FACTORS AFFECTING MATING, MONITORING AND PHENOLOGY OF GRAPE BERRY MOTH, *PARALOBESIA VITEANA*, IN MICHIGAN VINEYARDS

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ABSTRACT

FACTORS AFFECTING MATING, MONITORING, AND PHENOLOGY
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Paralobesia viteana (Clemens), the grape berry moth (GBM), is a major economic pest of cultivated grapes in Eastern North America. Although pheromone lures and traps are available for monitoring this pest, male moth captures in these traps are not consistent between Michigan grape-growing regions, and male captures decline as the infestation increases through the multiple generations that occur during a season. This makes it difficult to use traps to monitor this pest's population dynamics and complicates the timing of pest management activities.

Substantial regional variation exists in the magnitude of the response of male GBM to sex pheromone-baited traps in Michigan vineyards. Males are readily captured in traps in the southwest region, whereas in the northwest very few males are captured. However grape berry moth larval infestation is found in fruit in both regions. Using Y-tube choice tests and trapping trials with captive females, I determined that males from Southwest and Northwest Michigan responded similarly to the standard pheromone blend, and males did not preferentially choose females from the same population. From these results I conclude that the regional differences in male captures are not due to differential responses of males in these respective areas. I postulate that the reason fewer males are trapped in Northwest Michigan is because the *P. viteana* population is much smaller than in Southwest Michigan.

To test whether seasonal changes in the plant canopy affect captures of male grape berry moth, I manipulated grapevine fruit density or canopy structure in multiple growing seasons, and

measured male captures under these conditions. Removal of either 50 or 100% of the fruit clusters from vineyard plots did not consistently affect captures in pheromone traps. In a separate canopy manipulation experiment, I detected significant differences in male captures between unaltered and open canopies for some sample periods, and there was a trend toward numerically more male captures in unaltered than in open canopies. I conclude that fruit presence, fruit density and canopy fullness do not reduce male *P. viteana* captures late in the season, and thus do not explain the seasonal pattern of development and abundance of this insect.

Experiments that measured the frequency, intensity and duration of mating and reproductive behaviors in colonies held under different temperature and photoperiodic conditions were used to determine that temperature is the likely driving force behind the seasonal variation in male *P. viteana* captures, and thus shapes the observed phenology of this pest. The frequency of male flights, mating and oviposition increased with temperature. This amplified activity helps to explain the intensification of oviposition and subsequent larval feeding damage in vineyards during the summer and early fall when conditions are warm. My data also show the proportion of male flights that occur when females are not receptive to mating is greater at lower temperatures, which helps explain why more males are trapped in the spring when temperatures are cool.

Traps baited with lures that contained different quantities of *P. viteana* sex pheromone were used to determine that the increased amount of pheromone released by lures during hot periods can reduce male captures. My research shows that temperature is an important factor that governs the behaviors associated with mating and reproduction, and also influences the main tool for monitoring this pest, the pheromone trap. Taken as a whole, the effects of temperature on behavior and trapping strongly shape the observedphenology of this pest.

For my wonderful family, Julienne, Lindsey and Justin, and my parents, Gail and Charles Mason, I dedicate this dissertation to you with all my love and many thanks for your constant support and encouragement during this process.

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CHAPTER 1

NATURAL HISTORY, ECONOMIC IMPORTANCE, AND VARIATION IN MONITORING *PARALOBESIA VITEANA*

Taxonomy and biology

Nomenclature. The grape berry moth (GBM), *Paralobesia viteana* (Clemens) (Lepidoptera:Tortricidae), is a specialist herbivore found in woods and vineyards across Eastern North America (Johnson and Hammar 1912) that feeds primarily on wild and cultivated plants in the genus *Vitis* (Vitaceae). The original taxonomic determination was made in 1860 as *Endopiza viteana* Clemens. Prior to that it was considered to be the polyphagous fruit pest *Lobesia botrana* (Denis and Shiffermüller) known commonly as the European grape vine moth. It was determined that *P. viteana* was a separate species after observations that it was reared only from grapes, and in addition, *P. viteana* larvae pupated on rolled leaves and not on grape shoots, trunks or canes as is known for *L. botrana* (Slingerland 1904). Over time *P. viteana* has been classified in different genera including *Polychrosis, Lobesia, Endopiza and Paralobesia*, all with the specific epithet *viteana*. In a review of the discrepancies in the nomenclature of the Tortricidae (Brown 2006), the disparities within this species were clarified and it is currently classified as *Paralobesia viteana* (Clemens).

Hosts, habitat and distribution. Twelve species of wild grape (*Vitis*) occur in deciduous woods in Eastern North America (Galet 1979, Gilbert 2012) and these are thought to be ancestral hosts of *P. viteana* (Morano and Walker 1995). Wild grapes are common components of the woodland flora throughout North America, and are often found on the borders of wooded areas or in areas of disturbed habitat (Fergusson-Kolmes and Dennehy 1993, Morano and Walker 1995). In Michigan four species of *Vitis* occur naturally, *Vitis riparia* Michaux, *V. aestivalis* Michaux, *V. labrusca* L. and *V. vulpina* L., and of these species, *V. riparia* and *V. aestivalis* are the most

common in Michigan woodlands (Voss 1985, K. Mason personal observation). The presence of wild grapes is an important determinant of the large scale distribution of *P. viteana*, and records and reports of this insect from wild and cultivated sites range from Ontario to Texas and from the Rocky Mountains eastward to New England (Slingerland 1904, Johnson and Hammar 1912, Isley 1917, Pfeiffer et al. 1990). At the local scale, the abundance, distribution and risk of infestation of cultivated grapes is determined in large part by the density of wild grapes in the habitat adjacent to a vineyard (Hoffman and Dennehy 1989, Dennehy et al. 1990, Botero-Garcés and Isaacs 2003, 2004a, Jenkins and Isaacs 2007a).

Life cycle and reproductive biology. *Paralobesia viteana* exhibits multiple generations in a year, depending on the location and length of the growing season, e.g., three and sometimes four generations in Michigan and other parts of the Great Lakes region (Tobin et al. 2003, Teixeira et al. 2009, 2011). In these regions, first generation adults typically emerge from overwintering pupae beginning in late April or early May. Mated females begin to lay eggs around dusk on warm nights in early to mid-June (Clark and Dennehy 1988, Teixeira et al. 2009, 2011). After larvae hatch, they feed on multiple adjacent flowers or young berries and produce webbing that encloses the feeding site (Johnson and Hammar 1912, Jenkins and Isaacs 2007b, Isaacs et al. 2012b). Larvae of subsequent generations excavate a tunnel into the fruit, and also web together and feed on multiple berries.

Upon maturation, a larva leaves the feeding site, moves onto a leaf and forms a hibernaculum by using its mandibles to cut a small semi-circle on the leaf and then folding it over its body and sealing the edges with salivary silk. The larva then spins a cocoon in this parcel and pupates (Johnson and Hammar 1912, Taschenberg 1951). Eggs that are laid in the late summer and fall are increasingly likely to enter diapause, and eggs and young larvae that are exposed to day lengths shorter than 14 h (Nagarkatti et al. 2001, Tobin et al. 2002) will

overwinter as pupae on leaves. These leaves drop from the vine in late fall and may remain in the litter on the vineyard floor or be blown by wind into adjacent habitats (Johnson and Hammar 1912, Isley 1917, Taschenberg 1951, Botero-Garcés and Isaacs 2003, Matlock et al. 2016). These pupae then emerge the following spring to resume the cycle.

Economic importance

The economic impact of the first generation is minimal, but larvae of later generations can cause significant economic losses due to reduced fruit yield, lowered fruit quality, or rejection of contaminated fruit by fruit processors (Hoffman et al. 1992, Teixeira et al. 2009, Roubos et al. 2013). *Paralobesia viteana* infestation is higher in vineyards adjacent to deciduous woods that contain wild grape (Hoffman and Dennehy 1989, Botero-Garcés and Isaacs 2003, 2004a, Jenkins and Isaacs 2007a) with grape berry moth infestation consistently higher at wooded edges compared to the vineyard interior (Hoffman and Dennehy 1989, Trimble 1993, Botero-Garcés and Isaacs 2003, Mason et al. 2016). Left unmanaged, grape berry moth can infest 100% of clusters on vineyard borders by harvest, and larval contamination of the harvested fruit can lead to rejection of the crop by processors or wine makers. Larval feeding can also reducing cluster weight and increase susceptibility to fungal infections. Larval feeding by *P. viteana* is similar to that of the closely related European species, *Lobesia botrana* (Denis & Schiffermüller) that also affects yield and quality by increasing susceptibility to bacterial and fungal infections (Fermaud and Le Menn 1992, Mondy et al. 1998, Ioriatti et al. 2011). Because of the risks of direct yield loss, larval contamination of fruit and reduced quality due to pathogens, *P. viteana* is the target of the majority of insecticides used in vineyards east of the Rocky Mountains (Hoffman et al. 1992, Teixeira et al. 2009).

Paralobesia viteana was first noted as a pest soon after the start of commercial grape production in the Midwestern and Northeastern United States, when in 1869 yield losses of up to 50% of the crop were reported in Ohio, Missouri and Illinois (Johnson and Hammar 1912). It was noted that *P. viteana* infestation was not uniform at local or regional scales, and high levels of infestation were found only in a small number of vineyards, especially in Pennsylvania and New York (Isley 1917). In Michigan it was reported that this insect was found in many vineyards in Van Buren County, but there were few cases where *P. viteana* caused losses of economic significance (Isley 1917). It was not until 1922 that the first widespread *P. viteana* infestations were reported in Michigan, but the actual loss was not quantified in economic terms (Pettit 1922). By 1932 *P. viteana* was considered a "serious enemy of Michigan grapes" (Pettit 1932).

History of P. viteana management

Early measures used for *P. viteana* management were dominated by cultural controls to bury or damage the overwintering pupae by tilling, and mounding or hilling soil onto the vine trunk (Johnson and Hammar 1912, Isley 1917, Pettit 1932). The advent of chemical controls for agriculture began in the late 19th and early 20th century, and inorganic compounds containing toxic heavy metals such as lead arsenate and Paris green (copper acetoarsenite) added to either water or Bordeaux mixture (Clark 1902), proved to be very effective at controlling this pest (Johnson and Hammar 1912, Isley 1917). Starting in the immediate post-World War II era and continuing through the 1950s and early 1960s, highly toxic chlorinated hydrocarbon insecticides, including dichloro-diphenyl-trichloroethane (DDT), dieldrin and thiodan were used to control *P. viteana* and other pests. Later and into the 1980s organophosphates, such as azinphos-methyl and methyl parathion saw widespread use for pest control in grapes (Taschenberg 1948, 1950, Taschenberg and Avens 1960, Taschenberg et al. 1964, Dennehy et al. 1990). These compounds

were all part of the "Green Revolution" in which synthetic pesticides and fertilizers were seen as the solution to world hunger. During this period many cost-effective tools were developed, and global food production was increased greatly. However, high chemical inputs into some agricultural systems produced unforeseen adverse environmental consequences, such as surface water contamination, detrimental effects on non-target species and the development of pesticide resistance (Henny 1972, Devonshire and Moores 1982, Brattsten et al. 1986). The insecticides of this era typically had a broad spectrum of activity, and because they were contact poisons with high volatility and long residual activity, could be applied without precise timing either prophylactically, or as a "rescue" or "clean up" application after *P. viteana* infestation was established. Additionally these chemicals were deemed less hazardous and more economical than previous options such as lead arsenate, or Paris green, and this led to the overuse of insecticides for control of *P. viteana* and other pests (Pimentel 2012). As these compounds were lipophilic molecules, they were prone to bio-accumulate in sediments and then in fatty tissues in organisms at low trophic levels. These substances then bio-magnified upward into higher trophic levels resulting in widespread environmental effects including population reductions in fish, amphibians and birds of prey (Henny 1972).

The rise of IPM. The declines across many taxa that were linked to the overuse of pesticides were brought into public view in the seminal work, *Silent Spring,* (Carson 1962). In addition to the ensuing public backlash against existing agricultural practices that was incited by Carson (1962), multiple cases of insecticide resistance were linked to the indiscriminate and repeated use of some of these compounds (Devonshire and Moores 1982, Brattsten et al. 1986, Nagarkatti et al. 2002b). These led to the development of new insecticides including novel chemistries that were designed to be used in rotation to limit the buildup of insecticide resistance. However many of these chemicals had similar environmental and worker/consumer safety concerns as the

chlorinated hydrocarbons. This led to the eventual passage of the Food Quality Protection Act (FQPA) (Anon 1996), that mandated the review and ultimate restriction of several organophosphate and carbamate insecticides, not only in grapes, but in all food crops.

The decade following *Silent Spring* (Carson 1962) also saw the development and adoption of many new tools and strategies to control *P. viteana* and other pests. These tactics taken as a whole became known as integrated pest management (IPM) (Figure 1.1, from Fleischer et al., 2014). The components of IPM include: efforts to better understand pest biology and ecology to improve control tactics; implementation of methods for monitoring pest abundance and phenology; derivation of economic and action thresholds to determine if and when to apply pesticides; advancement of weather-based models to predict disease and insect pest development; formulation and use of novel chemistries that are selective for a narrow range of organisms with reduced risks to the environment and the health of agricultural workers and consumers; and the promotion of sustainable pest management approaches such as cultural and biological control (DeBach and Rosen 1991, Dent 2000, Jenkins and Isaacs 2007a, 2007b) (Figure 1.1). All of these control methods have seen some adoption in *P. viteana* management programs, however control of this insect remains dominated by the use of synthetic insecticides (Isaacs et al. 2012b).

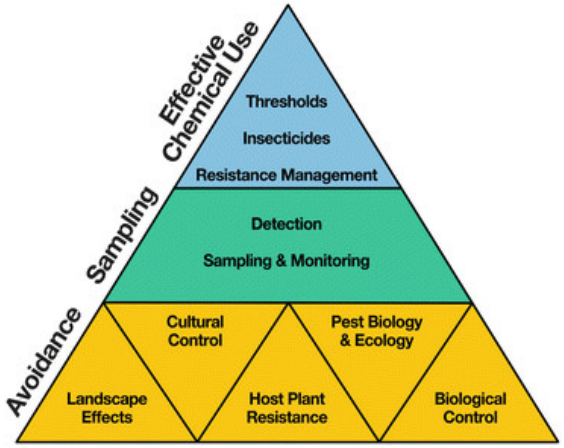

Figure 1.1. Pyramid of IPM tactics

Monitoring

Thresholds. Pest monitoring is an important component of IPM. Information gathered from regular visual sampling and the use of passive or baited traps can be used to determine when susceptible stages of a pest are present, and if pests are in high enough abundance to warrant control (Binns and Nyrop 1992, Knight and Light 2005a, Roubos et al. 2013). This is done by comparing the cost of applying a given treatment to the dollar value that the crop would lose if it was left untreated (economic threshold). In general, if the cost of treatment is less than the potential loss of crop value, then the treatment should be used. In practice the treatment is applied when the action threshold is reached. This is a level of pest damage or abundance that triggers treatments to be applied to maintain pest abundance below the economic threshold. Monitoring can also be used to develop predictive models that estimate when a pest is likely to reach the action threshold (Binns and Nyrop 1992, Knight and Light 2005a).

The MSU Enviroweather model. In the case of *P. viteana,* multiple years of scouting to determine when eggs and larval damage were present in clusters were used to create a model posted on Michigan State University's Agricultural weather online service, MSU Enviroweather (Isaacs 2017). This model predicts when oviposition will begin in different areas of the state based on the accumulation of growing degree days (base temperature 8.41ºC) after bloom of wild grape (*V. riparia*) at multiple remote weather stations in regions of grape production. This model has helped improve *P. viteana* management in Michigan using reduced-risk insecticides, such as the ecdysone agonist methoxyfenozide and the anthranilic diamide chlorantraniliprole. These compounds need to be ingested by the target insect, so they need to be applied to grapes at the start of the ovipositional period to be most effective (Isaacs et al. 2005, Jenkins and Isaacs 2007b, Teixeira et al. 2009, 2011, Mason et al. 2016).

The importance of pheromones in monitoring. Traps baited with sex pheromone are often used to monitor specific insects, and hundreds of insect sex pheromones have been identified (Arn et al. 1992, El-Sayed et al. 2009). The sex pheromones of several economically important tortricid fruit pests have been synthesized and are available commercially for use in traps to monitor males of specific species (Baker 2009, Witzgall et al. 2010). Most of these pheromones are either single hydrocarbon compounds with 9 to 16 carbon atoms, one or two double bonds and a single functional group; or they are blends of two or three such compounds (Arn et al. 1992, El-Sayed et al. 2009). Males of a given species are sensitive to not only the specific ratios of different pheromone components, but typically they only respond to sex pheromone when it is present in a range of acceptable concentrations (Roelofs and Comeau 1969, Arn et al. 1992, Linn and Roelofs 1995, El-Sayed et al. 2009).

The sex pheromone of *P. viteana.* The female sex pheromone of *P. viteana* is a mixture of a two monounsaturated acetates. The major component in this standard blend is (*Z*)-9-dodecenyl acetate (Roelofs et al. 1971), and the minor constituent is (*Z*)-11-tetradecenyl acetate. This blend is attractive to males in compositions from 20:1 to 5:1 (Witzgall et al. 2000), but the major component alone is also effective at attracting males (Roelofs et al. 1971, Witzgall et al. 2000).

Lures loaded with 100µg of the two-component blend of this pheromone are available for monitoring traps in the integrated management of this pest (Botero-Garcés and Isaacs 2003, Isaacs et al. 2012b, Jordan et al. 2013). However the consistency of male captures can vary greatly with time of year (Taschenberg et al. 1974, Taschenberg and Roelofs 1977, Hoffman et al. 1992, Mason and Isaacs 2018), lure manufacturer (Jordan et al. 2013), location in the vineyard, or in relation to adjacent habitat (Hoffman and Dennehy 1989, Botero-Garcés and Isaacs 2003, 2004a). Exploring the factors that affect male captures of this species are important to inform decision-making regarding the need for treatment, and may allow for the development

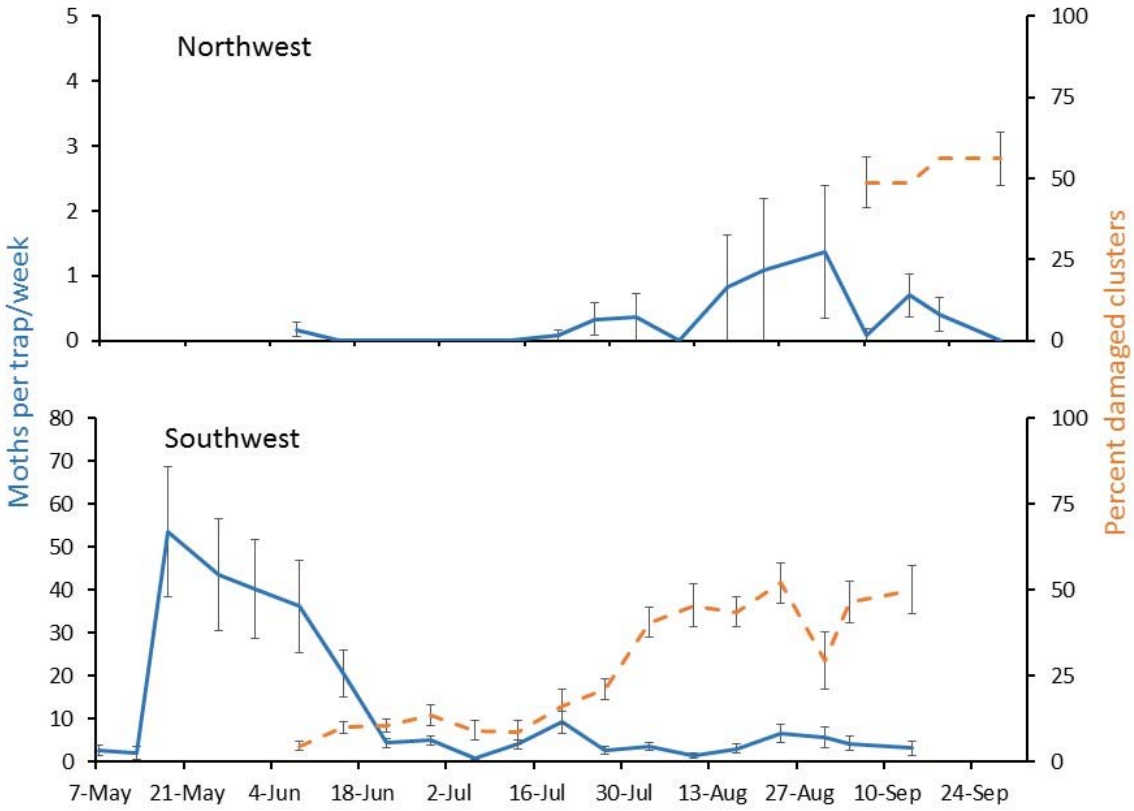

Figure 1.2 Geographic variation in the response of *P. viteana* males to pheromone-baited traps and incidence of grape berry moth feeding damage at representative vineyards in the major grape growing regions of Michigan in 2015. Note difference in Y-axes between graphs. Blue solid line indicates male moth capture and orange dashed line denotes *P. viteana* damaged clusters.

of lures or trapping strategies that improve moth monitoring, potentially improving timing of insecticide applications or other management strategies.

Geographic variation in sex pheromones

Variation between *P. viteana* populations. In the previous decade, variation in male response to *P. viteana* sex pheromone baited traps has been observed during routine scouting during 2015 in different regions of Michigan (Mason unpublished - Figure 1.2). In Northwest Michigan, standard pheromone traps capture very few male moths during the growing season, yet

infestation is found in some vineyards at harvest. These observations raises the possibility that Northern and Southern Michigan populations of this pest may have diverged physiologically or behaviorally, leading to the observed differential responses of males to the standard pheromone blend. Variation in *P. viteana* male captures between Michigan grape growing regions is yet to be reported in the literature.

Regional variation in other species. There are several examples of variation in composition of the pheromone released by females and in the male response between geographically separated populations of several species of lepidopteran pests. In the turnip moth *Agrotis segetum* (Denis and Schiffermüller), the relative proportions of the three pheromone components varies among European populations, and the relative abundance of sensillae on male antennae that are sensitive to these blends also varies between geographic areas (Hansson et al. 1986, Löfstedt et al. 1986). Pheromone trapping of *A. segetum* across Eurasia and Africa has shown that populations north of the Sahara desert have distinctly different pheromone composition to populations south of the Sahara, and males from these regions respond preferentially to the pheromone blend of females from the same region (Tòth et al. 1992). In field tests using pheromone blends from Japanese, Indian and Philippine populations of the rice leaffolder, *Cnaphalocrocis medinalis* Guenée, Kawazu et al.(2009) showed male *C. medinalis* from Balinese and Javanese populations were attracted to the Japanese blend, but were not attracted to the Indian or Philippine blends. While researching geographic variation in male captures of two tortricid pest species, *Planotortrix excessana* Walker and *Ctenopseutis obilquana,* Walker in New Zealand, Foster et al. (1986) showed that sibling species in each of these pests had become reproductively isolated, and sibling species had slightly different pheromone blends. In the obliquebaded leafroller, *Christoneura rosaceana* (Harris) male response to sex pheromone is stronger when males and females are from the same geographic regions in North America (El-Sayed et al. 2003, Stelinski

et al. 2007b). These studies and subsequent research led to the development of eastern and western pheromone lure blends that are used for this species in those respective regions of North America (El-Sayed et al. 2003, Stelinski et al. 2007b).

One objective of this dissertation was to explore the factors that may be involved in the differences in *P. viteana* male captures that have been observed between northern and southern growing regions in Michigan. Behavioral tests in the lab and the field were used to compare male responses to different pheromone sources to determine if males from different regions of Michigan respond similarly to a standard pheromone blend. This study also tested whether males from northern and southern regions respond preferentially to females from the same region.

