range.
CHAPTER 4
PHEROMONE ATTRACTIVENESS AND MATING DISRUPTION STUDIES

Introduction

Grape Root Borer

Grape root borer (GRB), *Vitacea polistiformis* Harris, was first reported attacking cultivated grapes in 1854 (Bambara and Neunzig 1977). Since then, GRB has become a widespread pest of grapes in the Eastern United States. Grape root borer is one of the most destructive pests of grapes in North (Pearson and Schal 1999) and South Carolina (Pollet 1975). It is considered the key grape pest in Georgia (Weihman 2005) and in Florida (Liburd and Seferina 2004). As the amount of land devoted to viticulture in Florida has increased over the past several years (Weihman 2005), GRB has become an increasing problem for commercial growers.

Grape root borer attacks European grapes, *V. vinifera*, muscadine grapes, *Vitis rotundifolia*, bunch grapes, *V. labrusca*, and hybrid bunch grapes, *Euvitis* spp. In addition, GRB are capable of completing their life cycle on wild grapes. When contemplating control methods, it is important to consider areas with wild grapes as a reservoir for the pest (Weihman 2005).

Grape root borer feed on and create gouge-like wounds in grape roots which can kill smaller roots and girdle large ones. These wounds cut off or restrict nutrient and water transportation from roots to the rest of the plant reducing vine vigor. Specifically, average leaf area, berry and cane yields are significantly decreased (Dutcher and All 1979). In addition, wounds in the roots make the plant more susceptible to freeze damage, drought, and pathogens (Pearson and Meyer 1996). A single larva is capable of causing 6% girdling of the vine trunk resulting in 47% reduction in yield. Two or three
larvae are capable of killing the entire vine (Dutcher and All 1979). Grape root borer
damage has resulted in enormous losses to the commercial grape industry. Entire
vineyards have been destroyed in Florida and GRB was cited as the cause of total
cessation of grape production in South Carolina (Pollet 1975). According to Dutcher and
All (1979), the economic injury level of GRB is extremely low (0.074 larvae per vine)
meaning that insecticide control measures should be used as soon as GRB are
detected.

**Chemical Control**

Currently in Florida only chlorpyrifos is registered for GRB control (Turner et al.
2006). Chlorpyrifos is an acetylcholinesterase inhibitor and is most commonly
formulated for use against GRB as a soil drench (Lorsban®) to prevent newly hatched
first instar larvae from reaching grape roots. Adlerz (1984) reported that Lorsban® is
effective against GRB in muscadines but not as effective in bunch grapes. The root
structures of these grape genera differ, with muscadine roots being shallower. This may
allow some roots to be within the thin layer of soil that retains Lorsban®, protecting them
from infestation. In Florida, most cultivated grapes are muscadine, so Lorsban® would
have some control potential.

Unfortunately, however, there are problems with Lorsban® use. Only one
application of Lorsban® is permitted per season and, which maintains toxicity in the soil
for only 4 weeks. The emergence period in Florida lasts from four to six months and
often has bimodal peaks; therefore application of Lorsban® in Florida can only control
part of the population. Lorsban® is also not ideal for control in Florida due to its 35 day
pre-harvest interval and the conflicts between GRB peak emergence and harvesting
dates (Weihman 2005, Weihman and Liburd 2006). In many parts of Florida, peak
emergence coincides with harvest; therefore, Lorsban® cannot be used when it would be most effective. Weihman and Liburd (2007) recommend applying Lorsban® after harvest to maximize mortality in both bunch and muscadine grapes in Florida.

In addition, Lorsban® is suspected of being carcinogenic and its future use is not guaranteed (Weihman and Liburd 2006). The Food Quality Protection Act (FQPA) of 1996 has resulted in the elimination or restriction of some broad-spectrum pesticides. It is unclear how the FQPA will affect Lorsban® use in the future, but it is necessary to find alternatives to Lorsban® in the mean time. Some alternative methods have been researched, including attract-and-kill and mating disruption, which have demonstrated potential (Weihman and Liburd 2006).

**Mating Disruption**

Adult female GRB attract males from long distances using a female-specific sex pheromone blend comprised of two major components. The primary component, which comprises 99% of the blend, is $(E,Z)$-2,13-octadecadien-1-ol acetate. The secondary component is $(Z,Z)$-3,13-octadecadien-1-ol acetate. Mating disruption is a technique used to interfere with pheromone-mediated female location by males. Inability to find mates results in decreased reproductive success rates and a potentially drastic reduction in population the succeeding season. Generally, the female sex pheromone is placed in dispensers which are then distributed throughout the growing area. It is unclear which mechanism is mainly responsible for the success of mating disruption, though several have been postulated. The main hypotheses are competitive attraction, desensitization, camouflage, and sensory imbalance. According to competitive attraction, males follow the pheromone plume produced by dispensers and when they locate the source are unable to mate and must search again. The desensitization
hypothesis states that males become less sensitive or less responsive to female sex pheromone than they normally would be through habituation or adaptation. The camouflage hypothesis states that males are unable to find calling females because the guiding boundaries, which are necessary for anemotaxis, of their natural pheromone plumes are obscured by synthetic pheromone plumes. The sensory imbalance theory is defined as “disrupting mate-finding not via adaptation or habituation but by interfering with the male’s ability to perceive (as opposed to receive) the normal sensory inputs associated with their species’ sex pheromone” (Miller et al. 2006).

Mating disruption has been used with success in multiple crops with various lepidopteran pests. Codling moth, *Cydia pomonella* L., is the key pest in western U.S. pear and apple orchards and mating disruption has proved successful with the number of hectares treated by mating disruption increasing from near zero in 1990 to nearly 40,000 in 2000 (Brunner et al. 2002). Mating disruption has also proven successful in vineyards with the vine moth, *Eupoecilia ambiuella*, and the grape berry moth, *Lobesia botrana* in Germany. In more than 98% of vineyards implementing mating disruption in the Wuerttemberg region, attack damage did not exceed the economic threshold (Kast 2001, Louis and Schirra 2001).

Mating disruption has also proven successful in controlling several Sesiid pests; including peach tree borer, *Synanthedon exitiosa* (Say), lesser peach tree borer, *S. pictipes* (Grote and Robinson) and currant clearwing moth, *S. tipuliformis* (Clerk). In a field study testing mating disruption on lesser peach tree borer, Pfeiffer et al. (1991) reported complete trap shutdown in pheromone treated plots and a 19 to 97% reduction in population compared with control over three years as measured by pupal case
counts. In this study, mating disruption outperformed pesticides recommended for lesser peach tree borer control and is recommended by the authors to replace pesticide treatments.

