

ABSTRACT

PRITCHARD, PATRICIA MARIE. REPRODUCTIVE CAPACITY OF GRAPE ROOT BORER, *Vitacea polistiformis* (Harris), AND IMPLICATIONS FOR PHEROMONE BASED MANAGEMENT. (Under the direction of John R. Meyer.)

The increasing incidence of insect resistance to insecticides and proposed governmental restrictions of pesticides on food crops have led many fruit growers to consider the use of synthetic pheromones as a method of managing lepidopteran pests. Synthetic pheromones used in mating disruption disrupt olfactory sexual communications and are believed to result in either delayed or greatly reduced numbers of matings for a proportion of females in the population. In an effort to improve the efficiency of pest management programs utilizing synthetic pheromone for *V. polistiformis* on grapes, experiments were conducted to examine four components of this insect's biology and behavior that may be influenced as a result of the use of synthetic pheromone. The components studied were the incidence of polygyny, the effect of polygyny and delayed female mating on reproductive capacity, the evaluation of pheromone trap placement on the capture of males in a vineyard, and oviposition behaviors of gravid females in an in-field wind tunnel.

Polygyny was observed in *V. polistiformis*. Males were able to mate at least one time daily for six consecutive days. Male mating history significantly increased the duration of mating. The number of matings per male reduced female fertility but not fecundity or reproductive capacity.

Virgin female *V. polistiformis* were mated on day of eclosion or delayed to 3, 5, or 7 days after eclosion. Fecundity, fertility and reproductive capacity of females mated on day of eclosion or 3 days after eclosion were significantly greater than females mated 5 or 7 days after eclosion. Average fertility and reproductive capacity of females mating 7 days after eclosion were significantly lower than all other treatments. Delaying mating by 7 d has the potential to reduce population densities over five generations.

Male capture in pheromone traps is known to be influenced by numerous factors. The placement of pheromone traps in vineyards and wind direction were examined to determine their influence on capture frequency. The frequencies of daily per trap capture were compared among traps located in an inner and outer array, and upwind, downwind, and crosswind sectors of the vector-average wind direction in a vineyard. Trap captures were higher with traps located in an inner array compared with the array that was located near the outer edge of the vineyard. Male capture was greater in downwind traps compared with upwind or crosswind traps.

Limited information is available on cues that influence the ovipositional behavior of *V. polistiformis*. Simultaneous choice trials were conducted in a wind tunnel to determine if gravid females discriminate between a grape and non-host plant of similar appearance, and oviposit on grape with greater frequency. Most females

oviposited indiscriminately near the release point, or remained motionless. Indiscriminate maternal choice of oviposition sites may be related to the random distribution of wild grape vines, the insect's primary host, in wooded areas. Random oviposition may reduce high larval populations at any single site, and hence the probability of resource depletion and cannibalism.

**REPRODUCTIVE CAPACITY OF GRAPE ROOT BORER,
Vitacea polistiformis (Harris), AND IMPLICATIONS FOR
PHEROMONE BASED MANAGEMENT**

By

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DEDICATION

I dedicate this work to my husband, Dr. Donald Pritchard. Your deep appreciation of the science of agriculture has been an inspiration to me. My accomplishment would not have been possible without your unwavering support and countless sacrifices.

If the dream of America is to be preserved, we must not waste the genius of one mind, the strength of one body, or the spirit of one soul. We need all our people -- men and women, young and old, individuals of every race to be healthy, happy, and whole. This is our goal. We will not rest until all Americans can reach as high as their vision and God-given talents take them.

Ronald Reagan
January 26, 1984

BIOGRAPHY

Patricia Pritchard received a Bachelor of Science in Horticulture from The Ohio State University under the direction of Dr. Harry Tayama. During her senior year she conducted an independent study under the direction of Dr. Peter Fynn, Agricultural Engineer, examining the use of computerized environmental management systems by commercial greenhouse operations in Ohio. The results of the study were published in Greenhouse Grower Magazine (August, 1993) and were also presented at the annual meeting of the American Society for Horticultural Science, where she was awarded first place in the collegiate poster competition. She graduated magna cum laude in 1993.

In 1995 she received a Master of Science in Plant Pathology from Michigan State University working under the direction of Dr. Mary Hausbeck in Plant Pathology and Dr. Royal Heins in Horticulture. She conducted research on the influence of diurnal greenhouse temperatures on the postharvest susceptibility of floral crops to the fungus *Botrytis cinerea*. Her research was presented to the Society of American Florists and the American Phytopathological Society, and was published in Plant Disease. While at Michigan State University, she gained experience working in environmental controlled greenhouses on the Michigan State University campus, teaching an introductory plant pathology class of 65 students, and plant disease diagnostics. In 1994 and 1995 she gained additional

experience in plant disease diagnostics by working in the North Carolina State University Plant and Insect Diagnostic Laboratory for two-weeks each spring.

In 1995 she accepted a position as an Urban IPM Specialist, Extension Associate at North Carolina State University working with Dr. Mike Linker. Her primary responsibilities were to collaborate with faculty, public agency personnel, industry representatives, and urban pest control professionals to design and implement educational programs in the area of Urban Integrated Pest Management. She also coordinated the production of IPM programs, educational materials, and websites for North Carolina municipalities, interiorscapes, and schools, as well as collaborated with university faculty from eight southern region states to form a southern region IPM advisory group.

In 2004 she received a Ph.D. in Entomology from North Carolina State University with a minor in Math and Science Education under the direction of Dr. John Meyer. She conducted research on the reproductive capacity of grape root borer, *Vitacea polistiformis*, and implications for the use of pheromone-based management. She taught classes in biology and turf and ornamental entomology. She also conducted a study of the teaching practices of selected professors recognized as outstanding teachers at North Carolina State University.

Her organizational memberships include Phi Kappa Phi Honor Society, Gamma Sigma Delta Honor Society of Agriculture, and Pi Alpha Xi Honor Society of Floriculture.

TABLE OF CONTENTS

	PAGE
LIST OF TABLES -----	ix
LIST OF FIGURES -----	xi
LITERATURE REVIEW -----	1
Viticulture History in North America and North Carolina -----	1
History of the Grape Root Borer -----	7
Taxonomy -----	12
Grape Root Borer Research -----	13
Formulation and Dispersal of Lepidopteran Sex Pheromones -----	22
Pheromone Plumes and Mate Finding in Lepidoptera -----	27
Courtship and Female Mate Choice -----	28
Copulation, Sperm Transfer (Insemination), and Fertilization -----	30
Oviposition Behavior -----	31
Pheromone Mediated Mating and Oviposition Behaviors in Sesiidae -----	33
Pheromone Mediated Management Programs -----	35
Effects of Delayed Female Mating on the Reproductive Capacity of Lepidopteran Species -----	40
Effects of Multiple Male Mating on Reproductive Capacity of Lepidoptera -----	45
References -----	47
THE EFFECT OF POLYGyny ON THE REPRODUCTIVE CAPACITY OF GRAPE ROOT BORER <i>Vitacea polistiformis</i> (Harris), (LEPIDOPTERA: SESIIDAE) -----	65
Introduction -----	65
Materials and Methods -----	68
Results -----	71
Discussion -----	76
Acknowledgements -----	80
References -----	81

THE EFFECT OF DELAYED FEMALE MATING ON THE REPRODUCTIVE CAPACITY OF GRAPE ROOT BORER <i>Vitacea polistiformis</i> (Harris), (LEPIDOPTERA: SESIIDAE): IMPLICATIONS FOR PHEROMONE BASED MANAGEMENT STRATEGIES-----	85
--	----

Introduction -----	85
Materials and Methods -----	90
Results -----	92
Discussion -----	98
Effects of Delayed Mating on Reproductive Capacity -----	98
Effect of Body Size on Fecundity -----	102
Effect of Mating Delays on Longevity -----	103
Effect of Mating Delays on Female Behavior -----	103
Effect of Female Mating Delay on Population Growth -----	105
Implications for Pheromone Based Management -----	106
Acknowledgements -----	107
References -----	108

EVALUATION OF PHEROMONE TRAP PLACEMENT AND WIND DIRECTION ON THE CAPTURE OF MALE GRAPE ROOT BORER <i>Vitacea polistiformis</i> (Harris), (LEPIDOPTERA: SESIIDAE) IN A VINEYARD -----	114
--	-----

Introduction -----	114
Materials and Methods -----	118
Results -----	123
Discussion -----	129
Acknowledgements -----	134
References -----	134

OVIPOSITION BEHAVIORS OF GRAVID FEMALE GRAPE ROOT BORER <i>Vitacea polistiformis</i> (Harris), (LEPIDOPTERA: SESIIDAE) IN AN IN-FIELD WIND TUNNEL -----	138
---	-----

Introduction -----	138
Materials and Methods -----	142
Results -----	145
Discussion -----	149
Acknowledgements -----	151
References -----	151

CONCLUSIONS	154
-------------------	-----

LIST OF TABLES

PAGE

LITERATURE REVIEW

1. A summary of the similarities found among sesiid reproductive behaviors. ----- 34

THE EFFECT OF POLYGyny ON THE REPRODUCTIVE CAPACITY OF
GRAPE ROOT BORER *Vitacea polistiformis* (Harris), (LEPIDOPTERA:
SESIIDAE)

1. The effect of the number of matings per male on fecundity, fertility, and reproductive capacity (mean \pm SEM) on female *V. polistiformis* in 2001 and 2003. ----- 71
2. Year effect seen in *V. polistiformis* reproductive capacity, female longevity, and female body length in 2001 and 2003. ----- 72

THE EFFECT OF DELAYED FEMALE MATING ON THE REPRODUCTIVE
CAPACITY OF GRAPE ROOT BORER *Vitacea polistiformis* (Harris),
(LEPIDOPTERA: SEsiidae): IMPLICATIONS FOR PHEROMONE BASED
MANAGEMENT STRATEGIES

1. The effects (mean \pm SEM) of female age at mating on longevity, and female body length of *V. polistiformis* in 2002 and 2003. ----- 93
2. Effect of female age at mating on fecundity and fertility of various Lepidoptera.----- 100

EVALUATION OF PHEROMONE TRAP PLACEMENT AND WIND
DIRECTION ON THE CAPTURE OF MALE GRAPE ROOT BORER
Vitacea polistiformis (Harris), (LEPIDOPTERA: SESIIDAE) IN A
VINEYARD

1. Weekly per trap capture of *V. polistiformis* by array in
pheromone trap placement study from 11 August -
7 September, 2003. ----- 123
2. Average daily wind direction and capture of *V. polistiformis* in
upwind, downwind, and crosswind sector traps in pheromone
trapping study during 11 August – 7 September, 2003. ----- 125
3. Sex ratios of adult male and female *V. polistiformis* captured at
emergence by week from a research vineyard during 25 July –
28 August, 2003. ----- 129

OVIPOSITION BEHAVIORS OF GRAVID FEMALE GRAPE ROOT BORER
Vitacea polistiformis (Harris), (LEPIDOPTERA: SESIIDAE) IN AN IN-FIELD
WIND TUNNEL

1. Responses of mated female *V. polistiformis* behaviors when
released into an in-field wind tunnel in August 2002. ----- 147

LIST OF FIGURES

	PAGE
LITERATURE REVIEW	
1. (E,Z)-2,13-ODDA -----	27
2. (Z,Z)-3,13-ODDA -----	27
THE EFFECT OF POLYGyny ON THE REPRODUCTIVE CAPACITY OF GRAPE ROOT BORER <i>Vitacea polistiformis</i> (Harris), (LEPIDOPTERA: SESIIDAE)	
1. Relationship between female body length and fecundity of <i>V. polistiformis</i> in 2001 and 2003. -----	73
2. Effect of the number of matings per male on duration of mating of <i>V. polistiformis</i> in 2001. -----	74
3. Relationship between copulation duration and fertility among <i>V. polistiformis</i> males mating one or six times in 2001. -----	75
THE EFFECT OF DELAYED FEMALE MATING ON THE REPRODUCTIVE CAPACITY OF GRAPE ROOT BORER <i>Vitacea polistiformis</i> (Harris), (LEPIDOPTERA: SEsiidae): IMPLICATIONS FOR PHEROMONE BASED MANAGEMENT STRATEGIES	
1. Effect of female age at mating on fecundity and fertility (mean \pm SEM) of female <i>Vitacea polistiformis</i> in 2002 and 2003. ---	94
2. Effect of female age at mating on reproductive capacity (mean \pm SEM) of <i>Vitacea polistiformis</i> in 2002 and 2003. Reproductive capacity = (fertility x fecundity)/female body length (cm). -----	96
3. Estimated trajectories of <i>V. polistiformis</i> population growth over five generations when experiencing mating delays of 0, 3, 5, or 7 d calculated from a starting population of 100 individuals. -----	106

PAGE

EVALUATION OF PHEROMONE TRAP PLACEMENT AND WIND
DIRECTION ON THE CAPTURE OF MALE GRAPE ROOT BORER
Vitacea polistiformis (Harris), (LEPIDOPTERA: SESIIDAE) IN A
VINEYARD

1. Schematic of polygonal arrays used in pheromone trap study
11 August – 7 September, 2003. ----- 122
2. Number of days average wind direction was reported in four
different sectors by week between 11 August –
7 September, 2003. ----- 126
3. Weekly per trap capture of *V. polistiformis* in down, up, and
crosswind sectors of average wind direction for weeks between
11 August – 7 September, 2003. ----- 127
4. Daily trap capture of *V. polistiformis* males between 11 August –
7 September, 2003 in perimeter placed pheromone traps. ----- 128
5. Cumulative emergence of male and female *V. polistiformis* during
25 July – 28 August, 2003. ----- 128

OVIPOSITION BEHAVIORS OF GRAVID FEMALE GRAPE ROOT BORER
Vitacea polistiformis (Harris), (LEPIDOPTERA: SESIIDAE) IN AN IN-FIELD
WIND TUNNEL

1. In-field wind tunnel used in *V. polistiformis* oviposition behavior
study in August 2002. ----- 143
2. Compartment and fan configuration inside the in-field wind tunnel
used in *V. polistiformis* oviposition behavior study in
August 2002. ----- 143

LITERATURE REVIEW

Viticulture History in North America and North Carolina

Viticulture history in North America and North Carolina traces back to the earliest exploration of our country in the 16th century. In 1584 English explorers commissioned by Sir Walter Raleigh landed at Roanoke Island in North Carolina. They reported that the coast of the island was so full of grapes that they covered every shrub and climbed to the tops of high cedars. Raleigh's third expedition to North America in 1587 included 150 colonists. The colonists are credited with discovering the "Scuppernong mother vine" on Roanoke Island, which had a trunk 61 cm thick and covered 0.2 ha (North Carolina Department of Agriculture and Consumer Services 2003). The colonists also reportedly returned an illustration of *Papilio turnus* to England: the first documentation of insect fauna found in the New World.

There is evidence that early settlers of the new colonies (Jamestown colony, Lord Baltimore's settlement in Maryland, John Winthrop, the governor of the Massachusetts Bay Colony, and William Penn's colony in Philadelphia) all tried unsuccessfully to establish vineyards with seeds and cuttings of the European grape, *Vitis vinifera*. John Brickell (1737) was commissioned to conduct a natural history study of North Carolina. In his account he stated that a few European vines were thriving in the new colonies, but few vineyards were present at that time. Colonists were making fermented beverages from

persimmon, cedar, Indian corn, and molasses. The native grapes which Brickell referred to as fox grapes, would later be described as *Vitis rotundifolia* or muscadine grapes. They were primarily used by settlers as table grapes and for making vinegar. The native grapes were not considered by the colonists to be palatable for wine due to their strong flavor. The colonists believed the failure to cultivate European grapes in America was due to differences in climate and soil. It would be nearly 200 yr before it was discovered that insects were contributing to the failure.

Thomas Jefferson attempted to establish *Vitis vinifera* vineyards at Monticello six times from 1766-1824. In 1773 he gifted an Italian wine merchant, Filippo Mazzei, 809.7 ha of Monticello to establish a vineyard of European grapes. However, freezing temperatures, fungus, rot, and the destruction of the vineyard by Hessian soldiers during the Revolutionary War kept Jefferson from establishing these vineyards. In 1816, 50 yr after his first attempt to establish vineyards, Jefferson requested cuttings of a native American grape from Maryland, hoping that it would lead to the first successful vineyard at Monticello. Three yrs later Jefferson's attention would be drawn to North Carolina, where the American Farmer newspaper reported many farmers were producing excellent wine from the native scuppernong grapes (Ensrud 1988).

The first commercial vineyard in North Carolina, Medoc Vineyard, was established by Sydney Weller in 1829. He developed a scuppernong vineyard in Halifax county on a dilapidated 202 ha farm. By 1840 Weller had converted the farm into the largest scuppernong vineyard in the state. In 1867 the Weller winery was sold to the Garrett family. The Garretts expanded the vineyard and wine cellars during reconstruction and renamed them the Medoc Winery. In subsequent years North Carolina would become the nation's leading wine-producing state (Jones 2001).

Attempts to cultivate European grape varieties continued in the United States and in North Carolina. A North Carolina newspaper, The Raleigh Register, reported circa 1840 that a Dr. Caldwell attempted to introduce imported French vines into North Carolina. After only a few years, the plants were unhealthy and died due to unknown causes. A public debate centering around the cause of unsuccessful attempts to cultivate European grapes was building. Disagreement existed among individuals as to whether the North Carolina climate and soil were conducive to growing French grape cultivars.

In 1853, Dr. F.J. Kron of Albermarle, NC reported in the Raleigh Register that the cause of the failure to cultivate European grapes in Stanly County NC was due to an insect that was yet to be identified. Dr. Kron determined the lifecycle of the unknown insect, as well as the fact that the larvae of the insect fed upon the

roots, causing the slow decline and eventual death of the plants. Kron claimed that the native scuppernong plant was resistant to these unknown insects, as well as other pests and diseases (Kron 1854). It was later proven that scuppernong was susceptible to grape root borer damage.

Dr. Kron submitted samples of the unknown insect in its different life stages to Dr. Thaddeus Harris, a Harvard librarian, for identification and classification. Dr. Harris determined that although the insect had been previously undescribed, it had been crudely illustrated by John Abbot of Georgia in 1830, and was a suspected pest of grapes in that state. In 1854 Dr. Harris provided a scientific name (*Aegeria polistiformis*) and a description of the pest, and published the information in the Raleigh Register (Harris 1854a). In September, 1854, Harris would report on the grape vine borer to the American Pomological Society at its fifth annual meeting in Boston (Harris 1854b). It is interesting to note that the proceedings of that meeting were printed by the Franklin Printing House. Benjamin Franklin's older brother James was known to have established a printing house in Boston in the mid 1700s.

Although America could be described as a primarily agrarian society during its early years, there were very few entomological resources available to the founders. Fabricius's taxonomic publication in 1775 included limited descriptions of American insects. In 1797 John Abbot published illustrations of 103

lepidopterous insects found in Georgia, including their metamorphosis and the plants on which they fed.

Entomology became more established in American during the nineteenth century. In 1805 William Peck gave the first informal lectures on entomology at Harvard. One of his students, Dr. Thaddeus Harris, would continue Dr. Peck's teaching tradition by presenting informal entomology courses at Harvard from 1837 until 1842. In 1824 Thomas Say published a seminal work entitled American Entomology or Description of the Insects of North America, in which he described insects found in North America. In 1841 the state of Massachusetts commissioned Dr. Thaddeus Harris to publish a Report on Insects Injurious to Vegetation. It wasn't until 1853 that extensive crop loss due to insects prompted the state of New York to name Asa Fitch as its first state entomologist (Mallis 1971, United States Department of Agriculture 2003).

In 1862 forty-eight percent of the American population claimed farming as their occupation. The prominence of agriculture in the country led President Abraham Lincoln to create the U.S. Department of Agriculture. The demand for agricultural education also led to the passage of the Morrill Land Grant College Act, which established our current land grant university system (United States Department of Agriculture 2003).

Political unrest in the country over the issue of slavery resulted in the civil war during 1861-1865. Fighting in the eastern seaboard and southern states devastated this agrarian region of the country. The country would be involved in reconstruction from 1865-1877, and the emancipation of slaves would result in the replacement of the slave plantation system with sharecropping.

By the early 1900's, North Carolina had 25 operational wineries. Medoc Winery had established a national reputation and was selling North Carolina wines from Maine to California. In 1906 the success of the Medoc Winery ended when the winery was destroyed by fire. Three years later North Carolina voted to approve prohibition, which would remain in effect until 1933. Congress passed the Volstead Act in 1919, enforcing a constitutional amendment which banned the manufacture, sale or transportation of intoxicating liquors in the U.S. Prohibition put virtually all wineries in the United States out of business until its repeal in December, 1933. It was also during this period that the American banking system collapsed, resulting in the great depression and an unemployment rate of over 24% (Weinstein and Rubel 2002). The Medoc Winery, which at one time received national recognition for excellence, is today obscured by a century of forest reclamation (Jones 2001).

Although there were 13 identified wineries in North Carolina in 1947, all had closed by 1950 due to decisions at county levels regarding alcohol prohibition.

While there was a resurgence in viticulture in the 1960's, the growth was not sustained due to declining grape prices. By 1968 North Carolina still did not have any established wineries. Although the viticulture industry in North Carolina has experienced some difficult challenges in the past, the 2002 statistics from the North Carolina Department of Agriculture and Consumer Services reported that 25 wineries and 250 vineyards throughout the state have again become established and were cultivating 344 bearing hectares of grapes. In 2002 North Carolina ranked 14th nationally for total grape production (2,086 metric tons with an estimated value of 2.9 million dollars), and 12th for total wine production (2,271 kiloliters with an estimated value of 30 million dollars). North Carolina vineyards and wineries have also become an important component of the tourism industry. The Biltmore Estates Wine Company, is the largest winery in North Carolina and the most visited winery in the United States (North Carolina Department of Agriculture 2003).