Seasonal variation in male captures

The paradox between moth captures and infestation. *Paralobesia viteana* pheromone lures are available commercially, however in Southwestern Michigan males of this species are only captured consistently during the first generation that is active around bloom. This generation causes little larval feeding damage, but during the second and third generations that occur in the early and late summer, respectively, the level of oviposition on berries, and subsequent larval infestations increase greatly, yet few males are caught in monitoring traps (Figure 1.3) (Snyder et al. 1992, Teixeira et al. 2009, Isaacs et al. 2012b, Mason and Isaacs 2018). This incongruence between male moth captures and larval infestation prevents development of action thresholds based on captures in pheromone traps as has been done for other lepidopteran fruit pests (Binns and Nyrop 1992, Bradley et al. 1998, Reddy and Manjunatha 2000, Knight and Light 2005a).

The temporal variation in *P. viteana* male response to traps is concurrent with substantial changes that occur in the grapevine during seasonal growth. One hypothesis is that the observed

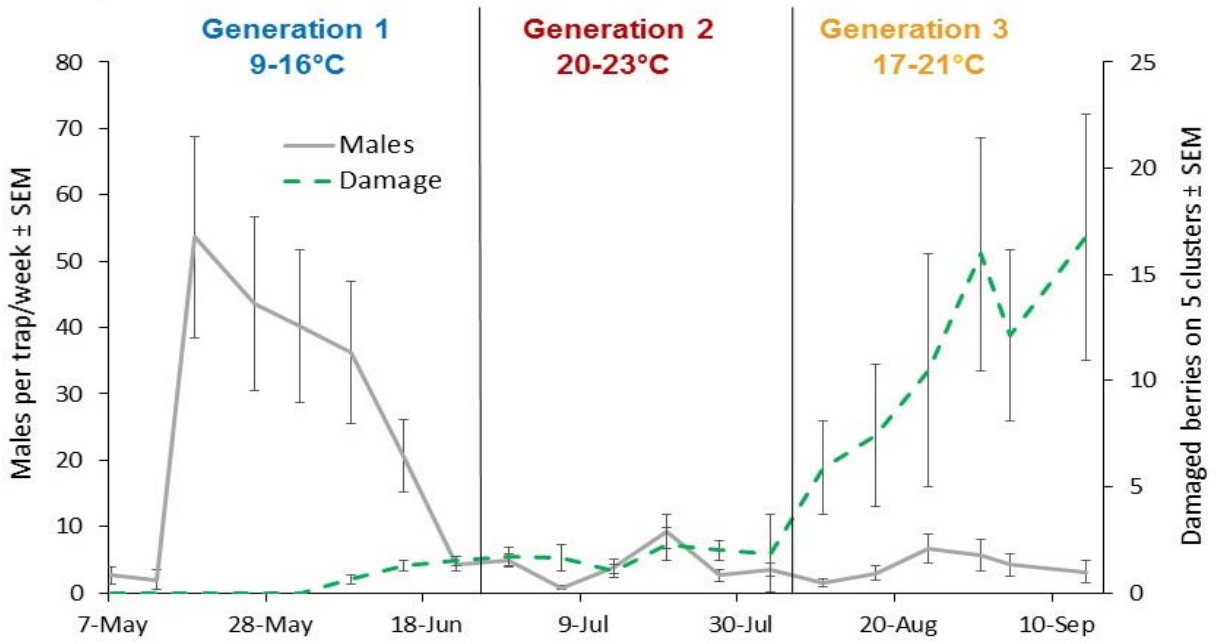

Figure 1.3. Phenology of *P. viteana* in southwest Michigan. Captures of males (gray solid line) are highest during the first generation, when berry infestation (green dashed line) is lowest. In the second and third generations, male capture is lower, but infestation increases until harvest. Data are averages from eight commercial vineyards during 2015.Temperatures at the top of the figure are the range of average daily temperatures for each generation in 2015.

differences in male captures are related to the phenology of the host plant. The underlying chemical and structural changes in the grapevine may affect the behavior of *P. viteana* such that the number of males that are caught in pheromone traps is reduced as the season progresses.

Plant volatiles as insect attractants. Volatile host plant chemicals can influence host-finding in some key crop pests (Bruce et al. 2005, Leskey et al. 2008, Piñero and Dorn 2009, Saveer et al. 2012). Most previous studies in this area have focused on the behavior of females, but either sex can respond to host plant volatiles in the laboratory or field (Stelinski et al. 2003, Knight and Light 2005b, Faraone et al. 2013). Within species, odorant receptors and neural pathways are also similar between sexes (Jordan et al. 2009, Varela et al. 2011). Female codling moths, *Cydia pomonella* L., are attracted to apple odors, and in laboratory studies show increased oviposition and flight response compared to an odorless control (Wearing et al. 1973, Yan et al. 1999,

Coracini et al. 2004). In addition, *C. pomonella* males are attracted to compounds derived from pear, and these can be used in monitoring traps resulting in higher captures than with pheromone alone (Light et al. 2001, Knight et al. 2005, Il'ichev et al. 2009).

In the oriental fruit moth, *Grapholita molesta* (Busck), females are attracted to a combination of green leaf volatiles and other aromatic compounds that are derived from peach shoots (Piñero and Dorn 2007). In addition, male and female *G. molesta* are attracted to monitoring traps baited with volatiles derived from peach and pear (Lu et al. 2012). Female European grapevine moth (*L. botrana*), consistently flew upwind and landed on unripe grape clusters in wind tunnel tests (Tasin et al. 2006). In subsequent studies, *L. botrana* females were also attracted to a four-component blend of volatile compounds collected from the headspace around confined grapes clusters, and females were attracted to synthetic grape volatiles in field studies (Tasin et al. 2009).

In similar studies, *P. viteana* females oriented toward grapevine components in wind tunnel tests, and this response was stronger for leaves and shoots than for flowers, unripe berries or mature fruit (Cha et al. 2008a). Male *P. viteana* were also attracted to a blend of grape shoot volatiles in the wind tunnel and in field tests (Cha et al. 2008b, Loeb et al. 2011). These latter studies show that grape shoot volatiles are important and attractive cues for both male and female *P. viteana*, and they also suggest that the volatile-rich grape canopy may influence the interaction between male *P. viteana* and monitoring traps.

Do grape clusters influence male captures? The size and chemical composition of grape clusters and the vine canopy change considerably during seasonal growth and development (Hrazdina et al. 1984, Mullins et al.1992, Schultz 1995). Early in the season, few leaves and small flower or berry clusters are present on vines. As the growing season proceeds, the clusters increase in weight 100 fold, and as the berries soften and become ripe, physiological changes

lead to increased soluble solids, lower organic acids (e.g. tartaric, malic and citric), and, depending on grape variety, an increase in pH to a final value between 2.8 and 3.7 at harvest (Hrazdina et al. 1984, Jackson and Lombard 1993). The concurrent increase in phenolic compounds, such as flavonoids and anthocyanins, can impart distinctive odors and flavors to ripening grape clusters (Singleton and Trousdale 1983), and volatile terpenes that increase greatly after veraison (fruit coloring) also change the aroma and flavor characteristics of grapes (Dimitriadis and Williams 1984, Rosillo et al. 1999). These physiological transformations contribute to an odor landscape that varies consistently through the growing season, and these changing host plant cues may provide information that *P. viteana* could use to orient to its host plant, and find potential mates and oviposition sites. If *P. viteana* mating occurs on the grape clusters, or if females spend the majority of their time on clusters, males would have an increased chance of finding females, and mating successfully, if they can detect and orient toward the volatiles produced by grape clusters. This could lead to a "competition" between clusters and traps that might lead to a reduction in the number of male captures in traps. In addition the combination of grape volatiles and sex pheromone may be more attractive to male *P. viteana* than sex pheromone alone, as has been shown for *C. pomonella* (Light et al. 2001). This preference could result in reduced *P. viteana* male captures in pheromone traps because the amount of grape cluster volatiles increases during the season due to the increase in cluster size and the ripening of the crop.

Does the grape canopy reduce male captures? In addition to changes that occur as clusters develop, the physical structure of the juice grapevine canopy is also transformed through each season. In varieties that are bred and managed to maximize crop yield, such as juice grapes (*V. labrusca,* cvs. Concord and Niagara), the vines are typically cane-pruned and trained to a single wire top-cordon system. The resulting trailing habit of shoot growth causes the canopy to

become increasingly dense, and it creates a thick matrix of shoots and leaves as the season progresses (Smart et al. 1982a, Miller and Howell 1998, Dry 2000, Bates 2008). The quantity and quality of grape shoot and leaf volatiles that are present in a vineyard change as the canopy grows and becomes more dense (Tasin et al. 2006). The dense canopy imposes challenges on disease and insect management by forming an impediment to air flow and reducing spray coverage (Wise et al. 2010). The increase in canopy density may also affect monitoring *P. viteana* with pheromone traps by altering trap findability (Miller et al. 2006), making it less likely that male moths find traps. The canopy could physically block males from reaching traps, it may disrupt the structure of the pheromone plumes from lures, or the naturally occurring grape shoot volatiles may draw moths away from traps.

Temperature and photoperiod effects on P. viteana

Relationships between male captures, temperature and photoperiod. In temperate regions, there are consistent patterns of variation in temperature and photoperiod over a growing season, and these correspond to the observed phenology of *P. viteana* (Figure 1.3). Typically in Michigan, the spring is cool with average daily temperatures around 10-15ºC, and the photoperiod increases from ~13 to 15 hours. Over this time *P. viteana* male captures are high and cluster infestation is low (Figure 1.3). The highest average daily temperatures (20 to 23ºC) occur in the middle to late summer and the longest day lengths (14.5 to 15.5 hours) also occur during this period. *P. viteana* male captures are typically at the lowest for the season, and in contrast cluster infestations begin to sharply increase (Figure 1.3). In the late summer and early fall cluster infestation is usually at its highest point and male captures increase, but remain much lower than in the spring (Figure 1.3). Average daily temperatures range from 17 to 21ºC and are

intermediate to those of spring and summer, and the photoperiod decreases during this time from about 14.5 to 12.5 hours (Table 4.1).

Temperature and insect physiology. Most insects are ectothermic, and consequently many aspects of insect physiology, development, behavior and reproduction are dependent on ambient temperature (Ratte 1985, Honek 1996, Frazier et al. 2006). For a diverse group of insects, the rate of enzymatic processes involved in physiological functions such as metabolism, hormone production, and digestion approximately doubles for each 10°C increase in ambient temperature (Rao and Bullock 1954, Ratte 1985). The range of temperatures where this Q10 doubling occurs is limited to temperatures between a critical minimum and a critical maximum, and although this differs somewhat between species and climatic regions, this temperature range is typically close to 10 to 30°C for species living in temperate zones (Nielsen et al. 1999, Nespolo 2003).

Longevity. The effect of temperature on adult longevity has been studied in several insect orders, and across all taxa, longevity decreases with increasing temperature (Graham et al. 1967, Emana 2007, Gómez et al. 2009). In field tests, Asaro and Berisford (2001) showed males of the first generation of the Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock) lived significantly longer than males in the second and third generations. The authors also demonstrated in the lab that there is a significant reduction of longevity with increasing constant temperature. Longevity of codling moth, *Cydia pomonella* (L.) in northern European populations was shorter for males and females held in a warm temperature regime (18°C day and 15°C night) compared to those in a cool temperature scheme (13°C day and 10°C night) (Sæthre and Hofsvang 2002).

Activity and movement

The frequency and intensity of insect movement and activity are also dependent on temperature. Between critical minimum and maximum temperatures, thermal performance, measured as cricket (*Acheta domesticus* L.) running speed and jumping distance, increases with temperature (Lachenicht et al. 2010). Similarly, locomotor activity of the tenebrionid, *Gonocephalum simplex* (Fabricius) increases with temperature up to a critical maximum temperature, and then activity decreases rapidly as the insect succumbs to heat stress (Klok et al. 2004).

Effects on flight. Insect flight shows a similar response to temperature, occurring above a critical minimum and decreasing at temperatures at and above a critical maximum. In early studies, Taylor (1963) found the minimum temperature for flight to be 15.5°C for *Vespula germanica* (L.) queens, 17.5°C for alates of the bean aphid *Aphis fabae* (Scopoli) and 16.5°C for the soldier beetle *Rhagonycha fulva* (Scopoli). However, (Taylor 1963) also found the minimum temperature for flight for the large bodied noctuids (~30-40mm wing span) *Agrochola lychnidis* (Denis & Schiffermüller) and *Amphipyra tragopoginis* (Clerck) to be considerably lower at 9 and 10.5°C, respectively. For smaller moths such as the tortricids (wing spans ~10-20mm) the critical minimum temperatures for flight are in the range of 12-15°C. For example the minimum temperature for flight in the eastern spruce bud worm, *Choristoneura fumiferana* (Clemens) is 14°C (Greenbank et al. 1980) and for oriental fruit moth *Grapholita molesta* (Busck), that temperature was 15°C (Rothschild and Minks 1974). Codling moth minimum flight temperature was found to be 12.7°C (Batiste et al. 1973), and for the light brown apple moth, *Epiphysis postvittana* (Walker) the minimum temperature for flight ranged from 8 to 11°C (Danthanarayana 1976).

The critical maximum temperature for flight has been quantified less often, but for codling moth the maximum was found to be 26.7°C (Batiste et al. 1973), and for the light brown apple moth, the maximum temperatures for flight ranged from 27 to 28°C (Danthanarayana 1976).

Oviposition and temperature. Oviposition is also highly dependent on temperature. In pink bollworm, oviposition increases 10-fold between females held at 15.5°C and those reared at 18.3°C. Over the range from 18.3 to 26.7°C oviposition was similar. At 32°C oviposition decreases by about half and at temperatures of 37.8°C and above oviposition is reduced a further 100-fold (Graham et al. 1967). Sæthre and Hofsvang (2002) showed northern European populations of codling moth begin to lay eggs at temperatures as low as 12.3°C, and the amount of oviposition increases steadily through a range of temperatures ending at 25°C (the highest temperature measured). Oriental fruit moth oviposition is highest from 22 to 30°C, and drops off rapidly at 33°C, while no egg-laying is observed at 35°C (Notter-Hausmann and Dorn 2010).

Effects of temperature and photoperiod on activity cycles.

The influence of temperature and photoperiod on daily and seasonal cycles of growth, development, activity, and reproduction have been studied in several insect families (Tauber and Tauber 1981, Beck 1983, Régnière et al. 2012, Tonnang et al. 2017). Moths in the family Tortricidae have been the subjects of much of this work owing to the status of some members of this family as economically important fruit pests.

Temperature and activity. In moth species where mating occurs at the beginning of the scotophase (dark phase), females emit pheromone (call) earlier in the day at lower temperatures. At higher temperatures, the female calling period is generally shorter, and occurs in the scotophase (Cardé et al. 1975b, Baker and Cardé 1979a, Delisle and McNeil 1987, Webster

1988). Male responsiveness to pheromone also occurs earlier in cool temperatures (Cardé et al. 1975a, b) Thus for some species the current air temperature, or changes in temperature dictate when mating behaviors will occur. In other species, ambient temperature in the period immediately before the scotophase determine the periods of female calling and male responsiveness (Baker and Cardé 1979a, Haynes and Birch 1986, Delisle and McNeil 1987).

Photoperiod and activity. Day length can also be an important cue for determining seasonal or daily patterns of activity (Delisle and McNeil 1987). For example decreasing day length in the autumn can trigger physiological changes that can alter insect development and lead to diapause (Riedl and Croft 1978, Gangavalli and Aliniazee 1985, Nagarkatti et al. 2001, Saunders 2001). Photoperiod can influence mating behavior, although relatively few studies have examined the effect of day length on mating, and the effect varies across species and sexes. As day length decreases, the female calling period is lengthened (Kanno 1981, Gerber and Howlader 1987), but decreasing day length in the late summer and fall reduce male responsiveness to sex pheromone (Delisle and McNeil 1986, 1987, Mulder et al. 1989). In cases where there is an effect of photoperiod on either male or female mating behavior, the effect is typically minor compared to the effect of temperature (McNeil 1991).

Identification of the patterns of daily mating activity can inform other management approaches such as mating disruption, mass trapping and attract and kill (McNeil 1991, El-Sayed and Suckling 2005, El-Sayed et al. 2006, 2009, Baker 2009, Rodriguez-Saona and Stelinski 2009). For example, in mating disruption of some tortricid pests, automated pheromone dispensers can be programmed to emit pheromone only during the period when females are calling, and this could reduce the costs of deploying mating disruption (Isaacs et al. 1999, Fadamiro and Baker 2002, Stelinski et al. 2007a, Jones and Wiman 2012).

Pheromone release and temperature. In developing sex pheromone lures for monitoring, the optimal amount of pheromone to use in a lure is often determined by comparing male captures in traps that contain a range of concentrations of an active compound (Roelofs et al. 1969, 1971, Witzgall et al. 2000, Suckling et al. 2005). Traps with too low of a pheromone concentration will not be attractive to males and lower captures will result (Roelofs et al. 1971, Taschenberg et al. 1974, Linn and Roelofs 1995). Adding more pheromone can increase the longevity of activity, but traps that have a higher than optimal concentration of pheromone can repel male moths, resulting in reduced catch compared to lower concentrations (Linn and Roelofs 1995, Witzgall et al. 2000, Suckling et al. 2005). The rate of pheromone release from lures increases with increasing temperature (McDonough et al. 1989), and the increase of pheromone release at high temperatures may suppress male catch in periods of hot weather (Sanders 1981, Cork et al. 2003, Cork 2016, Cardé et al. 2018).

The effect of temperature and photoperiod on the phenology and behavior of *P. viteana* were investigated to determine when mating activity and reproductive behaviors occur during the growing season. This is an essential component of integrated management of tortricid pests (Riedl et al. 1976, Damos and Savopoulou-Soultani 2010, Jones and Wiman 2012), yet little is known about the role of temperature and day length in regulating *P. viteana* mating activity or periodicity, and less is known about how temperature affects male response to sex pheromone traps.

Research goals

Some of the primary challenges of *P. viteana* management are due to the limited understanding of the factors that affect chemical communication between the sexes in this pest, and how these factors in turn govern mating and reproductive behaviors. This dissertation explored the variation in male *P. viteana* response to sex pheromone between geographic regions and over the course of a growing season. The results of this work help to explain some of the inconsistencies inherent in monitoring this important pest and how these factors interact to produce the observed phenology of *P. viteana*.

The specific goals of this research were to:

1) Determine the cause of low male captures in standard pheromone traps in northwest Michigan vineyards where *P. viteana* infestations occur.

2) Determine whether the seasonal changes in the grape canopy or cluster development influence male captures to produce the observed phenology of *P. viteana.*

3) Characterize the role of temperature and photoperiod in daily and seasonal patterns of *P. viteana* reproduction.

4) Improve understanding of how temperature can influence the biology of *P. viteana* and affect the function of pheromone traps, and how this ultimately produces the observed phenology.

CHAPTER 2

LOW CAPTURES OF MALE *PARALOBESIA VITEANA* IN MONITORING TRAPS ARE NOT DUE TO GEOGRAPHIC VARIATION IN MALE RESPONSE TO PHEROMONES

INTRODUCTION

Insect species with distributions that cover large geographic ranges often show variation in traits such as body size, physiology, coloration, or behavior among subpopulations that reside along that range (Futuyma and Moreno 1988, Hill et al. 1999, Goropashnaya et al. 2001, Eckert et al. 2008, Cruz-Esteban et al. 2017). Widespread planting of crops has helped to artificially increase the range of many pest insects, such as Colorado potato beetle, *Leptinotarsa decemlineata* (Hare 1990), fall armyworm, *Spodotera frugiperda* (Takahashi et al. 2012, Dávila et al. 2013, Chen et al. 2015), and tephritid fruit flies (Yee and Goughnour 2008, Hood et al. 2013). Pest populations can diverge so much across the host plant range that the phenotypic or genetic differences between geographically separated populations lead to the formation of races, subspecies or distinct species (Foster et al. 1986, Roderick 1996). These populations can show considerable variation in physical and behavioral characters, even though the sub-groups remain reproductively compatible (Hsiao 1978, Ramaswamy et al. 1988, Goropashnaya et al. 2001; Cruz-Esteban et al. 2017).

There are many examples of variation in the composition of the pheromone released by females and in the male response between geographically separated populations of several species of lepidopteran pests. In the turnip moth *Agrotis segetum* (Denis and Schiffermüller), the relative proportions of the three pheromone components varies among European populations, and the relative abundance of sensillae on male antennae that are sensitive to these blends also varies between geographic areas (Hansson et al. 1986, Löfstedt et al. 1986). Pheromone trapping of *A. segetum* across Eurasia and Africa has shown that populations north of the Sahara desert

have distinctly different pheromone composition to populations south of the Sahara, and males from these regions respond preferentially to the pheromone blend of females from the same region (Tòth et al. 1992). In field tests using pheromone blends from Japanese, Indian and Philippine populations of the rice leaffolder, *Cnaphalocrocis medinalis* Guenée, Kawazu et al. (2009) showed male *C. medinalis* from Balinese and Javanese populations were attracted to the Japanese blend, but were not attracted to the Indian or Philippine blends. While researching geographic variation in male captures of two tortricid pest species, *Planotortrix excessana* and *Ctenopseutis obilquana,* in New Zealand, Foster et al. (1986) showed that sibling species in each of these pests had become reproductively isolated, and sibling species had slightly different pheromone blends. In the obliquebanded leafroller, *Christoneura rosaceana* (Harris), male response to sex pheromone is stronger when males and females are from the same geographic regions in North America (El-Sayed et al. 2003, Stelinski et al. 2007b). These studies and subsequent research has led to the development of eastern and western pheromone lure blends that are used for this species in those respective regions of North America.

The female sex pheromone of *Paralobesia viteana* (Clemens) is a mixture of two monounsaturated acetates, with a major component of (*Z*)-9-dodecenyl acetate, and a minor constituent, (*Z*)-11-tetradecenyl acetate. This blend is attractive to males in compositions from 20:1 to 5:1 (Witzgall et al. 2000), but the major component alone is also effective at attracting males (Roelofs et al. 1971, Witzgall et al. 2000). Lures loaded with 100 µg of this pheromone are available for monitoring traps in the integrated management of this pest (Botero-Garcés and Isaacs 2003, Isaacs et al. 2012a, Jordan et al. 2013). The consistency of male captures can vary with time of year (Taschenberg et al. 1974, Taschenberg and Roelofs 1977, Hoffman et al. 1992, Mason and Isaacs 2018), lure manufacturer (Jordan et al. 2013), location in the vineyard, or in relation to adjacent habitat (Hoffman and Dennehy 1989, Botero-Garcés and Isaacs 2003,

2004a). However, variation in *P. viteana* male captures between growing regions has not been previously documented.

Variation in the response of males to *P. viteana* sex pheromone baited traps has been observed during routine scouting between different regions of Michigan (see Chapter 1, Figure 1.2). In Northwest Michigan, standard pheromone traps capture very few male moths during the growing season, yet infestation is found in some vineyards at harvest. These observations suggest that Northern and Southern Michigan populations of this pest may have diverged physiologically or behaviorally, leading to the observed differential responses of males to the standard pheromone blend.

Developing monitoring programs that consider the regional differences in male response to pheromone traps and allow accurate detection and monitoring of Northern Michigan populations would greatly improve management of this important pest. This study tested the hypothesis that the observed difference in the number of males captured in pheromone traps in Northwest and Southwest Michigan is due to differences between regions in male response to the standard pheromone. I posed two questions to test this hypothesis:

1) Do male *P. viteana* from Northwest and Southwest Michigan respond differently to a standard pheromone blend?

2) Do male *P. viteana* from Northwest and Southwest Michigan respond differently to females from these two regions?

METHODS

Study insects

Male and female *P. viteana* were obtained for behavioral testing and for captive female field trapping studies by opportunistically collecting *P. viteana*-infested fruit from six Southwest- and four Northwest-Michigan vineyards during July-September in 2014, 2015 and 2016. Following the methods of Taschenberg (1951, 1969), fruit from each site was held in a separate plastic bin containing strips of clear plastic for developing larvae to use as a substrate on which to pupate. Pupae were collected every three days from each bin, and then placed in individual 30 ml deli cups and held in an environmental chamber (28°C, 17:7 L:D and 56-60% RH) until the moths emerged. Upon emergence, moths were anesthetized with CO_2 and examined with a dissecting microscope to determine the sex. Adults were used within 5 days after emergence.