Mating disruption has also been shown to be an effective means for controlling GRB. Johnson et al. (1991) report that mating disruption using either of the two main components of the GRB female sex pheromone reduced GRB population levels, as assessed by exposed pupal casings, the following season. They also report that mating disruption using the minor component, \((Z,Z)\)-3,13octadecadien-1-ol, was more effective than using the major component, \((E,Z)\)-2-13octadecadien-1-ol. Commonly, trap shutdown is used to assess the success of mating disruption treatments. It is presumed that if males cannot find traps (trap shut down) baited with the synthetic blend of the female sex pheromone they are unable to successfully follow a pheromone plume to a female and consequently cannot mate with the same frequency. It is conceded that mating is still possible under these conditions, but it is unlikely and probably depends on population density (Weihman and Liburd 2006). Weihman and Liburd (2006) report successful trap shutdown using the sex pheromone of the Leopard moth, Zeuzera pyrina L., to control GRB. In behavioral studies conducted under field conditions, I have documented that GRB males exhibit anemotaxis in response to pheromone plumes produced by dispensers releasing Leopard moth pheromone in the field. It has also been shown that the synthetic form of the natural GRB sex pheromone has great potential for control of GRB (Webb and Mortensen 1990). Currently, the GRB sex pheromone is more expensive than the Leopard moth pheromone and is commercially unavailable for use as a mating disruptant.
Materials and Methods

Chemicals and Dispensers

Two different pheromone blends were used repeatedly throughout our experiments: GRB pheromone, which is 99% (E,Z)-2,13-octadecadien-1-ol: 1% (Z,Z)-3,13-octadecadien-1-ol, and Leopard moth pheromone, which is 95% (E,Z)-2,13-octadecadien-1-ol: 5% (E,Z)-3,13-octadecadien-1-ol. For all experiments, except when specified, monitoring traps were Pherocon® VI delta wing traps (Trécé Inc.; Adair, OK). Each trap was baited with a red rubber septum treated with 1 mg of GRB pheromone (Great Lakes IPM Vesterburg, MI). Wax dispensers used were made by mixing 99.9% pure pheromone purchased from (PHEROBANK; Wageningen, Netherlands) with the wax base, Specialized Pheromone & Lure Application Technology (SPLAT) release matrix obtained from ISCA technologies. Wax dispensers deployed in all experiments were approximately 1 g of SPLAT per deposit. Dispensers used in 2008 were deployed with a 1 g calibrated caulking gun while dispensers for the 2009 experiments were deployed using volumetric syringes.

Experimental Sites

Three different vineyards were used repeatedly for these experiments. Each is described here and referred to by location.

Citra

This vineyard is comprised of approximately 0.5 ha. The vineyard is divided into six plots; each plot is approximately 25 m by 21 m. Plots are separated by aisles approximately 5.25 m wide and each plot contains a different grape variety. Plot one contained the variety ‘Carlos bronze’, plot two ‘Noble black’, plot three ‘Triumph bronze’, plot four ‘Alachua black’, plot five ‘Blanc DuBois’, and plot six ‘Conquistador’. Plots 1 to
4 are planted using the bilateral cordon system while plots five and six are single cordon systems. This vineyard is not operated commercially.

**Lithia**

This commercially operated vineyard covers approximately 4 ha. The majority of this acreage is split nearly in half between Carlos black and Nobel black grapes with an aisle approximately 8 m wide separating them. Vines are spaced approximately 3.7 m apart both within and between rows. Disruption experiments were carried out in this part of the vineyard. Two plots, 33 x 70 m, are separated from the rest of the vineyard by approximately 61 m and were not used for disruption experiments. All grapes are grown using the bilateral cordon system.

**Bradenton**

This commercially operated vineyard covers approximately 4 ha. Unlike the two previously described locations, the number of grape varieties grown in this vineyard and their orientations prevented all blocks from containing only a single grape variety. Plants were spaced 3.7 m within rows and 2.7 m between rows. For the duration of the study, grapes were grown using the bilateral cordon system.

**Pheromone Attraction Experiments**

**Determining the relative attractiveness of pheromone blends with trapping**

Two weeks prior to the experiment, the grape root borer population was sampled for one week in the test vineyard located in Citra, Florida using four green universal moth (GUM) traps (Great Lakes IPM; Vesterburg, MI). Traps were baited with the previously described red septa (Great Lakes IPM; Vesterburg, MI) and placed in the corners of the vineyard at least 5 m from the edge of the plot, to avoid edge effects, and left for one week. After confirming catch of male GRB in traps, the four traps were
removed and the pheromone was allowed to dissipate for one week. The experiment began on September 14, 2007. Each plot contained four GUM traps, each baited with a red rubber septum with a different treatment; 1 mg of GRB pheromone, 1 or 10 mg of Leopard moth pheromone or a blank negative control. The 99% E\text{Z}: 1% Z\text{Z} lures were obtained from Great Lakes IPM (Vesterburg, MI) while the 95% E\text{Z}-2,13 : 5% E\text{Z}-3,13 were prepared as follows: red rubber septa (West Pharmaceutical, Lionville PA) were extracted for 24 hours in hexane, 24 hours in dichloromethane then air dried for 48 hours as per the methods of Knight (2002). Stock solutions of 10 mg/100 ul were prepared using the pheromone blend from Shin-Etsu Isonet Z fibers (E, Z-2,13-ODDA and E, Z-3,13-ODDA) in dichloromethane. A 1 mg/100 ul stock solution was also prepared from these dispensers. Septa were loaded with 100 ul of the respective stock (1 mg or 10 mg) and then another 100 ul of dichloromethane was added to each reservoir. They were allowed to air dry until there was no more solvent in the reservoir. Control septa received 200 ul dichloromethane. Sixteen septa were prepared of each type.

**Statistics.** Traps were checked weekly and the number of trapped male moths was recorded throughout the season. Trap catch was not normally distributed and was therefore modeled by negative binomial regression and differences among means were assessed using differences of least squares means ($P < 0.05$) (SAS/STAT v 9.2, SAS Institute 2009). This analysis was used for objectives I, IV, V, VI, and VII.

