

**PLANT-INSECT INTERACTIONS BETWEEN FEMALE DOGWOOD BORER  
AND APPLE**

Daniel Lee Frank

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State  
University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy  
In  
Entomology

J. Christopher Bergh  
Tracy C. Leskey  
Douglas G. Pfeiffer  
Scott M. Salom  
Aijun Zhang

December 8, 2009  
Blacksburg, Virginia

Keywords: *Synanthedon scitula*, *Malus domestica*, host plant volatiles, olfaction, rearing,  
geostatistics, kriging, antennal morphology, sensilla, GC-EAD

## **Plant-insect interactions between female dogwood borer and apple**

**Daniel Lee Frank**

### **ABSTRACT**

A rearing methodology for dogwood borer was developed, using standardized procedures at each developmental stage. These methods enabled the establishment of a laboratory colony and efficient production of synchronized cohorts of each of its lifestages throughout the year for specific experimental needs.

The behavioral repertoire shown by mated female dogwood borer in an apple orchard was characterized and quantified and the diel periodicity with which those behaviors occurred was determined. Mated females were easily recognized, based on their characteristic casting flight directed toward areas below the graft union of apple trees, and were observed more frequently during the late afternoon and evening. Casting flight, probing with the ovipositor, and oviposition were the most frequent behaviors observed, but the duration of those behaviors was relatively short compared with the much lengthier periods of resting behavior that typically occurred within the canopy.

Data from a previous, three-year study in two newly planted apple orchards were subjected to geostatistical analyses to examine the temporal and spatial patterns of infestation by larval dogwood borer and to gain further information about the spatial scales at which oviposition occurs. There were moderate to high degrees of aggregation of dogwood borer infestations on neighboring apple trees, with ranges of spatial dependence from 7.50–19.87 m. No directionality was observed in the spatial autocorrelation of infestation and it appears that females utilized oviposition sites equally along and across orchard rows. The aggregated nature of infestations requires that random, independent samples must be taken from a number of sample pairs at distances greater than the range of spatial dependence to ensure that sample data are not autocorrelated. Alternatively, an efficient sampling program for mapping dogwood borer infestation can be achieved by limiting sample points to distances within the range of spatial dependence. These sample points can be used in interpolating algorithms, such as

kriging, to predict infestation at unsampled locations in space for use in site-specific pest management programs.

The external morphology of male and female dogwood borer antennae and their sensilla were examined using light and scanning electron microscopy to characterize, measure and compare the types, number, and distribution of sensilla. Although the general shape and size of male and female antennae were similar, those from females possessed a greater number of generally smaller antennal flagellomeres. The flagellum of both male and female antennae contained seven sensillum types including auricillica, basiconica, chaetica, coeloconica, squamiformia, styloconica, and three subtypes of sensilla trichoidea. With the exception of sensilla basiconica, which were present in roughly equal numbers on male and female antennae, all other sensillum types were significantly more abundant on female antennae. The antennae of female dogwood borer appear well equipped to perceive olfactory stimuli, based on the types and number of sensilla present.

Coupled gas chromatography and electroantennogram detection (GC-EAD) analyses of headspace collections from damaged and undamaged tissues from apple and dogwood trees were conducted to examine and compare the antennal responsiveness of female dogwood borer to host plant volatiles. A total of 16 and 9 compounds from apple and dogwood tissues, respectively, consistently elicited an antennal response in females. There were no differences in the response of antennae from virgin and mated females, and the amplitude of the female response to host odors was greater than that of males. Six compounds were identified from the headspace collections from apple trees, four of which (octanal, nonanal, decanal, and methyl salicylate) were identified from all apple tissues sampled. A novel compound,  $\alpha$ -bergamotene, was identified from injured apple bark, from apple burr knots infested with dogwood borer larvae and from larval dogwood borer frass, and appears to be produced by apple trees in response to injury. Another novel compound, methyl-2,4-decadienoate, was identified from infested burr knot tissue and appears to be produced in response to an insect-plant interaction. Two compounds, hexanoic and nonanoic acid, were identified from headspace collections from dogwood trees.

Numerous approaches were used to examine the behavioral response of mated female dogwood borer to host plant headspace collections and to individual compounds from those collections that elicited a strong and repeatable antennal response. Under both natural and semi-natural conditions in the field and in laboratory bioassays, neither attraction/orientation or consistent oviposition were documented and it is apparent that correlating the electrophysiological and behavioral responses of mated female dogwood borer to olfactory stimuli from their host plants will require further research on bioassay development.

## ACKNOWLEDGMENTS

I would like to thank my major advisors, Dr. Chris Bergh and Dr. Tracy Leskey, for their support, guidance and assistance throughout this project. I have become a better scientist as a result of the training I have received in their labs. I thank Dr. Aijun Zhang for the training and assistance that he provided in GC-EAD, and for allowing me the opportunity to work in his lab whenever I needed. I am grateful for the invaluable advice and assistance provided by my committee members Dr. Douglas Pfeiffer and Dr. Scott Salom. I extend a big thank you to Dr. Carlyle Brewster for his assistance and critical review of Chapter 4. I am grateful to Jean Engelman, Starker Wright, and Torri Hancock for the expert technical assistance they provided during the course of my experiments. I would like to thank Sharon Jones for her assistance with antennal preparations and operation of the SEM. I am grateful to all the summer students at the AHS-AREC for their help during the field season. I thank J. Marker, J. Snapp, and R. Solenberger for allowing access to their orchards. I would like to thank the faculty, staff, and students of the Department of Entomology at Virginia Tech for their helpfulness and friendship. I would especially like to thank my family for all their encouragement and love. Finally, thank you Jessica Thompson for your patience, love, laughter and support.

## TABLE OF CONTENTS

	Page
ABSTRACT .....	ii
ACKNOWLEDGMENTS.....	v
TABLE OF CONTENTS .....	vi
LIST OF TABLES .....	x
LIST OF FIGURES.....	xi
CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW .....	1
Host Plant Volatiles and Their Role in Insect Behavior .....	1
Mechanisms of Host Plant Selection.....	1
The Role of Olfaction in Plant-Insect Interactions.....	1
Host Plant Volatiles.....	3
Behavioral Responses of Lepidoptera to Host Plant Volatiles .....	4
Behavioral Responses of Sesiidae to Host Plant Volatiles .....	5
The Role of Host Plant Volatiles in Monitoring and Managing Agricultural Pests.....	6
Biology and Pest Status of Dogwood Borer.....	7
Life History .....	7
Pest Status .....	9
Dogwood Borer Management and Control .....	10
Chemical Control .....	10
Cultural Control.....	10
Biological Control.....	11
Behavioral Manipulation.....	11
Justification for Research.....	11
Research Objectives .....	13
CHAPTER 2: DEVELOPMENT OF A REARING METHODOLOGY FOR THE DOGWOOD BORER .....	18
Abstract .....	18
Introduction .....	18
Materials and Methods .....	20
General Protocols .....	20
Rearing Field Collected Dogwood Borer Larvae.....	20
Establishing a Laboratory Colony of Dogwood Borer.....	20
Effect of Moth Age and Time of Day on Mating.....	20
Effect of Cage Size and Fading Light Intensity on Mating Success.....	21
Oviposition Substrates, Egg Developmental Duration and Survivorship .....	22
Larval Establishment and Development in Thinning Apples .....	22
Effect of Photoperiod on Pupation .....	23
Pupal Development and Synchronization of Adult Emergence.....	24

Results .....	25
Effect of Moth Age and Time of Day on Mating.....	25
Effect of Cage Size and Fading Light Intensity on Mating Success.....	25
Oviposition Substrates, Egg Developmental Duration and Survivorship.....	25
Larval Establishment and Development in Thinning Apples .....	25
Effect of Photoperiod on Pupation.....	26
Pupal Development and Synchronization of Adult Emergence.....	26
Discussion .....	26
<b>CHAPTER 3: POST-MATING BEHAVIOR OF FEMALE DOGWOOD BORER IN APPLE ORCHARDS.....</b>	<b>35</b>
Abstract .....	35
Introduction.....	35
Materials and Methods.....	37
Gender and Mating Status of Adults Orienting to Apple Trees Under Field Conditions .....	37
Orchard Site for Temporal and Behavior Studies.....	37
Temporal Survey of Female Activity.....	38
Characterization of Mated Behaviors.....	38
Statistical Analysis.....	39
Results.....	39
Gender and Mating Status of Adults Orienting to Apple Trees under Field Conditions .....	39
Temporal Survey of Female Activity.....	39
Characterization of Mated Behaviors.....	40
Discussion .....	41
<b>CHAPTER 4: FACTORS INFLUENCING THE TEMPORAL AND SPATIAL PATTERNS OF DOGWOOD BORER INFESTATIONS IN NEWLY PLANTED APPLE ORCHARDS.....</b>	<b>50</b>
Abstract .....	50
Introduction.....	50
Materials and Methods.....	52
Orchard Design .....	52
Orchard Management.....	53
Trunk Treatments and Infestation Surveys .....	53
Statistical Analysis.....	54
Geostatistical Analysis.....	55
Results.....	57
Factors of Infestation and Temporal Patterns .....	57
West Virginia Orchard .....	57
Virginia Orchard .....	58
Spatial Pattern of Infestation.....	59
West Virginia Orchard .....	59
Virginia Orchard .....	59
Discussion .....	60

CHAPTER 5: MORPHOLOGICAL CHARACTERIZATION OF THE ANTENNAL SENSILLA OF THE DOGWOOD BORER.....	74
Abstract .....	74
Introduction .....	74
Materials and Methods .....	76
Insects.....	76
Measurements of Antennae.....	76
Measurements of Sensilla.....	76
Statistical Analysis .....	77
Results .....	77
Antennal Morphology .....	77
Antennal Sensilla.....	78
Sensilla auricillica .....	78
Sensilla basiconica .....	78
Sensilla chaetica .....	78
Sensilla coeloconica .....	79
Sensilla squamiformia.....	79
Sensilla styloconica.....	79
Sensilla trichoidea .....	79
Discussion .....	80
CHAPTER 6: ELECTROPHYSIOLOGICAL RESPONSE OF FEMALE DOGWOOD BORER TO HOST-PLANT VOLATILE COMPOUNDS .....	91
Abstract .....	91
Introduction .....	91
Materials and Methods .....	93
Insects.....	93
Volatile Collections from Host-Plant Source Material .....	94
Volatile Collections from Dogwood Borer Larvae and Artificial Diet.....	95
SPME Collections of Dogwood Borer Frass.....	95
Electrophysiological Recordings.....	96
Results .....	96
Identification of Electrophysiologically Active Compounds.....	96
Discussion .....	98
CHAPTER 7: CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH .....	122
Research on the Behavioral Response of Mated Female Dogwood Borer to Host Plant Stimuli.....	125
Orientation Behavior and Attraction to Host Plant Stimuli .....	125
Orientation to Host Plants Under Semi-Field Conditions.....	125
Orientation to Host Volatiles in Laboratory Bioassays.....	126
Orientation to Host Volatiles Under Semi-Field Conditions .....	127
Oviposition Behavior .....	127
Oviposition in Response to Host Plants Under Field and Semi-Field Conditions .....	127
Oviposition in Response to Host Volatiles in Laboratory Bioassays.....	128



Possible Explanations for Why Behavioral Responses Were Not Observed.....	129
Female dogwood borer do not use host plant stimuli in their host-finding and oviposition site selection behavior .....	129
Inappropriate stimuli and/or lack of understanding of the possible ecological role of stimuli.....	129
Inappropriate bioassay methodology or apparatus.....	130
Lack of physiological readiness .....	131
REFERENCES.....	133

## LIST OF TABLES

Table	Page
3.1 Behaviors observed by mated female dogwood borer in an apple orchard .....	44
3.2 Transition matrix summarizing the frequency with which a behavior (column) was succeeded by another behavior (row). Transitions that occurred significantly more often than expected by chance ( $P < 0.05$ , $\chi^2$ test) are shown in grey boxes .....	45
3.3 Mean ( $\pm$ SE) duration of behaviors exhibited by mated female dogwood borer and the percentage of total time engaged in each .....	46
4.1 Mean ( $\pm$ SE) number of infestation sites per tree in West Virginia apple orchards with M.7 and M.26 rootstocks and three trunk treatments.....	64
4.2 Geostatistical description of larval dogwood borer infestation from 2002 to 2004 in West Virginia and Virginia orchards .....	65
5.1 Mean ( $\pm$ SE) length of the pedicel, scape and selected antennal flagella of dogwood borer.....	84
5.2 Mean ( $\pm$ SE) width of the pedicel, scape and selected antennal flagella of dogwood borer.....	85
5.3 Mean ( $\pm$ SE) number of sensilla on the ventral surface of male and female dogwood borer antennae .....	86
6.1 Volatile compounds present in headspace collections of selected host-plant tissues .....	101

## LIST OF FIGURES

Figure	Page
1.1	Adult female dogwood borer on an apple leaf .....14
1.2	Dogwood borer mating pair in copula. Genders can be distinguished by a wide yellow band on the fourth abdominal segment of females compared with a narrower band on the same segment of males.....15
1.3	Dogwood borer larva feeding on apple burr knot tissue .....16
1.4	Female dogwood borer pupa. Identification based on 4 rows of spines on the terminal abdominal segment .....17
2.1	In thinning apples, dogwood borer larvae construct cocoons from available loose materials (e.g. frass and pieces of apple) bound together with silk .....30
2.2	Dogwood borer pupa at the “dark-eye” stage. The eyes have now become fully pigmented and will increasingly darken from this point forward in development .....31
2.3	Mating success of dogwood borer using 30 cm <sup>3</sup> and 60 cm <sup>3</sup> cages under natural daylight and artificial light. N = 30 pairs per cage size and lighting combination.....32
2.4	Oviposition by dogwood borer in cups containing no substrate, cheesecloth, or apple branch wrapped in cheesecloth.....33
2.5	Proportion of dogwood borer larvae that pupated under natural daylight and artificial long-day photoperiods from August-November. N = 40 larvae per mo.....34
3.1	Mean ± SE proportion of female dogwood borer sightings at 2-h intervals in a) 2006 and b) 2007.....47
3.2	First-order Markov chain analysis of a transition matrix constructed from 1108 behavior patterns exhibited by 66 mated female dogwood borer. The size of bounding boxes is proportional to the frequency of a behavioral pattern. Sequences of behaviors are represented as arrows when transitions occur significantly more often than predicted by chance (P = 0.05, $\chi^2$ test). The size of arrows show the degree to which a specific transition is over-represented .....48

Figure	Page
3.3 Percentage of the total number of occurrences of each behavior exhibited by mated female dogwood borer at selected locations on apple hosts.....	49
4.1 Layout of the research apple orchard in Jefferson Co., West Virginia, showing rootstocks (M.7 and M.26), tree varieties ('Sun Fuji' and 'Gale Gala'), and trunk treatments.....	66
4.2 Layout of the research apple orchard planted on M.26 rootstock in Frederick Co., Virginia, showing tree varieties ('Buckeye Gala' and 'Idared') and trunk treatments.....	67
4.3 Temporal patterns of the mean number of infestation sites per tree infested by dogwood borer on two varieties of apples in orchards located in West Virginia. Soil mound trunk treatments were removed in May 2004.....	68
4.4 Temporal patterns of the mean number of infestation sites per tree infested by dogwood borer on apple trees subjected to three trunk treatments in the West Virginia orchard grown on a) M.7 and b) M.26 rootstock. Soil mound trunk treatments were removed in May 2004 .....	69
4.5 Temporal patterns of the mean proportion of trees infested by dogwood borer in the Virginia orchard in relation to a) variety and b) trunk treatment. Soil mound trunk treatments were removed in May 2004.....	70
4.6 Omnidirectional semivariograms and kriged surfaces of the mean number of infestation sites per tree in the West Virginia M.7 plot for 2002, 2003, and 2004 .....	71
4.7 Omnidirectional semivariograms and kriged surfaces of the mean number of infestation sites per tree in the West Virginia M.26 plot for 2002, 2003, and 2004 .....	72
4.8 Omnidirectional indicator semivariograms and kriged surfaces of infestation probabilities above a median threshold of 0.50 in the Virginia orchard for 2003 and 2004 .....	73
5.1 Scanning electron micrographs showing a) scape of the female dogwood borer antennae and location of the Böhm's bristles, b) close-up of the apical sensors on the female antennae, c) profile of the male dogwood borer antennae possessing numerous overlapping scales on the dorsal surface, large sensilla trichoidea on the ventral surface and apical sensors on the terminal flagellomere. AS, apical sensors; BB, Böhm's bristles; LTr, large trichoidea sensilla; Sc scales .....	87

Figure	Page
5.2	Scanning electron micrograph of a female dogwood borer antennal flagellomere showing most sensillum types. Au, auricillica sensillum; Ba, basiconica sensillum; Ch, chaetica sensillum; Co, coeloconica sensillum; Sq, squamiformia sensillum; St, styloconica sensillum; MTr, medium trichoidea sensillum; STr, small trichoidea sensillum .....88
5.3	Scanning electron micrograph of a male dogwood borer antennal flagellomere showing most sensillum types. Au, auricillica sensillum; Ba, basiconica sensillum; Ch, chaetica sensillum; Co, coeloconica sensillum; St, styloconica sensillum; LTr, large trichoidea sensillum; MTr, medium trichoidea sensillum; STr, small trichoidea sensillum .....89
5.4	Scanning electron micrograph showing a) close-up of auricillica sensilla, which had either a characteristic rabbit-ear shape or appeared dorsoventrally flattened, b) details of the basiconica sensillum, c) close-up of chaetica sensilla, which arose from a distinctive collar-like socket, d) details of the coeloconica sensillum, characterized by a ring of cuticular teeth surrounding a central peg-like structure, e) squamiformia sensilla located on the dorsal surface of an antennal flagellomere, f) details of the styloconica sensillum. Au, auricillica sensillum; Ba, basiconica sensillum; Ch, chaetica sensillum; Co, coeloconica sensillum; Sq squamiformia sensilla; St, styloconica sensillum.....90
6.1	Representation of the headspace collection set-up for potted apple trees..... 102
6.2	Dogwood borer antennae positioned on the EAD holding station..... 103
6.3	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Red Chief Delicious’ apple trees with infested burr knot tissue. * indicates all unidentified compounds ..... 104
6.4	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Red Chief Delicious’ apple trees with no burr knots present ..... 105
6.5	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Red Chief Delicious’ apple trees with uninfested burr knots ..... 106
6.6	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Red Chief Delicious’ apple trees with burr knots infested with dogwood borer larvae ..... 107

Figure	Page
6.7	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Red Chief Delicious’ apple trees with no burr knots present, but with 3-d-old cut bark ..... 108
6.8	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections of ‘Red Chief Delicious’ apple trees with no burr knots present, but with 30-d-old cut bark..... 109
6.9	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections of ‘Cherokee Princess’ dogwood trees with intact bark. * indicates all unidentified compounds ..... 110
6.10	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Cherokee Princess’ dogwood trees with intact bark ..... 111
6.11	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Cherokee Princess’ dogwood trees with 3-d-old cut bark ..... 112
6.12	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Cherokee Princess’ dogwood trees with 30-d-old cut bark ..... 113
6.13	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Granny Smith’ apple trees with no burr knots present..... 114
6.14	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Granny Smith’ apple trees with uninfested burr knots..... 115
6.15	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Granny Smith’ apple trees with burr knots infested by dogwood borer larvae..... 116
6.16	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Granny Smith’ apple trees with no burr knots present, but with 1-d-old cut bark..... 117
6.17	Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Granny Smith’ apple trees with no burr knots present, but with 30-d-old cut bark..... 118

Figure	Page
6.18 Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from a single dogwood borer larva .....	119
6.19 Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from a single dogwood borer larva feeding on lepidopteran diet.....	120
6.20 Representative GC-EAD recording of the responses of female dogwood borer to SPME collected volatiles from 1-d-old dogwood borer frass .....	121

## CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

### Host Plant Volatiles and Their Role in Insect Behavior

**Mechanisms of Host Plant Selection.** Many factors affect the selection of host plants by phytophagous insects, including sensory cues, physiological processes, and environmental stimuli that may act separately or interactively to elicit a sequence of behavioral responses that can lead to host location and acceptance (Harris and Foster 1995). The sequence of behaviors that results in feeding and/or oviposition generally begins with arousal, followed by searching and orientation to the potential host, landing and surface evaluation, and finally acceptance (Bernays and Chapman 1994, Renwick and Chew 1994). Various sensory modalities are involved with these processes, including vision, olfaction, mechanoreception, and contact chemoreception (Ramaswamy 1988, Bernays and Chapman 1994). External cues stimulate sensory receptors of different modalities, which provide information that is processed and integrated by the central nervous system, and result in a particular behavioral response elicited by the insect.

A number of visual and olfactory cues facilitate host habitat and plant location. Visual cues such as size, shape, and color can play a major role in the searching and orientation behavior of many insect species (Ramaswamy 1988, Bernays and Chapman 1994, Renwick and Chew 1994). Many plant-produced semiochemicals are also often critical during the host finding process (Hansson 1995). After host location and landing, short-range cues that are perceived upon contact may evoke behaviors leading to host acceptance. While plant surface features are often involved with host acceptance (Bernays and Chapman 1994, Renwick and Chew 1994), contact chemoreception of compounds associated with the host plant appears to be the primary modality involved with host acceptance (Ramaswamy 1988).

**The Role of Olfaction in Plant-Insect Interactions.** Although various sensory modalities are involved with the host selection behaviors shown by herbivorous insects, olfaction can be particularly important during the host-finding process and in the final stages of plant acceptance (Bernays and Chapman 1994), especially for monophagous or oligophagous species (Ramaswamy 1988). Consequently, olfactory mechanisms that enable an insect to detect and distinguish specific plant-derived volatiles from other



background odors in the environment can be crucial for survival and fitness. In insects, perception of volatile chemicals is mediated by the dendrites of bipolar olfactory receptor neurons housed in various morphologically distinct sensilla types present primarily on the antennae (Hansson 1995). Dendrites of these neurons project into the fluid filled lumen of the sensilla and their axons extend into the antennal lobes of the deutocerebrum. Within the antennal lobes, the axons terminate in specific glomeruli where integration of olfactory input occurs. Odor molecules enter the sensilla through pores that penetrate the cuticular wall where they then interact with hydrophilic odorant binding proteins present in the lumen (Vogt et al. 1991). It is proposed that these proteins bind and transport odor molecules to olfactory receptor proteins located in the membrane of olfactory receptor neurons, where a transduction mechanism is activated if binding of the odor molecules occurs. A second set of proteins present in the lumen, known as odorant degrading enzymes, are then responsible for modifying or degrading the odor molecules (Chapman 1998).

It is assumed that the specificity of an olfactory receptor neuron is largely dependent on the types of olfactory receptor proteins present and that different types of receptor proteins bind with specific odor molecules possessing certain molecular characteristics, which can include the number and location of specific functional groups, position of unsaturations and chirality (Hansson 1995). Olfactory receptor neurons that respond to a range of structurally different odor molecules presumably possess several types of receptor proteins, while more specific neurons possess fewer different types. Ultimately, the range and accuracy with which odor molecules can be identified is thus largely dependant on the number of sensilla present and the specificity of their olfactory receptor neurons (Chapman 1998).

Schneider (1957) developed the electroantennogram (EAG) technique to measure the response of insect antennae to olfactory stimuli. Although initially used widely as a tool to identify insect sex pheromones, the EAG technique is a powerful and standard procedure for identification of stimulatory odorants from a wide range of sources. An EAG measures the change in electrical potential across a whole antennal preparation that has been stimulated by a particular odor, and is thought to represent the summed action potentials of all the responding olfactory receptor neurons in the antennae (Mayer et al. 1984). To measure this change, an electrode is typically introduced to both ends of an

excised antenna and the potential difference following stimulus application is recorded. The amplitude of the EAG roughly indicates the insect's sensitivity to the stimulus. This technique has been improved upon by coupling gas chromatography with an electroantennographic detector (GC-EAD) (Arn et al. 1975), whereby the effluent from the GC column is split, then simultaneously passed through the flame ionization detector of the GC and over an antennal preparation on the EAD. Compounds exiting the GC column that an insect is able to detect elicit synchronous GC and EAD responses that are recorded as simultaneous peaks on the respective traces from both instruments. This technique enables rapid screening of the volatile compounds within complex mixtures, such as plant odors to which herbivorous insects respond. Coupled GC-mass spectrometry can then be used to characterize and identify the electrophysiologically active compounds.

**Host Plant Volatiles.** Plants release a large number of volatile compounds, some of which can act as a chemical message for an insect during the host selection process. Analyses of these emissions, (the plant's "headspace") has revealed a wide array of organic molecules, including alcohols, aldehydes, ketones, esters, phenolics, lactones, and terpenoids (Bernays and Chapman 1994). Although the concentration of volatile compounds is highest near the boundary layer of the plant surface, many compounds with small molecular weight can travel great distances downwind (Bernays and Chapman 1994).

A number of factors can influence plant volatile emissions, including species or cultivar (Loughrin 1996), tissue type (Bengtsson et al. 2001, Vallat and Dorn 2005), phenological stage (Rapparini et al. 2001, Casado et al. 2006), time of day (Agelopoulos et al. 2000, Piechulla and Pott 2003) and environmental factors such as drought stress (Ebel et al. 1995), temperature, rainfall or relative humidity (Vallat et al. 2005). Plants may also emit specific blends of volatiles in response to herbivory, which can vary according to the plant and herbivore species involved. These herbivore induced plant volatiles (HIPV) have gained considerable attention for their role in mediating specific interactions of plants with their herbivores, the natural enemies of their herbivores and other plants (Sabelis et al. 2007).

The study of plant-insect interactions as related to the use of host plant volatiles for host and oviposition site identification, location and acceptance has become an

important topic of research within entomology (Schoonhoven et al. 2005). Plant-derived volatiles can affect the behavior of an individual insect by serving as an attractant, repellent, stimulant, or deterrent (Bernays and Chapman 1994) and methods by which these volatile chemicals can be used to manipulate host-finding and acceptance behaviors for monitoring or management purposes has received increasing attention (Metcalf and Metcalf 1992, Rodriguez-Saona and Stelinski 2008). Investigation of the behavioral responses of insects to these stimuli is often challenging because numerous and potentially interacting signals, include those detected by other sensory modalities (e.g., vision) are often involved in host recognition, and synergism among sensory modalities or other chemical stimuli can occur (Viser 1986, Landolt and Phillips 1997). Furthermore, these behavioral responses may only be elicited during a particular physiological state of the insect (Bernays and Chapman 1994, Hern and Dorn 1999) or when an appropriate concentration or ratio of host plant volatiles is perceived (Bruce et al. 2005).

**Behavioral Responses of Lepidoptera to Host Plant Volatiles.** Host plant volatiles can serve as an attractant for many lepidopteran species (Bernays and Chapman 1994, Rodriguez-Saona and Stelinski 2008), including pests of tree fruit. For example, laboratory bioassays showed that butyl hexanoate, a major component of apple fruit volatiles, was attractive to both female *Cydia pomonella* L. (Hern and Dorn 2004) and *Grapholita molesta* (Busck) (Natale et al. 2004a). Similarly,  $\alpha$ -farnesene, another volatile constituent of apple, is attractive to *C. pomonella* larvae and adult females and can also stimulate oviposition (Wearing and Hutchins 1973, Hern and Dorn 1999). Field studies conducted with *C. pomonella* demonstrated that lures containing the pear ester kairomone ethyl (E,Z)-2,4-decadienoate was attractive to both genders (Light et al. 2001) and that traps containing these lures caught similar or greater numbers of moths than sex pheromone baited traps in orchards under mating disruption (Light et al. 2001, Thwaite et al. 2004, Knight et al. 2005).

Although attraction of lepidopteran insects has been documented using a single host plant volatile compound, most evidence suggests that a ratio-specific blend of volatiles is often more attractive (Bruce et al. 2005). For example, electrophysiological bioassays revealed that 11 compounds from grape shoots elicited antennal responses by female *Paralobesia viteana* (Clemens) (Cha et al. 2008a). Synthetic blends containing all

11 compounds, or a 7-component mixture, stimulated equivalent levels of attraction in a flight tunnel compared with host tissues, but removal of any one compound within the 7-component mixture resulted in a significant decrease in female upwind flight responses. Tasin et al. (2006) showed that 3 grape volatiles,  $\beta$ -caryophyllene, (E)- $\beta$ -farnesene and (E)-4,8-dimethyl-1,3,7-nonatriene, had a strong synergistic effect on female attraction of *Lobesia botrana* (Den. and Schiff.) in a wind tunnel, but omission of any one compound resulted in significant decrease in female upwind flight responses. Furthermore, Tasin et al. (2007) demonstrated that it was possible to substitute the three compounds with other grape volatiles that stimulated an antennal response to partially restore attraction. Hartlieb and Rembold (1996) showed that a blend of six sesquiterpenes mixed in the same proportions as those obtained from steam distillates of pigeonpea were responsible for stimulating attraction and oviposition by *Helicoverpa armigera* (Hubner) in the laboratory. Although one compound,  $\alpha$ -bulnesene, was attractive by itself, the response of female moths to it was less than to the complete mixture.

**Behavioral Responses of Sesiidae to Host Plant Volatiles.** Female sesiid moths appear to be intriguing candidates for investigating the use of host plant volatiles to manipulate orientation and acceptance behaviors because many species appear to oviposit preferentially near specific host tissues (Solomon 1995). Increased susceptibility to infestation due to damage or disease has been documented for several important *Synanthedon* species including *S. pictipes* (Grote and Robinson) (King 1917, Meyer 1982), *S. tipuliformis* (Clerck) (Hardy 1982), *S. sequoiae* (Hy. Edwards) (Furniss and Carolin 1977, Koehler et al. 1983), and *S. novaroensis* (Hy. Edwards) (Furniss and Carolin 1977, Johnson 1993, Rocchini et al. 1999). Early studies by Andersen et al. (1987) showed that volatiles from peach tree bark stimulated EAG responses in *S. pictipes* females and that guaiacol, methyl benzoate, and 1-phenyl-1,2-propanedione were most stimulatory. *S. pictipes* deposited significantly more eggs in response to stimuli associated with canker wounds (Swift 1986, Reed et al. 1988, Cottrell et al. 2008) and mechanically damaged bark (Cottrell et al. 2008) compared with non-damaged bark. Gentry and Wells (1982) showed that extracts from *S. exitiosa* (Say) cocoons, peach tree bark, and frass/gum mixtures from peach tree wounds contained substances that stimulated oviposition by gravid females within Plexiglas™ cages. Derksen et al. (2007) identified 21 compounds from gum-frass mixtures that elicited antennal responses by

male and female *S. exitiosa*. Furthermore, synthetic blends containing all 21 compounds, and synthetic blends containing all compounds except 3 acetates, stimulated significantly higher rates of oviposition, compared with untreated controls.

The dogwood borer, *S. scitula* (Harris), is an economically important sesiid native to eastern North America (Eichlin and Duckworth 1988). The olfactory responses of female dogwood borer to host-plant volatiles appear to play an important function in their host-finding and oviposition site selection behaviors. Numerous early studies indicated that dogwood borer infestation was associated with wounded host plant tissue (Herrick 1904, Underhill 1935, Pierce and Nickels 1941, Wallace 1945, Englehardt 1946, Pless and Stanley 1967). Potter and Timmons (1981) confirmed that increases in the severity of mechanical wounding significantly increased the severity of dogwood borer populations in flowering dogwood, *Cornus florida* L. The presence of burr knot tissue (Riedl et al. 1985, Warner and Hay 1985, Kain and Straub 2001, Kain et al. 2004, Leskey and Bergh 2005), insect galls (Eliason and Potter 2000) and insect- and disease-induced injuries to the exterior of trees (Engelhardt 1932, Pless and Stanley 1967) appear to increase the likelihood of infestation. Despite these observations, no studies have systematically examined the role of host plant volatiles on the host-finding and/or oviposition behavior of female dogwood borer.

**The Role of Host Plant Volatiles in Monitoring and Managing Agricultural Pests.** Much of the research investigating semiochemicals for use in insect pest management has focused on sex pheromones (Foster and Harris 1997). In Lepidoptera, female produced sex pheromones have been used extensively to monitor conspecific male populations and have enabled the development of pheromone-based management strategies for a number of important agricultural pests (Cardé and Minks 1995, Howse et al. 1998). A limitation of using sex pheromones is that egg-laying female populations are not targeted. The development of an effective female attractant would facilitate monitoring of their behavior and may present opportunities to develop control strategies complementary to male orientation disruption.

Utilization of attractive host plant volatiles is a method that has been used successfully to monitor female pest populations. In Lepidoptera, traps baited with the pear ester volatile ethyl (E,Z)-2,4-decadienoate were effectively used to track the seasonal flight patterns of female and male *C. pomonella* in apple orchards under mating

disruption (Knight and Light 2005). In combination with other visual or chemical stimuli, host plant volatiles have been used to monitor females and males of several Curculionidae species (Oehlschlager et al. 1993, Giblin-Davis et al. 1996, Leskey and Wright 2004) and female apple maggot flies, *Rhagoletis pomonella* (Walsh) (Zhang et al. 1999). In addition, host plant volatiles have the potential to be utilized as a component of behaviorally based management strategies targeting female populations in a manner similar to sex pheromone-based management strategies for male populations, such as mass trapping, attract-and-kill, push-pull, and disruption of host finding (Rodriguez-Saona and Stelinski 2008).

### **Biology and Pest Status of Dogwood Borer**

**Life History.** Dogwood borer adults are bluish-black with yellow bands on the second and fourth abdominal segments, narrow longitudinal yellow bands on opposite sides of the thorax and with clear wings bordered by black scales (Figs. 1.1 and 1.2). The sex ratio is 50:50 (Wallace 1945) and external morphological differences between the genders is typically expressed as a wide yellow band on the fourth abdominal segment of females compared with a narrower band on the same segment of males (Ayers 1966). Eggs are deposited singly in the cracks and crevices of host tissues and are ovate and light brown in color with hexagonal reticulations on the surface (Wallace 1945).

Upon emergence, larvae immediately search for an entrance point into the tree, where they will feed and develop (Wallace 1945, Bergh and Leskey 2003). Larvae are generally whitish to cream colored and have a brown head capsule and prothoracic shield (Fig. 1.3). Underhill (1935) and Wallace (1945) initially reported six larval instars, although subsequent studies documented seven instars (Ayers 1966, Neal 1984). Larvae do not have an obligatory diapause and can continue feeding during the winter when temperatures are above 4°C (Pless and Stanley 1967). Multiple larval stages may inhabit a given area of the tree at one time and appear to coexist well unless they are closely confined (Wallace 1945).

Pupation occurs in a silken cocoon within a puparium constructed of a mixture of plant material and frass (Herrick 1904, Wallace 1945, Pless and Stanley 1967). Pupae are brown and possess several rows of posteriorly projecting spines on the abdominal segments (Fig. 1.4) that can be used for gender differentiation (Leskey and Bergh 2003).

In males, segments 8-10 are fused and possess 3 rows of spines unlike females, which have segments 7-10 fused with 4 rows of spines. In the laboratory, moth emergence occurs during the morning, with a pronounced peak occurring between 06:00 and 07:00 h (Bergh et al. 2006).

A review of early dogwood borer monitoring studies (Bergh and Leskey 2003) showed that the seasonal emergence and duration of adult flight activity differed within its geographic range and that unimodal (Riedl et al. 1985, Warner and Hay 1985, Howitt 1993), bimodal (Potter and Timmons 1981, Rogers and Grant 1990, Pfeiffer and Killian 1999, Eliason and Potter 2000) and multimodal (Snow et al. 1985) flight patterns appeared to be based on latitude. Those studies relied on pheromone lures containing a generic blend of sex pheromone components identified from other sesiid species (Tumlinson et al. 1974, Nielsen et al. 1979). Identification of the female dogwood borer sex pheromone (Zhang et al. 2005), an 88:6:6 blend of (Z,Z)-3,13-octadecadienyl acetate (ODDA), (E,Z)-2,13-ODDA, and (Z,E)-3,13-ODDA, provided a monitoring tool that was significantly more attractive to and species-specific for male dogwood borer (Leskey et al. 2006) than the most attractive commercially available lure (Bergh et al. 2004). Using this pheromone blend, Bergh et al. (2009) established that the onset of flight occurred in early June in New York and in early to mid-May in West Virginia, Virginia, North Carolina and Tennessee, and continued into October in all states and confirmed that male captures in apple orchards and urban landscapes showed a bimodal flight pattern.

Despite a bimodal flight pattern, the dogwood borer has generally been considered univoltine, based on emergence studies using dogwood (Underhill 1935, Potter and Timmons 1981) and apple hosts (Riedl et al. 1985). Riedl et al. (1985) suggested that two years might be required for complete development of some larvae in apple trees in New York. In Germany, *S. myopaeformis* Borkh. larvae feeding in the bark of the trunk or branches of apple trees completed development in two years, while those feeding on burr knot tissue could complete development in one year (Dickler 1976). However, the duration of emergence of *S. myopaeformis* occurs from late June to August, a much shorter period than has been reported for dogwood borer. Assuming a temperature-dependent developmental rate of dogwood borer larvae, it has been suggested that more than one generation may occur in parts of its range (Bergh and Leskey 2003, Bergh et al. 2009).

**Pest Status.** The dogwood borer has been recognized as an economically important pest for over 100 years (Herrick 1904). Given that it has been recorded from 19 species of trees and woody shrubs belonging to 10 families, it is considered to have the broadest host range among the North American Sesiidae (Eichlin and Duckworth 1988). Larvae are the damaging stage, feeding predominately on the cambium and phloem tissues of host plants (Underhill 1935, Wallace 1945). Consecutive years of larval feeding can result in the destruction of vascular plant tissue and girdling of trunks and/or branches (Underhill 1935, Wallace 1945), which can result in the death of young trees (Weires 1986, Howitt 1993) or render deciduous ornamental trees unmarketable (Rogers and Grant 1990).

Dogwood borer was first considered primarily a major pest of dogwood in nurseries and urban landscapes (Underhill 1935, Wallace 1945, Potter and Timmons 1981). Since the mid 1980s, it has become an increasingly important pest of apple trees, *Malus domestica* Borkh. (Riedl et al. 1985). Bergh et al. (2009) showed that captures in apple orchards exceeded those in urban and woodland habitats by about 13 and 206 times, respectively. The increasing abundance of dogwood borer in apple orchards appears to have coincided with the adoption of size-controlling rootstocks (Riedl et al. 1985, Kain and Straub 2001, Bergh and Leskey 2003). These rootstocks limit scion growth (i.e. the fruiting portion of the tree), enabling increased tree density per acre and reduced time, labor and cost associated with their management. Many apple varieties propagated on size-controlling rootstocks show an increased propensity to produce burr knots, which are adventitious root initials that form primarily below the graft union and, in some varieties, on the scion (Rom 1970, 1973, Marini et al. 2003). Burr knot tissue appears to be an important resource for dogwood borer; in newly established apple orchards the quantity of available burr knot tissue has the greatest influence on the initiation, persistence, and extent of infestations (Leskey and Bergh 2005).

A number of factors can increase the severity and frequency of burr knot formation. Rom and Brown (1979) showed that increased humidity and low light intensity from shading by weeds, trunk protectors, or low hanging limbs can stimulate burr knot formation. In addition, some rootstocks (e.g. P.1, O.3, and M.26) and/or varieties (e.g. 'Idared', 'Empire' and 'Gala') may be more prone to burr knot formation than others (Howitt 1993, Marini et al. 2003, Leskey and Bergh 2005). The susceptibility



of certain apple rootstocks and/or varieties to infestation by dogwood borer was shown in several studies. For example, ‘Idared’ apple trees on M.26 rootstock had significantly higher levels of infestation compared with ‘Buckeye Gala’ on M.26 (Leskey and Bergh 2005). In New York, apple trees on M.9, M.26, MM.106, and M.9 interstem on MM.106 had significantly higher levels of infestation compared with trees on MM.111 and that the rootstock/variety combinations with highest infestation levels included ‘MacIntosh’ on M.26, ‘Golden Delicious’ on M.9, and ‘Idared’ on MM.106 (Riedl et al. 1985). The susceptibility of specific apple varieties and rootstocks to the congeneric species, *S. myopaeformis*, has also been reported. In southern Germany, infestation levels by this species among different varieties including ‘Mutsu’, ‘Marigold’, ‘Idared’ and ‘Granny Smith’ on M.9 were 70.6%, 55.9%, 50.8%, and 19.8%, respectively (Dickler 1976). In Jordan, ‘Mondial Gala’ trees grafted on M.9 and M.26 had higher levels of infestation by *S. myopaeformis* than trees on MM.106 (Ateyyat 2006).

### **Dogwood Borer Management and Control**

**Chemical Control.** The most effective and consistent control of dogwood borer in apple hosts has been provided by the organophosphate insecticide, chlorpyrifos (Riedl et al. 1985, Kain and Straub 2001, Kain et al. 2002, 2004). In apple, chlorpyrifos use is currently restricted to one application per season, regardless of the pest targeted, application method (foliar spray or trunk drench) or formulation. For control of dogwood borer, a drench spray can be applied only to the lower 4 ft of the trunk, which would not affect dogwood borer larvae feeding at sites higher on the tree (Pfeiffer 2009). A single trunk drench application during the prebloom or early postbloom periods is recommended for season-long control (Pfeiffer 2009).

**Cultural Control.** Mounding or berming soil around the base of apple trees to prevent female dogwood borer from ovipositing on burr knots can be an effective management tactic (Gut et al. 2005). However, covering burr knots with soil promotes scion rooting and tree vigor, often negating the size-controlling affects of these rootstocks (Young and Tyler 1983, Riedl et al. 1985, Kain and Straub 2001, Leskey and Bergh 2005). Furthermore, the longevity of mounds can vary due to slope, soil type, and/or environmental conditions (Bergh and Leskey 2003) and can be costly in established orchards (Gut et al. 2005). White paint applied to burr knot tissue has provided some

control of dogwood borer (Warner and Hay 1985), possibly by preventing pupal emergence or larval entrance (Wallace 1945, Warner and Hay 1985) or by deterring oviposition.

**Biological Control.** Entomopathogenic nematodes have shown promise for control of several important *Synanthedon* species, including *S. culiciformis* L. (Kaya and Brown 1986), *S. resplendens* H. Edwards (Kaya and Brown 1986), *S. tipuliformis* (Clerck) (Miller and Bedding 1982) and *S. pictipes* (Shapiro-Ilan and Cottrell 2006). In all North American studies, *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae) was more virulent on clearwing larvae than were other nematodes. Davidson et al. (1992) reported that *S. carpocapsae* reduced the number of dogwood trees infested with dogwood borer larvae and significantly reduced the number of larvae per tree.

**Behavioral Manipulation.** Pheromone-based management strategies have been used to control several important sesiid pests (Pfeiffer et al. 1991, Agnello and Kain 2002, Kittelson 2006). An initial, three-year attempt at dogwood borer mating disruption using a commercial formulation of the peachtree borer sex pheromone resulted in the elimination of male captures in traps but not reductions of infestations (Pfeiffer and Killian 1999), likely due to sub-optimally attractive disruption dispensers and pheromone lures for monitoring treatment effects (Leskey et al. 2009). Subsequently, Leskey et al. (2009) showed that mating disruption dispensers containing a combination of the behavioral antagonist, (E,Z)-3,13-ODDA, (Zhang et al. 2005) and the main dogwood borer sex pheromone component, (Z,Z)-3,13-ODDA, effectively disrupted captures of male dogwood borer in pheromone traps baited with lures containing the trinary pheromone blend, although effects on infestations were not evaluated. Despite repeated demonstrations that traps baited with the dogwood borer sex pheromone can remove large numbers of male moths from the population (Leskey et al. 2006, Bergh et al. 2009), mass trapping was not effective at reducing infestations in apple orchards (Leskey et al. 2009).

### **Justification for Research**

The dogwood borer is an increasingly important economic pest in eastern apple orchards in North America. Competition in the international market (Marshall and

Andrews 1994) has prompted apple growers to plant high density orchards on size-controlling rootstocks. As occurred earlier in Europe with *S. myopaeformis* (Dickler and Hoffman 1974), the increasing use of these rootstocks appears to have resulted in an increased pest pressure from dogwood borer in eastern orchards (Riedl et al. 1985, Kain and Straub 2001). In young trees, females appear to preferentially oviposit on or near burr knots (Riedl et al. 1985, Kain and Straub 2001, Leskey and Bergh 2005), which appear to be an important resource for developing dogwood borer larvae and may be associated with the greater abundance of dogwood borer in orchards than in other habitats containing its other host plants (Bergh et al. 2009).