Bioassays

A Y-tube olfactometer (Figure 2.1) was used in all experiments. This apparatus was custom built by the Michigan State University Chemistry Glass shop (East Lansing, MI, USA) using 1.5 cm i.d. glass tubing. The body of the Y-tube was made by heat-joining a 13 cm length of glass tubing to two 7 cm arms, each positioned 30º from the longitudinal axis of the 13 cm tube with all three tubes positioned in the same plane. The body of the Y-tube had ends with female connections, each with a ground glass interior surface to connect the upper arms of the Y-tube to sample chambers. A moth transfer tube connected to the third opening facilitated introduction of male moths into the apparatus. Sample chambers/transfer tubes were constructed from a single piece of 2 cm (o.d) glass tubing, a porous glass partition that divided the lumen of the tube so it could be used as a sample chamber or a moth transfer tube. The upper compartment

(sample chamber) was topped by a 3 cm threaded collar and this held either a pheromone sample or a female *P. viteana*. Threaded open-top bushings (McMaster-Carr, Elhurst, IL, USA) were used to connect the air supply to the sample chambers (Figure 2.1). The compartment below the glass partition was made with a ground glass outer surface that fit into the female ends of the Y-tube body. After each run the Y-tubes were washed in methanol and hexane and dried before the next set of runs. Five sets of Y-tube bodies and sample chambers/transfer tubes were constructed to allow for changing out the apparatus periodically without having to wash the Y-tube component parts and wait for them to dry.

Air from the pressurized building air supply was purified with activated carbon, and hydrated by passing the airflow through 0.83 cm food grade tubing (McMaster-Carr, Elhurst, IL, USA) into a glass air stone submerged in de-ionized water inside a stoppered 250 ml Erlenmeyer flask with an outflow port. The hydrated outflowing airstream was split with a branched connector (McMaster-Carr, Elhurst, IL, USA) and each branch flowed through additional similar tubing (~0.5 m) into a 300 ccm flowmeter (Analytical Research Systems, Gainesville, FL, USA). The flow through each sample chamber on the upper arms of Y-tube apparatus was held at 25 ml/min.

To test whether male moths from Northwestern and Southwestern Michigan respond differently to a standard pheromone blend, a 1 µg/ml stock solution of *P. viteana* pheromone (10:1 mix of (*Z*)-9-dodecenyl acetate and (*Z*)-11-tetradecenyl acetate) in hexane was blended from pure pheromone components (Bedoukian, Danbury, CT, USA). A 5µl aliquot (5ng) of this

Figure 2.1. Y-tube apparatus to test behavioral responses of male *P. viteana* to a standard pheromone blend or to females from different populations. Left) Y-tube set up showing experimental enclosure; middle) close-up of Y-tube; right) male moth in one arm of the Y-tube.

pheromone solution was pipetted onto a 0.5 x 0.5cm piece of filter paper and allowed to dry. The treated filter paper was then placed in a sample chamber attached to one arm of the Y-tube. A similar piece of filter paper treated with 5µl of hexane was placed in the sample chamber in the other arm of the Y-tube. A small ball of glass wool was placed at the top of each sample chamber to separate the end of the air supply tubing from the contents of the sample chamber and prevent contamination of the air-supply tubing.

All assays were performed in a walk-in environmental chamber set to 28°C and 60% RH and during the scotophase when males and females are most active (Cha et al. 2008a, Cha et al. 2008b, see also Chapter 4), and when female *P. viteana* have the highest pheromone titer (Witzgall et al 2000). A run began when a 2 to 3 day old male *P. viteana* moth was moved into a transfer tube that was then attached to the base of the Y-tube. The male was able to move freely in the Y-tube and chose an arm. A choice was scored when the test insect moved through the Y-tube, continued at least half way up one arm of the tube, and remained in the arm for at least 5 seconds. Males that did not move into an arm after 3 minutes were scored as “no choice”, and these runs were excluded from the analysis. Each male was used only once, and the location of

the arm where the pheromone sample was presented was alternated every 5 to 10 runs to reduce positional bias. The Y-tube apparatus was washed with methanol and then hexane and allowed to dry between trials. To test whether Northwest or Southwest Michigan males chose the Y-tube arm with the pheromone sample more or less often than by chance, a two-tailed Fisher's exact test was used (JMP v13.1.0, 2016 SAS Institute, Cary NC, USA).

To compare the responsiveness of Northwest and Southwest Michigan male *P. viteana* to females from the same or different region, a series of Y-tube bioassays were conducted. Individual moths were obtained in the collections described above, and the Y-tube olfactometer was the same as described previously (Figure 2.1). These assays were performed during the scotophase in a walk-in environmental chamber set to 28°C and 60% RH. One two to three day old female *P. viteana* from a Northwest Michigan vineyard and one similarly aged female from a Southwest Michigan vineyard were placed in sample chambers in separate arms of the Y-tube, and a small ball of glass wool was positioned in each sample chamber above the female. For each run, a two to three day old naïve male *P. viteana* moth was transferred to the base of the Y-tube and allowed move in the tube. Choices were scored as above, and the sides of the Y-tube that contained females from the two regions were alternated to reduce positional bias. Females were used for 5-10 runs and then replaced, and males were used only once. The proportion of moths that chose a female from the same population was compared using a two-tailed Fisher's exact test (JMP v13.1.0, 2016 SAS Institute, Cary NC, USA).

Figure 2.2. Captive female traps and lures for comparing the response of males in northwestern and southern Michigan vineyards. Left to right: Captive females in a mesh cage inside a trap. Standard pheromone lure loading set-up (inset in upper left shows orientation of septa for loading). Pherocon VI (Delta) trap with a standard pheromone lure deployed in a vineyard.

Field trials

To determine the response of resident males to a standard pheromone blend and to females from Northwestern and Southwestern Michigan, a trapping experiment was established in 2015, 2016 and 2017 at three Southwest and three Northwest Michigan vineyards, each with a history of grape berry moth infestations. At each vineyard, white Pherocon VI traps (Trece Inc, Adair, OK, USA) were baited with captive females or lures containing the standard *P. viteana* pheromone blend described above.

Female *P. viteana* for these experiments were obtained from the collections described previously. On the day prior to deploying traps, individual 2 to 3 day old female moths were anesthetized with CO_2 and transferred, in groups of 5, into a metal mesh cage (Figure 2.2) along with a 1.27 cm piece of moistened dental wicking (Absorbal, Wichita, Kansas, USA). Cages were then closed and held in an environmental chamber at 10°C, 60% RH overnight. The following day, these females were transported to field sites in a cooler with ice packs for deployment in traps

Lures for this experiment were made using the standard *P. viteana* pheromone blend described above. The stock solution of *P. viteana* pheromone in hexane (1µg/ml), a 10-fold dilution of this solution, and pure hexane as a negative control were used to make solutions of 1.0, 0.1, or 0µg/ml. Small rubber septa (West Pharmaceuticals, Exton, PA, USA) were arranged open side up in aluminum mesh racks with 0.64cm^2 openings (Figure 2.2), and septa were filled with 1 ml of the approriate dilution in four 0.25ml aliquots to produce a set of lures that contained 1.0, 0.1, or 0µg of pheromone per lure. These quantities are well below the 100 µg that is used in commercial lures (Jordan et al. 2013), and I chose these reduced levels to make the lures more comparable to the 1 ng of pheromone produced by female *P. viteana* (Witzgall et al. 2000). The mesh racks were held in a fume hood during loading, and then septa were stored in the hood until the solutions evaporated. An additional 0.25ml of hexane was then pipetted into each septum to help the pheromone adsorb onto the septum wall. The lures were dried in the fume hood and then grouped by pheromone load and each group was placed in a separate 200ml labeled glass jar, sealed with teflon tape and stored in a freezer (-5°C) until the lures were deployed in traps. On the day of deployment, the lures were transported to field sites in a cooler with ice packs separate from the females.

At each vineyard, white Pherocon VI traps (Trece Inc, Adair, OK, USA) were baited with one of the following treatments: five caged Northwest Michigan females, five caged Southwest Michigan females, a 1µg *P. viteana* pheromone lure, a 0.1µg pheromone lure or a blank lure as a negative control. Traps were hung from the trellis wire (1.5m above the ground) next to vineyard end posts that were adjacent to a woodlot. Traps were separated by 10m, and deployed for one week. Trapping dates varied between years with traps in the southwest deployed 11 to 25 August 2015; 17 to 31 May, 12 to 19 July and 5 to 12 August 2016, yielding a total of 17 vineyard x date combinations. Traps in the northwest were deployed for a total of 15 vineyard x date

combinations with dates of 22 to 29 August 2015, 17 June to 1 July 2016 and 9 to 29 August 2017. When traps were checked, sticky trap liners, caged females and lures were removed and replaced, and the location of traps was re-randomized.

Due to previous knowledge about the differences in captures between Northwestern and Southwestern Michigan vineyards, I expected region to be a strong and significant predictor of male captures, and inclusion of this factor in our statistical analysis would cause a significant interaction between lure treatment and region. In addition to complicating the interpretation of the results, this would reduce the main effect of bait type. Thus, I chose to compare traps within regions. The documented variation in male captures between times of year (Botero-Garcés and Isaacs 2003, Mason and Isaacs 2018), resulted in considerable variation between sample dates. Therefore, I chose to compare the sum of male captures in each lure treatment over the entire experiment. The total number of male captures was compared between treatments with a mixed model ANOVA with vineyard as a random factor and lure type as a fixed factor separately by region using vineyards as replicates (JMP Pro Ver 13.1 2016, SAS Institute Inc 2016).

RESULTS

Bioassays

In Y-tube tests of male regional response to a standard pheromone sample, 69% of the tested males moved in the Y-tube olfactometer and made a choice between arms containing pheromone or plain air. Males from northwestern vineyards chose the arm with the pheromone sample in 39% of the runs, while southwestern males chose the arm with the pheromone sample in 60% of the runs, and these percentages were not significantly different between regions (Figure 2.3, $\chi 2 = 1.8$, n=43 df =1, $P = 0.22$).

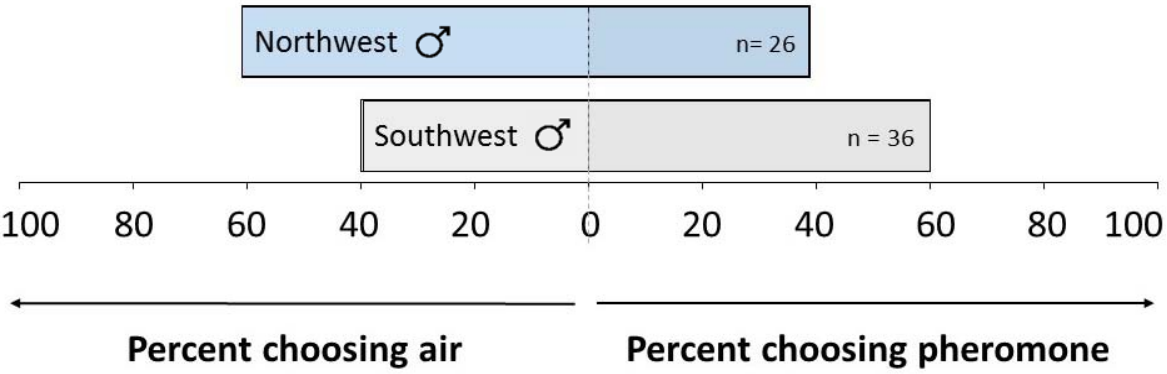

Figure 2.3. Percentage of *P. viteana* males from northwest and southwest Michigan that chose the Y-tube olfactometer arm containg a pheromone sample. This was not significantly different between regions.

In bioassays that tested whether males respond preferentially to females from the same region, 84% of the tested males moved in the Y-tube olfactometer and made a choice between arms that contained a northwestern or southwestern female. Northwestern and southwestern males responded similarly to females from either population in Y-tube assessments. Males from

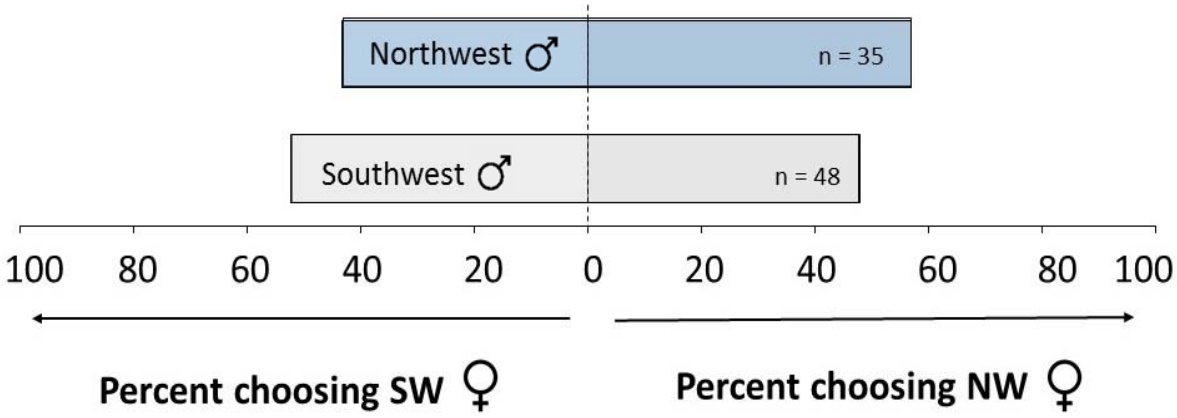

Figure 2.4. Percentage of *P. viteana* males from northwest and southwest Michigan that chose the Y-tube olfactometer arm containg a female from the same or different regions. Males did not prefer females from the same region.

northwestern vineyards chose the arm with the northwestern female in 57% of the runs, while southwestern males chose the arm with the southwestern female in 50% of the runs (Figure 2.4), and male response was not significantly different between regions ($\chi 2 = 0.42$, n=83, df =1, $P = 0.66$).

Field trials

In the field trial to test the response of resident males in different regions to traps baited with captive females from Northwest or Southwest Michigan and to a standard pheromone blend, the total number of males captured in traps deployed in Southwest Michigan vineyards was over 45 times higher than those in Northwest Michigan. In Southwest Michigan, male captures in the 1 µg lure treatment were at least 20 fold higher than captures in either of the captive female traps or in the control traps, and these differences were statistically significant ($F_{4, 8} = 14.84$, $P = 0.0009$). There was a similar pattern of captures across bait treatments in Northwest and Southwest Michigan vineyards. Traps containing the standard pheromone lure caught a greater

number of moths than the traps loaded with either Southwest or Northwest Michigan females or the control (0 µg) lure (Figure 2.5). However, male captures in the 1 µg lure in vineyards in Northwest Michigan were only 2 to 4 fold higher than in other traps and captures were not significantly different across trap treatments ($F_{4, 8} = 2.12$, $P = 0.17$; Figure 2.5).

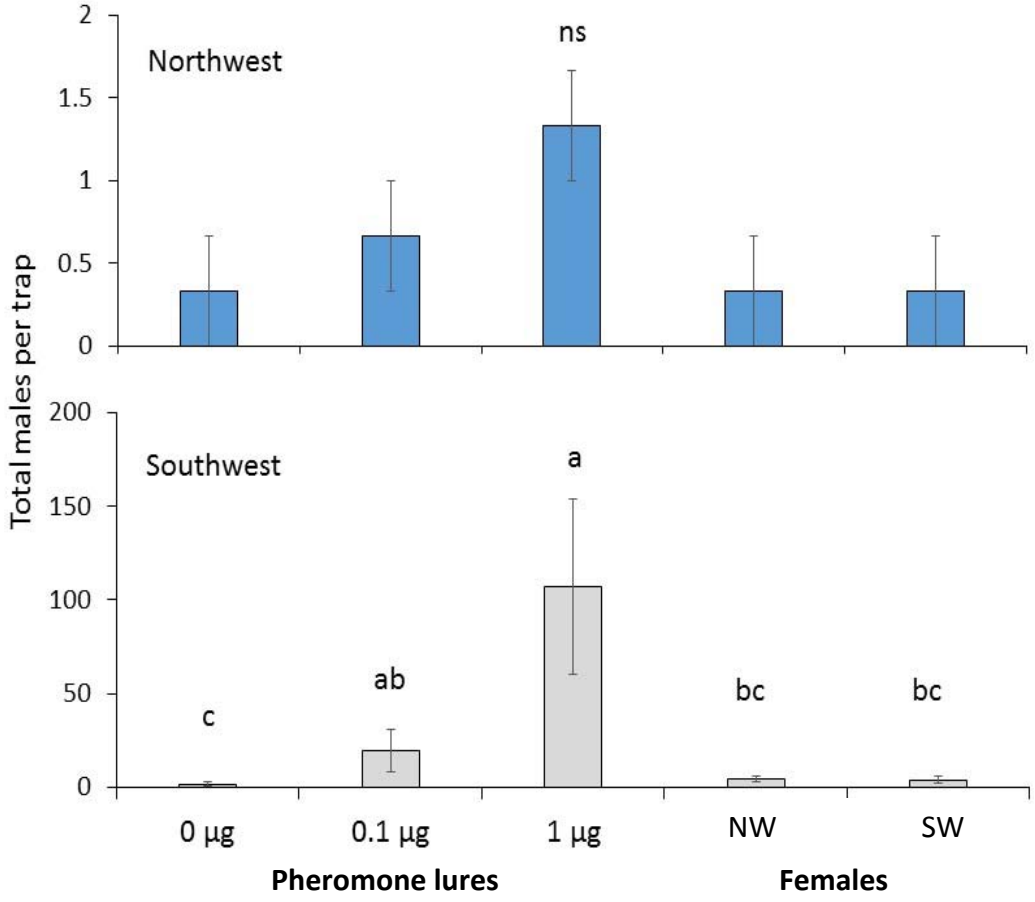

Figure 2.5. Response of resident male *P. viteana* to traps deployed in vineyards in Northwest- and Southwest-Michigan. Traps were baited with pheromone lures or captive *P. viteana* females from Northwestern or Southwestern Michigan. Columns labeled with the same letters are not significantly different ($P > 0.05$).

DISCUSSION

Paralobesia viteana males from different regions had similar responses to a standard pheromone, and they also responded similarly to females from their own and different geographical areas. In Y-tube choice tests, the percentage of Northwestern males that chose the standard pheromone sample was not different than that for Southwestern males. When allowed to choose between females from Northwestern and Southwestern Michigan in a Y-tube olfactometer, males showed no preference for females from the same region. In vineyards in Northwest and Southwest Michigan, there was a similar pattern in the relative numbers of resident males captured in traps baited with a standard pheromone blend or with captive females from Northwest or Southwest Michigan. In both regions, more males were caught in traps baited with a 1µg pheromone lure than in those baited with a 0.1µg lure, a 0µg lure or with captive females from either Northwest or Southwest Michigan. Although there were only significant differences in male captures among trap bait treatments in Southwest Michigan, there were no significant differences in the total number of males captured in traps baited with females from Northwestern or Southwestern Michigan. That is, resident males did not preferentially chose females from the same region, and males from both regions are captured in traps containing the standard pheromone blend. The fact that *P. viteana* males from different regions had similar responses to a standard pheromone and responded similarly to females from different geographical areas suggests that variation in male captures between Southwest and Northwest Michigan is not caused by regional differences in male response to pheromone or to regional differences in pheromone blend.

My results contrast other studies reporting geographic differences in pheromone composition and male response to pheromone in other tortricid species (Foster 1986, El - Sayed et al. 2003, Stelinski et al 2007b). In previous studies that showed divergence in pheromone composition or male response to pheromone, thousands of kilometers separated the observed

populations. For example, the populations of *C. rosaceana* in the Eastern and Western US that were compared by El - Sayed et al (2003) and Stelinski (2007b) were separated by over 4,000 km. Given there is a negative relationship between geographical distance and gene flow between populations (Manel et al. 2003), the likelihood of local adaptations that result in trait divergence increases with the distance between populations. Likewise, physical barriers that restrict movement between populations would be more likely to occur between widely separated populations compared to nearby populations. Northwestern and Southwestern *P. viteana* populations in Michigan are separated by 400km, and this may not have been sufficient to retard gene flow and movement of individuals between northwestern and southwestern populations, and therefore prevented differentiation of pheromone composition or male response to pheromones between these regions.

Additionally, the observation in Y-tube assays and field trapping that males do not prefer females from their region of origin supports the premise that the composition of the female sex pheromone has not diverged between southwestern and northwestern populations of *P. viteana* to such a degree that we can observe differential responses in males. Additional research that includes collecting and comparing the sex pheromone from females in northwest and southwest Michigan using gas chromatography and mass spectrometry would more directly address this question. If different pheromone compositions were present, the response of males to these different blends could be tested using electro-antennography and behavioral assays in flight tunnels, but these experiments were beyond the scope of the current study. The similar response of males to the standard pheromone and females in Y-tube assays, and the similar pattern of male captures in field studies suggest geographic variation in pheromone composition is unlikely to be present.

The results of bioassays and field studies do not support my prediction that the low *P. viteana* male captures observed in most vineyards in Northwestern Michigan are caused by regional differences in male response to the standard pheromone used in commercial lures, and the basis of this difference must lie elsewhere. There are several distinct differences between grape production areas in Michigan that align with the observed regional differences in male captures. The growing season is shorter and typically drier in Northwest Michigan, and these two factors may limit *P. viteana* population growth in that area relative to the warmer and wetter southwest portion of the state. In addition there are contrasts in the size and composition of the grape industry between northwest and southwest Michigan. There are about 13,100 acres of grapes in Michigan and approximately 75% of this acreage is planted in juice grapes (*Vitis labrusca* L. cv. Concord and cv. Niagara) in Southwest Michigan. The remaining acreage is planted in wine grapes with a little over half of the acreage is in Northwest Michigan and consists of a mix of European wine grape varieties of *V. vinifera* L. and French hybrids (Anon 2016). There are marked differences in the intensity and types of management practices used in wine and juice grape production in Michigan. The high value of wine grapes provides a margin of profit that allows growers to invest more capital in pest and disease management and vineyard maintenance than in lower value juice grape varieties. Although these differences may result in lower rates of cluster infestation in wine grape vineyards, it is unlikely to produce the observed difference in male captures between regions, because insecticide applications in grapes typically target larvae, and not adults (Isaacs et al. 2005, Isaacs et al. 2012b, Mason et al. 2016). It is also not likely that the difference in species and varieties of grape cultivated between Northwest and Southwest Michigan contributed to this phenomenon, because in areas where both juice and wine grapes are grown there are similar levels of *P. viteana* male captures and cluster infestation in

wine and juice grape vineyards (Slingerland 1904, Johnson and Hammar 1912, Biever and Hostetter 1989, K. Mason. personal observation).

Although the incidence of *P. viteana* infestation (i.e. % damaged clusters) can be similar between varieties or between regions as is shown in Chapter 1, (Figure 1.2), my personal observations over the past two decades show that the severity of *P. viteana* infestations (the number of damaged berries per cluster) in southwest Michigan is much higher than the severity of damage in northwest Michigan. My research results and observations lead me to conclude that the regional difference in the number of male *P. viteana* captured in pheromone traps in Michigan reflects the relative population sizes of *P. viteana* in these two production areas. Although population size was not exhaustively measured in the current study, the results of the field trial provide some evidence for this possibility. Across all bait types more males were captured in southwest Michigan compared to captures in traps in northwest vineyards. This includes captures in the control traps baited with a blank lure, which can be considered passive traps that would measure the activity/density of *P. viteana* in a given area without the possible interactive effect of an attractant. Over the course of the current study in traps that contained 0 µg lures, there was an average of 0.3 males per trap per vineyard in Northwest Michigan and 1.7 males per trap per vineyard in Southwest Michigan. This six fold difference in captures in un-baited traps indicates there is likely a difference in the size of *P. viteana* populations between regions.

The argument that the variation in *P. viteana* captures between regions in Michigan is a reflection of different population sizes is strengthened by considering the history of the grape industry in Michigan. Commercial grape production in southwest Michigan dates back to the late 19th century (Slingerland 1904, Johnson and Hammar 1912, Pinney 1989), whereas in Northwest Michigan, the first commercial plantings were established in 1970 (Anon 2016). Given that wild

grapes (*Vitis* spp.) are common in wooded areas throughout North America (Johnson and Hammar 1912, Galet 1979, Morano and Walker 1995), including Michigan (Voss 1996, Botero-Garcés and Isaacs 2004a, Jenkins and Isaacs 2007a), it is likely that *P. viteana* populations exist in these areas and these populations could increase if vineyards were planted near wooded areas.