History of the Grape Root Borer

Nearly 150 yr after Harris described the grape vine borer, the insect continues to cause damage to grapes in states from Ohio to Florida and as far west as Missouri and Arkansas. Over the last century entomologists have continued to acquire information about the pest's biology and reproduction, as well as effective control methods.

After identifying the grape root borer as *Aegeria polistiformis* and classifying it in the family Aegeriidae, Thaddeus Harris reported on the pest to the American Pomology Society in Boston in September (Harris 1854b). In that same year, Dr. Kron notified the U.S. Patent Office, Bureau of Agriculture, which served as a clearinghouse for agricultural information prior to the formation of the Department of Agriculture, of a new pest on grapes in North Carolina. Reporting on this new pest would likely have been one of Townsend Glover's first responsibilities after joining the Bureau in June of that year as an entomologist (Mallis 1971). Glover reported that *A. polistiformis* was observed on the wing from the middle of June through the middle of September, during which time the insect mated and laid eggs near the roots of vines. Immature stages bored into bark and wood during the summer and pupae formed within or adjacent to injured roots. No controls were recommended because the pest was so new. Because of its relationship to the peach tree borer, which had been identified by Thomas Say, recommendations given for controlling that pest were suggested until more details about the new grapevine pest were obtained. The scuppernong was reported as resistant, and grafting vines onto scuppernong rootstock was recommended (Glover 1855).

In 1856 Asa Fitch, the first state entomologist in New York, reported to the New York Agricultural Society on the grape vine borer. Although he referenced the Patent Office Report prepared by Glover, he erroneously reported the pest as

Trochilium polistiformis. He stated that the pest was destructive to both wild and cultivated grapes (Fitch 1856). During the next ten years there would be no mention of the grape vine borer in the literature while the country was involved in the Civil War. Following the civil war in 1867, Dr. Kron again submitted samples of the grape vine borer found in North Carolina to Glover. Glover again reported on the grape vine borer, but this time in his capacity as the United States Entomologist in the recently formed Department of Agriculture (Glover 1868). In his report, Glover reconfirmed the identity of the pest as *Aegeria polistiformis*, and indicated that the geographical range of the pest had spread to include Ohio, Kentucky, and Missouri. Growers were advised to examine the roots of unhealthy vines, and if the pest was present to cut out and destroy the infested roots. The scuppernong was still considered to be resistant to the pest. State entomologists in both Minnesota and Illinois would also mention the grape root borer in their annual reports. Over the next 30 yr reports of the grape root borer were primarily limited to reports from state entomologists and federal agencies. In a treatise on the cultivation of the native grape, damage by the grape root borer would be alluded to but not mentioned explicitly (Fuller 1886). Reports on the insect's biology and life cycle were brief and little information was added to that submitted by Kron before the civil war.

Missouri State Entomologist Charles V. Riley reported on that state's grape root borer population (Riley 1871). In Riley's estimation "THE" grape root borer

moth did not seem as destructive in Missouri vineyards as two beetle larvae (*Prionus laticollis* and *P. imbricornis*) referred to by Riley as gigantic root borers. Riley contributed information that further described the life cycle of the insect. Riley proposed that the life cycle was completed in one year and assumed the insect overwintered as a larva. He described the pupal stage as a spined or toothed chrysalis within a cocoon. Riley speculated the spines were used to assist the insect when working its way out of the cocoon. Adult emergence from the chrysalis occurred at the soil surface. Riley commented that soil mounding could be a possible method of control, as with the peach tree borer, but that he had not experimented with it because the insect pressure was low in Missouri. Riley's keen observational skills also led him to notice discrepancies in previous descriptions of this insect. In correspondence with LeBaron in 1850, Thaddeus Harris indicated he had captured an *Aegeria* in New England that had pectinate antenna. Riley noted, however, that Harris was not consistent with this description because he reported the insects having bipectinate antennae in specimens submitted by Kron and in his report to the American Pomological Society. Glover also received specimens from Kron but failed to describe the characteristic when he submitted his report from the patent office in 1855. Riley noted that Glover did, however, include the characteristic in his illustration of the insect.

It is interesting to note that Harris's incomplete description of the grape vine borer and his lack of attention to detail was criticized by Kron in 1853. Harris's identification of the grape root borer occurred during the last two years of his life when he suffered with pleurisy. In November, 1855 Harris was confined to his home due to his illness and died two months later (Mallis 1971).

An unusual report of injury to North Carolina grapes by the grape vine borer was published in 1873, during reconstruction, by Charles James Stewart Bethune, president of the Entomological Society of Ontario and editor of the Canadian Entomologist (Bethune 1873). Because Canada was outside of the insect's reported range, his report seems unusual until you understand that Dr. Bethune was reported to have inherited a strong sympathy for the Confederate States and an aversion for "Yankees" from his grandfather (Mallis 1971). Dr. Bethune's report was taken from the monthly Department of Agriculture report prepared by Townsend Glover in October, 1873.

For the year 1873 Glover reported on the grape root borer to both the U.S. Department of Agriculture (Glover 1873) and to the Commissioner of Agriculture (Glover 1874). In both reports he included an account of a crop loss in North Carolina of 5,000 vines of 107 varieties of French grapes.

Taxonomy

There were disagreements among taxonomists regarding the genus in which grape root borer should be classified. Both Harris (1854a) and Glover (1868) placed grape root borer in the genus *Aegeria*, while Fitch (1856) placed the insect in the genus *Trochilium*. Although Beutenmuller initially placed the insect in the genus *Sciapteron* (1890), he later placed the insect in the genus *Memythrus* (*Memythrus polistiformis*) when preparing a synopsis of food habits of larval Sesiidae (1900). Brooks (1907) also identified it as *Memythrus polistiformis*. McDunnough (1939), Brimley (1938), and Tietz (1959) placed the insect in the genus *Paranthrene*. Engelhardt (1946) published a complete taxonomic work of the Aegeriidae. In this work grape root borer was listed in a new genus, *Vitacea*, with the holotype being *Aegeria polistiformis* Harris. The currently recognized classification came from the work of Duckworth and Eichlin (1973). In their work the family Aegeriidae was renamed Sesiidae, and the type and holotype of *V. polistiformis* from Huron was identified from the United States in an area north of Mexico. In later work Duckworth and Eichlin (1977) described the genus *Vitacea* and four species in the genus. The host range of these species was restricted to plants in the family Vitaceae. Taft et al. (1991) prepared an illustrated guide of the Sesiidae, summarizing the host plant, flight period, and identified pheromone for approximately 50 of the known species in the north central United States. Taft et al. (1991) reported only one species of *Vitacea* occurring in the north central United States.

Grape Root Borer Research

Throughout the mid 1800's through the early 1900's the literature contained reports primarily on the description, biology, and occurrence of grape root borer. Management options during this time were labor intensive and representative of the use of plantation labor in agrarian societies and the pre-insecticidal era. Recommendations included vine digging and destruction of infested vines, handpicking, and soil mounding. In a manual for American grape growers, Hedrick (1919) expressed the certainty that eastern grape growers would sometime be able to grow European grapes. The grape vine root borer (*Memythrus polistiformis*) was identified as a destructive pest in West Virginia and neighboring states. Soil mounding was mentioned as a method of control, although inconsistently effective.

Adult grape root borer descriptions have been made by Harris (1854a), Riley (1871), and Brooks (1907, 1918). Descriptions of eggs were made by Brooks (1907), Sarai (1972), and Bambara and Neunzig (1977). Larval description and setal diagrams were published by MacKay (1968) and Bambara and Neunzig (1977). Pupal descriptions were made by Harris (1854a), Mosher (1969), and Bambara and Neunzig (1977).

The life history of the grape root borer was described by Packard (1889), Brooks (1907, 1918), Clark and Enns (1964), Sarai (1972), and Townsend (1980). The

first detailed record was reported by Brooks (1907) in West Virginia. Vineyard observations of adults resulted in Brooks reporting sexual dimorphism and protandry. Although more males than females were observed, Brooks did not report on the sex ratio of this insect. Female emergence occurred between 0900 - 1100 hours EDT. Differences in reported flight times by states were mentioned. Brooks estimated that the longevity of adults was not likely to exceed 10-14 d. Based on observations of larvae in roots, he suggested that the lifecycle took 2 yr for completion. Time of egg hatch was determined as 21-23 d by capturing two fertile females and allowing oviposited eggs to hatch. First-instars were determined to be capable of surviving for several days with little food or water while in search of grape roots. Two sizes of larvae were observed in roots, with no gradation in size between the classes. Larvae of the larger class emerged from the roots and pupated in mid-July, while larvae of the smaller class remained feeding and growing in the roots for the remainder of the season. Observations made after egg hatch in mid-August showed that newly hatched smaller larvae were found feeding along with larger larvae that had hatched the previous summer. Larger diameter roots were observed to be completely severed by larvae. In response to larval injury, the plant was producing a vigorous growth of young roots. The natural enemies of grape root borer were reported to be crested flycatchers and *Photuris pennsylvanica* larvae. Other methods of control suggested were swatting and cultivation.

In 1918 Brooks reported on the reproductive behavior of grape root borer. He had observed females calling and postulated that they were likely releasing a scent to attract males. Brooks observed copulation occurring during the afternoon of female emergence, and duration was 2-3 hours. Oviposition occurred between 0900 – 1600 hours the following day. Females were observed to oviposit on grape canes and leaves, grass, and weeds throughout a period of one week.

Research reports on grape root borer were absent in the literature from around 1919 through the mid 1950s, possibly as a result of World Wars I and II. In the early 1960s the heavy reliance on chemical insecticides for pest management was losing public favor due to reported problems with insect resistance and the effects of DDT on wildlife, as identified by Rachel Carson in her book Silent Spring (Carson 1962). Research efforts on grape root borer during the 1960s and 1970s were likely influenced by the movement towards integrated pest management because the majority of reports in the literature focused on identifying cultural controls, fungal and nematode biocontrol agents, and other natural enemies.

As a result of losses caused by this pest and concerns of commercial grape growers in Missouri, Clark and Enns (1964) conducted a study of grape root borer biology. Findings by Brooks (1907, 1918) were confirmed. In the Missouri

population, copulation occurred between 1230 - 1600 hours. Duration ranged from 45 min - 2 h 35 min. Adults lived an average of 7.4 d. Oviposition occurred from 1000 hours through early afternoon. The authors also determined that climatic conditions influenced emergence periods, with lack of rainfall likely resulting in shorter emergence periods. As many as 23 borers were found per vine in the crown and root system of grapevines. The existence of a sex pheromone was again suggested. Fungal pathogens, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarrhizium anisopliae* (Metchnikoff) Sorokin were identified as infecting larvae and pupae. Predation by barn swallows (*Hirundo rustica erythrogaster* Boddaert) and mockingbirds (*Mimus polyglottos polyglottos* (Linnaeus)) was also observed. Sarai (1972) confirmed the findings of Clark and Enns, and also found that mortality of newly hatched larvae increased as soil moisture decreased up to a depth of 15.2 cm.

In the late 1970's and early 1980's research focused on the development of control methods and identification of components that would lead to the development of integrated pest management programs. Dutcher and All (1978a) developed a degree day model (10° C base temperature) to predict adult emergence in Georgia. Female pupal development was determined to take 74 degree days longer than male pupal development. Emergence was significantly correlated with sugar accumulation in berries. The model developed by Dutcher and All was challenged by Webb et al. (1992) due to peak insect emergence in

Florida occurring in September while peak sugar accumulation in fruit occurred in July and August. Webb et al. (1992) speculated that emergence in Florida may be related to increasing amino acid levels in grape roots, synchronizing larval hatch with higher quality food sources. Larval host plant quality has been demonstrated to have an effect the fecundity of adult gypsy moth. Adult gypsy moths that fed as larvae on hosts with low nitrogen levels were less fecund than those reared on hosts with high nitrogen (Hough and Pimentel 1978).

Soluble nitrogen content of plant tissues vary during the year. Foliar nitrogen levels are high when leaves are growing. In the fall when leaves begin to senesce, nitrogen reserves are mobilized within the plant in preparation for leaf drop and colder temperatures. Andersen (1987) suggested that perennial plants use roots or below ground organs to store assimilate. Root herbivory can negatively affect plant sugar and carbohydrate resources in storage tissues such as roots and can alter the translocation of assimilates towards sink tissues. Reductions in subterranean herbivory have resulted in compensatory regrowth of root tissue above the site of feeding injury in some plants (Blossey and Hunt-Joshi 2003).

Dutcher and All (1978b) reported on the effects of cultural control on grape root borer survivorship. Since quantitative estimates of mortality had only been reported for first-instars (Sarai 1972), life tables were established for the life

stages of grape root borer in this study. No significant differences in survival of subterranean life stages were seen in vineyards receiving insecticides and annual tillage versus those receiving no insecticides or tillage. Mortality of the egg stage averaged 72%, while first-instar mortality was reported as 98%. Once the larvae were established in the feeding site mortality fell to 4.5%. Meyer (unpublished data) observed a 31% overall mortality in caged field-grown potted plants in North Carolina. Since there are no control methods available once an instar becomes established in a root, control measures are recommended for the egg and first-instar life stages.

Dutcher and All (1978c) examined the distribution of grape root borer larvae around grape roots in Georgia vineyards. They reported the male:female sex ratio as 0.62:1.27. Three distinct larval sizes were found throughout the season. Small larvae were distributed evenly around the grape roots, while medium and large larvae were more likely to be found in larger roots and near the base of the trunk. Brooks (1907) and Clark and Enns (1964) found two sizes of larvae in grape vine roots in West Virginia and Missouri, respectively. Sarai (1972) found three sizes of larvae in Missouri. Pupae were distributed evenly around the base of trunks and density decreased exponentially away from the trunk. Larvae were observed migrating in the soil between the roots.

The reproductive behavior was reported by Dutcher and All (1978d). In Georgia, female calling occurred between 1300 – 1800 hours EDT. Mating duration was between 1 - 4 h. Oviposition began between 800 – 900 hours the following morning and continued during the photophase for up to 8 d. Fifty-one percent of the eggs were oviposited within 24 h of mating. Male mate finding, courtship, and copulation behaviors were reported.

Dutcher and All (1979) estimated an economic injury level for grape root borer. Shoot growth and fruit yield were reduced by 90% when larvae fed on 21% of the outer circumference of the trunk base. The presence of 42 feeding sites on a root system resulted in vine death. Based on production costs and market values in 1979, Dutcher and All established an economic injury level of 0.074 larva/vine or 73 larvae/hectare. Townsend (1980) reported an approximate 50% reduction in yield in Missouri catawba vineyards when six grape root borer adults emerged per vine compared to vines with no adult emergence. He also noted a higher incidence of infestation around the perimeter of a 12-yr-old vineyard that he speculated was caused by migration of gravid females from an adjacent 30-yr-old infested vineyard.

Schmidt and All (1978) reported on an entomopathogenic nematode, *Neoaplectana carpocapsae* Weiser, an obligate parasite found in larvae of the grape root borer. Biological control studies by All et al. (1981) found that

application rates of nematodes which had been determined by lab bioassays to be infective to newly eclosed larvae, failed to infect larvae in the vineyard.

The effectiveness of foliar applied insecticides was examined by Dutcher and All (1978b). The authors found little or no effect on the number of surviving eggs and larvae when vineyards were treated weekly with methyl parathion and carbaryl. All et al. (1985) investigated the use of chlorpyrifos applied to the soil to control first-instars and found it to be effective for up to 4 wk. It was not toxic to pupae and did not inhibit adult emergence. Label restrictions permitted a single annual application no later than 35-d before harvest. However, cultivars with varying harvest times and temporal variations in grape root borer emergence in southern states made the use of this insecticide impractical for many growers.

The use of resistant root stock was investigated by Wylie and Johnson (1978) and Webb and Mortensen (1990). Wylie (1972) determined that the scuppernong vine was not resistant to grape root borer as was previously reported. Webb and Mortensen (1990) determined in a 10-yr field study that muscadine cultivars were more susceptible to grape root borer larval feeding than bunch grape rootstocks. The bunch grape, *V. shuttleworthii* House, was found to have resistance worthy of further investigation.

Painter (1951) defined plant tolerance as a type of resistance in which the plant shows an ability to grow and repair injury in spite of supporting an insect population causing damage. Plant tolerance to root herbivory is affected by stress factors experienced by the plant, such as heat and drought. Although root pruning caused by severe infestations can weaken the plant, regrowth of root tissue can compensate for damage (Blossey and Hunt-Joshi 2003). Brooks (1907) observed compensatory growth occurring in root tissue severely damaged by grape root borer larval feeding. Wylie (1968) also noted that heavily infested grapes in Arkansas were producing satisfactory crops and attributed plant response to cultural practices that permitted the plant to compensate for root damage caused by the larvae.

Johnson et al. (1991) emphasized that a control strategy applicable to all cultivars was critical to achieving control of the grape root borer. Although soil mounding reduced adult emergence, the procedure was labor intensive and not effective on muscadine grapes because of their shallow root systems. Label restrictions on chlorpyrifos limited use of the product by all growers. The identification and synthesis of a new sex pheromone for sesiid moths (Schwarz et al 1983) presented a new opportunity for control of this pest. The use of synthetic pheromones to disrupt mating showed promise in vineyard trials as a control method for grape root borer. Townsend (1990) speculated that if mating could be disrupted for two consecutive years, borer populations could be

reduced. However, sources of reinfestation would influence the success of controlling the pest.

Formulation and Dispersal of Lepidoptera Sex Pheromones

Sexual communication in Lepidoptera occurs when a member of one sex (usually female) releases an intraspecific chemical (pheromone) into the atmosphere intended to communicate to a conspecific individual of the opposite sex that they are receptive to mating. Gilbert (1976) postulated that in insects that mate shortly after emergence pheromone synthesis begins in the pupal stage. Organogenesis of pheromone glands in short-lived insects that emerge from the imaginal molt as reproductively mature adults is thought to be regulated by juvenile hormone and ecdysteroids that govern the development from immature to adult (Schal et al. 1992).

Little was understood about chemical communication among insects until 1959 when Butenandt et al. (1959) first isolated and identified the sex pheromone of the silk moth (*Bombyx mori*). Field studies using sex pheromones to control pink bollworm by disrupting mating were first conducted by Shorey et al. (1974). This study was soon followed by successful mating disruption field trials for gypsy moth *Lymantria dispar* (Carde et al. 1977), following the isolation and synthesis (Bierl et al. 1970) of disparlure.

Pheromone production in insects was first proposed by Riddiford and Williams (1971) to be under neuroendocrine control. Itagaki and Conner (1987) showed that pheromone emission in *Utetheisa ornatrix* was controlled by the brain. Pheromone synthesis in the corn earworm (*Helicoverpa zea* (Bodie)), gypsy moth (*L. dispar*) (Masler et al. 1994), and redbanded leafroller *Argyrotaenia velutinana* (Jurenka et al. 1991) have been shown to involve pheromone biosynthesis activating neuropeptide (PBAN). PBAN is synthesized in the subesophageal ganglion, stored in the corpora cardiaca (Raina and Klun 1984), and released into the hemolymph under the influence of circadian rhythms (Raina 1993). There have been reports that PBAN activity may require an intact ventral nerve cord. PBAN may be released directly from the corpora cardiaca into the hemolymph, or it may act on the terminal abdominal ganglion which innervates the pheromone gland. The pheromone gland in both *H. zea* and *H. virescens* is innervated by the terminal abdominal ganglion and pheromone production can be stimulated by electrical stimulation (Christensen et al. 1991). Teal et al. (1989) reported that nerve cord ligation of *H. zea* inhibited pheromone production, supporting neural involvement in pheromone production. However, Raina et al. (1991), Jurenka et al. (1991), and Roelofs et al. (1992) found that nerve cord ligation in *H. zea* had no effect on the action of PBAN. The pheromone gland of *Ostrinia nubilalis*, however, is not innervated (Roelofs et al. 1992).

Pheromone production and emission in females and male response to pheromone plumes have been shown to be under the control of circadian rhythms, entrained by photoperiodic cues, and influenced by exogenous factors such as temperature (Carde and Webster 1981). In insects without a pheromone reservoir, such as many Lepidoptera, pheromone is dispersed as it is synthesized.

Mating activities as well as pheromone production in *H. zea* occur during the scotophase. Raina et al. (1991) reported that pheromone production and calling in *H. zea* began at the onset of scotophase and calling was initiated (usually within 30 min) when pheromone titer reached 30 ng. Pheromone production could be induced in females during photophase when injected with PBAN, but the titer decreased rapidly after 1 h. Schal and Carde (1986) reported that female emission of pheromone in the tiger moth *Holomelina lamae* (Freeman) was controlled by an endogenous circadian clock that was entrained by the scotophase. Changes in temperature influenced the onset and mean hour of calling. The rate of evaporation and emission of pheromone varies with temperature. Gorsuch and Karandinos (1974) suggested that emergence of *Synanthedon pictipes* (G & R) was photoperiodically entrained at constant temperatures. Greenfield and Karandinos (1976a) determined that the mean time of oviposition in *S. pictipes* occurred earlier in insects held at 26.7° C day - 6.7° C night temperatures and synchronized with the photoperiod, compared to

insects held at a constant 26.9° C. The diel patterns of pheromone synthesis may result in calling and mating occurring only at certain times of the day and contribute to species isolation.