Current management strategies for dogwood borer rely exclusively on chlorpyrifos. Due to the increased awareness of the harmful effects of organophosphate insecticides on non-target organisms and the environment, as well as to increasing restrictions and regulations on the use of chlorpyrifos, the search for alternative methods to manage dogwood borer has become necessary. The integration of tactics that target multiple aspects of dogwood borer biology and life stages would provide a more sustainable management approach. Behavioral manipulation of pest insects is an environmentally benign tactic that has received increasing attention for pest control and that can be readily aligned with other management strategies (Foster and Harris 1997).

To date, the development of behaviorally-based management strategies for dogwood borer has focused on male attraction to female sex pheromone and disruption of mate-finding behaviors (Leskey et al. 2006, 2009) as has been the case for most Lepidoptera. A potential alternative approach to managing lepidopteran pests is based on the identification of host plant-derived attractants for female moths that may be used to remove females from the population and/or to prevent them from locating and infesting plants (Rodriguez-Saona and Stelinski 2008). Given that there is very little known about female dogwood borer behavior and chemical ecology, these topics must be broadly and thoroughly studied in order to determine if such a management strategy for female dogwood borer is biologically feasible.

To conduct rigorous studies focusing on the behavioral and chemical ecology of female dogwood borer, it is essential that females of known age and physiological state are available for experimentation. While rearing procedures have been developed for several sesiid species including *S. exitiosa* (Smith 1965), *S. pictipes* (Cleveland et al.

1968, Reed and Tromley 1985), *Podosesia syringae* (Harris) (Solomon 1983), and *Paranthrene dollii* (Neumoegen) (Forschler and Nordin 1989), efforts to investigate the biology and behavior of dogwood borer have been complicated by the lack of an effective rearing method. Although Leskey and Bergh (2002) reared field collected dogwood borer larvae to the adult stage using a bean-based Lepidoptera diet, field collections are time consuming and the high mortality of young larvae on this medium limited its utility. Leskey and Bergh (2002) were the first to report successful mating of dogwood borer in captivity; mating was observed in a 244 x 61 x 61 cm flight tunnel but not in smaller cages. Although laboratory-mated females oviposited on several substrates, eggs often failed to hatch. These limitations indicated the need for further research on the conditions required to successfully and continuously rear this species.

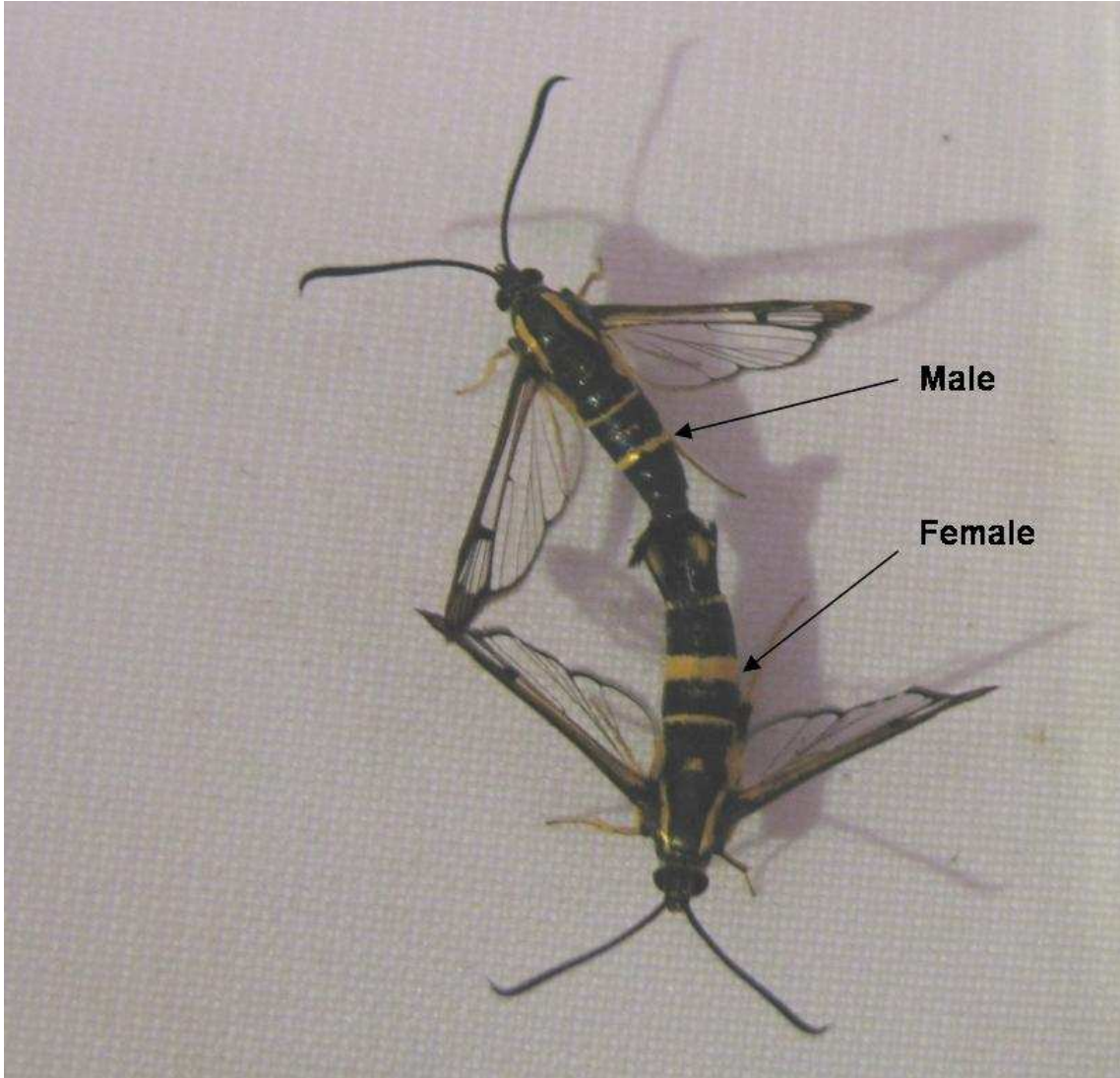
### **Research Objectives**

The research presented in this dissertation is intended to improve our understanding of the chemical ecology and insect-host plant interactions of female dogwood borer in apple orchards. Specific objectives addressed are: 1) Development of rearing methodology, 2) Characterization of the behavior of mated dogwood borer females in apple orchards, 3) Determination of the factors that influence the temporal and spatial patterns of dogwood borer infestations in apple orchards, 4) Characterization of the distribution and types of dogwood borer antennal sensilla and 5) Determination of the electrophysiological and behavioral response of dogwood borer females to host plant volatiles.

**Figure 1.1** Adult female dogwood borer on an apple leaf.



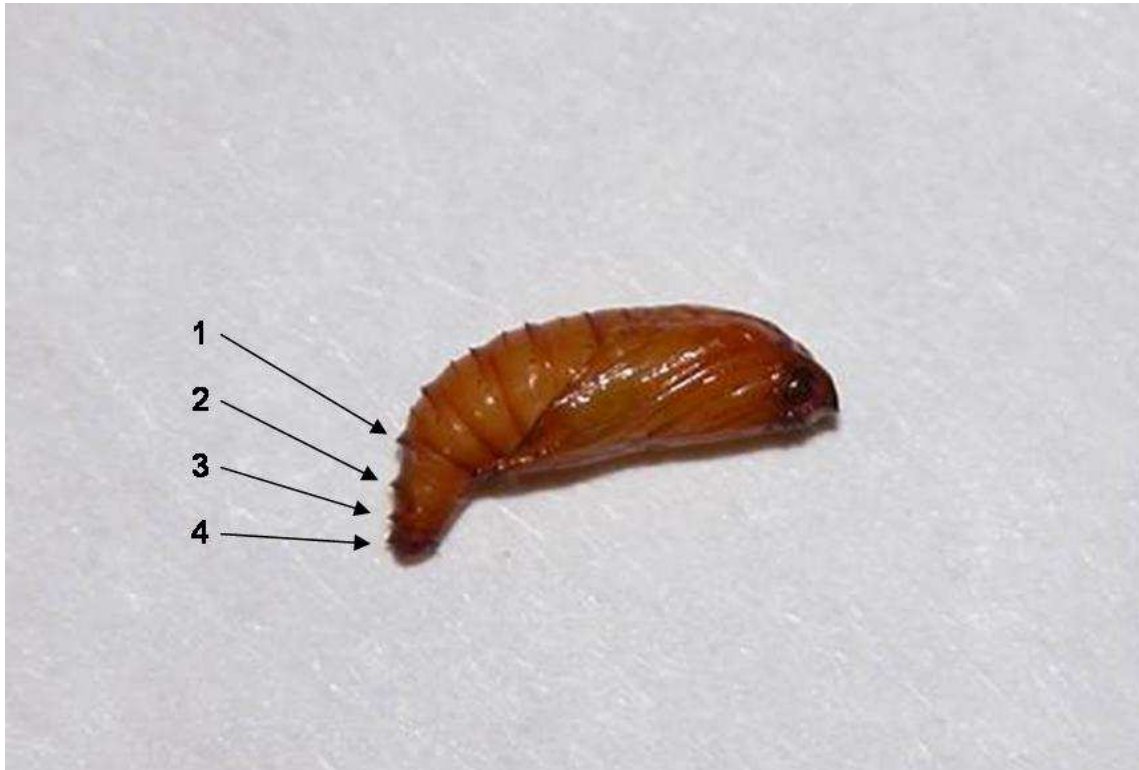
**Figure 1.2** Dogwood borer mating pair. Genders can be distinguished by a wide yellow band on the fourth abdominal segment of females compared with a narrower band on the same segment of males.



**Figure 1.3** Dogwood borer larva feeding on apple burr knot tissue.



**Figure 1.4** Female dogwood borer pupa. Identification based on 4 rows of spines on the terminal abdominal segment.





## **CHAPTER 2: DEVELOPMENT OF A REARING METHODOLOGY FOR THE DOGWOOD BORER**

**Frank, D. L., T. C. Leskey, and J. C. Bergh**

**This chapter was published by *Ann. Entomol. Soc. Am.* 103: *in press*.**

### **Abstract**

Studies examining the factors that influence the mating, oviposition and development of dogwood borer and that pertain to its efficient rearing in captivity are reported. The mating success of pairs of moths held in 30 or 60 cm<sup>3</sup> cages exposed to natural daylight or artificial light did not differ significantly. Under natural daylight and artificial light, the average time at which mating occurred was 19:50 h and 19:15 h, respectively, but mating could be triggered earlier in the day by gradually reducing the intensity of artificial light. Mated females held in waxed paper cups deposited significantly more eggs on 2-yr-old apple branches wrapped in cheesecloth than on cheesecloth alone or when no oviposition substrate was provided. Larvae fed on and pupated within small, immature apples and their establishment on this rearing medium was significantly improved on fruit with small perforations compared with those without perforations. Larvae introduced to these apples monthly between August and November completed development and pupated if exposed to constant long-day conditions using artificial light, but showed a significant reduction in pupation in September and a cessation of pupation by October if exposed to natural daylight and decreasing daylength. The “dark-eye” stage was established as a common point in pupal development that could be used to generate a cohort of pupae from which moths emerged over a period of 2-3 d. These procedures provide the basis for successful establishment of a laboratory-based colony of dogwood borer.

### **Introduction**

The dogwood borer, *S. scitula*, is a pest of 19 species of trees and woody shrubs throughout eastern North America (Eichlin and Duckworth 1988). Previous studies examining dogwood borer infestation in host trees have suggested that mated females preferentially oviposit near areas associated with damaged bark (Wallace 1945, Potter

and Timmons 1981), insect induced galls (Eliason and Potter 2000), and apple burr knot tissue (Riedl et al. 1985, Kain and Straub 2001, Leskey and Bergh 2005). Host damage occurs as developing larvae mine outward into the cambium (Underhill 1935, Wallace 1945) where extensive larval feeding can render trees unmarketable (Rogers and Grant 1990), increase their susceptibility to disease and damage from other pests (Walton 1986), as well as partially or completely girdle branches or the trunk (Underhill 1935).

Historically an economically important pest of dogwood, *C. florida* L., in nurseries and urban landscapes (Wallace 1945, Pless and Stanley 1967, Potter and Timmons 1981), its pest status in commercial apple orchards has increased in recent years (reviewed in Bergh and Leskey 2003). Management of dogwood borer in orchards is achieved via trunk-drench applications of the organophosphate pesticide, chlorpyrifos (Kain et al. 2004). However, this pest species remains abundant and widely-distributed (Bergh et al. 2009). To expedite on-going efforts to develop alternative management tactics for dogwood borer (Gut et al. 2005, Leskey et al. 2009), development of an efficient rearing method was considered necessary, as was the case for other important sesiid pests of orchard crops, including *S. exitiosa* (Say) (Smith 1965) and *S. pictipes* (Grote and Robinson) (Cleveland et al. 1968, Reed and Tromley 1985). Previous obstacles to rearing dogwood borer in captivity have included inconsistent mating (Wallace 1945, Ayers 1966) and oviposition (Underhill 1935, Pless and Stanley 1967), unreliable egg hatch (Underhill 1935, Leskey and Bergh 2002), poor establishment of neonate larvae on artificial diet (Leskey and Bergh 2002), and an inability to generate a cohort of pupae from which relatively synchronous moth emergence would occur.

Late instar dogwood borer larvae collected from apple trees can be reared on commercial lepidopteran diet (Leskey and Bergh 2002), but such collections are time-consuming and restricted to a few months per year. Furthermore, high mortality of young larvae on this medium limits its utility. Here, I describe the results of experiments examining factors that influence mating, oviposition and some aspects of larval development and pupation that led to the development of protocols enabling continuous culture of dogwood borer in captivity.

## **Materials and Methods**

### **General Protocols**

Unless otherwise specified, conditions in the controlled environment chamber (Percival Scientific, Perry, IA) were kept at a constant 25°C and 16:8 (L:D) h. Larvae and pupae were held individually in clear, 30 ml plastic cups with clear lids (Polar Plastics, St.-Laurent, Quebec) placed in plastic diet cup trays (BioServ, Frenchtown, NJ). Cups containing pupae contained a 1.5 cm piece of moist cotton dental wick to prevent desiccation. Moths were held individually in 300 ml, white, waxed paper cups (Sweetheart Cup Co., Owings Mills, MD) provisioned with a cotton ball saturated with a 10% sucrose solution in a small glass dish, topped with a clear plastic lid, and held in the chamber under the conditions described above.

### **Rearing Field Collected Dogwood Borer Larvae**

Several hundred dogwood borer larvae were extracted from apple trees in commercial orchards in Frederick Co., Virginia, USA in May 2007 and 2008. Larvae were placed in cups containing general lepidopteran diet (BioServ, Frenchtown, NJ) that had the surface scraped lightly with a metal probe to loosen the material and facilitate feeding and tunneling by larvae. Cups with larvae were placed in the environmental chamber and checked weekly for cocoons. Pupae were removed by using a metal probe to peel away the dorsal side of the cocoon and sexed according to the number of rows of spines on the fused, terminal abdominal segment (Leskey and Bergh 2003). Pupae were held in cups in the environmental chamber until eclosion.

### **Establishing a Laboratory Colony of Dogwood Borer**

**Effect of Moth Age and Time of Day on Mating.** Mating was evaluated from July to August, 2008 using pairs of virgin (<1-d-old) male and female moths. For all mating studies, only moths that were able to fly and showed no physical deformities were used. Individual pairs of moths were placed in ventilated 60 cm<sup>3</sup> plywood cages with Plexiglas panels comprising the top and front side. Twenty-seven moth pairs were examined in cages held outdoors under ambient temperature and indirect sunlight (photoperiod approx. 15:9 L:D h, with sunset at about 20:30 h). In the laboratory, 27

moth pairs were examined in cages at room temperature (~24-28°C), illuminated (~650 lux) by a 200-w incandescent lamp at 60 cm above them and exposed to a photoperiod of 16:8 (L:D) h, with abrupt darkness at 21:00 h. Moths were placed in cages at 16:00 h and observed at 15 min intervals until 21:00 h. Only females observed in copula for  $\geq 2$  h were considered mated. Moths not in copula by 21:00 h were returned to the environmental chamber until 16:00 h the next day, then placed in their respective mating cages. This was repeated until each female had been mated or died. The age of moths at mating and the time of day at which mating occurred were recorded. Data did not conform to the assumptions of normality so the mean age at mating under natural and artificial light was compared using the non-parametric Wilcoxon-Mann-Whitney test (SAS Institute 2003). The proportion of moth pairs that mated in indoor and outdoor cages was compared using Fisher's exact test (SAS Institute 2003). Results of all analyses were considered statistically different at  $P < 0.05$ .

**Effect of Cage Size and Fading Light Intensity on Mating Success.** From June to August, 2008, individual pairs of virgin male and female moths (1- to 3-d-old) were placed in 30 cm<sup>3</sup> metal screen cages (BioQuip Products, Inc., Gardena, CA) and in the 60 cm<sup>3</sup> cages described above, at 16:00 h. The cages were held outdoors under indirect sunlight and in the laboratory under the conditions described previously (n = 30 pairs per cage size, lighting combination). All moth pairs were observed at 1-h intervals until 21:00 h and only females observed in copula for  $\geq 2$  h were considered mated. Pairs not in copula by 21:00 h were handled as previously described. The proportion of moth pairs that mated in each cage size, lighting combination was compared using Fisher's exact test (SAS Institute 2003).

To determine if mating could be elicited earlier in the day, I examined the response of pairs of moths held indoors in 60 cm<sup>3</sup> cages and exposed to fading light during the afternoon. From August to October, 2008, individual pairs of 1- to 2-d-old virgin males and females were introduced into the cages and exposed to artificial light. A light meter (3252 Traceable, Friendwood, TX) was used to calibrate the initial light intensity inside cages to 600 lux. Moths were placed in the cages at 13:00 h or 14:00 h and acclimated to these conditions for 1 h, after which the light intensity was reduced using a dimmer in increments of 50 lux per 10 min over 2-h. In total, 20 pairs of moths were tested during each 2-h period. Those that did not mate were returned to the

environmental chamber until the following day, and then placed together in cages at the same time as on the previous day. This was repeated until each female had been mated or died. The proportion of moth pairs that mated was compared between the 2-h periods using Fisher's exact test (SAS Institute 2003). The duration (d) mating pairs remained in copula was recorded at 15 min intervals from the onset of mating.

**Oviposition Substrates, Egg Developmental Duration and Survivorship.** In July and August, 2008, female dogwood borer (1- to 3-d-old) that had mated on the previous evening were placed in cups containing either no oviposition substrate, a 3 cm<sup>2</sup> piece of cheesecloth or a freshly cut 10 cm long piece of 2-yr-old apple branch wrapped in cheesecloth (n = 20 females/treatment). The cups were held on a laboratory bench at room temperature (24-28°C) and a 16:8 (L:D) photoperiod under four, 40-w fluorescent bulbs (Sylvania, Danvers, MA) positioned 35 cm above containers. Each cup and oviposition substrate was examined daily for eggs under a 10X stereomicroscope (Olympus SZ40, Center Valley, PA) until each female had died. The number of days to the onset of oviposition and the number of eggs deposited on each substrate by each female was determined. Data did not conform to the assumptions of normality so the numbers of eggs deposited were compared among substrates using non-parametric statistics (Kruskal-Wallis one-way ANOVA and Wilcoxon-Mann-Whitney test) (SAS Institute 2003).

To measure the developmental duration and survivorship of eggs, seven recently mated females (1- to 2-d-old) were held individually in cups containing a piece of 2-yr-old apple branch wrapped in cheesecloth. The cups were held in the controlled environment chamber and examined daily for eggs under a 10X stereomicroscope. If eggs were deposited, the female was transferred to a cup containing a new oviposition substrate and this was repeated until each female died. A total of 372 eggs were held in the environmental chamber and the percentage egg hatch and number of days to larval eclosion was determined from daily observations.

**Larval Establishment and Development in Thinning Apples.** Preliminary observations suggested that dogwood borer larvae fed and developed on thinning apples, which are small, immature, green apples removed from the tree via mechanical or chemical thinning as an annual horticultural practice. A preliminary study also revealed that larval establishment in these apples could be significantly improved by making small

perforations (ca.  $\leq 1$  mm diam and 1-2 mm deep) in the skin of the fruit near the stem end, using a pointed metal probe. Ninety five percent of larvae ( $n = 20$ ) established in fruit that had been punctured compared with 25% of larvae ( $n = 20$ ) in fruit without punctures.

To determine the survivorship and developmental duration of dogwood borer on this medium, thinning apples (~3 cm diam) were collected in June and stored at 4°C. Fruit were removed from cold storage in July, allowed to warm to room temperature for 30 min and punctured (6-8 perforations per fruit) as described above. Under a stereomicroscope at 10X magnification, a moistened, fine-tipped brush was used to transfer individual neonate larvae (1- to 4-h-old) from cups in which they had emerged to each of 384 apples, which were placed into several 3.7 L clear plastic containers (Rubbermaid, Fairlawn, OH) covered loosely with a clear plastic lid to prevent moisture accumulation. One group of larvae was exposed to natural daylight through a south-facing window with an approximate photoperiod of 15:9 (L:D) h and room temperature of 24-28°C. The remaining group of larvae was exposed to artificial light from a bank of four, 40-w fluorescent bulbs 35 cm above the containers, a photoperiod of 16:8 (L:D) h and an ambient temperature of 24-28°C. After 30 d, apples under both light conditions were cut open and any cocoons found were collected and opened (Fig. 2.1). Pupae and cocoons containing larvae were placed in cups under the same light conditions described above and examined daily for pupation. Larvae that had not yet constructed a cocoon were transferred to new apples with a single perforation (~4 mm diam) extending into the core. Those fruit were held for 1 wk under the same light conditions described above then opened and examined for cocoons and pupae. The number of larvae that pupated under each light condition was compared using a chi-square test (SAS Institute 2003). For both natural daylight and artificial light, the duration (d) of the period between introducing each larva to an apple and its emergence as an adult was compared using the Wilcoxon-Mann-Whitney test (SAS Institute 2003).

**Effect of Photoperiod on Pupation.** To determine if photoperiod influenced pupation, neonate larvae were established in thinning apples monthly from August to November, 2008 and exposed to natural daylight (decreasing photoperiod) ( $n = 40$  larvae/mo) or artificial light (constant 16:8 L:D photoperiod) ( $n = 40$  larvae/mo) and held at room temperature ranging from 22-28°C. After 30 d, apples were opened and examined for cocoons. Using the same methods described above, the number of larvae

that pupated under each light condition each month was recorded and compared using Fisher's exact test (SAS Institute 2003).

**Pupal Development and Synchronization of Adult Emergence.** Field collected larvae reared on artificial diet in a controlled environment chamber were examined daily. When each larva had constructed a cocoon, a metal probe was used to remove a small section of the dorsal side of the cocoon and the larva was monitored twice daily for pupation. Recently pupated individuals were identified by a lack of cuticular tanning and scleritization. One day after pupation, pupae were removed from cocoons, placed in cups and held in the environmental chamber. Pupae were sexed (n = 35 males and 35 females) and examined twice daily under a stereomicroscope at 10X magnification. The developmental duration (d) between initial pupation and the "dark-eye" stage (i.e. the point at which the eyes of the pupae first became entirely pigmented; Fig. 2.2) and between pupation and adult emergence were determined.

To determine if a cohort of pupae could be generated from which adult emergence would occur during a limited and predictable period, I examined the use of the "dark-eye" stage to predict the period between that developmental point and adult emergence. Pupae (1- to 3-d-old) collected in 2007 (n = 90 per gender) and 2008 (n = 135 per gender) were examined daily under a stereomicroscope at 10X magnification until they reached the "dark-eye" stage. Upon reaching this developmental point, 30 and 45 pupae of each gender in 2007 and 2008, respectively, were allowed to continue developing for 0, 2, or 4 d. Each group was then placed in a refrigerator at ~ 5°C for 1 wk, after which they were returned to the environmental chamber and examined daily for emergence. Data did not conform to the assumptions of normality, so the number of days to adult emergence between genders and years was compared using the Wilcoxon-Mann-Whitney test (SAS Institute 2003). A Kruskal-Wallis one-way ANOVA was used to compare the number of days to adult emergence among pupae chilled at 0, 2, and 4 days, respectively, with means of significant effects separated using the Wilcoxon-Mann-Whitney test (SAS Institute 2003). The survivorship of pupae chilled at 0, 2, and 4 days, respectively, to adult emergence was recorded and compared using Fisher's exact test (SAS Institute 2003).

## Results

**Effect of Moth Age and Time of Day on Mating.** The mean ( $\pm$  SE) age at mating of moths exposed to natural daylight ( $1.8 \pm 0.2$  d) and artificial light ( $1.8 \pm 0.1$  d) did not differ significantly ( $z = -0.02$ ;  $P = 0.985$ ). The proportion of pairs that mated under natural daylight (0.93) and artificial light (0.85) was not significantly different ( $P = 0.669$ , Fisher's exact test). On average, mating occurred at 19:50 h under natural daylight (range = 18:45 – 20:45 h) and at 19:15 h under artificial light (range = 16:30 – 21:00 h). Under natural daylight 9, 13 and 3 pairs mated on the first, second and third evening after emergence, respectively. Under artificial light 8, 12, and 3 pairs mated on the first, second and the third evening after emergence, respectively.

**Effect of Cage Size and Fading Light Intensity on Mating Success.** Mating success in 30 or 60 cm<sup>3</sup> cages under natural daylight and artificial light was not significantly different ( $P = 0.339$ , Fisher's exact test) (Fig. 2.3). Mating success of moths introduced to cages in the laboratory at 14:00 or 15:00 h and then exposed to a gradual reduction in light intensity was 85% and 90%, respectively, and not significantly different ( $P = 0.329$ , Fisher's exact test). Combined data from each 2-h period showed that mating pairs remained in copula for  $3.3 \pm 0.5$  h.

**Oviposition Substrates, Egg Developmental Duration and Survivorship.** The mean ( $\pm$  SE) number of days to the onset of oviposition by mated female dogwood borer in cups containing no substrate ( $2.0 \pm 0.3$  d), cheesecloth ( $2.2 \pm 0.3$  d) and apple branch wrapped in cheesecloth ( $2.2 \pm 0.4$  d) was not significantly different ( $H = 0.14$ ;  $df = 2$ ;  $P = 0.930$ ). However, significantly more eggs were deposited in cups containing an apple branch wrapped in cheesecloth compared with cheesecloth alone or no substrate ( $H = 12.81$ ;  $df = 2$ ;  $P = 0.002$ ) (Fig. 2.4). The average time required for dogwood borer eggs to hatch was  $8.8 \pm 0.4$  d and 82.3% of eggs hatched.

**Larval Establishment and Development in Thinning Apples.** Larval survivorship to the pupal stage in thinning apples was 88% and 93 % under natural daylight and artificial light, respectively, and not significantly different ( $\chi^2 = 2.92$ ;  $df = 1$ ,  $P = 0.088$ ). The mean ( $\pm$  SE) duration of the period between introducing larvae to apples and their emergence as adults was not significantly different ( $z = -1.13$ ;  $P = 0.257$ ) under



natural daylight ( $38.1 \pm 0.2$  d) or artificial light ( $39.0 \pm 0.3$  d). Pooled data revealed that the male:female ratio of moths emerging from thinning apples was 54.5:45.5.

**Effect of Photoperiod on Pupation.** Under constant long-day conditions using artificial light there was no significant difference in the number of larvae that pupated between August and November ( $P = 1.00$ , Fisher's exact test); most larvae that were established on thinning apples each month during this period completed development and pupated (Fig. 2.5). However, exposing larvae to natural daylight and decreasing daylength during the same period had a significant effect on pupation ( $P < 0.0001$ , Fisher's exact test). Larvae established in September showed a reduction in the number that pupated and those established in October or November did not pupate (Fig. 2.5), although they continued to feed.

**Pupal Development and Synchronization of Adult Emergence.** The mean ( $\pm$  SE) number of days between pupation and the "dark-eye" stage and between pupation and adult emergence was  $5.0 \pm 0.4$  and  $12.36 \pm 0.59$  d, respectively. For pupae chilled for 1 wk at 0, 2, and 4 days following the "dark-eye stage", there was no significant difference in the time to adult emergence based on year of collection ( $z = -0.19$ ;  $P = 0.853$ ) or gender ( $z = -0.37$ ;  $P = 0.715$ ). The number of days to adult emergence among pupae chilled at 0 ( $7.5 \pm 0.1$  d), 2 ( $5.2 \pm 0.1$  d), and 4 ( $3.4 \pm 0.1$  d) d following the "dark-eye stage" was significantly different ( $H = 126.93$ ;  $df = 2$ ;  $P < 0.0001$ ). However, excluding the 1-wk period of chilling, the average total developmental duration between the "dark-eye stage" and adult emergence for each group was similar ( $7.5 \pm 0.1$ ,  $7.2 \pm 0.1$ , and  $7.4 \pm 0.1$  d for pupae at 0, 2, and 4 d following the "dark-eye stage", respectively). Moths emerged from 94, 96, and 94% of pupae chilled at 0, 2, and 4 days, respectively, which was not significantly different ( $P = 1.0000$ , Fisher's exact test).

## Discussion

These studies provide specific guidelines for rearing dogwood borer in captivity. Under both laboratory and outdoor conditions, most females were mated by the second evening after eclosion. Although calling by females was not monitored, my data are consistent with those of Bergh et al. (2006), who reported that 89% of virgin female dogwood borer initiated calling by the second evening after emergence. Buda and Karalius (1985) noted that 1- to 2-d-old virgin female *S. tipuliformis* (Clerck) called more

intensively the first few days after emergence than individuals that were more than two days old. Similarly, Wong et al. (1969) showed that virgin *S. pictipes* females remain attractive to males throughout their life, but that females attracted the greatest number of males within the first 2 days after emergence.

While, Reed and Tromley (1985) suggested that cages  $\geq 82$  cm<sup>2</sup> should be used to mate *S. pictipes* in the laboratory, smaller cages can be used to mate dogwood borer. Mating success was similar in 30 cm<sup>3</sup> or 60 cm<sup>3</sup> cages exposed to natural daylight or artificial light. Mating in outdoor cages occurred from 18:45 – 20:45 h, consistent with a previous study (Bergh et al. 2006) showing that males were captured in traps baited with pheromone lures or virgin females during a 2-h interval that began near sunset. Mating in laboratory cages occurred within a 4 h period beginning shortly after moth pairs were introduced into cages and continuing until lights were shut off, although moths could be induced to mate earlier in the day by gradually reducing light intensity, presumably mimicking fading daylight.

Oviposition by dogwood borer females generally occurred within 2 d of mating and often continued until females died. Since eggs were affixed to cups and substrates by a thin, glue-like secretion and easily damaged by attempts to remove them, they were left in place until hatch when neonates could easily be transferred. In contrast, oviposition by *S. pictipes* in the laboratory occurs within 2-3 h after mating (Reed and Tromley 1985) and eggs can easily be collected by gently tapping substrates over a piece of waxed paper (Cottrell et al. 2008). Although dogwood borer females deposited significantly more eggs on apple substrates wrapped in cheesecloth, the variability in daily and total oviposition among females was high, and accurate assessments of the rate of oviposition await the results of ongoing studies of the stimuli that trigger egg deposition.

Although thinning apples are not a food source for dogwood borer larvae in nature, they were an effective rearing medium and have been used to rear larvae of two closely related species, *S. pictipes* (Cleveland et al. 1968, Reed and Tromley 1985) and *S. exitiosa* (Smith 1965). Although dogwood borer has generally been considered univoltine (Underhill 1935, Potter and Timmons 1981, Riedl et al. 1985), my results showed that dogwood borer larvae reared on thinning apples can complete a generation (egg to adult) in <50 d. Preliminary studies (Leskey unpublished data) showed that dogwood borer larvae feeding on burr knots on potted apple trees can complete one

generation in approximately 50 d. Similarly, larvae of *S. pictipes*, a bivoltine species, can complete one generation within 50 d on thinning apples in the laboratory (Reed and Tromley 1985) or in the field on peach hosts (Bobb 1959). Pupation of dogwood borer reared on thinning apples occurred through November if larvae were exposed to long-day conditions, while exposing them to decreasing daylength resulted in reduced pupation in September and its cessation thereafter. In nature, dogwood borer larvae overwinter in various instars (Underhill 1935, Bergh and Leskey 2003) that do not exhibit an obligatory diapause and can continue to feed on warm winter days (Pless and Stanley 1967). However, several studies have shown that in nature they do not pupate after the first week of October (Underhill 1935, Pless and Stanley 1967, Bergh and Leskey 2003).

Use of the “dark-eye” stage as a common point in pupal development enabled the synchronization of adult dogwood borer emergence over a limited and predictable period. Although Bergh et al. (2006) attempted to synchronize moth emergence by generating cohorts of pupae whose development was arrested by chilling when they were deemed to have reached the “black-eye” stage, adult emergence spanned 7 d. I believe that the variability in adult emergence reported by Bergh et al. (2006) was due to their use of an ambiguous point in pupal development. As pupae develop, the eyes increasingly darken and it becomes difficult to accurately determine the point at which they become truly black. Instead, use of the point at which their eyes first become entirely pigmented appears to provide a more consistent and reliable outcome. Furthermore, chilling pupae did not affect their subsequent development and pupae chilled for up to 2 weeks produced adults showing no physical deformities. This shows that chilling can be used to accumulate dogwood borer pupae as they reach the dark-eye stage and that pupae can be brought out of chilling to successfully yield a cohort of adults of identical or similar age.

During these studies I noticed that larvae reared on thinning apples produced adults that were often larger than those from field-collected larvae reared on artificial diet. Examination of females from two successive laboratory reared generations revealed that their egg loads were often ~25% greater than from field-collected females reared on artificial diet. In addition, there were no apparent adverse effects on the development or mating success of dogwood borer reared for five generations in the laboratory on thinning apples.

Although thinning apples are a suitable medium for rearing dogwood borer larvae, development of a meridic diet would be desirable. Thinning apples are a very seasonal resource and problems with *Drosophila* spp. and other microorganisms can occur if good sanitation is not practiced (Antonio et al. 1975, Reed and Tromley 1985). In addition, thinning apples deteriorate relatively quickly under humid conditions in an environmental chamber, limiting my ability to systematically examine the effects of temperature on the survivorship and developmental rates of larvae. Finally, identification of the stimuli, including plant-produced semiochemicals, that mediate oviposition site selection and elicit oviposition by female dogwood borer may reduce the variability in oviposition among females and improve my ability to consistently garner maximal output of eggs from them.

The procedures that I have developed enable reliable and efficient rearing of synchronized cohorts of distinct lifestages of dogwood borer for specific experimental needs. Previous studies examining the biology, ecology, and behavior of dogwood borer were limited to the summer months when adult moths were active or field collections of larvae were possible. These methods will greatly expedite future laboratory and field studies by providing a relatively rapid and continuous supply of dogwood borer throughout the year.

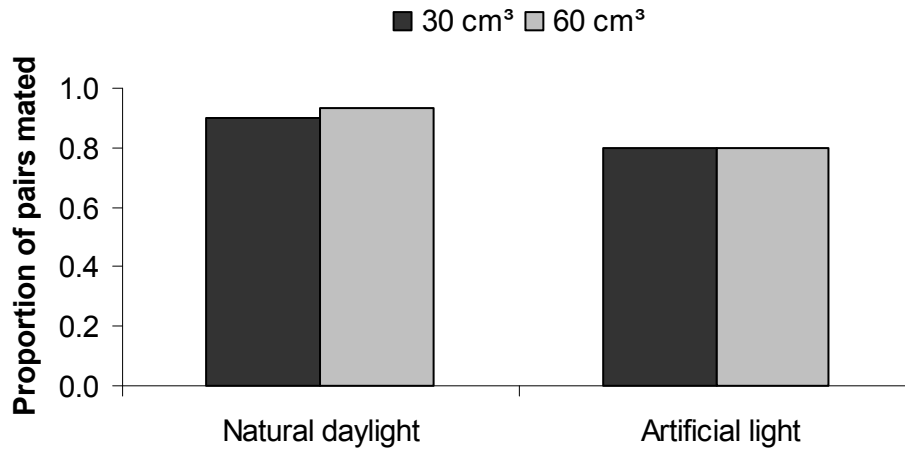
**Figure 2.1** In thinning apples, dogwood borer larvae construct cocoons from available loose materials (e.g. frass and pieces of apple) bound together with silk.



**Figure 2.2** Dogwood borer pupa at the “dark-eye” stage. The eyes have now become fully pigmented and will increasingly darken from this point forward in development.



**Figure 2.3** Mating success of dogwood borer using 30 cm<sup>3</sup> and 60 cm<sup>3</sup> cages under natural daylight and artificial light. N = 30 pairs per cage size and lighting combination.

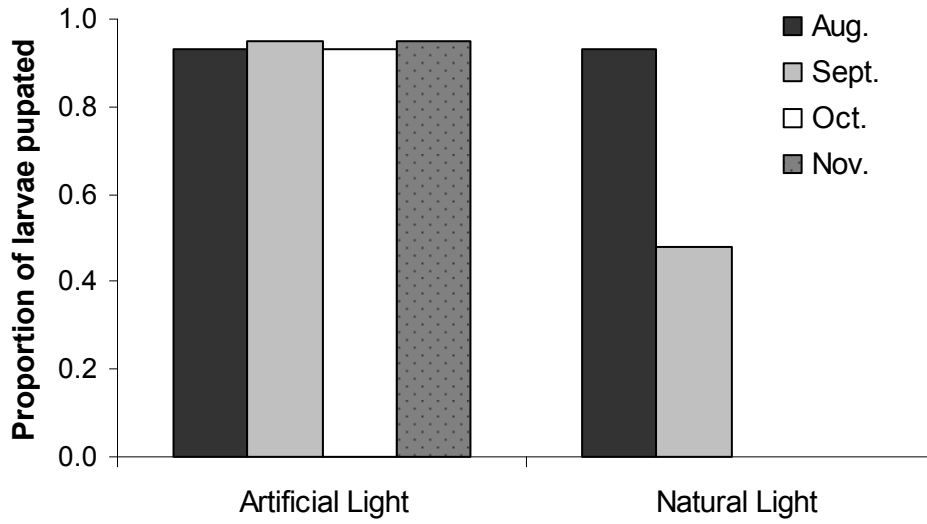


**Figure 2.4** Oviposition by dogwood borer in cups containing no substrate, cheesecloth, or apple branch wrapped in cheesecloth.





**Figure 2.5** Proportion of dogwood borer larvae that pupated under natural daylight and artificial long-day photoperiods from August-November. N = 40 larvae per mo.



## **CHAPTER 3: POST-MATING BEHAVIOR OF FEMALE DOGWOOD BORER IN APPLE ORCHARDS**

**Frank, D. L., T. C. Leskey, and J. C. Bergh**

**This chapter was published by *Environ. Entomol.* 38: 1219-1225.**

### **Abstract**

The post-mating behavior of female dogwood borer was examined in a young apple orchard planted on size-controlling rootstock in Virginia. All female dogwood borers captured while exhibiting casting flight near the base of trees were mated, based on the presence of a spermatophore. Surveys of female activity within orchards were conducted at regular intervals throughout the daylight hours, revealing a diel periodicity that peaked between 1700 and 1900 hours, with most females located below the graft union of trees. A transition matrix based on 1108 behavioral sequences exhibited by 66 females was used to produce a first-order Markov chain of behavioral events that occurred significantly more often than expected by chance. Casting flight, probing with ovipositor, and oviposition were the most frequent behaviors observed, representing 31.7, 30.0, and 18.1% of all behaviors recorded, respectively. My observations revealed that 88, 99, 99% of casting flight, probing with the ovipositor, and oviposition, respectively, occurred below the graft union. Observed behaviors considered not directly related to oviposition site selection or oviposition included antennal grooming, non-casting flight, and resting, representing 1.3, 8.3 and 10.6% of all behaviors recorded, respectively. Mated females spent significantly more time resting than in other behaviors and significantly more time in that state within the apple tree canopy than on other parts of the tree. Results are discussed in relation to the influence of insect-host plant interactions on oviposition site selection by female dogwood borer.

### **Introduction**

The dogwood borer, *S. scitula*, attacks a broad range of ornamental, fruit, and nut trees throughout eastern North America (Eichlin and Duckworth 1988, Johnson and Lyon 1991). Historically recognized as an important pest of dogwood, *C. florida* L. (Underhill 1935, Wallace 1945, Pless and Stanley 1967), it has become increasingly problematic in

apple orchards since the 1980's, coinciding with the adoption of size-controlling rootstocks (Riedl et al. 1985, Kain and Straub 2001, Leskey and Bergh 2005). A common characteristic of these rootstocks is the development of adventitious root initials, or burr knots, below the graft union or on the scion of trees (Rom 1970, 1973, Marini et al. 2003). The presence of burr knot tissue on host trees increases the likelihood of dogwood borer infestation (Riedl et al. 1985, Warner and Hay 1985, Kain and Straub 2001, Leskey and Bergh 2005). In newly established apple orchards, the quantity of available burr knot tissue has the greatest influence on the initiation, persistence, and extent of infestations (Leskey and Bergh 2005). Consecutive seasons of infestation and larval feeding on burr knot tissue and the surrounding cambium can result in the decline of tree health and vigor, or in severe cases cause tree death from girdling (Weires 1986, Howitt 1993).

Oviposition by many *Synanthedon* species occurs near wound sites on the host surface (Solomon 1995). Increased susceptibility to infestation due to mechanical injury or pruning scars were documented for several species including *S. tipuliformis* (Clerck) (Hardy 1982), *S. novaroensis* (Hy. Edwards) (Johnson 1993), and *S. sequoiae* (Hy. Edwards) (Koehler et al. 1983). Rocchini et al. (1999) suggested that the presence of fungal-induced cankers and galls in lodgepole pine influenced the susceptibility of trees to attack by *S. novaroensis*. In peach, *S. pictipes* (Grote and Robinson) were shown to deposit significantly more eggs in response to stimuli associated with canker wounds (Swift 1986, Reed et al. 1988, Cottrell et al. 2008) and mechanically damaged bark (Cottrell et al. 2008) compared with non-damaged bark. Furthermore, volatile compounds emanating from the gum and frass of infested peach trees were shown to stimulate oviposition by both *S. pictipes* (Reed et al. 1988, Cottrell et al. 2008) and *S. exitiosa* (Say) (Gentry and Wells 1982, Derksen et al. 2007).

Numerous early studies suggested that dogwood borer infestation was associated with wounded host plant tissue (reviewed in Bergh and Leskey 2003). Potter and Timmons (1981) confirmed that increases in the severity of mechanical wounding significantly increased the probability of dogwood borer attack in flowering dogwoods and Pless and Stanley (1967) noted that previously infested dogwood trees were highly prone to subsequent infestation by dogwood borer. Bergh et al. (2009) captured significantly more dogwood borer males in pheromone traps deployed in apple orchards

than in urban or forest environments. The proximate and ultimate reasons for higher densities of dogwood borer in orchards remain speculative but may be associated with aspects of the insect-plant relationship, including host preference or attraction and enhanced fitness.

Electrophysiological studies have shown that both virgin and mated female dogwood borer respond to volatile compounds collected from apple burr knot tissue (Chapter 6, Frank et al. 2007) and I am exploring the ecological role of these stimuli, including their use in host plant location and oviposition site selection by mated female dogwood borer. An important first step in the interpretation of their responses to these sources of stimuli is to observe and quantify their behavior under field conditions. While Bergh et al. (2006) characterized pre-mating behaviors of male and female dogwood borer, previous attempts to measure the post-mating behaviors of female dogwood borer have failed. For example, observations of post-mating behavior of females in cages containing potted dogwood trees (Wallace 1945, Pless and Stanley 1967) or blueberry plants (Ayers 1966), or in heavily infested dogwood nurseries (Pless and Stanley 1967), were unsuccessful.

Here I report the results from field studies that used quantitative descriptions of behaviors and transition matrices to characterize the behavioral repertoire of mated female dogwood borer within apple orchards under natural conditions and the periodicity with which those behaviors occurred.

## **Materials and Methods**

**Gender and Mating Status of Adults Orienting to Apple Trees Under Field Conditions.** To determine if casting flight (see Table 1) toward apple trees was unique to mated female dogwood borer, 25 moths orienting to the base of young apple trees on size-controlling rootstock in four orchards in Frederick Co., VA were collected using a sweep net from June-August in 2006 and 2007 during the afternoon. Moths were dissected under a 10X stereomicroscope (Olympus SZ40, Center Valley, PA) to confirm their gender and to determine their mating status, based on the presence or absence of a spermatophore within the bursa copulatrix.