I postulate that *P. viteana* populations are much larger in commercial grape plantings in Southwest Michigan because of the increased amount of available resources in the form of cultivated grapes.

Landscape composition varies between production regions. In the northwest region of Michigan (Leelanau and Grand Traverse counties) 12% of the land area is forested, and this is much greater than in the southwestern region (Allegan, Van Buren and Berrien counties) where 2% of the land area is forested (Homer et al. 2015). Wild grapevine density may also vary between regions, and this could contribute to different population sizes in Northwest and Southwest Michigan. This premise could be further explored with a trapping and mapping study that compares *P. viteana* captures in commercial vineyards and adjacent woodlots in Southwest Michigan to similar measures in vineyards and adjacent woodlots in Northwest Michigan. Traps would also need to be placed in wooded areas away from any grape production to estimate the background population of *P. viteana*. I would expect that captures in woodlots in Northwest Michigan would be similar to those in wooded areas that are not close to commercial vineyards. In addition, captures in the northern region would be much lower than those in wooded areas adjacent to vineyards in Southwest Michigan. Given that there is a large difference between regions in the percentage of area covered in woodland, it would be critical to conduct vegetation surveys and landscape analysis to measure the abundance and density of wild grape and characterize the landscape surrounding the areas where grape berry moth traps are deployed.

Independent of any underlying factors that may be responsible for potential regional differences in *P. viteana* population sizes, my results have important implications for future management of *P. viteana* in Michigan. This is particularly salient in Northwest Michigan, and in other areas where commercial grape production is beginning or expanding into uncultivated areas with high proportions wooded habitat where wild grape (*Vitis* spp.) is prevalent. *Paralobesia viteana* is a native insect that will likely be present at low levels in these areas, and if left unchecked can develop into a major economic pest.

Given that southwestern and northwestern males respond similarly to the standard pheromone, my advice to growers and fruit extension agents in areas where grape plantings are increasing would be to start monitoring for this pest by using pheromone traps and by quantifying infestation in newly planted vineyards. Effective control tactics have already been developed and tested in areas where *P. viteana* is a serious pest, and these tools could be used when population buildup is first detected to reduce the likelihood that *P. viteana* causes substantial economic losses. Educational programming in areas where viticulture is expanding should start while wild populations of *P. viteana* are at low levels. This could easily be accomplished with minimal funding through university extension or grower industry collaboratives that are already in place.

CHAPTER 3

JUICE GRAPE CANOPY STRUCTURE AND CLUSTER AVAILABILITY DO NOT REDUCE MIDDLE AND LATE SEASON CAPTURES OF MALE GRAPE BERRY MOTH, *PARALOBESIA VITEANA* (LEPIDOPTERA: TOTRICIDAE) IN SEX PHEROMONE TRAPS

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INTRODUCTION

The grape berry moth, *Paralobesia viteana* (Clemens) (Lepidoptera:Tortricidae), is a specialist herbivore found in woods and vineyards across eastern North America, feeding primarily on wild and cultivated plants in the genus *Vitis* (Johnson and Hammar 1912). This moth is a primary pest of cultivated grapes (*Vitis labrusca* L. and *Vitis vinifera* L. and their hybrids), and it has challenged grape growers since the beginning of commercial grape production in this region (Johnson and Hammar 1912, Hoffman et al. 1992, Isaacs et al. 2012b).

There are multiple generations of *P. viteana* in a growing season depending on location and the length of the growing season, with three and sometimes four generations in Michigan (Tobin et al. 2003, Teixeira et al. 2009, 2011). First generation adults begin to emerge from overwintering pupae in late April or early May, and mated females start to lay eggs on warm nights in early to mid-June (Clark and Dennehy 1988, Teixeira et al. 2009, 2011). After larvae hatch, they feed on multiple adjacent flowers or young berries and produce webbing that encloses the feeding site (Johnson and Hammar 1912). Larvae of subsequent generations excavate tunnels into the fruit, and web together and infest multiple berries (Johnson and Hammar 1912). The economic impact of the first generation is minimal, but larvae of later generations can cause significant economic losses due to reduced yield, lowered fruit quality or rejection of contaminated fruit by fruit processors (Hoffman et al. 1992, Teixeira et al. 2009,

Roubos et al. 2013) (Hoffman et al. 1992. Larval feeding by *P. viteana* is similar to that of the closely related European species, *Lobesia botrana* (Denis & Schiffermüller) that also affects yield and quality by increasing susceptibility to bacterial and fungal infections (Fermaud and Le Menn 1992, Mondy et al. 1998, Ioriatti et al. 2011). Because of the risks of direct yield loss, larval contamination of fruit and reduced fruit quality due to pathogens, *P. viteana* is the target of the majority of insecticide applications to vineyards east of the Rocky Mountains (Hoffman et al. 1992, Teixeira et al. 2009).

Flight activity of male grape berry moth can be monitored using traps containing lures baited with synthetic sex pheromone, but the males of this species are only captured in pheromone traps consistently during the first generation that is active around bloom. The first generation causes little larval feeding damage, but in the second and third generations in the early and late summer, respectively, the level of oviposition on berries, and subsequent larval infestations increase greatly, yet few males are caught in monitoring traps (Snyder et al. 1992, Teixeira et al. 2009 - Figure 1, Isaacs et al. 2012a). This disconnect between male moth captures and larval infestation at harvest prevents development of action thresholds based on captures of male moths as has been done for other lepidopteran fruit pests (Binns and Nyrop 1992, Bradley et al. 1998, Reddy and Manjunatha 2000, Knight and Light 2005a). Exploring factors that affect male captures of this species will inform the development of lures or trapping strategies that improve moth monitoring, potentially allowing for better timing of insecticide applications and improving decision-making about the need for treatments.

Volatile host plant chemicals can influence host-finding in some key crop pests (Bruce et al. 2005, Leskey et al. 2008, Piñero and Dorn 2009, Saveer et al. 2012). Most previous studies in this area have focused on the behavior of females, but either sex can respond to host plant volatiles in the laboratory or field (Stelinski et al. 2003, Light and Knight 2005, Faraone et al.

2013). Within species, odorant receptors and neural pathways are also similar between sexes (Jordan et al. 2009, Varela et al. 2011). Female codling moth, *Cydia pomonella* L., are attracted to apple odor, and show increased oviposition and flight response in laboratory studies (Wearing et al. 1973, Yan et al. 1999, Coracini et al. 2004). In addition, *C. pomonella* males are attracted to compounds derived from pear, and these can be used in monitoring traps resulting in higher captures than with pheromone alone (Light et al. 2001, Knight and Light 2005b, Il'ichev et al. 2009)). In the oriental fruit moth, *Grapholita molesta* (Busck), females are attracted to a combination of green leaf volatiles and other aromatic compounds that are derived from peach shoots (Piñero and Dorn 2007). In addition, male and female *G. molesta* are attracted to monitoring traps baited with volatiles derived from peach and pear (Lu et al. 2012). Female European grapevine moth (*L. botrana*), consistently flew upwind and landed on unripe grape clusters in wind tunnel tests (Tasin et al. 2006). In subsequent studies, *L. botrana* females were also attracted to a four-component blend of volatile compounds collected from the headspace around confined grapes clusters, and females were attracted to synthetic grape volatiles in field studies (Tasin et al. 2009). In similar studies, *P. viteana* females oriented toward grapevine structures in wind tunnel tests, and this response was stronger for shoot tips and mature leaves than that for flowers, unripe berries or mature fruit (Cha et al. 2008a). In further tests using wind tunnels, females consistently flew to lures loaded with blends of grape shoot volatiles that were collected from the headspace around grape shoots (Cha et al. 2008b). Male *P. viteana* were also attracted to this blend as males were captured in traps baited with the same volatile blends in field tests (Cha et al. 2008b, Loeb et al. 2011). These latter studies show that grape shoot volatiles are important and attractive cues for both male and female *P. viteana*, and they also suggest that the volatile-rich grape canopy may influence the interaction between male *P. viteana* and monitoring traps.

The size and chemical composition of grape clusters and the vine canopy change considerably during seasonal growth and development (Hrazdina et al. 1984, Mullins et al. 1992, Schultz 1995). Early in the season, few leaves and only small flower or berry clusters are present on vines. As the growing season proceeds, the clusters increase in weight 100 fold, and as the berries soften and become ripe, physiological changes lead to increased soluble solids, lower organic acids (e.g. tartaric, malic and citric), and, depending on grape variety, an increase in pH to a final value between 2.8 and 3.7 at harvest (Hrazdina et al. 1984, Jackson and Lombard 1993). The concurrent increase in phenolic compounds, such as flavonoids and anthocyanins, can impart distinctive odors and flavors to ripening grape clusters (Singleton and Trousdale 1983), and volatile terpenes that increase greatly after veraison (fruit coloring) also change the aroma and flavor characteristics of grapes (Dimitriadis and Williams 1984, Rosillo et al. 1999). These physiological transformations contribute to an odor landscape that varies consistently through the growing season, and these changing host plant cues may provide information that *P. viteana* could use to orient to its host plant, and find potential mates and oviposition sites. If *P. viteana* mating occurs on the grape clusters, or if females spend the majority of their time on clusters, males would have an increased chance of finding females, and mating successfully, if they can detect and orient toward the volatiles produced by grape clusters. This could lead to a "competition" between clusters and traps that might lead to a reduction in the number of male captures in traps. In addition the combination of grape volatiles and sex pheromone may be more attractive to male *P. viteana* than sex pheromone alone, as has been shown for *C. pomonella* (Light et al. 2001). This preference could result in reduced *P. viteana* male captures in pheromone traps because the amount of grape cluster volatiles increases during the season due to the increase in cluster size and the ripening of the crop.

In addition to changes that occur as clusters develop, the physical structure of the juice

grapevine canopy is also transformed through each season. In varieties that are bred and managed to maximize crop yield, such as juice grapes (*Vitis labrusca,* cvs. Concord and Niagara), the vines are typically cane-pruned and trained to a single wire top-cordon system. The resulting trailing habit of shoot growth causes the canopy to become increasingly dense, and it creates a thick matrix of shoots and leaves as the season progresses (Smart et al. 1982b, Miller and Howell 1998, Dry 2000, Bates 2008). The quantity and quality of grape shoot and leaf volatiles that are present in a vineyard change as the canopy grows and becomes more dense (Tasin et al. 2006). The dense canopy imposes challenges on disease and insect management by forming an impediment to air flow and reducing spray coverage (Wise et al. 2010). The increase in canopy density may also affect monitoring *P. viteana* with pheromone traps by altering trap findability (Miller et al. 2006), making it less likely that male moths find traps. The canopy could physically block males from reaching traps, it may disrupt the structure of the pheromone plumes from lures, or the naturally occurring grape shoot volatiles may draw moths away from traps.

To better understand the observed phenology of *P. viteana* in vineyards, this study explored the interaction between *P. viteana* and two host plant characteristics, cluster density and canopy structure. We determined if these factors reduce moth captures in pheromone traps by testing two hypotheses: 1) grape clusters reduce captures of *P. viteana* males in pheromone traps, and 2) the juice grape canopy reduces male *P. viteana* moth captures in pheromone traps.

METHODS

Field trials measured the effect of the presence and density of grape clusters on captures of male *P. viteana* at six (2013) or five (2014 and 2015) commercial juice grape vineyards (c.v. 'Concord') in Van Buren and Berrien counties in southwest Michigan (42.25 to 41.75ºN, 86.5 to 85.75ºW). All vineyards were grown on a 1.8m (6ft) tall trellis with a single-wire, top-cordon training system, and vineyards were cane pruned to ~75 buds per vine. At each vineyard, three 15x15m plots (5 rows x 5 vines), separated by at least 15m, were set up on the vineyard border adjacent to a woodlot containing wild grape (*Vitis* spp). Plots received one of the following randomized treatments: 100% of the clusters removed, 50% of the clusters removed or 0% of the clusters removed (control). Pruning shears were used to remove clusters between the 26th of May and the 18th of June, before the end of bloom in all three years.

One Pherocon VI monitoring trap baited with a sex pheromone lure (Trece Inc, Adair, OK) was hung inside the grape canopy with the long axis of the trap parallel to the row. Traps were attached to the trellis wire in the middle row of each plot between the third and fourth vines in the row when clusters were removed in late May or early June. Captures of males were recorded weekly until the end of the experiment. For each season, lures from a single lot were used, and lures were changed every 6 weeks to coincide with the start of each generation. Sticky trap liners were changed every 2 to 4 weeks when they became water-logged or excessively soiled. *P. viteana* generations were determined using the MSU Enviroweather grape berry moth model (Isaacs 2017) that employs an egg to adult development time of 423.9 $DD_{8.41^{o}C}$ and local bloom of wild grape (*Vitis riparia* L.) as the biofix (Tobin et al. 2001, Teixeira et al. 2009). Using this model, moths that emerged from overwintering pupae and were caught in May and June were classified as the first generation, those caught in July through mid-August comprised the second generation, and the third generation consisted of moths that were captured from mid-

August until harvest in late September. In each year, the total number of males captured during each of three generations and the total number captured per season were square root transformed before analysis to stabilize error variance in order to meet the normality assumptions of the analysis of variance. Captures were then compared among treatments with analysis of variance using vineyard as a random variable and plots as replicates followed by means separation using Tukey's HSD test (JMP Pro ver 13.1.0, SAS Institute Inc 2016).

To determine whether mid and late-season *P. viteana* male captures are reduced by a dense juice grape canopy, a field trial was established during the spring of 2012 at a commercial Concord vineyard in Lawton, MI (42.15°N, 85.83°W). This experiment was repeated in 2014 and 2015 in a vineyard (cv Niagara) at the Trevor Nichols Research Center in Fennville, MI (42.58°N, 86.14°W). Ten 15 x 15m plots (5 rows x 5 vines) were marked on the edge of the vineyard adjacent to a woodlot containing wild grape (*Vitis* spp.). The vineyard was grown on a

Figure 3.1. Vineyard plot containing a triangular tunnel designed to keep grape canopy open. Photograph was taken early in the season before the canopy was fully developed.

1.8m (6ft) tall trellis and trained to a single-wire, top-cordon system, and vines were cane pruned to ~75 buds per vine. We did not collect canopy measurements such as shoot length, cluster density leaf density, etc., as our study only included two levels of canopy density, "Open" or "Unaltered". Treatments were arranged in blocked pairs with the two different treatments applied in adjacent plots that were separated by at least 9m. In the center row of one randomly chosen plot in each pair, we used bamboo stakes, snow fence and zip ties to hold the canopy open by constructing a 15m long triangular tunnel (0.5m on a side) that was open at the bottom (Figure 3.1), and this treatment was designated as "Open". The second plot in the pair was left unaltered, and this treatment was referred to as "Unaltered", and was used as a control. Treatments were re-randomized each year to reduce location effects. In mid-May of each year, a monitoring trap as described above was hung on the trellis wire with the long axis of the trap parallel to the row and between the 3rd and 4th vines in the middle row of each plot. Traps were within the canopy in "Unaltered" plots, or inside the tunnel in "Open" plots. The number of *P. viteana* males captured was recorded each week from mid-May through September. Each year, lures came from a single lot and were changed every 6 weeks to coincide with the start of each generation. Sticky trap liners were changed at least every 4 weeks when they became waterlogged or soiled with insects. Generations were determined as described above, and the total number of males captured during each generation and the total captured in a season were compared between treatments with analysis of variance using square-root transformed data and included blocked pairs as a random variable and vineyards as replicates (JMP Pro 13.1.0, SAS Institute Inc, 2016). In 2015, data transformation did not satisfy the normality assumptions for analysis of variance, so these data were not transformed and were compared between treatments using a Wilcoxon Test of summed ranks (JMP Pro 13.1.0, SAS Institute Inc 2016).

RESULTS

In 2013, significantly more *P. viteana* males were caught in traps in plots where clusters were retained compared to plots where they were removed. This was true for the number of moths trapped during each generation (Generation 1: $F_{2,10} = 4.67$, $P < 0.037$; Generation 2 $F_{2,10} = 7.48$, $P < 0.01$; Generation 3: $F_{2,10} = 7.73$, $P < 0.01$), and for the total captured during the entire season ($F_{2,10} = 9.04$, $P < 0.006$; Figure 3.2). However, in 2014 and in 2015 after experimental plots were re-randomized, no significant differences were detected among cluster removal treatments in the total number of male *P. viteana* captured during the entire season ($F_{2,8} = 2.53$, $P > 0.14$, Figure 3.2). There was also no difference in the number of males captured during any of the three generations in 2014 ($F_{2,8} < 2.52$, $P > 0.14$), or 2015 ($F_{2,8} < 0.86$, $P > 0.45$). The contrasting results between the first year and subsequent years indicate there was a confounding location effect in 2013, most likely because our control plots were inadvertently situated in areas of high grape berry moth pressure.

No significant differences were found between male captures in plots where all of the clusters were removed compared to captures in plots where 50% of the clusters were removed. The absence of a cluster removal rate effect suggests that removing clusters triggered vegetative growth, and this may have led to a similar increase in leaf volatiles in the canopy in the 50% and 100% cluster removal treatments. This may have led to similar male moth captures between those treatments.

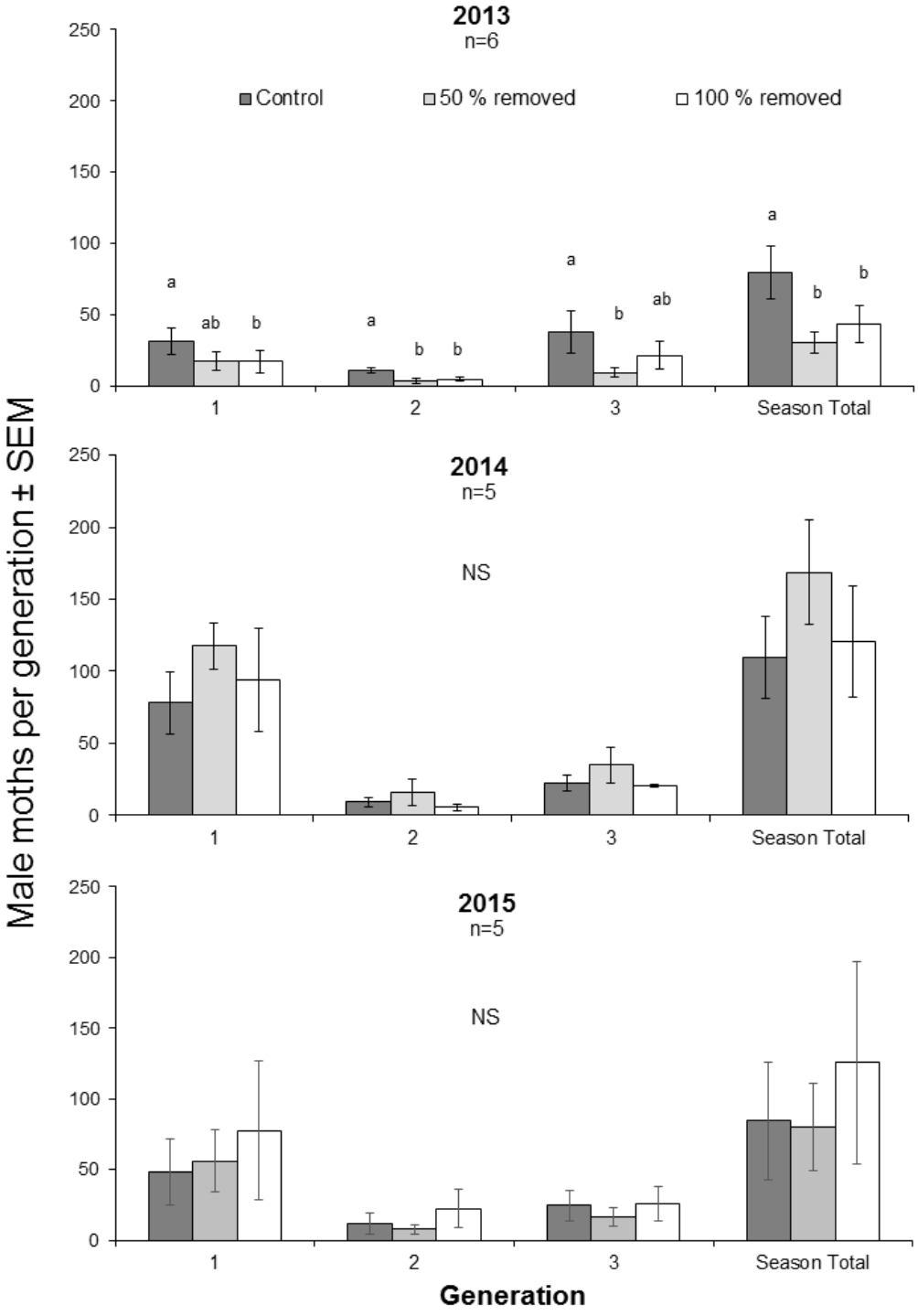


Figure 3.2. Effect of cluster removal on capture of male *P. viteana.* Captures of male *P. viteana* in plots with 0, 50% or 100% of clusters removed in 2013, 2014 and 2015. Columns with the same letter are not significantly different; "NS" indicates no significant differences were found among treatments, $P > 0.05$.

The number of male moths captured in open canopies was similar to moth capture in unaltered canopies in 2012 (Figure 3.3). No significant differences in total number of male moths captured during the entire season were detected between canopy treatments in 2012 ($F_{1,4} = 0.22$, $P > 0.65$). Similarly, there were no significant differences between treatments in the number of moths caught during any of the three generations in 2012 (Generation 1: $F_{1,4} = 0.05$, $P > 0.84$; Generation 2: $F_{1,4} = 0.65$, $P > 0.46$; Generation 3: $F_{1,4} = 0.12$, $P > 0.74$). In 2014, significantly more male moths were captured in unaltered canopies than in open canopies during the second generation ($F_{1,4} = 15.22$, $P < 0.018$; Figure 3.3). However we found no significant differences in the number of male moths captured between canopy treatments during the first ($F_{1,4} = 5.74$, $P > 0.07$) or third generation ($F_{1,4} = 3.59$, $P > 0.13$). The sum of moth captures over the entire season was also similar between canopy treatments ($F_{1,4} = 5.72$, $P > 0.07$). Moth captures in unaltered canopies were significantly greater than captures in open canopies only during the third generation in 2015 (Figure 3.3 Generation 3: $X^2 = 5.12$, df = 1, $P < 0.03$). Although the average number of males captured in unaltered canopies was arithmetically greater than captures in open canopies in the second generation, these differences were not statistically significant because of considerable variability within treatments (Generation 1: $X^2 = 2.45$, df = 1, $P > 0.11$; Generation 2: $X^2 = 3.55$, df = 1, $P < 0.06$). Similarly, total captures for the season were not different between canopy treatments (Figure 3.3, Season total: $X^2 = 0.53$, df = 1, $P > 0.46$). The consistent pattern of numerically higher male captures in unaltered canopies during the second and third generations shows the canopy does not reduce moth capture, and it suggests that shoots and leaves may be used by males to find traps or calling females.