The sex pheromone of most female Lepidoptera are straight chain carbon compounds. The carbons are usually composed of a minimal number of carbons (ranging from 10-18) due to short chain compounds having a higher volatility than long chain compounds. Tumlinson (1988) speculated that the commonality of straight chain compounds could be due to the method of biosynthesis of pheromone. In many Lepidoptera *in vivo* synthesis of pheromone involves the modification of fatty acid functional groups. Pheromone diversity within the order occurs as a result of the number of carbons in the chain, the diversity of functional groups, and the number, position, and configuration (cis or trans) of the double bonds. Nielsen and Balderston (1973), however, reported on evidence of intergeneric attraction among aegeriids (sesiidae) to sex pheromones. It was later determined that pheromones were comprised of different compound blends, and the ratio of compounds in the formulation was responsible for species specificity.

The earliest work conducted on chemical analysis of sesiid sex pheromones was done when Tumlinson et al. (1974) identified the pheromones of the most economically important members of the family, the lesser peachtree borer,

Synanthedon pictipes (Grote and Robinson), and the peachtree borer, *Synanthedon exitiosa* (Say) as 18-carbon straight chain acetates, (E,Z)- and (Z,Z)-3-13-octadecadien-1-ol acetate, respectively. In 1979 the USDA Science and Education Administration organized a symposium on sesiid pheromones. In that report, pheromone baited trapping used in field studies resulted in the reported range of many sesiids being extended, as well as the identification of seasonal occurrence and the presence of sesiid genera previously unknown in certain regions. Additionally, it was recognized that the use of chemically similar sex pheromones by several species of sesiids suggested that other factors such as temporal (diel or seasonal), geographic, or host plant separation serve as mechanisms to ensure reproductive isolation of these species (Greenfield and Karandinos 1979). The extent of infestations of grape root borer in Ohio was unknown until grape root borer pheromone was available (Alm et al. 1989). Variation in seasonal occurrence of grape root borer in the Eastern United States was determined using sex pheromone baited sticky traps (Snow et al. 1991).

Brooks (1918) was the first to observe that grape root borer females seemed to be emitting a scent which almost instantly attracted males and resulted in copulation. It would be 65 yr until Schwartz et al. (1983) identified the grape root borer pheromone (E,Z)-2-13 octadecadienyl acetate (Figure 1), a new chemical structure of sesiid pheromones. Because this compound attracted only low numbers of males, Schwartz postulated that it was likely the compound did not

represent all the components of the pheromone. Snow et al. (1987) determined that a blend of 1% (Z,Z)-3,13 octadecadienyl acetate (Figure 2) to the pheromone identified by Schwartz increased the capture rate of grape root borer males by up to 50 times. In a 3 yr field trial, 1,067 (46%) of all grape root borer males trapped over the 3 yr period (2,302) were captured using the pure (E,Z)-2,13 ODDA isomer. Snow et al. (1989) identified combinations of E,Z, Z,Z, and Z isomers of 2,13 and 3,13 ODDA and ODDOH as species specific attractants for 13 different sesiids, increasing the understanding of chemotaxonomic relationships among the Sesiidae. Johnson et al. (1986, 1991) reported successful disruption of mating of grape root borer in Arkansas vineyards.



Figure 1. (E,Z)-2,13-ODDA



Figure 2. (Z,Z)-3,13-ODDA

Pheromone Plumes and Mate Finding in Lepidoptera

Pheromone emission from reproductively receptive females diffuses into the atmosphere and is circulated by air currents and eddies to conspecific males. When airborne pheromone molecules come into contact with receptor sites on male antennae, an action potential (depolarization of the dendrite membrane) results in a nerve impulse. This impulse is transmitted to the macroglomerular complex of the deutocerebrum where the information is integrated and may result in a sequence of behaviors, such as flight and orientation to the pheromone

plume. Once an impulse initiates a behavioral change, male flight typically occurs upwind in a zigzag pattern towards higher concentrations of pheromone, and ultimately to the point source, the calling female.

Courtship and Female Mate Choice

A major influence on the behavior of insects with a short life expectancy is the temporal requirements of mating. Courtship behaviors vary among Lepidoptera. Male chemical communication during courtship has been reported in several families, suggesting the existence of male pheromones associated with mating. The stimuli responsible for intromission, or permission to mate, is generally unknown.

In many insects, females often determine the acceptance or rejection of males. This concept is generally accepted because female investment in the production of gametes is greater than that of males. Female mate choice, however, is generally not well understood. Greenfield (1981) suggested that low release rates of pheromone by females represents a type of female sexual selection because females are selecting for males that are strong flyers over a great distance. Gypsy moth males can detect female pheromone up to 100 m. Male lesser peach tree borers were released and recovered from as far as 800 m downwind of synthetic pheromone baited traps (Karandinos 1974a).

The sex ratio in insect populations is typically 1:1 (male:female). Sex ratios have been shown to affect female fecundity and fertility in adult Lepidoptera. In *Heliothis virescens*, fecundity increases when the sex ratio favors females (Guerra et al. 1974). Stewart and Philogene (1983) reported that fecundity in *Manduca sexta* is optimal when the sex ratio is 1:1. However, the greatest fertility was seen at 5:1 sex ratio. The reported sex ratio of grape root borer favored males in West Virginia (Brooks 1907). Meyer (unpublished) observed that the sex ratio favored males the first year (1:0.59) and females the following year (1:1.65). Dutcher and All (1978c) reported sex ratios in populations in five different north central Georgia vineyards as 1:1 (1978b) and 1:1.60, 1:1, 1:1.78, and 1:1. The concept of sex ratio modification to reduce female fecundity and fertility is the basis of mass trapping and mating disruption.

Protandry, or male emergence prior to females, occurs in many insects that have discrete non-overlapping generations. Protandry is generally believed to be a mechanism that maximizes the number of males available for mating when females become receptive. It also minimizes female delays in mating due to mate finding. This phenomenon suggests that males have a shorter developmental time than females since both sexes are thought to grow at a similar rate. Singer (1982) proposed that protandry can only be achieved if the male sex becomes smaller, resulting in sexual dimorphism.

Copulation, Sperm Transfer (Insemination), and Fertilization

Copulation duration and frequency varies among Lepidoptera. Spermatozoa, or mature sperm, are present at eclosion in adult males that are short lived, suggesting that spermatogenesis occurs in the pupal stage (Gillott 1995). Male Lepidoptera transfer both eupyrene (nucleated) and apyrene (anucleated) sperm, as well as accessory gland secretions in a spermatophore at the time of copulation. Only eupyrene sperm are capable of fertilizing eggs. Seth et al. (2002b) reported that the testes of newly emerged adult male *S. litura* contained approximately 3,300 eupyrene sperm bundles and 10,000 apyrene sperm bundles. In several species of Lepidoptera, the release of sperm from testes has been shown to be controlled by circadian rhythms with the clock and photoreceptor located in the testis-vas deferens complex (Giebultowicz et al. 1989). Sperm is stored in the duplex until copulation occurs. Spermatophores containing sperm are formed directly in the female bursa copulatrix during copulation when the aedeagus contacts the opening of the female bursa copulatrix. The duration of mating is determined by the time required for spermatophore formation. Seth et al. (2002a) observed spermatophore formation and deposition of sperm and secretions in *S. litura* over an average 75 min mating period. Osanai et al. (1987) reported that the spermatophore of *Bombyx mori* was formed entirely within the female bursa copulatrix during copulation.

Spermatophores, which are composed of amino acids and proteins, often contain enough sperm to fertilize numerous eggs. They are produced from male accessory gland and simplex (ejaculatory duct in Lepidoptera) secretions, filled with sperm from the duplex (seminal vesicle), and deposited into the bursa copulatrix of the female at the time of mating. Sperm are released from the spermatophore within the female as a result of abrasive movements of spines within the bursa copulatrix. Spermatophores may serve as a nutrient resource for females during egg formation. After being released from the spermatophore and released into the female reproductive tract, spermatozoa are transported to the spermatheca for storage, although the manner of transport is not known. Seth et al. (2002a) reported that 30 minutes after the conclusion of mating, 82% of eupyrene sperm bundles were dissociated, and within 45 minutes after mating the bundles were detected in the spermatheca in *S. litura*. Fertilization occurs when spermatozoa enter the egg through a single micropyle as chorionated eggs pass through the median oviduct during ovulation. The micropyle of *V. polistiformis* eggs was described by Bambara and Neunzig (1977).

Oviposition Behavior

Successful insemination results in changes in female behaviors. Production and release of pheromone cease and females are no longer receptive to copulation. Reports of mating-induced termination of pheromone production and female receptivity varies in Lepidoptera species. Foster and Ayers (1995) reported on

three different substances transferred from males during mating that were responsible for changes in female mating behavior in lightbrown apple moth (*Epiphyas postvittana* (Walker)). Raina (1989) identified a factor in mated female hemolymph that was responsible for the termination of pheromone production in *H. zea*. Giebultowicz et al. (1990) reported that hemolymph factors were not responsible for postmating termination of pheromone production in *L. dispar*, but rather a neural connection of the spermatheca with the central nervous system. Kingan et al. (1993) reported that *H. zea* females ceased sex pheromone production and were unreceptive to other males immediately following the transfer of a spermatophore. Kingan et al. (1994) suggested that neural signals to the brain via the ventral nerve cord inhibited PBAN release in mated females.

To increase the fitness of their offspring, females often oviposit eggs on or near the larval host plant. Although the exact behaviors used by females to select a site for oviposition can vary by species, component behaviors include flight orientation towards a host, landing on the host, contact evaluation, and acceptance or rejection of the host. Female moths may employ olfactory, visual, and contact chemoreception methods to receive information about their ovipositional substrates. Ramaswamy (1988) reviewed the literature and found that most evaluate and prefer substrate surface textures that are rugose or rough as oviposition sites. Reed et al. (1988) reported that lesser peach tree borer

females deposited significantly more eggs on substrates that had been treated with mixtures of peach tree wood volatiles.

Pheromone Mediated Mating and Oviposition Behaviors in Sesiidae

Reports on the reproductive behavior of sesiids have been published for the peach tree borer (Smith 1970, Barry and Nielsen 1984, Gentry and Wells 1982, Jacklin and Yonce 1969) grape root borer (Dutcher and All 1978d), rhododendron borer (Neal 1984), and the lesser peach tree borer (Wong et al. 1969, Greenfield and Karandinos 1976a, 1976b, Nielsen et al. 1975, Gorsuch et al. 1975, Karandinos 1974a, 1974b), and *Carmenta haematica* (Cordo et al. 1995). Table 1. summarizes the similarities found among sesiid reproductive behaviors.

Table 1. A summary of the similarities found among sesiid reproductive behaviors.

SESIID	PROTANDRY	CALLING TIME	MATING DURATION	COURTSHIP BEHAVIOR DESCRIBED	OVIPOSITION BEHAVIOR	OVIPOSITION DURATION	AVG. FECUNDITY	MALE MATING FREQUENCY	AUTHOR
Grape Root Borer	Yes	1300-1800 hours EST	1-4 hr	Yes	0800-0900 hours EST following mating	Up to 8 d 51% eggs laid by 24 hr post mating	354	Not known	Dutcher & All (1978)
Rhododendron Borer	Yes	1000-1400 hours EST sunny days	1+ hr	Yes	Female move into canopy in pm to oviposit.	--	40 in field collected mated females	Not known	Neal (1984)
Peach Tree Borer	Yes (Jacklin & Yonce 1969)	Synchronized with changing light cycles; avg. 4 hr after emergence (Jacklin & Yonce, 1969)	1.3 hr (Gentry & Wells (1982) 1.5 hr mate 15 min After lights on at any time of day or night (Smith, 1970).	Yes (Gentry & Wells, 1982; Barry & Nielsen, 1984)	Stimulated by extracts of peach tree bark, cocoons, frass & tree gum (Gentry & Wells, 1982) Start time varied; ovipositor moved side to side, eggs laid in bark crevices (Barry & Nielsen, 1984)			2 times (Smith, 1970)	
Lesser Peach Tree Borer	Yes (Gorsuch & Karandinos, 1974)	0745-1815 hours CST Peak calling from 1045-1315 hrs CST (Gorsuch et al., 1975) 1000-1300 hrs CST	1.4 hr	Female less attractive to males as they age.	During photophase In presence of host point volatiles (Reed, et al., 1988) Ovipos. Optimal at temps between 26.9-33.6 C (Greenfield & Karandinos, 1976)	Egg laying in afternoon of day following mating (Wong et al., 1969)		3.8 times; capable of spermatophore transfer daily (Wong, et al., 1969)	

Pheromone Mediated Management Programs

Initial experiments involving the use of female sex attractants were conducted by baiting traps with live virgin females. The opportunities for utilizing behavioral modification as a management tool became possible as a result of the work of Butenandt et al. (1959). Since the mid 1960's pheromones have been identified and synthesized for numerous moth pests including codling moth, oriental fruit moth, gypsy moth, and pink bollworm. Synthetic pheromones are now available for use in monitoring pest outbreaks, determining emergence times, mass trapping, attracting and killing, and/or mating disruption management programs for many insects. There are currently over 5,000 pheromones and semiochemicals that have been identified for numerous insect orders (El-Sayed 2004). The discussion here will be limited to mass trapping and mating disruption as management tools.

The principle of mass trapping involves modification of the male:female sex ratio within a population. Removal of males in the population results in mating reductions and a decline in the population of the subsequent generation. Pest species are good candidates for mass trapping when the population occurs in relatively low densities, has limited dispersal ability, is restricted to a narrow host range, and existing control methods are not effective or are too labor intensive. Factors limiting the effectiveness of this approach are the immigration of gravid females from outside the treated area and the inability to remove a high

proportion of males in the population. The effectiveness of mass trapping is dependent on the population density of the pest, with lower densities being more effective. Knipling (1979) suggested that mass trapping may be effective against the peach tree borer due to economic damage caused by low-population densities.

The concept of eliminating male insects from a population in an effort to reduce reproduction is considered an effective approach to pest management in theory. However, the reported results of mass trapping have been mixed. Mass trapping efforts in moth pests have been reported in *Spodoptera littoralis*, cocoa pod borer (Beever et al. 1993), redbanded leafroller (*Argyrotaenia velutinana* (Walker)), and grape berry moth (*Paralobesia viteana* (Clemens)) (Taschenberg et al. 1974). Wong et al. (1972) reported on mass trapping of male lesser peachtree borer.

The effect of mass trapping on the Cocoa Pod Borer (*Conopomorpha cramerella*), using pheromone baited mass traps, resulted in a small residual population of males and a daily emerging male/female population with a 1:1 sex ratio (Beever et al. 1993). The authors estimated that the effect of early male removal from the population resulted in the remaining males having to mate six times to maintain the population density. A corresponding reduction in cocoa pod damage was observed and attributed to a reduction in female fecundity as a result of delayed or prevented mating of females in the mass trapped area.

While pheromone baited mass trapping systems were able to have an effect on fecundity, a residual population existed that could not be reduced further. Roelofs et al. (1970) reported that in the redbanded leaf roller, a high proportion of males would have to be captured before reductions in female fecundity would occur. Granett (1974) discussed the impact of male capture in gypsy moth as a function of the potential number of females a male could mate with and the population density. Determination of optimal trap density and placement is also an important factor in successful pheromone based management programs.

The principle of mating disruption is to create confusion in males during flight orientation towards calling females as a result of competing synthetic and natural pheromone plumes. Sanders (1997) postulated that mating disruption results in a reduction of the response of chemoreceptors on the antenna (adaptation) and a reduction in response to continuous signals presented to the insect central nervous system (habituation). Pearson and Meyer (1996) reported that in mating disruption trials of *V. polistiformis* using either 254 or 508 rope or rubber septa dispensers per hectare, releasing either a 99:1 blend of (EZ)- 2-13 ODDA : (ZZ)- 3,13 ODDA or 100% (ZZ)-3,13 ODDA significantly reduced female ability to attract males. Females in the treated vineyards called significantly longer than control females. Although interruption of mate location by disrupting pheromone communication can result in delays in female mating, it should be considered that it does not completely inhibit mating and some offspring should be expected.

Mating disruption success is influenced by pest density and migration. High pest populations increase the probability of mate finding, and can reduce the efficiency of synthetic pheromone sources. Meissner et al. (2001) and Borchert and Walgenbach (2000) reported greater feeding damage of tufted apple bud moth in North Carolina apple orchards using mating disruption compared with conventional insecticidal treatments when moth population densities were high. Increases in the number of virgin females due to dispersal, as well as movement of gravid females into the treated area, will also reduce the efficiency of mating disruption. Perimeter effects have been reported in numerous mating disruption studies, suggesting the likelihood of female migration to locations that could increase their chances of mating. Sauer et al. (1992) reported that pheromone density decreased sharply within a distance of 10 m in a treated vineyard. Pearson (1992) observed perimeter effects in mating disruption experiments with *V. polistiformis*. Townsend (1980) reported an increase in the number of adults emerging along the perimeter of an uninfested vineyard adjacent to a 30-yr-old infested vineyard, and concluded that the migration of gravid females caused a perimeter effect in the new vineyard.

Assessment of mating disruption efficiency has been reported using several methods. A comparison of trap captures can be made by placing traps both within and outside the treated areas. An indication of successful mating disruption occurs when trap catches within the plot are reduced by 98-100%

compared with those outside the treated area. An additional method of evaluation reported by Fadamiro and Baker (1999) in European corn borer trials is the evaluation of the mean number of spermatophores present in females. Females averaged two spermatophores in control plots, while the number present in females in plots utilizing mating disruption were significantly reduced to 1.33-1.58. A reduction in the amount of crop damage caused by the pest has also been used to assess the efficiency of mating disruption.

Carde and Minks (1995) reported that mating disruption has been as successful as conventional pesticide programs in some moth pests such as pink bollworm, oriental fruit moth, lightbrown apple moth, and grape berry moth. Mating disruption in the sesiid, *Synanthedon tipuliformis*, was also reported to be successful in black currants on the South Island of New Zealand (Carde and Minks 1995). Mating disruption is widely used to control European grapevine moth (*Lobesia botrana*) and has been reported to be at least as effective as conventional insecticides (Arn and Louis 1997). Barclay and Judd (1995) developed a model for mating disruption which included the effects of male confusion, false trail following, and emigration of males prior to mating as influenced by mating disruption.

Effects of Delayed Female Mating on the Reproductive Capacity of Lepidopteran Species

The use of synthetic pheromone to manage lepidopteran pest populations has led to numerous reports in the literature examining the effects of delayed female mating and multiple male matings on the subsequent reproductive capacity of females.

Most lepidopteran females have two ovaries, each containing four ovarioles (Proshold et al. 1982, Imms 1977). Vitellogenesis, or yolk deposition in the egg, occurs in the terminal oocyte within the ovariole. Wheeler (1996) reported that Lepidoptera usually use energy reserves accumulated during the larval stage to supply energy for egg production. In moths that do not feed as adults, (*H. cecropia*, *B. mori*, *L. dispar*, and *M. sexta*) juvenile hormone does not, however, appear to be involved in vitellogenin synthesis. Many female moths mate within 24 h after eclosion (Webster and Carde 1982). Oogenesis has been reported to occur during metamorphosis and egg development (maturation) has begun at the time of adult eclosion but is not yet complete. Giebultowicz et al. (1990) reported that at eclosion >50% of gypsy moth eggs are mature, and the remaining eggs matured very rapidly. Within 48 h of eclosion, females mated and oviposited up to 80% of their total eggs within the first day after mating. Proshold et al. (1982) reported that in tobacco budworm, all potential eggs were present in females at emergence but the most developed oocyte in each ovariole

had matured only as far as vitellogenesis. Tobacco budworms required 12-18 h post emergence to reach sexual maturity, as determined by the onset of female calling. Greenfield and Karandinos (1976b) reported that in the lesser peachtree borer over 50% of visible eggs were immature on the day of emergence. Greenfield and Karandinos (1976b) reported that females required approximately 4 h after emergence before peak calling was observed. The authors concluded that oogenesis (egg maturation) may continue during adult life in *S. pictipes*, and likely was affected by temperature. Herman and Barker (1977) reported that male factors acquired at copulation may induce oogenesis (egg maturation) through hormonal effects. Bali et al. (1996) found that mating in *Helicoverpa zea* (Hubner) stimulated egg maturation and oviposition. In addition to egg maturation, pheromone production, calling, and oviposition are also processes that require significant energy consumption by females (Fadamiro and Baker 1999).

The retention of eggs, as a result of delayed mating, can be estimated by comparing reductions in fecundity among females experiencing mating delays with females mating at or near the time of emergence. Proshold et al. (1982) reported that mating had to occur early for the greatest egg production in tobacco budworm. Mated females oviposited nearly all mature eggs within 24 h after mating, and subsequent mature eggs with each following 24 h period. Proshold (1996) reported that gypsy moth females that mated within the first 2 d of

eclosion laid nearly all their eggs, compared with females that mated on day five, which retained approximately 33% of their eggs. Torres-Vila et al. (2002) reported that 87% of 16-d-old European grapevine moth females retained their eggs in anticipation of mating. Proshold (1996) postulated that as female age increased, fewer nutrients were dedicated to maturing ova or some eggs had been reabsorbed. Reabsorption of the oocytes (oosorption) occurs when females decrease their allocation of energy resources to oocyte production, or protein and lipids are reabsorbed from oocytes to maintain somatic function (Bell and Bohm 1975). Oosorption may occur at any stage of egg development.