**Orchard Site for Temporal and Behavior Studies.** Behavioral studies were conducted in a 0.65 ha research apple orchard containing 5-yr-old 'Buckeye Gala' and

'Idared' apple trees on M.26 rootstock at the Alson H. Smith Jr. Agricultural Research and Extension Center (AHS-AREC), near Winchester, VA. Tree varieties occurred in two adjacent blocks, each consisting of 60 trees in 3 rows with 6.1 m between rows and 2.4 m between trees. Trees were approximately 2.7 m tall x 2.4 m wide with an average trunk diameter of 6.5 cm directly above the graft union. Orchard drive rows were mowed frequently throughout the study, and 1.75 m wide weed-free strips were maintained beneath trees with herbicides. Disease management in the orchard was based on recommended guidelines for commercial fruit growers in the mid-Atlantic states (Pfeiffer 2007). In 2006, insecticide evaluation trials conducted in the orchard included foliar sprays to 20% of the Idared trees through late April and to 33.3% of the Gala trees through mid-August. In 2007, a standard management program for other apple pests used only foliar sprays from mid-April through late August. None of the products used would be expected to reduce the infestation of dogwood borer at the base of trees.

**Temporal Survey of Female Activity.** A survey of the temporal patterns of female dogwood borer activity in the orchard involved two observers walking abreast on opposite sides of each orchard row while recording the presence and location of female dogwood borer on each tree. The number of female sightings was recorded at 2-h intervals in non-inclement weather from 09:00 h until 19:00 h in 2006 (11 surveys), and from 08:00 h until 20:00 h in 2007 (10 surveys) from July-September. Temperature, relative humidity, wind speed, barometric pressure, and solar radiation data for each 2-h period were collected from a weather station at the AHS-AREC.

**Characterization of Mated Behaviors.** Orientation and oviposition site selection behaviors exhibited by 28 female dogwood borer in 2006, and 38 females in 2007, were characterized within the orchard described above. Observations were recorded by a single observer during peak periods of activity determined from results generated by my temporal survey. Observations of individual females lasted for a minimum of 1 min and a maximum of 60 min with a mean duration of  $15.2 \pm 12.8$  (SE) min. Specific behaviors recorded included resting, grooming, probing with the ovipositor, oviposition, casting flight, and non-casting flight (Table 3.1). The location of all females observed was also recorded, based on the following categories; (1) within the canopy, (2) above the graft union (i.e. between graft union and lower scaffold limb), (3) below the graft union, and (4) on the ground.

A behavioral ethogram was developed and programmed into The Observer (Noldus Information Technology, Version 3.0, Leesburg, VA). Behaviors were recorded using a portable cassette recorder and subsequently transcribed into the The Observer for calculation of the frequency and duration of each behavior observed and the location at which it occurred. After recording the behavior of a female, the observer moved to a different area of the orchard (minimum distance of 15 m away from the previous observation site) to minimize the possibility of recording the same individual a second time.

**Statistical Analysis.** The number of female sightings during surveys at each 2-h interval in 2006 and 2007 were converted to proportions, transformed using arcsine square-root to stabilize variance, then compared using ANOVA and Fisher's LSD test (SAS 2003). The influence of abiotic factors on the numbers of female sightings was analyzed using stepwise regression (SAS 2003). A transition matrix was constructed by transferring behavioral data from The Observer into a matrix of preceding and succeeding behaviors. To examine whether transitional behaviors were more likely to occur than expected by chance, a  $\chi^2$  test was applied to all transitions with a frequency greater than 1% of the total. Data from the transition matrix were used to construct a first-order Markov chain (Gottman and Roy 1990, Bishir et al. 2004) depicting the probabilities of transitions between behavioral activities. The duration of each behavior was analyzed using ANOVA (SAS 2003) with means separated using Fisher's LSD. Results from all tests were considered statistically different at  $P < 0.05$ .

## Results

**Gender and Mating Status of Adults Orienting to Apple Trees under Field Conditions.** Dissection of 25 moths captured while exhibiting casting flight at the base of apple trees confirmed that all were mated females, based on the presence of a spermatophore in the bursa copulatrix.

**Temporal Survey of Female Activity.** Time of day had a significant effect on the number of female sightings recorded at 2-h intervals in 2006 ( $F = 7.18$ ;  $df = 5, 129$ ;  $P < 0.0001$ ) and 2007 ( $F = 5.91$ ;  $df = 6, 139$ ;  $P < 0.0001$ ) (Fig. 3.1a,b). In 2006 and 2007, significantly more sightings occurred at 19:00 h and 18:00 h, respectively compared with all other intervals. There was no significant difference in the number of sightings in

‘Buckeye Gala’ and ‘Idared’ cultivars in 2006 ( $F = 0.58$ ,  $df = 1$ , 129,  $P = 0.4487$ ) or 2007 ( $F = 1.21$ ,  $df = 1$ , 139,  $P = 0.2726$ ). Combined data from both years revealed non-random distribution of females among tree locations: 89.7% of females were observed near the graft union of trees compared with 8.6% within the canopy and 1.7% flying away. There was a significant inverse relationship between solar radiation and the number of sightings in 2006 ( $F = 9.23$ ;  $df = 1$ , 64;  $P = 0.0035$ ), although this relationship explained only 13% of the variation and was not significant in 2007. No significant effects were detected among all other environmental factors measured.

**Characterization of Mated Behaviors.** The sequence of behaviors established by analysis of a transition matrix (Table 3.2) was used to produce a first-order Markov chain of behavioral events that occurred significantly more often than expected by chance (Fig. 3.2). The sequence, or chains, of behaviors are represented as arrows and the relative frequency of a specific behavioral pattern as bounding boxes. Casting flight was the behavior most frequently observed and began as a loop that was followed by probing with the ovipositor and oviposition, representing 31.7, 30.0, and 18.1% of all behaviors recorded, respectively. When exiting this loop, females transitioned into non-casting flight from which they again entered the loop or proceeded to the resting state, followed by grooming, representing 8.3, 10.6, and 1.3% of all behaviors recorded, respectively. Although the frequency of casting, probing, and oviposition represented 80% of total occurrences, dogwood borer females spent a significantly greater percentage of time resting (Table 3.3) compared with all other behavioral activities. Fifty percent of resting occurrences were recorded below the graft union compared with 32.3% in the canopy, 15.3% on the ground, and 2.4% above the graft union (Fig. 3.3). However, the average duration of a resting bout was significantly greater within the canopy compared with on the ground or above and below the graft union ( $F = 6.94$ ;  $df = 3$ , 122;  $P = 0.0005$ ). Although females engaged in behaviors directly involved with oviposition site selection (i.e. casting flight, probing with the ovipositor, and ovipositing) above and below the graft union, the frequency of casting, probing, and oviposition occurrences below the graft union was 88%, 99%, and 99% of the total for each behavioral category, respectively (Fig. 3.3).

## Discussion

Mated female dogwood borer were easily recognized in the field, based on their characteristic casting flight directed toward apple trees, enabling us to determine their daily period of activity and to quantify specific behaviors in an apple orchard. Mated female dogwood borer were recorded more frequently during the late afternoon and evening, conforming with the results of laboratory studies of *S. pictipes* showing that the mean rate of oviposition by this species peaked during the late afternoon and ceased during scotophase (Greenfield and Karandinos 1976) and with field observations of other sesiid species showing that peak oviposition occurred mainly from mid- to late afternoon (Solomon 1975, Barry and Nielsen 1984). Since it was very difficult to track between-tree movements of individual females, my observations were confined to the periods during which each female remained at a particular tree; inter-tree movements of females were observed but not recorded. The duration of observing and recording the behavior of each female was as short as 1 min, but I also observed individual females repeatedly searching for oviposition sites on a single tree for up to 60 min. I did not attempt to record the number of eggs on host tissues of trees on which females were observed, since it would not have been possible to differentiate between eggs laid by females observed and those laid previously. However, some females exhibited what I interpreted to be repeated instances of oviposition on an individual tree. The sequences of casting flight, probing, and ovipositing were interspersed with relatively long periods of resting behavior within the canopy, as has been observed for other *Synanthedon* species (Bobb 1959, Barry and Nielsen 1984).

Relative to other major sesiid pests, little is known about the stimuli responsible for host location or oviposition site selection by female dogwood borer. Gentry and Wells (1982) showed that volatiles from *S. exitiosa* cocoons and peach tree bark, and gum-frass mixtures obtained from wounds on peach trees, contained substances that stimulated oviposition by gravid females. Electrophysiological bioassays conducted by Derksen et al. (2007) revealed 21 compounds from gum-frass mixtures that elicited antennal responses by male and female *S. exitiosa*. Synthetic blends containing all 21 compounds, and synthetic blends containing all compounds except 3 acetates, stimulated significantly higher rates of oviposition compared with unbaited controls. Cottrell et al.



(2008) showed that surrogate peach branches that were mechanically wounded, actively infested with larvae, or that possessed fungal-induced wounds were more attractive to gravid females than undamaged branches or decoys. Similarly, Reed et al. (1988) showed that natural and chemically fractionated peach wood volatiles, and hosts possessing cankers as well as non-hosts treated with canker-bark extracts, stimulated oviposition by *S. pictipes* females even when blinded. Although chemosensory stimuli were strongly implicated for stimulating oviposition, field-cage studies revealed that size and texture of host surfaces were also significant factors, and that mechanoreceptors located on the ovipositor may further aid oviposition site selection.

Previous studies examining dogwood borer infestation in apple and other hosts have suggested that mated females preferentially oviposit near areas associated with pruning cuts (Herrick 1904, Pierce and Nickels 1941), mechanical wounds (Potter and Timmons 1981, Rogers and Grant 1990), insect-induced galls (Eliason and Potter 2000), apple burr knot tissue (Riedl et al. 1985, Warner and Hay 1985, Kain and Straub 2001, Kain et al. 2004, Leskey and Bergh 2005), as well as insect- and disease-induced injuries to the exterior of trees (Pless and Stanley 1967). My observations showed that mated female dogwood borer most frequently engaged in casting flight, probing with the ovipositor, and oviposition below the graft union of trees. The orchard used for these studies contained trees with cracks and crevices below the graft union and also burr knots below the union that were either previously infested or actively infested by dogwood borer larvae. While I did not attempt to relate the searching behavior of mated females with the presence of previously infested or infested burr knots, or other factors that might have influenced their response to individual trees, an increasing body of evidence supports the hypothesis that females respond to volatile stimuli associated with these plant features. Leskey and Bergh (2005) showed that greater amounts of burr knot tissue on newly established apple trees resulted in higher dogwood borer infestation rates. Furthermore, several volatile compounds collected from infested burr knot tissue evoked a strong and repeatable electrophysiological response by dogwood borer females (Chapter 6, Frank et al. 2007).

Although I have yet to determine whether the electrophysiologically active compounds are plant- or insect-derived, or a combination of the two, or whether these compounds elicit attraction and orientation to apple burr knots, this tissue appears to be

an important resource for dogwood borer. Burr knots are rather soft and spongy compared with apple bark and often possess many small crevices associated with root primordia. Female dogwood borer provided burr knot tissue commonly deposit their eggs tightly within these crevices and neonate larvae quickly enter and begin feeding on the tissue (Frank personal observation). In Europe, Dickler (1976) showed that larvae of the congeneric species, *S. myopaeformis*, developed more quickly on apple burr knot tissue than on other apple tissues and preliminary evidence (Leskey, unpublished data) has suggested a shortened developmental duration of dogwood borer larvae on this tissue as well.

By observing individual female dogwood borers, I have revealed distinct behavioral patterns common to mated females in nature. Further studies are needed to fully understand the chemical ecology and insect-host plant interactions of female dogwood borer in apple. A complete understanding of the mechanisms involved with oviposition site selection and acceptance will create opportunities to assess female responses to manipulated stimuli toward the development of monitoring or management programs that specifically target female dogwood borer.

**Table 3.1** Behaviors observed by mated female dogwood borer in an apple orchard.

---

Resting	Body motionless with abdomen parallel to resting surface
Probing	Lateral movement of the abdomen including brief periods (< 1 s) of direct contact of extruded ovipositor tip with host tissue
Grooming	Female grasps an antennae with tibia of foreleg and scraped the antennae in an anterior direction (sometimes repeatedly)
Ovipositing	Female abdomen slightly flexed towards substrate surface with extruded ovipositor tip contacting host tissue for periods < 1 s
Observed flight patterns of mated females within the apple orchard	
Non-casting	Observed flight that does not include casting flight patterns
Casting	Female executes a rapid series of counterturning flight patterns in the horizontal plane directed toward, and > 20 cm away from, the host tissue (including burr knot tissue, cracks in bark, or wound sites)

---

**Table 3.2** Transition matrix summarizing the frequency with which a behavior (column) was succeeded by another behavior (row). Transitions that occurred significantly more often than expected by chance ( $P < 0.05$ ,  $\chi^2$  test) are shown in grey boxes.

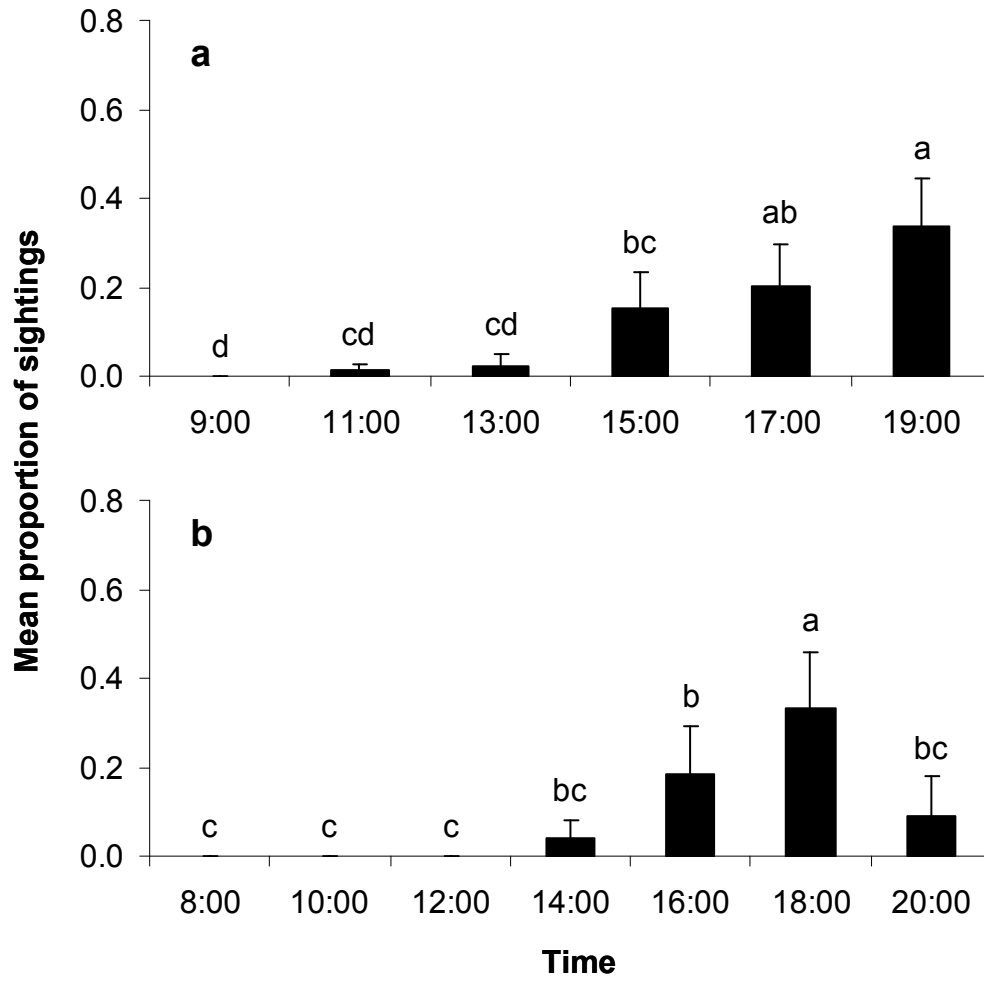
	Resting	Probing	Grooming	Ovipositing	Non-casting	Casting	$\Sigma$
Resting	-	22	15	1	41	38	117
Probing	22	-	0	200	7	104	333
Grooming	15	0	-	0	0	0	15
Ovipositing	22	89	0	-	6	83	200
Non-casting	35	0	0	0	-	57	92
Casting	23	218	0	0	110	-	351
$\Sigma$	117	329	15	201	164	282	1108

**Table 3.3** Mean ( $\pm$  SE) duration of behaviors exhibited by mated female dogwood borer and the percentage of total time engaged in each.

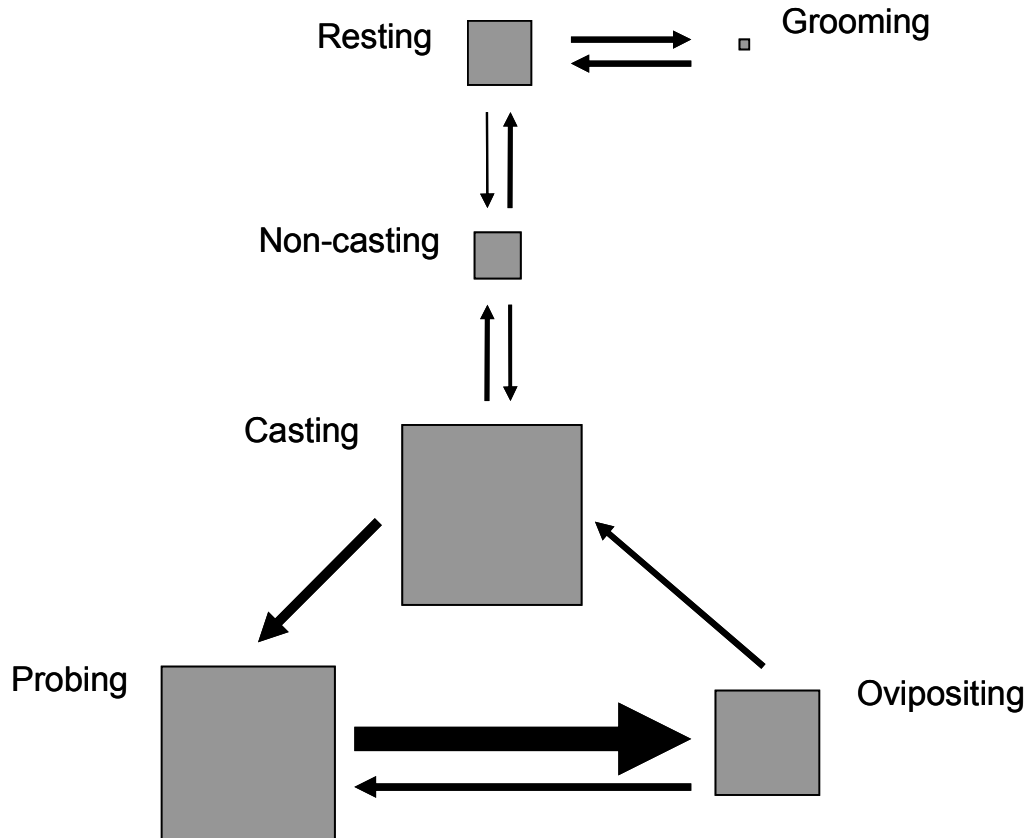
Behavior	Mean $\pm$ SE (sec.)	% of Total Time
Resting	160.90 $\pm$ 30.77 a	65.76
Probing	15.25 $\pm$ 0.77 b	16.79
Grooming	11.96 $\pm$ 1.81 b	0.59
Ovipositing	5.29 $\pm$ 0.26 b	3.49
Non-casting	6.14 $\pm$ 0.25 b	3.81
Casting	7.21 $\pm$ 0.25 b	9.56

Means followed by the same letter are not significantly different ( $P = 0.05$ , LSD test)  $F = 33.05$ ;  $df = 5, 70$ ;  $P < 0.0001$ .

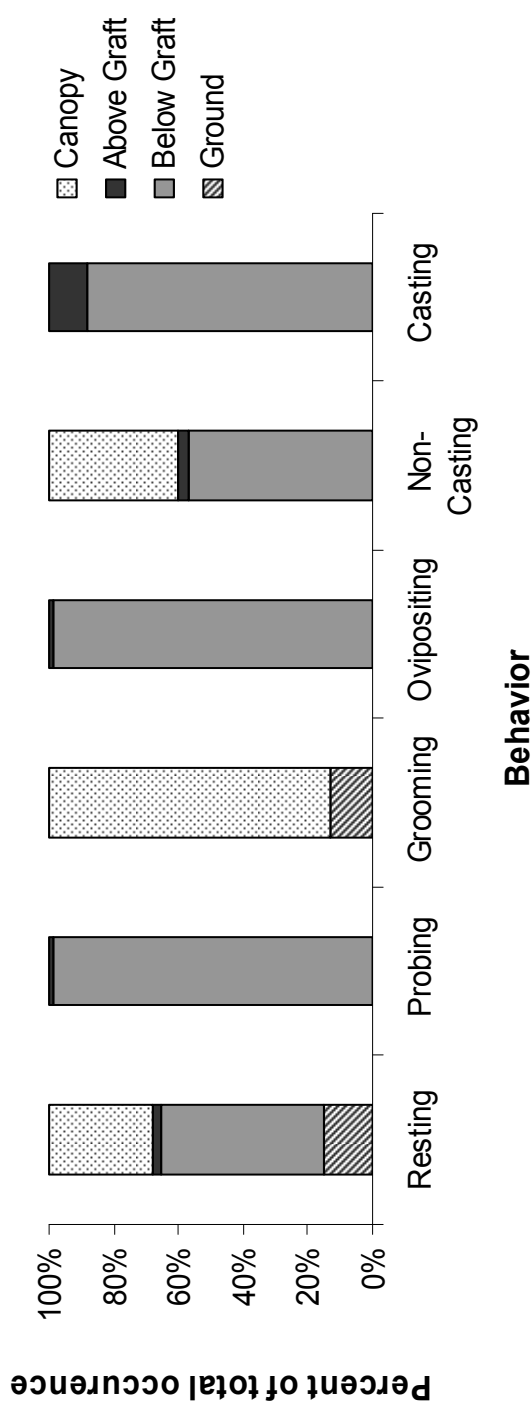
**Figure 3.1** Mean  $\pm$  SE proportion of female dogwood borer sightings at 2-h intervals in a) 2006 (n = 11) and b) 2007 (n = 10).



**Figure 3.2** First-order Markov chain analysis of a transition matrix constructed from 1108 behavior patterns exhibited by 66 mated female dogwood borer. The size of bounding boxes is proportional to the frequency of a behavioral pattern. Sequences of behaviors are represented as arrows when transitions occur significantly more often than predicted by chance ( $P = 0.05$ ,  $\chi^2$  test). The size of arrows show the degree to which a specific transition is over-represented.



**Figure 3.3** Percentage of the total number of occurrences of each behavior exhibited by mated female dogwood borer at selected locations on apple hosts.





## **CHAPTER 4: FACTORS INFLUENCING THE TEMPORAL AND SPATIAL PATTERNS OF DOGWOOD BORER INFESTATIONS IN NEWLY PLANTED APPLE ORCHARDS**

### **Abstract**

The temporal and spatial patterns of infestation by larval dogwood borer were studied from 2002–2004 in two newly planted apple orchards in West Virginia and Virginia. The orchards contained several rootstock-variety combinations grown under different cultural management regimes. Rootstock, tree variety, and cultural management practice were significantly associated with the presence and extent of dogwood borer infestation. In West Virginia, infestation was significantly greater on trees planted on M.26 rootstock compared with M.7 rootstock, and on trees fitted with spiral wrap tree guards compared with other trunk treatments. In Virginia, the proportion of infested trees was significantly greater for ‘Idared’ tree varieties compared with ‘Buckeye Gala’, and on trees fitted with spiral wrap tree guards compared with other trunk treatments. The results of geostatistical analysis indicated that there were moderate to high degrees of aggregation in dogwood borer infestations on neighboring apple trees with ranges of spatial autocorrelation from 7.50–19.87 m. The spatial patterns of the aggregation of infestation in the West Virginia and Virginia orchards were best described by the spherical and exponential semiovariogram models, respectively. Interpolated surface maps of infestation revealed local hot spots, which were generally more prevalent within tree guard treatments, and were typically found where dogwood borer infestations originated. Results are discussed in relation to the development of sampling plans for management of dogwood borer larvae and for site-specific agriculture.

### **Introduction**

The dogwood borer, *S. scitula*, is an important indirect pest of apple trees throughout eastern North America, particularly in high-density apple orchards in which trees are planted on size-controlling rootstocks (Riedl et al. 1985, Kain and Straub 2001, Bergh and Leskey 2003). These rootstocks often promote the formation of burr knots, or adventitious root initials, below the graft union or scion (Rom 1970, 1973, Marini et al. 2003). Burr knots are attractive oviposition sites for female dogwood borer and appear to

be an important resource for developing larvae (Riedl et al. 1985, Kain and Straub 2001, Bergh and Leskey 2003, Leskey and Bergh 2005). Over consecutive seasons of larval feeding, reductions in tree health and yield (Howitt 1993) and tree death from girdling (Weires 1986) can occur as larvae consume available burr knot tissue and mine outward into the surrounding cambium. It is likely that the effect of larval feeding may be most severe during the initial years of orchard establishment and growth (Leskey and Bergh 2005).

The development of effective management programs for the dogwood borer in apple orchards is needed to reduce damage caused by this pest. A prerequisite for such programs is an understanding of the temporal and spatial population dynamics of the insect and the factors that affect these dynamics. Several studies have examined the effects of host plant status, such as the presence and amount of burr knot tissue, on dogwood borer population development, and incidence of infestation (Riedl et al. 1985, Warner and Hay 1985, Kain and Straub 2001, Leskey and Bergh 2005). The use of clonal rootstock, tree guards, and mounding of soil and inadequate weed control around the rootstock are some of the factors that have been implicated in the development of burr knot tissue (Leskey and Bergh 2005). However, although there appears to be a positive relationship between the amount of burr knot tissue and the level of dogwood borer infestation (Leskey and Bergh 2005), data presented in Riedl et al. (1985) suggest that the mere presence of burr knot tissue on individual trees does not guarantee that affected trees will be attacked by the insect. The precise reasons for this are unclear, but might be related to factors such as the level of adult dogwood borer populations in the vicinity of the orchard and variability of within-orchard factors (e.g., temperature, humidity, and management practices) that affect the temporal and spatial occurrences of burr knot tissue and probability of dogwood borer attack. An assessment of the temporal changes in insect-host plant interaction, therefore, is needed to better understand the dynamics of localized dogwood borer populations and infestations of burr knot tissue by the insect.

It is to be expected that temporal changes in insect density in an area will be influenced by the spatial dynamics of the population (Schotzko and Knudsen 1992). As such, our understanding of the factors that could potentially influence species dynamics is usually strengthened by knowledge of the spatial structure of the population within the management unit (Strother and Steelman 2001, Tobin and Pitts 2002). Currently,

however, very little is known about the spatial structure of dogwood borer populations and infestations of burr knot tissue within apple orchards.

Several approaches can be used to describe the spatial structure of plant and animal populations within defined areas (Isaaks and Srivastava 1989, Cressie 1993, Perry 1995, Dale 1998, Haining 2003). One approach that has been applied to insect populations is geostatistics (Schotzko and O’Keeffe 1989, Midgarden et al. 1993). Geostatistical analysis can provide valuable information on the spatial dependence of individuals in relation to their resources within a defined area. This information can be used in developing efficient sampling programs (Kemp et al. 1989, Schotzko and O’Keeffe 1989, Midgarden et al. 1993) and for obtaining spatially independent samples that satisfy the assumption of random sampling for the design and analysis of statistical experiments (Williams et al. 1992, Wright et al. 2002). In addition, knowledge of the spatial structure of the insect, or of its effects, can be used to create distribution maps for the development of management support systems that lead to optimized insecticide spray programs by way of spatially precise and targeted applications for current and/or future infestations within the target area. This type of spatially referenced approach to insect management, formerly referred to as precision integrated pest management (PIPM), has been described for several insect pests (Weisz et al. 1995, Midgarden et al. 1997, Ellisbury et al. 1998, Blom and Fleischer 2001, Blom et al. 2002).

In this study, I investigated the factors associated with and their influence on the temporal pattern of dogwood borer infestations within apple orchards. The data I examined were collected as part of a larger effort to study the initiation and level of infestation by the dogwood borer in two newly planted apple orchards grown under different cultural management regimes (Leskey and Bergh 2005). In addition, I used geostatistics to explore the effects of within-orchard factors on the spatial variability of dogwood borer infestations so as to understand the seasonal spatial patterns of larval populations across years.

## **Materials and Methods**

**Orchard Design.** Data on infestation of burr knot tissue by dogwood borer larvae were collected in two experimental apple orchards located in Jefferson Co., West Virginia (Fig. 4.1) and Frederick Co., Virginia (Fig. 4.2) USA from 2002–2004. The

West Virginia orchard (Fig. 4.1) consisted of two 0.625 ha plots separated by a spacing of 7.6 m. ‘Gale Gala’ and ‘Sun Fuji’ trees were planted on size-controlling rootstocks in each plot in December 2001. The northern-most plot consisted of 12 rows of 20 trees planted on M.7 rootstock. Tree varieties were separated into two equal blocks consisting of 10 trees in six rows with 4.9 m between rows and 3.7 m between trees. The southwestern plot consisted of 12 rows of 30 trees planted on M.26 rootstock. Tree varieties were separated into two equal blocks consisting of 15 trees in six rows with 4.9 m between rows and 2.4 m between trees. Crabapple pollenizers were planted in both plots, which consisted of ‘Manchurian’ on M.7 rootstock planted every two trees in every other row in the northeastern plot, and ‘Snow Drift’ on M.26 rootstock planted every three trees in every other row in the southwestern plot.

The Virginia orchard (Fig. 4.2) consisted of a 0.65 ha plot (6 rows x 60 trees) of ‘Idared’ and ‘Buckeye Gala’ trees planted on M.26 rootstock in March 2002. Tree varieties were separated into two neighboring blocks consisting of 60 trees in 3 rows with 6.1 m between rows and 2.4 between trees.

**Orchard Management.** The orchard drive rows were mowed frequently throughout the study, and 1.2 and 1.75 m wide herbicide strips were maintained beneath trees in the West Virginia and Virginia orchards, respectively. Disease management in both orchards was based on recommended guidelines for commercial fruit growers in the mid-Atlantic states (Pfeiffer 2004). No insecticides were applied to the West Virginia orchards during the study. The Virginia orchard received minimal insecticide inputs (4 spray applications) in 2002. In 2003 and 2004, 7 and 9 insecticide applications were carried out, respectively, including three pyrethroid applications in 2004 to suppress damage from a severe infestation of the 17-yr periodical cicada (*Magicicada* spp.). None of the products used as foliar sprays to control arthropod pests in the tree canopy would be expected to reduce the infestation of dogwood borer at the base of trees.

**Trunk Treatments and Infestation Surveys.** Four replicates of each of three trunk treatments consisting of bare trunk, tree guard, and soil mound were randomly assigned to groups of trees as described in Leskey and Bergh (2005). Bare trunk trees received no protective treatment during the study, while spiral wrap rodent guards (OESCO, Conway, MA) were used on trees in the tree guard treatment. For mounded

trees, soil barriers were used to maintain coverage of the entire graft union throughout 2002 and 2003, but were removed in May 2004.

Infestation of burr knots and rooting tissue by dogwood borer larvae was evaluated based on the presence of fresh frass below the graft union at the base of trees (Leskey and Bergh 2005). In West Virginia, the number of infested trees and the number of infestation sites per tree were recorded monthly from June to October, March to November, and May to September in 2002, 2003, and 2004, respectively. In Virginia, I recorded each tree as infested (1) or not infested (0) at approximately monthly intervals from October to November, May to October, and May to November in 2002, 2003, and 2004, respectively.

**Statistical Analysis.** The data on the number of infestation sites per tree within the West Virginia orchards were analyzed by repeated measures Analysis of Variance (ANOVA) on three main factors, rootstock, trunk treatment, and variety, and with time as the repeated measures factor. The response variable was  $\log(y + 1)$  transformed for the analysis, which examined two effects; between-subject effect of the three main factors and within-subject effect that included the time factor. I followed the method outlined in Ott and Longnecker (2001) for analyzing three-factor experiments by first determining whether there was a significant interaction among the three main factors before looking at the two-factor interactions. In the presence of a significant two-factor interaction, each of the levels of one of the factors was examined separately at each of the levels of the other factor. From the analysis of the within-subject effect, the significance of the time  $\times$  main factor interactions were also assessed to determine whether the patterns of infestations across time differed with respect to each of the treatments. As suggested in Ott and Longnecker (2001), I used the adjusted  $F$ -values from the within-subject  $F$ -test to determine significance, and these values are reported. In the presence of significant differences ( $\alpha = 0.05$ ), multiple comparisons of the mean responses of the factor levels were carried out by orthogonal contrast (Ott and Longnecker 2001).

For the Virginia orchard, data on the proportion of trees infested by the dogwood borer within the treatment combinations (variety and trunk treatment) and across all sampling dates were also analyzed using repeated measures ANOVA. The data were arcsine square-root transformed for analysis to stabilize variances, and statistical

differences were determined at  $\alpha = 0.05$ . In the absence of a significant two-factor interaction, the main effects of the factors were tested and orthogonal contrast was used for the multiple comparisons of the mean proportion of tree infested for each of the factor levels (Ott and Longnecker 2001). All statistical analyses were carried out with JMP 7.0.1 (SAS Institute, Inc., Cary NC, 2005).

**Geostatistical Analysis.** The small-scale spatial structure of dogwood borer infestation within the West Virginia and Virginia orchards was examined by geostatistical analysis. Specific details of theory of geostatistics and its applications can be found in Isaaks and Srivastava (1989) and Rossi et al. (1992). Briefly, geostatistics involves two basic techniques, variography and kriging (Rossi et al. 1992). Variography is used to develop an experimental semivariogram for the sampled data, which describes the degree of spatial dependence (autocorrelation) between sample values with distance and/or direction within the sampling space. The semivariogram is developed using a function of the form,

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i) - z(x_i + h)]^2, \quad (1)$$

where  $\hat{\gamma}(h)$  is the estimated semivariance for the entity of interest ( $z$ ) at all sampling points ( $x_i$ ) separated by lag distance  $h$ , and  $N(h)$  is the number of pairs of samples separated by lag distance  $h$ . When the function in equation (1) is applied to spatially referenced sampling data at several lag distances a graph (semivariogram) is generated that shows the estimated semivariance values ( $\hat{\gamma}(h)$ ) at different lag distances ( $h$ ) between sampling points. If values at  $z(x_i)$  and  $z(x_i + h)$  are autocorrelated, equation (1) will give a small semivariance relative to pairs of points that are uncorrelated (Robinson and Metternicht 2006).

Once the semivariogram has been developed, a variogram model (e.g., spherical, exponential, Gaussian, or linear) can be fitted that best describes the spatial structure in the sampled data. The best fit model is usually determined by the coefficient of determination ( $r^2$ ) and/or residual sums of squares (RSS) for each of the models (Cressie 1993, Park and Tollefson 2005a, Robinson and Metternicht 2006). The analysis also generates three useful parameters, the nugget ( $C_0$ ), sill ( $C_0 + C$ ), and range ( $A$ ) that describe the shape of a variogram model, which are used in the kriging process. The

nugget is the value of the semivariance at which the lag distance equals zero (i.e., where the model intercepts the y-axis of the semivariogram graph); the sill is the value of the semivariance at which the model levels off and is equivalent to the variance of the data (Farias et al. 2004); the range is the average distance at which the semivariogram levels off, or the lag distance beyond which spatial dependence decreases. The nugget-to-sill ratio, or  $C_0/(C_0 + C)$ , provides a measure of the degree of spatial dependence in the variable and can be used to compare variograms developed for different situations (Farias et al. 2004, Liu et al. 2006).

The shape of the fitted variogram model can also be used to determine whether the spatial structure of the sampling data are uniform, random, or aggregated (Schotzko and O’Keefe 1989). In the case of aggregated sample data, for example, the semivariance is expected to increase with an increase in the separation distance between pairs of points up to some distance after which it will level off at the sill (Farias et al. 2004).

Kriging is an interpolation process based on a theoretical weighted moving average,

$$\hat{z}(x_0) = \sum_{i=1}^n \lambda_i z(x_i) \quad (2),$$

where  $\hat{z}(x_0)$  is the value to be estimated at an unsampled location  $x_0$ ,  $z(x_i)$  is the known value at the sampling point  $x_i$ ,  $n$  is the number of sampling points within a defined search area that are used for the estimation, and  $\lambda_i$  are the weights associated with each measured value (Farias et al. 2004). Kriging uses the information generated by the semivariance analysis to provide estimates of the values of the phenomena at unsampled locations (Rossi et al. 1992).

For the West Virginia orchard (M.7 and M.26 blocks) semivariograms were calculated separately for each of the three years using the log-transformed pooled data with a standard back-transformation applied to the output data (Isaaks and Srivastava 1989, Ellisbury et al. 1998). Pooling of the data collected over time has been shown to be an efficient method for determining spatial structure in many cases, but particularly when sample sizes are relatively small (Cressie 1993, Park and Tollefson 2005b). For the Virginia orchard, indicator variograms were developed based on the presence (1) or

absence (0) of larval infestation within trees. The indicator value (1 or 0) at each sampled location was determined by redefining the probability of infestation for each tree during the season based on a cutoff or threshold value of 0.50. That is, if the probability that the tree at a sampled location was infested  $\geq 0.50$ , the location was assigned a value of 1; otherwise the location was assigned a value of 0. The value of 0.50 was chosen as the cutoff because it was close to the median of the occurrence values (Isaaks and Srivastava 1989) within the orchards. I also considered a cutoff value of 0.75 based on the suggestion of Isaaks and Srivastava (1989) that other cutoffs should be examined. However, this cutoff value provided insufficient data for spatial analysis, and therefore is not discussed further.

Because no evidence of directionality was detected at 0, 45, 90, or 130°, only omnidirectional semivariograms are reported. After selecting the best variogram model for each spatial pattern, the parameters of the model were used in a block kriging process to create an interpolated surface map depicting infestation of dogwood borer larval throughout the orchard. All geostatistical analyses were performed using GS<sup>+</sup> version 7.0 (Gamma Design Software, Plainwell, MI).

## Results

### Factors of Infestation and Temporal Patterns

**West Virginia Orchard.** The analysis detected no significant effect of the interaction of rootstock  $\times$  trunk treatment  $\times$  variety ( $F = 0.21$ ;  $df = 2,580$ ;  $P > 0.05$ ) on number of infestation sites per tree. The only significant two-factor interaction was that of rootstock  $\times$  trunk treatment ( $F = 61.76$ ;  $df = 2,580$ ;  $P < 0.0001$ ). Because variety was not included in the significant two-way interaction, this main factor was examined alone as suggested by Ott and Longnecker (2001). The results of this analysis showed that although variety did not have a significant effect on the mean number of infestation sites per tree ( $F = 3.13$ ;  $df = 1,590$ ;  $P > 0.05$ ), the time  $\times$  variety interaction was significant ( $F = 7.29$ ;  $df = 17, 10030$ ;  $P < 0.0001$ ) suggesting that the two varieties, ‘Sun Fuji’ and ‘Gale Gala’, had different patterns of infestation sites per tree across time (Fig. 4.3).

Because of the significant rootstock  $\times$  trunk treatment interaction, each of the levels of rootstock (M.7 and M.26) was examined separately at each of the levels of trunk



treatment (bare trunk, tree guard, and soil mound). The results are presented in a two-way table of treatment means for the data collected across all sampling dates (Table 4.1). Essentially, regardless of the rootstock, the mean number of infestation sites per tree was significantly greater on trees with the spiral wrap tree guard, followed by the bare trunk treatment, and soil mound treatment. In addition, the mean number of infestation sites per tree was significantly greater on M.26 than M.7 rootstock with respect to the bare trunk ( $F = 136.79$ ;  $df = 1,195$ ;  $P < 0.0001$ ) and spiral wrap tree guard ( $F = 113.76$ ;  $df = 1,194$ ;  $P < 0.0001$ ) treatments. However, no significant difference between the rootstocks in the number of infestation sites per tree was observed with the soil mound treatment ( $F = 0.006$ ;  $df = 1,197$ ;  $P > 0.05$ )

The patterns of infestation by the dogwood borer across time were significantly different among the three trunk treatments on both M.7 ( $F = 34.16$ ;  $df = 34,4029$ ;  $P < 0.0001$ ) and M.26 ( $F = 79.72$ ;  $df = 34,5933$ ;  $P < 0.0001$ ) rootstocks (Fig. 4.4a,b). Likewise, the patterns of infestation by the dogwood borer across time were significantly different between the two rootstocks with respect to bare trunk ( $F = 19.83$ ;  $df = 17, 3315$ ;  $P < 0.0001$ ) and tree guard ( $F = 41.11$ ;  $df = 17, 3298$ ;  $P < 0.0001$ ) treatments, but not significantly different for the soil mound treatment ( $F = 0.20$ ;  $df = 17, 3349$ ;  $P > 0.05$ ; Fig. 4.4a,b).

**Virginia Orchard.** No significant effect of the interaction of variety  $\times$  trunk treatment ( $F = 3.42$ ;  $df = 2,12$ ;  $P > 0.05$ ) was detected on the proportion of trees infested by dogwood borer during the 2002–2004 study period in the Virginia orchard. However, both variety ( $F = 25.60$ ;  $df = 1,12$ ;  $P < 0.05$ ) and trunk treatment ( $F = 92.65$ ;  $df = 2,12$ ;  $P < 0.0001$ ) alone had significant effects on dogwood borer infestation. The mean proportion of infested trees was significantly greater within the ‘Idared’ variety ( $0.47 \pm 0.03$ ) compared with the ‘Buckeye Gala’ variety ( $0.32 \pm 0.03$ ). Furthermore, the mean proportion of infested trees was significantly greater within the tree guard treatment ( $0.58 \pm 0.03$ ) compared with the bare trunk ( $0.45 \pm 0.04$ ) and soil mound ( $0.15 \pm 0.03$ ) treatments.

The significant time  $\times$  variety ( $F = 3.04$ ;  $df = 14,168$ ;  $P < 0.001$ ) and time  $\times$  trunk treatment ( $F = 17.74$ ;  $df = 28,168$ ;  $P < 0.0001$ ) interactions suggested that the patterns of dogwood borer infestation across time within the Virginia orchard differed with respect to the levels of the two main factors (Fig. 4.5a,b).

## Spatial Pattern of Infestation

**West Virginia Orchard.** The semivariograms of dogwood borer infestation within the West Virginia orchards are shown in Figs. 4.6 and 4.7. A spherical model provided the best fit to the semivariance in both the M.7 and M.26 plots in all years (Table 4.2) indicating some degree of aggregation of infestation with the orchard. The range of spatial autocorrelation of infestations in the M.7 and M.26 plots varied from 7.79–17.98 m and 14.50–19.87 m, respectively, during the study (Table 4.2). This suggests that the farthest distance at which infestation levels remained spatially dependent, or autocorrelated, was ~18.0 and ~20.0 m for the M.7 and M.26 plots, respectively. The nugget-to-sill ratio,  $C_0/(C_0 + C)$ , provides a measure of spatial dependence and varies from 0 to 1 (Cambardella et al. 1994). Values of this ratio ranged from 0.00–0.50 (Table 4.2), indicating strong to moderate spatial aggregation in infestation among trees at the distances and directions tested.

The interpolated surface maps of the number of infestation sites per tree show that infestation in the M.7 plot was not readily apparent until the 2003 season (Fig. 4.6). Areas of relatively high mean number of infestation sites per tree (i.e. ‘hot spots’) that were initially confined to the tree guard treatments became noticeably more intense encompassing successively broader areas of the orchard in 2004 (Fig. 4.6). In the M.26 plot, some infestation was noticeable during the first season after trees were planted. Hot spots, which also developed primarily within the tree guard treatments in 2002, moved outward into the bare trunk treatments during 2003 and 2004 (Fig. 4.7).

**Virginia Orchard.** In 2002, infestation probabilities were below the threshold of 0.50, so the data for this year could not be used to model the spatial pattern of infested trees. Indicator variograms of the 2003 and 2004 data at a threshold of 0.50 suggested that dogwood borer infestation was spatially aggregated (Fig. 4.8). In both years, an exponential model provided the best fit to the semivariance with spatial dependence in larval densities ranging from 7.50–10.50 m (Table 4.2). During 2003 and 2004 the nugget-to-sill ratio was 0.12, indicating a strong degree of spatial aggregation in infestation among trees (Table 4.2). Interpolated surface maps based on the probability of trees being infested showed that during the 2003 season, hot spots of infestation developed primarily within the ‘Idared’ trees, whether or not they were fitted with spiral

wrap tree guards. However, in 2004 infestations had radiated outward into the ‘Buckeye Gala’ trees until much of the orchard was infested (Fig. 4.8).