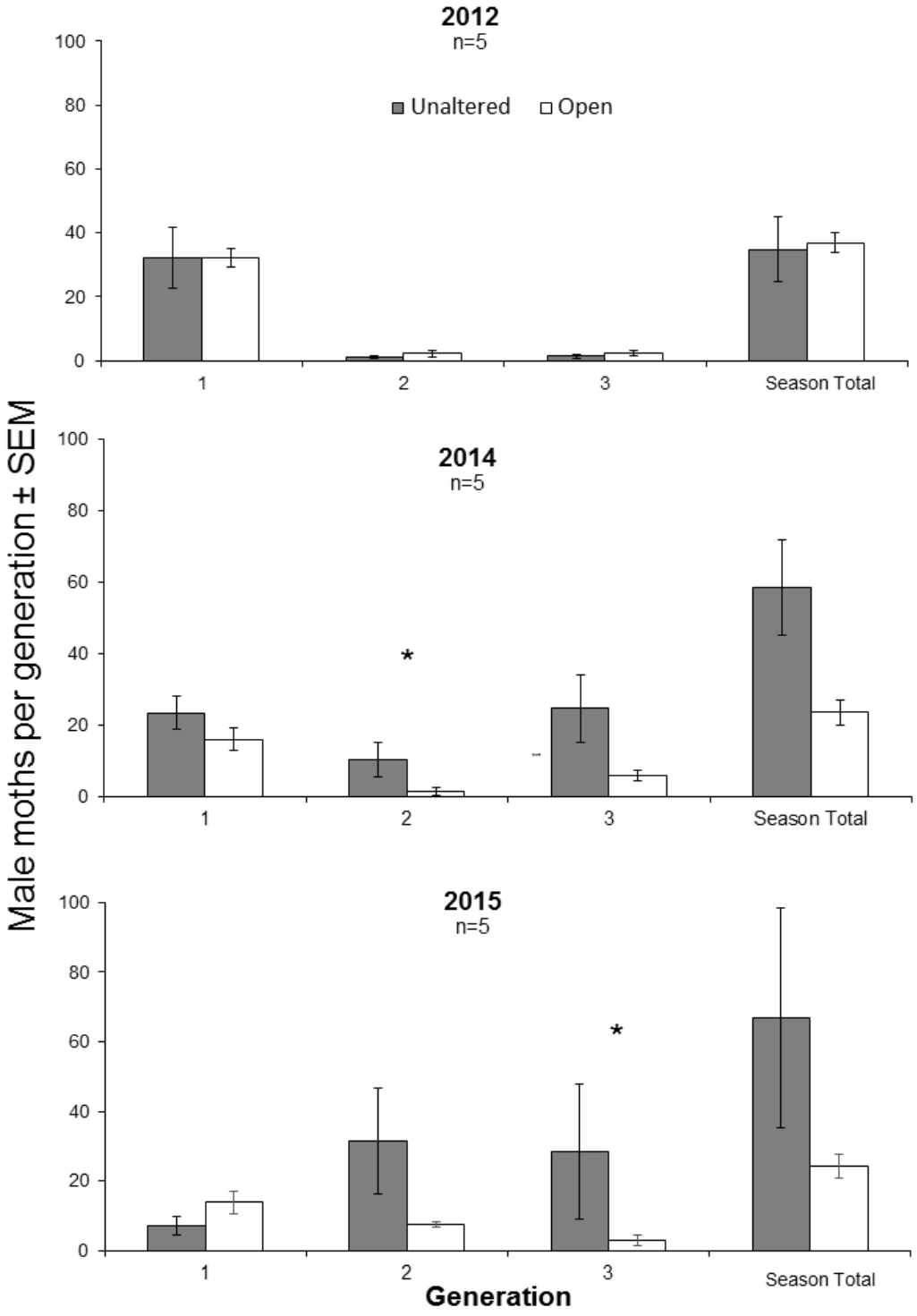


Figure 3.3. Effect of manipulating canopy structure on captures of male *P. viteana.* Male captures in plots with unaltered, dense juice grape canopies (Unaltered) or canopies held open with a triangular tunnel (Open) in 2012, 2014 and 2015. Asterisks indicate treatments were significantly different (P <0.05). This experiment was run in a Concord vineyard in 2012 and in a Niagara vineyard in 2014 and 2015.

DISCUSSION

These experiments showed that captures of male *P. viteana* are not consistently affected by cluster density or by the vine canopy structure in juice grape (cv Concord and cv Niagara) vineyards. When we did observe treatment effects on moth captures, the results did not help to explain the previously documented reduction in male *P. viteana* captures in monitoring traps in the middle and late season.

In the cluster density experiment in 2013, we caught significantly more males in plots where clusters were left intact than in plots with 50 or 100% of the clusters removed. This effect was not present in the following years when treatment locations were re-randomized, as there were no significant differences between cluster removal treatments in 2014 or 2015. We interpret this as evidence of a location effect in 2013, likely because by chance our control plots were near areas of high male moth activity/density. The results from all three years of our study falsified our original hypothesis that cluster density reduces male *P. viteana* capture in traps. Therefore, the presence or density of fruit does not cause the reduction of male captures of *P. viteana* in pheromone traps that occurs during the second and third generations (Snyder et al. 1992, Teixeira et al. 2009). Even though clusters increase greatly in size and the volatile chemicals produced by fruit clusters change markedly through the season, it appears they are not a critical cue used by male *P. viteana.* This contrasts the behavior of some other fruit feeding tortricids such as *C. pomonella, G. molesta* and *L. botrana*, that are attracted to volatiles derived from fruit (Knight and Light 2005b, Piñero and Dorn 2007, Tasin et al. 2009). However these three species all feed on multiple hosts, so we cannot rule out some contribution of diet breadth to the difference between these pests and the specialist, *P. viteana.* A meta-analysis comparing the responses of males of different species to host plant volatiles derived from fruit to those present

in shoots may help explain these differences, but that is beyond the scope of the present work. Our results are also consistent with previous research that shows fruit is less attractive than grape shoot tips and leaves to *P. viteana* (Cha et al. 2008a). Males are strongly attracted to shoot tips and leaves in addition to sex pheromone, and we saw that a change in the density of fruit does not affect male moth capture. We would expect an increase in vegetative growth when clusters are removed, and this presumably would lead to an increase in the amount of leaf volatiles in areas where clusters are removed. It is conceivable that an increase in leaf volatiles could draw more males into the cluster removal plots, but these males may be attracted to leaves and not to traps. Unfortunately, we did not measure vegetative growth or leaf volatile titers in our research plots, so we cannot directly assess the effect these factors had on male capture.

In two of the three years of our canopy structure study, we found consistent numerical differences between male captures in vineyard plots with an unaltered canopy and those that were opened using an artificial tunnel. In 2012, the effects of these treatments did not differ, whereas in the second generation in 2014 and in the third generation in 2015, the number of males captured in unaltered canopies was significantly higher than in open canopies. These results show that the canopy does not impede the movement of male moths to traps. Captures of *P. viteana* in dense, unaltered grape canopies were higher than in open canopies, which highlights the possible importance of shoots and leaves in male trap finding behavior. As volatile compounds are attractive to male *P. viteana*, (Cha et al. 2008b), we may have caught more males in unaltered canopies because traps were in closer proximity to leaves in the unaltered vs open treatments. We were unable to assess the effect of the seasonal increase in leaf volatile titer on male captures because we did not manipulate the amount of leaf area in experimental plots with pruning, leaf removal, etc., but we expect the titer of leaf volatiles would have been similar between treatments. Still the results of our experiment refute the hypothesis that canopy structure

reduces male moth capture, and does not support the prediction that low captures of male *P. viteana* in traps at the end of the season are due to physical interference from the canopy.

It may be advantageous for a grape specialist such as *P. viteana* to respond more strongly to leaf volatiles than to fruit volatiles due to the climbing, spreading growth of grapevines in a natural setting. The total area of leaves of a grapevine is much greater than the area of the fruiting zone, and growing shoots may be several meters away from fruit clusters, particularly in uncultivated habitats (Mullins et al. 1992). We would therefore argue that *P. viteana* moving through wooded areas are more likely to encounter leaves than fruit, so detecting leaf volatiles may be the optimal strategy for finding host plants within the dense deciduous habitats of eastern North America.

Paralobesia viteana has limited capacity for long flights and it seems well adapted to movement within the dense vegetation of its natural habitat (Botero-Garcés and Isaacs 2004b). A series of short flights between shoots may be advantageous for an organism like *P. viteana* that must find mates and food resources in a structurally complex environment such as a contiguous forest or vineyard canopy. If *P. viteana* uses short flights between shoots to move around its environment, we would expect to see lower numbers of males in traps in open canopies, because the trap is outside of the space where the moths are moving. Movement in the canopy was not addressed in our study, but it could be explored using video recordings (Grieshop et al. 2012) in open and unaltered canopies to compare the movement behavior of moths in relation to varying habitat structure.

Some possible confounding factors may have influenced the canopy experiment, but it is unclear how important these were for our results. The experiment was performed at two different locations over three years, and two closely related grape varieties were used. In 2012, the trial was performed in a commercial vineyard (*Vitis labrusca* L. cv 'Concord') in Lawton Michigan,

while in 2014 and 2015, we used a *V. labrusca* L. (cv 'Niagara') vineyard 80 km away at the Trevor Nichols Research Center in Fennville, MI. High temperatures (25 - 30°C) in the spring of 2012 led to an early start of growth, and made this one of the warmest years on record for the region. Subsequent spring freezes reduced the crop load by 30- 40% in the section of the vineyard we used, and the owner of the vineyard reduced his disease and insect management program, as is common practice in freeze years. The 2014 and 2015 growing seasons did not have a major freeze event, but they were some of the coolest seasons in the previous twenty years, and the experimental vineyard did not receive insecticide in 2014 or 2015. In any given year, all of the treated plots were exposed to these factors to the same degree, so we conclude that there were no confounding effects from weather, location or variety.

The fencing material that we used to construct the tunnels to create open canopies may have produced repellent odors, or created a visual pattern that could have deterred male moths from entering plots that contained the tunnels. Although we did not directly test for the effect of the fencing on male moth captures, our data suggest there was not a repellent effect. There were no significant differences in captures between plots with fencing (Open) and plots without this material (Unaltered) in the first generation. During this period, the grape canopy was not fully grown and the tunnels were visible and any odors from the fencing would also be present (Figure 3.1). Our results show the juice grape canopy affects captures of male grape berry moth, but the observed effect does not explain the previously reported reduction of male *P. viteana* captures in pheromone traps during the second and third generations (Teixeira et al. 2009, Isaacs et al. 2012a). We postulated that the unaltered juice grape canopy interferes with a male's ability to find traps. However, we caught more males in traps that we placed in unaltered as opposed to open canopies, and from this, we propose that the canopy is an important component of trap and presumably mate finding. Surprisingly, fruit cluster presence or density did not consistently

affect male captures, so we conclude that any effect the fruit may have does not contribute to the reduction of male captures during the middle and late season generations. There remains a need to explore the factors that cause reduced moth captures at the time when oviposition by *P. viteana* is increasing, and this could help to improve monitoring of this key vineyard pest. Additional studies that examine the reduction of captures during the second and third generation flights when oviposition and infestation increase will be reported later as separate publications. Other areas of research focus on testing the effects of temperature on some important aspects of mating behavior such as: female calling/receptivity to mating; male diurnal activity; the timing and frequency of mating; the duration of the mating period; moth longevity; and oviposition. We are also testing the effect of seasonal temperature changes on the release rate of sex pheromone lures to determine if second and third generation males are repelled by monitoring traps due to higher pheromone release rate at high temperatures.

CHAPTER 4

RESPONSE OF *PARALOBESIA VITEANA* TO TEMPERATURE HELPS DETERMINE SEASONAL PATTERNS OF MOTH FLIGHT AND VINEYARD INFESTATION

INTRODUCTION

Most insects are ectothermic, and consequently many aspects of insect physiology, development, behavior and reproduction are dependent on ambient temperature (Ratte 1985, Honek 1996, Frazier et al. 2006). For a diverse group of insects, the rate of enzymatic processes involved in physiological functions such as metabolism, hormone production, and digestion approximately doubles for each 10°C increase in ambient temperature (Rao and Bullock 1954, Ratte 1985). The range of temperatures where this Q_{10} doubling differs somewhat between species and climatic regions, but is typically close to 10 to 30°C for species living in temperate zones (Nielsen et al. 1999, Nespolo 2003).

The effect of temperature on adult longevity has been studied in several insect orders, and across all taxa, longevity decreases with increasing temperature (Graham et al. 1967, Emana 2007, Gómez et al. 2009). In field tests, Asaro and Berisford (2001) reported males of the first generation of the Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock) lived significantly longer than males in the second and third generations. The authors also demonstrated in the lab that there is a significant reduction of longevity with increasing temperature. Longevity of codling moth, *Cydia pomonella* (L.) in northern European populations was about 25% shorter for males and females held in a warm temperature regime (18°C day and 15°C night) compared to those in a cool temperature scheme (13°C day and 10°C night) (Sæthre and Hofsvang 2002).

The frequency and intensity of insect movement and activity are also dependent on temperature. Between critical minimum and maximum temperatures, thermal performance, measured as cricket (*Acheta domesticus* L.) running speed and jumping distance, increases with

temperature (Lachenicht et al. 2010). Similarly, locomotor activity of the tenebrionid, *Gonocephalum simplex* (Fabricius), increases with temperature up to a critical maximum temperature, and then activity decreases rapidly as the insect succumbs to heat stress (Klok et al. 2004).

Insect flight shows a similar response to temperature above a critical minimum and decreasing at temperatures at and above a critical maximum. In early studies, Taylor (1963) found the minimum temperature for flight to be 15.5°C for *Vespula germanica* (L.) queens, 17.5°C for alates of the bean aphid *Aphis fabae* (Scopoli) and 16.5°C for the soldier beetle *Rhagonycha fulva* (Scopoli). However, (Taylor 1963) also found the minimum temperature for flight for the large bodied noctuids (~30-40mm wing span) *Agrochola lychnidis* (Denis & Schiffermüller) and *Amphipyra tragopoginis* (Clerck) to be considerably lower at 9 and 10.5°C, respectively. For smaller moths such as the tortricids (wing spans ~10-20mm) the critical minimum temperatures for flight are in the range of 8-15°C. For example the minimum temperature for flight in the eastern spruce bud worm, *Choristoneura fumiferana* (Clemens) is 14°C (Greenbank et al. 1980) and it is 15 ºC for oriental fruit moth *Grapholita molesta* (Busck) (Rothschild and Minks 1974). Codling moth minimum flight temperature was found to be 12.7°C (Batiste et al. 1973), and for the light brown apple moth, *Epiphysis postvittana* (Walker) the minimum temperature for flight ranged from 8 to 11°C (Danthanarayana 1976). The critical maximum temperature for flight has been quantified less often, but for codling moth the maximum was found to be 26.7°C (Batiste et al. 1973), and for the light brown apple moth, the upper limit of temperatures for flight ranged from 27 to 28°C (Danthanarayana 1976).

Oviposition is also highly dependent on temperature. In pink bollworm, oviposition increases 10-fold (from 22 to 236 eggs per female) between females held at 15.5°C and those reared at 18.3°C. Over the range from 18.3 to 26.7°C oviposition was similar (range 228 to 280

eggs per female). At 32°C, oviposition decreases to 157 eggs per female and at temperatures of 37.8°C and above oviposition is less than one egg per female (Graham et al. 1967). Særthre and Hofsvang (2002) showed northern European populations of codling moth begin to lay eggs at temperatures as low as 12.3°C, and the amount of oviposition increases steadily through a range of temperatures ending at 25°C (the highest temperature measured). Oriental fruit moth oviposition is highest from 22 to 30°C, and drops off rapidly at 33°C while no egglaying is observed at 35°C (Notter-Hausmann and Dorn 2010).

The influence of temperature and photoperiod on daily and seasonal cycles of growth, development, activity, and reproduction have been studied in several insect families (Tauber and Tauber 1981, Beck 1983, Régnière et al. 2012, Tonnang et al. 2017). Moths in the family Tortricidae have been the subjects of much of this work owing to the status of some members of this family as economically important fruit pests.

In moth species where mating occurs at the beginning of the scotophase (dark phase), females emit pheromone (call) earlier in the day at lower temperatures. At higher temperatures, the female calling period is generally shorter, and occurs in the scotophase (Cardé et al. 1975b, Baker and Cardé 1979a, Delisle and McNeil 1987, Webster 1988). Male responsiveness to pheromone also occurs earlier in cool temperatures (Cardé et al. 1975a). Thus for some species the current air temperature, or changes in temperature, dictate when mating behaviors will occur. In other species, ambient temperature in the period immediately before the scotophase limit the periods of female calling and male responsiveness (Baker and Cardé 1979a, Haynes and Birch 1986, Delisle and McNeil 1987). Photoperiod can also be an important cue for determining seasonal or daily patterns of activity (Delisle and McNeil 1987). For example decreasing day length in the autumn can trigger physiological changes that can alter insect development and trigger diapause (Riedl and Croft 1978, Gangavalli and Aliniazee 1985, Nagarkatti et al. 2001,

Saunders 2001). Photoperiod can influence mating behavior, although relatively few studies have examined the effect of day length on mating, and the effect varies across species and sexes. As day length decreases, the female calling period is lengthened (Kanno 1981, Gerber and Howlader 1987), but decreasing day length in the late summer and fall reduce male responsiveness to sex pheromone (Delisle and McNeil 1986, 1987, Mulder et al. 1989). In cases where there is an effect of photoperiod on either male or female mating behavior, the effect is typically minor compared to the effect of temperature (McNeil 1991).

Identification of the patterns of daily mating activity can inform other management approaches such as mating disruption, mass trapping and attract and kill (McNeil 1991, El-Sayed and Suckling 2005, El-Sayed et al. 2006, 2009, Baker 2009, Rodriguez-Saona and Stelinski 2009). For example, in mating disruption of some tortricid pests, automated pheromone dispensers can be programmed to emit pheromone only during the period when females are calling, and this could reduce the costs of deploying mating disruption (Isaacs et al. 1999, Fadamiro and Baker 2002, Stelinski et al. 2007a, Jones and Wiman 2012).

In developing sex pheromone lures for monitoring, the optimal amount of pheromone to use in a lure is often determined by comparing male captures in traps that contain a range of concentrations of an active compound (Roelofs et al. 1969, 1971, Witzgall et al. 2000, Suckling et al. 2005). Traps with too low of a pheromone concentration will not be attractive to males and lower captures will result (Roelofs et al. 1971, Taschenberg et al. 1974, Linn and Roelofs 1995). In addition, the rate of pheromone release from lures increases with increasing temperature (McDonough et al. 1989), and increased pheromone release at high temperatures may also suppress male catch in periods of hot weather (Sanders 1981, Cork et al. 2003, Cork 2016, Cardé et al. 2018). In this study, the effect of temperature and photoperiod on the behavior of *P. viteana* were investigated to determine how daily periods of mating activity and reproductive behaviors

change during the growing season, and how these changes can influence *P. viteana* phenology. This is an essential component of integrated management of tortricid pests (Riedl et al. 1976, Damos and Savopoulou-Soultani 2010, Jones and Wiman 2012), yet little is known about the role of temperature and day length in *P. viteana.*

The grape berry moth, *Paralobesia viteana* (Clemens) (Lepidoptera:Tortricidae), is a specialist herbivore found in woods and vineyards across eastern North America, feeding primarily on wild and cultivated plants in the genus *Vitis* (Johnson and Hammar 1912). This moth is a primary pest of cultivated grapes (*V. labrusca* L. and *V. vinifera* L. and their hybrids), and it has challenged grape growers since the beginning of commercial grape production in this region (Johnson and Hammar 1912, Hoffman et al. 1992, Isaacs et al. 2012b).

Flight activity of male grape berry moth can be monitored using traps that contain lures baited with synthetic sex pheromone, but the males of this species are only captured in pheromone traps consistently during the first generation that is active around bloom. The first generation causes little larval feeding damage, but in the second and third generations in the early and late summer, respectively, the level of oviposition on berries, and subsequent larval infestations increase greatly, yet few males are caught in monitoring traps (see Chapter 1, Figure 1.3) (Snyder et al. 1992, Teixeira et al. 2009, Isaacs et al. 2012b). This complicates *P. viteana* management because action thresholds based on captures of male moths cannot be used for all generations as has been done for other lepidopteran fruit pests (Binns and Nyrop 1992, Bradley et al. 1998, Reddy and Manjunatha 2000, Knight and Light 2005a). Exploring factors that affect male captures of this species will inform decision-making about the need for treatment and the development of lures or trapping strategies that improve moth monitoring could lead to better timing of insecticide applications or other management strategies.

A model was developed to predict how different temperatures during the three grape

berry moth generations produce the observed pattern of *P. viteana* phenology (Figure 4.1). Male response through the diurnal cycle is shown by the solid black lines, and it is assumed that the period of this response changes little between the three generations. The period of female calling changes with temperature and is the shortest when temperatures are the lowest, during Generation 1. The female calling period is expected to be longest in the second generation (Figure 4.1) when temperatures are the highest. The area between the lines of male response and female calling indicates the time that males are responsive to pheromone and females are not emitting pheromone. This is the period when males would be most likely to be caught in traps, and this would the longest in Generation 1 (Figure 4.1). Thus this model predicts the highest male catch in this generation. Conversely, the lowest number of males would be caught in Generation 2 (Figure 4.1) because females are calling for the entire period that males are responsive, and traps would thus be in competition with calling females as is seen the tea tortrix (Noguchi and Tamaki 1985) and spruce budworm (Palanisawamy and Seabrook 1978, Sanders 1987). Male capture in Generation 3, would be lower than in Generation 1, but higher than in Generation 2. This is expected to occur because the lower temperatures during this time would create periods in Generation 3 when males are responsive but females are not calling. Thus the effect of temperature on female calling period may be responsible for reduced male captures

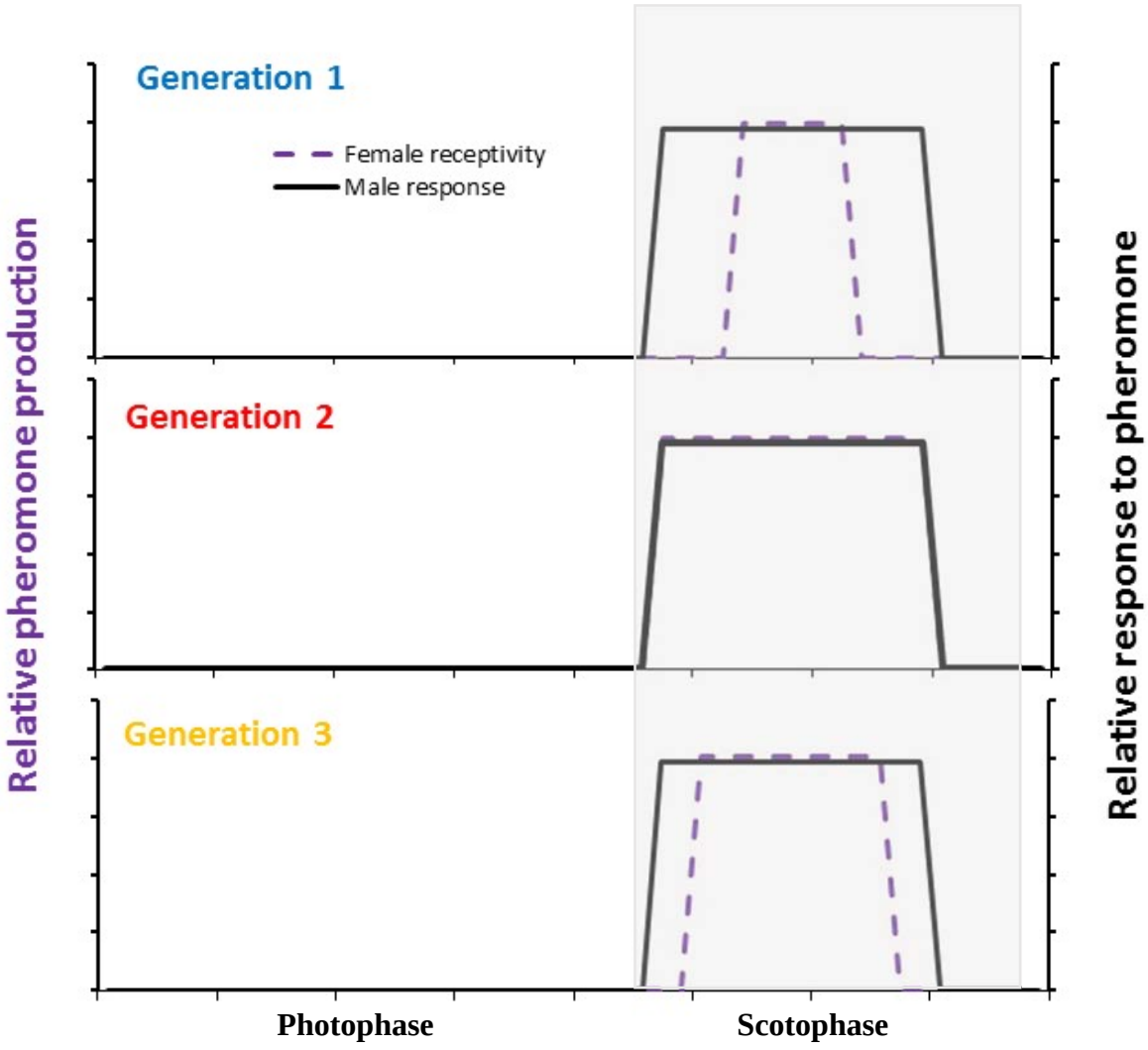


Figure 4.1 Model of changing female pheromone production (purple dashed line) and male response to pheromone (solid black line). The gray rectangle denotes the dark phase of the light cycle. The area between the purple dashed and solid black line represents the proportion of time males are receptive to pheromone, but females are not emitting pheromone (i.e. not receptive to mating).

during the second and third grape berry moth generations that occur in the middle and late season, respectively.