**Direct observation of male GRB response to pheromone blends in the field**

The relative attractiveness of two pheromone blends were assessed using slightly modified versions of three behavioral criteria previously used by Stelinski et al. (2004) in addition to the total number of GRB visitors per observation period. Behaviors
monitored included: flying within 0.5 m of the lure, as ascertained by crossing over the table’s edge, approach within approximately 10 cm, and physical contact with the lure. A circular observational arena one meter in diameter was established between vine rows within a small vineyard on the University of Florida campus with known GRB infestation. A pheromone lure was suspended above the circular surface, 1.5 meters above the ground. Two types of pheromone lures were observed: 1) a rubber septum loaded with 1 mg GRB pheromone (Great Lakes IPM; Vesterburg, MI) and 2) a hollow polyethylene tube containing 70 mg of leopard moth pheromone (Shin-Etsu Isonet Z; Tokyo). The study consisted of consecutive observation sessions, each 1.5 h long. The study began on September 10th and was repeated four times that week with observation sessions beginning at 1 pm and ending at 4 pm (Clark and Enns 1964). Treatments under observation were rotated and randomized daily. The observer maintained constant visual contact with experimental setup and recorded data via tape recorder and stopwatch to be transcribed later (Stelinski et al. 2004).

Statistics. Data means were analyzed by ANOVA and differences among means were determined by Tukey’s means separation test (P<0.05) (SAS/STAT v 9.2, SAS Institute 2009).

Determining the relative attractiveness of SPLAT vs. septa

The objective of this experiment was to compare the attractiveness of rubber septum lures used to monitor GRB in all tests with SPLAT dispensers used in mating disruption studies described below. This experiment was established on August 24th, 2009 in the Citra, FL vineyard and data were recorded biweekly from September 2nd to October 12th. The experiment was arranged as a 2x2 factorial design. The factors tested were: 1) dispenser type (septum vs. SPLAT), and 2) trap density (one or two
lures per plot). Two of the six plots were assigned two monitoring traps, one of each lure type, and these were placed in the central row, approximately 8 m apart and at least 5 m from the plot edge. The other four plots were assigned with one lure treatment per plot; two plots receiving a SPLAT baited monitoring trap and two receiving a septum baited monitoring trap. Monitoring traps in plots receiving only one lure treatment were hung 1.5 m above the ground on the central plant. Each trap was checked biweekly when trapped GRB males were counted and the sticky cards were replaced.

**Statistics.** Two sample unpaired t-tests were used to determine whether catch values from traps in the double trap plots were different from single trap plots. Once trap catch was determined independent from number of traps per plot, trap catch data was pooled for similar lure treatments and trap catch means were analyzed using ANOVA. Differences among means were determined by Tukey’s means separation.

**Pheromone Disruption Experiments**

**Determining the effects of pheromone blend on disruption efficacy**

It was hypothesized that a more effective pheromone blend should result in lower male moth counts per trap as compared with the control and result in lower future trap catches as compared with each other and the control. An experiment to test this hypothesis was established on August 23, 2008 in the Bradenton, FL vineyard and data were recorded weekly from September 7th to October 26th. The experiment was arranged as a randomized complete block with three treatments replicated four times. Wax dispensers were prepared as previously described with one treatment containing GRB pheromone, one treatment containing leopard moth pheromone and the control receiving no pheromone. Dispensers were deployed in rectangular plots of 25 grape vines, five plants per row in five rows, at a density of five per plant (125 per plot). Each
plot contained one monitoring trap hung on the centermost plant 1.5 m above the crown. The traps were checked weekly and the number of trapped males was counted.

**Determining the effects of dispenser density on disruption efficacy**

This experiment was established on August 22, 2008 in the Lithia, FL vineyard and data was recorded weekly from September 7\textsuperscript{th} to October 26\textsuperscript{th}. The experiment was arranged as a complete randomized block design with four treatments that were replicated four times. Each plot consisted of 25 grape plants in five rows (five plants per row). Treatments consisted of wax dispensers containing GRB pheromone, prepared as described previously, deployed at different densities: one dispenser per five plants (five total), one dispenser per plant (25 total or 735 per ha), 10 dispensers per plant (250 total) or zero dispensers, and a no pheromone control. One monitoring trap was deployed per plot on the centermost plant 1.5 m above the crown. Traps were checked weekly by counting the number of GRB captured and replacing sticky card inserts. The degree of trap catch disruption was determined by comparing trap catch to the control as described in Weihman and Liburd (2006).

**Determining the effect of pheromone point source aggregation on GRB disruption**

Because competitive attraction was expected to be the most important underlying mechanism for mating disruption in GRB, it was hypothesized that a treatment with a high number of deployment sites would result in lower trap catch than a treatment with few deployment sites. The experiment was established on August 21\textsuperscript{st}, 2009 in the Bradenton, FL vineyard and data were recorded weekly from August 23\textsuperscript{rd} to October 24\textsuperscript{th}. The experiment was arranged as a randomized complete block with four treatments replicated four times. Wax dispensers, prepared using GRB pheromone, were deployed at a total density of 25 to 26 per plot; distribution of these dispensers
was varied from a highly clumped distribution to one that was highly dispersed. Plots were rectangular and consisted of 25 grape vines, five plants per row in five rows. Treatments were: untreated control (without dispensers), one dispenser per plant (for a total of 25 dispensers per 25 vines), two dispensers on every other plant (for a total of 26 dispensers per 13 vines), and five dispensers on each corner plant and five on the most central plant (for a total of 25 dispensers per five vines). One monitoring trap was deployed on the centermost plant 1.5 meters above the crown per plot. For the five dispenser location/plot treatment, the trap was not placed on the centermost plant because there were five dispensers deployed on it. In this case, the trap was placed one vine from the centermost location in any arbitrary direction. Each trap was checked weekly and the degree of trap catch disruption was assessed as described earlier.

**Determining load rate of pheromone in dispensers for optimal disruption**

This experiment was established on August 22\textsuperscript{nd}, 2009 in the Lithia, FL vineyard and data were recorded weekly from August 23\textsuperscript{rd} to October 24\textsuperscript{th}. The experiment was arranged as a randomized complete block with four treatments replicated four times. The treatments were wax (SPLAT, as described above) dispensers loaded with 0, 0.5, 2.5, and 5 mg of pheromone. Each treatment was deployed at the density of one per plant (735/ha) for a total of 25 dispensers per plot. Each square plot consisted of 25 grape plants in five rows, with five plants per row. One monitoring trap was deployed on the centermost plant 1.5 m above the crown. The traps were checked weekly when the number of trapped was males counted, and the sticky card was replaced. The degree of orientation disruption was determined by comparing catch of traps in treated plots to that of traps in control traps of the same block (Weihman and Liburd 2006). It was
hypothesized that disruption of male orientation to traps would increase as pheromone load rate per dispenser was increased.