### **Discussion**

Rootstock, tree variety, and cultural management practices were significantly associated with the presence and extent of dogwood borer infestation in the apple orchards throughout the study period. In West Virginia, infestation was significantly higher within the plot containing trees on M.26 rootstock compared with the M.7 plot. The number of infestation sites was also significantly greater on trees fitted with spiral wrap tree guards compared with other tree treatments. These overall levels of dogwood borer infestations of burr knot and rooting tissue are also reflected in the patterns of infestations across time. In Virginia, the proportion of infested trees was significantly greater for ‘Idared’ compared with ‘Buckeye Gala’ varieties and for trees fitted with spiral wrap tree guards compared with other tree treatments. All of these results are consistent with previous research, which suggest that female dogwood borer preferentially oviposit near areas associated with apple burr knot tissue (Riedl et al. 1985, Warner and Hay 1985, Kain and Straub 2001, Leskey and Bergh 2005). Also, as reported by Leskey and Bergh (2005), different types of rootstock, tree variety, and management regime can affect the amount of burr knot and rooting tissue and significantly increase the levels of infestation by dogwood borer.

A feature in the patterns of dogwood borer infestation in the West Virginia orchards that should be highlighted was the rapid build up of infestation sites during the first season in the M.26 plot on trees that received the bare trunk and tree guard treatments, and the delayed infestation until the second season in the M.7 plot. It has been shown that rootstock is one of the determining factors in the number of burr knots per tree (Marini et al. 2003, Leskey and Bergh 2005). A likely explanation for the difference in infestation between the rootstocks during the first season, therefore, is that trees on the M.26 rootstock not only develop more burr knots, but have the propensity to do so more quickly than do trees on M.7 rootstock. Trees on M.26 rootstock, therefore, appear to be susceptible to dogwood borer attack much earlier than trees on M.7 rootstock. Other factors, such as tree variety also should be considered. For example, although the analysis detected no interaction between variety and rootstock in the West

Virginia orchard, I might speculate that burr knots on ‘Sun Fuji’ trees planted on M.26 rootstock were more susceptible initially to dogwood borer infestation than burr knots on ‘Gale Gala’ trees on the same rootstock.

Another factor that may have resulted in the difference in number of infestation sites per tree between the M.7 and M.26 rootstocks in the West Virginia orchard during the first season is the location of the plots. Infestation in the M.26 plot may have been caused by the early movement into and build up of dogwood borer population from the south. As such, it is likely that if the locations of the plots of the two rootstocks were reversed, with the M.7 rootstock located to the south of the orchard, the temporal patterns of infestations might be different. No data, however, are available to verify the movement pattern of dogwood borer adults into the orchards during the first season after planting.

Variogram analyses enabled us to assess the spatial dependence of dogwood borer infestations on neighboring trees. The range of spatial dependence or autocorrelation in infestation among the orchards varied from 7.50–19.87 m, with a mean ( $\pm$  SE) range of  $13.47 \pm 4.52$  m. The degree of spatial dependence in infestation within each orchard is reflected by the values of the nugget-to-sill ratios. In general, strong spatial dependence in a variable results in a nugget-to-sill ratio that is  $<0.25$ , and moderate spatial dependence is indicated when the ratio is between 0.25 and 0.75; values greater than 0.75 indicate weak spatial dependence in the variable (Farias et al. 2004, Liu et. al. 2006). If I ignored the results of the spatial analysis for the first season, it is clear that there was a high degree of spatial dependence in infestation within the Virginia orchard, and a moderate degree of spatial dependence within the West Virginia orchard, during both the second and third seasons. The difference in the degree of spatial dependence between the Virginia and West Virginia orchards may be due to differences between the regions in the level of dogwood borer populations, in the design of the orchards, in the susceptibility of the varieties tested to the development of burr knots and dogwood borer infestation, and with respect to the characteristics of the response variables that were analyzed. For the West Virginia orchard, the response variable was the actual number of infestation sites per tree at each location, while for the Virginia orchard, an indicator variable of either 1 (infested) or 0 (not infested) was analyzed for each sampling location. Although Isaaks and Srivastava (1989) noted that spatial structure based on indicator variables is easier to

model and interpret than those based on the original variable, they warned that the spatial structure of the variable from indicator variograms may not always reflect spatial continuity or dependence of the variable, but rather the clustering of the sample data.

Nevertheless, it appears that there was some level of aggregation of dogwood borer infestation within the orchards, which might be explained by the close proximity of susceptible trees and female moth post-mating behavior. Although dogwood borer females are normally swift and active fliers, Frank et al. (2009) found during behavioral observations in the field that mated females were often observed searching for oviposition sites and ovipositing on individual rather than on multiple trees. Similar observations have been reported for other *Synanthedon* species. For example, Barry and Nielsen (1984) reported that *S. exitiosa* (Say) females generally oviposited on the same peach trees on which they were found mating, but later flew to nearby trees within the orchard. In addition, *S. pictipes* (Grote and Robinson) females often oviposited numerous eggs within close proximity to each other on a single peach tree host (Bobb 1959).

No directionality was observed in the spatial autocorrelation of infestation among neighboring infested trees within the orchards. This suggests that dogwood borer females are able to locate suitable oviposition sites equally well along and across orchard rows (i.e. infestations are equally likely to spread along and across rows). In addition, the interpolated surface maps of infestation for each orchard revealed local hot spots for each year of the study. Hot spots were generally more prevalent within tree guard treatments and were typically found where dogwood borer infestations originated. Tree guards likely led to increased infestation rates by promoting burr knot development (Leskey and Bergh 2005) and perhaps by offering developing larvae protection from desiccation and natural enemies (Bergh and Leskey 2003). As expected, areas with the lowest level of infestation occurred within the soil mound treatments throughout the study. Leskey and Bergh (2005) indicated that soil mounds appeared to promote undesirable horticultural effects in these trees as a result of scion rooting and that mounds were difficult to maintain. Furthermore, prior to the removal of soil mounds in 2004, the trees developed a large amount of rooting tissue, which provided a suitable habitat for subsequent infestation by larvae.

An understanding of the spatial dependence of dogwood borer infestations is an important first step for developing accurate and efficient sampling plans for larvae and for the design of experiments (Williams et al. 1992). To obtain unbiased estimates of population totals, independent samples must be taken at random so that every sample location has an equal chance of being selected (Pedigo 1999). Because of the aggregated nature of dogwood borer infestations, random, independent samples can only be obtained at distances greater than the range of spatial dependence to ensure that these samples are not autocorrelated. It is clear from the results that the distance between sample points will vary depending on the age of the orchard at which sampling is conducted. Alternatively, an efficient sampling program for mapping dogwood borer infestation can be achieved by limiting sample points to distances within the calculated range of spatial dependence. An understanding of the sampling distances required to assess larval populations can lead to a reduction in the number of samples needed within an orchard while maintaining the accuracy and precision of sample data. The sample points can also be used in interpolating algorithms, such as kriging, to predict infestation at unsampled locations in space. The interpolated surface maps could then be used in a dogwood borer management program to indicate areas where larval infestations are more likely to occur, where scouting should begin, and where management intervention is necessary. Currently, dogwood borer populations in apple orchards are mainly controlled using truck drench applications of an organophosphate insecticide (e.g. Lorsban), which are often labor-intensive and costly to apply. The development of this type of site-specific management (Weisz et al. 1995, Midgarden et al. 1997) offers the advantage of limiting the amount of insecticide used within an orchard, as well as the time and labor involved with its application, by targeting sprays at potential hot spots or aggregated infestation sites. Furthermore, understanding the arrangement and pattern of infestations may offer clues for development of alternative management strategies for dogwood borer control.

**Table 4.1** Mean ( $\pm$  SE) number of infestation sites per tree in West Virginia apple orchards with M.7 and M.26 rootstocks and three trunk treatments.

Rootstocks	Trunk Treatments			
	n	Bare Trunk	Tree Guard	Soil Mound
M.7	1440	0.24 $\pm$ 0.02 b,B	0.94 $\pm$ 0.05 a,B	0.12 $\pm$ 0.01 c,A
M.26	2142	1.35 $\pm$ 0.04 b,A	2.35 $\pm$ 0.06 a,A	0.10 $\pm$ 0.01 c,A

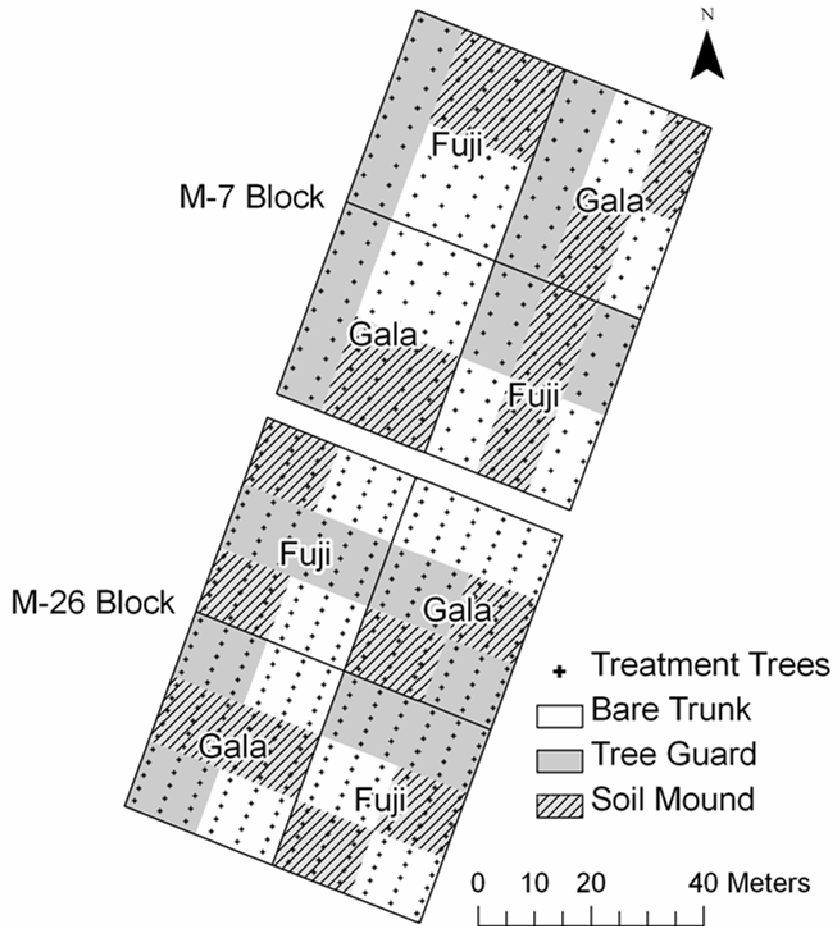
<sup>a</sup> Means across each row (rootstock) followed by the same lowercase letter are not significantly different ( $P > 0.05$ ); means within each column (trunk treatment) followed by the same uppercase letter are not significantly different ( $P > 0.05$ ).

**Table 4.2** Geostatistical description of larval dogwood borer infestation from 2002 to 2004 in West Virginia and Virginia orchards.

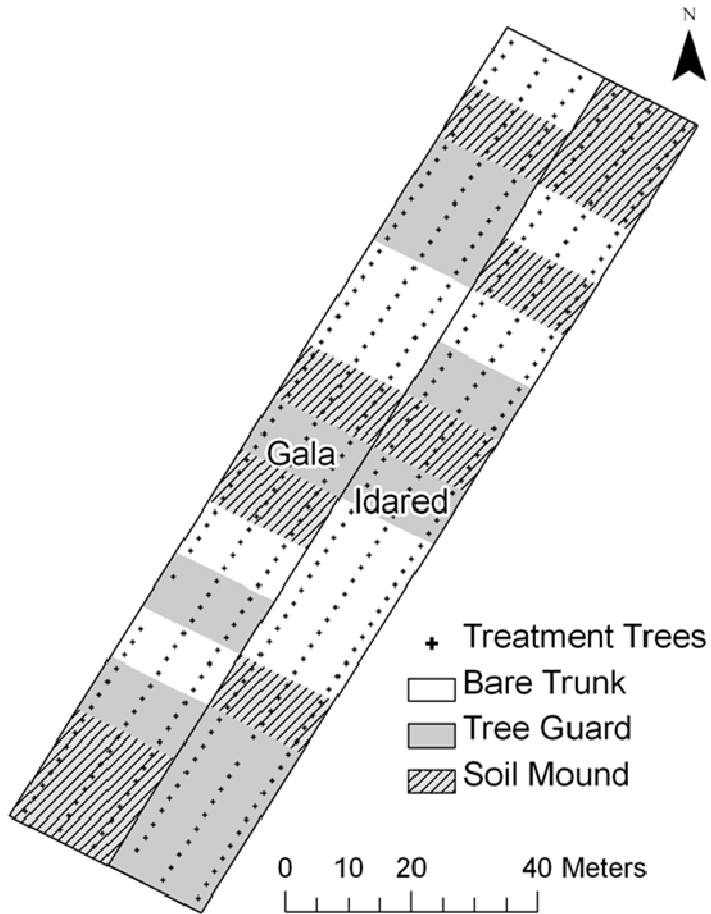
Data Set	Model	Nugget ( $C_0$ )	Sill ( $C_0 + C$ )	Range (m) ( $A$ )	$R^2$	Nugget/Sill Ratio $C_0 / (C_0 + C)$
West Virginia; M.7						
2002	Spherical	0.000	0.003	7.79	0.58	0.00
2003	Spherical	0.044	0.088	17.98	0.81	0.50
2004	Spherical	0.125	0.343	15.03	0.85	0.36
West Virginia; M.26						
2002	Spherical	0.055	0.129	19.87	0.87	0.43
2003	Spherical	0.105	0.397	14.56	0.93	0.26
2004	Spherical	0.122	0.446	14.50	0.87	0.27
Virginia; M.26						
2003	Exponential	0.029	0.236	7.50	0.69	0.12
2004	Exponential	0.027	0.232	10.50	0.77	0.12



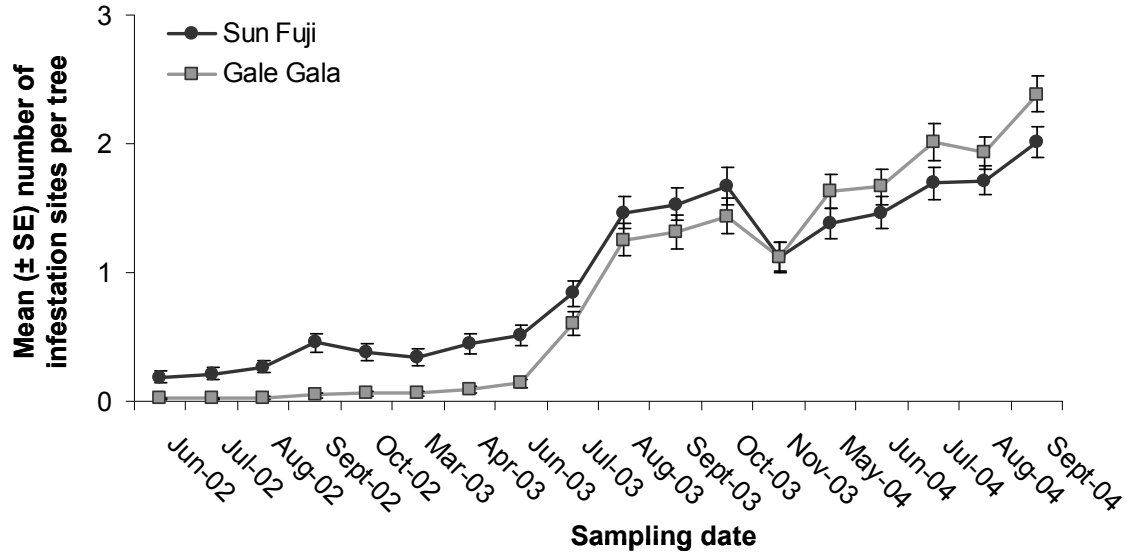
**Figure 4.1** Layout of the research apple orchard in Jefferson Co., West Virginia, showing rootstocks (M.7 and M.26), tree varieties ('Sun Fuji' and 'Gala Gala'), and trunk treatments.



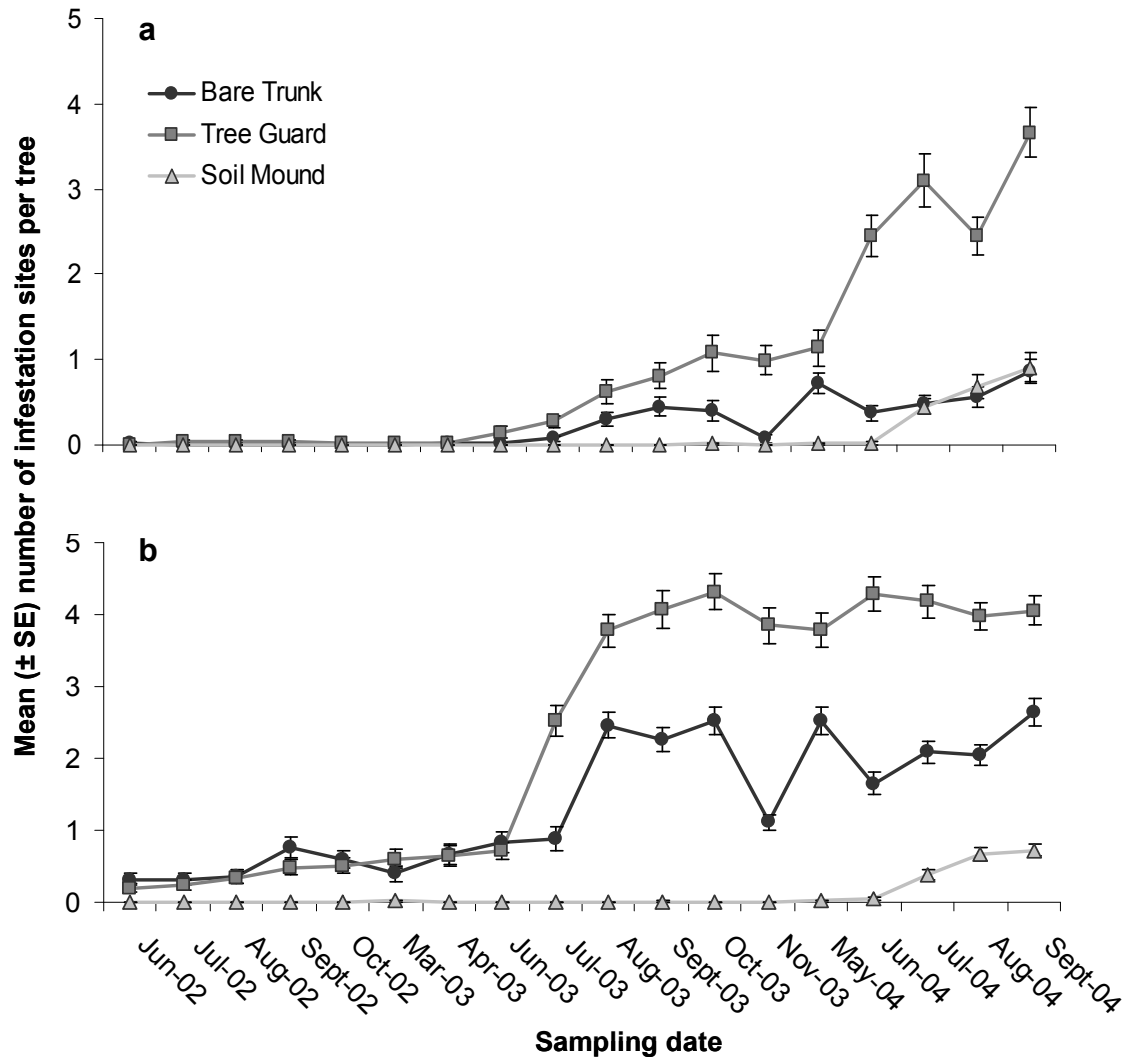
**Figure 4.2** Layout of the research apple orchard planted on M.26 rootstock in Frederick Co., Virginia, showing tree varieties ('Buckeye Gala' and 'Idared') and trunk treatments.



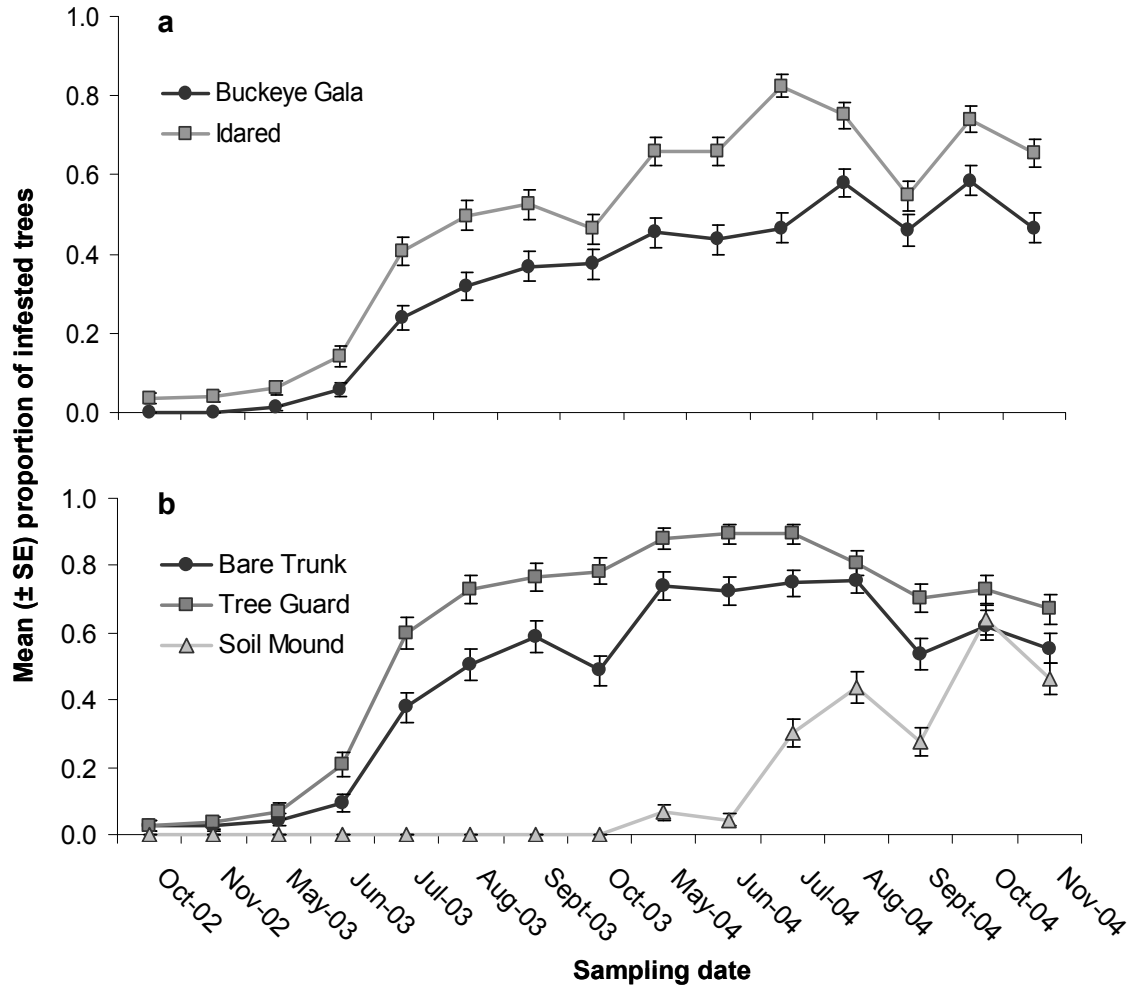
**Figure 4.3** Temporal patterns of the mean number of infestation sites per tree infested by dogwood borer on two varieties of apples in orchards located in West Virginia. Soil mound trunk treatments were removed in May 2004.



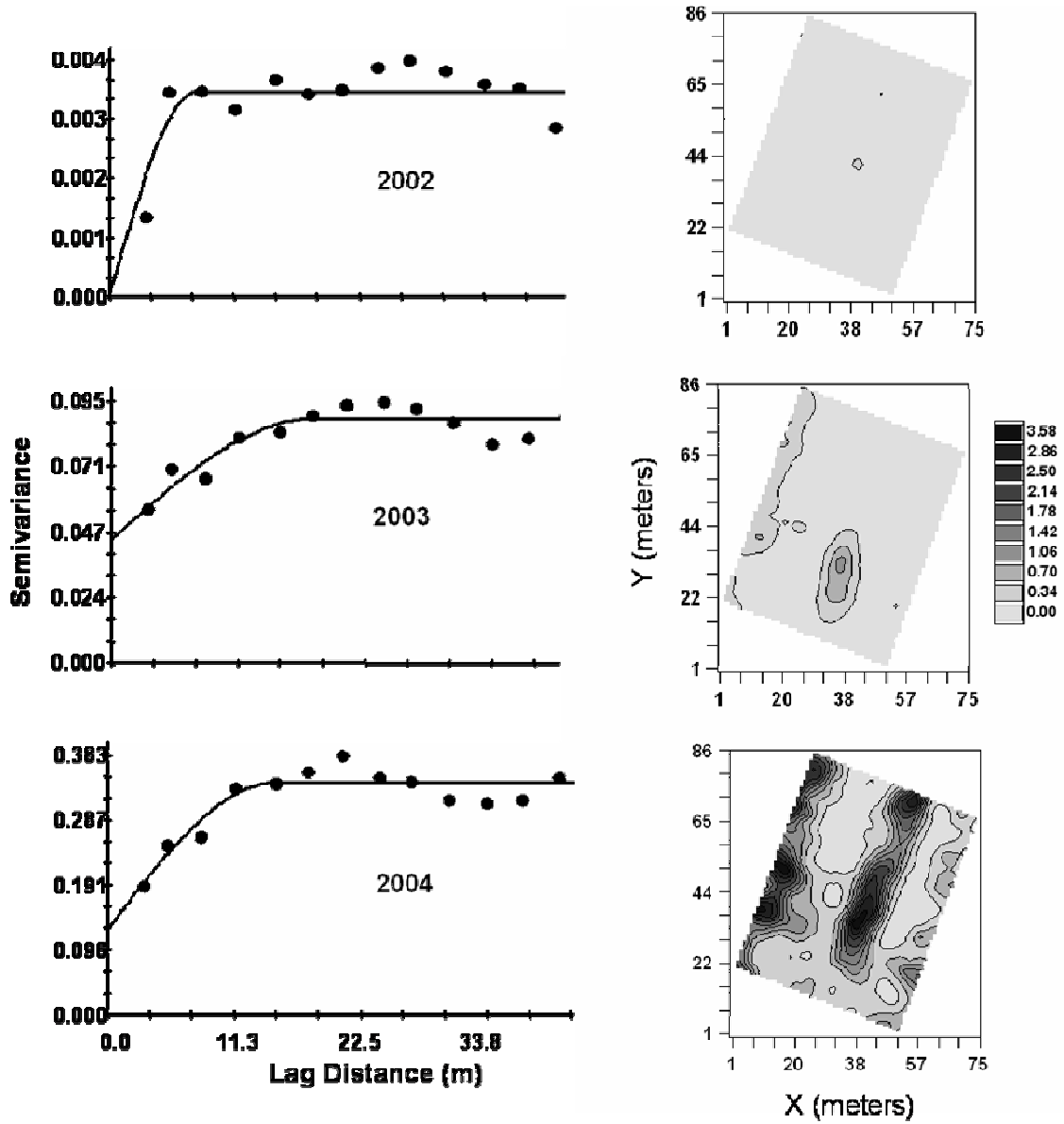
**Figure 4.4** Temporal patterns of the mean number of infestation sites per tree infested by dogwood borer on apple trees subjected to three trunk treatments in the West Virginia orchard grown on a) M.7 and b) M.26 rootstock. Soil mound trunk treatments were removed in May 2004.



**Figure 4.5** Temporal patterns of the mean proportion of trees infested by dogwood borer in the Virginia orchard in relation to a) variety and b) trunk treatment. Soil mound trunk treatments were removed in May 2004.



**Figure 4.6** Omnidirectional semivariograms and kriged surfaces of the mean number of infestation sites per tree in the West Virginia M.7 plot for 2002, 2003, and 2004.



**Figure 4.7** Omnidirectional semivariograms and kriged surfaces of the mean number of infestation sites per tree in the West Virginia M.26 plot for 2002, 2003, and 2004.

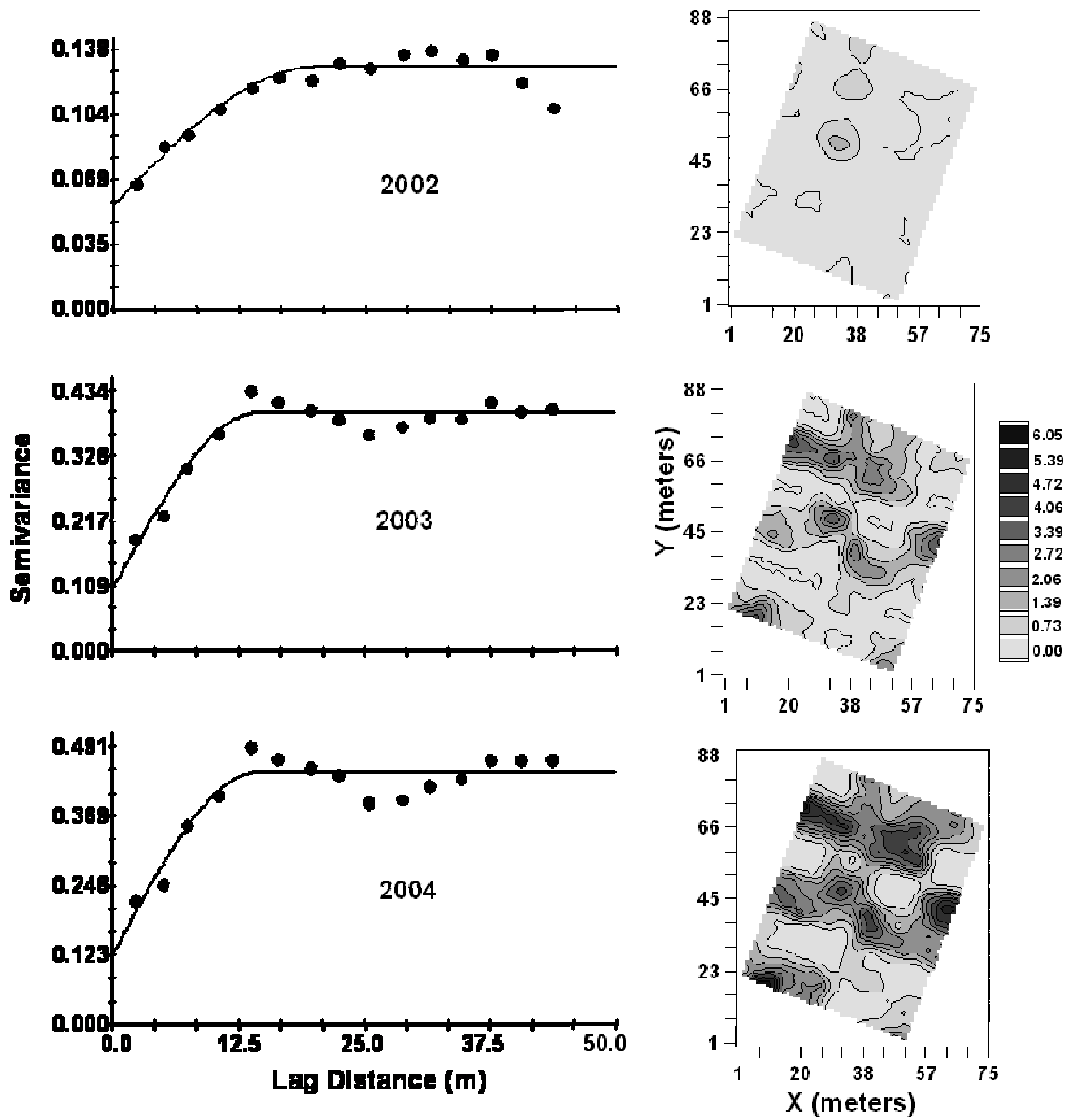
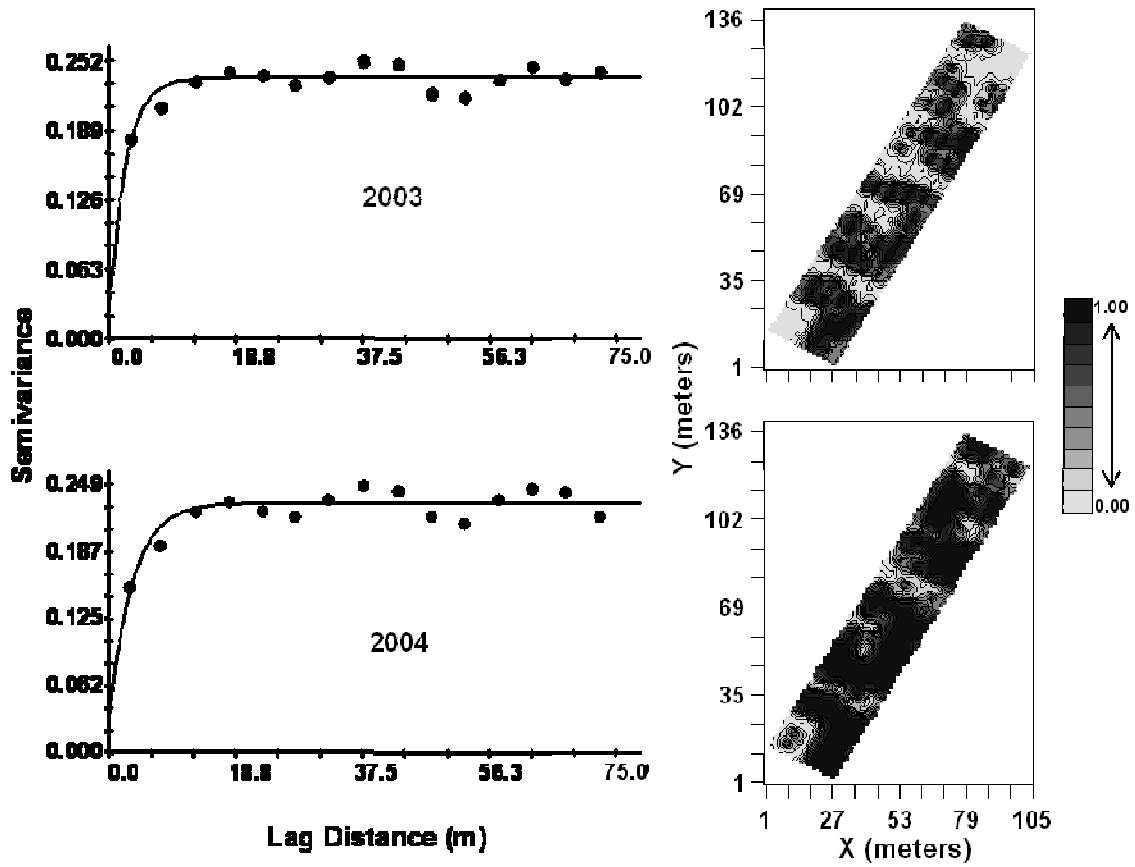


Figure 4.8 Omnidirectional indicator semivariograms and kriged surfaces of infestation probabilities above a median threshold of 0.50 in the Virginia orchard for 2003 and 2004.





## **CHAPTER 5: MORPHOLOGICAL CHARACTERIZATION OF THE ANTENNAL SENSILLA OF THE DOGWOOD BORER**

### **Abstract**

The external morphology of the dogwood borer antennae and their sensilla was investigated using light and scanning electron microscopy. Male and female antennae were clavate before tapering to an apical point and consisted of three main segments; the scape, pedicel, and flagellum. Although there was no significant difference in the length of the flagellum between genders, the number of flagellomeres was significantly greater in females than in males and the length and width of individual flagellomeres was significantly greater in males than in females, except near the distal end of the antennae. The antennal flagellum of both male and female dogwood borer contained seven sensillum types: auricillica, basiconica, chaetica, coeloconica, squamiformia, styloconica, and trichoidea (3 subtypes). The mean number of sensilla basiconica did not differ between female and male antennae, but all other sensillum types were significantly more abundant on female antennae. The morphology and purported function of each sensillum type are discussed in relation to the host and oviposition site finding and acceptance behaviors exhibited by dogwood borer.

### **Introduction**

Defining plant-insect interactions related to the use of host plant compounds for host and oviposition site location, identification and acceptance has become an important topic of entomological research (Renwick 1989, Bernays and Chapman 1994). Host plant compounds can play an important role in host selection processes of female insects, thus affecting the survival and distribution of their offspring (Mayhew 1997). Although investigation of the behavioral responses of insects to host plant compounds for development of monitoring or management strategies has often been difficult because of the numerous signals used by insects in host location and mate finding and the potential synergistic interactions between them (Viser 1986, Landolt and Phillips 1997), some success in the integration of these chemical stimuli in behaviorally based management strategies has been documented (Van Steenwyk and Barnett 1987, Prokopy et al. 1992, Yang et al. 2004, Leskey et al. 2008).

Females of many species of Sesiidae appear to respond to particular host plant odors, as they often oviposit preferentially near damaged host tissues (Solomon 1995). For example, oleoresin exudates from pruning wounds served as attractive oviposition stimuli for *S. novaroensis* (Hy. Edwards) in managed Douglas-fir stands (Johnson 1993). Rocchini et al. (1999) suggested that the occurrence of fungal-induced cankers and galls in lodgepole pine influenced the susceptibility of trees to attack by *S. novaroensis*. Cottrell et al. (2008) showed that *S. pictipes* (Grote and Robinson) deposited significantly more eggs on peach limbs that were mechanically damaged or possessed fungal-induced wounds compared with non-damaged limbs. In addition, Derksen et al. (2007) showed that volatile compounds emanating from the gum and frass of infested peach trees induced oviposition by *S. exitiosa* (Say).

The dogwood borer, *S. scitula*, is a polyphagous clearwing moth that occurs throughout much of eastern North America (Eichlin and Duckworth 1988) and is much more abundant in commercial apple orchards than in the forest or managed urban landscapes (Bergh et al. 2009). In apple orchards, females preferentially oviposit on or nearby burr knot tissue on host trees, which appear to be an important resource for developing larvae (Riedl et al. 1985, Kain and Straub 2001, Leskey and Bergh 2005). Frank et al. (2009) showed that mated female dogwood borer frequently engaged in casting flight toward, and oviposition near, sites below the graft union of apple trees where there were burr knots and cracks in the bark. Furthermore, both virgin and mated female dogwood borer respond electrophysiologically to volatile compounds collected from this tissue (Chapter 6, Frank et al. 2007). Although the response of male dogwood borer to its sex pheromone (Zhang et al. 2005, Leskey et al. 2006) has been examined, the investigation of the role of host plant compounds on the behavioral responses of females and males has largely been neglected.

As a fundamental companion study to my on-going research on the use of plant-derived semiochemicals for host-finding and oviposition by female dogwood borer, I examined the antennal morphology of male and female dogwood borer and compared the types, number, and distribution of antennal sensilla of both genders.

## Materials and Methods

**Insects.** In May 2009, a laboratory colony of *S. scitula* was initiated with late instar larvae from commercial apple orchards in Frederick Co., VA, then propagated and maintained on green thinning apples as described in Chapter 2, with periodic introductions of field collected larvae. Adults (1- to 2-d-old) from the colony were frozen until prepared for examination.

**Measurements of Antennae.** Excised heads of 35 male and 35 female dogwood borer were placed in a 10% potassium hydroxide solution at 80°C for 1 h until the scales were removed and the antennae cleared. The heads were transferred to a bath of distilled water at 80°C for 1 h, then dehydrated in 70 and 100% alcohol for 30 min each.

Antennae were excised from the heads and photographed at 20X magnification using a Paxcam (MIS Inc., Villa Park, IL) digital camera mounted on a Nikon SMZ1500 (Nikon Instruments Inc., Melville, NY) microscope. Pax-it Version 7.0 (MIS Inc., Villa Park, IL) imaging software was used to examine the images for measurements of the length of the antennal flagellum, the number of antennal flagellomeres and the length and width of the scape, pedicel and selected flagellomeres.

**Measurements of Sensilla.** Excised heads of 8 male and 8 female dogwood borer were placed in a 10% potassium hydroxide solution at 100°C for about 2 min until the antennal scales were removed. The heads were rinsed with distilled water, then dehydrated through a graded series of 70, 80, 90, and 100% ethanol for 30 min each. Antennae were removed from the heads and mounted on specimen stubs with adhesive, so that either the ventral (5 antennae/gender) or dorsal (3 antennae/gender) surface was visible. After air drying for 5 min, the antennae were sputter coated with gold/palladium in a Hummer VI (Anatech Ltd., Alexandria, VA) sputtering system followed by examination under a Stereoscan 120 (Cambridge Scientific Instruments Ltd., Cambridge, United Kingdom) scanning electron microscope at 10 kV. Images of each antennal flagellomere were recorded using an Orion 5.0 (E.L.I. Microscopy, Brussels, Belgium) imaging system from which the types and number of sensilla from each gender was determined. Using Pax-it Version 7.0 imaging software the average length and basal diameter of each type of sensillum from each gender was calculated from 30 measurements taken along the length of the antennae from the images of five individuals.

**Statistical Analysis.** All data were analyzed using an unpaired t-test (SAS 2003). When necessary, the results were square root transformed to meet the assumptions of normality and homogeneity of variances. Results from all tests were considered statistically different at  $P < 0.05$ .

## Results

### Antennal Morphology

Dogwood borer antennae were situated between the compound eyes and consisted of two basal segments, the scape and pedicel, followed by a distal flagellum consisting of subsegments, or flagellomeres. The scape was the largest part of the antennae (Tables 5.1 and 5.2) and was bulbous in form following a constriction distal to its insertion in the antennal socket. The pedicel was smaller than the scape and more elongated on the dorsal side compared with the ventral surface. Both the scape and pedicel were covered in overlapping scales and possessed numerous spine-like Böhm's bristles near the proximal end of each (Fig. 5.1a). Male and female antennae did not differ significantly in the length of the scape and pedicel (Table 5.1), although the width of both was significantly larger in males (Table 5.2). For both genders, the flagellum was clavate before tapering to an apical point. Individual flagellomeres were typically cylindrical in shape and could be divided into two main areas, the ventral and dorsal surfaces (Fig 5.1c). The ventral surface was highly textured with many grooves and ridges and possessed most of the sensilla. The dorsal surface was generally smoother in texture, and possessed numerous rows of overlapping scales along its length. No sensilla were located on the penultimate flagellomere. The most distal, conical shaped, flagellomere was devoid of scales and possessed numerous spike-like apical sensors (Fig. 5.1b,c). The mean ( $\pm$  SE) number of flagellomeres was  $46.8 \pm 0.3$  for males (range = 43-53), and  $49.3 \pm 0.4$  for females (range = 46-55), which was significantly different ( $t = 5.06$ ,  $df = 68$ ,  $P \leq 0.0001$ ). Although female antennae possessed more flagellomeres, the length of the flagellum was  $5834.8 \pm 53.5 \mu\text{m}$  and  $5759.0 \pm 62.6 \mu\text{m}$  for males and females, respectively, which was not significantly different ( $t = -0.92$ ,  $df = 68$ ,  $P = 0.359$ ). The length and width of individual flagellomeres was significantly greater in males than in females except near the distal end (Tables 5.1 and 5.2).

## Antennal Sensilla

Sensilla morphology was based upon the descriptions and terminology used by Sellier (1977) and Blum (1985). The antennal flagellomeres (except the most distal two) of the dogwood borer possessed seven different sensillum types; auricillica, basiconica, chaetica, coeloconica, squamiformia, styloconica, and trichoidea (three subtypes). The size and structure of each sensillum type was nearly identical between male and female moths. Unless otherwise noted, the following morphological descriptions apply to both genders.

**Sensilla auricillica.** These sensilla were the second most abundant of all sensillum types on the antennae of both males and females (Table 5.3), occurring primarily on the ventral surface along the length of the flagellum (Figs. 5.2 and 5.3), but also on the dorsal surface among the scales of the distal flagellomeres. Their distribution on the flagellomere was random and their number increased distally along the flagellum, generally reaching their maximum abundance before the 35<sup>th</sup> flagellomere, at which point the antennae began to taper in size. These sensilla had a rabbit-ear shape, but could also appear dorsoventrally flattened (Fig. 5.4a). They were 9.1 - 17.6  $\mu\text{m}$  long and had a basal width of 1.4 - 3.3  $\mu\text{m}$ . Sensilla auricillica were significantly more abundant on female antennae ( $t = 8.44$ ,  $df = 8$ ,  $P \leq 0.0001$ ) (Table 5.3).