The purpose of this study was to determine the roles of temperature and day length in the levels and timing of mating behavior, oviposition, and activity of *P. viteana* and to ascertain whether these relationships explain the phenology observed in Michigan vineyards. My research focused on the following eleven questions:

1) How are longevity, flight ability and oviposition affected by temperature?

2) Does the frequency of mating or male flights increase with temperature?

3) Does the time of first mating or the time of first male flight change with temperature?

4) Do the durations of the mating period and the period of male flight vary with temperature?

5) Are there more male flights when females are not receptive in colonies reared at low temperatures than in colonies reared at high temperatures?

6) Does day length affect the frequency of mating frequency of male flights, the time of first mating or the time of first male flight, the duration of the male flight or mating period?

7) Does day length affect the number of male flights when females are not receptive?

8) Do diurnal temperature regimes affect timing or duration of mating period and male flights?

9) Do diurnal temperature regimes affect the proportion of male flights that occur when females are not receptive to mating?

10) Does the rate of pheromone release from lures change with temperature?

11) Do high pheromone release rates reduce male capture?

METHODS

To determine a range of temperatures for testing their effects on mating behaviors, average daily temperatures were determined for April to September based on 2010-2014 weather data from Lawton, Michigan (enviroweather.msu.edu). Those values were used to choose the temperatures used in all experiments (Table 4.1). I also selected day length values that encompass the range of day lengths in Michigan during the growing season (Table 4.1).

Table 4.1 Average daily temperatures and day lengths for April to October 2010 – 2014 for Lawton, Michigan.

Month	Temperature °C	SEM	Day length*
April	9.1	0.9	13:22
May	16.1	0.4	14:35
June	20.5	0.3	15:15
July	22.8	1.2	14:58
August	21.4	0.4	13:53
September	16.7	0.3	12:30

*Day length on 15th day of the month

Insect rearing

Insects used in these experiments were obtained from a colony of freely mating *P. viteana* that has run continuously for over a decade. The rearing methods were based on those of (Taschenberg 1951, 1969). *Paralobesia viteana* infested fruit was collected from multiple agricultural and natural sites in Berrien and Van Buren counties in southwest Michigan and adults reared from these collections were used to start the colony. In the summer and fall of each year, individuals from similar collections were added to the colony to reduce potential selective effects of laboratory rearing (Chambers 1977, Cha, et al. 2008a). Adult moths (100 – 400 individuals in a 50:50 sex ratio) were held in 30cm^3 Bugdorm-1 cages (MegaView Science Co., Ltd, Taichung City, Taiwan) and reared in a walk-in chamber set at 28°C; 60-75% RH and a 17L:7D light cycle. Deionized water and a 10% sucrose solution were provided *ad libitum* in 200

ml glass bottles each containing a 15 cm piece of dental wicking (Absorbal Inc. Wheat Ridge, CO, USA) and sealed with Parafilm so that a 3 cm piece of wicking extended above the bottleneck. Fluorescent lamps provided all lighting in the rearing chamber except for two 10W incandescent bulbs that were continually illuminated to provide twilight conditions to encourage mating and egglaying in the colony chambers during the scotophase. To provide oviposition sites and food for hatching larvae, I used fresh seedless grape clusters that were purchased weekly from a local grocer. To prepare them for the colony, I soaked the clusters for 5 min in 5% bleach solution, and then rinsed exhaustively and allowed them to air dry. Three times per week, 2 to 3 grape clusters (300-400g total) were hung in the rearing chambers and the previous set of clusters were removed and placed in 17L plastic rearing bins with fine nylon mesh lids. The grapes were placed in the bins on 25 x 30 x 5 cm platforms made from aluminum metal mesh with 0.64cm^2 openings. Pieces of cellulose sponge (5 x 8cm) that were placed under the metal mesh absorbed liquid that leaked from the grapes. Ten to fifteen strips of clear plastic (2 x 10cm) were placed on the bottom of the bin and around the grapes to provide pupation substrate for mature larvae that leave the clusters. Rearing bins were held in the same walk-in chamber as the adult cages. After 2-3 weeks mature larvae start to drop from the fruit and form a pupal cocoon by cutting a small disc from a plastic strip and wrapping themselves up and sealing it using salivary silk. These pupae were then collected every two or three days and put in small bins until the adults emerged. Adults were aspirated out of these bins and divided up among the active oviposition cages.

Moth longevity

To quantify the effect of temperature on moth longevity, pupae from the colony were placed in 29.6ml plastic deli cups (Solo, Urbana, IL, USA). A 1cm piece of moist dental wicking

(Absorbal, Wheat Ridge, CO, USA) was placed in the cup, and was covered with a perforated lid and held at 28°C, 60% RH and 17L:7D until adults emerged. Newly emerged moths were then moved to an environmental chamber set at either 10°C, 18°C or 28°C, and 60% RH and 17L:7D. Moths were checked daily for viability and touched with a small paintbrush to determine if they were still alive. At least 15 (maximum 34) replicate moths of each sex were assessed at each temperature. The sex of each moth and the number of days from emergence to death were recorded, and their longevity was compared between the sexes and across temperatures with a two way analysis of variance (ANOVA) followed by means separation with Tukey's Honest Significant Difference test (HSD) (JMP 13.1.0, SAS Institute. Cary, NC).

Minimum flight temperature

The minimum temperature required for flight by male and female *P. viteana* was determined using a catapult to propel moths into the air at different temperatures and record their flight behavior. This experiment was performed in a walk-in environmental chamber that was set to temperatures between 10 and 19°C and under 50-60% RH, fluorescent lighting on a 17:7 L:D cycle.

The catapult (Figure 4.2) was constructed from a 42 x 24 x 2.5cm wooden platform, and a 4.5 x 23 x 0.2cm strip of semi- rigid plastic with one end attached to the platform with wood screws. The other end of the plastic strip was raised above the platform using a large binder clip attached to the platform with wood screws so it was positioned underneath the strip to serve as a fulcrum. This allowed the suspended end of the plastic strip to be pressed down to a standard height above the platform (3cm). An inverted 29.6ml plastic deli cup was glued to the platform so the strip could be set before releasing the strip to launch a moth into the air. A scintillation

vial cap was glued to the upper side of the suspended end of the strip to serve as a holding basket for a moth before it was launched.

Before testing, one to five day old moths from the colony were held in an environmental chamber at 10°C, 75% RH and 17:7 L:D in individual 29.6ml (1oz) plastic deli cups (Solo, Urbana, IL, USA) containing moist cotton wicking and covered with a perforated lid. On the day of testing, moths were held in the walk-in chamber at test temperatures for at least 30 min prior to measurement to allow their bodies to equilibrate with that temperature. I launched individual moths at temperatures between 10 and 19°C at 0.5°C increments starting at the lowest temperature to be tested. In total, 147 female and 155 males were tested and at least 5 moths of each sex were tested at each temperature during each round of tests.

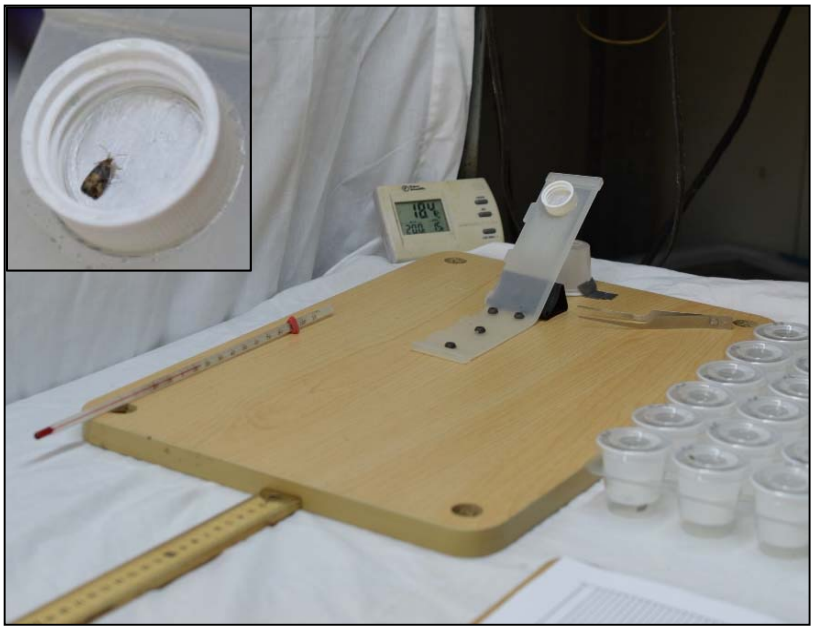


Figure 4.2 Catapult used to propel moths into the air at different temperatures to determine the lower temperature threshold for flight. Inset in upper left shows a moth ready to be launched.

For each moth, I classified the flight behavior as either: 1) Glide – following a parabolic path without moving wings. 2) Flutter – wing movement is visible but moth follows a parabolic path without a change of direction. 3) Partial – wing beating is visible and moth flight veers from the parabolic path, or changes direction, but sustained or upward flight is not seen. 4) Full – moth shows wing flapping and non-parabolic upward or extended flight.

The threshold temperature for flight was defined as the temperature at which 50% of moths were able to fly (FT_{50}). I determined the FT_{50} by fitting a line to the average proportion of moths that were able to fly in the range of 14°C to 16°C, and calculated the FT_{50}. This was determined separately for males and females, and I pooled male and female flight data to determine the FT_{50} for all moths.

Oviposition

The effect of temperature on *P. viteana* oviposition was determined using a series of bioassays in mesh cages made by rolling a 30 x 60cm piece of aluminum window screen into cylindrical 30 x 15cm diameter mesh cage. The vertical seam of the cage was sealed with hot glue and the cage was covered on the top and bottom with 15cm plastic Petri dishes. I placed five female and five male 3 to 5 day old lab reared *P. viteana* adults along with three table grapes into the cages. Grapes were either threaded on a wire, fixed in place by applying hot glue between the grapes and then suspended in the middle of the cage, or they were glued, stem end down, onto a 6cm Petri dish and placed on the cage bottom. I randomly assigned cages to one of three different temperature treatments, and placed them in environmental chambers at 10, 18 or 28°C with 60% RH and a 17L:7D light cycle, and with lighting provided as described above. The trial using grapes suspended in cages was replicated at least 27 times at each temperature, and the trial where grapes were placed on the cage floor was replicated 10 times at each temperature. After 48

hours the berries from each cage were removed and, using a dissecting microscope, the number of eggs were counted and recorded. I compared the average number of eggs laid per female among temperatures with one-way ANOVA followed by means separation with Tukey's Honest Significant Difference test (JMP 13.1.0, SAS Institute. Cary, NC). I did this separately for cages with grapes suspended and those with them on the bottom.

Duration and frequency of the mating period and male flight period

To determine whether the frequency, timing and length of the mating period, or the male flight period are affected by temperature, day length or patterns of diurnal temperature changes, I conducted timed observations of freely mating *P. viteana* moths in colonies held under specific conditions. A colony consisted of 150 to 200 adults with an approximate 1:1 sex ratio held in a $30cm^3$ Bugdorm-1© clear plastic cage with mesh walls (MegaView Science Co., Ltd, Taichung City, Taiwan). Newly emerged moths were added to the rearing cages and dead moths were removed every other day to keep colonies at a standard density of 150 to 200 moths. Fresh seedless table grapes, water and sucrose solution were available in the colony, and these were replaced and handled as described above.

Experimental conditions were similar to the conditions for colony rearing described above except that I varied temperature or day length according to experimental requirements. I used walk-in (EGC, Chagrin Falls OH, USA) and reach in (Percival Scientific, Perry IA, USA) environmental chambers so that I could collect data simultaneously from multiple colonies under different conditions. I kept the intensity of light at comparable levels between all chambers (550 lux - light phase, 0.5 lux - dark phase).

The insects in the cages were video recorded during different temperature and day length regimes through multiple daily cycles using Panasonic HC-V160 (Panasonic Corp. Newark, NJ,

USA) portable camcorders with low light recording capabilities. I was not able to record at all hours of the daily cycle in a single recording session due to video memory and staffing constraints, so to ensure that data were collected during each hour of the 24-hour cycle, I periodically shifted the start of the dark phase in the rearing chambers by 6 or 12 hours. Colonies were allowed to acclimate to new conditions for 3 to 5 days before video recording. I standardized the time of observations relative to the dark phase of the sampling period by subtracting the time the sample occurred from the start time of the dark phase using this simple equation: $T_{rdp} = 24 – (T_{dp} - T_s)$. Where T_{rdp} is time relative to dark phase, T_{dp} is the time the dark phase starts, and T_s is the time the sample was taken. To simplify data interpretation by humans, all times were then standardized to a 24 hour cycle in which the dark phase began at 20:00.

The video recordings were sampled at approximately 30 min intervals by replaying the recording and observing the colony for 30 second samples. I used Windows Media Player version 10 or VLC Media Player version 2.26 for video playback, and made a minimum of 12 samples from different cages of moths for each hour and temperature or day length combination. At each 30 second sample, the number of mating pairs, the number of male flights, and the time that each sample was taken (calculated from the video file information and the video timer) was recorded. Similarly, the start and end of the dark phase of the light cycle was recorded from the video timer. For each recording session, mating pairs and male flights were averaged for each h of observation to yield matings per hour (mph) and flights per hour (fph). Observations that occurred during the 24h period from 12:00 on the first day of recording to 11:59 the following day were grouped into replicates for statistical analyses. I counted all observed flights as male flights based on the results of a pilot test where I used 5 *P. viteana* colonies and captured 10 moths in each colony that were flying during the scotophase. Captured moths were anesthetized

with CO_2, and after examination using a dissecting microscope, all of the captured moths were determined to be male.

The data collection protocol described below was used for all observations in three experiments. In the first experiment, the effect of temperature on male flight and mating frequency and period was tested by placing colonies in environmental chambers set at constant temperatures of 10, 15, 18, 23, 28 or 35°C, 60% RH and a 17L:7D light cycle. In the second experiment the effect of day length on moth mating and male flight was tested by placing colonies in chambers set at 28°C and 60% RH and programmed with either a long, 17 hour light:7 hour dark (17L:7D), moderate (14L:10D), or short (12L:12D) day length regime. In the final set of experiments, colonies were observed under three diurnal temperature regimes that were loosely based on temperatures that occur during the growing season. The diurnal regimes consisted of a hot weather cycle (28°C day - 18°C night), a warm weather regimen (28°C day - 10°C night) and a cool weather treatment (20°C day - 10°C night), and all colonies experienced the same photoperiod 17L:7D and 60% RH. This was repeated for 3 colonies and each one received one of the three diurnal weather treatments on successive weeks, and I randomized the order that the diurnal temperature regimes were presented. For each of the three experiments explained above, I compared the start time, end time, length of the mating period and mating frequency across temperature, day length and diurnal pattern treatments in experiment sets 1, 2 and 3, respectively, with one-way ANOVAs followed by Tukey's HSD for mean separations. I did similar comparisons of start time, end time, period length and frequency for male flight activity in those same experiments. In all analyses, data were square root or natural log transformed as needed to stabilize error variance and to meet the assumptions of analysis of variance (ANOVA). In cases where transformations did not satisfy the assumptions of ANOVA, a Wilcoxon ranked sums test was used on untransformed data to determine if treatment

differences existed, and to separate the mean treatment effects (JMP 13.1.0, SAS Institute. Cary, NC). Specific transformations that were used for statistical analyses are reported in the Results section.

To determine how often male flights occurred when females were not receptive to mating (i.e. outside of the mating period), I calculated the proportion of all male flights and mating pairs that occurred during each hour of the 24 cycle for each experimental treatment. These data were graphed together by treatment, and then the area under each portion of the graph where male flight occurred but mating pairs were not observed was integrated using Microsoft Excel. For each experiment (constant temperature, day length or diurnal temperature regime), I then compared the proportion of male flights in each treatment that occurred outside of the mating period across treatments using the comparison of multiple proportions test (Zar 1999). These proportions will be discussed as percentages for clarity.

Response to pheromone lures at different times of the season

A field trial to test the response of male *P. viteana* to sex pheromone lures at different times of the season was conducted from May to October 2017. Lures with custom pheromone loads were made for this experiment using a 10:1 ratio of analytical grade (*Z*)-9-dodecenyl acetate and (*Z*)-11-tetradecenyl acetate (Bedoukian, Danbury, CT, USA). I first mixed stock solutions of 300 and 500µg/ml using hexane as the solvent, and then further diluted these with hexane to yield loading solutions of 60, 300, 400 and 500µg/ml (Table 4.2). Small rubber septa (West Pharmaceuticals, Exton, PA, USA) were arranged open side up in aluminum mesh racks with 0.64 cm^2 openings and filled with either 0.20 ml or 0.25ml of the approriate dilution to produce lures loaded with 0, 15, 60, 75, 100 or 125 µg of *P. viteana* pheromone.

The mesh racks were held in a fume hood until the solutions evaporated. An additional 0.25ml of hexane was then pipetted into each septum to help the pheromone adsorb onto the septum wall. The lures were dried in the fume hood and then grouped by pheromone load and each group was placed in a separate 200ml labeled glass jar, sealed with teflon tape and stored in a freezer (-5°C) until the lures were deployed in traps.

Table 4.2. Stock solutions, dilutions and loading volumes to make lures for field testing.

Lure load (µg/lure)	Stock solution (µg/ml)	Dilution factor	Loading concentration (µg/ml)	Loading aliquot (ml)
0	0	1	0	0.25
15	300	5	60	0.25
60	300	1	300	0.20
75	300	1	300	0.25
100	500	1	500	0.20
125	500	1	500	0.25

The field trial to test the response of male *P. viteana* to traps baited with different pheromone loads was performed in seven juice grape vineyards in Lawton, Michigan. Each vineyard had a history of grape berry moth infestation and was adjacent to a woodlot containing wild grape (*Vitis* spp.). At each site, the trial was established along a 50m section of vineyard border adjacent to a woodlot. All vineyards were planted on single wire, top-cordon trellis, with 3 or 3.5m between vineyard rows and 3 or 3.5m between vines. Six vineyard posts situated at least 10m (3 rows) apart and along the border were flagged and these locations were used to hang traps throughout the season. To record the temperature and calculate accumulated growing degree days at each site, a Hobo UA-001-08 data logger (Onset Computer Corp., Bourne, MA, USA) was hung on the inside of an inverted 240ml (8oz) white styrofoam cup and suspended from the trellis wire 1.7m (5.5ft) above the ground. Hobo data-loggers were deployed at the approximate center of the 50m section of vineyard border used for this experiment. White

Pherocon VI large plastic delta traps (Trece Inc., Bend, OR, USA) were baited with one of the lures listed in Table 4.2. A 4cm sewing pin was inserted through the side wall of each trap and a lure was skewered on the pin and held near the middle of the open space inside the trap. Sticky trap liners were inserted into the bottom of baited traps and these were then hung on the vineyard trellis wire. Traps were removed from the trellis each week and the number of *P. viteana* males captured on the sticky trap liners were recorded. Trap liners were replaced, and the relative location of the traps was re-randomized before the traps were reattached to the trellis wires. Traps were set up on 9 May (early vine growth) and removed from the vineyards on 14 November 2017 (postharvest). Lures were changed approximately every 3-4 weeks, except that one set of lures was deployed for 8 weeks during the harvest period in September and October. The length of time each lure set was deployed constituted a trapping period, so there were five trapping periods (May, June, July, August and September/October). As the length of the trapping period was not the same for all lure sets, prior to analysis, male captures were divided by the number of days in the trapping period to give captures per day.

For each lure change, the jars of lures described above were transported to the field in a cooler filled with ice packs. After traps were checked, the used lures were removed, placed separately in labeled 29.34 ml plastic deli cups (Solo, Urbana, IL, USA), and a new lure of the same pheromone load was hung on the pin inside the trap. The cups with used lures were then placed in a cooler, returned to the laboratory and stored in a freezer (-5°C) until the pheromone in the lures could be extracted and analyzed using GC-MS. Nitrile gloves were used for all trap and lure handling, and gloves were changed between checking individual traps and between changing different lures.

To estimate the quantity of pheromone released from lures during each trapping period, I soaked each lure in 5 ml of hexane in individual 20ml glass scintillation vials (RPI, Mt. Prospect,

IL, USA), for 10 days to extract the pheromone remaining in the lure. For each lure set, one unused lure from each level of lure load was selected to be used as a control on the day that the extractions were set up, and the pheromone was extracted using the same method. A one ml sample of solvent from each lure extraction was then pipetted into separate 2ml clear glass screw top GC vials (Wheaton, Millville, NJ, USA). To calculate the concentration of target compunds in each sample, a 50µl aliquot of methyl myristate (methyl tetradecanoate) (Bedoukian, Danbury, CT, USA) in hexane (500µg/ml) was added to each GC vial as an internal standard with a final concentration of 25µg/ml (Rodriguez-Saona and Stelinski 2009). The amount of pheromone in each lure was analyzed using an Agilent 7890A gas chromatograph (GC) paired with an Agilent 5975C mass spectrometer (MS) (Agilent Technologies, Santa Clara, CA, USA) following the methods reported in Hufnagel et al. (2017). An Agilent HP-5 column (30m length, 0.320mm ID, film thickness 0.25µm) was used for all GC-MS analyses with helium used as the carrier gas at 30cm s^{-1} flow velocity. Aliquots (1 µl) of each sample were injected into the GC-MS and separated using a program of 1 min at 40°C followed by increasing temperature at a rate of 10°C min^{-1} to 260°C. Isobutane was used as the reagent gas for chemical ionization. Ion source temperature was 250°C in chemical ionization mode and was 220°C in electron impact mode. GC-MS results were analyzed using MSD ChemStation v.2.00 (Agilent Technologies, Santa Clara, CA, USA). (*Z*)-9-dodecenyl acetate, (*Z*)-11-tetradecenyl acetate and methyl myristate were identified from the samples by comparing the mass spectrum of each compound to those in a reference library, NIST 11 (National Institute of Standards and Technology, Springfield VA, USA). Compound identifications were confirmed by analyzing standard solutions of single compounds and mixtures of (*Z*)-9-dodecenyl acetate, (*Z*)-11-tetradecenyl acetate and methyl myristate with concentrations ranging from 0.5 to 500µg/ml using the GC method described above to determine retention times, and the range of response for each of these compounds. The

amount of each target compound in each sample was then calculated using the ratio of the peak area of that compound to the peak area of the known amount of internal standard in the sample using this equation:

Amount of compound A(µg) = (Peak area A/Peak area IS) x [IS] (µg/ml) x extraction volume (ml).

I then determined the amount of pheromone components, (*Z*)-9-dodecenyl acetate and (*Z*)-11-tetradecenyl) released from each lure during the trapping period by subtracting the amounts of these compounds in the lure sample from the average amounts of these compounds extracted from the corresponding unused control lures. Only the major component, (*Z*)-9-dodecenyl acetate was used in subsequent analyses because the GC-MS was not able to accurately measure the very low amounts of the minor pheromone component, (*Z*)-11-tetradecenyl acetate) in these samples. The amount of (*Z*)-9-dodecenyl acetate released was calculated as the difference in the mass of pheromone in the deployed lures and the average mass in the undeployed control lures. As the length of the trapping period was not the same for all lure sets, values are presented as µg released per day.