Delays in female mating have been shown to reduce fecundity (total eggs laid), fertility (percent egg hatch) and mating success of numerous Lepidoptera species, as well as increase female longevity. Reports of reductions in the fecundity and fertility as a result of delays in female mating vary by family and age of female. Fecundity and fertility reductions due to female age have been reported in several moth species. In Noctuidae, the number of eggs was reduced approximately 50% and the percent hatch approximately 67%, respectively, in 11-d-old beet armyworm (*Spodoptera exigua* (Huber)), compared to females mating on day one (Rogers and Marti 1997). Similar reductions of 40 and 5%, respectively, were seen in 7-d-old tobacco budworm (*Heliothis virescens*) compared to females mating on day one (Proshold et al. 1982); 15 and 35%, respectively, in 5-d-old pine beauty moth (*Panolis flammea* (Denis &

Schiffermuller)) compared with females mating on the day of emergence (Leather et al. 1985); and 87 and 99%, respectively, in 8-d-old *Copitarsia consueta* (Walker) compared with females mating on day two (Rojas and Cibrian-Tovar 1994). In Tortricidae, reductions of approximately 30% in fecundity and 31% in fertility occurred in 4-d-old codling moth (*Cydia pomonella* (L.)) compared with females mating on the day of emergence (Vickers 1997); 46 and 74% reductions, respectively, in 16-d-old European grapevine moth (*Lobesia botrana*) compared with females mating on day of emergence (Torres-Vila et al. 2002); 41 and 12% reductions, respectively, in 6-d-old koa seedworm (*Cryptophelbia illepida* (Butler)) compared with females mating one day after emergence (Jones and Aihara-Sasaki 2001); and a 49% reduction in both fecundity and fertility in 10-d-old Oriental fruit moth (*Grapholita molesta* (Busck)) compared with females mating on day of emergence (Fraser and Trimble 2001). In Gelechiidae, 89 and 81% reductions, respectively, were seen in 15-d-old pink bollworm females (*Pectinophora gossypiella* (Saunders)) compared with females mating on day of emergence (Lingren et al. 1988). In Lymantriidae, 47 and 28% reductions, respectively, were seen in 5-d-old gypsy moths (*Lymantria dispar* (L.)) compared to females that mated on day of emergence (Proshold 1996). In Crambidae, 42 and 99.7 % reduction, respectively, were seen in 7-d-old European corn borer (*Ostrinia nubilalis*) compared with females that mated one day after emergence (Fadamiro and Baker 1999). In Pyralidae, reductions of 58% in fecundity and 83% in fertility were seen in 6-d-old *Chilo partellus* (Swinhoe) females compared

with females that mated on day of emergence (Unnithan and Paye 1991). As a result of delays in mating, reductions in fecundity and fertility serve to reduce the number of viable eggs produced by females and will impact the ensuing generation.

Female longevity generally increases with an increase in mating delays. In the reported studies of female delayed mating, the longevity of females mating on day of emergence ranged from approximately seven in *Chilo partellus* (Unnithan and Paye 1991) to 17 in beet armyworm (Rogers and Marti 1997). Females mating at least 7 d after emergence could result in a doubling of longevity. Longevity of virgin females ranged from 11-21 d, and represented a doubling of longevity in many species. Virgin females may obtain additional energy from egg reabsorption (Bell and Bohm 1975). Ramaswamy et al. (1997) hypothesized that increases in longevity for virgin females may be due to egg resorption as a result of the absence of a male-derived gonadotropic signal.

Researchers have concluded that the use of synthetic pheromones can result in significant reductions in Lepidoptera populations as a result of mating delays experienced by females. Although declines in reproductive potential have been observed in controlled studies, the ability to implement these delays in the field and cause reductions in pest numbers significant enough to reduce crop damage to an acceptable level is unknown. In crops where acceptable injury levels are

lower, supplemental pest management practices that may reduce pests to an acceptable level may be required. Pest behavior modifications in response to synthetic pheromones are also not fully understood.

Effects of Multiple Male Mating on Reproductive Capacity of Lepidoptera

Male mating success is measured as the capacity to transfer an appropriate number of sperm to the female bursa copulatrix during copulation. In Lepidoptera, both eupyrene (nucleated) and apyrene (anucleated) sperm are produced and transferred to females along with accessory gland secretions in a spermatophore. Only eupyrene sperm are capable of fertilizing eggs.

Gamete formation in Lepidoptera occurs during metamorphosis. Sperm descent into the duplex has been shown to be under the influence of a circadian rhythm and is synchronized with female pheromone release. The presence of eupyrene sperm bundles in the duplex is an indication of a male's mating readiness. Seth et al. (2002b) reported that the presence of sperm and secretions in the duplex of *S. litura* were an indicator of male mating readiness. Sperm are produced daily and move into the duplex at the end of a male's daily period of receptivity to female sex pheromone where they are held for copulation the following day (Giebultowicz et al. 1989). Thorson and Riemann (1977) found that sperm release and movement in the Mediterranean flour moth occurred daily at the end of the male's receptivity period to female sex pheromone.

The consequences of multiple male matings reported in the literature include a decrease in spermatophore size as mating frequency increases, and a loss in the ability to change a female's mating behavior to "mated" status. Multiple male matings have been shown to decrease spermatophore size in European corn borer (*Ostrinia nubilalis*) (Royer and McNeil 1993), *Plodia interpunctella* (Hubner) (Cook 1999), and turnip moth (*Agrotis segetum* (Schiff.)) (Svensson et al. 1998). The transfer of eupyrene sperm has been reported to decrease in tobacco budworm *H. virescens* (F) (Henneberry and Clayton 1984) and the monarch butterfly (Oberhauser 1989) as the number of male matings increased. Female alfalfa caterpillars, *Colias eurythene* Boisduval, that mated with males that had previously mated within 1 h, showed both reduced reproductive output and longevity. Additionally, male spermatophores were shown to be reduced in size (Rutowski et al. 1987).

Seth et al. (2002b) reported that the number of sperm bundles descending into the duplex in *S. litura* was not affected by previous matings, and concluded that the negative effects of multiple matings could be due to a delayed recovery of secretions from the simplex (ejaculatory duct), accessory gland, and vas deferens associated with spermatophore formation.

Despite all the reported consequences, male mating frequency has not been shown to negatively affect female reproductive fitness in *H. virescens*, the

monarch butterfly, or the cabbage looper, *Trichoplusia ni* (Hubner). However, Hou and Shang (1999) reported that male mating frequency did affect the fecundity of *H. armigera*. Foster and Ayers (1995) reported that in lightbrown apple moth (*Epiphyas postvittana* (Walker)), 54% of females that mated with males that had a previous history of seven matings, showed a fertility of zero, as compared with only 6% of females having zero fertility when mating with males that had mated only one time previously. Seth et al. (2002a) reported that lack of eupyrene sperm in the spermatheca resulted in zero fertility in *S. litura*.

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THE EFFECT OF POLYGyny ON THE REPRODUCTIVE CAPACITY OF GRAPE ROOT BORER *Vitacea polistiformis* (Harris), (LEPIDOPTERA: SESIIDAE)

Introduction

Diurnal moths in the family Sesiidae are serious pests of cucurbit crops, fruit trees, forest trees, and ornamentals (Taft et al. 1991). Although the reproductive behaviors of sesiids have been reported by Barry & Nielsen (1984), Wong et al. (1969), Gorsuch et al. (1975), Neal (1984), and Cordo et al. (1995), little has been reported on the reproductive biology of these moths. Wong et al. (1969) reported on the frequency of male and female lesser peach tree borer (*Synanthedon pictipes* (Grote and Robinson)) matings, and the ability of aged females to attract males. Dutcher and All (1978) reported on reproductive behaviors of *V. polistiformis*, identifying females as monandrous. The number of times males are capable of mating, however, has never been described in the literature. In a review of published evidence, Ridley (1988) stated that in species where females are normally monandrous, males typically transfer enough sperm at a single mating to fertilize all the female's eggs. This would suggest that multiple copulations by males may not affect the reproductive potential of females.

Current pest management recommendations for grape root borer are limited. Soil mounding is labor intensive and may damage shallow muscadine grape roots.

Chemical control of *V. polistiformis* is currently limited to chlorpyrifos (Lorsban, Dow AgroSciences LLC). Preharvest label restrictions make applications against early hatching larva difficult due to varying harvest dates of different cultivars (Johnson et al 1991). Grape producers face the restriction or elimination of this product as a result of the Food Quality Protection Act of 1996 (United States Environmental Protection Agency, 1996). The identification and synthesis of *V. polistiformis* sex pheromone (Schwartz et al. 1983, Snow et al. 1987) led to efforts to use mating disruption in vineyards (Johnson et al. 1986, 1991), which resulted in a 92.7% reduction of pupal skins over a 2-yr period (life cycle) compared with untreated vineyards. The use of pheromone-based pest management may result in delayed female mating and/or multiple matings per male.

Polygyny, serial copulation of a male with more than one female, is common in Lepidoptera species. As the use of synthetic pheromone based management programs has increased, there has been an increasing number of reports in the literature on the effects of polygyny on the subsequent reproductive capacity of numerous female moth species. Reports, however, differ with regard to the effects of multiple male copulations on female reproductive potential. Male Lepidoptera transfer a spermatophore to the female bursa copulatrix during copulation. This structure contains eupyrene (nucleated) and apyrene (anucleated) sperm, as well as accessory gland secretions, although only

eupyrene sperm are capable of fertilizing eggs. Seth et al. (2002a) reported that both mating status and male age influenced sperm transfer to spermatophores and consequent fertility in *Spodoptera litura*. Henneberry and Clayton (1984) reported no significant difference in the fertility or fecundity of *Heliothis virescens* females when mated with previously mated males. However, the authors later determined that there was a decrease in the number of mated females (from 100-34%) that had eupyrene sperm in the spermathecae after mating as male mating increased from one to eight times (Henneberry and Clayton 1985). The number of viable eggs was reduced when 1- or 2-d-old female moths mated with 6-d-old males. In turnip moths (*Agrotis segetum*), (Svensson et al. 1998) reported a negative correlation between the number of times a male had previously mated and both spermatophore size and the number of fertile eggs. Foster and Ayers (1996) reported a reduction in the number of fertile eggs and an increase in the proportion of infertile matings in light brown apple moths (*Epiphyas postvittana* (Walker)) as the number of times a male had mated increased. Cook (1999) reported the number of sperm and mass of spermatophores in *Plodia interpunctella* decreased with successive matings. However, females that received a single small spermatophore containing fewer sperm were still able to fertilize all their eggs.

The objectives of this study were to determine if polygyny occurs in *V. polistiformis*, and if multiple copulations would have an effect on the reproductive

capacity and behavior of female *V. polistiformis*. This information could increase the understanding of the effect of pheromone based management programs on the reproductive biology of *V. polistiformis*, and result in improved efficiency of control.

Materials and Methods

In 2001 newly eclosed 2-yr-old male and female *V. polistiformis* adults were collected daily (31 July–23 August) from infested 4-yr-old grape vines (*Vitaceae labrusca* ‘Concord’) potted in 33 by 38 by 25 cm plastic file crates lined with nylon fabric. Insects were considered to be newly eclosed when they were captured as they emerged from their pupal skins, or after they had emerged but before their wings were fully inflated. Pupal skins were recovered from the soil for each insect. Vines were located at the North Carolina State University Lake Wheeler Horticultural Field Laboratory in Raleigh, North Carolina, and housed in outdoor cages covered with mosquito netting to prevent natural infestation by feral grape root borer populations. In 2003 newly eclosed 1- and 2-yr-old males and females were collected (14 August–26 August) from a 21-yr-old muscadine and bunch grape research vineyard at the North Carolina State University Lake Wheeler Field Laboratory in Raleigh, North Carolina. In both years, insects were collected between 0900 - 1200 hours EDT.

Individual adults were placed in a 946 ml plastic holding container (Ziploc Brand) with a grape leaf. Small holes in the lid allowed circulation of air in the container. Containers with insects were kept in shade at ambient temperature and misted daily in the morning with distilled water to simulate dew.

Newly eclosed male *V. polistiformis* were permitted to mate once daily with newly eclosed virgin females for up to six consecutive days. As females emerged, they were randomly assigned to male mating treatments. All matings were conducted inside a 140 by 140 by 140 cm cage covered with mosquito netting in a semi-shaded area. The cage contained a 2-yr-old grape vine (*Vitis labrusca* “Concord”) potted in a 33 by 38 by 25 cm plastic file crate lined with nylon fabric. The grape leaf from each holding container was used to transfer insects onto the potted grape plant. No more than three females were placed on the plant at one time. When a female commenced calling, a male that had previously mated from zero to five times was randomly selected and released into the cage downwind of the calling female. Observations of pheromone mediated flight, courtship, and copulation behaviors were recorded. Pairs in copula were returned to holding containers using the grape leaf and remained there until copulation was complete. In 2001 mating pairs were observed every 10 min to record copulation duration. Males were removed from the holding containers within 1h of completion of copulation. Both males and gravid females remained in holding containers at ambient temperatures in a shaded holding area until their death.

Two weeks after mating or at the time of female death, whichever occurred first, 100 eggs were randomly sampled from the container holding a mated female and placed in 14.8 ml glass vials with plastic caps. Vials were kept at ambient temperature in a shaded location. This subsample was collected to estimate percent of egg hatch (fertility). The total number of eggs laid (fecundity) and dates of male and female death were recorded. Male and female body lengths were measured from the anterior of the head to the posterior of the abdomen (cm) at the time of death. The percentage of sub-sampled eggs that hatched (fertility) was recorded after a minimum of 21 d following oviposition. The number of viable eggs produced by each female was calculated by multiplying the total number of eggs laid by the percent egg hatch. The number of viable eggs was divided by female body length to standardize for female size. In this paper the term 'reproductive capacity' will be defined as the standardized number of viable eggs per female.

Data were subjected to analysis of variance using the general linear models procedure (PROC GLM) in SAS. Significantly different means were separated by the LSD t test. Linear regression analysis was performed on female body length and fecundity and number of male matings and duration of mating (SAS Institute 1999).

Results

There was no interaction between the number of matings per male and years for fecundity, fertility, or reproductive capacity permitting all treatment means to be pooled over years. The number of matings per male significantly affected female fertility ($F = 2.51$; $df = 5,184$; $P < 0.05$), but did not affect fecundity or reproductive capacity of females (Table 1).

Table. 1. The effect of the number of matings per male on fecundity, fertility, and reproductive capacity (mean \pm SEM) on female *V. polistiformis* in 2001 and 2003.

No. Matings Per Male	n	Average Fecundity (total eggs)	Average Fertility (% egg hatch)	Reproductive Capacity (no. viable eggs/cm female body length)
1	39	508.7 \pm 22.3	90.3 \pm 1.6 a	206.9 \pm 7.6
2	37	556.6 \pm 25.2	84.9 \pm 1.7 abc	208.1 \pm 9.0
3	32	500.2 \pm 29.1	87.6 \pm 2.1 abc	198.7 \pm 10.2
4	33	528.4 \pm 28.3	81.6 \pm 2.9 c	189.5 \pm 10.4
5	29	511.9 \pm 27.9	87.7 \pm 2.1 ab	205.3 \pm 10.4
6	26	540.4 \pm 32.1	82.9 \pm 2.5 bc	197.5 \pm 11.9

Means in a column not followed by the same letters are significantly different ($P < 0.05$) based on LSD t test.

The average percentage of eggs that hatched as a result of males that mated for the first time (90%) versus males that had mated four times (82%) or six times (83%) were significantly different ($P < 0.05$). There were no significant differences at the 95% confidence level in percent egg hatch among treatments when males had mated 1, 2, 3, or 5 times (Table 1). Fertility resulting from the 4th or 5th matings were significantly different from each other ($P < 0.05$).

A significant year effect was seen in reproductive capacity ($F = 16.91$; $df = 1,184$; $P < 0.0001$), female longevity ($F = 35.77$; $df = 1,184$; $P < 0.0001$), and female body length ($F = 17.10$; $df = 1,184$; $P < 0.0001$) (Table 2).

Table 2. Year effect seen in *V. polistiformis* reproductive capacity, female longevity and female body length in 2001 and 2003.

Year	Reproductive Capacity (no. viable eggs/cm female body length)	Female Longevity (days)	Female Body Length (cm)
2001	217.8 ± 5.2	$11.7 \pm .3$	$2.14 \pm .02$
2003	186.0 ± 5.6	$14.6 \pm .4$	$2.27 \pm .02$

Although females in 2003 were significantly larger than females in 2001, fecundity did not differ significantly between years ($F = 3.66$; $df = 1,184$; $P = 0.057$). The number of times a male mated also did not have a significant effect on fecundity ($F = 0.66$; $df = 5,184$; $P = 0.654$). Female reproductive capacity was significantly greater in smaller females in 2001 compared with larger females in 2003. A significant linear relationship existed between fecundity and female body length (Figure 1) in 2001 ($y = 628.28x - 800.43$ $r^2 = 0.64$) and 2003 ($y = 529.2x - 696.2$; $r^2 = .59$), suggesting that in each year larger females laid more eggs.

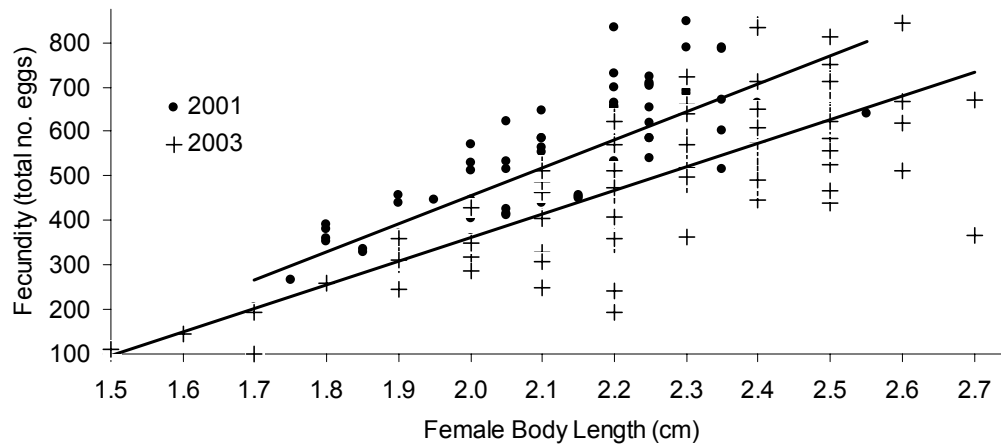


Figure 1. Relationship between female body length and fecundity of *V. polistiformis* in 2001 and 2003.

In 2001 the number of times a male had previously mated significantly affected the duration of mating ($F = 4.67$; $df = 5,89$; $P < 0.001$). The copulation duration increased linearly with the number of times a male mated ($y = 15.2x + 166.9$; $r^2 = 0.16$) (Figure 2). Copulation duration was significantly reduced among males that had not previously mated (averaged $163 \pm \text{SEM } 10.4$ min). Copulation duration among males mating for the 6th time ($260 \pm \text{SEM } 17.8$ min) was significantly greater than all other matings (Figure 2).

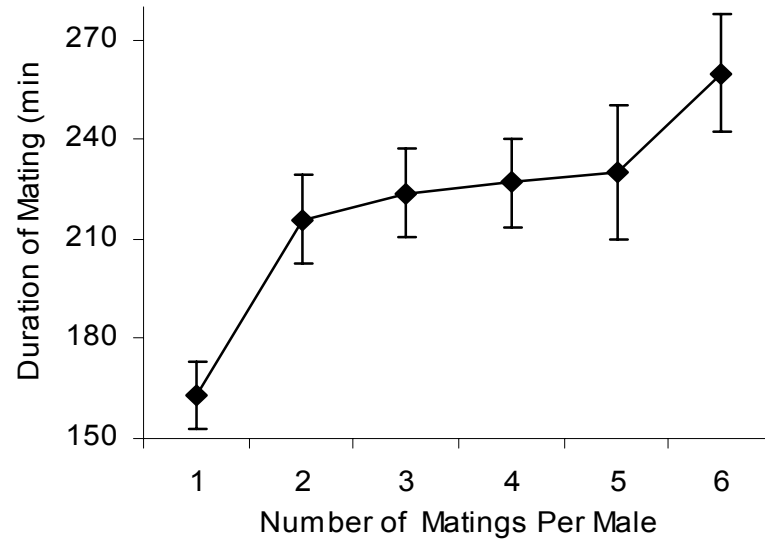


Figure 2. Effect of the number of matings per male on duration of mating of *V. polistiformis* in 2001.

Males mating for the first time had a significantly greater percentage of eggs hatch compared with males mating for the sixth time. Copulation duration was also significantly different between these groups (Figure 3).

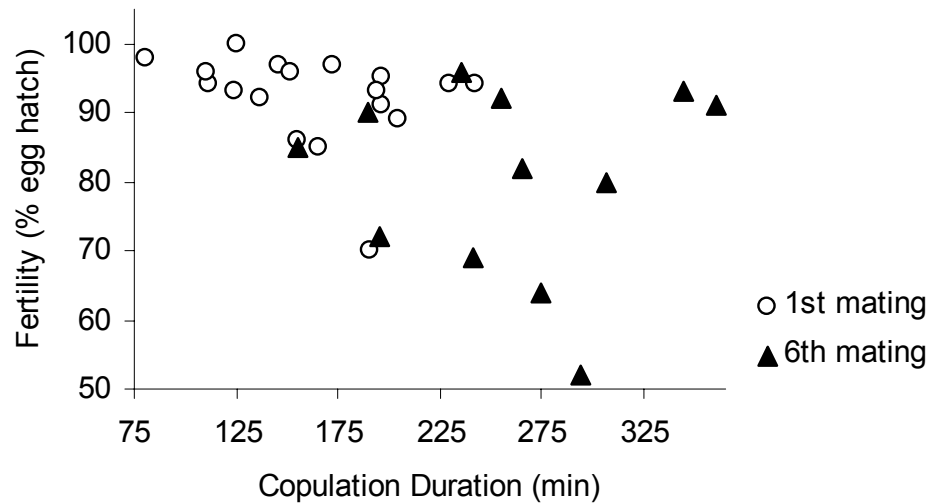


Figure 3. Relationship between copulation duration and fertility among *V. polistiformis* males mating one or six times in 2001.