**Sensilla basiconica.** These sensilla were the least abundant of the sensillum types on the antennae of both males and females (Table 5.3). They were found randomly along the length of the flagellum before the 35<sup>th</sup> flagellomere, at which point the antennae began to taper in size and were typically located near the distal, lateral region of the ventral surface of the flagellomere (Figs. 5.2 and 5.3). These sensilla had a conical, peg-like appearance (Fig. 5.4b) and were 1.9 - 4.5  $\mu\text{m}$  long and had a basal width of 0.9 - 1.7  $\mu\text{m}$ . The mean number of sensilla basiconica was similar in both genders ( $t = 1.50$ ,  $df = 8$ ,  $P = 0.173$ ) (Table 5.3).

**Sensilla chaetica.** These sensilla were distributed evenly along the flagellum, near the center or lateral region of the ventral surface, and typically numbered from 1 - 4 per flagellomere (Figs. 5.2 and 5.3). They had a straight, needle-like appearance and arose from a distinctive round collar-like socket (Fig. 5.4c). They were 12.6 - 38.3  $\mu\text{m}$

long and had a basal width of 1.5 – 3.3  $\mu\text{m}$ . Sensilla chaetica were significantly more abundant on the female antennae ( $t = 3.83$ ,  $df = 8$ ,  $P = 0.005$ ) (Table 5.3).

**Sensilla coeloconica.** These sensilla were found along the ventral surface of the entire flagellum and typically numbered from 0 – 6 per flagellomere (Figs. 5.2 and 5.3). They consisted of a submerged central peg surrounded by a ring of spine-like cuticular teeth (Fig. 5.4d). These sensilla were 4.5 – 12.9  $\mu\text{m}$  in diameter and were significantly more abundant on female antennae ( $t = 7.05$ ,  $df = 8$ ,  $P \leq 0.0001$ ) (Table 5.3).

**Sensilla squamiformia.** These sensilla were found along the length of the flagellum near the center or lateral region of the dorsal surface among the scales, near the lateral region of the ventral surface and among the scales on the scape and pedicel (Fig. 5.2). They were scale-like in appearance with a distal end tapering to a forked point (Fig. 5.4e). These sensilla were 29.5 – 58.1  $\mu\text{m}$  long and had a basal width of 0.9 – 1.9  $\mu\text{m}$ . The number of sensilla squamiformia was not counted.

**Sensilla styloconica.** These sensilla occurred along the entire flagellum near the upper middle region of the ventral surface (Figs. 5.2 and 5.3). A single sensillum of this type was typically found on each flagellomere, except the most proximal few. They consisted of a solid cylindrical base with an apically protruding conical structure (Fig. 5.4f) and were 6.5 – 18.5  $\mu\text{m}$  long, with a basal width of 4.0 – 10.2  $\mu\text{m}$ . Sensilla styloconica were significantly more abundant on female antennae ( $t = 4.14$ ,  $df = 8$ ,  $P = 0.003$ ) (Table 5.3).

**Sensilla trichoidea.** These sensilla were the most abundant of all sensillum types on the antennae of both males and females (Table 5.3), occurring along the length of the flagellum on the ventral surface. Their distribution on flagellomeres was random (Figs. 5.2 and 5.3) and their number increased distally along the flagellum, generally reaching their maximum abundance before the 35<sup>th</sup> flagellomere, at which point the antennae began to taper in size. These sensilla had a hair-like appearance and could be divided into three subtypes (i.e. large, medium and small) according to their size (Fig. 5.3). The size of all sensilla trichoidea decreased distally along the flagellum, which made accurate counts of each subtype difficult, however the total mean number was significantly higher on female than on male antennae ( $t = 5.27$ ,  $df = 8$ ,  $P = 0.001$ ) (Table 5.3). Large sensilla trichoidea were 53.2 – 164.9  $\mu\text{m}$  long, had a basal width of 3.0 – 5.7  $\mu\text{m}$  and were present only on male antennae. Medium sensilla trichoidea were 24.7 – 59.1  $\mu\text{m}$  long

and had a basal width of 1.7 – 3.4  $\mu\text{m}$  and small sensilla trichoidea were 11.3 – 29.1  $\mu\text{m}$  long and had a basal width of 0.8 – 2.3  $\mu\text{m}$ . Large sensilla trichoidea were found along the entire flagellum of male antennae. Although medium sensilla trichoidea were found along the entire flagellum of female antennae, they were only located on the middle and distal regions of the flagellum of male antennae. For both genders, small sensilla trichoidea were found along the entire flagellum except for the most proximal and distal flagellomeres.

### **Discussion**

The general shape and structure of the antennae of male and female dogwood borer were similar to that reported for a related species, *S. tipuliformis* (Clerck) (Karalius 1994). Although similar in size to male antennae, the antennae of female dogwood borer possessed a greater number of antennal flagellomeres, which were typically smaller in size near the distal end compared with males. The antennal flagellomeres of both males and females were cylindrical in shape and possessed a ventral surface on which most of the sensilla were located, and a dorsal surface possessing numerous overlapping scales. This arrangement of a distinct “sensory” and “scale” surface has been reported for several lepidopteran families including the Sesiidae (Sellier 1977, Karalius 1994), Noctuidae (Mochizuki et al. 1992, Koh et al. 1995), Pyralidae (Hansson et al. 1995, Castrejón-Gómez et al. 2003), and Tortricidae (Castrejón-Gómez and Carrasco 2008). The supposed function of this arrangement may include the ability to detect stimulus direction (Van der Pers et al. 1980) and protection of the antennae and their sensilla from damage (Koh et al. 1995). An additional feature of dogwood borer antennae was the highly textured ventral surface of each flagellomere. The numerous ridge-like processes surrounding individual sensilla were seen in both male and female dogwood borer and may act as a mechanism by which odorant molecules are trapped and concentrated along the sensory surface.

The sensilla auricillica of the dogwood borer were similar in appearance and distribution to those described from *S. tipuliformis* (Karalius 1994). In Lepidoptera, this sensillum type is generally innervated by 2-3 sensory neurons and possesses thin multiporous walls (Anderson et al. 2000, Ansebo et al. 2005). Studies of Noctuidae (Anderson et al. 2000) and Tortricidae (Den Otter et al. 1978, Ansebo et al. 2005) species

showed that these sensilla are involved with plant odor detection, and in the case of *C. pomonella*, the detection of minor sex pheromone components (Ebbinghaus et al. 1998). The large number of sensilla auricillica on the antennae of dogwood borer suggests that these sensilla play an important role in the olfactory response of this species. Since more of these sensilla were located on the individual flagellomeres of female than male antennae, and since females do not respond electrophysiologically to components of their sex pheromone (Zhang personal communication), but do respond to host plant volatiles (Chapter 6), it is probable that they function mainly as olfactory receptors for plant odors.

Sensilla basiconica were the only sensillum type present in roughly equal numbers on the flagellum of male and female dogwood borer and were similar in appearance to those described for *Sesia apiformis* (Clerck) (Sellier 1977). Studies of other lepidopteran species suggest that these sensilla may function as chemoreceptors because they have multiple sensory neurons and thin multiporous walls (Koh et al. 1995, Anderson et al. 2000). I was unable to confirm the presence of wall pores in the sensilla basiconica of dogwood borer. Since a small number of these sensilla are located on the antennae of dogwood borer, and they are present in roughly equal numbers on each gender, their role in olfaction in this species is unclear.

Several studies have suggested that sensilla chaetica serve both a contact chemo- and mechanoreceptive function because they arise from a socket and possess a terminal pore (Altner and Prillinger 1980, Van der Pers et al. 1980). In studies of other sesiid species, the presence of a terminal pore was not documented and the primary function of these sensilla was considered mechanoreceptive (Sellier 1977, Karalius 1994). Although a terminal pore was not confirmed on the sensilla chaetica of dogwood borer, the behavior of females suggests that the role of these sensilla may involve both mechano- and contact chemoreception. Female dogwood borer searching for oviposition sites walk on the surface of host plants, probe it with their ovipositor and antennate (Chapter 3, Frank 2009). Although more sensilla chaetica were observed on female antennae, I should note that the presence of numerous large sensilla trichoidea located near the proximal end of male antennae may have obstructed the view of additional sensilla chaetica on those flagellomeres, possibly leading to lower counts in males.

In many lepidopteran insects, sensilla coeloconica have been described as possessing sensory neurons receptive to olfactory stimuli (Hansson et al. 1995, Hunger



and Steinbrecht 1998) that may be sensitive to plant odors (Altner and Prillinger 1980). In the American cockroach, *Periplaneta americana* L., these sensilla can function as thermo- or hygrosensory structures (Altner et al. 1977). Since more of these sensilla were recorded from individual flagellomeres of female than from male dogwood borer antennae, it is probable that they function as olfactory receptors for plant odors.

The sensilla squamiformia on dogwood borer antennae were similar in appearance to those of *S. tipuliformis* (Karalius 1994). These sensilla are thought to have a mechanoreceptive function (Schneider 1964, McIver 1975). These sensilla were easily detached from dogwood borer antennae during removal of scales, limiting my ability to make accurate assessments of their number.

The sensilla styloconica on dogwood borer antennae were distributed on the antennae in a similar arrangement as described for several other lepidopteran species (Sellier 1977, Mochizuki et al. 1992, Castrejón-Gómez et al. 2003). These sensilla are considered thermo- and hygrosensitive in *Bombyx mori* L. (Steinbrecht 1989) and the Pyralidae (Hallberg et al. 1994, Hansson et al. 1995), however the presence of an apical pore in the Tortricidae have suggested a contact chemoreceptive function (Wall 1978, Castrejón-Gómez and Carrasco 2008). No apical pore was observed on the sensilla styloconica of dogwood borer, which suggests they may have a thermo- and hygrosensory function. Although more of these sensilla were observed on female antennae, based on their distribution and number on individual flagellomeres, I believe that the difference between males and females may be due primarily to the greater number of flagellomeres on female antennae.

Three distinct subtypes of sensilla trichoidea, differentiated according to their size, were found on the antennae of dogwood borer. Studies conducted by Sellier (1977) showed that antennae of the sesiid, *Sesia vespiformis* Hüfnagel, similarly possessed large, medium and small sensilla trichoidea. These sensilla were of particular interest because the absence of large sensilla trichoidea on female dogwood borer antennae was the only characteristic that could be used to easily differentiate between male and female antennae under low magnification. Several studies have shown that large-type sensilla trichoidea of male lepidopterans are involved with olfactory reception of pheromone components (Mochizuki et al. 1992, Hansson et al. 1995, Ebbinghaus et al. 1998). The presence of numerous large sensilla trichoidea on male antennae and their complete absence on the

antennae of females suggests that their main role is detection of the female-produced sex pheromone. However, the function of medium and small sensilla trichoidea is unclear. Medium sensilla trichoidea were present on both male and female antennae, although female antennae possessed a significantly greater number of this sensillum subtype, which likely lead to greater overall numbers of sensilla trichoidea compared with males. Small sensilla trichoidea appeared to be in roughly equal numbers on the antennae of both males and females. Although it is possible that the sensilla trichoidea on the antennae of female moths can detect their own sex pheromone (Ljungberg et al. 1993, Pearson and Schal 1999), it is more likely that they function as general olfactory receptors (Mochizuki et al. 1992, Koh et al. 1995).

Other sensory structures observed on the antennae of dogwood borer but that were not studied in further detail included the Böhm's bristles and apical sensors. Böhm's bristles are mechanoreceptive sensilla (Schneider 1964) and their placement near the proximal end of the scape and pedicel suggest a proprioceptive function (Chapman 1998). Apical sensors were the only sensory structure observed on the terminal flagellomere. They have similarly been observed on the terminal flagellomere of other sesiid species and are thought to function as mechanoreceptors for sensing air currents (Karalius 1994).

This study has provided fundamental background information on the sensory structures present on the antennae of male and female dogwood borer and will enhance my ability to resolve the electrophysiological and behavioral responses of females to host plant volatiles. An understanding of the mechanisms involved with host-finding and oviposition acceptance behaviors will create opportunities to assess the possibility of using plant-derived compounds for the monitoring or management of female dogwood borer in apple orchards or managed urban landscapes.

**Table 5.1** Mean ( $\pm$  SE) length of the pedicel, scape and selected antennal flagella of dogwood borer.

Flagellum number	Length ( $\mu\text{m}$ )			
	Male	Female	<i>t</i>	df
Scape	236.2 $\pm$ 2.6	237.3 $\pm$ 3.1	0.25	68
Pedicel	143.1 $\pm$ 2.8	145.1 $\pm$ 2.3	0.59	68
5	135.2 $\pm$ 3.1	110.7 $\pm$ 1.9	-6.93	68
10	148.5 $\pm$ 2.4	126.2 $\pm$ 2.3	-6.67	68
15	154.8 $\pm$ 2.6	135.3 $\pm$ 2.0	-5.94	68
20	152.2 $\pm$ 2.3	132.4 $\pm$ 2.6	-5.66	68
25	151.2 $\pm$ 2.9	130.6 $\pm$ 1.9	-6.03	68
30	142.9 $\pm$ 2.7	123.4 $\pm$ 2.2	-5.66	68
35	125.0 $\pm$ 2.7	116.4 $\pm$ 2.1	-2.55	68
40	85.3 $\pm$ 3.2	95.6 $\pm$ 2.7	-2.53	68
Penultimate	37.5 $\pm$ 1.5	38.4 $\pm$ 1.0	-0.92	68

**Table 5.2** Mean ( $\pm$  SE) width of the pedicel, scape and selected antennal flagella of dogwood borer.

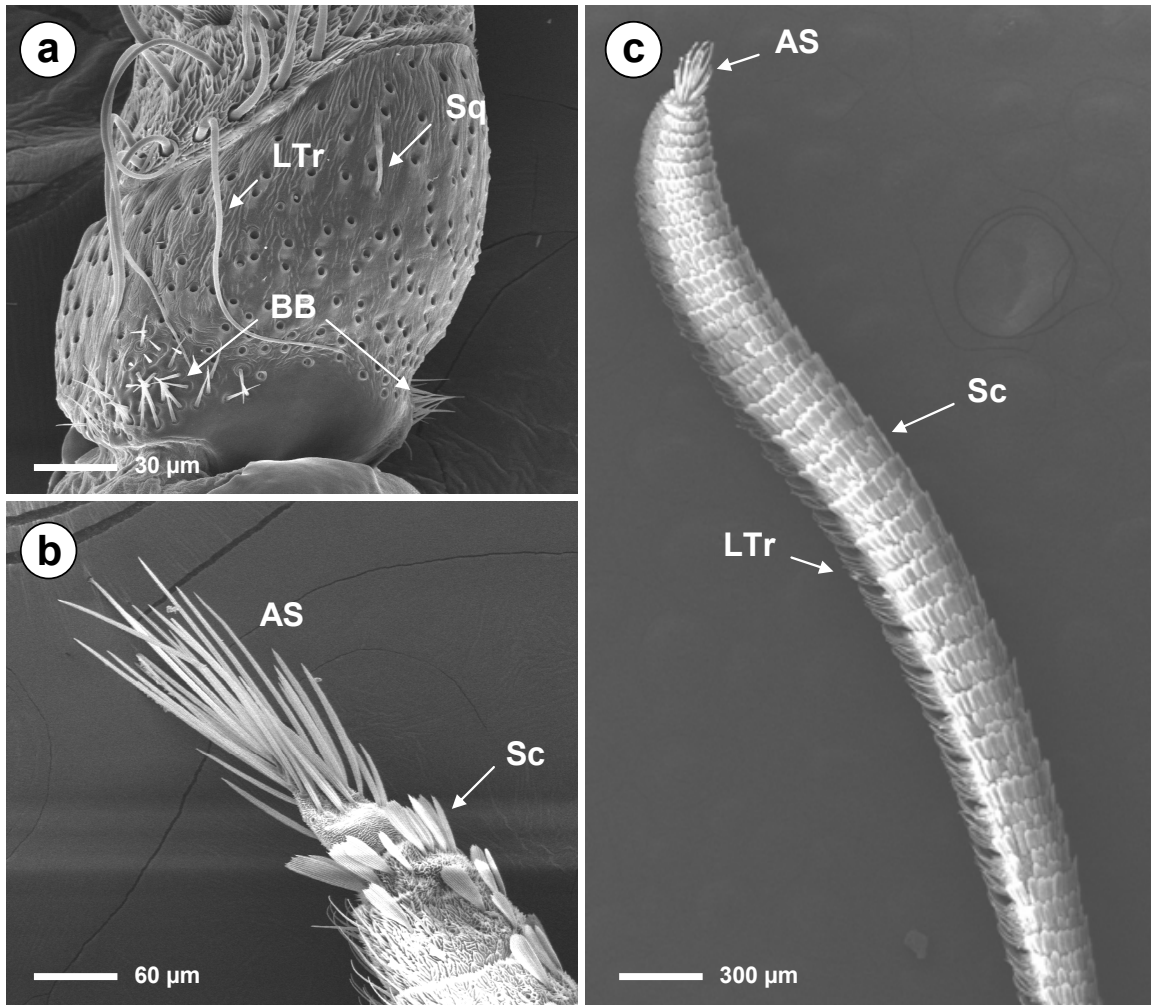
Flagellum number	Width ( $\mu\text{m}$ )				<i>t</i>	df	<i>P</i>
	Male	Female					
Scape	230.2 $\pm$ 3.8	215.4 $\pm$ 3.8			-2.72	68	0.0086
Pedicel	165.6 $\pm$ 3.9	154.2 $\pm$ 2.7			-2.38	68	0.0205
5	143.2 $\pm$ 3.2	137.6 $\pm$ 2.5			-1.39	68	0.1707
10	147.7 $\pm$ 3.5	139.6 $\pm$ 2.4			-1.92	68	0.0581
15	158.1 $\pm$ 3.8	144.9 $\pm$ 1.9			-3.12	68	0.0027
20	180.4 $\pm$ 5.0	157.9 $\pm$ 2.1			-4.19	68	0.0001
25	215.5 $\pm$ 5.3	178.2 $\pm$ 2.3			-6.69	68	0.0001
30	254.6 $\pm$ 5.2	207.0 $\pm$ 3.2			-7.91	68	0.0001
35	256.5 $\pm$ 4.3	227.5 $\pm$ 3.1			-5.49	68	0.0001
40	204.5 $\pm$ 5.0	207.2 $\pm$ 4.4			0.42	68	0.6736
Penultimate	75.5 $\pm$ 1.4	72.2 $\pm$ 1.3			-1.76	68	0.0824

**Table 5.3** Mean ( $\pm$  SE) number of sensilla on the ventral surface of male and female dogwood borer antennae.

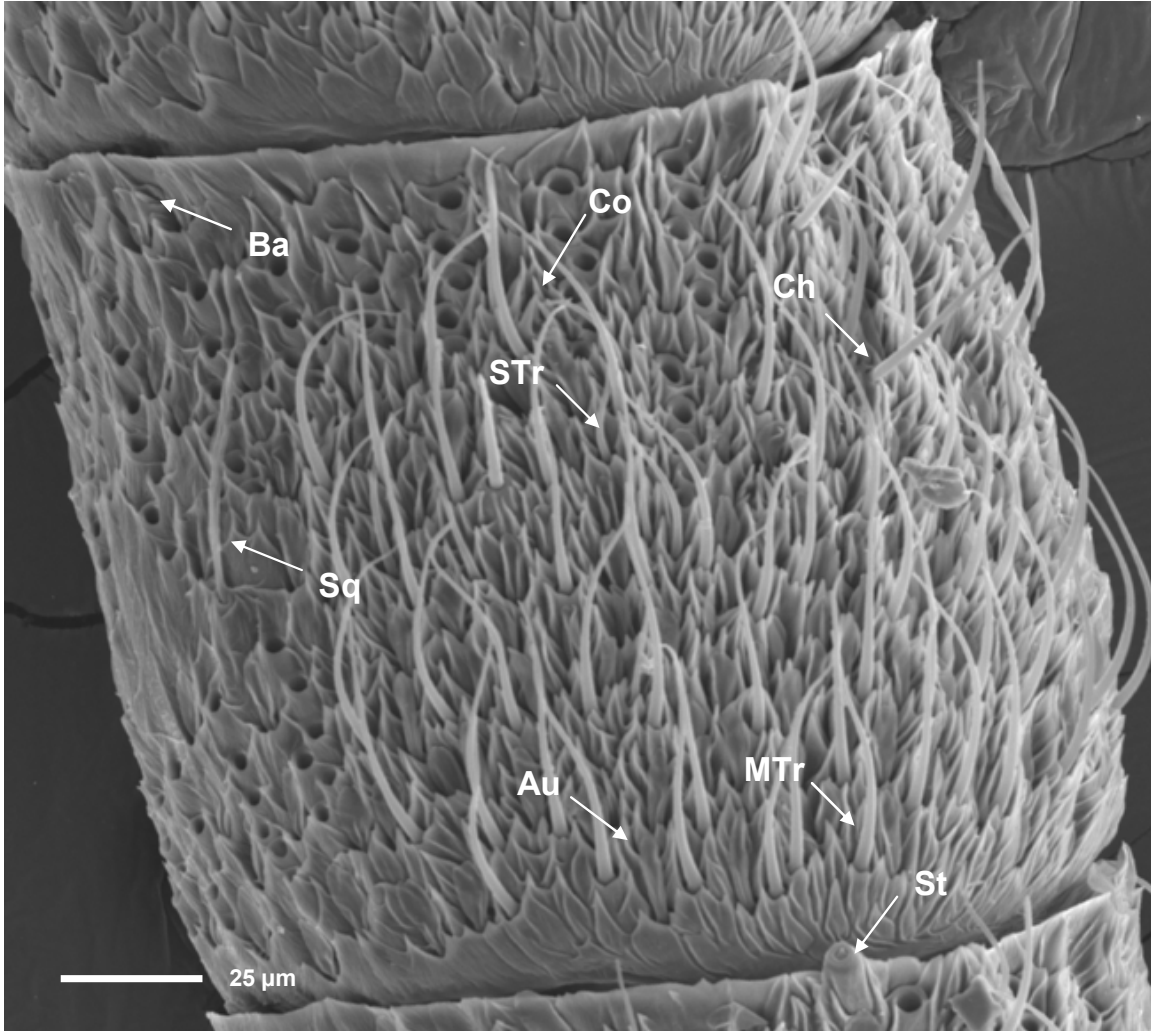
Type of sensilla	Number of sensilla	
	Male	Female
Auricillica	434.6 $\pm$ 49.9 b	1322.4 $\pm$ 92.6 a
Basiconica	6.2 $\pm$ 2.6 a	11.2 $\pm$ 2.1 a
Chaetica	83.2 $\pm$ 8.3 b	128.8 $\pm$ 8.6 a
Coeloconica	36.6 $\pm$ 8.0 b	120.4 $\pm$ 8.8 a
Styloconica	32.4 $\pm$ 0.8 b	37.8 $\pm$ 1.0 a
Trichoidea (all subtypes)	2319.0 $\pm$ 25.5 b	2827.0 $\pm$ 92.9 a

Means across rows followed by the same letter are not significantly different ( $P = 0.05$ ,  $t$ -test).

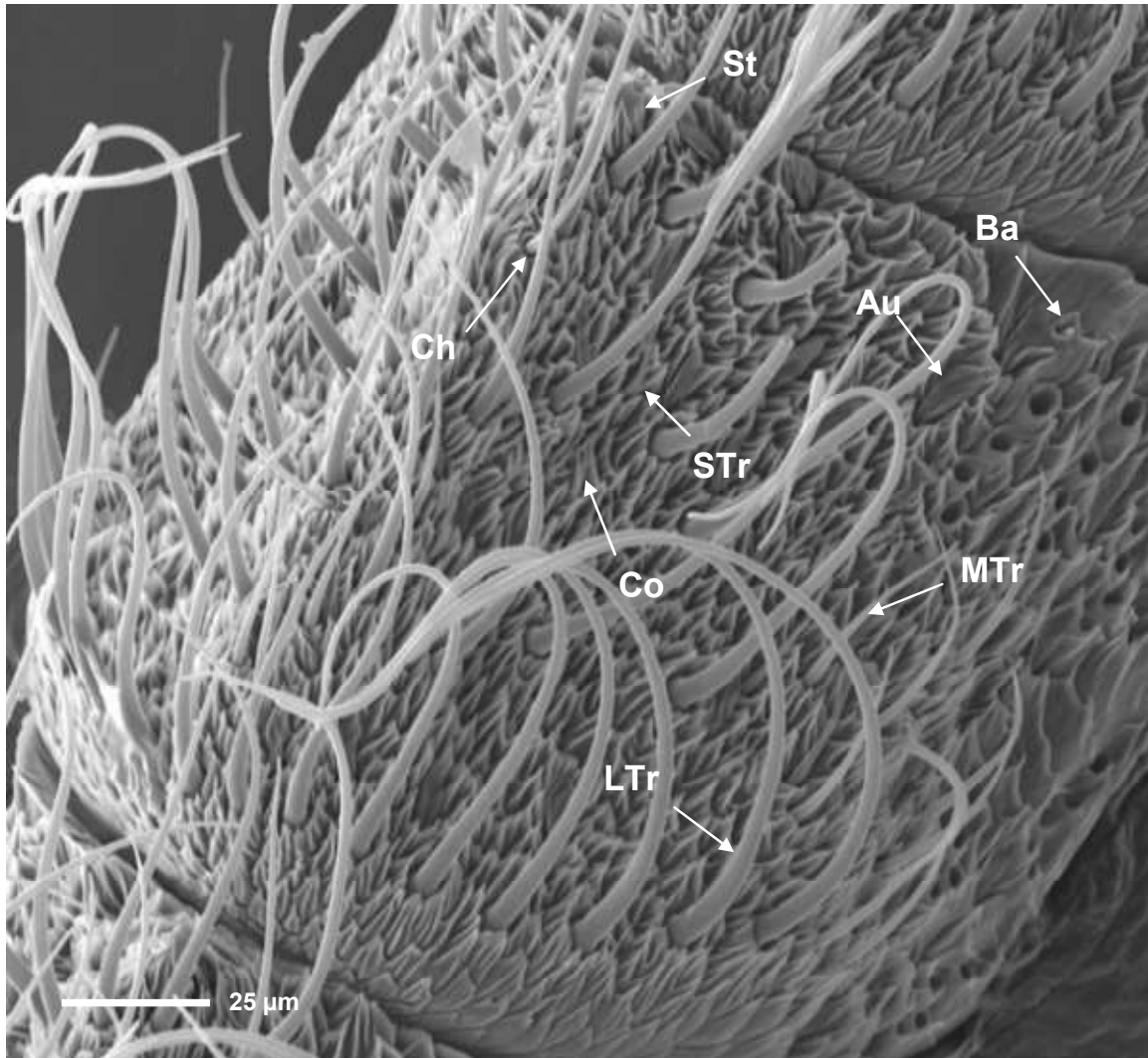
**Figure 5.1** Scanning electron micrographs showing a) scape of the female dogwood borer antennae and location of the Böhm's bristles, b) close-up of the apical sensors on the female antennae, c) profile of the male dogwood borer antennae possessing numerous overlapping scales on the dorsal surface, large sensilla trichoidea on the ventral surface and apical sensors on the terminal flagellomere. AS, apical sensors; BB, Böhm's bristles; LTr, large trichoidea sensilla; Sc scales, Sq squamiformia sensillum.



**Figure 5.2** Scanning electron micrograph of a female dogwood borer antennal flagellomere showing most sensillum types. Au, auricillica sensillum; Ba, basiconica sensillum; Ch, chaetica sensillum; Co, coeloconica sensillum; Sq, squamiformia sensillum; St, styloconica sensillum; MTr, medium trichoidea sensillum; STr, small trichoidea sensillum.

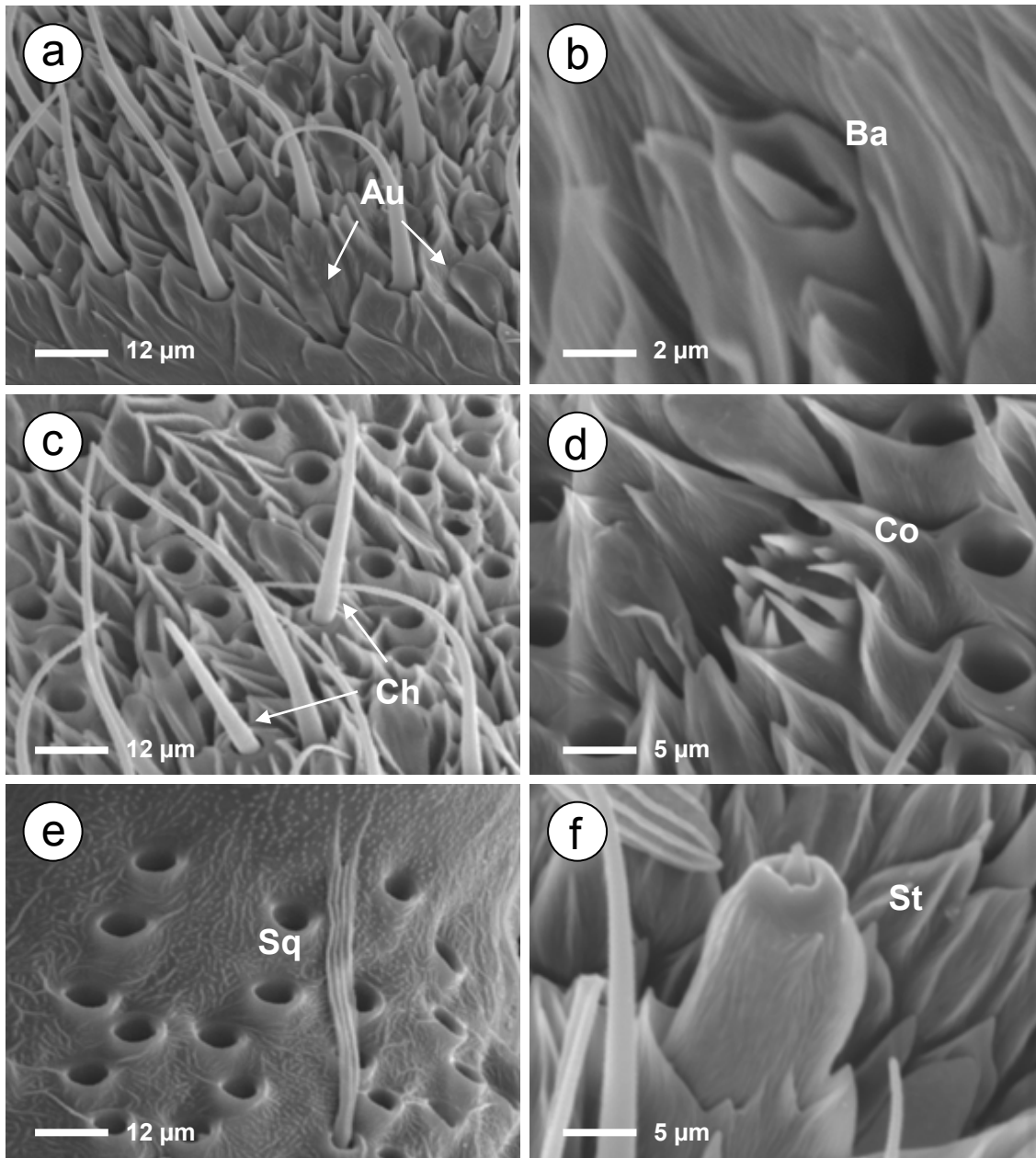


**Figure 5.3** Scanning electron micrograph of a male dogwood borer antennal flagellomere showing most sensillum types. Au, auricillica sensillum; Ba, basiconica sensillum; Ch, chaetica sensillum; Co, coeloconica sensillum; St, styloconica sensillum; LTr, large trichoidea sensillum; MTr, medium trichoidea sensillum; STr, small trichoidea sensillum.





**Figure 5.4** Scanning electron micrograph showing a) close-up of auricillica sensilla, which had either a characteristic rabbit-ear shape or appeared dorsoventrally flattened, b) details of the basiconica sensillum, c) close-up of chaetica sensilla, which arose from a distinctive collar-like socket, d) details of the coeloconica sensillum, characterized by a ring of cuticular teeth surrounding a central peg-like structure, e) squamiformia sensilla located on the dorsal surface of an antennal flagellomere, f) details of the styloconica sensillum. Au, auricillica sensillum; Ba, basiconica sensillum; Ch, chaetica sensillum; Co, coeloconica sensillum; Sq squamiformia sensilla; St, styloconica sensillum.



## CHAPTER 6: ELECTROPHYSIOLOGICAL RESPONSE OF FEMALE DOGWOOD BORER TO HOST-PLANT VOLATILE COMPOUNDS

### Abstract

Coupled gas chromatography and electroantennogram detection (GC-EAD) analyses of headspace volatiles from apple and dogwood host tissues revealed a total of 16 and 9 antennal responses, respectively, to which female dogwood borer responded. There were no differences in the response of antennae from virgin and mated females, and the amplitude of the antennal response of females to host odors was greater than that of males. Four compounds, including octanal, nonanal, decanal, and methyl salicylate, were identified from all headspace collections from apple trees. Use of the solid-phase microextraction (SPME) technique revealed that a single volatile compound,  $\alpha$ -bergamotene, emanating from larval dogwood borer frass elicited a strong female antennal response. This compound was also present in headspace collections from apple trees with infested burr knot tissue and ‘Granny Smith’ trees with 1-d-old cut bark, suggesting that it is produced by apple trees in response to injury. An additional compound, methyl-2,4-decadienoate, present only in headspace collections from burr knots infested with dogwood borer larvae on ‘Granny Smith’ trees elicited a strong female antennal response. Two stimulatory compounds, hexanoic acid and nonanoic acid, were identified from all headspace collections from dogwood trees.

### Introduction

The dogwood borer, *S. scitula*, is a polyphagous clearwing moth that feeds on 19 species of fruit, nut and ornamental trees throughout eastern North America (Eichlin and Duckworth 1988). Despite its large host range, dogwood borer is significantly more abundant in apple orchards than in woodlands or managed urban landscapes (Bergh et al. 2009) and ongoing larval infestations in apple orchards can result in the death of young trees (Weires 1986, Howitt 1993). Although dogwood borer infestations in apple orchards can be effectively controlled using the organophosphate insecticide, chlorpyrifos (Riedl et al 1985, Kain and Straub 2001, Kain et al. 2004), increased restrictions on its use have emphasized the need for development of alternative management options.

Pheromone-based management strategies have been used to control a number of insect pests (Novak and Roelofs 1985, Cardé and Minks 1995, El-Sayed et al. 2006), including sesiids (Pfeiffer et al. 1991, Agnello and Kain 2002, Kittelson 2006). Identification of the female produced dogwood borer sex pheromone and a behavioral antagonist (Zhang et al. 2005) have resulted in the establishment of a more effective monitoring system (Leskey et al. 2006) and has created opportunities to evaluate the potential of mass trapping and mating disruption for dogwood borer control (Leskey et al. 2009). However, these management strategies are typically less effective at high population densities and require large areas under treatment to combat the effects of immigrating females (Gut and Brunner 1998, Thomson et al. 1998, Hughes and Dorn 2002).

Methods to also attract and/or manipulate the behavior of female insects for pest management purposes are receiving increasing attention. Host-plant derived volatile compounds have been used effectively to monitor or manage several important orchard pests (Rodriguez-Saona and Stelinski 2008). Zhang et al. (1999) showed that red sticky spheres used in combination with a synthetic blend of 5 fruit volatiles was highly attractive to *R. pomonella* in laboratory and field studies. Traps baited with a pear ester kairomone were used to track the seasonal flight patterns of female and male *C. pomonella* in apple orchards under mating disruption (Knight and Light 2005). Leskey et al. (2008) showed that *Conotrachelus nenuphar* (Herbst) was effectively controlled in apple orchards by applying insecticides only to perimeter-row trap trees possessing a synergistic blend of the male-produced aggregation pheromone and host-plant volatile, benzaldehyde. Van Steenwyk and Barnett (1987) showed that an application of 5% crude almond oil to almond trees could be used to disrupt host-finding by female *Amyelois transitella* (Walker).

Female sesiid moths appear to be intriguing candidates for investigating the use of host-plant compounds to manipulate host-finding and acceptance behaviors. Derksen et al. (2007) reported that 21 compounds in gum-frass mixtures from peach trees elicited antennal responses from male and female *S. exitiosa* and that synthetic blends containing all 21 compounds or all but 3 acetates, stimulated significantly higher rates of oviposition compared with untreated controls. Similarly, *S. pictipes* deposited significantly more

eggs in response to stimuli associated with mechanically damaged peach bark and peach bark possessing canker wounds compared with non-damaged bark (Cottrell et al. 2008).

The olfactory responses of female dogwood borer to host-plant volatiles appear to be an important component of their host-finding and oviposition site selection behaviors. Female dogwood borer appear to preferentially oviposit near areas associated with apple burr knot tissue (Riedl et al. 1985, Kain and Straub 2001, Leskey and Bergh 2005), injured bark on dogwood trees (Wallace 1945, Potter and Timmons 1981) and insect-induced galls on pin oak trees (Eliason and Potter 2000). In apple orchards, mated female dogwood borer repeatedly engaged in casting flight toward and oviposition near burr knots and cracks in the bark below the graft union of apple trees (Chapter 3, Frank et al. 2009). Furthermore, female dogwood borer antennae possess a greater number of olfactory sensilla than do male antennae (Chapter 5).

In this study, I characterized and compared the responses of dogwood borer antennae to volatiles from headspace collections from several apple, *M. domestica*, and dogwood, *C. florida*, tissues. The effects of gender and female mating status on antennal responses were compared and the identity of selected stimulatory compounds from host-plant headspace collections was determined.

## **Materials and Methods**

**Insects.** Adult dogwood borers were obtained by collecting late instar larvae from apple trees in commercial orchards in Frederick Co., VA and rearing them to the adult stage or from a continuous colony of dogwood borer maintained in the laboratory (Chapter 2). Mated females were obtained by introducing a pair of ~1- to 2-d-old male and female moths into a ventilated 60 cm<sup>3</sup> plywood cage with Plexiglas panels comprising the top and front side and which were located outdoors during the evening hours when mate-finding occurs (Bergh et al. 2006). Only females observed in copula for at least 2.5 h were considered mated. Mated females were held in 300 ml, white, waxed paper cups (Sweetheart Cup Co., Owings Mills, MD) provisioned with a cotton ball saturated with a 10% sucrose solution in a small glass dish, and topped with a clear plastic lid, and held in a controlled environment chamber (Percival Scientific, Perry, IA) at 25°C and 16:8 (L:D) h until used. All females used for GC-EAD analysis were 1- to 5-d-old.

**Volatile Collections from Host-Plant Source Material.** Headspace volatiles were collected from potted apple trees ('Red Chief Delicious' and 'Granny Smith') on M.26 rootstock and potted dogwood trees ('Cherokee Princess') from 2006-2009. All trees were 2- to 3-yr-old, approximately 2 m tall with healthy branches and foliage, and were maintained outdoors at Virginia Tech's Alson H. Smith Jr., Agricultural Research and Extension Center (AHS-AREC) in Winchester, VA.

All volatile collections from 'Red Chief Delicious' apple trees were made under field conditions at the AHS-AREC by sampling from the shank of the rootstock between the soil line and the graft union. Tree treatments included those with; (1) burr knots infested with dogwood borer larvae, (2) uninfested burr knots, (3) no burr knots, (4) no burr knots, but with 3-d-old cut bark and (5) no burr knots, but with 30-d-old cut bark. All trees with cut bark had a rectangular piece of bark (~ 2 x 5 cm) excised using a pocket knife. A large polyethylene bag (48.26 x 58.42 cm, Reynolds Oven Bags, Reynolds, Richmond, VA) was used as a containment vessel, placed around the collection area of the tree and sealed with plastic ties. Air was drawn into the bag through 2-4 perforations (~ 3 mm in diameter) in it, and drawn out through a glass collection column (10/30:24/40; Ace Glass, Inc, Vineland, NJ) containing 50 mg of Super Q (Alltech Associates, Inc., Deerfield, IL) connected to a GilAir5 (Sensidyne, Clearwater, FL) battery powered vacuum pump by flexible PVC tubing (Fig. 6.1). Airflow through the bags was regulated at ~1 liter/min by individual flow meters. After each 24-h period, volatiles collected were eluted from the trap using 1 ml of methylene chloride (repeated 3 times) into 4 ml glass vials with Teflon lined screwcaps (Kimble Glass Inc., Vineland, NJ). The eluates (~ 3 ml each sample) were concentrated under a nitrogen stream to approximately 50  $\mu$ l and stored at 4°C until analysis.

Volatiles from 'Cherokee Princess' dogwood trees were collected near the base of the tree just above the soil line, as described above. Tree treatments included those with; (1) intact bark, (2) 3-d-old cut bark, and (3) 30-d-old cut bark. After each 24-h period, volatiles collected were eluted as described above.

Volatiles from 'Granny Smith' apple trees were collected at the Invasive Insect Biocontrol and Behavior Laboratory (IIBBL) in Beltsville, MD. Sampling and treatment protocols were similar to those for 'Red Chief Delicious' trees. Tree treatments included those with; (1) burr knots infested with dogwood borer larvae; (2) uninfested burr knots;

(3) no burr knots, (4) no burr knots, but with 1-d-old cut bark, and (5) no burr knots, but with 30-d-old cut bark. i.e. same as previous format. A large polyethylene bag was placed around the collection area of the tree as described above. A filter trap and glass collection column was attached to each bag at two ends through reducing unions fitted into a small hole in the bag. Air was drawn into the bag through 6-14 mesh activated charcoal (Fisher Scientific, Pittsburgh, PA) and out through the collection column containing 200 mg of Super Q connected to a vacuum in the laboratory by flexible PVC tubing. Airflow through the bags was regulated at ~1 liter/min by individual flow meters. Volatiles were collected continuously for 24 h at room temperature and 16:8 (L:D) photoperiod. After each 24-h collection period, volatiles collected were eluted as described above.

#### **Volatile Collections from Dogwood Borer Larvae and Artificial Diet.**

Headspace volatiles were collected from late instar dogwood borer larvae at the Appalachian Fruit Research Station (AFRS) in Kearneysville, WV in 2007. All larvae were recently collected from the field and had been feeding on general lepidopteran diet (BioServ, Frenchtown, NJ) for ~ 3 wk. Treatments included: (1) single larva, (2) larva feeding on a disc (~ 20 g) of lepidopteran diet, and (3) a disc (~ 20 g) of lepidopteran diet. A 1-liter, 4 necked glass container was used as the source containment vessel, following Zhang et al. (1994). Filter traps and collection columns were attached to each collection vessel at two ends on the cover. Air was drawn into the vessel through 6-14 mesh activated charcoal and out of the vessel through collection columns containing 200 mg of Super Q connected to a vacuum in the laboratory by flexible PVC tubing. Airflow through the vessels was regulated at ~1 liter/min by individual flow meters. Volatiles were collected continuously for 24 h at room temperature and 16:8 (L:D) photoperiod. After each 24-h collection period, volatiles collected were eluted as described above.

**SPME Collections of Dogwood Borer Frass.** The solid phase microextraction (SPME) technique was performed as described by Zhang et al. (1999). Dogwood borer frass was collected from infested burr knots on potted apple trees and from burr knots on apple trees in commercial orchards. Frass was held in 250 ml amber jars with Teflon<sup>TM</sup> lined screwcaps (Fisher Scientific, Pittsburgh, PA) and stored in a refrigerator for 1 or 3 d before being taken to the IIBBL. A small hole was made through the cap, through which a poly-dimethyl siloxane-coated SPME fiber (film thickness 100 µm; Supelco Inc.,

Bellefonte, PA) was passed for 5 min to absorb the volatiles. The SPME fiber was then immediately injected into the GC port where volatiles were thermally desorbed and analyzed for GC-EAD activity.

**Electrophysiological Recordings.** GC-EAD analyses was performed at the IIBBL using a Hewlett Packard 5890 Series II gas chromatograph equipped with a 60 m x 0.25-mm i.d., 0.25- $\mu$ m film-thickness DB-WAXETR capillary column (J&W Scientific Inc., Folsom, CA, 80°C 2 min, 10°C /min to 250°C, hold for 10 min) in splitless mode with hydrogen carrier gas (1.4 ml/min). The column effluent was split 1:1 in the oven of the flame ionization detector and the EAD. Both antennae from individual moths were removed and positioned between two gold wire electrodes, which were immersed in saline-filled wells in a small acrylic holding station. The base of each antenna was placed in one well while the distal tip of each antenna was removed and maneuvered to make contact with the saline in the other well (Fig. 6.2). The temperature of the antennae on the acrylic station was maintained at  $\sim$ 5°C by flushing 0°C water from a benchtop refrigerated circulator (RTE-100, NESLAB Instruments, Inc., Portsmouth, NH) through the insulation layer of the modified condenser containing the acrylic station mounted on top of the GC. An HP 3390A integrator was used for the EAD recordings. Three  $\mu$ l of sample volatiles from headspace collections were injected into the GC port for each antennal recording. At least 10 virgin and mated females and 5 males were used for analysis of volatiles (2-3 runs/individual) from ‘Red Chief Delicious’ source materials. At least 10 virgin females (2-3 runs/individual) were used to analyze volatiles from all other source materials.