**Assessing effect of orientation disruption on next season’s GRB population**

It was hypothesized that the orientation disruption experiments would have a negative effect on GRB population as males’ ability to find mates would diminish or disappear, and that this decrease in population would be detectable via decreased catch in the control traps located within blocks of the orientation disruption experiments from 2008 to 2009. In the two 33m x 75m plots that offset from the rest of the Lithia, FL vineyard by >60 meters, four monitoring traps (2 per plot) were hung at least 20 m apart. These traps were checked weekly, the trapped GRB counted and the sticky card replaced. This monitoring study was repeated for both years. In 2008, the traps were deployed on August 30th and data collection began a week later on September 7th. In 2009, these traps were deployed on September 2nd and data collection began a week later on September 9th. In 2008, these monitoring traps were removed after four weeks of data had been collected. In 2009 the traps were maintained in the field for the entire season. It was expected that these two plots, >60 meters away from the rest of the vineyard, would not receive any significant synthetic pheromone plume penetration from experiments in the main vineyard and orientation would be relatively unaffected. Any decline resulting from orientation disruption experiments in the main vineyard therefore might not occur in these plots.

**Statistics.** Data means were analyzed by ANOVA and differences among means were determined by Tukey’s means separation test (P<0.05) (SAS/STAT v 9.2, SAS Institute 2009).
Quantification of pheromone release rate from dispensers used in mating disruption investigations

The objective of this experiment was to quantify the release rate of pheromone from SPLAT dispensers used in mating disruption and trapping studies. The experiment was established on August 21\textsuperscript{st}, 2008. Individual dispensers containing the GRB pheromone blend were dispensed as 1 mg dollops (as described above) onto acetate strips. Each dispenser was weighed and the acetate strip stapled to a numbered wooden board. Five blocks of 14 pheromone treated of wax dispensers were prepared in this manner as well as 12 blank (negative control) dispensers, which were deployed in the Lithia, FL vineyard in an area separated from the main vineyard by >60m for the duration of GRB flight.

Initially samples were collected once each day to allow detection of an exponential decay release rate at test onset. Thereafter, the sampling frequency decreased, finally reaching one sample collection per week. One week’s sampling consisted of one treated dispenser per block, for a total of five replicates, and one untreated control dispenser from a randomly selected block. After removal, the dispensers were placed into separate glass vials and transported on ice from the experimental site in Lithia, FL to the lab in Gainesville, FL where each vial received 5 ml of acetonitrile and internal standard, hexadecyl acetate (193.4 ng/\textmu l, Sigma chemical, St. Louis Missouri. 99\% purity). The samples were then stored in the freezer until they could be tested for pheromone retention using gas chromatography. In addition to the collected dispensers, data on humidity, rainfall, wind speed, as well as temperature high, low, and average were recorded by a nearby weather station in Lithia, FL.
Pheromone within samples was quantified using a gas chromatograph (GC) (HP-6890, Hewlett-Packard, Palo Alto, CA). The GC was equipped with a DBWAXETR polar column (model 122-7332, J&W Scientific Folsom, CA) of length 30 m and internal diameter 250µm. The initial GC temperature was held at 130°C for 2 min and then ramped at a rate of 2.5 C/min to 160°C, where it was held for 2 min. The program then ran at 40°C/min to a final temperature of 230°C. The carrier gas, He, entered the column at 20 psi. The pheromone content of the samples was calculated using the internal standard method.

Statistics. Release rate was modeled with multiple linear regression (SAS/STAT v 9.2, SAS Institute 2009). As the initial model contained non-significant parameters, the model was revised multiple times. At each revision, the single parameter with the highest non-significant p-value was removed (α=.05), and the model run again until no non-significant parameters were left.

Results

Pheromone Attraction Experiments

Determining the relative attractiveness of pheromone blends with trapping

Both the control and the 1 mg of leopard moth pheromone treatment resulted in zero catch of male GRB for the duration of the experiment; therefore, these data were not included in the analysis. The negative binomial model fit the data adequately well (Pearson $\chi^2 = 32.55$, $X^2 = 40.11$). Weekly mean male catch was significantly affected by treatment (Figure 4.1) ($\chi^2 = 31.92$, d.f. = 1, $P < .0001$) but not by week ($\chi^2 = 2.66$, d.f. = 2.66, $P=.264$) or block ($\chi^2 = 3.45$, d.f. = 5, $P=.631$). GRB pheromone attracted
significantly ($\chi^2 = 47.32$, d.f. = 1, $P < .0001$) more GRB males per trap per week than the LM pheromone (Table 4-1).

**Direct observation of male GRB response to pheromone blends in the field**

**Visits.** We found that the total number of GRB male visits to dispensers was significantly ($F=33.64$, d.f. = 1, $P=.0285$) affected by treatment (Figure 4-2). GRB pheromone attracted >3 times more male moths (9.5±2.5 vs. 2.5±0.58) than the MP pheromone ($T=6.08$). Neither day of observation ($F=1.11$, d.f.=3, $P=.51$) nor the order in which the pheromone treatments were presented ($F=1.00$, d.f.=1, $P=.42$) affected the number of visits by moths.

**Visit duration.** The log transformation of visit duration differed significantly as a result of treatment ($F=5.25$, d.f.=1, $P=.0269$). The logs of visit durations of males to GRB pheromone lures were significantly higher than those to LM pheromone lures (Figure 4-3). Both day of observation ($F=2.35$, d.f.=3, $P=.086$) and the order of treatment presentation ($F=0$, d.f.=1, $P=.976$) were non-significant.

**Proximity.** The number of moths approaching within 10 cm of the lure was not significantly different between treatments ($F=3.5$, d.f.=1, $P=.0680$) and that neither order of pheromone treatment presentation ($F=0.47$, d.f.=1, $P=.496$) nor day of observation ($F=1.53$, d.f.=3, $P=.221$) affected frequency of this behavior during observation periods.