Reproductive behaviors of males and females were similar to that reported by Dutcher and All (1978). In 2001 female rejection of males was observed in 3% of all matings. Rejection behaviors included physical aggression by the female towards the male using front legs. Females also repositioned themselves on the plant away from the male. Two different virgin females rejected 3-d-old virgin males but mated with males that were 5- and 12-d-old that had previously mated four and five times, respectively.

Average body length of males that mated on six consecutive days was 1.8 cm in 2001 (range from 1.5 – 1.9 cm, n=12) and 1.7 cm in 2003 (range from 1.6-1.9 cm, n=14).

Discussion

This study provides evidence that male *V. polistiformis* are capable of mating once daily for at least six consecutive days when given access to newly eclosed virgin females. It is possible that males could mate more frequently than six consecutive days. This supposition is supported by the observation that one male in 2003 mated eight times, and died attempting to mate for the ninth time. Multiple mating of the male lesser peach tree borer was reported by Wong et al. (1969). Male borers were able to mate an average of 3.8 times, and were capable of transferring a spermatophore at least daily in greenhouse studies. While there is evidence that sesiid moths are capable of consecutive matings, the occurrence of daily matings under field conditions is not known.

The reproductive capacity of *V. polistiformis* was not affected by the number of matings per male in this study. However, there was a year effect associated with reproductive capacity and female size. Although females were significantly larger in 2003, there was no significant difference in fecundity compared with females in 2001 which were smaller, suggesting that insect size, as proposed by Gilbert (1984), may not be the only factor influencing the fecundity of *V.*

polistiformis. Difference in female size between years could be attributed to cultivation methods used for grapevines, variation in the duration of life cycle completion (1-yr vs. 2-yr-old adults), or weather patterns that may have delayed emergence. Although the quantity and quality of larval food available from the two cultivation methods could have influenced the size of the adults, the effects of larval nutrition on adult body size has not been reported in sesiids. Torres-Vila et al. (1999) reported that in *Lobesia botrana*, egg viability was positively correlated with female body weight, but the number of spermatophores produced by males was not affected by body weight.

The period of time a female takes to complete development and emerge may also contribute to fecundity. In caged field studies conducted in North Carolina, Meyer (unpublished data) determined that the duration of the *V. polistiformis* life cycle can be 1 or 2 yr. In 2001 insects were taken from plants whose root growth was restricted to a 33 by 38 by 25 cm crate and a known age of 2 yr (Meyer, unpublished data), while insects in 2003 were taken from vines in a 21-yr-old vineyard and presumed to be both a mixture of 1- and 2-y-old.

In August 2003 adult emergence was influenced for several days by above normal rainfall (24.64 cm) for the month. Daily emergence during 3-7 August averaged 15.4 individuals. On 8 August the vineyard received 6.35 cm of rain, saturating the ground. Average daily emergence during 8-12 August was

reduced to 6.2 individuals. During 13-17 August, after the soil had dried, daily emergence nearly tripled to an average of 17.8 individuals. Female *V. polistiformis* experiencing delayed emergence during 8-12 August would have required energy for somatic maintenance during this time, and could have obtained it by reabsorbing nutrients from eggs, reducing the number of viable eggs and thus reproductive capacity observed in 2003.

While there was a significant difference in percentage of egg hatch among treatment groups as a result of the number of times a male mated, it was not a linear effect as might be expected, and the differences were not large enough to result in a reduction of the reproductive capacity of females. Hou and Sheng (1999) reported similar results in egg viability with *Helicoverpa armigera*, and Cook (1999) in *Plodia interpunctella*. Although polygyny has been reported to influence fertility in other Lepidoptera, the differences reported in this experiment were only approximately 10% and could be attributed to biological variation, making it difficult to conclude whether the reductions were the result of a male or female effect.

Thornhill and Alcock (1983) stated that there is considerable indirect, but almost no direct, evidence that females actually chose certain conspecific males over others under natural conditions. From a statistical standpoint, the behavioral observations in this study did not occur with enough frequency to justify statistical

analysis. However, they are worthy of mention in this document for the benefit of other researchers. It was observed that a 3 d-old virgin male was rejected by two virgin females, but the females mated with older males that had previously mated 4 or 5 times. The mechanism by which females detect male age and sperm availability is not known. Royer and McNeil (1993) proposed that female rejection of males may be influenced by a close range male sex pheromone, which may provide information regarding age and mating history. Bebas and Cymborowski (1999) compared the number of eupyrene sperm bundles in 1- versus 10-day-old *S. littoralis*, and found a reduction in the number of sperm in the older insects.

Average male body length was similar in both years (1.8 cm (SEM \pm 0.04) in 2001 and 1.7 cm (SEM \pm 0.03) in 2003). One individual in 2003 that was smaller (1.4 cm) than the average mated successfully eight times. The body size of this male did not appear to influence a female's choice to mate because female rejection of this individual was not observed. The fertility resulting from the eight matings ranged from 71 to 92%.

The results of this experiment suggest that in *V. polistiformis* there is an increase in copulation duration and a decrease in female fertility when males mate for six consecutive days. Seth et al. (2002b) reported similar results with *S. litura*, and determined that the number of eupyrene sperm transferred to females by 2-d-old

males mating for the second time was reduced by 77% compared with 1-d-old males mating for the first time. Fertility, however, was reduced by only 27%. In 2001, four matings (4.2% of total matings) resulted in zero fertility, one of which was the 6th mating of a male in which copulation occurred for 5 h 36 min. In 2003 there was only one mating that resulted in zero fertility. This was also a 6th mating and copulation lasted for only 1h.

If pheromone based management programs result in an increase in the occurrence of polygyny, these results suggest that this factor will not have as great an effect on reducing the reproductive capacity of *V. polistiformis* as does delayed female matings, which will be discussed in the following chapter. Beevor et al (1993) speculated that polygyny would have to occur in mass trapped cocoa pod borers to maintain the population density. However, since pod damage decreased steadily, it was concluded that polygyny could not occur at the frequency required in the mass trapped area. Identification of methods that could decrease eupyrene sperm numbers to a level that would result in reduced female reproductive capacity are desirable.

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THE EFFECT OF DELAYED FEMALE MATING ON THE REPRODUCTIVE CAPACITY OF GRAPE ROOT BORER *Vitacea polistiformis* (Harris), (LEPIDOPTERA: SESIIDAE): IMPLICATIONS FOR PHEROMONE BASED MANAGEMENT STRATEGIES

Introduction

Diurnal moths in the family Sesiidae are serious pests of cucurbit crops, fruit orchards, forest trees, and ornamentals (Taft et al. 1991). Adults reportedly do not feed and their longevity ranges from 2-10 d (Cordo et al. 1995, Clark and Enns 1964, Wong et al. 1969). Larval development occurs inside roots and woody plant tissue and can take as long as 2 yr, making management of this damaging life stage difficult once it becomes established inside the plant.

The behavioral responses of sesiids to sex pheromone have been reported in the literature. Barry & Nielsen (1984) reported on the peachtree borer (*Synanthedon exitiosa* (Say)), Wong et al. (1969), and Gorsuch et al. (1975) on the lesser peachtree borer (*S. pictipes* (G. & R.)), and Neal (1984) on the rhododendron borer (*S. rhododendri* (Beutenmuller)). Cordo et al. (1995) reported on the biology of *Carmenta haematica* (Ureta) in Argentina. The primary component of the sex pheromone of Sesiidae has been identified as 3,13-octadecadien-1-ol acetate or alcohol. Intergeneric attraction to the pheromone has been reported (Pfeiffer and Killian 1999, Snow et al. 1989, 1991), but temporal, geographical, seasonal and diel mechanisms are likely to contribute to reproductive isolation (Greenfield and Karandinos 1979).

The grape root borer, *Vitacea polistiformis* (Harris), is regarded as an important sessile pest of all commercially grown grapes, including the European grape (*Vitus vinifera*), the fox grape (*V. labrusca*), and muscadine grapes (*V. rotundifolia*). The pest is distributed in the United States east of the Mississippi River as far north as Michigan and south to Florida. In 2003 the United States viticulture industry produced approximately 5.9 million metric tons of grapes with a value of 26 million dollars (United States Department of Agriculture 2004). In 2002 North Carolina ranked 14th in the U.S. for grape production and acreage, producing approximately 2,086 metric tons of grapes, valued at 2.9 million dollars, and 2,271 kiloliters of wine valued at 30 million dollars (North Carolina Department of Agriculture and Consumer Services 2002). Despite its importance as a viticulture pest, economic losses from *V. polistiformis* have not been reported in the literature.

Adult *V. polistiformis* eclosion, as reported by Snow et al. (1991), occurs over a 2-3 mo period in northern states beginning in June or early July. Eclosion begins in late July in the central states, and in August in the lower southern states where flight can occur for up to 6 mo. Dutcher and All (1978b) reported that the female pupal stadium is 74⁰ DD (Base =10⁰ C) greater than that of the male, supporting the occurrence of protandry. Peak male flight occurs in August in the South and late July in the North (Snow et al. 1991). Calling has been reported between 1230-1600 hours CST in Missouri (Clark & Enns 1964) and 1300-1800 hours

EST in Georgia (Dutcher and All 1978d) on the day of eclosion. Copulation time ranges from 1-4 h (Dutcher and All 1978a). Females oviposit eggs on weeds, grass, fence posts, and grape plants. Neonates burrow into the soil in search of grape roots. Larval feeding occurs in the root cortex and the crown of grapes for up to 24 mo. Pupation requires 30-45 d and occurs within 5 cm of the soil surface.

Dutcher and All (1978a) reported on the calling, mating, and oviposition behavior of laboratory reared *V. polistiformis* females. Female *V. polistiformis* calling occurred daily during the photophase (1400-1800 hours), beginning on the day of eclosion. Females are reportedly monandrous. Wong et al. (1969) reported single spermatophores in dissections of mated *S. pictipes* females. Polygyny in *V. polistiformis* was demonstrated in the previous chapter.

Although a variety of cultural, chemical, and biological control strategies have been examined for this pest, most have resulted in limited effectiveness (Olien et al. 1993). Chemical control is currently limited to chlorpyrifos (Lorsban, Dow AgroSciences LLC). Label restrictions include a 35 d pre-harvest interval. This can be problematic for growers in southern states since harvest dates vary by grape cultivars, but often coincide with peak egg hatch. The Food Quality Protection Act of 1996 proposes the restriction or elimination of chlorpyrifos on

food crops, which could leave grape producers with no chemical control options (United States Environmental Protection Agency 1996).

The use of synthetic pheromone for mating disruption and mass trapping has been reported as a management strategy for numerous lepidopteran pests of field and fruit crops. Results have varied due to pest species, pest density, distance from untreated areas, and movement of gravid females into the treated areas. Although mass trapping may reduce population growth, damage to high-value commercial crops may still exceed acceptable levels. Mating disruption is widely used for the European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae) in French vineyards, and has been reported to be at least as effective as chemical controls (Arn & Louis 1997). Charmillot et al. (1995) reported that both male capture and matings of tethered female *L. botrana* were reduced by more than 90% inside the vineyard when compared with perimeter pheromone baited dispensers.

Schwarz et al. (1983) identified the *V. polistiformis* sex pheromone as (E,Z)-2-13 octadecadienyl acetate (ODDA). Snow et al. (1987) determined that a 99:1 blend of (E,Z)-2,12 ODDA to (Z,Z)-3,13 ODDA increased male capture by up to 50 times. Snow et al. (1989) reported trapping various sesiids using pheromone baited Pherocon sticky traps. The study resulted in a capture of over 2,300 male *V. polistiformis* over a three year period in locations without regard to the

presence of vineyards or wild grapes. Johnson et al. (1991) reported that *V. polistiformis* matings were reduced, as measured by a reduction in pupal skins, by 93% in vineyards treated with (Z,Z)-3,13-ODDA and 66% in those treated with (E,Z)-2,13-ODDA.

Increasing the effectiveness of synthetic pheromones as a management strategy for *V. polistiformis* requires a better understanding of how mating delays can influence the reproductive potential of this insect. In most holometabolous insects the quantity and quality of food ingested during larval stages determines the amount of energy available for reproduction and somatic maintenance in adult insects (Boggs 1981). Lepidoptera that are short-lived and do not feed as adults are thought to utilize reproductive energy reserves when mating is delayed. Torres-Vila et al. (2002) summarized the literature examining the effects of delayed female mating on fecundity, fertility, mating success, oviposition periods, and longevity in numerous lepidopteran species. Reports on the effects of female age at mating on fecundity and fertility among sesiids are limited. Wong et al. (1969) reported that aging of virgin female lesser peach tree borers, *S. pictipes* (Grote & Robinson), reduced their ability to attract males.

The objective of this study was to determine the effects of delayed mating on the reproductive capacity and behavior of female *V. polistiformis* that may result from the use of pheromone based management. It was hypothesized that delayed

female mating would result in a negative effect on the reproductive capacity of *V. polistiformis* by reducing the fertility and fecundity of females.

Materials and Methods

Newly eclosed female and male *V. polistiformis* adults were collected from 2 August - 19 August 2002 and from 25 July - 17 August 2003 from a 21-yr-old muscadine and bunch grape research vineyard located at the North Carolina State University Lake Wheeler Field Laboratory in Raleigh, North Carolina between 0900-1200 hours EDT. Insects were considered to be newly eclosed when they were captured as they emerged from their pupal skins, or after they had emerged but before their wings were fully inflated. Pupal skins were recovered from the soil for each insect. Individual adults were placed in a 946 ml plastic holding container (Ziploc Brand) with a grape leaf. Small holes in the container lid allowed circulation of air in the container. Containers were kept in shade at ambient temperature and misted daily in the morning with distilled water to simulate dew. Each day female *V. polistiformis* were randomly assigned to one of five treatments: non-mated, mated on day one (day of eclosion), and mated on day three, five, or seven after eclosion. Because it was shown in the previous chapter that male mating history did not have a significant effect on reproductive capacity of females, males were randomly assigned to mating treatments.

All matings were conducted in a 140 by 140 by 140 cm galvanized-pipe framed cage covered with mosquito netting in a semi-shaded area. The cage contained a 2-yr-old grape vine (*Vitis labrusca* 'Concord') potted in a 33 by 38 by 25 cm plastic file crate lined with nylon fabric. The grape leaf from each holding container was used to transfer insects onto the potted grape plant. No more than three females were placed on the plant at one time. When females commenced calling, a male was released into the cage. Observations of pheromone mediated flight, courtship, and copulation behaviors were recorded. Mating pairs were returned to holding containers using a grape leaf and remained until copulation was complete. Males were removed from the holding containers within 1 h of completion of copulation, and females were returned to a sheltered holding area.

Two weeks after mating or at time of female death, whichever occurred first, 100 eggs were randomly sampled from the container holding each mated female and divided evenly between two 14.8 ml glass vials. Vials were kept at ambient temperature in a shade location. The subsample was collected to estimate percentage of egg hatching (fertility). The total number of eggs laid (fecundity) and date of female death were recorded. Female body length was measured from the anterior of the head to the posterior of the abdomen (cm) at the time of death. The percentage of sub-sampled eggs that hatched (fertility) was recorded a minimum of 21 d after being oviposited. The number of viable eggs produced

by each female was calculated by multiplying the total number of eggs laid by the percent egg hatch. The number of viable eggs was divided by female body length to standardize for female size. In this paper the term reproductive capacity will be used to define the standardized number of viable eggs per female.

Data were subjected to analysis of variance using the general linear models (PROC GLM) procedure in SAS, and significantly different means were separated by the LSD t test (SAS Institute 1999).

Results

There was no interaction between female age at mating and years for fecundity ($F = 0.98$; $df = 3,174$; $P = 0.4057$), fertility ($F = 0.1422$; $df = 3,174$; $P = 0.1422$), or reproductive capacity ($F = 1.05$; $df = 3,174$; $P = 0.3732$) permitting all mating delay treatment means to be pooled over years. Additionally, there was no significant year effect on female age at mating for any of the factors.

Female age at mating significantly affected fecundity ($F = 5.80$; $df = 3,174$; $P < 0.0008$) and fertility ($F = 22.78$; $df = 3,174$; $P < 0.0001$) (Table 1). A mating delay of 7 d had a greater effect on fertility (31% reduction) than on fecundity (25% reduction) when compared with females mating on the day of eclosion.

Table 1. The effects (mean \pm SEM) of female age at mating on longevity, and female body length of *V. polistiformis* in 2002 and 2003.

Female Age at Mating (d)	n	Longevity (d)	Female Body Length (cm)
1	43	15.1 \pm 0.6	2.27 \pm 0.04
3	47	14.4 \pm 0.5	2.27 \pm 0.04
5	48	15.5 \pm 0.5	2.22 \pm 0.04
7	44	16.6 \pm 0.6	2.21 \pm 0.05
Unmated*	16	16.6 \pm 1.2	2.37 \pm 0.07

* Not included in statistical analysis

Both fecundity and fertility declined linearly as a result of mating delays (Figure 1). A linear contrast of the mean number of eggs laid ($F = 15.19$; $P < 0.0001$) and % egg hatch ($F = 65.68$; $P < 0.0001$) as a result of mating delays was significant. Average fecundity of unmated females (157.3 ± 13.0 SEM) was not included in the analysis because they represented a total of only 16 females and was obtained in 2003 only; hence, direct comparisons could not be made with the averaged data for two years. Fertility of eggs from all unmated females was zero.

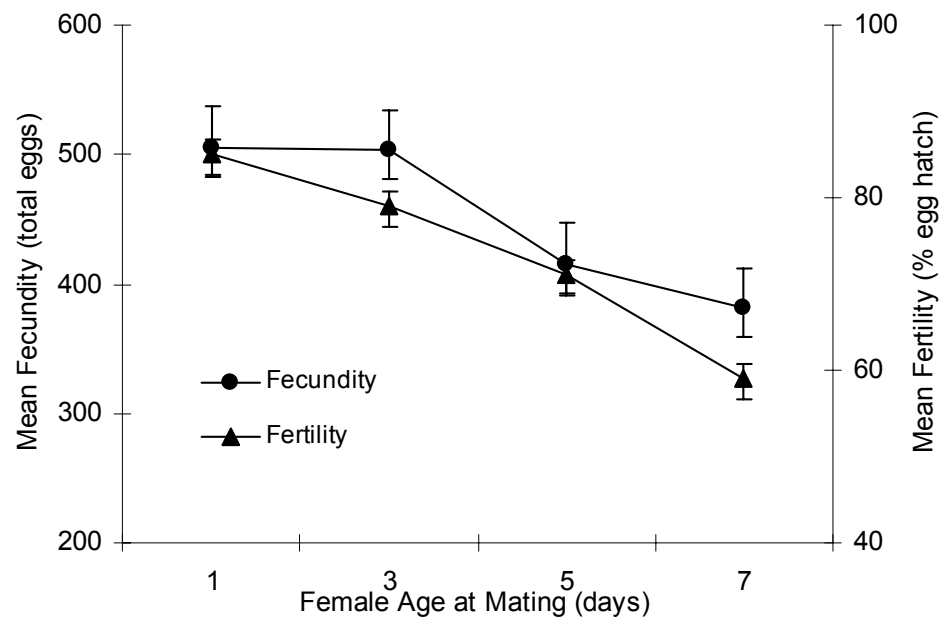


Figure 1. Effect of female age at mating on fecundity and fertility (mean \pm SEM) of female *Vitacea polistiformis* in 2002 and 2003.

Female age at mating did not have a significant affect on longevity ($F = 2.53$; $df = 3,174$; $P = 0.0586$) (Table 1). The average longevity was 15.4 d across both years, and ranged from 14.4 in 3-d-old mated females to 16.6 in 7-d-old mated females. Longevity of unmated virgin females was 16.6 d and was similar to that of females mated 7 d after eclosion.

There were no significant differences in female body length among mating delay treatments ($F = 0.57$; $df = 3,174$; $P = 0.6339$) or between years ($F = 0.68$; $df = 1,174$; $P = 0.4104$). Average female length was $2.23 \pm \text{SEM } 0.03$ cm in 2002 and $2.26 \pm \text{SEM } 0.03$ cm in 2003. The average body length of unmated females

in 2002 and 2003 was $2.03 \pm \text{SEM } 0.05$ cm and 2.37 ± 0.07 cm, respectively. Unmated females in 2002 were obtained during the last 2 wk of eclosion. They were not included in treatment comparisons because they were not randomly assigned to treatments throughout the eclosion period.

Female age at mating had a significant effect on reproductive capacity ($F = 20.68$; $df = 3,174$; $P < 0.0001$). The combined effects of fecundity and fertility reductions due to delays in female mating resulted in a 47% reduction in reproductive capacity of females mated on day seven compared with those mated on the day of eclosion. A linear contrast of the means of mating delays and reproductive capacity was significant ($F = 56.66$; $P < 0.0001$). The number of viable eggs declined from $184 \pm \text{SEM } 10.21$ in females mated on the day of eclosion to $177 \pm \text{SEM } 10.56$, $131 \pm \text{SEM } 7.90$, and $98 \pm \text{SEM } 6.45$ in females mating 3-, 5-, and 7-d-after eclosion, respectively (Figure 2). Average number of viable eggs of females mated on the day of eclosion and three days later was significantly higher than females mated 5 and 7 d after eclosion. Average number of viable eggs from females mating on day seven was significantly lower than all other groups.