GC-mass spectrometry (GC-MS) was carried out with a Hewlett Packard 6890 gas chromatograph coupled to an HP 5970B Mass Selective Detector (EI) with 60 m DB-WAXETR capillary column with helium as the carrier gas. The oven temperature was programmed at 40°C for 5 min, then 5°C/min to 250°C, and held for 20 min.

## Results

**Identification of Electrophysiologically Active Compounds.** GC-EAD analyses of volatiles collected from ‘Red Chief Delicious’ apple tissues under field conditions revealed 16 compounds to which female dogwood borer antennae responded (Fig. 6.3). There was no difference in antennal sensitivity between virgin and mated

females to those compounds. No consistent responses were generated from male antennae and therefore they were not subject to further study.

Five volatile compounds were consistently most stimulatory to female antennae. These were identified by A. Zhang and confirmed by comparing their GC retention times, mass spectra, and EAD activity with those of synthetic standards. Three aldehydes, octanal, nonanal, and decanal, and the organic ester, methyl salicylate, were identified from all collections (Figs. 6.4-6.8, Table 6.1). A sesquiterpene,  $\alpha$ -bergamotene, was identified only from trees with infested burr knot tissue (Fig. 6.6, Table 6.1). GC-EAD analyses of volatiles collected from dogwood trees under field conditions revealed 9 compounds to which female dogwood borer antennae responded (Fig. 6.9, Table 6.1). Two compounds, hexanoic acid and nonanoic acid, were identified from all dogwood collections (Figs. 6.10-6.12, Table 6.1).

GC-EAD analyses of volatiles collected from 'Granny Smith' apple tissues in the laboratory were similar to those from 'Red Chief Delicious'. The same four compounds, octanal, nonanal, decanal, and methyl salicylate, were present in all collections (Figs. 6.13-6.17, Table 6.1). However,  $\alpha$ -bergamotene was present in volatiles collected from trees with burr knots infested with dogwood borer larvae (Fig. 6.15, Table 6.1) and 1-d-old cut bark (Fig. 6.16, Table 6.1). An additional compound, methyl-2,4-decadienoate, was identified from volatiles from infested burr knot tissue (Fig. 6.15, Table 6.1).

GC-EAD analyses of volatiles from dogwood borer larvae revealed the presence of two compounds that were previously identified from apple host tissues, nonanal and decanal, and that consistently elicited an antennal response (Fig. 6.18). Similarly, nonanal and decanal were present in volatiles from a dogwood borer larva feeding on a disc of lepidopteran diet (Fig. 6.19). Volatile samples from lepidopteran diet evoked antennal responses in female antennae, but did not share any compounds with those from other source materials and are not reported.

SPME sampling and GC-EAD analysis of volatiles from dogwood borer frass consistently revealed the presence of  $\alpha$ -bergamotene from 1-d-old samples (Fig. 6.20). This compound was present in high concentrations and consistently elicited a strong antennal response in females, but was not detected in 3-d-old samples.



## Discussion

Female dogwood borer antennae responded to volatile compounds emanating from host tissues. There was no difference in antennal sensitivity between virgin and mated females to host volatiles tested and no consistent responses were generated from male antennae. Characterization of the antennal sensilla of dogwood borer has shown that female antennae possess significantly greater numbers of olfactory sensilla that may be responsible for host odor perception compared with male antennae (Chapter 5). It seems likely that the sexual dimorphism observed in the overall number of antennal sensilla could result in greater sensitivity to host volatiles in females.

For both apple varieties evaluated, 5 volatile compounds were repeatedly among the most stimulatory compounds identified. Four of these compounds, octanal, nonanal, decanal, and methyl salicylate, were identified from all apple treatments and have previously been reported from headspace collections from ‘Smoothie Golden’ apple (Casado et al. 2006) and pear trees (Scutareanu et al. 1997). Furthermore, studies conducted by Casado et al. (2006) showed that these same 4 compounds elicited an antennal response in male and female *C. pomonella*. To my knowledge, the compound  $\alpha$ -bergamotene is reported for the first time from apple trees and appears to be plant produced in response to injury. This compound was present only in volatiles from apple trees with infested burr knot tissue and ‘Granny Smith’ trees with 1-d-old cut bark. Furthermore, use of the SPME technique revealed that  $\alpha$ -bergamotene was the primary volatile compound detected in freshly collected frass from apple trees infested with dogwood borer larvae. The absence of this compound in volatiles from apple trees with 30-d-old cut bark, ‘Red Chief Delicious’ with 3-d-old cut bark and frass samples held for 3 d suggests  $\alpha$ -bergamotene is highly volatile and will readily decompose.

The compound, methyl-2,4-decadienoate, was identified only in volatiles from ‘Granny Smith’ with infested burr knot tissue, suggesting that it is produced in relation to a larval/plant interaction. The presence of this compound was interesting, since it has been identified as an aggregation pheromone component for the bark beetle, *Pityogenes chalcographus* (Kupferstecher) (Byers et al. 1990) as well as for pentatomids belonging to the genus *Euschistus* (Aldrich et al. 1991). Whether dogwood borer use this compound for some form of species-specific communication remains uncertain. It is

unclear as to why methyl-2,4-decadienoate was absent from volatiles from ‘Red Chief Delicious’ with infested burr knots. However, I speculate that this difference between cultivars may have resulted from a combination of small quantities of this compound emanating from infested burr knot tissues and the limitations of the equipment used for headspace collections from ‘Red Chief Delicious’ in the field versus that used for ‘Granny Smith’ in the laboratory. Field collections relied on small battery powered pumps that could only reliably draw air through a collection column containing 50 mg of Super Q while laboratory collections used 4 times more adsorbent to collect volatiles.

Nine volatile compounds collected from dogwood treatments elicited a measurable antennal response in female dogwood borer and two compounds, hexanoic and nonanoic acid, were identified. The low concentration of volatiles in dogwood samples limited my ability to identify other stimulatory compounds. Collection of headspace samples from dogwood trees with 1-d-old cut bark were not conducted and my attempts to infest potted dogwood trees were unsuccessful. Consequently, the presence of  $\alpha$ -bergamotene or methyl-2,4-decadienoate in volatile emissions from dogwood tissues with these sources of damage remains unknown.

Several compounds identified in this study were stimulatory to sesiid pests of other hosts. Andersen et al. (1987), showed that 25 volatiles present in peach tree bark stimulated antennal responses in *S. pictipes* females and that nonanal, decanal, methyl salicylate and hexanoic acid were among the most stimulatory compounds. Similarly, Derksen et al. (2007) reported that 21 compounds, including nonanal and nonanoic acid, from gum-frass mixtures from peach trees elicited antennal responses from male and female *S. exitiosa*.

Compounds that elicit an antennal response in a particular insect species may not necessarily evoke a behavioral response from males or females of that species. Many of the compounds identified from headspace collections from apple and dogwood trees are also emitted by other plants that are not hosts of dogwood borer. Although, I have demonstrated that female dogwood borer respond electrophysiologically to headspace volatiles from two of their important host plants, my attempts to correlate this with a behavioral response were not successful. These attempts are described in Chapter 7 and it is clear that the development of suitable bioassays will be required to investigate the behavioral responses to the volatile blends and individual components that elicit an

electrophysiological response. Methyl-2,4-decadienoate and  $\alpha$ -bergamotene are two intriguing candidate compounds that should be targeted for evaluation in such experiments. Knowledge of the stimuli involved with host-plant selection behaviors will create future opportunities to assess female responses to manipulated stimuli toward the development of monitoring or management programs that specifically target female dogwood borer.

**Table 6.1** Volatile compounds present in headspace collections of selected host-plant tissues.

Treatment	Compound									
	Hexanoic acid	Nonanoic acid	Octanal	Nonanal	Decanal	$\alpha$ -bergamotene	Methyl-2,4-decadienoate	Methyl salicylate		
<b>'Red Chief Delicious'</b>										
No burr knots			X	X	X			X		
Uninfested burr knots			X	X	X			X		
Infested burr knots			X	X	X	X		X		
Cut bark (3-d-old)			X	X	X			X		
Cut bark (30-d-old)			X	X	X			X		
<b>'Granny Smith'</b>										
No burr knots			X	X	X			X		
Uninfested burr knots			X	X	X			X		
Infested burr knots			X	X	X	X		X		
Cut bark (1-d-old)			X	X	X	X		X		
Cut bark (30-d-old)			X	X	X	X		X		
<b>'Cherokee Princess'</b>										
<b>dogwood</b>										
Intact bark	X									
Cut bark (3-d-old)	X	X								
Cut bark (30-d-old)	X	X								

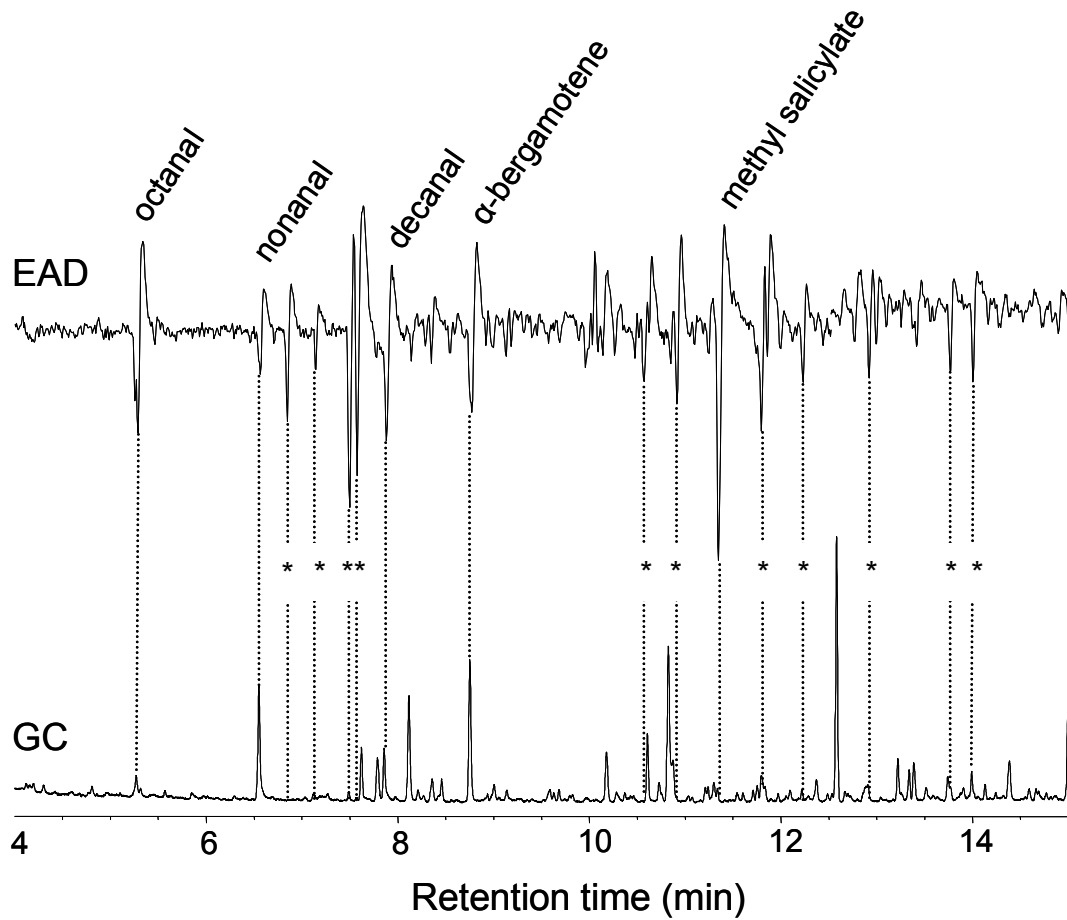
**Figure 6.1** Representation of the headspace collection set-up for potted apple trees in the field.



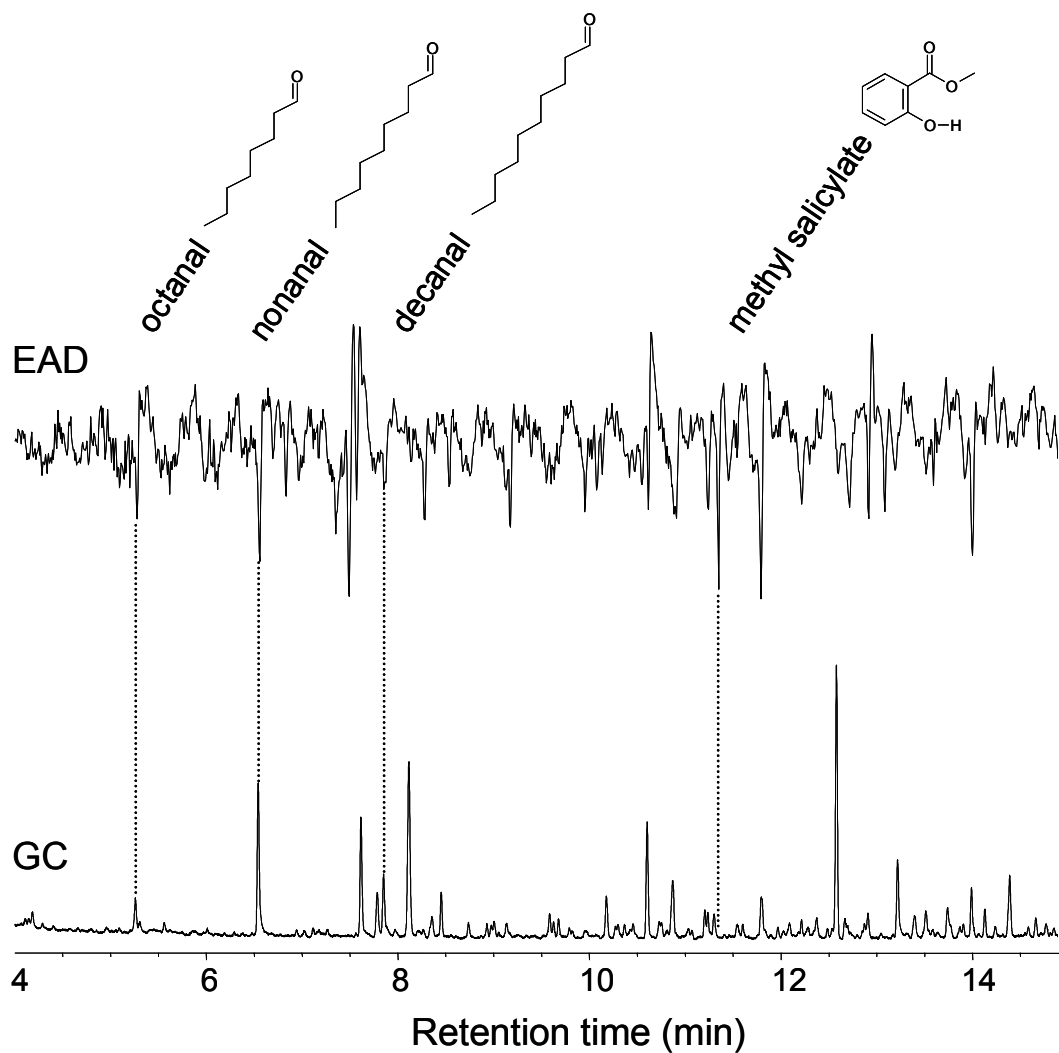
**Figure 6.2** Dogwood borer antennae aligned together and positioned on the EAD holding station.



**Figure 6.3** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from 'Red Chief Delicious' apple trees with infested burr knot tissue. \* indicates all unidentified compounds.

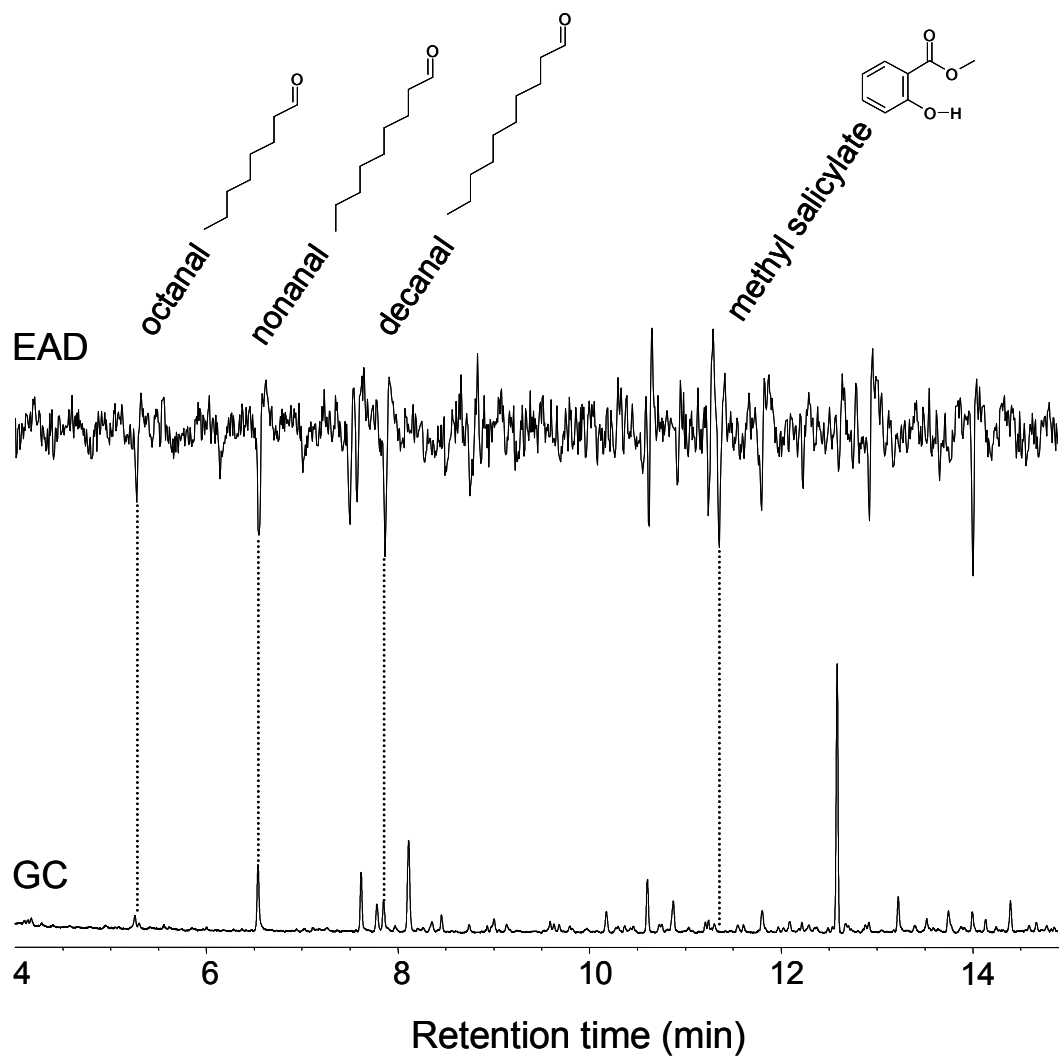


**Figure 6.4** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from 'Red Chief Delicious' apple trees with no burr knots present.

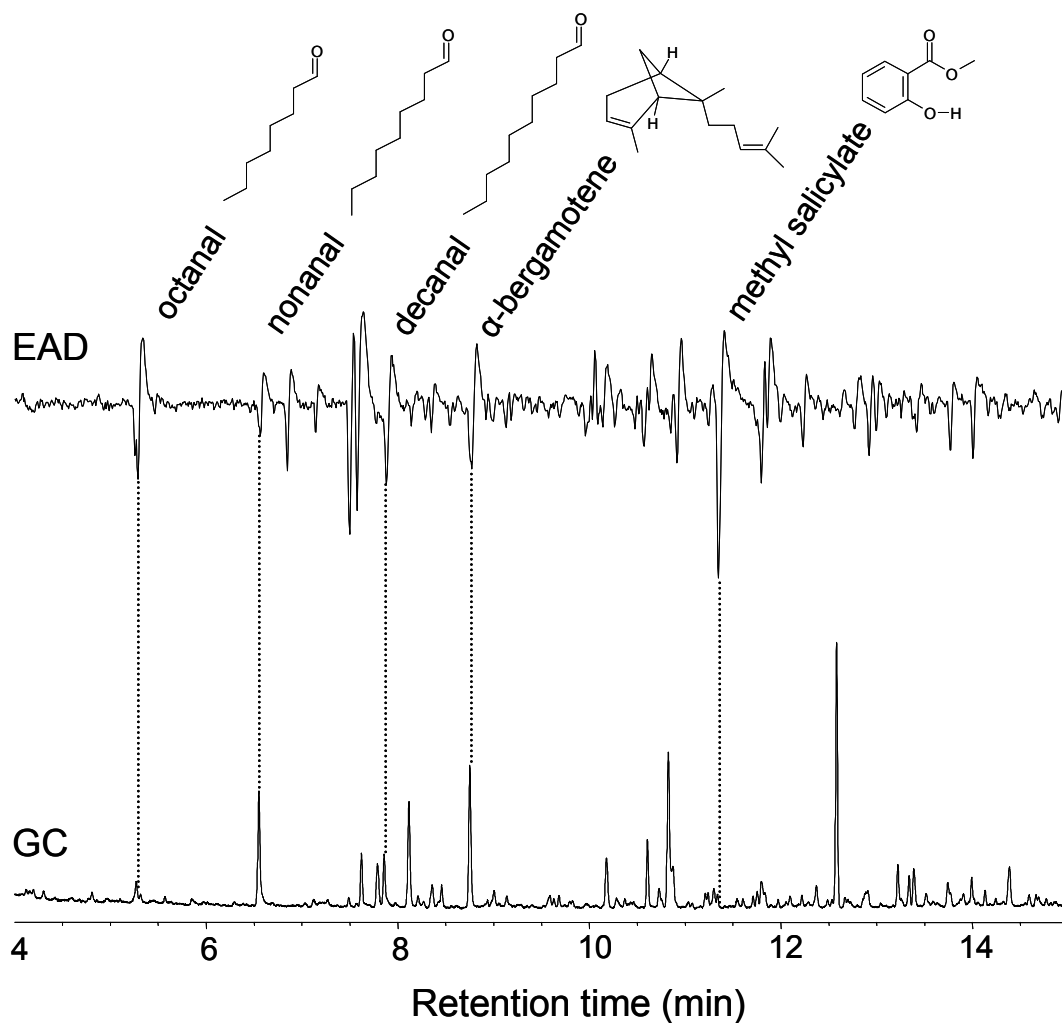




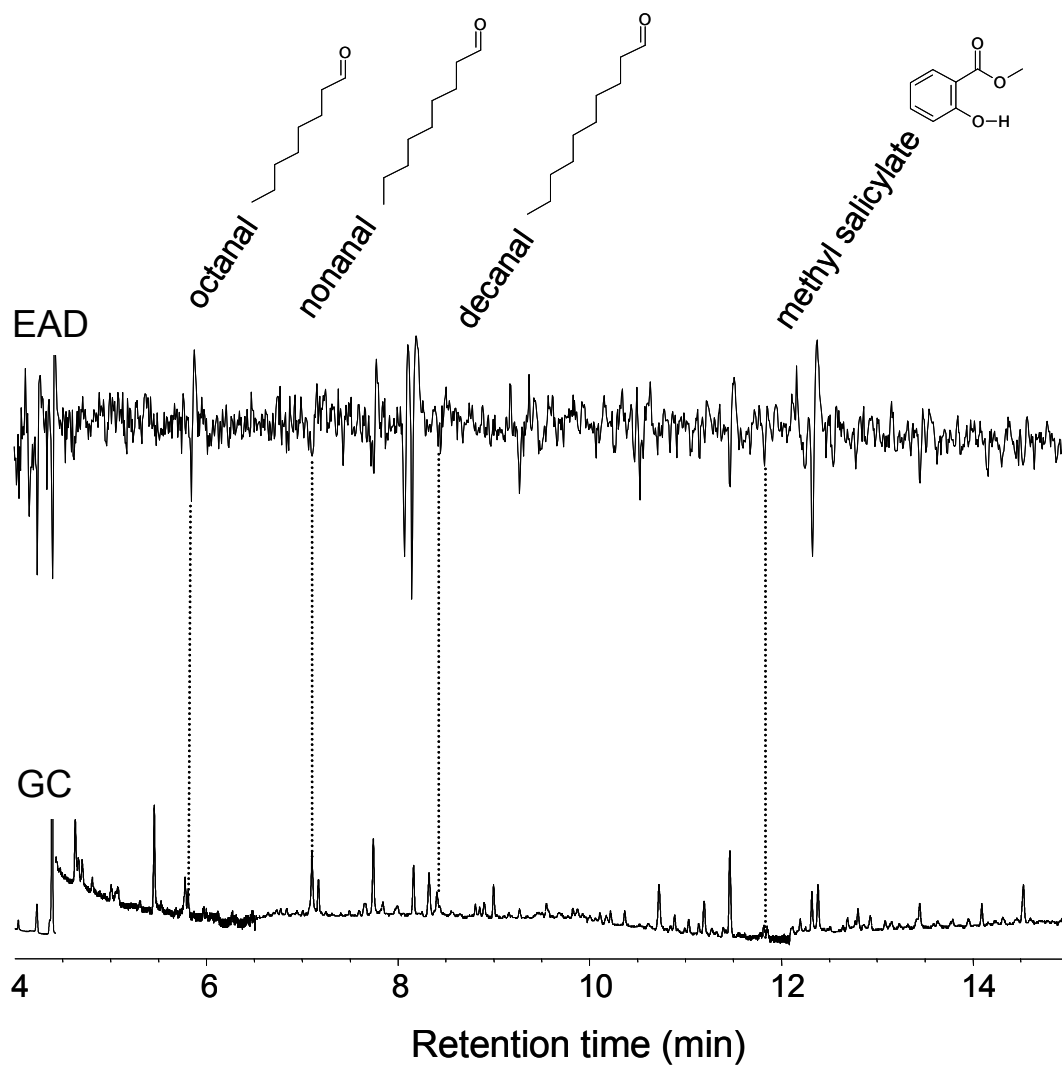
**Figure 6.5** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from 'Red Chief Delicious' apple trees with uninfested burr knots.



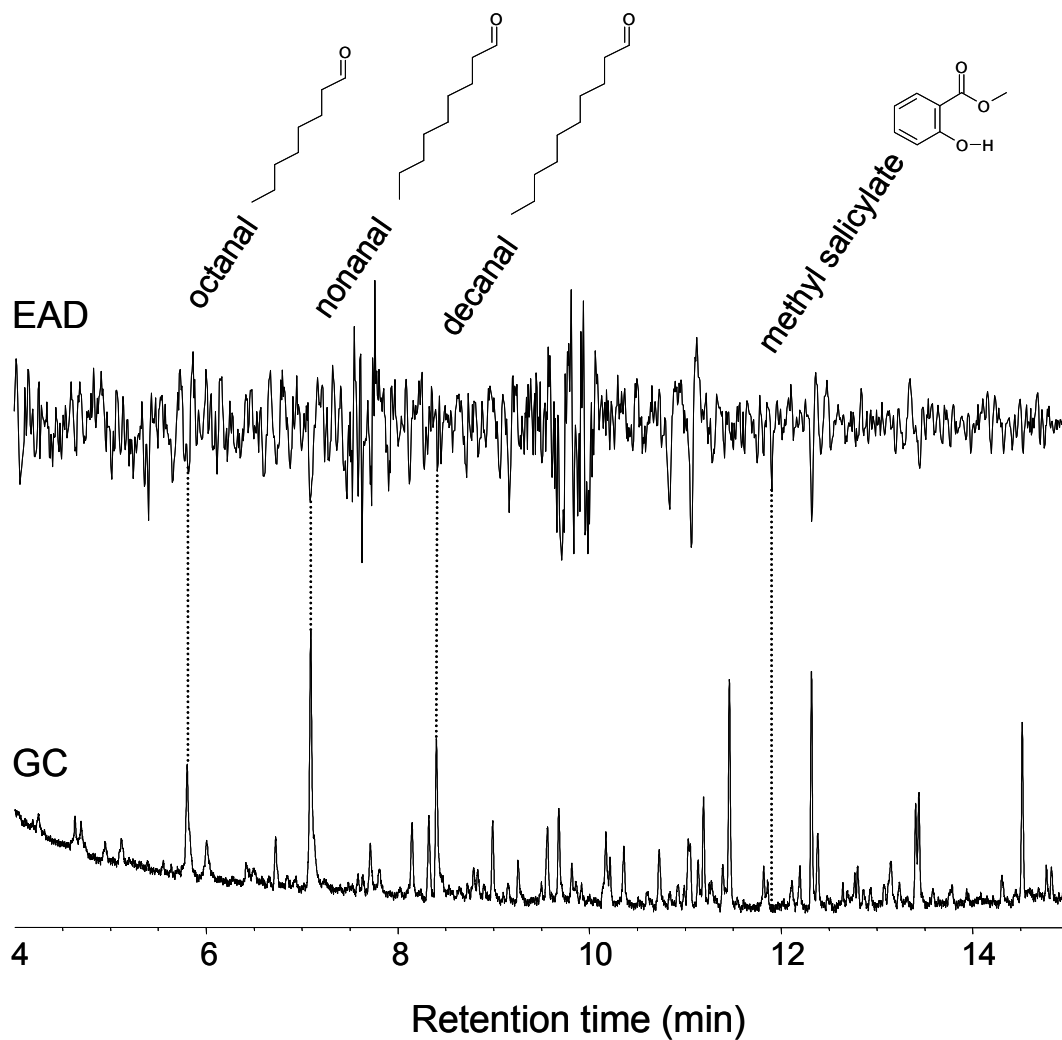
**Figure 6.6** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from 'Red Chief Delicious' apple trees with burr knots infested with dogwood borer larvae.



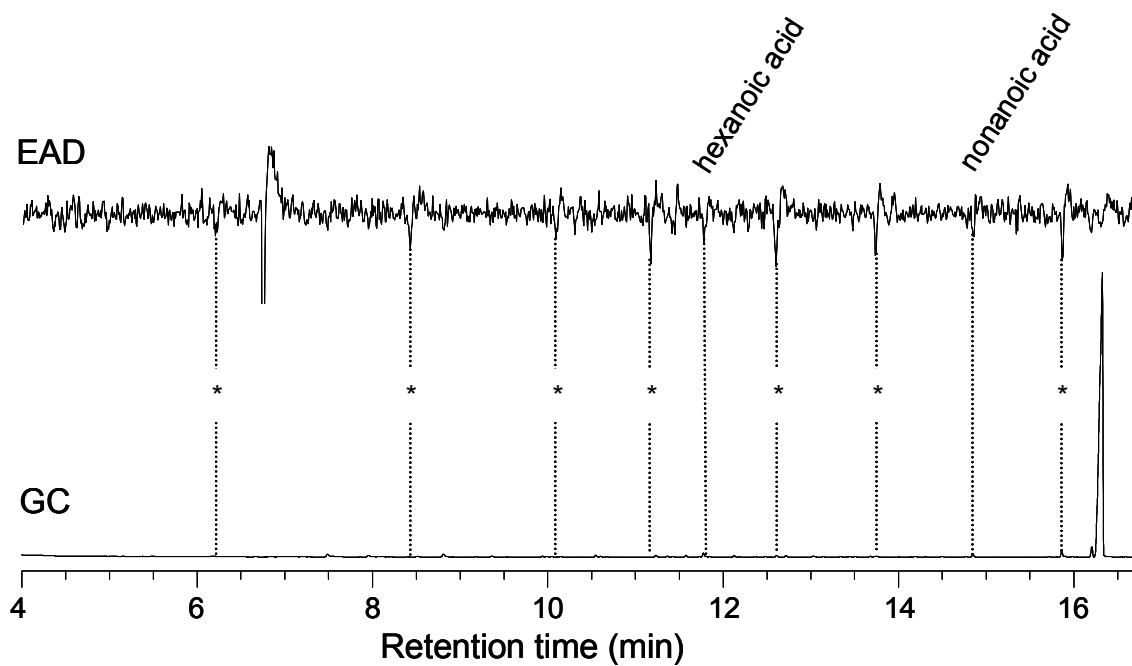
**Figure 6.7** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from 'Red Chief Delicious' apple trees with no burr knots present, but with 3-d-old cut bark.



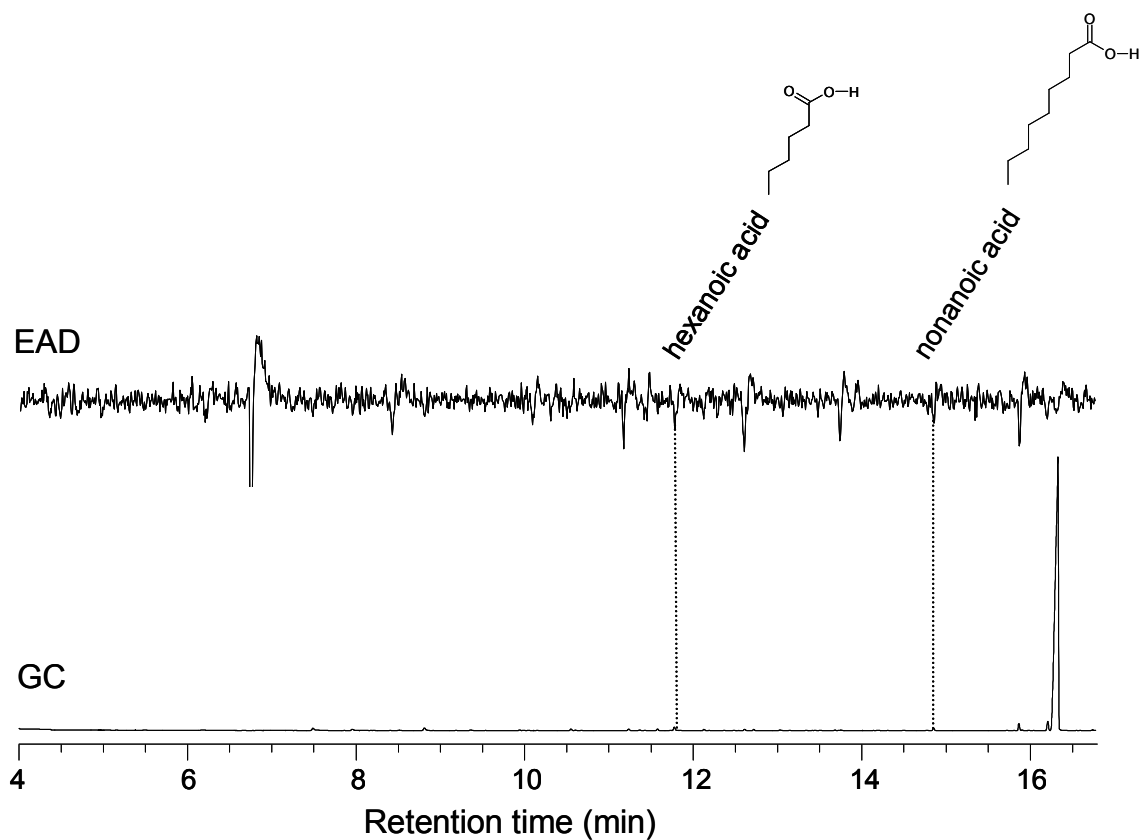
**Figure 6.8** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections of ‘Red Chief Delicious’ apple trees with no burr knots present, but with 30-d-old cut bark.



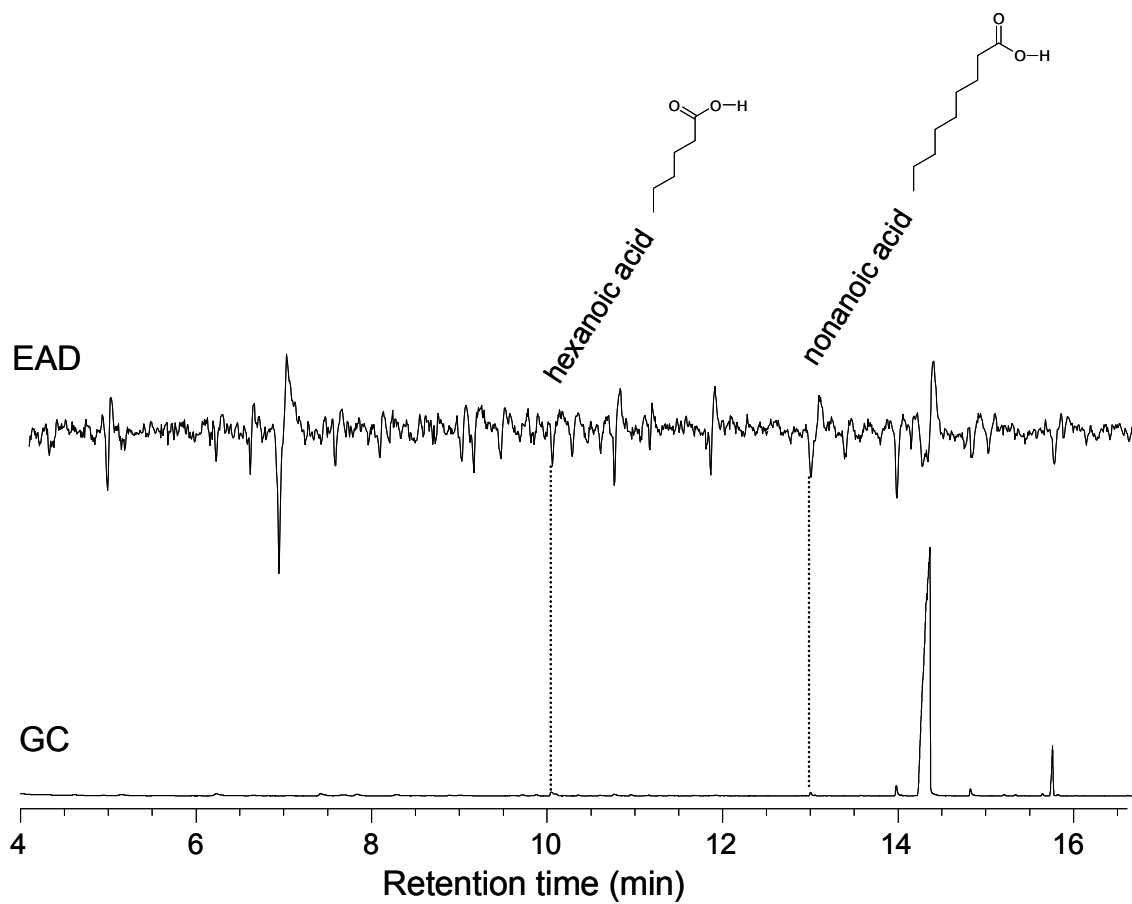
**Figure 6.9** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections of ‘Cherokee Princess’ dogwood trees with intact bark. \* indicates all unidentified compounds.



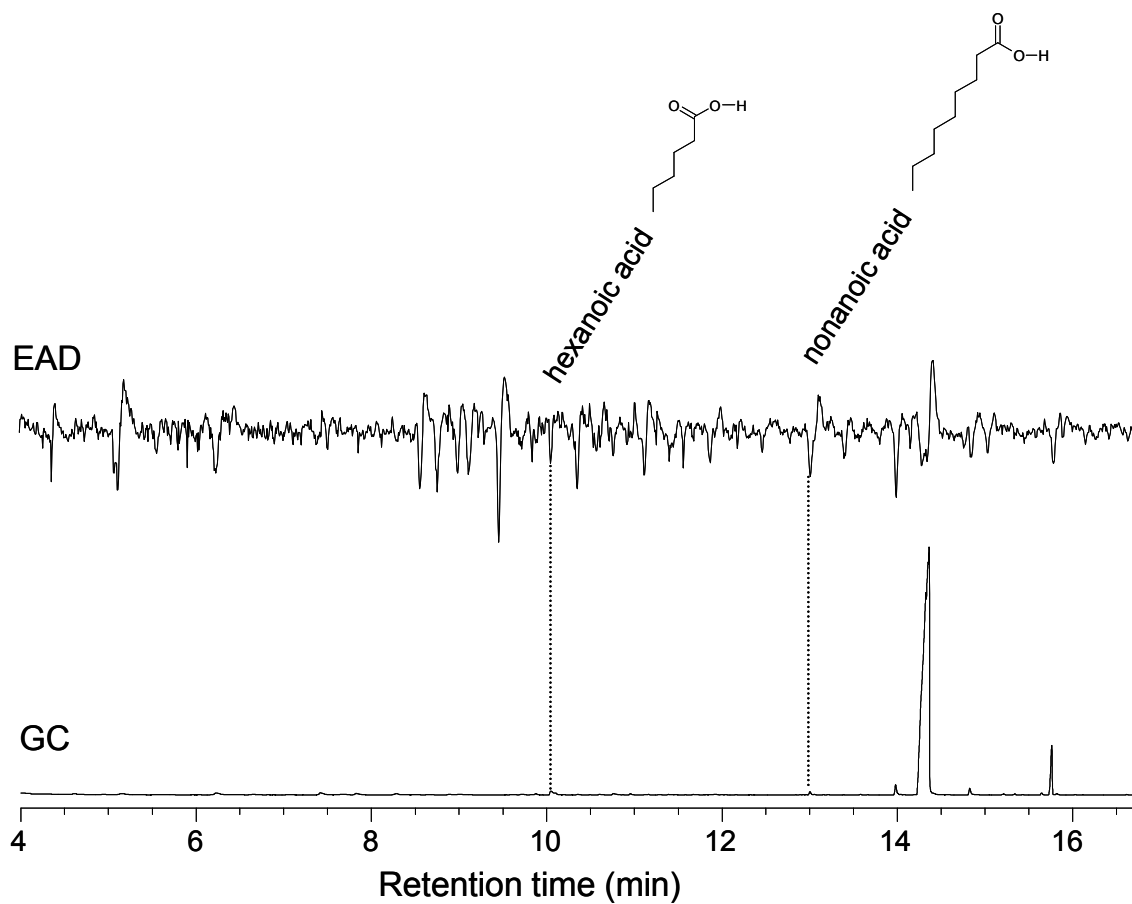
**Figure 6.10** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Cherokee Princess’ dogwood trees with intact bark.



**Figure 6.11** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Cherokee Princess’ dogwood trees with 3-d-old cut bark.

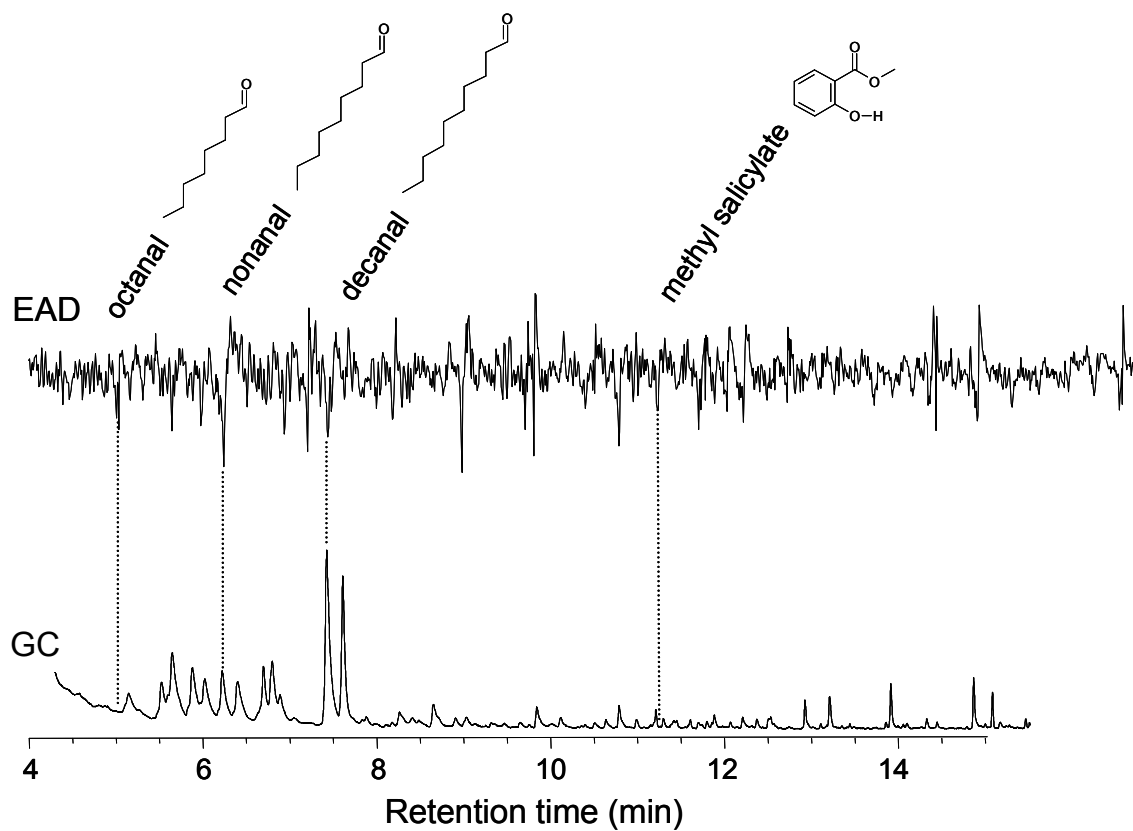


**Figure 6.12** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Cherokee Princess’ dogwood trees with 30-d-old cut bark.