**Contact.** The number of moths making physical contact with pheromone lures was not affected by treatment ($F=1.57$, d.f.=1, $P=.218$), order of pheromone treatment presentation ($F=0.08$, d.f.=1, $P=.781$) or day of observation ($F=0.87$, d.f.=3, $P=.463$).
**Determining the relative attractiveness of splat vs. septa**

Traps baited with rubber septa and deployed singly within plots caught an average of 5.5±5.1 males/trap while paired septum-baited traps caught a similar (t= -0.242, d.f.=2, P=.813) average number of males (5.5±2.9) (Table 4-2). SPLAT-baited traps caught fewer moths than septum-baited traps in both single trap (1.8±1.2) and 2-trap plots (2.3±2.7). The difference between SPLAT-baited trap catch in single and 2-trap plots was non-significant (t= -0.479, d.f.=14, P=.639). Dispenser type was significant (F=12.15, d.f.=1, P= .002) while block (F=2.5, d.f = 5, P=.061) and week (F=3.02, d.f.=3, P=.052) were not significant. Overall, septum-baited traps caught significantly (T=1.93, d.f. = 22, α=.05) more moths (5.25±3.93) than SPLAT-baited traps (2.00±1.49 traps) (Figure 4-4).

**Pheromone Disruption Experiments**

**Determining the effects of pheromone blend on disruption efficacy**

The GRB and LM pheromone treatments both resulted in complete trap shutdown for the entire season. Given a dispenser density of five per plant, or approximately 3000 per hectare, and a load rate of 5 mg pheromone per 1 g of SPLAT compound, GRB and LM pheromone are equally effective. Both treatments were determined to be significantly different (0±0) (χ²= 87.27, d.f.=1, P<.0001) from the control (Table 4-3).

**Determining the effects of dispenser density on disruption efficacy**

Mean weekly trap catch declined with increasing dispenser density and all treatments resulted in >95% mating disruption (Table 4-4). The negative binomial model fit the data adequately well (Pearson χ²= 126.1, d.f.=114, α=.05). The effect of both week (χ²=39.88, d.f.=7, P<.0001) and dispenser density (χ²=123.1, d.f.=3, P<.0001) on
catch of male GRB was significantly different from zero. The effect of block was not significantly different from zero ($\chi^2=6.21$, d.f.=3, $P=.102$). Traps within the control plots caught significantly more moths ($7.06 \pm 3.51$) than traps in plots of all other treatments (Figure 4-5). Traps in plots containing five pheromone dispensers caught more moths ($0.44 \pm 0.82$) than traps in control plots, but fewer than traps in plots receiving 250 dispensers ($0\pm0$). Moth catch in plots receiving 25 dispensers ($0.03 \pm 0.06$) was numerically higher than that of traps in plots with five dispensers, but not significantly so. Moth catch of plots with 25 and 250 dispensers could not be compared statistically because sufficient variance was missing. They therefore cannot be considered different from one another and are considered part of the same mean group. Traps in plots with 250 dispensers caught the fewest moths, but not significantly fewer than traps in 25 dispenser plots (Table 4-4).

**Determining the effect of pheromone point source aggregation on GRB disruption**

The negative binomial model fit the trap capture data adequately (Pearson $\chi^2=143.38$, d.f.=129, $\alpha=.05$). The effect of dispenser aggregation ($\chi^2=39.73$, d.f.=3, $P<.0001$), week ($\chi^2=39.73$, d.f.=8, $P=.0029$), and block ($\chi^2=11.29$, d.f.=3, $P=.0103$) on catch of male GRB was significantly different from zero. Pairwise comparisons were made using lsmeans in the Genmod procedure. We found that traps in control plots caught significantly more moths than traps in all other treatment groups (Figure 4-6). Traps in plots that received the 25 dispenser site treatment caught significantly fewer (0.03±0.06) than all other treatments and resulted in a seasonal average of 97.2% mating disruption. (Table 4-5). Traps in plots with five dispenser sites (mean catch 0.47±0.071) and 13 dispenser sites (0.28 ± 0.49) caught significantly more moths than
traps in plots with 25 dispensers but significantly fewer than traps in control plots. The five dispenser sites treatment resulted in 51.9% mating disruption while 13 dispenser sites treatment resulted in 80.61% mating disruption, but these treatments were not significantly different from one another (Table 4-5). Traps in control plots caught significantly more moths than any other treatment.

**Determining load rate of pheromone in dispensers for optimal disruption**

The negative binomial model fit the data adequately (Pearson $\chi^2 = 123.73$, d.f.=144, $\alpha=.05$). The effect of load rate ($\chi^2 = 28.93$, d.f.=3, $P<.0001$) and block ($\chi^2 = 30.88$, d.f.=3, $P<.001$) on catch of male GRB was significantly different from zero while week was not ($\chi^2 = 14.42$, d.f.=8, $P=.1080$). Pairwise comparisons were made using the lsmeans subroutine in the Genmod procedure. The control was significantly different from all treatments (Figure 4-7). Dispensers loaded with 0.5 mg of GRB pheromone caused an average of 60.4% orientation disruption, which was significantly higher than the control but not significantly different from dispensers loaded with 2.5 mg and significantly lower than dispensers treated with 5 mg of pheromone. Dispensers loaded with 2.5 mg caused 90% orientation disruption which was similar to load rates 0.5 and 5 mg. Dispensers treated with 5 mg of GRB pheromone caused significantly higher orientation disruption than the control and dispensers with the 0.5 mg loading rate, but this level of disruption was not significantly higher than obtained with dispensers containing the 2.5 mg load rate (Table 4-6).

**Assessing effect of orientation disruption on next season’s GRB population**

Monitoring traps were deployed in the side vineyard and data were collected for only 4 weeks, so only the data taken from orientation experiments during the same four
weeks was included in this analysis. In 2008, control traps of the orientation disruption experiment in the main vineyard at the Lithia, FL location caught 7.88 ± 4.23 per week while control traps of the experiment in Bradenton, FL caught 7.19 ± 4.29 (Figure 4-8). The difference in weekly mean moth catch was not significant. The monitoring traps in the two side plots in Lithia, FL caught an average of 15.31 ± 6.74 moths which was significantly more than both experimental control groups. In 2009, control traps of the orientation disruption experiment in the main vineyard at the Lithia, FL location caught 2.81±0.53 GRB per week while control traps of the experiment in Bradenton caught 2.38 ± 0.58 (Figure 4-9), the difference between them was not significant, but the difference between the 2008 and 2009 weekly mean catch was significantly different. The traps in the two side plots in Lithia, FL caught an average of 15.19 ± 2.02 in 2009. This was not significantly different from the catch in the same plots the year before, but was significantly different from the means of both experimental control groups that year (Table 4-7).