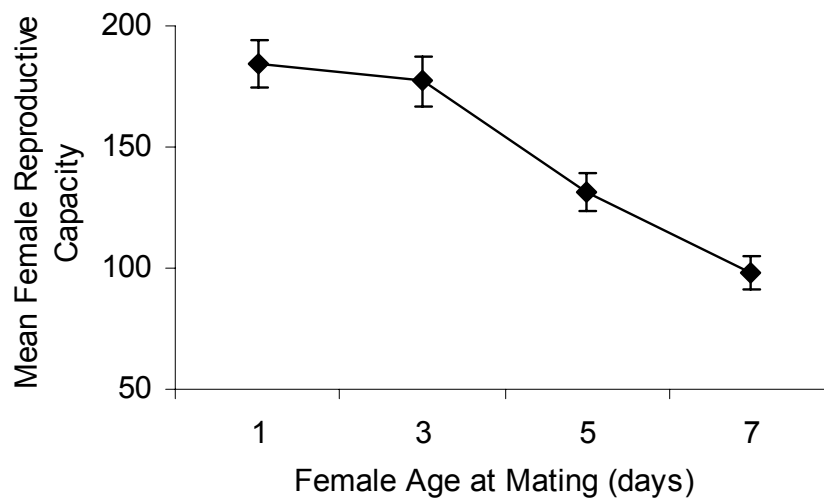


Figure 2. Effect of female age at mating on reproductive capacity (mean \pm SEM) of *Vitacea polistiformis* in 2002 and 2003. Reproductive capacity = (fertility \times fecundity)/female body length (cm).

In 2002, 9.7% of mated females (11 individuals) across all treatments had zero fertility. The average fecundity of these eleven females was 280.6 (\pm 41.8 SEM) eggs, with a range of 32-443 eggs per female. These individuals were not included in the analysis. In 2003 there were no individuals with zero fecundity.

Observations of mating behaviors were similar to that reported by Dutcher and All (1978). Mate rejection was observed in both females and males. Females were observed to physically reject males by striking them with their tarsi and relocating away from the males. Males were less likely to mate with 5- and 7-d-old females. Opportunistic calling was observed in aged females when they were in close proximity to younger calling females. When aged females were near

young females that had attracted males, aged females began calling in response to male presence.

Visual cues appeared to play a role in mate finding by males. Males oriented their flight towards dark contrast areas on the grape plant. Other behavior modifications observed as a result of mating delays during this 2 yr study included females mating for a second time and females calling after mating. Across both years a small percentage (2.7%) of the females called after mating. In 2002, a single female in the 7-d-old mated treatment group called after mating and mated a second time. The second mating occurred 9 d after the first mating. From the first mating, this female laid 377 eggs and 78% hatched. After the second mating, the female laid 16 eggs and 56% hatched. Also in 2002, a virgin female in the 7-d-old treatment group oviposited numerous eggs prior to mating. Although this individual did not begin calling until 1900 hours, she successfully mated and laid a total of 749 eggs. However, only 31% of the eggs hatched. In 2003 three females were observed calling after copulation. Following the completion of a 2 h 20 min copulation, a 5-d-old female began calling for a second time. Males responded to her pheromone release, but once they came into close proximity they did not mate with her.

Regardless of the age of females at mating, calling occurred between 1300-1730 hours. Temperature at time of mating ranged from 22 - 35⁰ C. Aging virgin

females that were not calling were observed to commence calling when a male came in close proximity to them. Newly eclosed virgin males were more likely to mate with aged virgin females than were the older virgin males. Weather effects on mating behaviors were also observed. Interestingly, all females were observed to call simultaneously regardless of their age prior to afternoon thunder storms.

Discussion

Effects of delayed mating on reproductive capacity

The results of these experiments support the hypothesis that increased female age at mating negatively affects the reproductive capacity of *V. polistiformis*, which has also been observed with numerous other Lepidoptera (Table 2). Torres-Vila et al. (2002) suggested that reduced fecundity as a consequence of delayed mating is due to the attenuated production of oocytes and/or the reabsorption of eggs to meet energy needs. Oosorption can occur in previtellogenic or vitellogenic oocytes as well as chorionated eggs. Lepidoptera typically use energy reserves accumulated during larval development to supply energy for egg production (Wheeler 1996). Many moths, including *V. polistiformis*, have a short, non-feeding adult stage and mate soon after eclosion. It is likely that energy reserves from larval nutrition are being partitioned in these insects for both somatic and reproductive purposes. Pheromone production, calling, and oviposition also require significant energy consumption by females

(Fadamiro and Baker 1999). Wong et al. (1969) reported that 1-d-old female lesser peach tree borer were most attractive to males, and their ability to attract males declined as they aged. The reduction estimates reported from our experiments were obtained from females confined to containers. In the field, flight associated with oviposition would likely have an additional influence on these estimates due to increased energy consumption.

Table 2. Effect of female age at mating on fecundity and fertility of various Lepidoptera.

Family	% Fecundity Reduction	Reduction in % Fertility	Female Age at Mating
<i>Noctuidae</i>			
Beet Armyworm (1)	50	67	1-d vs. 11-d-old
Tobacco budworm (2)	40	5	1-d vs. 7-d-old
Pine beauty moth (3)	15	35	Day of eclosion vs. 5-d-old
<i>Copitarsia consueta</i> (4)	87	99	2-d vs. 8-d-old
<i>Tortricidae</i>			
Codling moth (5)	30	31	Day of eclosion vs. 4-d-old
European grapevine moth (6)	46	74	Day of eclosion vs. 16-d-old
Koa seedworm (7)	41	12	1-d-old vs. 6-d-old
Oriental fruit moth (8)	49	49	Day of eclosion vs. 10-d-old
<i>Gelechiidae</i>			
Pink bollworm (9)	89	81	Day of eclosion vs. 15-d-old
<i>Lymantriidae</i>			
Gypsy moth (10)	47	28	Day of eclosion vs. 5-d-old
<i>Crambidae</i>			
European Corn Borer(11)	42	100	1-d-old vs. 7-d-old
<i>Pyralidae</i>			
Stem borer (12)	58	83	Day of eclosion vs. 6-d-old

(1) Rogers and Marti 1997; (2) Proshold et al 1982; (3) Leather et al. 1985; (4) Rojas and Cibrian-Tovar 1994; (5) Vickers 1997; (6) Torres-Vila et al. 2002; (7) Jones and Aihara-Sasaki 2001; (8) Fraser and Trimble 2001; (9) Lingren et al 1988; (10) Proshold 1996; (11) Fadamiro and Baker 1999; (12) Unnithan and Paye 1991.

There was no attempt to determine a relationship between weather and fecundity. Sarai (1972) and Clark and Enns (1964) did report on such a relationship. Weather data are reported here as a record of the conditions

under which the experiments were conducted. In 2002 the average daily and average daily high temperatures in July was 27⁰ C and 32.6⁰ C, respectively and 25.9⁰ C and 32.1⁰ C, respectively in August. The monthly precipitation at 2 m in July and August was 8.00 and 14.83 cm, respectively. In 2003 the average daily and average daily high temperatures in July was 26.4⁰ C and 31.5⁰ C, respectively, and 26.3⁰ C and 31.5⁰ C, respectively in August. The monthly precipitation at 2 m in July and August was 12.45 and 24.64 cm, respectively. (State Climate Office of North Carolina 2004).

There are no reports of the feeding habits of *V. polistiformis* in the literature. All adults in this study extended their proboscis and took up water when receiving daily misting of water. Several females extended their proboscis during afternoon matings. When offered water from leaf surfaces, females took up water and discontinued the behavior.

Although it is not possible to state conclusively the cause of the incidence of zero fertility in mated females in 2002, it could be attributed to the failure of males to transfer eupyrene sperm in the spermatophore during copulation. Seth et al. (2002) reported that matings of *Spodoptera litura* that resulted in zero fertility were the result of a failure to transfer eupyrene sperm. In the current study, the duration of all matings was within the range observed in fertile female matings, suggesting adequate time necessary for successful sperm transfer. Body length

of infertile females ranged from 2.1 - 2.9 cm and was comparable to fertile females, suggesting that poor larval nutrition was not a factor affecting fertility. The average age of the mated infertile females was 19 d, greater than that of both 7-d-delay and unmated virgin females. Four of the eleven infertile females lived from 22-25 d.

Effect of body size on fecundity

Body size is generally recognized as an intrinsic factor that influences fecundity, with larger females consistently laying more eggs than smaller females (Gilbert 1984). Proshold (1996) reported on work by Maksimovic that identified a strong correlation between female size and reductions in fecundity in gypsy moth, as well as other authors that have reported the effects of larval nutrition and overcrowding on gypsy moth size and fecundity. Torres-Villa et al. (2002) reported a significant positive linear correlation between the number of viable eggs and female weight in European grapevine moth, however the correlation declined as mating delays increased. Average body length of females in 2002 and 2003 were not significantly different, suggesting that all females would have nearly equal fecundity. The average body length of unmated virgin females in the 2003 study was 2.37 cm, and was similar to that of mated females (2.26 cm). Variation in female body length over the 2 yr study could be attributed to females completing their larval development in either 1 or 2 yr within the population.

Meyer (unpublished data) determined that the duration of *V. polistiformis* life cycle is 1 or 2 yr in North Carolina.

Effect of mating delays on longevity

Female longevity in Lepidoptera generally increases with an increase in mating delays. The effect of mating delay on female longevity in this study was not significant. Average longevity ranged from 14 – 17 d. Unmated females lived about the same length of time as 7-d-old females. There are few reports of longevity in the literature with which to compare the results of this study. Clark and Enns (1964) reported a longevity of 7.4 d in Missouri, Sorensen (1975) a 7 d life in North Carolina, and Brooks (1907) a 6 d life in West Virginia. Because there is no mention of how the insect were treated to obtain longevity estimates, direct comparisons cannot be made.

Effect of mating delays on female behavior

From a statistical standpoint the frequency of behavior observations in this study were not adequate to be significant, but were considered worthy of mentioning here as documentation for the benefit of other researchers. Adaptive mating behaviors were observed in a small percentage (<10%) of the females observed over the 2 yr study. Opportunistic calling, defined as aged females calling in response to male presence once a younger female had attracted a male into close range, as well as females relocating on the plant when in close proximity

to another calling female was seen among females of various ages. Whether these behaviors were a result of mating delays is unknown. Wong et al. (1969) reported that as lesser peach tree borer females aged, their ability to attract males in the field diminished, with 4- and 7-d-old females experiencing a 50 and 70% reduction in the ability to attract a male, respectively. All virgin *V. polistiformis* females continued to call regardless of mating delays. Wong et al. (1969) reported that virgin lesser peach tree borer females attracted males until death and lived longer than mated females. Torres-Villa et al. (2002) observed the same behavior in European grapevine moths.

Pheromone production and emission has been shown to be under the control of circadian rhythms, entrained by photoperiodic cues, and influenced by exogenous factors such as temperature (Carde and Webster 1981). Females experiencing mating delays did not appear to consistently alter their time of calling as a result of competition with younger females. A small number of older females were observed to commence calling proceeding the calling and mating of younger females. Opportunistic calling was observed in females experiencing 5- or 7-d mating delays. This may serve as a competitive strategy for increasing the probability of mating. This strategy also suggests that as females age they conserve pheromone and the energy required to produce and/or release it.

The behaviors of females calling after mating and mating multiple times were not anticipated. While these observations were only noted in individuals whose mating was delayed 5 or 7 d, they may occur in females of other ages. It is unknown if similar behaviors would be observed in females in field populations that experience mating delays.

Effect of female mating delay on population growth

Using estimates for reductions in fecundity and fertility due to delayed mating found in this study (Table 1), and natural mortality estimates in vineyards proposed by Dutcher and All (1978c) (77% mortality of the egg stage and 97% mortality of the first-instar), estimates of the cumulative effects of mating delays and mortality on the intrinsic rate of increase in the population can be estimated as shown in Figure 3. The intrinsic rate of increase (r) was calculated as the slope of each line. When comparing the rate of population increase for a female mated 5 d after eclosion a female mated on day of emergence, the number of viable eggs per female was reduced by approximately 31% (295 compared with 430 in females mating on the day of emergence). Assuming 295 viable eggs laid by the 5 d delayed female, a 77% egg mortality, a 97% first-instar mortality, and a 1:1 sex ratio, approximately two larvae would survive (1 male and 1 female) as a result of a successful mating. The number of larvae resulting from a female mating on the day of emergence using the same calculation procedures would be three. The population experiencing a 5 d delay would remain nearly constant

over a 60 mo period while the population experiencing no delay in mating would increase exponentially during the same period. Delaying mating by 7 d would result in a decline in the population over the 60 mo period (Figure 3).

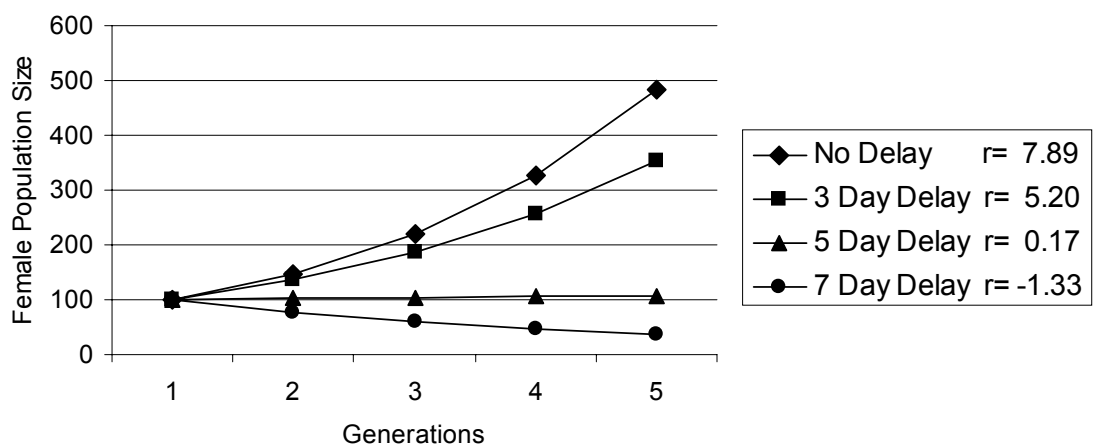


Figure 3. Estimated trajectories of *V. polistiformis* population growth over five generations when experiencing mating delays of 0, 3, 5, or 7 d. calculated from a starting population of 100 individuals.

Implications for pheromone based management

Circumstantial evidence suggests that delays of female mating have occurred in the field in codling moth using mating disruption (Vickers 1997; Knight 2000) and in the cocoa pod borer using mass trapping (Beever et al. 1993).

V. polistiformis is a good candidate for pheromone based management strategies because its host range is restricted to grapes, there is a single discrete non-overlapping generation per year, eclosion typically occurs during an identified 3

mo period, females mate only once, and protandry would presumably increase male capture rates. Although male mating frequency does not appear to influence female reproductive capacity, further research to examine the possible cumulative effects of delayed female and multiple male matings on the reproductive capacity of this insect may be warranted. The establishment of a correlation between trap catch and reduction in damage, while complicated by the subterranean nature and a 2 yr life cycle, also needs to be established.

Although female mating delays will likely not result in complete control of *V. polistiformis*, it does offer promise as a method for reducing population densities and their negative effects on plant yield. Considering the fact that growers are facing the impending loss of the only registered chemical control option for this pest, increased research in pheromone based management could result in more successful management of *V. polistiformis* and increased opportunities for viticulture production in the Eastern United States.

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EVALUATION OF PHEROMONE TRAP PLACEMENT AND WIND DIRECTION ON THE CAPTURE OF MALE GRAPE ROOT BORER *Vitacea polistiformis* (Harris), (LEPIDOPTERA: SESIIDAE) IN A VINEYARD

Introduction

The increased incidence of insect resistance and proposed governmental restrictions of pesticides on food crops have led many fruit growers to consider the use of synthetic pheromones as a method for managing lepidopteran pest populations on their crops. Mass trapping and mating disruption are two methods that employ synthetic pheromones to capture or disrupt olfactory sexual communications of moths and can result in mating delays or reductions in the proportion of mated females in the population. The mechanisms of how disruption works are largely unknown (Sanders 1997).

Although there are numerous factors that influence male capture in pheromone-based systems, trap density, trap placement, and wind direction are three factors that have been shown to influence the capture distribution of insects in pheromone traps.

In mating disruption studies, reports of pheromone trap density needed to sufficiently encompass a vineyard with pheromone as well as optimal placements of traps within treated areas in vineyards are varied. Sauer et al. (1992) used a portable electroantennogram (EAG) to measure concentrations of

Lobesia botrana pheromone in a research vineyard. At a density of 500 dispensers/ha, the authors reported that there was a nearly constant pheromone concentrations present within the boundaries of treated vineyard, and concluded that pheromone was homogenously distributed. Charmillot et al. (1995) reported reductions in tethered female grape vine moth (*Lobesia botrana*) matings and male captures within the vineyard when 4224 dispensers were hung along a 10 ha rectangle as well as a large grid of dispensers 50 m from one another both inside and outside of the perimeter dispensers.

Optimal trap placement is generally recognized as being influenced by prevailing wind direction such that plume coverage should pass downwind over the crop. The use of pheromone-regulated anemotaxis by male moths to locate sources of pheromone was demonstrated by Kennedy and Marsh (1974). Wind direction and speed fluctuate frequently in the environment (Murlis et al. 1992) and have also been reported to influence trap capture of moths in other crops. Novak and Roelofs (1985) conducted release/capture studies in small orchards with male red banded leaf rollers and determined that trap catch in perimeter traps was dependent on wind direction. Wall and Perry (1978, 1980) reported male pea moth (*Cydia nigricana*) trap catches were greater in upwind traps than in center or downwind traps. In a 6 x 6 grid of traps spaced 80 m apart, Elkinton and Carde (1988) reported higher male gypsy moth (*Lymantria dispar*) captures in

perimeter traps than in center traps. More males were also captured in upwind and downwind traps compared with crosswind and central traps.

Although there is limited information regarding perimeter placement of traps in the literature, reports suggest that a perimeter approach to trap placement may provide additional protection to crops by serving as a downwind capture point for immigrating males before they orient to sexually receptive females. Charmillot et al. (1995) reported an average reduction in damage of 68.5% in the center of a vineyard and a 90% reduction of male captured inside the vineyard of grape vine moth when pheromone dispensers were distributed along the edge of a 10 ha rectangular vineyard and spaced within a vineyard. Lawson et al. (1996) reported no edge effect around apple orchards treated with pheromone to control oblique banded leaf rollers. Although the authors found an increased number of captured males along the perimeter, they attributed the increase to uneven pheromone coverage in that area. Sauer et al. (1992) reported a sharp decrease in pheromone density within a distance of 10 m from a pheromone-treated vineyard and concluded that to prevent mating of pests around the perimeter of a pheromone treated area, trap placement must exceed the boundaries of the treated area.

Several characteristics of *V. polistiformis* populations suggest that mass trapping and/or mating disruption could be effective controls. The sex pheromone has

been identified and synthesized (Schwartz et al. 1983, Snow et al. 1987) and is available commercially. The host range of *V. polistiformis* is restricted to grapes. Protandry has been reported by Dutcher and All (1978a) creating the possibility of removal of a proportion of males early in the season. Characteristics of the mating behaviors (Dutcher and All 1978b), the diel periodicity of female calling (Dutcher and All 1978c) and the sex ratio of a Georgia population (0.62:1.27 male:female) have also been described.

Although Johnson et al. (1986, 1991) reported successful mating disruption of *V. polistiformis* using synthetic pheromone in Arkansas vineyards, as measured by a reduction in the number of pupal skins in subsequent years, there are no other reports in the literature substantiating these results.

A better understanding of the factors influencing the capture of male *V. polistiformis* in pheromone traps is needed to improve the efficiency of this management approach. The objectives of this study were to determine the distribution of capture of male *V. polistiformis* using an array placement of traps, and to determine if wind direction influences trap capture. Observations of the sex ratio of the population are also documented.

Materials and Methods

All trapping experiments were conducted in a 21-yr-old muscadine and bunch grape research vineyard that was approximately 0.33 ha (64 by 51 m), located at the North Carolina State University Lake Wheeler Field Laboratory in Raleigh, North Carolina. Vines were spaced in eight rows approximately 7 m apart.

On 11 August, 2003 25 Pherocon[®] 1C wing traps were baited with commercially prepared grape root borer pheromone, a 99:1 blend of (E,Z)-2,13-octadecadien-1-ol acetate : (Z,Z)-3,13- octadecadien-1-ol acetate (Great Lakes IPM, Vestaburg, MI) impregnated in rubber septa, and hung at a height of 1.5 m on a metal pole anchored in concrete and covered with a chicken wire cone that was covered with a rubber camouflage material to provide diffused shade under the traps. The poles were located adjacent to the grapevine canopy.

A modification of the array design described by Elkinton and Carde (1988) was used for trap placement. A schematic drawing of the trap placement used in the study is shown in Figure 1. Twenty-four traps were placed in two polygonal arrays within the vineyard resulting in a density of 76 traps/ha. The inner array had a diameter of approximately 15.24 m, and included six traps located at 30, 90, 150, 210, 270, and 330 degrees clockwise from due north with an intertrap distance of 7.6 m. The outer array, located 15.24 m outside of the inner array, and had a diameter of 45.70 m and included 18 traps located every 20 degrees

starting at 10 degrees clockwise from due north and ending at 350 degrees from due north with an intertrap distance of 7.6 m. A single trap was positioned in the center of the first array.

The number of males captured in each trap was recorded the day following capture between 0900 -1000 hours EDT. Captured males were removed from the sticky trap bottoms daily and destroyed.

To test the null hypothesis that males captured per trap in the inner and outer array would occur with equal frequency, a Wilcoxon signed-rank test by the UNIVARIATE procedure in SAS (SAS Institute, 1999) was performed.

Average hourly wind direction and wind speed at 10 m above ground level were obtained for the Lake Wheeler Field Laboratory for the period between 11 August – 7 September from the State Climatic Office of North Carolina (2004). The average daily wind direction for the period that *V. polistiformis* adults are known to be reproductively active (between 1100 – 1700 hours EDT) was obtained by calculating a vector average wind direction during the 7 h period.