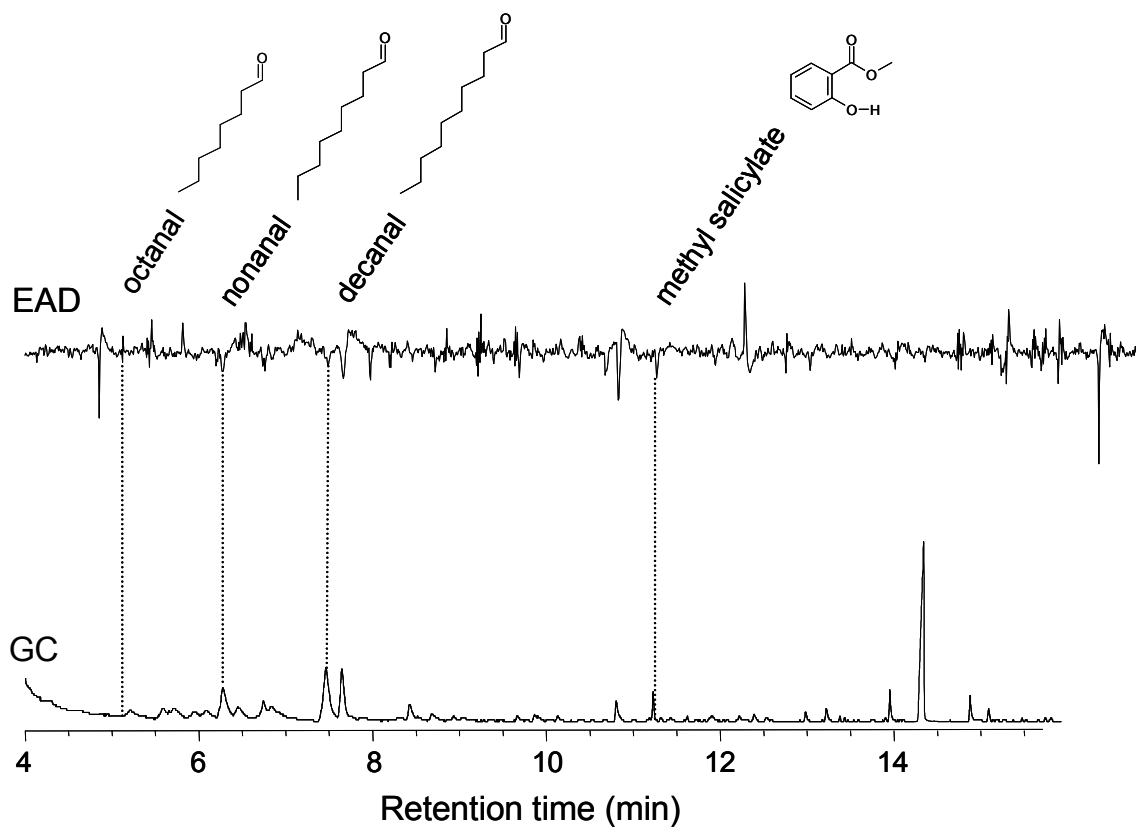




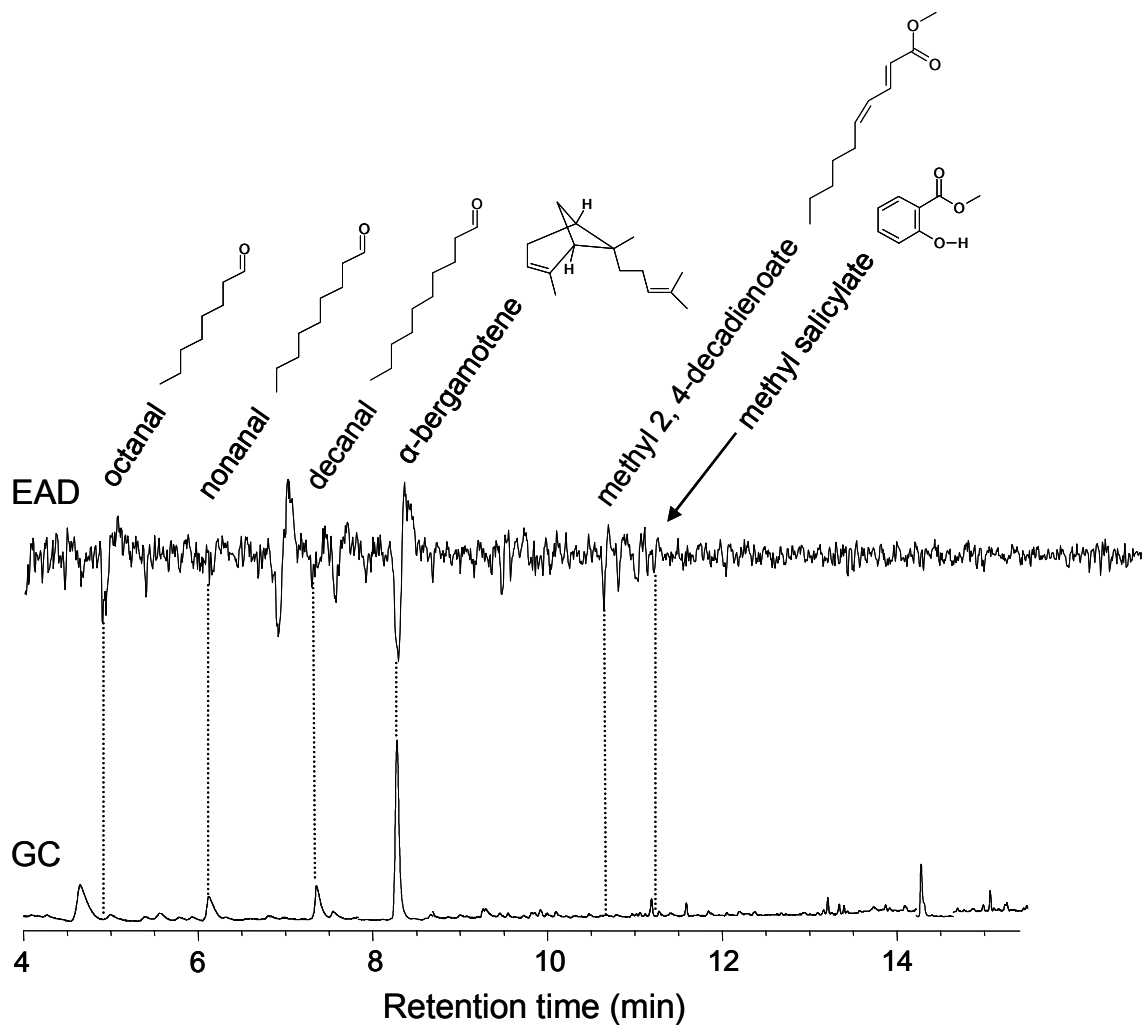
**Figure 6.13** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Granny Smith’ apple trees with no burr knots present.



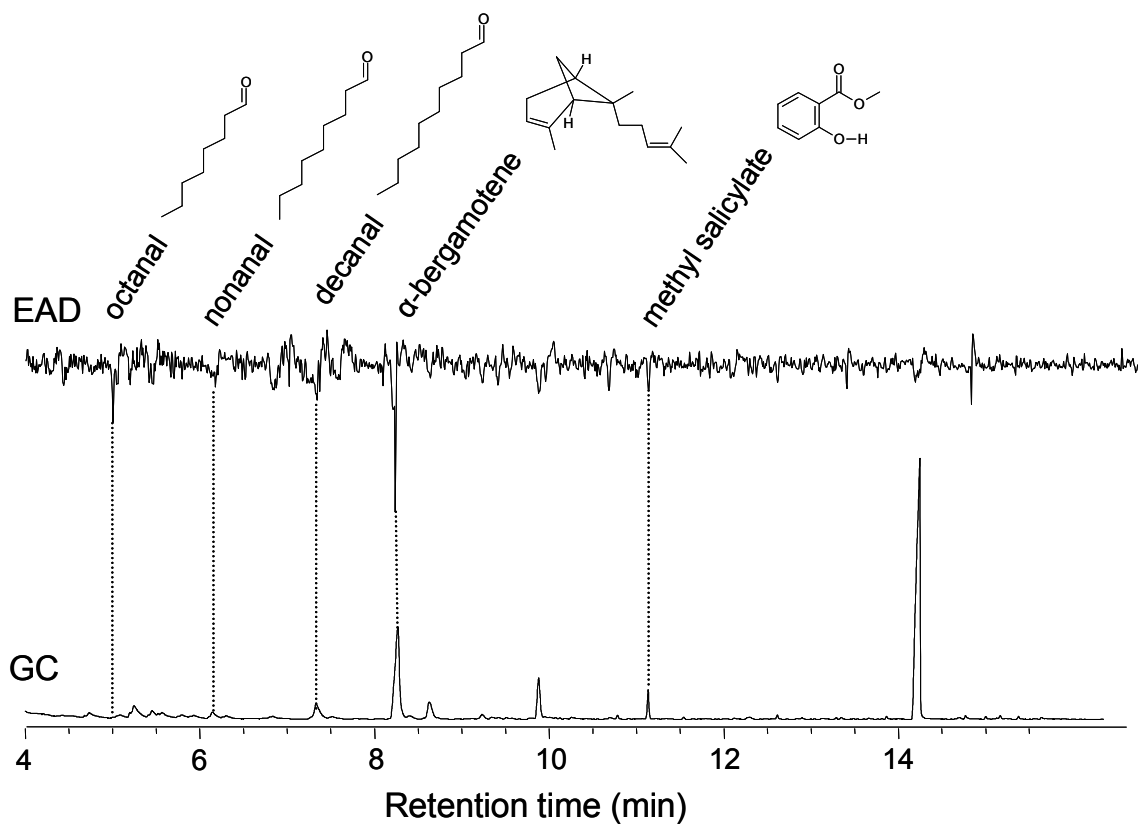
**Figure 6.14** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Granny Smith’ apple trees with uninfested burr knots.



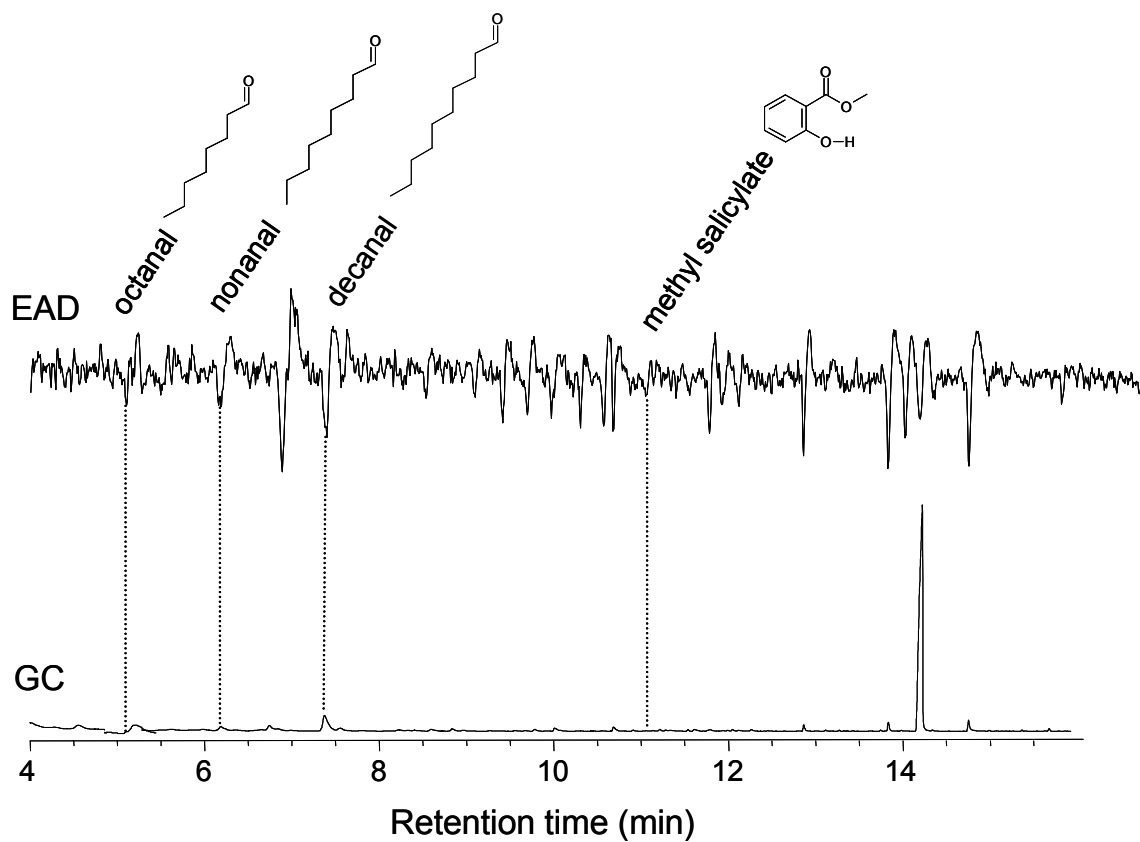
**Figure 6.15** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Granny Smith’ apple trees with burr knots infested by dogwood borer larvae.



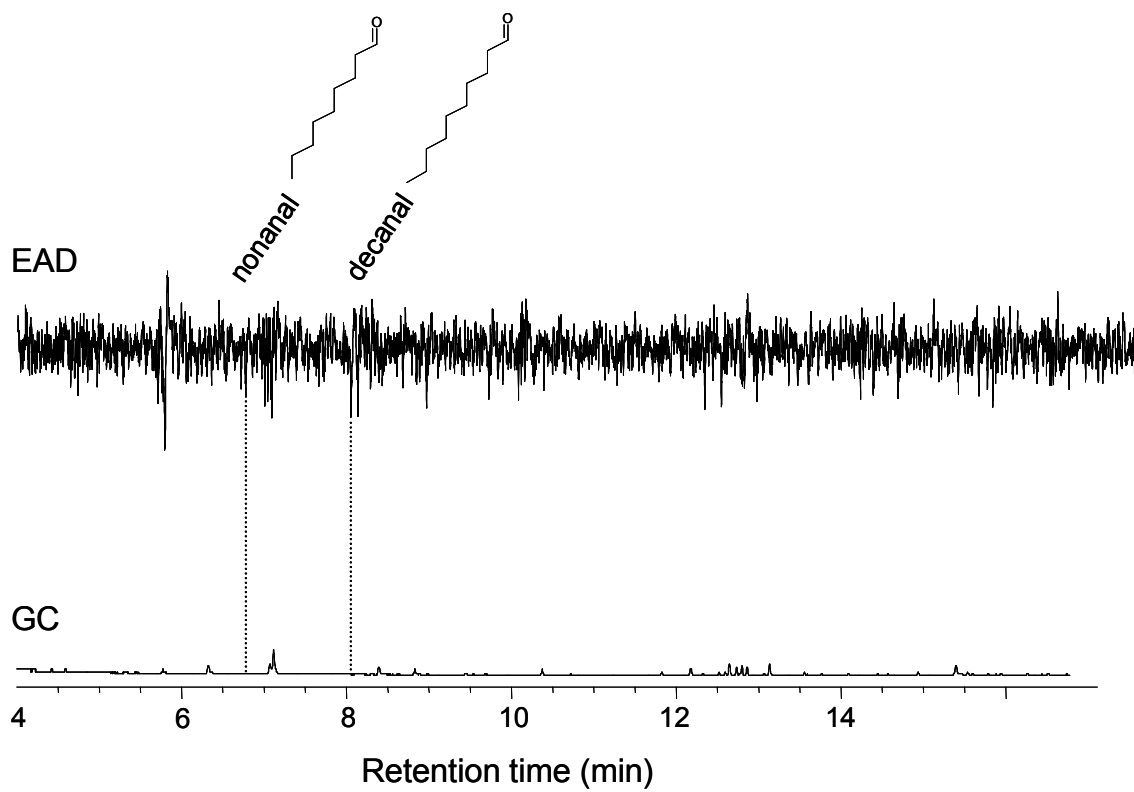
**Figure 6.16** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Granny Smith’ apple trees with no burr knots present, but with 1-d-old cut bark.



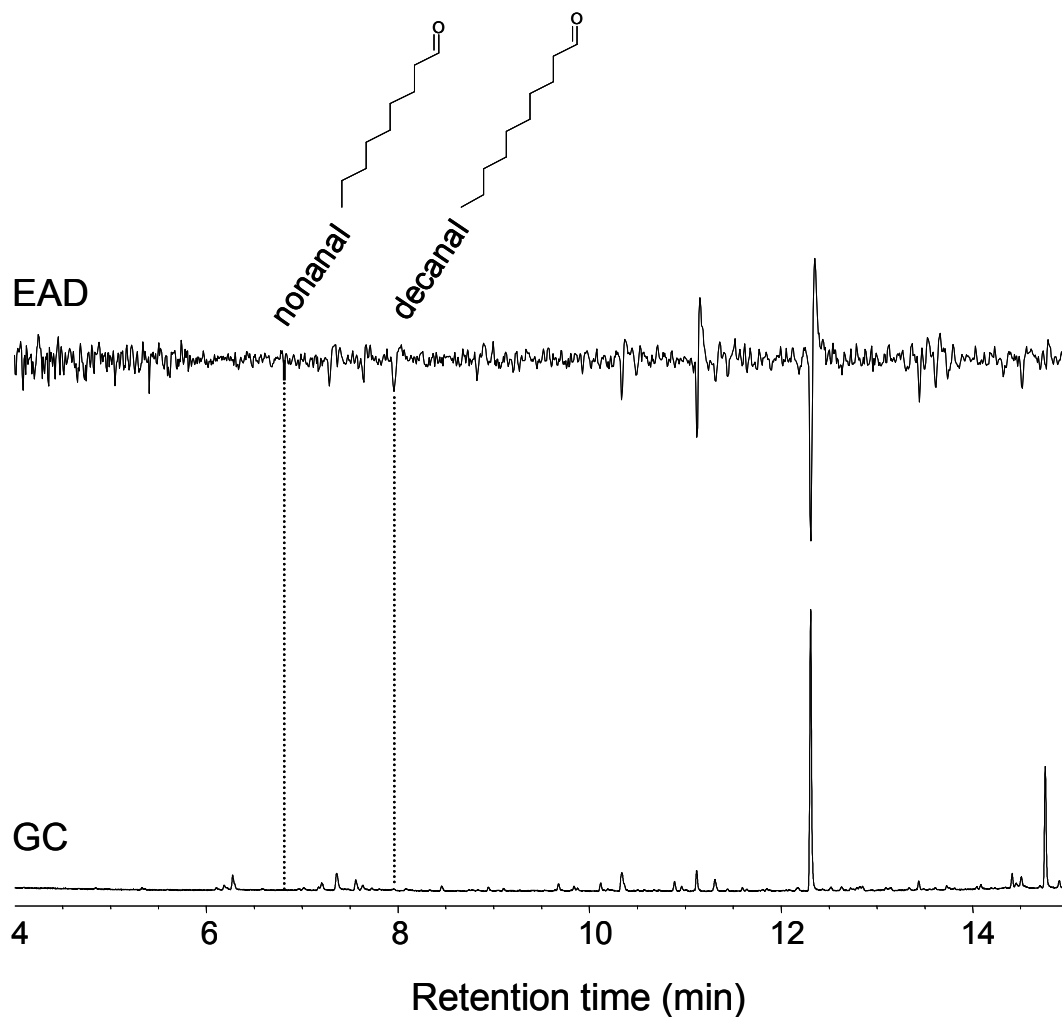
**Figure 6.17** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from ‘Granny Smith’ apple trees with no burr knots present, but with 30-d-old cut bark.



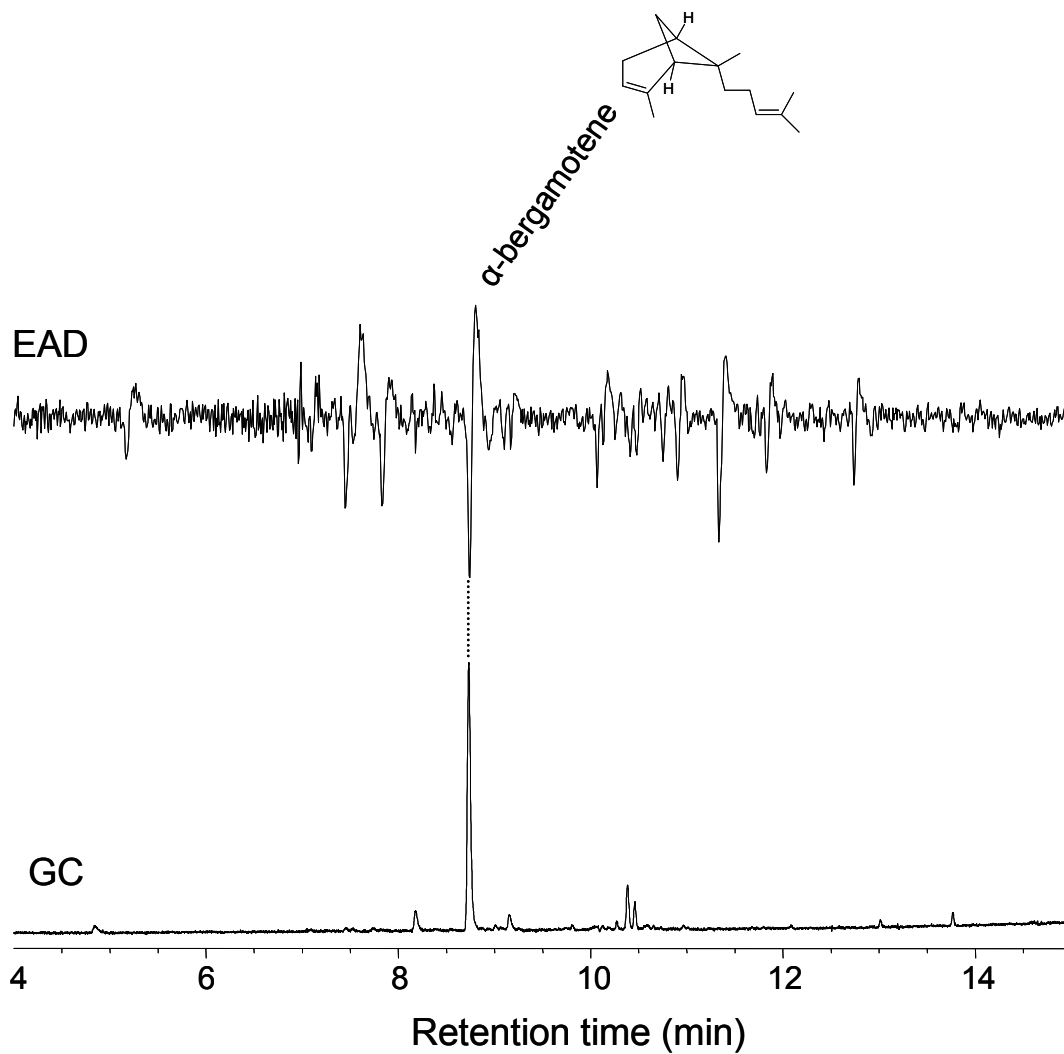
**Figure 6.18** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from a single dogwood borer larva.



**Figure 6.19** Representative GC-EAD recording of the response of female dogwood borer antennae to volatile collections from a single dogwood borer larva feeding on lepidopteran diet.



**Figure 6.20** Representative GC-EAD recording of the responses of female dogwood borer to SPME collected volatiles from 1-d-old dogwood borer frass.





## CHAPTER 7: CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

To enhance our research on the behavior and plant-insect interactions of female dogwood borer, a rearing methodology was developed using standardized procedures at each developmental stage. These procedures enabled the establishment of a laboratory-based colony of dogwood borer and reliable and efficient rearing of synchronized cohorts of distinct lifestages throughout the year for specific experimental needs. Aside from their logistical practicality, the results from rearing studies revealed new information about some basic aspects of dogwood borer biology. Complete development of dogwood borer from egg to adult can occur in < 50 d when reared on thinning apples, indicating the possibility of a much faster developmental rate than was previously recognized in apple and dogwood host tissues. Rapid deterioration of thinning apples in a controlled environment chamber precluded our ability to investigate the relationship between temperature and developmental rate. Future work on this question, especially with respect to their development on different host plants, may yield important insights into the voltinism of dogwood borer and provide an explanation for its bimodal annual flight pattern. Photoperiod is an important factor triggering the cessation of the pupal period and results in adult flight activity ceasing in October. Dogwood borer is not considered to have an obligatory diapause and overwinters as larvae in various stages of development. Factors that trigger the onset of pupation in the spring remain unknown.

Females were typically mated by the second evening after eclosion and it appears that fading daylight is an important stimulus for triggering mating. The onset of oviposition generally occurred within 2 d of mating and is an important consideration in the design of bioassays to address questions regarding the stimuli eliciting orientation toward host plants and/or critical to oviposition site selection. Consistent and pronounced variability in daily and total egg production among females was an impediment to optimal rearing, measurements of lifetime fecundity and attempts to assess female response to host plant stimuli that may elicit oviposition. Although additional research will be required to determine the stimuli that trigger egg deposition by mated females, the results discussed below should provide strong guidance for any such future efforts.

Quantification of the post-mating behaviors of dogwood borer under natural conditions showed that mated females exhibit orientation and oviposition behaviors if presented with appropriate stimuli. Areas below the graft union of apple trees were highly attractive to mated females based on the frequency of occurrences of casting flight, probing with the ovipositor and oviposition at those parts of the tree. The mating status of females was easily determined, based on the presence of a spermatophore in the bursa copulatrix. My results indicated that bioassays investigating female post-mating behavior should be conducted during the late afternoon and early evening hours, when females most frequently engaged in orientation and oviposition behaviors.

The temporal and spatial patterns of larval infestations in newly planted apple orchards showed a rapid increase of infestation sites during the first season after planting. Females were able to locate suitable oviposition sites equally well along and across orchard rows. Factors promoting burr knot formation such as rootstock, variety, and cultural management practice were significantly associated with the presence and extent of infestations and should be considered before and during the establishment of new orchards to mitigate injury by dogwood borer. The range of spatial dependence in infestation between the orchards varied from 7.50–19.87 m, which has important implications for the development of sampling programs for dogwood borer. The aggregated nature of infestations require that random, independent samples must be taken from sample points at distances greater than the range of spatial dependence to ensure that sample data are not autocorrelated. Alternatively, an efficient sampling program for mapping dogwood borer infestation can be achieved by limiting sample points to distances within the range of spatial dependence. These data can be used in interpolating algorithms, such as kriging, to create interpolated surface maps that can be used in a dogwood borer management program to indicate areas where larval infestations are more likely to occur, where scouting should begin, and where management intervention is necessary.

The sensilla present on antennae of male and female dogwood borer indicate that both genders readily perceive olfactory stimuli. The antennal flagellum of both male and female dogwood borer contained seven different sensillum types. However, female antennae possessed a greater number of olfactory sensilla compared with male antennae and, based on research conducted with other lepidopteran species (see Chapter 5), several

of these (auricillica, coeloconica and trichoidea) are likely involved with host odor perception. Future research investigating the response of these olfactory sensilla to host plant volatiles using single sensillum recordings may offer further insight into the specificity of olfactory receptor neurons and the multi-component blends that may lead to synergism or suppression of responses.

My exploration of the olfactory stimuli responsible for eliciting orientation and/or oviposition by mated females involved headspace collections from apple and dogwood host tissues. Results from GC-EAD analyses revealed that female dogwood borer perceive several volatile compounds emanating from these tissues, regardless of their mating status. Importantly, the response of female antennae to host odors was greater than that of males, which suggests that host odors may play an important role in evoking or guiding some female behaviors. Six compounds that elicited a strong and consistent antennal response were identified from apple host tissues. Four compounds (octanal, nonanal, decanal and methyl salicylate) were identified from all headspace collections from apple. Two novel stimulatory compounds that appear to be identified from apple trees for the first time were  $\alpha$ -bergamotene and methyl-2,4-decadienoate. The compound  $\alpha$ -bergamotene was identified from injured apple bark, from apple burr knots infested with dogwood borer larvae and from larval dogwood borer frass, and appears to be produced by apple trees in response to injury because of its absence from volatile collections from intact apple tissues and dogwood borer larvae feeding on a meridic diet. The compound methyl-2,4-decadienoate was identified from infested burr knot tissue and appears to be produced in response to an insect-plant interaction because of its absence from volatile collections from either damaged apple tissue or dogwood borer larvae. Two additional stimulatory compounds, hexanoic and nonanoic acid, were identified from all headspace collections from dogwood tissues.

Characterization of the olfactory stimuli that dogwood borer females are able to perceive has great potential utility, although the relationship between their antennal and behavioral responsiveness is imperative before further conclusions can be made. My numerous attempts to develop these relationships were unsuccessful. Further research investigating the stimuli involved with host and oviposition site identification, location and acceptance by female dogwood borer will require the development of behavioral bioassays that will satisfy the, as yet undetermined, physiological or abiotic conditions

necessary for successful assessment of female dogwood borer responses to behavioral stimuli under controlled conditions. As reference for future efforts in this regard, I describe below the general bioassay methods and approaches that were conducted, and their outcome.

### **Research on the Behavioral Response of Mated Female Dogwood Borer to Host Plant Stimuli.**

Numerous approaches to measuring the orientation or oviposition behaviors of mated female dogwood borers involved presenting them with various stimuli under natural, semi-natural or laboratory conditions. All field studies were conducted from June-September, while those in the laboratory occurred throughout the year. Based on the results of my direct observations of their activity in the field throughout the day, observations of female behavior were conducted during the late afternoon and evening hours. Only mated females were used in these assays; their mating status was confirmed by the presence of a spermatophore upon dissection following every experiment. Both laboratory-mated and field-collected females were used in the following studies. Females collected from the field were captured in apple orchards while exhibiting orientation and/or oviposition behavior at the base of apple trees, suggesting their physiological readiness to oviposit. Based on my rearing experiments, females mated in the laboratory were used at 1 to 2-d after copulation and should also have been in a state of physiological readiness to oviposit.

#### **Orientation Behavior and Attraction to Host Plant Stimuli**

**Orientation to Host Plants Under Semi-Field Conditions.** Initial attempts to measure the orientation of female dogwood borer to host plants under semi-field conditions were conducted in a circular, screened field cage (3 m high x 3 m diam) erected in a grassy area adjacent to forest. A single potted apple tree was positioned at the upwind edge of the cage. Treatments included trees with no burr knots, uninfested burr knots or infested burr knots below the graft union. A small platform (~1 m high) at the downwind edge of the cage served as a release site for female moths. Females that had been mated in the laboratory (n = 24) and those that had been collected while exhibiting orientation behavior toward apple trees in the field (n = 9) were used. A single

female was placed in a wire mesh cage on the platform and allowed to acclimate for 30 min. The lid of the cage was removed and observations of female behavior began immediately thereafter, for  $\geq 1$  h. All females eventually exited the release cage, but none showed oriented flight to the potted trees. Most time was spent either stationary on the side or top of the screened cage or flying within it, apparently attempting to escape. This experiment was conducted again by providing females a choice of 2 or 5 apple trees, with similar results.

In an attempt to create more natural conditions, the field cage was erected around single apple tree within a 5-yr-old orchard at the AHS-AREC. During the study, trees confined within the cage included those with actively infested burr knots or previously infested burr knots below the graft union. Using the methods described above, a single laboratory-mated or field-collected female was introduced into the cage and observations of its behavior were conducted for  $\geq 1$  h. Again, females ( $n = 32$ ) typically spent the majority of their time resting on the sides of the screened cage or in the canopy of the tree or trying to escape. One field-collected female briefly exhibited casting behavior toward the base of a tree after release, and then spent the remainder of time stationary or trying to escape.

**Orientation to Host Volatiles in Laboratory Bioassays.** Attraction to stimulatory host volatiles was measured using a Y-tube olfactometer ( $n = 15$  females), dual-choice arena (Natale et al. 2004b) ( $n = 50$  females) and a Plexiglas<sup>TM</sup> flight tunnel (183 cm long and 61  $\times$  61 cm square) ( $n = 50$  females). All experiments were conducted as choice tests. Treatments included filter papers treated with extracts from headspace collections of apple trees with no burr knots, uninfested burr knots or infested burr knots in methylene chloride, crude extracts of larval dogwood borer frass in methylene chloride, and pieces of excised burr knot tissue. Each treatment was paired with a blank control treated with hexane and prepared 15 min prior to the start of each bioassay, to allow solvent evaporation. Laboratory-mated females were tested individually in each bioassay and allowed to acclimate for 15 min before treatments were presented and observations began. Females showed no consistent response to any of the volatiles presented in any of the bioassays, spending most time stationary or flying within the bioassay apparatus, apparently attempting to escape.

**Orientation to Host Volatiles Under Semi-Field Conditions.** Attraction to stimulatory host volatiles was quantified using screened cages measuring 30 cm<sup>3</sup> (n = 56 females) and in those measuring 0.9 x 0.9 x 1.8 m (n = 56 females). Source stimuli consisted of crude extracts of burr knot tissue in hexane or ethanol, larval dogwood borer frass in methylene chloride, and several synthetic compounds in hexane (nonanal, hexanoic acid, nonanoic acid, methyl-2,4-decadienoate and methyl salicylate) that elicited a strong and repeatable EAD response. A single mated female was introduced into a cage and allowed to acclimate for 15 min before a filter paper containing one of the stimulatory host volatiles (prepared 15 min prior) was presented at the upwind side of the cage and direct observations of female behavior were commenced. Again, females spent most time stationary or flying within cages and showed no consistent or directed response to any of the volatiles presented.

### **Oviposition Behavior**

**Oviposition in Response to Host Plants Under Field and Semi-Field Conditions.** Initial attempts to measure oviposition by mated females involved confining them in a screened enclosure around the base of potted apple trees that had no burr knots, uninfested burr knots or infested burr knots below the graft union. Periodic observations of 15 females over several days revealed that they spent the majority of their time stationary or flying within the enclosure and egg-laying was never observed. Assessments of whether eggs were deposited by females at the end of each experiment were unsuccessful due to the small size and color of eggs and to the highly textured nature of the host surface.

Female dogwood borer will oviposit on burr knots surrounded by white plastic spiral wrap tree guards (Chapter 4) and on pieces of apple branch wrapped in white muslin cloth (Chapter 2). Eggs laid in muslin can be readily located with the aid of a dissecting microscope. To assess oviposition by free-flying female dogwood borer in an orchard, white muslin was wrapped around the base of trees, from the soil line to the graft union. Treatments included trees with uninfested or infested burr knots that were growing within four rows of an orchard (n = 20 trees/treatment) or in pots placed between trees in an orchard (n = 10 trees/treatment). The muslin from each tree was examined and replaced weekly for 4 wk.

The same approach was used for laboratory-mated females ( $n = 30$ ) placed in 0.9 x 0.9 x 1.8 m screened cages containing a potted apple tree with uninfested or infested burr knots. The muslin and female in each cage were examined and replaced at 3-d intervals for 30 d. Although eggs were occasionally found on muslin from orchard trees and those in cages, no consistent pattern among oviposition responses were detected. In the orchard, eggs were recovered from the muslin on one tree with infested burr knots on 2 consecutive occasions (12 and 8 eggs, respectively). No eggs were recovered from muslin on potted trees deployed in an orchard. In cages, eggs were recovered from the muslin on 3 occasions from trees with infested burr knots (15, 7 and 2 eggs) and on 1 occasion from a tree with uninfested burr knots (6 eggs).

#### **Oviposition in Response to Host Volatiles in Laboratory Bioassays.**

Laboratory bioassays were conducted to determine the influence of host plant volatile stimuli on oviposition by female dogwood borer. Stimuli included crude extracts and headspace collections from host tissues and synthetic compounds that elicited an antennal response in GC-EAD studies (Chapter 6). Responses were evaluated in 500 ml straight-sided glass jars with a screw cap, provisioned with a cotton ball saturated with 10% sucrose solution in a small glass dish and held on a laboratory bench at room temperature and a 16:8 (L:D) photoperiod under four, 40-w fluorescent bulbs positioned 35 cm above containers. An experimental replicate was initiated by randomly assigning a treatment stimulus to oviposition substrates placed in jars, after which one mated female was introduced to each. Treatments consisted of crude extract of burr knot tissue in hexane, ethanol, and water, and frass in methylene chloride, headspace collections from apple trees with no burr knots, uninfested burr knots, infested burr knots and 1-d-old cut bark in methylene chloride, and serial dilutions of synthetic compounds in hexane that elicited an antennal response (nonanal, hexanoic and nonanoic acid, methyl-2,4-decadienoate and methyl salicylate). Volatile treatments were placed on filter paper wrapped around oak dowels and then wrapped in muslin or on 5 cm pieces of cotton dental wick. All sources were prepared 15 min prior to their placement in jars. Using daily observations, the latency to the onset of oviposition and the number of eggs deposited by each female ( $n = 8$ /treatment) was determined. At the end of each experiment, each female was dissected, the number of eggs remaining in its abdomen was counted and the proportion of its total

egg load deposited in response to each treatment was calculated. There were no significant differences among treatments in the number or proportion of eggs deposited.

### **Possible Explanations for Why Behavioral Responses Were Not Observed**

It remains uncertain as to why female dogwood borer that were either mated in the laboratory or collected from the field while exhibiting orientation and oviposition behaviors did not demonstrate clear behavioral responses to host plant stimuli. Discussed below are possible explanations that may account for these results, which should be considered before future behavioral research is conducted.

**Female dogwood borer do not use host plant stimuli in their host-finding and oviposition site selection behavior.** Many polyphagous insect species do not depend on olfactory cues during the host-selection process (Ramaswamy 1988). Although I cannot conclude that this is not true for dogwood borer, evidence suggests strongly that females do respond behaviorally to particular host plant odors. Infestations are most commonly observed on specific host tissues such as apple burr knots (Riedl et al. 1985, Kain and Straub 2001, Leskey and Bergh 2005), injured bark on dogwood trees (Wallace 1945, Potter and Timmons 1981) and insect-induced galls on pin oak trees (Eliason and Potter 2000). Furthermore, my research has shown that females frequently exhibited orientation and oviposition behaviors toward areas below the graft union of apple trees (Chapter 3) and that factors promoting burr knot development were significantly associated with the presence and extent of infestations over both time and space (Chapter 4). Finally, given that insect olfactory capabilities are generally “hardwired” to perceive primarily stimuli that are ecologically relevant, the results of my electrophysiological studies (Chapter 6) suggest such relevance of compounds like  $\alpha$ -bergamotene and others.

**Inappropriate stimuli and/or lack of understanding of the possible ecological role of stimuli.** It has been suggested that most phytophagous insects rely on a ratio-specific blend of commonly occurring volatiles to recognize host plants (Bruce et al. 2005), which may account for the lack of significant behavioral responses by female dogwood borer to individual synthetic compounds that elicited an antennal response. Several of the compounds identified from headspace collections from apple and dogwood trees are also emitted by other plants that are not hosts of dogwood borer and can similarly elicit an antennal response in other lepidopteran species (Andersen et al. 1987,



Tasin et al. 2005, Cha et al. 2008a, Casado et al. 2008). The response to widespread, rather than host-specific compounds can result in the utilization of other host plants by insects (Bengtsson et al. 2006) and may account for the polyphagous nature of dogwood borer. Furthermore, it is possible that synergism between general host plant volatile components is required to induce a behavioral response in females or that individual compounds or blends of compounds may trigger specific behaviors at different points in the host selection process such as attraction over long or short distances, or elicitation of landing or oviposition

If dogwood borer females respond to multi-component blends of host volatiles, it remains unclear why orientation behavior and attraction to intact host plants and headspace collections were not observed. Although there appears to be a positive relationship between the amount of burr knot tissue and the level of dogwood borer infestation (Leskey and Bergh, 2005), data presented in Riedl et al. (1985) suggest that the mere presence of burr knot tissue does not guarantee that affected trees will become infested. Plant volatile emissions can vary among individuals due to a number of abiotic and physiological factors and it is possible that the volatiles emitted from trees selected for bioassays or headspace collections did not release the appropriate ratio and/or concentration of stimulatory compounds necessary to elicit attraction or oviposition when utilized. If an attractive host odor is composed of a mixture, its identity can change with minor alterations in the ratio of volatile components and consequently cause variations in insect responses. Future bioassays and/or volatile collections may need to be conducted using trees to which attraction by females in the field has recently been observed, so that it is clear that stimulatory host plant volatiles are emitted.

**Inappropriate bioassay methodology or apparatus.** It may be possible that the design of bioassays were inadequate to effectively measure female dogwood borer behaviors under the conditions tested and that other sensory and/or environmental stimuli were required to elicit consistent and repeatable behavioral responses. Field and laboratory bioassays were conducted during the late afternoon and evening when females were observed most frequently (Chapter 3). However, the possible effects of other abiotic stimuli on female behavior and how they should be presented during bioassays remains unclear. Cha et al. (2008b) demonstrated that several abiotic factors including time of day, light intensity, patterns on the flight tunnel floor and ceiling and wind speed

influenced attraction of a tortricid, the grape berry moth, *P. viteana*, to host plant stimuli. Additional studies should be conducted to determine whether similar factors influence the attraction of dogwood borer to host stimuli. Size and texture of host surfaces can be a significant factor influencing the oviposition behavior of sesiids (Bobb 1959, Hardy 1982, Reed et al. 1988, Brown et al. 1991) and may need to be investigated further to generate consistent oviposition responses from female dogwood borer. Although muslin cloth provided a textured surface for egg deposition to occur (Chapter 2), other substrates or surfaces may provide more optimal conditions to encourage oviposition. Furthermore, visual or chemosensory cues associated with muslin may have discouraged oviposition by females under field and semi-field conditions.

**Lack of physiological readiness.** Physiological factors may have influenced the behavioral responses of females. For example, many insects adjust their host-finding and oviposition behavior in response to egg-load (Minkenberget al. 1992). Our initial assumption that the egg load of field-collected females used in these bioassays would vary from nearly complete to significantly depleted was not confirmed by dissections of them following experiments. Somewhat surprisingly, all field-collected females contained a greatly reduced egg complement, which may have accounted for their lack of an orientation response to host trees in cages. Although laboratory-mated females contained a full egg complement when used in experiments, it may be that their physiological readiness to seek host plants and oviposit follows a mandatory period of dispersal flight or other behavioral requirement.

The oviposition choices made by female dogwood borer are vital to their reproductive success because neonate larvae have limited ability to relocate from areas where they are placed. The preference-performance hypothesis proposed by Jaenike (1978), predicts that in this situation there will be a strong selection pressure on adults to choose oviposition sites that maximize the survival of their offspring. An increasing body of evidence supports the hypothesis that female dogwood borer respond to volatile stimuli associated with apple burr knots and that this tissue is an important resource for dogwood borer larvae. Although research investigating the olfactory stimuli responsible for mediating host and oviposition site identification, location and acceptance by dogwood borer should continue to focus on host plant volatiles emanating from apple burr knots, other host tissues should also be investigated and compared. The presence of

$\alpha$ -bergamotene and methyl-2,4-decadienoate were unique to damaged and/or infested apple tissue and are candidate compounds that should be included in future bioassays. However, several other compounds may likely be involved in stimulating a behavioral response in females. A greater understanding of the host plant volatiles involved with host selection behaviors in dogwood borer may provide a foundation for their future application in management strategies and offer additional insight into the role of these compounds in plant-insect interactions.