**Quantification of pheromone release rate from dispensers used in mating disruption investigations**

In the development of the multiple linear regression model for GRB pheromone release rate, the initial model ($F= 37.06$, d.f.=8, $P<.001$, adj $r^2= 0.85$), containing days passed since initial deployment, initial dispenser weight, intercept, and multiple weather parameters, underwent several revisions. At each revision, the single parameter with the highest non-significant p-value was removed ($\alpha=.05$), and the model was run again. In this way, weekly mean low temperature ($t=0.07$, d.f.=1, $P=.942$), total weekly rainfall ($t=-0.39$, d.f.=1, $P=.698$), average wind speed ($t=-0.38$, d.f.=1, $P=.708$), average weekly high temperature ($t=1.17$, d.f.=1, $P=.248$), and humidity ($t=-0.69$, d.f.=1, $P=.495$) were
removed from the model. The final model ($F=104.63$, d.f.=3, $P<.0001$, adj. $r^2 = .859$) (Figure 4-10) shows release rate of GRB pheromone is dependent on three of the parameters tested; number of days passed since dispenser deployment ($t=-17.42$, d.f.=1, $P<.0001$), average weekly temperature ($t=-2.67$, d.f.=1, $P=.01$) and initial dispenser weight ($t=4.92$, d.f.=1, $P<.0001$). The intercept was also significant ($t=5.84$, d.f.=1, $P<.0001$). The release rate predicted by the model is $77.44\pm75.82 \mu g$ per gram of impregnated SPLAT compound, given similar average weekly temperatures.

**Discussion**

All dispenser densities, two load rates and one level of aggregation tested were sufficient for $\geq 90\%$ orientation disruption. Two different densities, approximately 700 and 7000 dispensers/ha, of dispensers treated with GRB pheromone caused $100\%$ orientation disruption. The lower density, of the two, was shown to be effective several times during both years of the study; causing $100\%$, $99\%$, $97\%$ and $92\%$ orientation disruption. Other treatments using GRB pheromone shown to be effective were approximately 150/ha loaded with 5 mg per dispenser and approximately 700/ha with load rate of 2.5 mg per dispenser (Table 4-8).

The experiment in 2008 comparing GRB and LM pheromone blends showed that both are equally effective disruptants when deployed at approximately 3500 dispensers per hectare and with an initial load rate of approximately 5 mg per 1 g dollop (Table 4-8). It was also shown in multiple experiments in 2007 and 2008 (Table 4-1 and Figures 4-1 to 4-3). These results show that, given similar population levels, as dispenser density increases the attractiveness of each dispenser becomes less important. A male can only visit an unknown finite number of pheromone point sources, synthetic or natural, in his lifetime, $M_v$. With a finite number of males, $M_n$, only an unknown number
of visits, \( V_p \), is possible \( (M_v \cdot M_n = V_p) \). If each dispenser is less attractive for any reason (ie: decreased load rate, less attractive pheromone blend), the number of visits each dispenser will generate, \( D_v \), will decrease. As long as this decrease is made up for by the number of dispensers in the field, \( D_n \), (or as long as \( D_v \cdot D_n \geq V_p \)) the total amount of orientation disruption should remain unaffected. This reasoning follows the assumption that the main mechanism for mating disruption is competitive attraction.

These experiments support that assumption. Miller et al (2006) cite three main predictions for the competitive attraction model: that 1) target males will be attracted to pheromone point sources 2) increasing the number of point sources will increase orientation disruption, defined as 1- (mean catch per trap in treated plot/ mean catch per trap in control plot, and 3) this increase will not be linear. Our experiments show that all three of these predictions are true for our system.

The prediction that males will be attracted to the pheromone point sources is true. The pheromone point sources used by Weihman (2006) to cause 100% orientation disruption were shown to be attractive (Figures 4-2 and 4-3) as were the dispensers responsible for orientation disruption in our experiments (Table 4-2 and Figure 4-4). Miller et al. (2006) predict that under the competitive attraction model, initially the impact of small increases of dispenser density is great but that the return for a given increment in dispenser density progressively diminishes. For the camouflage hypothesis, the efficacy would increase slowly as a function of dispenser density, and then increase dramatically as plume coverage approached 100% (Figure 4-11). Our dispenser density experiment shows both predictions two and three of the competitive attraction model are accurate for our system; increasing dispenser density increases orientation disruption.
and in the non-linear fashion predicted (Figure 4-12). Therefore, it is likely that competitive attraction is mainly responsible for the orientation disruption observed in our experiments and that camouflage is not a factor in our system.

The experiment run in 2009 testing the effects of dispenser aggregation used a dispenser density of approximately 700/ha. This experiment showed that the effectiveness of the dispenser density was dependant on the degree of dispersal throughout the experimental plot. The trend found was that as dispensers were more evenly dispersed, or less aggregated, the more effective the same dispenser density became; the most aggregated treatment was 52% effective while the most dispersed treatment was 97% effective. It is acknowledged that in this experiment, the treatment giving 52% effectiveness was not found to be significantly different from one that gave 81% orientation disruption, however, even a 3% decrease in orientation disruption is enough to have profound impacts on the degree of mating disruption of a lepidopteran pest (Miller et al. 2006).

In 2008, the treatment of approximately 140/ha was responsible for 95% orientation disruption while in 2009, a treatment with the same load rate and a density of approximately 700/ha caused 52% disruption (Table 4-8). While plots of both treatments had the same number of treated plants, five per plot, the number of dispensers per treated plant was five times higher in the treatment that resulted in lower orientation disruption. This could be a result of the five dispensers as a cluster being less attractive to GRB males than a single dispenser and therefore decreasing orientation disruption. An experiment comparing the number of visits and visit duration of GRB males to these two treatments would be sufficient to disprove this hypothesis.
The number of moths caught in control plots of experimental areas of both vineyards decreased from 2008 to 2009. Control traps within the experimental area of Lithia’s vineyard caught 7.88 ± 4.23 in 2008 and 2.81 ± 0.53 in 2009. The difference between the two was considered significant and amounts to a 64% reduction. Control traps in Bradenton’s vineyard caught 7.19 ± 4.29 in 2008 and 2.38 ± 0.58 in 2009. The difference was significant and amounts to a 67% reduction. The control traps 60m outside Lithia’s vineyard caught 15.31 ± 6.74 in 2008 and 15.19 ± 2.02 in 2009. The weather conditions, grape varieties, cultural practices, and proximity to nearby wooded areas were the same for both areas of the Lithia vineyard. The only perceivable difference between the two areas was the presence or absence of experimental mating disruption plots. Given the similar decrease in control trap catch at the Bradenton, it seems likely that amount of orientation disruption resulting from the mating disruption experiments responsible for a decrease in reproductive success and a subsequent decrease population.