To evaluate the influence of wind direction on trap capture, comparisons were made between upwind, downwind, and crosswind per trap captures. The total number of traps were divided into orthogonal sectors, each containing six traps;

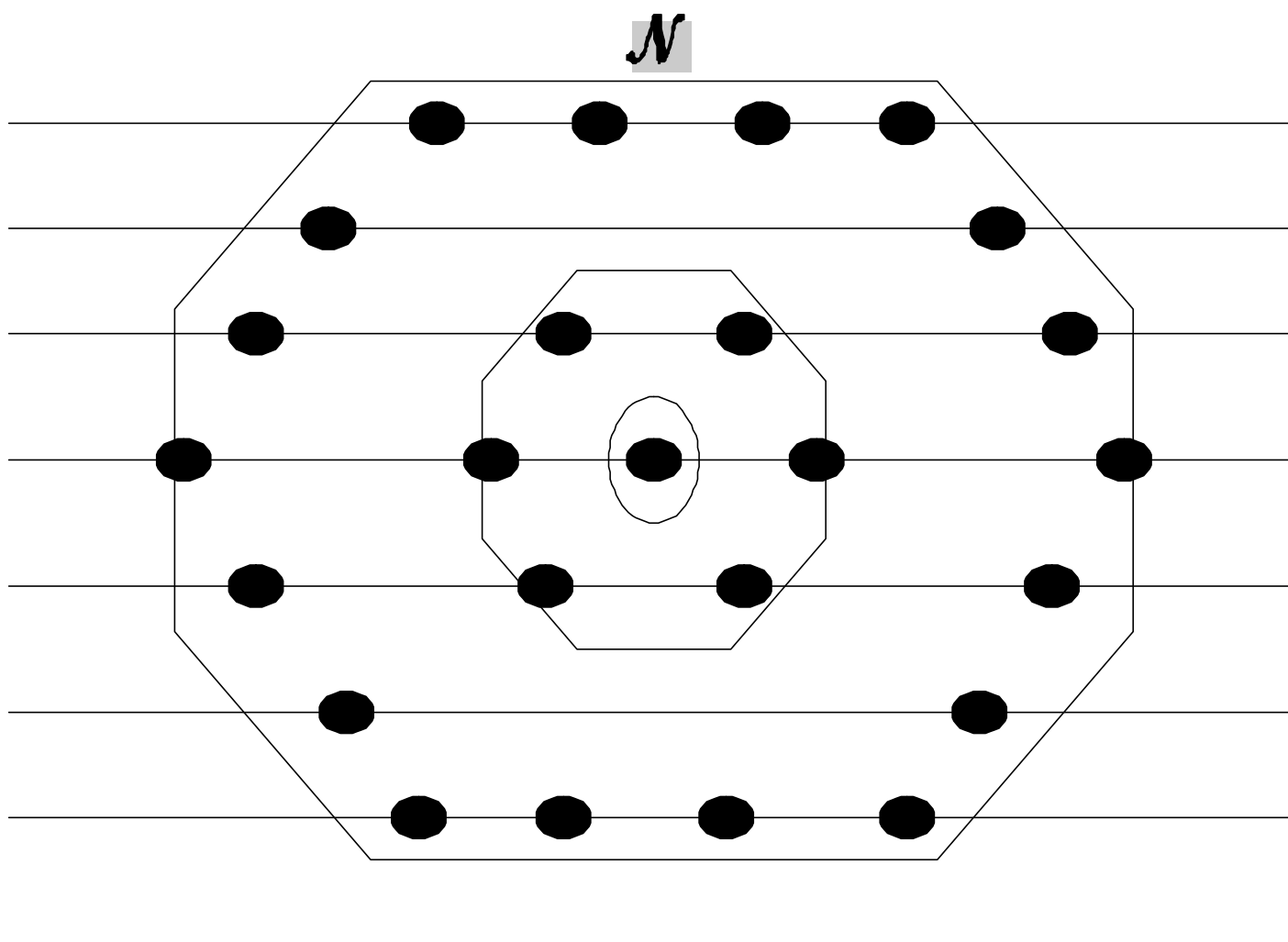
the up and downwind sectors contained two traps on either side of the average wind direction in the outer array, and one trap on either side of the average wind direction in the inner array. The crosswind sector contained two traps in the inner and four traps in the outer array that were closest to the angle that was 90° clockwise from the average wind direction in the upwind and downwind sectors.

The following hypotheses regarding wind direction and trap capture were tested using a Wilcoxon signed-rank test by the UNIVARIATE procedure in SAS: to test the null hypothesis that male capture per trap in the up and downwind sectors would occur with equal frequency, sectors were identified using the calculated daily average wind direction. A second hypothesis that trap capture of crosswind sectors would occur with a frequency equal to that of upwind or downwind sectors was also tested.

To determine if a relationship between standard deviation in average wind direction could explain variation in the overall percent trap catch in up and downwind sectors, a spearman's rank correlation procedure by the UNIVARIATE procedure in SAS was used.

The sex ratio of the GRB population in the research vineyard was estimated by collecting newly eclosed adults from the entire vineyard daily between 0900 – 1200 hours EDT from 25 July – 28 August, 2003. Adults were collected from the

same vineyard used for the trapping study. Adults were captured at emergence from their pupal skins from the soil underneath the grapevines or on the trunk of the grapevine following emergence but before their wings were fully expanded. Pupal skins were recovered from the soil for each insect. Insects were placed in 946 ml plastic holding container (Ziploc Brand) with a grape leaf. Small holes in the lid allowed circulation of air in the container. Adults were removed from the vineyard for use in other experiments.



Vineyard = 0.33 ha (64 by 51 m), 8 rows each 64 m long

Figure 1. Schematic of polygonal arrays used in pheromone trap study 11 August – 7 September, 2003.

Results

During the 4 wk period of the trapping study the number of males captured per trap was higher in the inner array traps compared with the outer array traps (Signed rank; $n = 28$; $s = 110$; $P < 0.0002$). The largest per trap capture of males occurred in the center trap. Per trap capture of males was greatest during the week of 18-24 August. A weekly summary of per trap captures by array is shown in Table 1.

Table 1. Weekly per trap capture of *V. polistiformis* by array in pheromone trap placement study from 11 August – 7 September, 2003.

Week	Center Trap (1 trap)	Inner Array (6 traps)	Outer Array (18 traps)	Total (25 traps)
1 (Aug. 11-17)	28	12.0	9.0	10.5
2 (Aug 18-24)	30	15.5	9.6	11.8
3 (Aug 25-31)	16	4.3	2.8	3.7
4 Sept 1-7	6	2.5	1.7	2.0
Total	80	34.3	23.1	28.1

When using the average daily wind direction to determine down and upwind sectors, the number of males captured per trap was higher in downwind sector traps compared with upwind sector traps (Signed rank; $n = 28$; $s = 119.5$; $P < 0.0004$). When comparing per trap captures of males in downwind with crosswind

sectors, a greater number of males were captured per trap in downwind sectors (Signed rank; $n = 28$; $s = 136$; $P < 0.0001$). However, there was no significant difference between capture in upwind traps compared to crosswind traps (Signed-rank; $n = 28$; $s = 20$; $P = .4350$). Although, there was a weak correlation (Spearman's rank correlation; $n = 28$; $r = 0.0375$) between standard deviation of the average daily wind direction and the percent of total daily capture in the down and upwind sectors, it was not significantly different from zero ($P = 0.8497$). A summary of wind direction and male per trap capture data are presented in Table 2.

Table 2. Average daily wind direction and capture of *V. polistiformis* in upwind, downwind, and crosswind sector traps in pheromone trapping study during 11 August – 7 September, 2003.

Date (2003)	Wind		Sector Per Trap Capture		
	Avg. Direction (deg.)	Std. Dev.	Upwind	Downwind	Crosswind
8/11	228.1	24.3	0.7	2.8	0.3
8/12	162.2	13.5	0.2	1.0	1.3
8/13	143.6	16.4	1.8	0.8	1.0
8/14	195.5	56.7	0.7	2.3	1.7
8/15	237.0	44.0	0.8	2.7	0.8
8/16	228.6	38.4	0.8	2.7	1.5
8/17	256.5	37.1	1.5	1.3	0.3
8/18	120.2	64.4	1.7	2.2	1.7
8/19	79.5	16.3	2.8	2.5	0.7
8/20	130.1	13.5	1.7	0.5	1.7
8/21	218.2	10.2	1.2	3.8	2.0
8/22	258.2	87.9	1.2	2.5	0.5
8/23	57.8	12.7	0.7	3.0	0.7
8/24	78.7	15.4	1.2	2.5	0.5
8/25	252.7	8.0	0.5	1.8	0.2
8/26	285.3	19.9	0.3	1.5	0.7
8/27	230.4	22.2	0.0	1.7	0.0
8/28	229.6	21.8	0.3	1.2	0.2
8/29	224.9	15.1	0.3	0.5	0.3
8/30	236.5	4.5	0.0	0.8	0.2
8/31	294.8	39.4	0.2	0.3	0.0
9/1	227.0	16.9	0.5	0.8	0.5
9/2	254.2	4.8	0.3	1.0	0.2
9/3	227.1	13.7	0.0	0.2	0.2
9/4	242.7	39.1	0.7	0.7	0.0
9/5	58.8	8.0	0.2	0.2	0.2
9/6	48.7	9.7	0.0	0.0	0.0
9/7	53.7	8.0	0.5	0.0	0.2

The percentage of time during the 4 wk trapping study that the average wind direction came from 1-90, 91-180, 181-270, 271-360° was 21, 14, 57, and 7%, respectively. The average daily wind direction during the predominant period of male flight (1100 – 1700 hours EDT) was from between 180-270° in half of the days of the study (Figure 2) resulting in downwind sector traps being located between 0-90°.

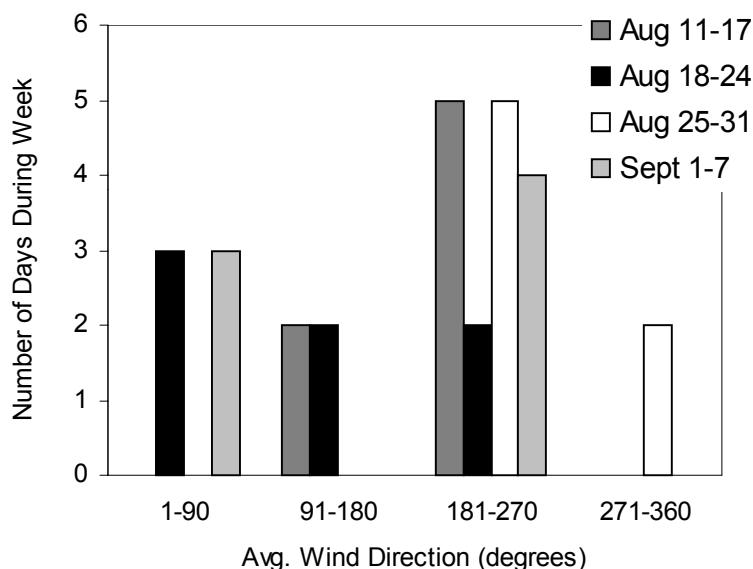


Figure 2. Number of days average wind direction was reported in four different sectors by week between 11 August – 7 September, 2003.

For the 4 wk period of the trapping study, 52% of the males were captured in traps located in the downwind sector, 26% in the upwind sector, and 22% in the

crosswind sector of the daily average wind direction. The weekly per trap capture of down, up, and crosswind traps are shown in Figure 3.

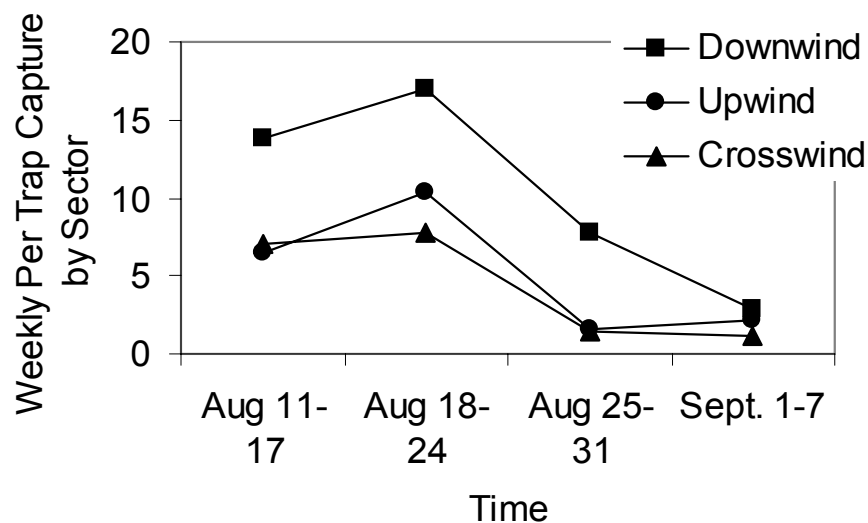


Figure 3. Weekly per trap capture of *V. polistiformis* in down, up, and crosswind sectors of average wind direction for weeks between 11 August – 7 September, 2003.

A total of 1114 adult *V. polistiformis* were removed from the research vineyard by either trapping or captured at emergence. Between 11 August – 7 September, 702 males were caught in the 24 traps that made up the two arrays, and 80 males were caught in the center trap (Figure 4). A total of 416 newly eclosed adults (168 males and 248 females) and their pupal cases were captured and removed from the entire vineyard between 25 July – 7 September 2003.

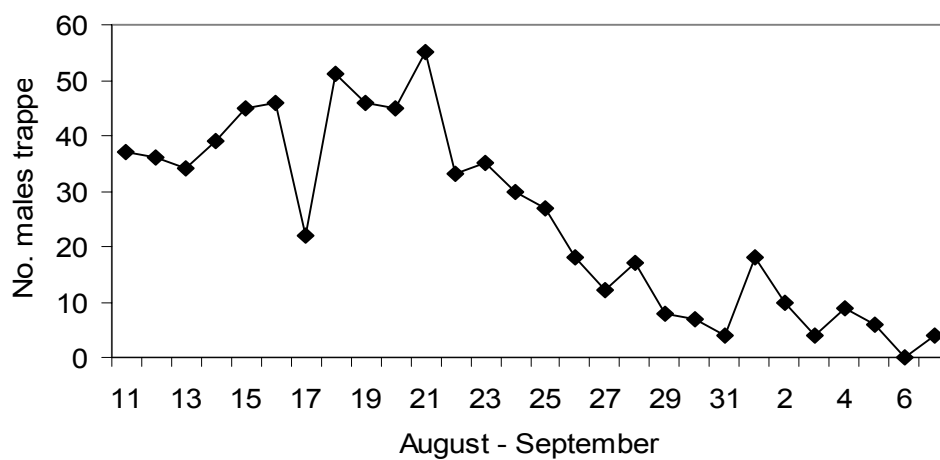


Figure 4. Daily trap capture of *V. polistiformis* males in pheromone trap study between 11 August – 7 September, 2003.

Based on the capture of newly eclosing males and females, cumulative emergence patterns observed in *V. polistiformis* were calculated and are shown in Figure 5.

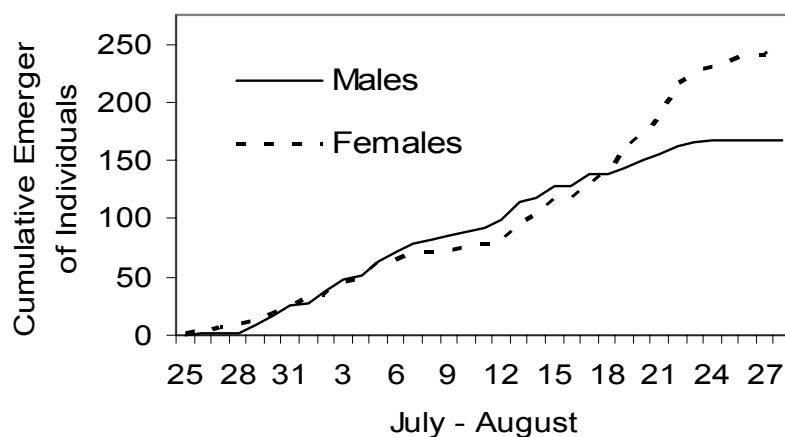


Figure 5. Cumulative emergence of male and female *V. polistiformis* during 25 July – 28 August, 2003.

Adult emergence was likely influenced by above normal rainfall (24.6 cm) for the month. Daily emergence during 3-7 August averaged 15.4 individuals. On 8 August the vineyard received 6.4 cm rain which saturated the soil beneath the plants. Average daily emergence during 8-12 August was reduced to 6.2 individuals. During 13-17 August, after the soil had dried, daily emergence nearly tripled to an average of 17.8 individuals.

The male:female sex ratio of captured adults is shown in Table 3.

Table 3. Sex ratios of adult male and female *V. polistiformis* captured at emergence by week from a research vineyard during 25 July - 28 August, 2003.

Week	Male	Female	Sex Ratio
July 25-31	25	24	1:0.96
Aug. 1-7	54	46	1:0.85
Aug. 8-14	39	32	1:0.82
Aug. 15-21	37	84	1:2.27
Aug. 22-28	13	62	1:4.77

Discussion

The results of this experiment suggest that per trap capture occurs more frequently in the inner array of traps compared with the outer array of traps. It seems likely that males bypassed traps in the outer array to be captured in inner array traps, however the reasons for this are not known. Howse et al. (1998) stated that when pheromone concentration levels adequate to prohibit males from finding and mating with females are released, trap shutdown occurs (trap

catch is decreased by 98-100%). Although pheromone levels were not measured, the number of dispensers/ha used in this study (76) were considerably lower (70-95% fewer) than reported in other vineyard studies (Charmillot et al. 1995, Johnson et al. 1991, 1986, Sauer et al. 1992). An insufficient amount of pheromone in the vineyard may account for the increase in inner array capture.

The results also suggest that wind direction has an influence on trap capture. Standard deviation of the average wind direction, however, failed to account for significant variation in percent trap capture in down and upwind sectors. Elkinton and Carde (1988), and Perry and Wall (1984) reported similar results. As a result of their work with the pea moth (*Cydia nigricana*), Wall and Perry (1978) determined that the use of several outer traps made statistical analysis of trap capture data complex due to the multiplicity or interactions between the traps. The authors later reported (1980) that the number of traps in a line had a greater effect on the interpretation of capture than the spacing between the traps. In their studies, traps located along the mean wind direction resulted in larger catches due to interference or overlap of pheromone plumes.

The concentration or distribution of pheromone in the vineyard as a result of the placement used in this study is unknown. The use of portable EAG equipment has been used in other studies and could potentially be helpful in quantifying

pheromone concentrations. Further research is needed to determine if the intertrap distance used in trap placement was sufficient to result in a continuous pheromone plume that encompassed the entire vineyard.

Dispersal of male moths has been reported to occur over many kilometers, and may result in elevated trap catches that do not reflect the local population density. Among sesiids, Gentry et al. (1979) reported that 97% of lesser peachtree borers released from the center of a pheromone treated area were captured within a distance of 0.6 km radius of the release point. However, little has been reported about male *V. polistiformis* movement within or dispersal outside the vineyard in response to calling females or synthetic pheromone. Townsend (1980) reported increased adult emergence along the perimeter of an uninfested vineyard adjacent to a 30-yr-old infested vineyard. The author concluded that movement of gravid females was occurring. Pearson (1992) also reported perimeter effects in mating disruption studies. In this study since the nearest known cultivated grapevines were located approximately 1.6 k to the SW, the research vineyard was considered isolated. Although *V. polistiformis* is considered to occur on indigenous wild grape vines in wooded areas, it has not been conclusively demonstrated. Furthermore, the population density and movement of gravid females from wild populations is unknown. Since a wooded area was located approximately 0.8 k to the SE of the vineyard, there is a possibility that feral insects from wild grape vines could have been present.

However, the distance that *V. polistiformis* males can detect female pheromone is unknown. Lesser peach tree borer males have been shown to detect synthetic pheromone traps from as far away as 800 m downwind (Karandinos 1974).

The incidence of males captured in traps in this study was much greater than newly emerging males captured daily. The sex ratio of newly eclosing adults captured during this study favored females (1:1.48), similar to the results reported by Dutcher and All (1978c). The male capture rate was verified by recovery of pupal skins daily from the entire vineyard, and there were very few captured adults for which pupal skins were not recovered. These findings suggest that immigration of feral males may be occurring. Wall and Perry (1978) speculated that a large number of pheromone traps in a small area could attract insects without catching them. The post-emergence movement of males should be investigated. The mark and recapture procedure used by Sharov et al. (2002) to estimate the distance gypsy moth males were able to detect pheromone treated areas could be used as a guideline.

It is difficult to make conclusions about the effects of trap placement used in this study and the potential implications for pheromone-based management. Knight and Croft (1987) cautioned that male capture rates in pheromone traps should be interpreted carefully. Captures may not coincide with female emergence or sexual receptivity, or even provide an accurate representation of pest population

density. In southern states, a bimodal flight has been reported (Snow et al. 1989, 1991) for *V. polistiformis* and a second peak emergence of males should be anticipated when interpreting trap captures.

The removal of nearly 250 newly emerging females from the vineyard would confound trap capture results obtained in this study. These females represent a source of competition in the vineyard with the synthetic pheromone. Their removal could serve to reduce competing sources of natural pheromone and could have potentially resulted in a greater trap capture.

The vigor of vines in the vineyard varied considerably and could also have contributed to the increased trap capture in particular areas of the vineyard. Vines that were more vigorous were assumed to have an increased root area capable of sustaining greater infestations. Differences in plant infestation levels could also be reflected in trap count variation.