To test the effect of different lure load treatments on the total number of males captured in a trapping period and all season, I used a mixed model ANOVA with male captures/d as the dependent variable, vineyard as a random or blocking factor and lure load as a fixed factor. In this analysis vineyards were considered replicates, and a separate analysis was performed for each trapping period. The mixed model analysis was followed by mean separation with Tukey's Honestly Significant Difference test (JMP 13.1.0, SAS Institute. Cary, NC).

To determine the relationships between male captures and pheromone release at different times of the season, I first regressed the average captures for each lure load on the average amount of pheromone released from those lures during each sampling period. To determine if the

relationship between captures and pheromone release was similar during different times of the year, I used analysis of covariance with male captures as the dependent variable, pheromone release as the main factor and sampling period as the covariate, and this analysis was followed by Tukey's HSD on least square means for sampling period to detect differences in the effect of pheromone release on captures across periods. As male capture is likely to be correlated with both temperature and pheromone release, I used residual regression analysis to tease apart the relationship between moth captures, pheromone release and temperature. I first used linear regression to determine the relationships between captures and pheromone loss and captures and temperature. I used growing degree day (GDD) accumulations based on the developmental threshold for *P. viteana* of 8.41°C (Tobin et al. 2001) as the measure of temperature. GDDs are a sum of accumulated heat during a sample period, and this better represents the totality of temperature conditions that lures and moths experienced during a sample period than does average daily temperature. I used averages pooled across replicate vineyards of male captures and pheromone release for each lure load/sampling period. I saved the residuals from the regressions of captures on $GDD_{8.41°C}$ and from the regression of pheromone release on $GDD_{8.41°C}$. By regressing these residuals, the effect of pheromone release on captures was assessed with the effect of temperature removed (JMP® 13.1.0., SAS Institute, Cary, NC, USA).

RESULTS

Moth longevity

Average *P. viteana* longevity was 57.5 ± 3.5 days at 10°C, 17.8 ± 0.9 days at 18°C, and 8.9 ± 0.7 days at 28°C. This represents an almost six-fold decrease in survival length from lowest to the highest temperature; and, moths held at 18°C lived twice as long as those held at 28°C. There was a strong statistically significant effect of temperature on longevity, and the effect was similar for males and females ($F_{1,136} = 0.0006$; $P = 0.94$, log transformed data). Longevity data were pooled for the two sexes for one-way analysis of variance (Figure 4.3). Longevity at 10°C was significantly longer than at 18°C, and longevity at 18°C was significantly lower than at 28°C ($F_{2,135} = 134.0$; $P < 0.0001$, log transformed data, Figure 4.3).

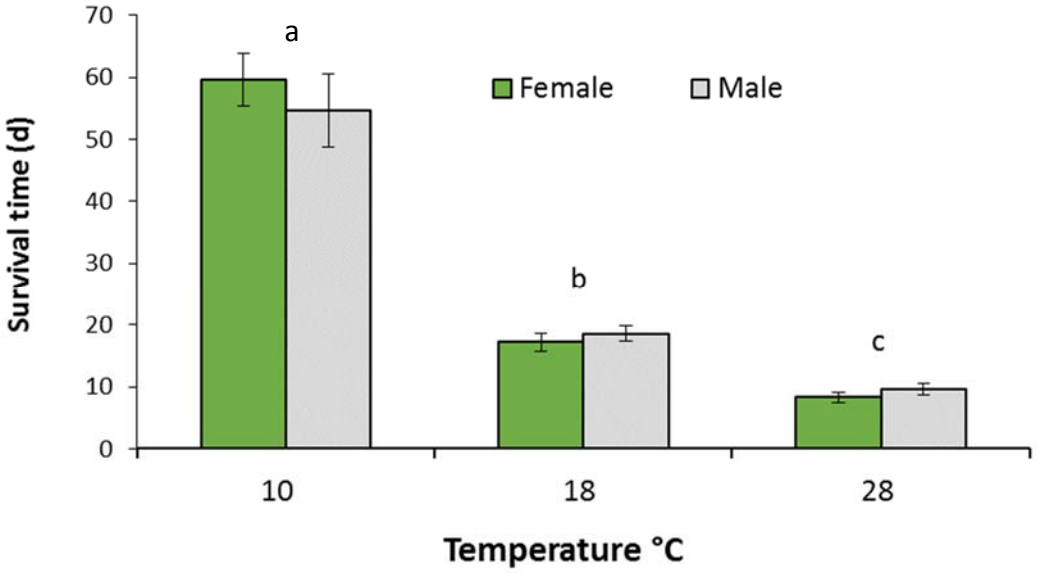
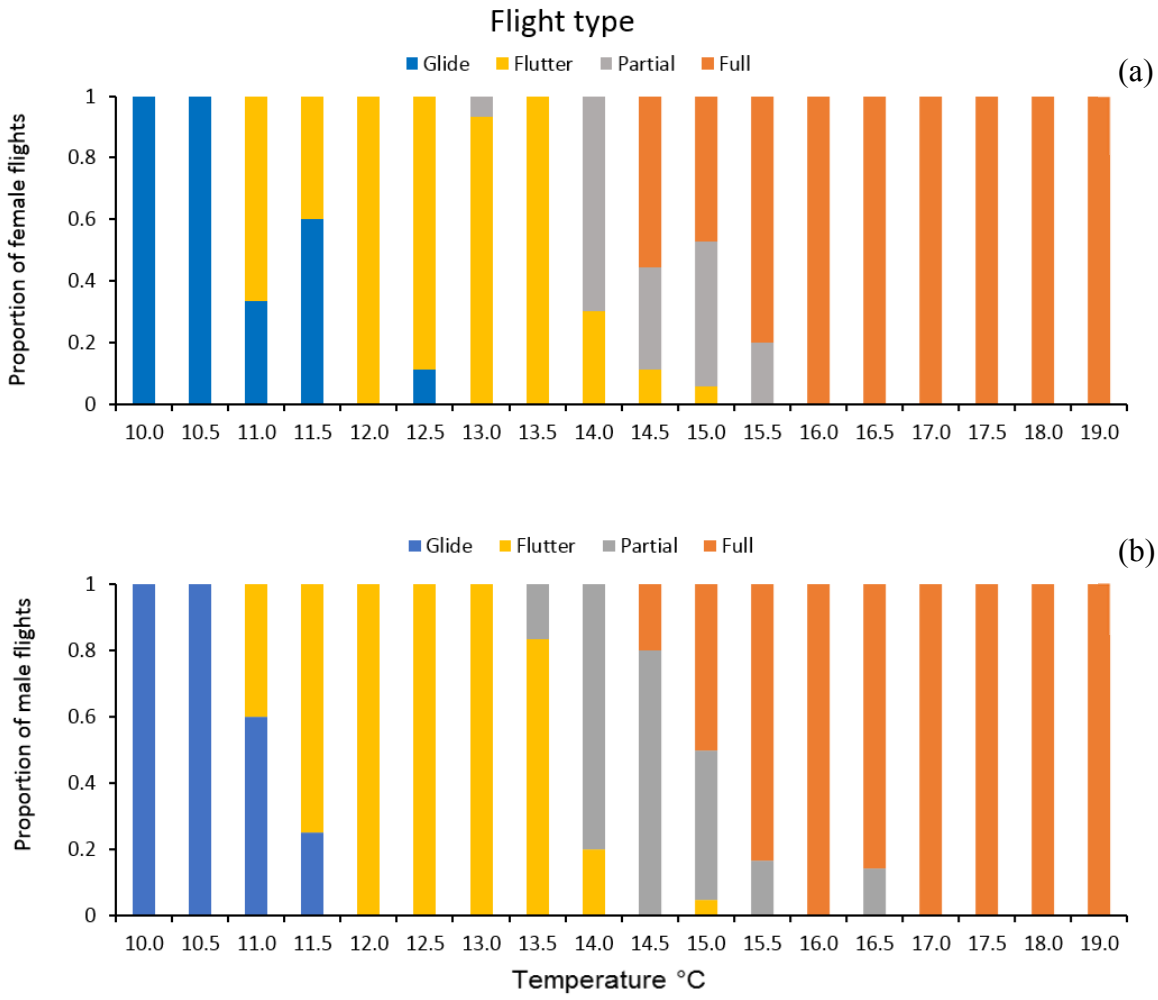


Figure 4.3. Longevity of moths held at different temperatures. Survival of males and females were not significantly different so data from the sexes were pooled, and all data were log transformed before analysis. Columns headed with different letters are significantly different (P < 0.05).

Minimum flight temperature

Male and female *P. viteana* showed similar characteristic flight behaviors when launched into the air at different temperatures (Figure 4.4). They were unable to fly at temperatures below 11°C, and at this temperature all launches resulted in gliding on a parabolic path in the vertical plane of the launching strip, with no wing movements. In the range from 11 to 13.5°C, catapulted moths displayed wing movements and fluttering flight, without deviating from a similar parabolic trajectory. Partial flight, where moths were able to move their wings



sufficiently to change direction from the normal parabolic path but without powered level or upward movement, occurred from 13°C to 15.5°C for female moths and from 13.5°C to 16.5°C for males. Full powered flight, where moths were able to change direction and fly upward or

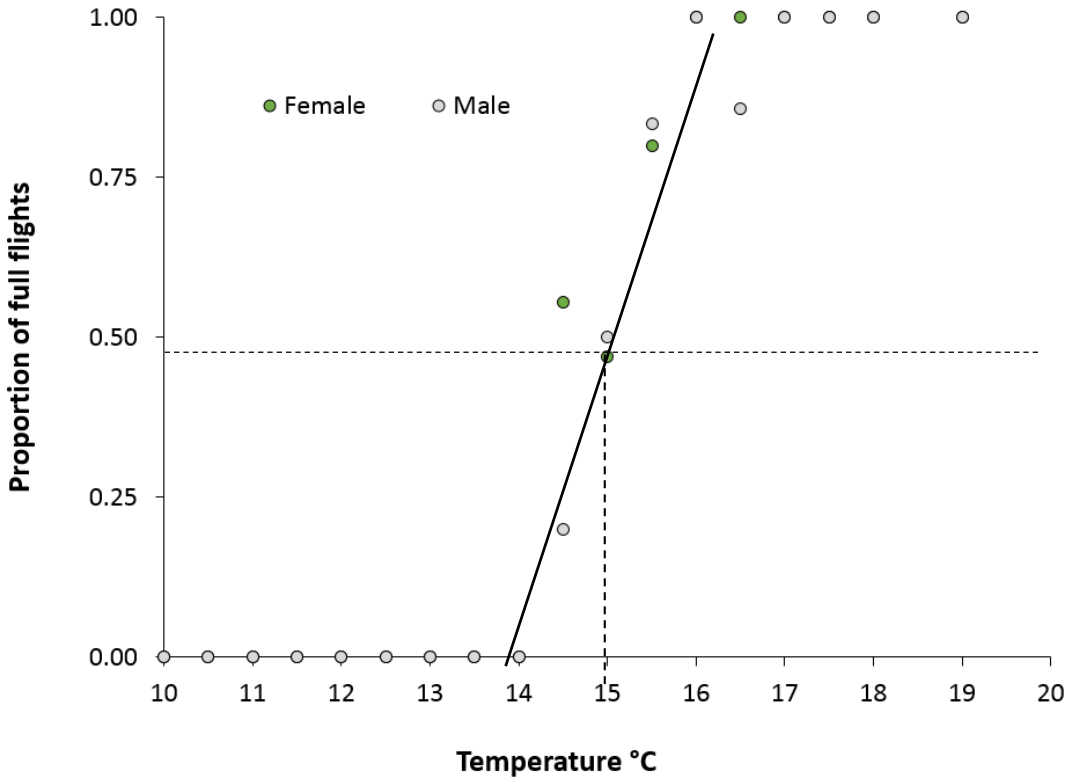


Figure 4.5. Average proportion of female and male *P. viteana* that exhibited full flight in the temperature range 10 to 19°C. The solid line indicates the line of best fit for pooled male and female data. The vertical dashed line shows the FT_{50} for all moths (pooled) is estimated to be 15ºC.

level after launch was first seen at 14.5°C for both sexes. The FT_{50} or the temperature at which 50% of moths were able to fly upward, level or change direction after launch, was determined to be 15°C for males and 14.9°C for females (Figure 4.5).

Oviposition

Paralobesia viteana female moths held with grapes suspended in cylindrical cages laid significantly more eggs on grapes at 28°C than at either 18°C or 10°C ($\chi^2 = 69.3$, $P < 0.0001$). At 28°C, 4.5 ± 0.8 eggs were laid per female, while at 18°C there was less than one egg per female (0.05 ± 0.05). Female moths maintained at 10°C did not oviposit. In trials with grapes affixed to a Petri dish that was placed on the cage floor, the results were very similar to the experiment above, as temperature had a strong significant effect on oviposition ($F_{2,27} = 25.8$; $P < 0.0001$, log-transformed data). At 28°C, I found females laid an average of 7.9 ± 1.6 eggs per moth, and that was reduced to 1.4 ± 0.7 eggs per female when cages were held at 18°C. No eggs were laid on grapes in the 10°C cages (Figure 4.6).

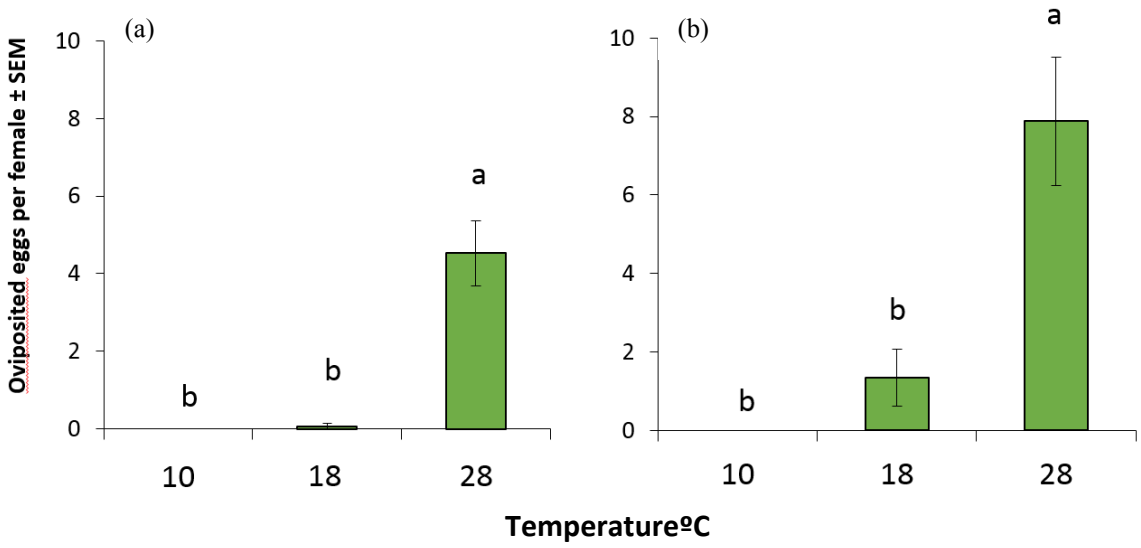


Figure 4.6 Oviposition at different temperatures in bioassay cages with (a) grapes suspended, or (b) attached to the floor of the cage. Columns with the same letter within each graph are not significantly different ($P > 0.05$).

Diel patterns of mating and male activity in colonies

Constant temperature. In colonies held at constant temperatures, the frequency of mating, expressed as matings per hour (mph) averaged over the entire 24-hour cycle, ranged from 0.01 mph at 10°C to 0.52 mph at 28°C. This is an exponential increase from 10 to 28°C, but mating frequency was reduced at 35°C (Figure 4.7a). At 28°C the frequency of mating was significantly higher than mating at any of the other temperatures ($\chi^2 = 62.9$, $P < 0.0001$, Figure 4.7a). The number of male flights observed per hour (fph) also increased significantly in the range of tested temperatures ($F_{4,122} = 27.9$; $P < 0.0001$, log transformed data, Figure 4.7b). The frequency of male flights ranged from 0.001 at 10°C to 9.8 fph at 28°C, and there were significant differences in male flight frequency between treatments.

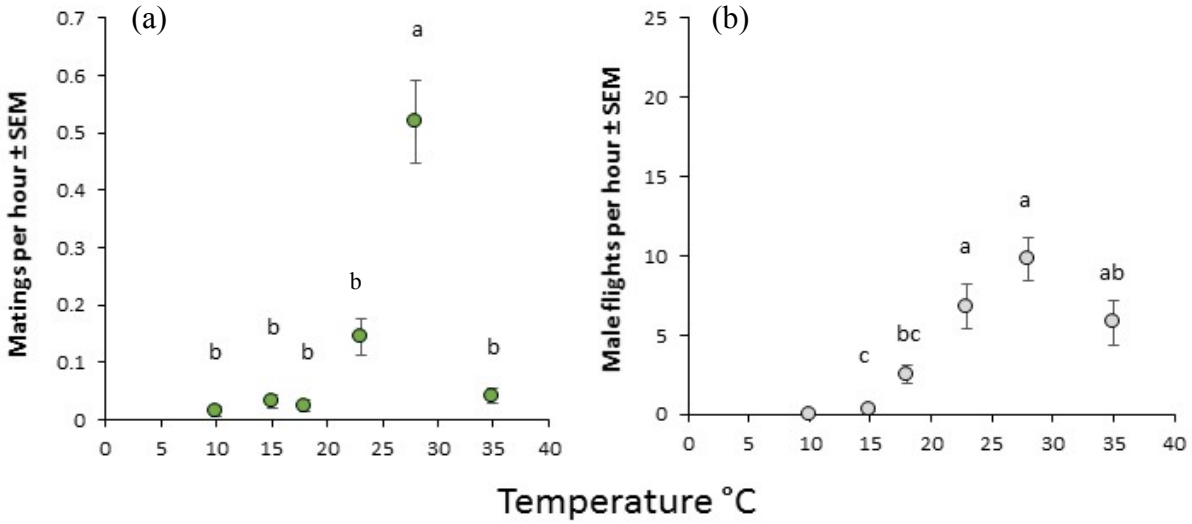


Figure 4.7. Effect of temperature on mating frequency (a) and flight frequency (b) in colonies held at different temperatures. Data are the average number of behaviors observed per hour averaged over all sampling hours. Points with different letters are significantly different ($P < 0.05$). Only one flight was observed in colonies at 10ºC so those data were excluded from the analysis.

Across all temperatures, the average time of the first observed mating pair occurred during the dark phase (20:00 to 03:00), and this time ranged from 21:26 to 0:34. Despite this large range, temperature had no significant effect on the time of first mating ($F_{5,102} = 2.29$; $P = 0.052$, log transformed data, Figure 4.8a). The average time of the first male flight ranged from 06:59 to 14:14, and thus occurred much earlier in the day than the first mating (Figure 4.8b). Significant differences among temperature treatments were detected in the time of first male flight ($\chi^2 = 17.3$, $P = 0.0017$, Figure 4.8b). Flights began significantly later in colonies reared at 23°C and 35°C compared to flights in colonies held at 18°C or 28°C. The last mating pair was observed

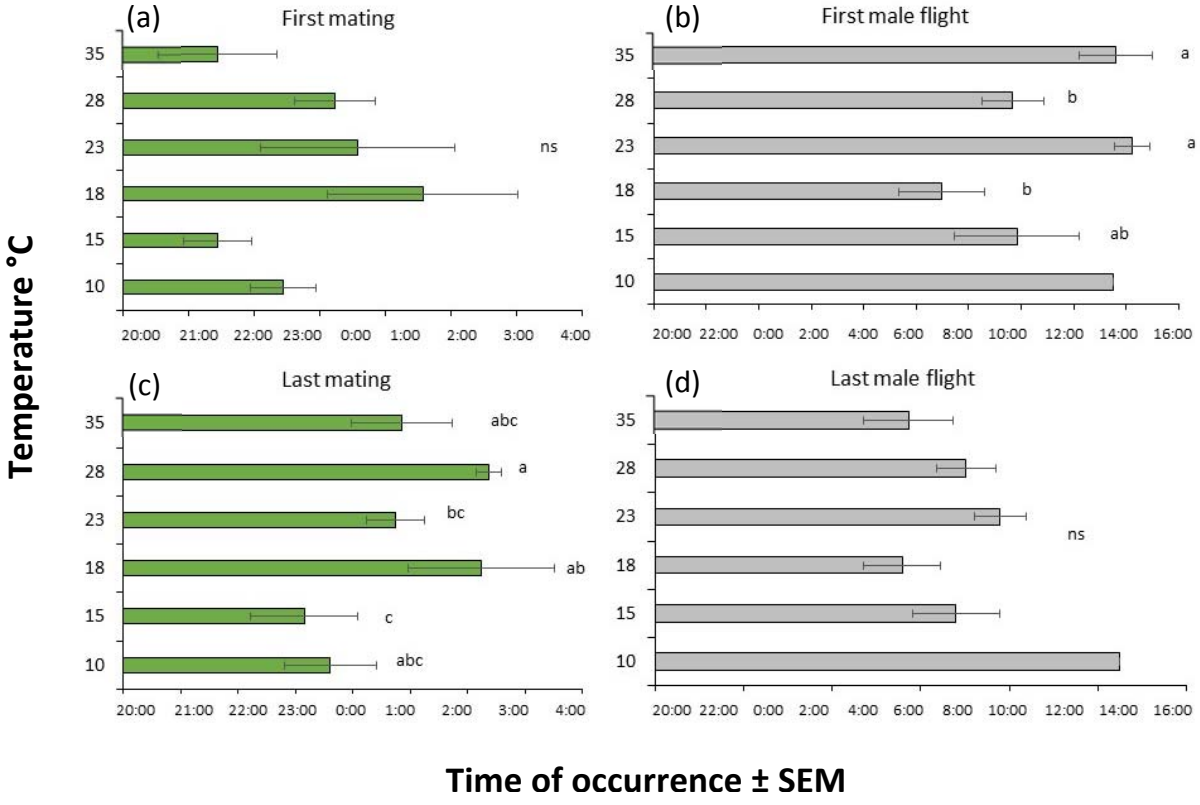


Figure 4.8. Average time of the first observation of mating pairs (a) and male flights (b), and average time of last mating pair (c) and male flight (d) in colonies reared at different temperatures. Times of first and last behaviors were averaged over all days where at least 2 behaviors were observed in the 24 h period from 12:00 on the first day of recording to 11:59 the following day. Male flight data from colonies reared at 10ºC were not included in the analysis because only one flight was observed across all samples. In each graph, columns with different letters are significantly different ($P < 0.05$).

from 03:09 to 06:22, and mating was observed significantly later in colonies reared at 28°C compared to colonies held at 23°C or 15°C. The last mating in colonies held at 18°C occurred later than in colonies reared at 15°C ($F_{5,102} = 5.51$; $P = 0002$, log transformed data, Figure 4.8c). The average time of the last male flight ranged from 09:18 to 17:30, and there were no significant differences among temperature treatments ($F_{4,97} = 1.97$; $P = 0.10$, log transformed data, Figure 4.8d).