It is possible the reason treatment in only portions of the vineyard caused such reduction in overall population is that a large amount of the pheromone released by dispensers remained in the vineyard by continually being adsorbed and reemitted on the surface of grape leaves (Sauer and Karg 1998) or that dispensers were more attractive than GRB females. A study examining the relative attractiveness of a female to the disruption dispensers deployed as well as the lures used in our monitoring traps would be incredibly useful to tease apart the effects of these two hypotheses, as well as explore discrepancies in the estimation of orientation disruption occurring in pheromone treated areas. If females are more attractive than monitoring traps, orientation disruption
could be overestimated while the opposite may happen if monitoring traps are more attractive than females (Miller et al. 2006).

The most obvious explanation for the decrease in population would be the reduction in the total number of mating GRB pairs; however, there is another explanation. Several studies have shown that fecundity significantly diminishes with the age of both males and females (Miller et al 2006, references therein). This was also shown to be the case specifically for GRB by Pritchard (2004). Her study showed that females mated seven days after eclosion laid, on average, fewer than 400 eggs with lower than 60% fertility while females mating on the day of eclosion laid approximately 500 eggs with over 80% fertility. These explanations are not mutually exclusive, and it seems likely that both bear some degree of responsibility for our results.

In conclusion, these experiments show that mating disruption of GRB has great potential for controlling this pest. Deployment of pheromone into portions of a vineyard resulted in approximately 65% decrease in population the subsequent year. Our release rate data suggest that 77.44±75.82 µg of pheromone are released per day and that this was sufficient for season long control. In order to maintain that release rate for a season ten weeks long, this would require a minimum initial load rate of 5.39 mg of pheromone per gram of release matrix. However, our experiments show an initial load rate of between 2.5 and 0.5 mg per gram of release matrix is enough to be effective. The load rate experiment showed no difference between 5 mg and 2.5 mg (Table 4-8) and no difference between 2.5 and 0.5 mg initial pheromone load. There is likely a load rate between 2.5 and 0.5 that would be found to be no different from 5mg per gram of release matrix and this value may be the most cost effective for grape growers looking
to adopt this system to control their pest populations. However, when decreasing initial load rates, it must be kept in mind that there must be enough pheromone to last until and through peak emergence in order to maximize efficacy. This can be done by increasing load rate or and deploying as soon as GRB are detected or by monitoring for the beginnings of peak emergence, and the deployment of release matrix with lower initial load rates. If the rate of pheromone release does not depend on how much pheromone remains, an initial load rate of 0.5 mg will only provide approximately 6.5 days of disruption. In our load rate experiment, 0.5 mg of pheromone only totally suppressed catch in three of ten weeks while 2.5 mg/g totally suppressed catch in 7 of 10 weeks.

Our experiments also show that as few as one dispenser every five plants, approximately 140/ha, is enough to cause 95% orientation disruption. This is a lower dispenser density than is reported for control of Oriental fruit moth (Stelinksi et al. 2005, Trimble et al. 2001) and Codling moth (Epstein et al. 2006) in apple orchards and GRB in Arkansas vineyards (Johnson et al. 1991). The success of mating disruption, however, depends on pest population density and therefore when choosing a dispenser density for pest suppression, current pest population levels must be considered. Our experiments also showed that when deploying pheromone dispensers, it is best to disperse them throughout the treatment area as evenly as possible. This seems likely to be true for any mating disruption program with competitive disruption as the main mechanism.
Table 4-1. Effect of pheromone blend on season mean GRB catch in 2007

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Catch ± STD</th>
<th>Mean Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 ± 0</td>
<td>n/a</td>
</tr>
<tr>
<td>1 mg LM</td>
<td>0 ± 0</td>
<td>n/a</td>
</tr>
<tr>
<td>10 mg LM</td>
<td>0.94 ± 0.9</td>
<td>A</td>
</tr>
<tr>
<td>1 mg GRB</td>
<td>7 ± 4.3</td>
<td>B</td>
</tr>
</tbody>
</table>

Note: Difference among means was determined using differences of least squares means (P < 0.05)

Figure 4-1. Effect of pheromone blend on weekly mean GRB male catch in 2007. Treatments marked with different letters were found to be significantly different at the α=0.05 level.
Figure 4-2. Effect of pheromone type on mean number of visits per observation session 2007. Treatments marked with different letters were found to be significantly different at the $\alpha=0.05$ level.

Figure 4-3. Effect of pheromone blend on mean log of visit duration per observation session in 2007. Treatments marked with different letters were found to be significantly different at the $\alpha=0.05$ level.
Figure 4-4. Effect of dispenser type on weekly mean GRB catch per trap in 2009. Treatments marked with different letters were found to be significantly different at the $\alpha=0.05$ level.

Table 4-2. Effect of dispenser type on mean GRB catch per trap in 2009

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Catch ± STD</th>
<th>Mean Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septum</td>
<td>5.25 ± 4</td>
<td>A</td>
</tr>
<tr>
<td>SPLAT</td>
<td>2 ± 2</td>
<td>B</td>
</tr>
</tbody>
</table>

Note: Differences were determined by Tukey’s means separation test ($\alpha=0.05$).

Table 4-3. Effect of GRB and LM pheromones on season mean catch per trap of GRB males and orientation disruption in 2008

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Catch ± STD</th>
<th>% disruption</th>
<th>Mean Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.97 ± 5.4</td>
<td>0</td>
<td>A</td>
</tr>
<tr>
<td>GRB</td>
<td>0 ± 0</td>
<td>100</td>
<td>B</td>
</tr>
<tr>
<td>LM</td>
<td>0 ± 0</td>
<td>100</td>
<td>B</td>
</tr>
</tbody>
</table>

Note: Differences were determined by differences of least squares means ($\alpha=0.05$).
Table 4-4. Effect of pheromone point source density on season mean catch per trap of GRB males and orientation disruption in 2008

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Catch ± STD</th>
<th>% Disruption</th>
<th>Mean Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.06 ± 3.51</td>
<td>0</td>
<td>A</td>
</tr>
<tr>
<td>5</td>
<td>0.44 ± 0.82</td>
<td>95</td>
<td>B</td>
</tr>
<tr>
<td>25</td>
<td>0.03 ± 0.06</td>
<td>99</td>
<td>B C</td>
</tr>
<tr>
<td>250</td>
<td>0 ± 0</td>
<td>100</td>
<td>C</td>
</tr>
</tbody>
</table>

Note: Differences were determined by differences of least squares means (α=0.05).