Additional research is needed before conclusions can be made about the most effective placement of perimeter traps for *V. polistiformis* in vineyards.

Acknowledgements

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OVIPOSITION BEHAVIORS OF GRAVID FEMALE GRAPE ROOT BORER *Vitacea polistiformis* (Harris), (Lepidoptera: Sesiidae) IN AN IN-FIELD WIND TUNNEL

Introduction

The close proximity of food is important for the survival and growth of newly hatching Lepidoptera larvae (Renwick and Chew 1994). Sensory stimuli that influence maternal choice of oviposition sites is of great interest to entomologists because an understanding of these behaviors and environmental cues may contribute to improved methods of managing Lepidoptera pests.

Although the exact behaviors used by females to select a site for oviposition varies by species (Thompson and Pellmyr 1991), component behaviors have generally been described as flight orientation towards a host, landing on the host, contact evaluation, and acceptance or rejection of the host.

Insect orientation to host plants is likely to be influenced by both visual and olfactory cues. Plant spectral quality, particularly hues in the wavelength range of 500-580 nm, as well as intensity of reflected or transmitted light, has been reported as principle stimuli inducing landing of many insects on living plants (Prokopy and Owens 1983). Shorey (1964) showed that mated *Trichoplusia ni* females laid more eggs on yellow substrates than any other color, and Landholt (1989) showed that mated females exhibited a greater chemically mediated anemotaxis to host plants than did virgin females or males. Visual and

chemical recognition and avoidance of conspecific eggs have also been reported in Heliconidae, Pieriidae, and Danaidae (Thompson and Pellmyr 1991).

Observations of oviposition on artificial models of different colors and shapes are useful to study the role of vision in host selection. Hirota and Kato (2001) examined the influence of visual cues on host finding in mated *Eurema hecabe*. When given choices of models treated with methanol extracts of *Lespedeza cuneata* (Fabaceae), females chose to oviposit on models that resembled the color, or spectral reflectance, and leaf pattern of the host plants, leading the authors to conclude that visual cues influenced oviposition responses of this insect.

Olfaction, or the perception of gaseous molecules, primarily occurs on antennal receptors. Raguso et al. (1996) conducted electroantennogram studies on *Hyles lineata* and concluded that female moths exhibited a strong EAG response to a variety of compounds in floral and vegetative tissue of plants that were used as food and oviposition resources. Haynes and Baker (1989) compared flight patterns in male and female navel orangeworm moths, *Amyelois transitella* (Walker), in response to pheromone and host odor, respectively, and found that both exhibited similar anemotactic flight during upwind flight towards an odor source. Tichenor and Seigler (1980) conducted electroantennogram (EAG) and cage studies to determine the response of mated female *Manduca sexta* to plant

volatiles of tobacco and tomato. Extracts of both plants induced oviposition in gravid moths. However, when extracts were fractionated, EAG-active fractions did not consistently induce oviposition. The authors concluded that the mechanisms used by females to locate host plants as well as gravid female discrimination between potential hosts, involves many factors and a complex of chemical signals. EAG responses, however, do not provide information about natural flight orientation or anemotactic behaviors. As a result, flight tunnels or wind tunnels have been used to simulate insect responses to air currents. Spencer et al. (1999) constructed a large walk-in wind tunnel to observe free flight of Western corn rootworm to corn or soybean plants, and Foster and Howard (1998) investigated female discrimination between hosts and behaviors of *Epiphyas postvittana* in wind tunnel studies. Studies conducted by Tingle et al. (1990) showed that gravid *H. subflexa* and *H. virescens* females oriented towards extracts of host plants in wind tunnels.

The most common method of physical host plant acceptance used by moths is contact chemoreception (Ramaswamy 1988). Contact chemoreceptors of moths are located on the antennae, tarsi, probosces, and ovipositors. Mechanoreception also appears to play an important role in physical evaluation of host acceptance. Ramaswamy (1988) reviewed the literature and found that moths evaluate and prefer substrate surface textures that are rugose or rough as ovipositional sites.

Ramaswamy (1988) modified models of general insect host finding developed by Kogan to specific strategies observed in moth host finding. Kogan's model suggests that selective specialists use odor conditioned anemotactic flight, contact chemoreception, and vision to discriminate among hosts. Ramaswamy also speculated that day flying moths were likely to use vision for host-habitat location much like butterflies.

Limited information is available on cues that influence the oviposition behavior of *V. polistiformis*. Brooks (1918) described females as making short flights and laying a small number of eggs indiscriminantly on grass, fence posts, and grapes. Both Brooks (1918) and Dutcher and All (1978) reported that oviposition occurred between 0900 – 1200 hours EDT the day following mating. Olfaction studies by Pearson and Schal (1999) reported on EAG responses of male and female *V. polistiformis* to sex pheromone. Both virgin male and female moths exhibited similar responses to the 99:1 blend of (E,Z)-2,13 : (Z,Z)-3,13 ODDA. (EZ:ZZ) Female antennae were more responsive to (E,Z)-2-13 ODD OH than male antennae, and mated female antennae responded significantly to (E,Z) and 99:1 EZ:ZZ blend. Although numerous volatiles associated with grapes have been identified (Hardie and O'Brien 1988, Bravado 2001), neither EAG studies of *V. polistiformis* responses to grape volatiles nor wind tunnel studies to determine anemotactic behaviors have been conducted for this pest.

A better understanding of the behaviors and environmental cues that influence oviposition of gravid *V. polistiformis* may contribute to the development of methods for managing this insect. Because *V. polistiformis* are selective specialists and larvae feed only on grape roots, it was hypothesized that females would discriminate between grape and non-host plants of similar appearance, and oviposit on grape with greater frequency.

Materials and Methods

An in-field wind tunnel was constructed in an open field located at the North Carolina State University Lake Wheeler Horticultural Field Laboratory in Raleigh, North Carolina. The wind tunnel was framed with galvanized piping and covered with gray-military-issue-nylon parachute fabric. It measured 6.1 m wide by 9.1 m long by 2.7 m high. The northern end of the wind tunnel was fitted with mosquito netting (Figure 1). The southern end was fitted with a 1.07 m diameter fan (model DBD 4223, Triangle Engineering Company, Jacksonville, Arkansas) that was situated 0.6 m off the ground on a wooden framed platform, which drew air through the wind tunnel at a rate of 396 m³/min (Figure 2). The volume of air movement over the release platform was not measured in the wind tunnel. Two compartments were formed at the northern end of the wind tunnel by constructing a 2.4 m by 2.7 m panel made of nylon parachute fabric.



Figure 1. In-field wind tunnel used in *V. polistiformis* oviposition behavior study in August 2002.



Figure 2. Compartment and fan configuration inside the in-field wind tunnel used in *V. polistiformis* oviposition behavior study in August 2002.

A potted 3-yr-old grape and morning glory plant were positioned at the north end of the tunnel and separated by the panel. The location of the plants were rotated daily between the two compartments prior to insect release.

Newly eclosed females were mated on the afternoon of emergence. Males were removed within 1 h after mating. Females were held overnight in a sheltered location at ambient temperature. Each morning females received a mist of distilled water to simulate dew formation.

Oviposition experiments were carried out as simultaneous choice trials between the grape and morning glory plants. A total of 26 releases were conducted in the wind tunnel during a 4 d period between 1-7 August. Twenty females were released individually directly from their holding containers beginning at 0930 hours EST the morning following mating. The containers of mated females were placed on a plastic file crate platform approximately 1.8 m from the southern end of the wind tunnel. Observations of ovipositioning behaviors, female plant choice, landing substrates and contact evaluation behaviors were recorded during a 15 min period. The number and location of eggs oviposited were also recorded.

The results of observations are presented in table format. Data were not statistically analyzed due to a low number of observations.

Results

During the trials, females displayed very little activity when released. During ten of the trials, females took short flights into the grass in close proximity to the release platform and then remained motionless for the remainder of the observation period. Eight females did not leave their container upon release or remained motionless during the entire observation period. Only four of the 20 females landed on a plant at the end of the wind tunnel, with three landing on morning glory and one on grape. Females that displayed activity were indiscriminant in oviposition behavior, laying eggs on the mosquito netting, the release platform, the grass inside the wind tunnel, as well as the parachute material. On the 2nd day of the study temperatures inside the wind tunnel reached 42^o C. Two insects released that day were found dead the following morning.

Due to a lack of response by females, observational periods were modified and females were released at either 0900 hours or 1900 hours EDT to determine if ambient temperature was limiting the responses. Although temperatures were approximately 6^o C lower at these modified release times, insects exhibited no response when released into the wind tunnel. The experiment was suspended until 6 August when the weather was partly cloudy and high temperatures were below 37^o C in the wind tunnel. Although more activity was observed, females still did not fly towards plants or oviposit more than 14 eggs. The experiment

was terminated on 8 Aug due to a lack of female response. A summary of the results is shown in Table 1.

On 9 August five females mated the previous day were released simultaneously into the wind tunnel beginning at 0930 hours EDT. Temperatures were not recorded. Insects were observed to walk, fly, void meconium, make short flights into grass, fan wings, and fly in a clockwise pattern from the point of release. Eggs were also oviposited. Four of the five females landed on plants at the end of the wind tunnel, three on morning glory and one on grape. The time of flight orientation was between 1300 – 1400 hours EDT.

Table 1. Responses of mated female *V. polistiformis* behaviors when released into an in-field wind tunnel in August 2002.

Date 2002	Female/ DOB	Release Time	Temp. °C	Stop Time	Temp.	Choice	Observational Notes
8-1	U2 (7/31)	0935	32.2	0943	-	None	No response
	EY9 (7-31)	0944	32.2	0951	34.4	None	Flew to grass from platform. No response following.
	EY17 (7-31)	0952	34.4	1009	35.0	Morning glory	Fanned wings. Flew towards fan then mg.
	U2 (7-31)	1009	35.6	1024	36.7	Morning glory	Cleaned antennae. Flew to grass laid 2 eggs. Flew to mg.
	EY9	1043	36.1	1104	38.9	Grape	Flew to grass from platform.
8-2	EY33 (8-1)	0950	32.8	1005	36.7	None	Flew into grass, no further activity
	U2 (8-1)	1006	36.7	1021	38.3	-	Flew into grass, lost.
	U2 (8-1)	1021	38.3	1024	38.3	None	Flew to top of cage over mg.
	EY20 (8-1)	1027	38.9	1042	39.4	None	No activity.
	EY? (8-1)	1052	40.6	1108	40.6	None	Short flight onto platform. No further activity.
	EY9(2 nd trial)	1110	40.6	1125	41.1	None	Flew into fan. No further activity.
	EY17(2 nd trial)	1127	41.7	1148	42.2	None	No activity
	U2 (2 nd trial)						Female dead.
8-3	EY? (2 nd trial)						Female dead.
	U2 (8/2) (1 st trial)	1053	36.7	1100	38.3	Morning Glory	Fanned wings. Flew to grass. Flew to morning glory.
	U2 (8/2) (1 st trial)	1029	35.0	1044	35.6	None	Flew to grass when container opened. Laid 1 egg. Short flight into grass. No additional activity.

Table 1. Continued

Date 2002	Female/ DOB	Release Time	Temp.	Stop Time	Temp.	Choice	Observational Notes
8-3 cont'd	U2 (8/1) 2 nd trial	1013	35.0	1028	34.4	None	Did not leave platform or container.
	EY33 (8-1) 2 nd trial	1115	38.9	1130	38.9	None	Slight antennation. Did not leave container.
	U2 (8/5) 1 st trial	0954	31.1	1009	33.3	None	Flew from container. No additional activity.
	U2 (8/5)	1010	33.9	1025	35.6	None	Laid 8 eggs in container on leaf. Laid 6 addtnl eggs under leaf during obser. Flew to grass.
	U2 (8/3)	1040	34.4	1055	37.2	None	3 short flights to grass (2) and platform (1). Laid 3 eggs on platform.
	OH54 (8/5)	1056	37.2	1111	37.2	None	Flew to grass. Moved 3x within grass no additnl activity
8-7	U2 (8/6)	1005	27.8	1020	31.1	None	Cleaned antennae
	U2 (8/6)	1021	31.1	1036	32.2	None	No activity
	U2 (8/6)	1150	36.1	1205	36.7	None	Eggs in container at release. Flew to side of container laid 7 eggs. Antennation but no movement.
	U2 (8/6)	1207	36.7	1222	36.7	None	Cleaned antennae & touched to surface. No eggs laid.

Discussion

The lack of response by gravid females in the wind tunnel was unexpected. Efforts to determine the cause of the lack of response included varying release time and days when temperatures were below 37⁰ C in the wind tunnel. Because two females died after being released when temperatures exceeded 42⁰ C, it is possible that the upper limits of the insect's temperature range had been exceeded. The experiment will be repeated another year. The wind tunnel will be assembled in a partly shaded location to reduce the potential heat load within the structure. Average ambient air temperature inside the tunnel at the time of female release on each of the four days were 32.9, 34.1, 35.3, 38.5⁰ C. Consideration should be given to replicating the insect's natural habitat when reconstructing the wind tunnel. Since the insect is believed to be found in wooded areas, perhaps a contrast of light and dark visual cues overhead are needed to stimulate oviposition behavior.

It is interesting to note that releases made on 9 August resulted in 80% of females making a choice between plants in the wind tunnel. When comparing conditions under which females did and did not exhibit activity, one difference was the time of release. The literature suggests that oviposition occurs between 0900 – 1200 hours EDT. However, insects in this experiment exhibited very little activity during the reported hours. Response times of insects were consistent and restricted to the hours between 1200 – 1400 EDT. This time period coincides

with the time of calling of unmated females. It is difficult to determine from such a small number of responses if the time of oviposition was truly different from that reported in the literature. More replications are needed before conclusions can be made about the observations reported.

The reported lack of discrimination in site selection for ovipositing by *V. polistiformis* seems unusual in view of the fact that grapes are the only reported host of this pest. However, considering that this insect's primary host is thought to be wild grapevines indigenous to wooded areas, the indiscriminant maternal choice of ovipositing sites may not be so unusual. Wild grape vines in the genus *Vitis* are North American in origin (Hardie and O'Brien 1988), and plants and their root systems are extensively distributed randomly in the understory of wooded areas. It follows that random ovipositing of a small number of eggs by *V. polistiformis* would increase the probability of eggs hatching close to a larval food source. Additionally, this behavior would reduce excessively high populations of larvae at any single site, and hence the probability of resource depletion and cannibalism. Thompson and Pellmyr (1991) referenced mathematical models that suggested Lepidopteran clutch size generally decreases as the number of females in an area increases or as larval competition increases. Viticulture in the eastern United States has only been established since the early 1800s, and vineyards have likely been exploited opportunistically by *V. polistiformis*. Considering the heterogeneous nature of

host plants in the natural habitat of *V. polistiformis*, maternal ovipositional behaviors seem unusual only when the insect is exploiting a homogenous habitat such as a cultivated vineyard.

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CONCLUSIONS

The increasing incidence of insect resistance to insecticides, and proposed governmental restrictions of pesticides on food crops have led many fruit growers to consider the use of synthetic pheromones as a method of managing lepidopteran pests of fruit crops. Synthetic pheromones used in mating disruption disrupt olfactory sexual communications and are believed to result in either delayed or greatly reduced numbers of matings for a proportion of females in the population. In an effort to improve the efficiency of pest management programs utilizing synthetic pheromone for *V. polistiformis* on grapes, experiments were conducted to examine four components of this insect's biology and behavior that may be influenced by the use of synthetic pheromone. The components studied were the incidence of polygyny, the effect of polygyny and delayed female mating on reproductive capacity, the evaluation of pheromone trap placement on the capture of males in a vineyard, and oviposition behaviors of gravid females in an in-field wind tunnel.

Polygyny was observed in *V. polistiformis*. Males were capable of mating one time daily for at least six consecutive days. Copulation duration (163 min) and average egg hatch (90.2%) were significantly different for males that had not previously mated compared with males mating for the 6th time (260 min and 83% average egg hatch). Although there were significant differences in egg hatch

due to polygyny, the results of this study suggest that it does not significantly reduce the number of viable eggs/cm of female body length of *V. polistiformis*.

Female Lepidoptera that are short-lived and do not feed as adults, such as *V. polistiformis*, are believed to utilize reproductive energy reserves through oosorption when mating is delayed, thus reducing the number of viable eggs they can produce if mating occurs at a later age. Aging virgin females also show a reduction in the ability to attract males when calling. As has been reported for many other moths, the age of female *V. polistiformis* at mating significantly affected fecundity, fertility, and reproductive capacity. Both fecundity and fertility declined linearly as mating delays increased. The combined effects of fecundity and fertility reductions due to delays in female mating resulted in a 47% reduction in the reproductive capacity of females mating seven days after eclosion compared with those mating on day of eclosion. All virgin females continued to exhibit diel periodicity in calling regardless of mating delays. Cumulative effects of mating delays and mortality reported in the literature suggest that when females experience a 5 d delay, population densities would remain nearly constant over five generations. Females that delayed mating until 7 d following eclosion would experience a population decline over five generations. If pheromone based management can result in female delays in mating, it offers promise for reducing population densities and their negative effects on plant yield over time.

If pheromone-based management programs result in an increase in the occurrence of polygyny, the results of this study suggest that this factor will not have as great an effect on reducing the reproductive capacity of *V. polistiformis* as would delayed female matings. Additional research to examine the possible cumulative effects of polygyny and delayed female mating on the reproductive capacity of this insect is warranted. The combined effects of delays in mating for both males and females may also result in delays in mating due to aging males attempting to mate with aging females.

Behavioral observations were made of both males and females during the polygyny and female mating delay experiments. The following observations were observed in a small percentage (<10%) of the matings, and did not occur with enough frequency to justify statistical analysis. However, they are worthy of mention in this document for the benefit of other researchers. In the polygyny study, the rejection of males by newly eclosed virgin females included physical aggression by females towards the male using their front tarsi. Females also repositioned themselves on the plant away from the male. Two different virgin females rejected 3-day-old virgin males but mated with males that were 5- and 12-d old that had previously mated four and five times, respectively.

Male body length did not appear to influence female mate choice. One individual in 2003 that was smaller (1.4 cm) than the average male (1.75 cm) mated

successfully eight times and died attempting to mate for the ninth time. Female rejection of this individual was not observed, and fertility resulting from the eight matings ranged from 71 to 92%.

In the delayed female mating study, mate rejection was observed in both females and males. Males were less likely to mate with 5- and 7-day old females. Opportunistic calling was observed in aged females when they were in close proximity to younger calling females. When aged females were near young females that had attracted males, aged females began calling in response to male presence. Visual cues appeared to play a role in mate finding by males. Males oriented their flight towards dark contrast areas on the grape plant. Other behavior modifications observed as a result of mating delays during this 2 yr study included females mating for a second time and females calling after mating. Across both years, a small percentage (2.7%) of the females called after mating. In 2002, a single female in the 7-d-old mated treatment group called after mating and mated a second time. The second mating occurred 9 d after the first mating. From the first mating, the female laid 377 eggs and 78% hatched. After the second mating, the female laid 16 eggs and 56% hatched. Also in 2002, a virgin female in the 7-d-old treatment group oviposited numerous eggs prior to mating. Although this individual did not begin calling until 1900 hours, she successfully mated and laid a total of 749 eggs. However, only 31% of the eggs hatched. In 2003 three females were observed calling after

copulation. Following the completion of a 2 h 20 min copulation, a 5-d-old female began calling for a second time. Males responded to her pheromone release, but once they came into close proximity they did not mate with her.

Regardless of the age of females at mating, calling occurred between 1300-1730 hours. Temperatures at the time of mating ranged from 22 - 35⁰ C. Aging virgin females that were not calling were observed to commence calling when a male came in close proximity to them. Aged virgin females were more likely to mate with newly eclosed virgin males than with aging virgin males. Weather effects on mating behaviors were also observed. Interestingly, all females were observed to call simultaneously regardless of their age just prior to afternoon thunder storms.

Male capture in pheromone traps is known to be influenced by numerous factors. The placement of pheromone traps in vineyards and wind direction were examined to determine their influence on capture frequency. The frequency of daily per trap capture was compared among traps located in an inner and outer array, and upwind, downwind, and crosswind sectors of the vector-averaged wind direction over a 7 hr period in a vineyard. Trap capture was higher in a placement of traps located in an inner array compared with the array that was located near the outer edge of the vineyard. Male capture was greater in downwind traps compared with upwind or crosswind traps. The correlation

between the standard deviation of the average daily wind and total percent trap capture of up and downwind sectors was not significant. It is difficult to make conclusions about the effect of trap placement on male capture from this study. Additional research is needed to describe the movement of males within and outside a vineyard in response to calling females or pheromone traps. It should also be determined how calling females compete with pheromone traps and influence trap capture.

The objective of the oviposition behavior study was to determine if gravid females would land and oviposit on a grape plant more frequently than on a non-host plant having a similar appearance. Females displayed very little response upon release into a in-field wind tunnel containing the binary choice of plants. Females that displayed activity were indiscriminant in oviposition behavior, ovipositing eggs on the mosquito netting, the release platform, the grass, and the wind tunnel material. Wild grape vines in the genus *Vitis* are North American in origin, and the plants and their root systems are randomly distributed in the understory of wooded areas. It follows that random oviposition of eggs in this habitat would increase the probability that eggs would hatch close to a larval food source (grape roots). Additionally, this behavior would reduce excessively high populations of larvae at any single site, and hence the probability of resource depletion and cannibalism. Considering the heterogenous nature of the natural habitat of *V. polistiformis*, maternal ovipositional behaviors seem unusual only

when the insect is exploiting a homogenous habitat such as a cultivated vineyard.