## REFERENCES

- Agelopoulos, N. G., K. Chamberlain, and J. A. Pickett. 2000.** Factors affecting volatile emissions of intact potato plants, *Solanum tuberosum*: Variability of quantities and stability of ratios. *J. Chem. Ecol.* 26: 497-511.
- Agnello, A. M. and D. P. Kain. 2002.** Evaluation of pheromone disruption in combination with insecticide applications for control of peachtree borers in peaches. *NY Fruit Quart.* 10: 29-31.
- Aldrich, J. R., M. P. Hoffmann, J. P. Kochansky, W. R. Lusby, J. E. Eger, and J. A. Payne. 1991.** Identification and attractiveness of a major pheromone component for nearctic *Euschistus* spp. stink bugs (Heteroptera: Pentatomidae). *Environ. Entomol.* 20: 477-483
- Altner, H. and L. Prillinger. 1980.** Ultrastructure of invertebrate chemo-, thermo-, and hygroreceptors and its functional significance. *Int. Rev. Cytol.* 67: 69-139.
- Altner, H., H. Sas, and I. Altner. 1977.** Relationship between structures and function of antennal chemo-, hygro-, and thermo-receptive sensilla in *Periplaneta americana*. *Cell. Tiss. Res.* 176: 3889-3405.
- Andersen, J. F., K. L. Mikolajczak, and D. K. Reed. 1987.** Analysis of peach bark volatiles and their electroantennogram activity with lesser peachtree borer, *Synanthedon pictipes* (Grote and Robinson). *J. Chem. Ecol.* 13: 2103-2114.
- Anderson, P., E. Hallberg, and M. Subchev. 2000.** Morphology of antennal sensilla auricillica and their detection of plant volatiles in the herald moth, *Scoliopteryx libatrix* L. (Lepidoptera: Noctuidae). *Arthro. Struct. Dev.* 29: 33-41.
- Ansebo, L., R. Ignell, J. Löfqvist, and B. S. Hansson. 2005.** Response to sex pheromone and plant odours by olfactory receptor neurons housed in sensilla auricillica of the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae). *J. Insect Physiol.* 51: 1066-1074.
- Antonio, A. Q., J. R. McLaughlin, N. C. Leppla, and C. W. Green. 1975.** Culturing the lesser peachtree borer. *J. Econ. Entomol.* 68: 309-310.
- Arn, H., E. Städler, and S. Rauscher. 1975.** The electroantennographic detector a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. *Z. Naturf.* 30c: 722-725.
- Ateyyat, M. A. 2006.** Effect of three apple rootstocks on the population of the small red-belted clearwing borer, *Synanthedon myopaeformis*. *J. Insect Sci.* 6: 1-5

- Ayers, G. S. 1966.** The bionomics of the dogwood borer, *Thamnospectia scitula* (Harris), attacking blueberry in Michigan. MSc thesis, Michigan State University, Ann Arbor.
- Barry, M. W. and D. G. Neilsen. 1984.** Behavior of adult peachtree borer (Lepidoptera: Sesiidae). *Ann. Entomol. Soc. Am.* 77:246-250.
- Bengtsson, M., A. C. Bäckman, I. Liblikas, M. I. Ramirez, A. K. Borg Karlson, L. Ansebo, P. Anderson, J. Löfqvist, and P. Witzgall. 2001.** Plant odor analysis of apple: Antennal response of codling moth females to apple volatiles during phenological development. *J. Agric. Food Chem.* 49: 3736-3741.
- Bengtsson, M., G. Jaastad, G. Knudsen, S. Kobro, A. C. Bäckman, E. Pettersson, and P. Witzgall. 2006.** Plant volatiles mediate attraction to host and non-host plant in apple fruit moth, *Argyresthia conjugella*. *Entomol. Exp. Appl.* 118: 77-85.
- Bergh, J.C. and T.C. Leskey. 2003.** Biology, ecology and management of dogwood borer in eastern apple orchards. *Can. Entomol.* 135: 615-635.
- Bergh, J. C., T. C. Leskey, and A. Zhang. 2004.** Discrimination by male dogwood borer, *Synanthedon scitula* (Lepidoptera: Sesiidae), among traps baited with commercially available pheromone lures. *J. Econ. Entomol.* 97: 344-352.
- Bergh, J. C., T. C. Leskey, J. M. Sousa, and A. Zhang. 2006.** Diel periodicity of emergence and pre-mating reproductive behaviors of adult dogwood borer (Lepidoptera: Sesiidae). *Environ. Entomol.* 35: 425-432.
- Bergh, J. C., T.C. Leskey, J.F. Walgenbach, W.E. Klingeman, D.P. Kain, and A. Zhang. 2009.** Dogwood borer (Lepidoptera: Sesiidae) abundance and seasonal flight activity in apple orchards, urban landscapes and woodlands in five eastern states. *Environ. Entomol.* 38: 530-538.
- Bernays, E. A. and R. F. Chapman. 1994.** Host-plant selection by phytophagous insects. Chapman and Hall, New York, NY.
- Bishir, J., J. H. Roberds, and B. L. Strom. 2004.** On-bark behavior of *Dendroctonus frontalis*: A Markov chain analysis. *J. Ins. Behav.* 17: 281-301.
- Blom, P. E. and S. J. Fleischer. 2001.** Dynamics in the spatial structure of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Environ. Entomol.* 30: 350-364.
- Blom, P. E., S. J. Fleischer, and Z. Smilowitz. 2002.** Spatial and temporal dynamics of Colorado potato beetle (Coleoptera: Chrysomelidae) in fields with perimeter and spatially targeted insecticides. *Environ. Entomol.* 31: 149-159.
- Blum, M. S. 1985.** Fundamentals of insect physiology. John Wiley and Sons, Inc., New York, NY.

- Bobb, M. L. 1959.** The biology of the lesser peach tree borer in Virginia. *J. Econ. Entomol.* 52: 634-636.
- Brown, S. L., C. C. Reilly, J. R. McVay, C. S. Gorsuch, R. Gentry, and J. A. Payne. 1991.** Evaluation of peach cultivars as peachtree borer (Lepidoptera: Sesiidae) hosts. *Environ. Entomol.* 20: 1108-1112.
- Bruce, T. J. A., L. J. Wadhams, and C. M. Woodcock. 2005.** Insect host location: A volatile situation. *Trends Plant Sci.* 10: 269-274.
- Buda, V. and V. Karalius. 1985.** Calling behavior of females of currant clearwing moth, *Synanthedon tipuliformis* (Clerck) (Lepidoptera: Sesiidae). *Z. Angew. Entomol.* 100: 297-302.
- Byers, J. A., G. Birgersson, J. Löfqvist, M. Appelgren, and G. Bergström. 1990.** Isolation of pheromone synergists of bark beetle, *Pityogenes chalcographus*, from complex insect-plant odors by fractionation and subtractive-combination bioassay. *J. Chem. Ecol.* 16: 861-876.
- Cambardella, C. A., T. B. Moorman, J. M. Novak, T. B. Parkin, D. L. Karlen, R. F. Turco, and A. E. Konopka. 1994.** Field-scale variability of soil properties in Central Iowa soils. *Soil Sci. Soc. Am. J.* 58: 1501-1511.
- Cardé, R. T. and A. K. Minks. 1995.** Control of moth pests by mating disruption: Successes and constraints. *Annu. Rev. Entomol.* 40: 559-585.
- Casado, D., C. Gemenó, J. A. Avilla, and A. Riba. 2006.** Day-night and phonological variation of apple tree volatiles and electroantennogram responses in *Cydia pomonella* (Lepidoptera: Tortricidae). *Environ. Entomol.* 35: 258-267.
- Casado D., C. Gemenó, J. Avilla, and M. Riba. 2008.** Diurnal variation of walnut tree volatiles and electrophysiological responses in *Cydia pomonella* (Lepidoptera: Tortricidae). *Pest Manag. Sci.* 64: 736-747.
- Castrejón-Gómez, V. R. and J. V. Carrasco. 2008.** Morphological characteristics of antennal sensilla in *Talponia batesi* (Lepidoptera: Tortricidae). *Ann. Entomol. Soc. Am.* 101: 181-188.
- Castrejón-Gómez, V. R., G. Nieto, J. Valdes, F. Castrejón, and J. C. Rojas. 2003.** The antennal sensilla of *Zamagiria dixolophella* Dyar (Lepidoptera: Pyralidae). *Ann. Entomol. Soc. Am.* 96: 672-678.
- Cha, D. H., S. Nojima, S. P. Hesler, A. Zhang, C. E. Linn Jr., W. L. Roelofs, and G. M. Loeb. 2008a.** Identification and field evaluation of grape shoot volatiles attractive to female grape berry moth (*Paralobesia viteana*). *J. Chem. Ecol.* 34: 1180-1189.

**Cha, D. H., S. P. Hesler, C. L. Moser, S. Nojima, C. E. Linn Jr., W. L. Roelofs, and G. M. Loeb. 2008b.** Flight tunnel responses of female grape berry moth (*Paralobesia viteana*) to host plants. *J. Chem. Ecol.* 34: 622-627.

**Chapman, R. F. 1998.** *The Insects: Structure and Function*, 4<sup>th</sup> edition. Cambridge University Press, Cambridge, United Kingdom.

**Cleveland, M. L., T. T. Y. Wong, and K. W. Lamansky. 1968.** Rearing methods and biology of the lesser peach tree borer, *Synanthedon pictipes*, in the laboratory. *Ann. Entomol. Soc. Am.* 61: 809-814.

**Cottrell, T. E., J. Fuest, and D. L. Horton. 2008.** Influence of *Prunus* spp., peach cultivars, and bark damage on oviposition choices by the lesser peachtree borer (Lepidoptera: Sesiidae). *Environ. Entomol.* 37: 1508-1513.

**Cressie, N. A. C. 1993.** *Statistics of Spatial Data*, Revised edition. Wiley, New York, NY.

**Dale, M. R. T. 1998.** *Spatial Pattern Analysis in Plant Ecology*. Cambridge University Press, Cambridge, United Kingdom.

**Davidson, J. A., S. A. Gill, and M. J. Raupp. 1992.** Controlling clearwing moths with Entomopathogenic nematodes: The dogwood borer case study. *J. Arboric.* 18: 81-84.

**Den Otter, C. J., H. A. Schuil, and A. S. van Oosten. 1978.** Reception of host-plant odors and female sex pheromone in *Adoxophyes orana* (Lepidoptera: Tortricidae): electrophysiology and morphology. *Entomol. Exp. Appl.* 24: 370-378.

**Derksen, S., M. Chatterton, R. Gries, M. Aurelian, G. Judd, and G. Gries. 2007.** Semiochemical-mediated oviposition behavior by female peachtree borer, *Synanthedon exitiosa*. *Entomol. Exp. Applic.* 123: 101-108.

**Dickler, V. E. 1976.** Zur Biologie und Schadwirkung von *Synanthedon myopaeformis* Brkh. (Lepid., Aegeriidae), einem neuen Schädling in Apfeldichtpflanzungen. *Z. Angew. Entomol.* 82: 259-266.

**Dickler, V. E. and K. Hofmann. 1974.** Zur Massenaufreten des Apfelbaumglasflügers *Synanthedon myopaeformis* Brkh., Lepid. aegeriid., in Apfeldichtpflanzungen: Negative Auswirkung von Kulturmassnahmen. *Nachrichtenbl. Dtsch. Pflanzenschutzd.* 26: 52-54.

**Ebbinghaus, D., P. M. Lösel, M. Lindemann, J. Scherckenbeck, and C. P. W. Zebitz. 1998.** Detection of major and minor sex pheromone components by the male codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). *J. Insect Physiol.* 44: 49-58.

**Ebel, R. C., J. P. Mattheis, and D. A. Buchanan. 1995.** Drought stress of apple trees alters leaf emissions of volatile compounds. *Plant Physiol.* 93: 709-712.

- Eichlin, T. D. and W. D. Duckworth. 1988.** Sesiioidea: Sesiidae. pp. 1-176 in: R. B. Dominick et al. eds. The moths of America north of Mexico, including Greenland, fascicle 5.1. Wedge Entomological Research Foundation, Washington D.C.
- Eliason, E. A. and D. A. Potter. 2000.** Dogwood borer (Lepidoptera: Sesiidae) infestation of horned oak galls. *J. Econ. Entomol.* 93: 757-762.
- Ellisbury, M. M., W. D. Woodson, S. A. Clay, D. Malo, J. Schumacher, D. E. Clay, and C. G. Carlson. 1998.** Geostatistical characterization of the spatial distribution of adult corn rootworm (Coleoptera: Chrysomelidae) emergence. *Environ. Entomol.* 27: 910-917.
- El-Sayed, A. M., D. M. Suckling, C. H. Wearing, and J. A. Byers. 2006.** Potential of mass trapping for long-term pest management and eradication of invasive species. *J. Econ. Entomol.* 99: 1550-1564.
- Engelhardt, G. P. 1932.** Business proceedings of the eastern branch of the American Association of Economic Entomologists. *J. Econ. Entomol.* 25: 293-294.
- Engelhardt, G. P. 1946.** The North American clearwing moths of the family Aegeriidae. *U. S. National Museum Bull.* 190: 1-222.
- Farias, P. R. S., S. R. Roberto, J. R. S. Lopes, and D. Perecin. 2004.** Geostatistical characterization of the spatial distribution of *Xylella fastidiosa* sharpshooter vectors on citrus. *Neotropical Entomol.* 33: 13-20.
- Forschler, B. T. and G. L. Nordin. 1989.** Techniques for rearing the wood borers *Prionoxystus robiniae* (Lepidoptera: Cossidae) and *Paranthrene dollii* (Lepidoptera: Sesiidae). *Fla. Entomol.* 72: 224-226.
- Foster, S. P. and M. O. Harris. 1997.** Behavioral manipulation methods for insect pest-management. *Annu. Rev. Entomol.* 42: 123-146.
- Frank, D. L., A. Zhang, T. C. Leskey, and J. C. Bergh. 2007.** Electrophysiological response of the dogwood borer, *Synanthedon scitula* (Lepidoptera: Sesiidae), to apple tissue volatiles. ESA Annual Meeting, Behavior and Ecology Session CD4, Abstract 0429. San Diego, CA, 9-12 December 2007.
- Frank, D. L., T. C. Leskey, and J. C. Bergh. 2009.** Post-mating behavior of female dogwood borer (Lepidoptera: Sesiidae) in apple orchards. *Environ. Entomol.* 38: 1219-1225.
- Furniss, R. L. and V. M. Carolin. 1977.** Western forest insects. U. S. Dep. Agric. Misc. Publ. No. 1339.
- Gentry, C. R. and J. M. Wells. 1982.** Evidence of an oviposition stimulant for peachtree borer. *J. Chem. Ecol.* 8: 1125-1132.



- Giblin-Davis, R. M., J. E. Peña, A. C. Oehlschlager, and A. L. Perez. 1996.** Optimization of semiochemicalbased trapping of *Metamasius hemipterus sericeus* (Olivier) (Coleoptera: Curculionidae). *J. Chem. Ecol.* 22: 1389-1410.
- Greenfield, M. D. and M. G. Karandinos. 1976.** Oviposition rhythm of *Synanthedon pictipes* under a 16:8 L:D photoperiod and various thermoperiods. *Environ. Entomol.* 5: 712-713.
- Gottman, J. M. and A. K. Roy. 1990.** Sequential analysis: A guide for behavioral researchers. Cambridge Press, Cambridge, United Kingdom.
- Gut, L. J. and J. F. Brunner. 1998.** Pheromone-based management of the codling moth (Lepidoptera: Tortricidae) in Washington apple orchards. *J. Appl. Entomol.* 15: 387-405.
- Gut, L. J., P. H. McGhee, and R. Perry. 2005.** Soil mounding as a control for dogwood borer in apple. *HortSci.* 40: 2066-2070.
- Haining, R. 2003.** Spatial data analysis. Theory and practice. Cambridge University Press, Cambridge, United Kingdom.
- Hallberg, E., B. S. Hansson, and R. A. Steinbrecht. 1994.** Morphological characteristics of antennal sensilla in the European corn borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Tiss. Cell.* 26: 489-502.
- Hansson, B. S. 1995.** Olfaction in Lepidoptera. *Experientia* 51: 1003-1027.
- Hansson, B. S., A. Blackwell, E. Hallberg, and J. Löfqvist. 1995.** Physiological and morphological characteristics of the sex pheromone detecting system in male corn stemborers, *Chilo partellus* (Lepidoptera: Pyralidae). *J. Insect Physiol.* 47: 171-178.
- Hardy, R. J. 1982.** The biology and behaviour of currant borer moth, *Synanthedon tipuliformis* (Clerck) (Lepidoptera: Aegeriidae) in Tasmania. *J. Aust. Entomol. Soc.* 21: 103-109.
- Harris, M. O. and S. P. Foster. 1995.** Behavior and integration. pp: 3-46 in: R. T. Cardé and W. J. Bell, eds. *Chemical ecology of insects 2*. Chapman and Hall, New York, NY.
- Hartlieb, E. and H. Rembold. 1996.** Behavioral response of female *Helicoverpa (Heliothis) armigera* HB. (Lepidoptera: Noctuidae) moths to synthetic pigeonpea (*Cajanus cajan* L.) kairomone. *J. Chem. Ecol.* 22: 821-837.
- Hern, A. and S. Dorn. 1999.** Sexual dimorphism in the olfactory orientation of adult *Cydia pomonella* in response to  $\alpha$ -farnesene. *Entomol. Exp. Appl.* 92: 63-72.

- Hern, A. and S. Dorn. 2004.** A female-specific attractant for the codling moth, *Cydia pomonella*, from apple fruit volatiles. *Naturwissenschaften* 91: 77-80.
- Herrick, G. W. 1904.** Insects injurious to pecans. *Miss. Agric. Exp. Stn. Bull.* 86: 11-15.
- Howitt, A. H. 1993.** Common tree fruit pests. Michigan State University, North Central Regional Extension Publication 63.
- Howse, P. E., I. D. R. Steven, and O. T. Jones. 1998.** Insect pheromones and their use in pest management. Chapman and Hall, London, United Kingdom.
- Hughes, J. and S. Dorn. 2002.** Sexual differences in the flight performance of the oriental fruit moth, *Cydia molesta*. *Entomol. Exp. Appl.* 103: 171-182.
- Hunger, T. and R. A. Steinbrecht. 1998.** Functional morphology of a double-walled multiporous olfactory sensillum: the sensillum coeloconicum of *Bombyx mori* (Insecta, Lepidoptera). *Tiss. Cell.* 30: 14-29.
- Issaks, E. H. and R. M. Srisvastava. 1989.** An introduction to applied geostatistics. Oxford University Press, New York, NY.
- Jaenike, J. 1978.** On optimal oviposition behavior in phytophagous insects. *Theoretical Pop. Biol.* 14: 350-356.
- Johnson, J. M. 1993.** Flight, emergence, and oviposition patterns of the Douglas-fir pitch moth *Synanthedon novoensis* (Hy. Edwards) (Lepidoptera: Sesiidae), in western Washington. MSc. Thesis, University of Washington, Seattle, WA.
- Johnson, W. T. and H. H. Lyon. 1991.** Insects that Feed on Trees and Shrubs, 2<sup>nd</sup> edition. Comstock, Ithaca, NY.
- Kain, D. P. and R. W. Straub. 2001.** Status of borers infesting apple burr knots and their management in New York orchards. *NY Fruit Quart.* 9: 10-12.
- Kain, D. P., R. W. Straub, and A. Agnello. 2002.** Apple, evaluation of various trunk sprays to control borers infesting burr knots, 2001. *Arth. Manag. Tests* 27: A42.
- Kain, D. P., R. W. Straub, and A. M. Agnello. 2004.** Incidence and control of dogwood borer (Lepidoptera:Sesiidae) and American plum borer (Lepidoptera: Pyralidae) infesting burrknots on clonal apple rootstocks in New York. *J. Econ. Entomol.* 97: 545-552.
- Karalius, V. 1994.** Types location and morphometry of antennal sensilla of currant clearwing moth (*Synanthedon tipuliformis* Cl.) (Lepidoptera, Sesiidae). *Pheromones.* 4: 93-108.

**Kaya, H. K. and L. R. Brown. 1986.** Field application of entomogenous nematodes for biological control of clearwing moth borers in alder and sycamore trees. *J. Arboric.* 12: 150-154.

**Kemp, W. P., T. M. Kalaris, and W. F. Quimby. 1989.** Rangeland grasshopper (Orthoptera: Acrididae) spatial variability: Macroscale population assessment. *J. Econ. Entomol.* 82: 1270-1276.

**King, J. L. 1917.** The lesser peach tree borer. *Ohio Agr. Exp. Stn. Bull.* 307: 399-448.

**Kittelson, N. T. 2006.** Biology and control of the western poplar clearwing moth, *Paranthrene robiniae* (Hy. Edwards), in hybrid poplars. PhD dissertation, Washington State University, Pullman, WA.

**Knight, A. L. and D. M. Light. 2005.** Seasonal flight patterns of codling moth (Lepidoptera: Tortricidae) monitored with pear ester and codlemone-baited traps in sex pheromone-treated apple orchards. *Environ. Entomol.* 34: 1028-1035.

**Knight, A. L., P. Van Buskirk, R. Hilton, B. Zoller, and D. M. Light. 2005.** Monitoring codling moth (Lepidoptera: Tortricidae) in four cultivars of pear. *Acta Hortic.* 594: 120-125.

**Koehler, C. S., G. W. Frankie, W. S. Moore, and V. R. Landwehr. 1983.** Relationship of infestation by the sequoia pitch moth (Lepidoptera: Sesiidae) to Monterey pine trunk injury. *Environ. Entomol.* 12: 979-981.

**Koh, Y. H., K. C. Park, and K. S. Boo. 1995.** Antennal sensilla in adult, *Helicoverpa assulta* (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 88: 519-530.

**Landolt, P. J. and T. W. Phillips. 1997.** Host plant influences on sex pheromone behavior of phytophagous insects. *Annu. Rev. Entomol.* 42: 371-391.

**Leskey, T. C. and J. C. Bergh. 2002.** Rearing methods for the dogwood borer, *Synanthedon scitula* (Harris). pp: 31-32 in: P. W. Shearer, ed. Proceedings of the 77<sup>th</sup> Annual Cumberland-Shenandoah Fruit Workers Conference, Winchester, VA, 15-16 November 2001.

**Leskey, T. C. and J. C. Bergh. 2003.** A simple character for sex differentiation of pupae and pupal exuviae of the dogwood borer (Lepidoptera: Sesiidae). *Fla. Entomol.* 86: 379-381.

**Leskey, T. C. and J. C. Bergh. 2005.** Factors promoting infestation of newly planted, non-bearing apple orchards by dogwood borer, *Synanthedon scitula* Harris (Lepidoptera: Sesiidae). *J. Econ. Entomol.* 98: 2121-2132.

- Leskey, T. C. and S. E. Wright. 2004.** Monitoring plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae), populations in apple and peach orchards in the mid-Atlantic. *J. Econ. Entomol.* 97: 79-88.
- Leskey, T. C., J. C. Bergh, J. F. Walgenbach, and A. Zhang. 2006.** Attractiveness and specificity of pheromone-baited traps for male dogwood borer, *Synanthedon scitula* (Harris) (Lepidoptera: Sesiidae). *Environ. Entomol.* 35: 268-275.
- Leskey, T. C., J. C. Piñero, S. Wood, and R. J. Prokopy. 2008.** Odor baited trap trees: A potential management tool for the plum curculio. *J. Econ. Entomol.* 101: 1302-1309.
- Leskey, T. C., J. C. Bergh, J. F. Walgenbach, and A. Zhang. 2009.** Evaluation of pheromone-based management strategies for dogwood borer (Lepidoptera: Sesiidae) in commercial apple orchards. *J. Econ. Entomol.* 102:1085-1093.
- Light, D. M., A. L. Knight, C. A. Henrick, D. Rajapaska, B. Lingren, J. C. Dickens, K. M. Reynolds, R. G. Buttery, G. Merrill, J. Roitman, and B. C. Campbell. 2001.** A pear derived kairomone with pheromonal potency that attracts male and female codling moth, *Cydia pomonella* (L.). *Naturwissenschaften.* 88: 333-338.
- Liu, D., Z. Wang, B. Zhang, K. Song, X. Li, J. Li, F. Li, and H. Duan. 2006.** Spatial distribution of soil organic carbon and analysis of related factors in croplands of the black soil region, Northeast China. *Agric. Ecosys. Environ.* 113: 73-81.
- Ljungberg, H., P. Anderson, and B. S. Hansson. 1993.** Physiology and morphology of peromone-specific sensilla on the antennae of male and female *Spodoptera littoralis* (Lepidoptera: Noctuidae). *J. Insect Physiol.* 39: 253-260.
- Loughrin, M. E. 1996.** Volatile compounds from crabapple cultivars (*Malus* spp.) differing in susceptibility to the Japanese beetle (*Popillia japonica* Newman). *J. Chem. Ecol.* 22: 1295-1305.
- Marini, R. P., M. L. Parker, J. A. Barden, and C. R. Unrath. 2003.** The effect of eight dwarf rootstocks on burr knot development on 'Gala' apple trees at two locations. *J. Am. Pomol. Soc.* 57: 93-96.
- Marshall, D. W. and P. K. Andrews. 1994.** Trends in Washington state's apple industry. *HortTechnol.* 4: 1-16.
- Mayer, M. S., R. W. Mankin, and G. F. Lemire. 1984.** Quantitation of the insect electroantennogram: Measurement of sensillar contributions, elimination of background potentials and relationship to olfactory sensation. *J. Insect Physiol.* 30: 757-763.
- Mayhew, P. J. 1997.** Adaptive patterns of host-plant selection by phytophagous insects. *Oikos* 79: 417-428.

**McIver, S. B. 1975.** Structure of cuticular mechanoreceptors of arthropods. *Annu. Rev. Entomol.* 20: 381-397.

**Metcalf, R. L. and E. R. Metcalf. 1992.** Plant kairomones in insect ecology and control. Chapman and Hall, New York, NY.

**Meyer, R. H. 1982.** Biological characteristics that make the lesser peachtree borer (Lepidoptera: Sesiidae) a pest on peach trees. *Great Lakes Entomol.* 15: 189-192.

**Midgarden, D. G., R. R. Youngman, and S. J. Fleischer. 1993.** Spatial analysis of counts of western corn rootworm (Coleoptera: Chrysomelidae) adults on yellow sticky traps in corn: Geostatistics and dispersion indices. *Environ. Entomol.* 22: 1124-1133.

**Midgarden, D. G., S. J. Fleischer, R. Weisz, and Z. Smilowitz. 1997.** Site-specific integrated pest management on development of esfenvalerate resistance in Colorado potato beetle (Coleoptera: Chrysomelidae) and on densities of natural enemies. *J. Econ. Entomol.* 90: 855-867.

**Miller, L. A. and R. A. Bedding. 1982.** Field testing of the insect parasitic nematode, *Neoaplectana bibioniz* (Nematoda: Steinernematidae) against currant borer moth, *Synanthedon tipuliformis* (Lepidoptera: Sesiidae) in blackcurrants. *Entomophaga* 27: 109-114.

**Minkenber, O. P. J. M., M. Tartar, and J. A. Rosenheim. 1992.** Egg load as a major source of variability in insect foraging and oviposition behavior. *Oikos* 65: 134-142.

**Mochizuki, F., N. Sugi, and T. Shibuya. 1992.** Pheromone sensilla of the beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae). *Appl. Entomol. Zool.* 27: 547-556.

**Natale, D., L. Mattiacci, E. Pasqualini, and D. Dorn. 2004a.** Apple and peach fruit volatiles and the apple constituent butyl hexanoate attract female oriental fruit moth, *Cydia molesta*, in the laboratory. *J. Appl. Entomol.* 128: 22-27.

**Natale, D., L. Mattiacci, A. Hern, E. Pasqualini, and S. Dorn. 2004b.** Bioassay approaches to observing behavioural responses of adult female *Cydia molesta* to host plant odour. *J. Appl. Ent.* 128: 182-187.

**Neal, J. W. 1984.** Bionomics and instar determination of *Synanthedon rhododendri* (Lepidoptera: Sesiidae) on rhododendron. *Ann. Entomol. Soc. Am.* 77: 552-560.

**Nielson, D. G., F. F. Purrington, and G. F. Shanbaugh. 1979.** EAG and field responses of sesiid males to sex pheromones and related compounds. pp. 11-26 in: *Pheromones of the Sesiidae.* SEA-AR, ARR-NE-6. Washington, D. C.: USDA.

**Novak, M. A. and W. L. Roelofs. 1985.** Behavior of male redbanded leafroller moths, *Argyrotaenia velutinana* (Lepidoptera: Tortricidae), in small disruption plots. *Environ. Entomol.* 14: 12-16.

**Oehlschlager, A. C., C. M. Chinchilla, L. M. Gonzalez, F. Jiron, R. Mexzon, and B. Morgan. 1993.** Development of a pheromone-based trapping system for *Rhynchophorus palmarum* (Coleoptera: Curculionidae). *J. Econ. Entomol.* 86: 1381-1392.

**Ott, R. L. and M. Longnecker. 2001.** An introduction to statistical methods and data analysis. 5<sup>th</sup> edition. Duxbury, Thompson Learning Inc., Pacific Grove, CA.

**Park, Y. L. and J. J. Tollefson. 2005a.** Characterization of the spatial dispersion of corn root injury by corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 98: 378-383.

**Park, Y. L. and J. J. Tollefson. 2005b.** Spatial predictions of corn rootworm (Coleoptera: Chrysomelidae) adult emergence in Iowa cornfields. *J. Econ. Entomol.* 98: 121-128.

**Pearson, G. A. and C. Schal. 1999.** Electroantennogram responses of both sexes of grape root borer (Lepidoptera: Sesiidae) to synthetic female sex pheromone. *Environ. Entomol.* 28: 943-946.

**Pedigo, L. P. 1999.** Entomology and Pest Management, 3<sup>rd</sup> edition. Prentice-Hall, Inc, Upper Saddle River, NJ.

**Perry J. N. 1995.** Spatial analysis by distance indices. *Ecology.* 64: 303-314.

**Pfeiffer, D. G. (ed.). 2004.** 2004 Spray bulletin for commercial tree fruit growers. Virginia, West Virginia, and Maryland Coperative Extension. Virginia Polytechnic Institute and State University. Publication 456-419.

**Pfeiffer, D. G. (ed.) 2007.** 2007 Spray bulletin for commercial tree fruit growers. Virginia, West Virginia, and Maryland Coperative Extension. Virginia Polytechnic Institute and State University. Publication 456-419.

**Pfeiffer, D. G. (ed.) 2009.** 2009 Spray bulletin for commercial tree fruit growers. Virginia, West Virginia, and Maryland Coperative Extension. Virginia Polytechnic Institute and State University. Publication 456-419.

**Pfeiffer, D. G. and J. C. Killian. 1999.** Dogwood borer (Lepidoptera: Sesiidae) flight activity and an attempt to control damage in 'Gala' apples using mating disruption. *J. Entomol. Sci.* 34: 210-218.

- Pfeiffer, D. G., J. C. Killian, E. G. Rajotte, L. A. Hull, and J. W. Snow. 1991.** Mating disruption for reduction of damage of lesser peachtree borer (Lepidoptera: Sesiidae) in Virginia and Pennsylvania peach orchards. *J. Econ. Entomol.* 84: 218-223.
- Piechulla, B. and M. B. Pott. 2003.** Plant scents-mediators of inter- and intraorganismic communication. *Planta* 217: 687-689.
- Pierce, W. C. and C. B. Nickels. 1941.** Control of borers on recently top-worked pecan trees. *J. Econ. Entomol.* 34: 522-526.
- Pless, C. D. and W. W. Stanley. 1967.** Life history and habits of the dogwood borer, *Thamnosphesia scitula* (Lepidoptera: Aegeriidae) in Tennessee. *J. Tenn. Acad. Sci.* 42: 117-123.
- Potter, D. A. and G. M. Timmons. 1981.** Factors affecting predisposition of flowering dogwood trees to attack by the dogwood borer. *HortSci.* 16: 677-679.
- Prokopy, R. J., D. R. Papaj, J. Hendrichs, and T. T. Y. Wong. 1992.** Behavioral responses of *Ceratitis capitata* flies to bait spray droplets and natural food. *Entomol. Ex. Appl.* 64: 247-257.
- Ramaswamy, S. B. 1988.** Host finding by moths: Sensory modalities and behaviors. *J. Insect Physiol.* 34: 235-249.
- Rapparini, F., R. Barladi, and O. Facini. 2001.** Seasonal variation of monoterpenes emission from *Malus domestica* and *Prunus avium*. *Phytochem.* 57: 681-687.
- Reed, D. K. and N. J. Tromley. 1985.** *Synanthedon pictipes*. pp. 477-482 in: P. Singh and R. F. Moore, eds. *Handbook of insect rearing vol. II.* Elsevier, New York, NY.
- Reed, D. K., K. L. Mikolajczak, and C. R. Krause. 1988.** Ovipositional behavior of lesser peachtree borer in presence of host-plant volatiles. *J. Chem. Ecol.* 14: 237-252.
- Renwick, J. A. A. 1989.** Chemical ecology of oviposition in phytophagous insects. *Experientia* 45: 223-228.
- Renwick, J. A. A. and F. S. Chew. 1994.** Oviposition behavior in Lepidoptera. *Annu. Rev. Entomol.* 39: 377-400.
- Riedl, H., R. W. Weires, A. Seaman, and S. A. Hoying. 1985.** Seasonal biology and control of the dogwood borer, *Synanthedon scitula* (Lepidoptera: Sesiidae) on clonal apple rootstocks in New York. *Can. Entomol.* 117: 1367-1377.
- Robinson, T. P. and G. Metternicht. 2006.** Testing the performance of spatial interpolation techniques for mapping soil properties. *Comput. Electron. Agr.* 50: 97-108.

**Rocchini, L. A., K. J. Lewis, B. S. Lindgren, and R. G. Bennett. 1999.** Association of pitch moths (Lepidoptera: Sesiidae and Pyralidae) with rust diseases in a lodgepole pine provenance trial. *Can. J. For. Res.* 29: 1610-1614.

**Rodriguez-Saona C. and L. L. Stelinski. 2008.** Behavior-modifying strategies in IPM: Theory and practice. pp. 261-311 in: R. Peshin and A.K. Dhawan, eds. *Integrated Pest Management: Innovation-development Process*. Springer, New York, NY.

**Rogers, L. E. and J. F. Grant. 1990.** Infestation levels of dogwood borer (Lepidoptera: Sesiidae) larvae on dogwood trees in selected habitats in Tennessee. *J. Entomol. Sci.* 25: 481-485.

**Rom, R.C. 1970.** Burr knot observations on clonal apple rootstocks in Arkansas. *Fruit Var. Hort. Dig.* 24: 66-68.

**Rom, R.C. 1973.** Burr knot characteristics of six clonal apple rootstocks. *Fruit Var. J.* 27: 8-86.

**Rom, R. C. and S. A. Brown. 1979.** Factors affecting burr knot formation on clonal *Malus* rootstocks. *HortSci.* 14: 231-232.

**Rossi, R. E., D. J. Mulla, A. G. Journel, and E. H. Franz. 1992.** Geostatistical tools for modeling and interpreting ecological spatial dependence. *Ecol. Monogr.* 62: 277-314.

**Sabelis M. W., J. Takabayashi, A. Janssen, M. R. Kant, M. van Wijk, B. Sznajder, N. S. Aratchige, I. Lesna, B. Belliure, and R. C. Schuurink. 2007.** Ecology meets plant physiology: Herbivore-induced plant responses and their indirect effects on arthropod communities. pp. 188-217 in: T. Ohgushi, T. P. Craig, and P. W. Price eds. *Ecological communities: Plant mediation in indirect interaction webs*. Cambridge University Press, Cambridge, United Kingdom.

**SAS Institute. 2003.** Version 9.1 SAS institute Inc., Cary, NC.

**Schneider, D. 1957.** Elektrophysiologische Untersuchungen von chemo- und Mechanorezeptoren der Antenne des Seidenspinners *Bombyx mori* L. *Z. vergl. Physiol.* 40: 8-41.

**Schneider, D. 1964.** Insect antennae. *Annu. Rev. Entomol.* 9: 103-122.

**Schoonhoven, L. M., J. J. A. van Loon, and M. Dicke. 2005.** *Insect-plant biology*. Oxford University Press Inc., New York, NY.

**Schostzko, D. J. and L. E. O’Keeffe. 1989.** Geostatistical description of the spatial distribution of *Lygus hesperus* (Heteroptera: Miridae) in lentils. *J. Econ. Entomol.* 82: 1277-1288.



- Schotzko, D. J. and G. R. Knudsen. 1992.** Use of geostatistics to evaluate a spatial simulation of Russian wheat aphid (Homoptera: Aphididae) movement behavior on preferred and nonpreferred hosts. *Environ. Entomol.* 21: 1271-1282.
- Scutareanu, P., B. Drukker, J. Bruin, M. A. Posthumus, and M. W. Sabelis. 1997.** Volatiles from *Psylla*-infested pear trees and their possible involvement in attraction of anthocorid predators. *J. Chem. Ecol.* 23: 2241-2260.
- Sellier, R. 1977.** Étude ultramorphologique des sensilles antennaires des sésiidés essai sur l'origine et les étapes de la morphogenèse des sensilles antennaires des lépidoptères. *Ann. Soc. Entomol. Fr.* 13: 347-367.
- Shapiro-Ilan, D. I. and T. E. Cottrell. 2006.** Susceptibility of the lesser peachtree borer (Lepidoptera: Sesiidae) to entomopathogenic nematodes under laboratory conditions. *Environ. Entomol.* 35: 358-365.
- Smith, E. H. 1965.** Laboratory rearing of the peachtree borer and notes on its biology. *J. Econ. Entomol.* 58: 228-236.
- Snow, J. W., T. D. Eichlin, and J. M. Tumlinson. 1985.** Seasonal captures of clearwing moths (Sesiidae) in traps baited with various formulations of 3,13-octadecadienyl acetate and alcohol. *J. Agric. Entomol.* 2: 73-84.
- Solomon, J. D. 1975.** Biology of an ash borer, *Podosesia syringae*, in green ash in Mississippi. *Ann. Entomol. Soc. Am.* 68: 325-328.
- Solomon, J. D. 1983.** Culturing the lilac borer, *Podosesia syringae* (Harris), a serious pest of green ash. *J. Ga. Entomol. Soc.* 18: 300-303.
- Solomon, J. D. 1995.** Guide to insect borers of North American broadleaf trees and shrubs. *Agric. Handbk.* 706. Washington D.C.: U.S. Dep. Agric., Forest Service. 735 p.
- Steinbrecht, R. A. 1989.** The fine structure of thermo-/hygrosensitive sensilla in the silkworm *Bombyx mori*; receptor membrane substructure and sensory cell contacts. *Cell Tiss. Res.* 255: 49-57.
- Strother, K. O. and C. D. Steelman. 2001.** Spatial analysis of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in broiler production facilities. *Environ. Entomol.* 30: 556-561.
- Swift, F. C. 1986.** *Cytospora* canker and the establishment of lesser peachtree borer (Lepidoptera: Sesiidae) in peach trees. *J. Econ. Entomol.* 79: 537-540.
- Tasin, M., G. Anfora, C. Ioriatti, S. Carlin, A. De Cristofaro, S. Schmidt, M. Bengtsson, G. Versini, and P. Witzgall. 2005.** Antennal and behavioral responses of grapevine moth *Lobesia botrana* females to volatiles from grapevine. *J. Chem. Ecol.* 31: 77-87.

- Tasin, M., A. C. Bäckman, M. Bengtsson, C. Ioriatti, and P. Witzgall. 2006.** Essential host plant cues in the grapevine moth. *Naturwissenschaften* 93: 141–144.
- Tasin, M., A. C. Bäckman, M. Coracini, D. Casado, C. Ioriatti, and P. Witzgall. 2007.** Synergism and redundancy in a plant volatile blend attracting grapevine moth females. *Phytochem.* 68: 203-209.
- Thomson, D. R., L. J. Gut, and J. W. Jenkins. 1998.** Pheromones for insect control: Strategies and successes. pp. 385-412 in: F. R. Hall and J. Menn eds. *Biopesticides: Use and Delivery*. Jumana Press, Totowa, NJ.
- Thwaite, W. G., A. M. Mooney, M. A. Eslick, and H. L. Nichol. 2004.** Evaluating pear-derived kairomone lures for monitoring *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in Granny Smith apples under mating disruption. *Gen. Appl. Entomol.* 33: 56-60.
- Tobin, P. C. and C. W. Pitts. 2002.** Geostatistical analysis and the impact of moisture on the spatial and temporal distribution of larval *Musca domestica* (Diptera: Muscidae). *Environ. Entomol.* 31: 273-280.
- Tumlinson, J. H., C. E. Yonce, R. E. Doolittle, R. R. Heath, C. R. Gentry, and E. R. Mitchell. 1974.** Sex pheromones and reproductive isolation of the lesser peachtree borer and the peachtree borer. *Science* 185: 614-616.
- Underhill, G. W. 1935.** The pecan tree borer in dogwood. *J. Econ. Entomol.* 28: 393-396.
- Vallat, A. and S. Dorn. 2005.** Changes in volatile emissions from apple trees and associated response of adult female codling moths over the fruit-growing season. *J. Agric. Food Chem.* 53: 4083–4090.
- Vallat, A., H. Gu, and S. Dorn. 2005.** How rainfall, relative humidity and temperature influence volatile emissions from apple trees in situ. *Phytochem.* 66: 1540-1550.
- Van der Pers, J. N. C., P. L. Cuperus, and C. J. den Otter. 1980.** Distribution of sense organs on male antennae of small ermine moths, *Yponomeuta* spp. (Lepidoptera: Yponomeutidae). *Int. J. Insect Morphol. Embryol.* 9: 15-23.
- Van Steenwyk, R. A. and W. W. Barnett. 1987.** Disruption of navel orangeworm (Lepidoptera: Pyralidae) oviposition by almond-byproducts. *J. Econ. Entomol.* 80: 1291-1296.
- Viser, J. H. 1986.** Host odor perception in phytophagous insects. *Annu. Rev. Entomol.* 31: 121-144.

- Vogt, R. G., G. D. Prestwich, and M. R. Lerner. 1991.** Odorant binding protein subfamilies associate with distinct classes of olfactory receptor neurons in insects. *J. Neurobiol.* 22: 74-84.
- Wall, C. 1978.** Morphology and histology of the antenna of *Cydia nigricana* (F.) (Lepidoptera: Tortricidae). *Int. J. Insect Morphol. Embryol.* 7: 237-250.
- Wallace, P. P. 1945.** Biology and control of the dogwood borer, *Synanthedon scitula* Harris. *Conn. Exp. Stn. Bull.* 488: 373-395.
- Walton, G. S. 1986.** Association of dogwood borer with the recent decline of dogwood. *J. Arboric.* 12: 196-198.
- Warner, J. and S. Hay. 1985.** Observations, monitoring, and control of clearwing borers (Lepidoptera: Sesiidae) on apple in central Ontario. *Can. Entomol.* 117: 1471-1478.
- Wearing, C. H. and R. F. N. Hutchins. 1973.**  $\alpha$ -Farnesene, a naturally occurring oviposition stimulant for the codling moth, *Laspeyresia pomonella*. *J. Insect Physiol.* 19: 1251-1256.
- Weires, R. 1986.** Five years research and experience with control of dogwood borer and related burr knot problems. *Compact Fruit Tree.* 19: 86-89.
- Weisz, R., S. Fleischer, and Z. Smilowitz. 1995.** Site-specific integrated pest management for high value crops: Sample units for map generation using the Colorado potato beetle (Coleoptera: Chrysomelidae) as a model system. *J. Econ. Entomol.* 88: 1069-1080.
- Williams, L., D. J. Schotzko, and J. P. McCaffrey. 1992.** Geostatistical description of the spatial distribution of *Limonius californicus* (Coleoptera: Elateridae) wireworms in the northwestern United States, with comments on sampling. *Environ. Entomol.* 21: 983-995.
- Wong, T. T. Y., M. L. Cleveland, and D. G. Davis. 1969.** Sex attraction and mating of lesser peach tree borer moths. *J. Econ. Entomol.* 62: 789-792.
- Wright, R. J., T. A. Devries, L. J. Young, K. J. Jarvi, and R. C. Seymour. 2002.** Geostatistical analysis of the small-scale distribution of European corn borer (Lepidoptera: Crambidae) larvae and damage in whorl stage corn. *Environ. Entomol.* 31: 160-167.
- Yang, Z., M. Bengsston, and P. Witzgall. 2004.** Host plant volatiles synergize response to sex pheromone in codling moth, *Cydia pomonella*. *J. Chem. Ecol.* 30: 619-629.
- Young, E. and R. H. Tyler. 1983.** Burrknot control on apple. *HortSci.* 18: 921-922.

**Zhang, A., H. T. Facundo, P. S. Robbins, C. E. Linn, J. L. Hanula, M. G. Villani, and W. L. Roelofs. 1994.** Identification and synthesis of female sex pheromone of Oriental beetle, *Anomala orientalis* (Coleoptera: Scarabaeidae). *J. Chem. Ecol.* 20: 2415-2427.

**Zhang, A., C. E. Linn, S. Wright, R. Prokopy, W. Reissig, and W. L. Roelofs. 1999.** Identification of a new blend of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella*. *J. Chem. Ecol.* 25: 1221-1232.

**Zhang, A., T. C. Leskey, J. C. Bergh, and J. F. Walgenbach. 2005.** Sex pheromone of the dogwood borer, *Synanthedon scitula*. *J. Chem. Ecol.* 31: 2463-2479.