Figure 4-5. Effect of pheromone point source density on weekly mean catch per trap of GRB males in 2008

Table 4-5. Effect of pheromone point source aggregation on season mean catch per trap of GRB male and orientation disruption in 2009

<table>
<thead>
<tr>
<th># of deployment sites</th>
<th>Mean Catch ± STDV</th>
<th>% Disruption</th>
<th>Mean Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.97 ± 1.1</td>
<td>0</td>
<td>A</td>
</tr>
<tr>
<td>5</td>
<td>0.47 ± 0.71</td>
<td>52</td>
<td>B</td>
</tr>
<tr>
<td>13</td>
<td>0.28 ± 0.49</td>
<td>81</td>
<td>B</td>
</tr>
<tr>
<td>25</td>
<td>0.03 ± 0.06</td>
<td>97</td>
<td>C</td>
</tr>
</tbody>
</table>

Note: Differences were determined by differences of least squares means (α=0.05).
Table 4-6. Effect of load rate on season mean catch per trap and % orientation disruption in 2009

<table>
<thead>
<tr>
<th>Load rate (mg)</th>
<th>Mean Catch ± STD</th>
<th>% Disruption</th>
<th>Mean Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.13 ± 1.62</td>
<td>0</td>
<td>A</td>
</tr>
<tr>
<td>0.5</td>
<td>0.35 ± 0.52</td>
<td>60</td>
<td>B</td>
</tr>
<tr>
<td>2.5</td>
<td>0.13 ± 0.25</td>
<td>90</td>
<td>B,C</td>
</tr>
<tr>
<td>5</td>
<td>0.08 ± 0.15</td>
<td>92</td>
<td>C</td>
</tr>
</tbody>
</table>

Note: Differences were determined by differences of least squares means (α=0.05).
Figure 4-7. Effect of load rate on weekly mean catch per trap of GRB males in 2009

Table 4-7. Mean catch per trap for all control traps in both vineyards for both years of mating disruption experiments

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatments</th>
<th>Mean catch ± STDV</th>
<th>Mean group</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>Lithia main</td>
<td>7.88 ± 4.23</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Lithia side</td>
<td>15.31 ± 6.74</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>Bradenton</td>
<td>7.19 ± 4.29</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Lithia main</td>
<td>2.81 ± 0.53</td>
<td>C</td>
</tr>
<tr>
<td>2009</td>
<td>Lithia side</td>
<td>15.19 ± 2.02</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>Bradenton</td>
<td>2.38 ± 0.58</td>
<td>C</td>
</tr>
</tbody>
</table>
Figure 4-8. Mean catch per trap of untreated plots for all experiments in 2008. Treatments marked with different letters were found to be significantly different at the $\alpha=0.05$ level.

Figure 4-9. Mean catch per trap of control plots for all experiments in 2009. Treatments marked with different letters were found to be significantly different at the $\alpha=0.05$ level.
Figure 4-10. Release rate of GRB pheromone from 1g SPLAT dispensers in 2008

Table 4-8. Results of all orientation disruption experiments

<table>
<thead>
<tr>
<th>Disruptant</th>
<th>Dispenser density (approximate number per ha)</th>
<th>Load rate (mg)</th>
<th>No. of deployment sites</th>
<th>Orientation disruption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LM</td>
<td>700</td>
<td>5</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>GRB</td>
<td>700</td>
<td>5</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>GRB</td>
<td>140</td>
<td>5</td>
<td>25</td>
<td>95</td>
</tr>
<tr>
<td>GRB</td>
<td>700</td>
<td>5</td>
<td>25</td>
<td>99</td>
</tr>
<tr>
<td>GRB</td>
<td>7000</td>
<td>5</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>GRB</td>
<td>700</td>
<td>5</td>
<td>5</td>
<td>52</td>
</tr>
<tr>
<td>GRB</td>
<td>700</td>
<td>5</td>
<td>13</td>
<td>81</td>
</tr>
<tr>
<td>GRB</td>
<td>700</td>
<td>5</td>
<td>25</td>
<td>97</td>
</tr>
<tr>
<td>GRB</td>
<td>700</td>
<td>0.5</td>
<td>25</td>
<td>60</td>
</tr>
<tr>
<td>GRB</td>
<td>700</td>
<td>2.5</td>
<td>25</td>
<td>90</td>
</tr>
<tr>
<td>GRB</td>
<td>700</td>
<td>5</td>
<td>25</td>
<td>92</td>
</tr>
</tbody>
</table>
Figure 4-11. Expected effect of increasing dispenser density on orientation disruption

Figure 4-12. Effect of increasing dispenser density on orientation disruption
LIST OF REFERENCES


Dutcher, J.D. and J.N. All. 1978b. Survivorship of the grape root borer in commercial grape vineyards with contrasting cultural practices. J. Econ. Entomol. 71:751-754.

Dutcher, J.D. and J.N. All. 1979. Damage impact of larval feeding of the grape root borer in a commercial Concord grape vineyard. J. Econ. Entomol. 72:159-161.


Louis, F. and K.J. Schirra. 2001. Mating disruption of Lobesia botrana (Lepidoptera: Tortricidae) in vineyards with very high population densities. IOBC wprs Bulletin 24(2):75-79


http://www.wineamerica.org/newsroom/docs/Wine%20Industry%20Fact%20Sheet%202009.pdf

BIOGRAPHICAL SKETCH

Will Sanders is a M.S. student in the Entomology and Nematology Department at the University of Florida, Gainesville. He earned his bachelor’s degree at New College of Florida in biology with a focus on entomology in August 2006. His thesis investigated possible explanations for susceptibility of European honey bees to small hive beetle. Presently he is pursuing a M.S. in Integrated Pest Management (IPM). The focus of his research is to explore sustainable control methods to develop a comprehensive IPM plan for control of grape root borer (GRB) in Florida. Will has presented his research findings in regional conferences, formal extension meetings, and numerous informal consultations. In the fall of 2008, he obtained a grant from The Center for Viticulture and Small Fruit Research at Florida A&M University to fund the behavioral portion of his research and in September 2009 Will obtained a grant from SARE to fund a portion of his research. Will taught ENY 3005L, the Principles of Entomology Lab, for two years.