The average duration of the mating period ranged from 69 minutes at 10°C to 294 minutes at 28°C and there was a significant effect of temperature on the duration of the mating period ($F_{5,102} = 5.7$, $P < 0.0001$, log transformed data, Figure 4.9a). The mating period was significantly longer at 28°C than at 10°C, 15°C or 18°C. Temperature significantly affected the duration of the male flight period ($F_{4,98} = 5.11$, $P = 0.0009$, log transformed data, Figure 4.9b). Male flight period

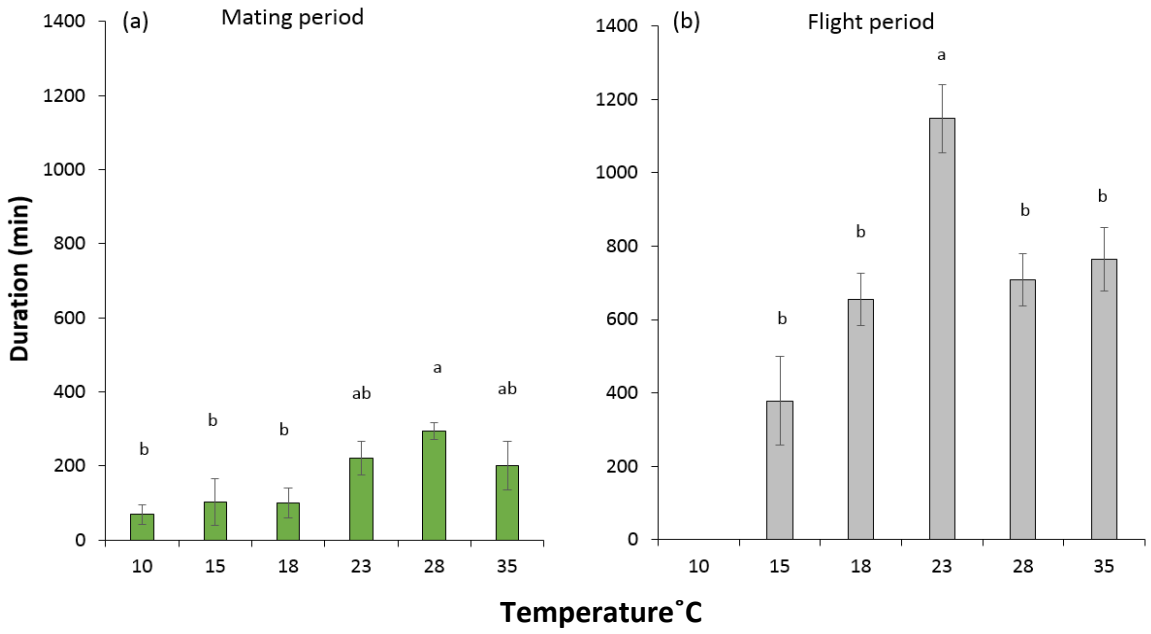


Figure 4.9. Average duration of the mating period (a) and flight period (b). In each graph, columns with different letters are significantly different ($P < 0.05$). The flight period was significantly longer than the mating period for all temperatures except 15ºC ($P < 0.05$). See text for statistics. Male flight data from colonies reared at 10ºC were not included because only one flight was observed across all samples.

duration ranged from 0 to 1147 minutes, and flight period duration for colonies held at 18, 23, 28 and 35°C were all significantly longer than for colonies reared at 15°C (Figure 4.9b). Figure 4.9 shows that the male flight period was longer than the period when mating pairs were observed. For all constant temperature treatments except 15°C, the flight period was significantly longer than the mating period (15°C - $F_{1,18} = 1.09$, $P = 0.3107$; 18°C - $F_{1,33} = 30.5$, $P < 0.0001$; 23°C - $F_{1,42} = 14.5$, $P = 0.0005$; 28°C - $F_{1,85} = 5.5$, $P = 0.0215$; 35°C - $F_{1,20} = 12.8$, $P = 0.0019$; all analyses used log transformed data). The duration of the mating period and flight period at 10°C was not used in the analysis because only one male flight was observed.

Table 4.3. Summary of mating and male flight measurements for colonies held at constant temperatures. Values in the same column with different letters are significantly different ($P < 0.05$).

Temp °C	Proportion male flights outside mating period	mph*	# times behavior observed	Hours observed	fph**	# times behavior observed	Hours observed
10	1	0.01	7	763	0.002	1	405
15	0.58 a	0.03 b	19	584	0.31 c	375	798
18	0.35 a	0.04 b	30	737	2.51 bc	1666	569
23	0.34 ab	0.14 a	114	833	6.82 a	5985	1089
28	0.12 b	0.52 a	639	1227	9.78 a	8751	650
35	0.63 a	0.06 b	30	495	5.79 ab	2864	472

* mph = Average number of mating pairs per hour averaged across all hours.
** fph = Average number of male flights per hour averaged across all hours.

The proportion of male flights that occurred outside of the mating period was lowest in colonies reared at 28°, and this was significantly lower than the proportion of male flights that occurred outside the mating period in colonies reared at 15, 18 or 35°C (Table 4.3). Data collected from colonies reared at 10°C were not included in this analysis. At 15°C, 58% of male flights occurred during periods when no mating pairs were observed, and this was statistically similar to male flight behavior in colonies at 18°C, 23°C and 35°C, where 35%, 34% 63% and of

flights were observed outside of the mating period, respectively (Figure 4.10, Table 4.3). Few mating pairs were observed at 35°C (Figure 4.8), so the high proportion of male flights that occurred at this temperature are contributing to this result (Table 4.3).

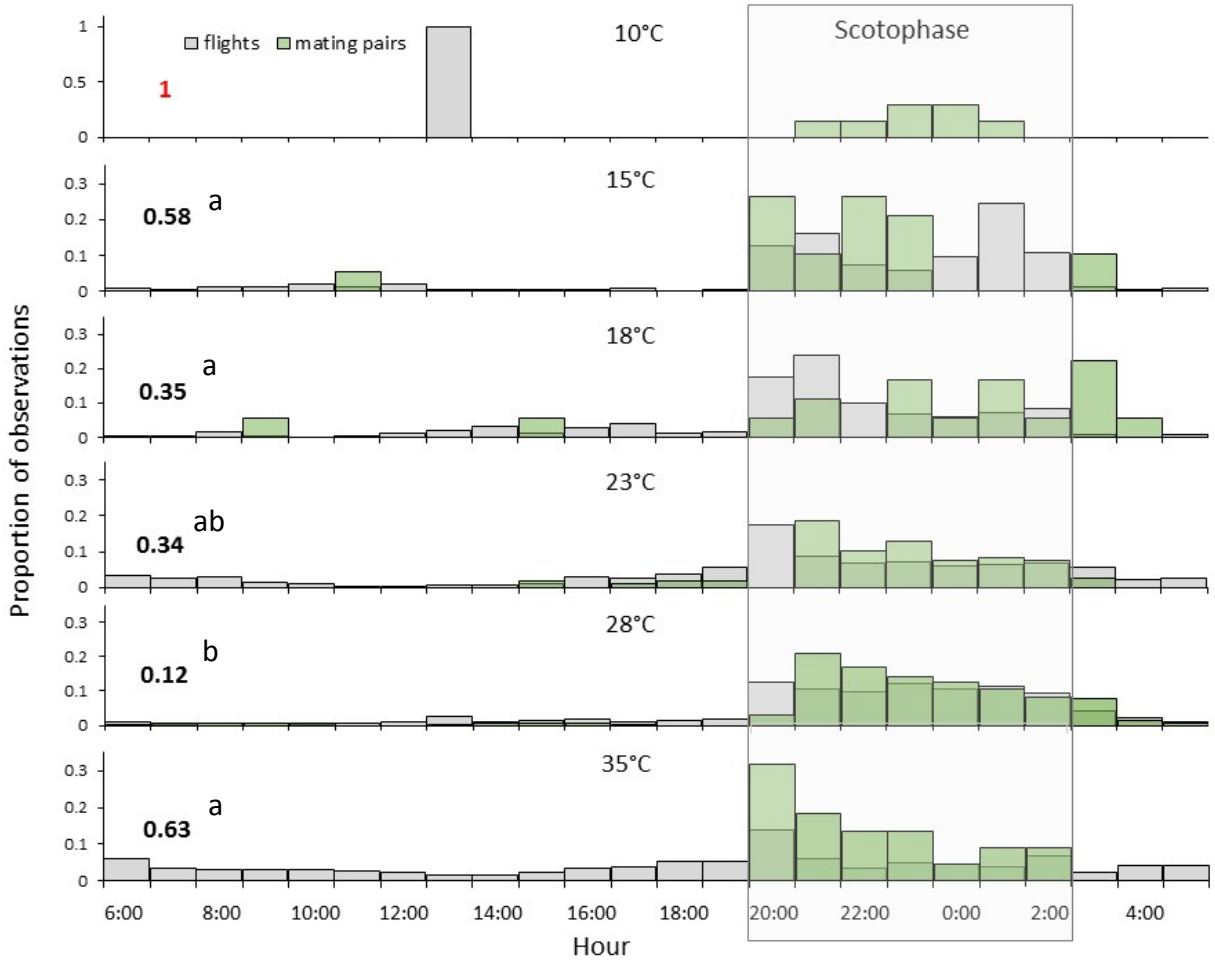


Figure 4.10 Proportions of male flights and mated pairs of *P. viteana* observed each hour in colonies held at constant temperatures under 17:7 L:D and 60-75% RH. The shaded rectangle covering the period from 2000 to 0300 hours on the x-axis indicates the scotophase. The proportion of male flights that occurred when mating was not observed is given in the bold numbers on the left hand side of each graph. Proportions with the same letter are not statistically different ($Q_{0.05,\infty,6} > 4.03$, $P < 0.05$, arcsine transformed data, using multiple comparison of proportions (Zar 1999)). Data from colonies reared at 10°C (shown in red) were not used in this analysis because only one flight was observed in all samples.

Day length. In colonies that were reared in short (12L:12D), moderate (14L:10D) or long (17L:7D) day length cycles, the frequency of mating ranged from 0.08 to 0.21 matings per hour (mph), and this frequency increased with increasing day length (Table 4.4). Mating frequency in the long day length treatment was significantly higher than that in the short or moderate day length treatments, but mating in the short and moderate day length treatments was similar ($F_{2,114}$ = 21.3; P <0.0001, log transformed data, Figure 4.11a). The number of male flights observed per hour (fph) also increased as day length increased, and significant differences were detected between day length treatments ($F_{2,72}$ = 12.1; P <0.0001, log transformed data, Figure 4.11b). Male flights per hour ranged from 8.51 fph under a short day length regime to 16.52 fph under the long day length treatment. Male flight frequency under this day length treatment was significantly higher than male flight frequency in either moderate or short day lengths (Figure 4.11b).

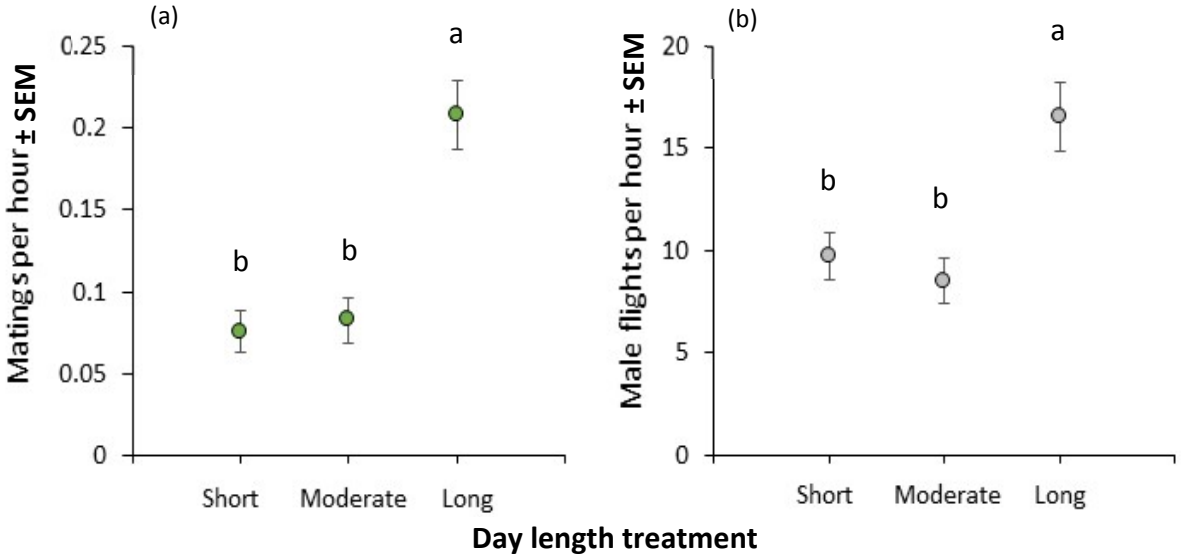


Figure 4.11. Matings per hour (a) and male flights per hour (b) in *Paralobesia viteana* colonies reared under short (12h), moderate (14h), or long (17h) day lengths. Values labeled with different letters are significantly different (P < 0.05).

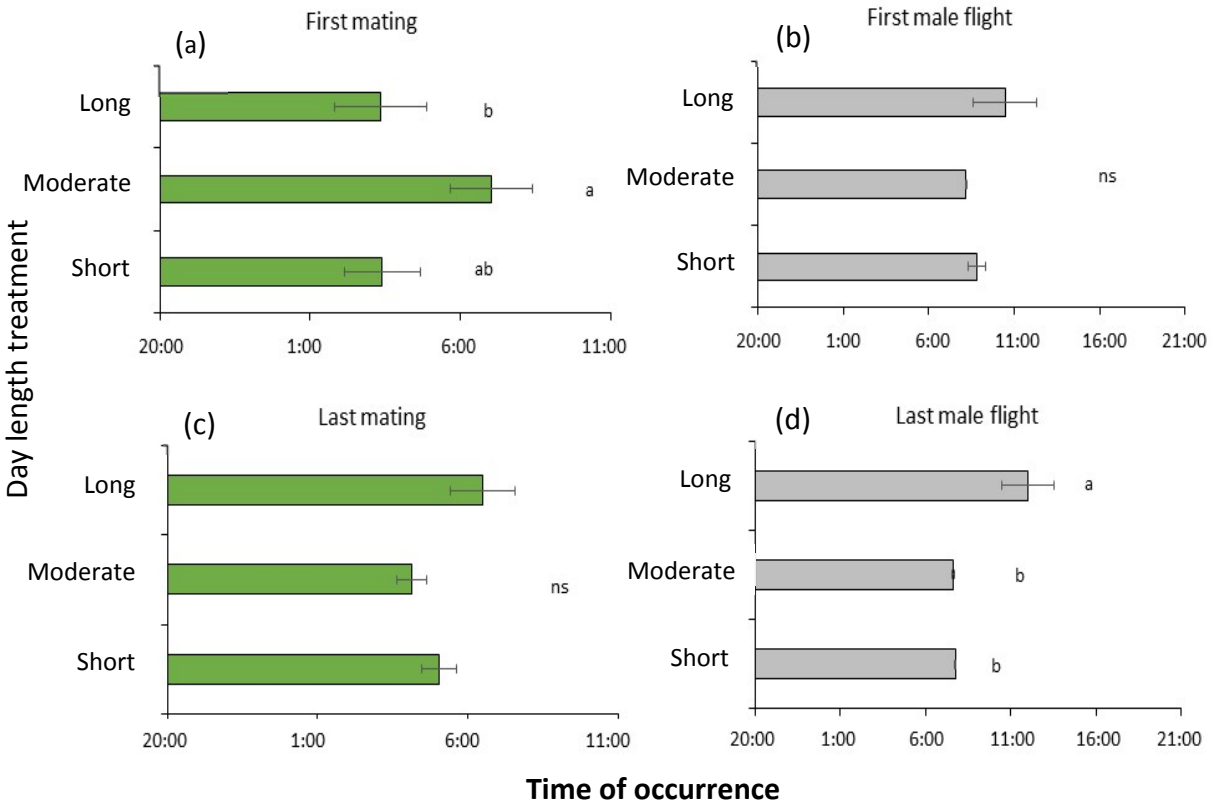


Figure 4.12. Average time of the first observation of mating pairs (a) and male flights (b), of *Paralobesia viteana* and average time of last mating pair (c) and male flight (d) in colonies reared under 3 different day length treatments. Times of first and last behaviors in the 24 h period from 12:00 on the first day of recording to 11:59 the following day on all days the behavior was observed. All colonies were reared at 28°C. Columns with different letters are significantly different ($P < 0.05$).

The average time of the first observed mating pair in colonies held under different day lengths ranged from 05:22 to 07:03. The first mating pair in the moderate day length treatment occurred significantly later than the first mating pair in the long day length treatment, but no differences were detected between the short and long day length treatments ($F_{2,96} = 3.35$; $P = 0.039$, log transformed data, Figure 4.12a). The average time the last mating pair was observed ranged from 04:08 to 06:30, but the end of the mating period was not significantly different among treatments ($F_{2,96} = 0.34$; $P = 0.47$, log transformed data, Figure 4.12c). The average time of the first male flight ranged from 08:12 to 10:28, and there were no significant differences

detected between day length treatments ($F_{2,55}$ = 1.64; P =0.2019, log transformed data, Figure 4.12b). The average time of the last observed flight across all treatments ranged from 07:38 to 12:01. The last flight in colonies reared in the long day length treatment was significantly later than that in either the short or moderate day length treatments (χ^2 = 23.03, P < 0.0001, Figure (4.12d).

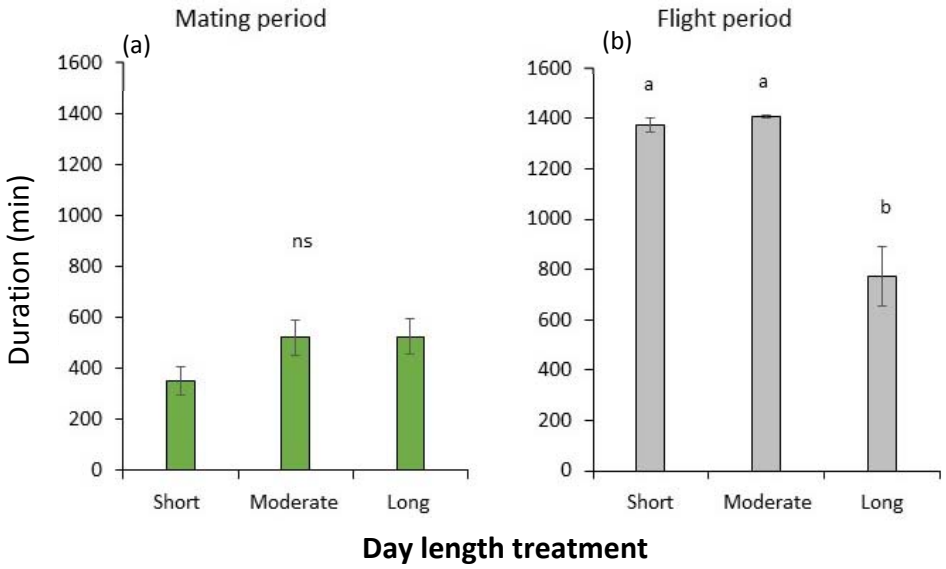


Figure 4.13. Mating period duration (a), and flight period duration (b) of *Paralobesia viteana*, in colonies reared under different day lengths. Averages are taken only where a given behavior was observed at least twice during the 24 hour period from 12:00 on the first day to 11:59 on the second day of recording. Columns labeled with different letters are significantly different (P < 0.0001).

Table 4.4. Summary of mating frequency and male flight measurements for colonies of *Paralobesia viteana* held under different day length regimes. Values in a given column with different letters are significantly different ($P < 0.05$).

Day length	Proportion male flights outside mating period	mph*	# times behavior observed	Hours observed	fph**	# times behavior observed	Hours observed
Short	0.11	0.08 b	7	763	9.68 b	8221	932
Moderate	0.06	0.08 b	19	584	8.51 b	7328	890
Long	0.02	0.21 a	30	737	16.52a	10676	778

* mph = Average number of mating pairs per hour averaged across all hours.
** fph = Average number of male flights per hour averaged across all hours.

The duration of the mating period ranged from 353 minutes (5h, 53m) in the short day length treatment to 526 minutes (8h, 46m) for colonies reared under a long day length, but no significant effect of day length on the duration of the mating period was detected ($\chi^2 = 3.77$, df =2, $P = 0.15$ Figure 4.13a). There was a significant effect of temperature on the duration of the male flight period ($\chi^2 = 23.03$, df =2, $P < 0.0001$, Figure 4.13b). Male flight period duration ranged from 774 to 1406 min (12h 54m to 23h 26m), and the flight period for colonies held at short or moderate day length was significantly longer than for colonies reared under the long day day length treatment (Figure 4.13b). In colonies reared under the short day length treatment, the duration of the flight period was 774 minutes and this was significantly longer than the mating period of 353 minutes ($\chi^2 = 36.2$, df =2, $P < 0.0001$). Similarly, in the moderate day length treatment the male flight period was 1406 minutes, and this was significantly longer than the mating period of 552 minutes ($\chi^2 = 34.8$, df =2, $P < 0.0001$). In the long day length treatment, the lengths of the flight period (774 minutes) and the mating period (526 minutes) were not different ($\chi^2 = 2.88$, df =2, $P = 0.09$). This indicates that in the short and moderate day length

treatments, males were flying when females were not receptive to mating, but this is not true in colonies reared in the long day length treatment.

The proportions of male flights that occurred outside of the mating period were similar among day length regimes. This proportion was lowest in colonies under the long day length treatment: under these conditions only 2% of male flights occurred when mated pairs were not visible. In colonies reared under a moderate day length, 6% of male flights occurred during periods when no mating pairs were observed. The highest proportion of male flights occurring outside of the mating period (11%) was recorded in colonies reared under a short day length treatment (Figure 4.14, Table 4.4).

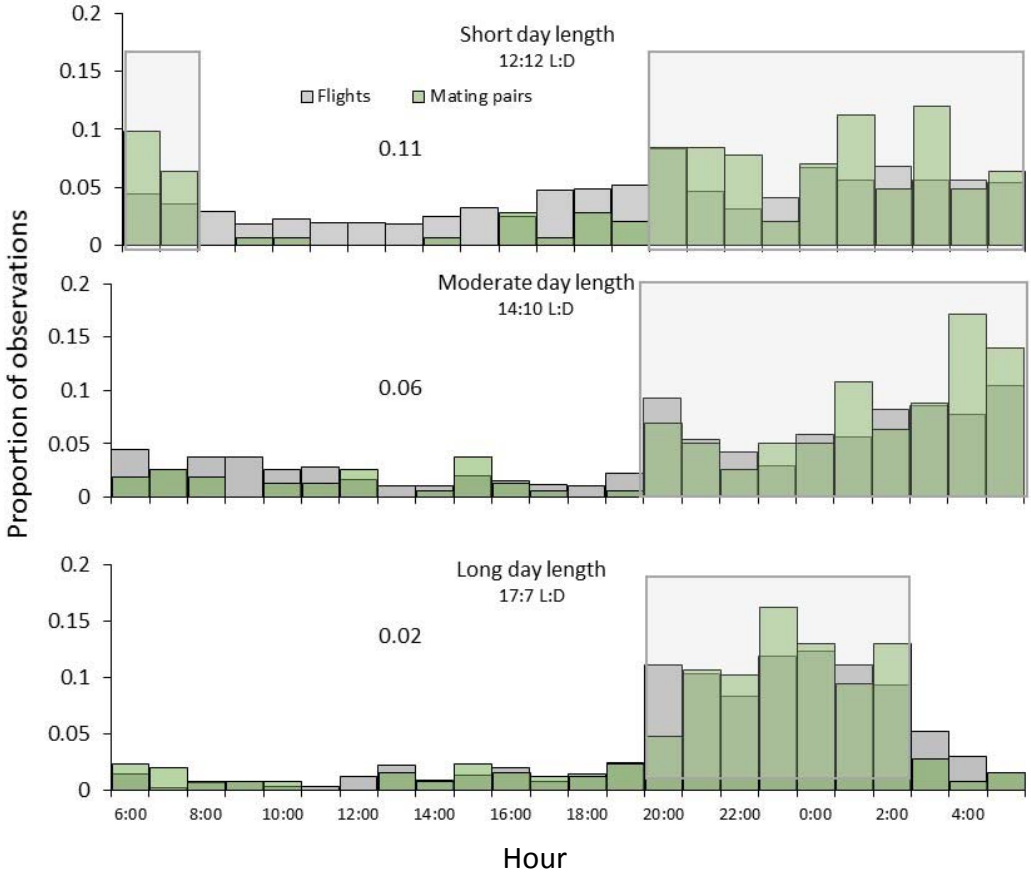


Figure 4.14. Proportion of mated pairs and male flights of *P. viteana* observed each hour in colonies reared under different day length treatments at 28°C and 60 - 75% RH. Numbers in the plot area of each graph indicate the proportion of male flights that occurred when no mating pairs were detected. The shaded rectangles in each graph indicate the scotophase of the light: dark cycle. No significant differences in the proportion of male flights that occurred when mating was not observed were detected among day length treatments ($Q_{0.05, \infty, 3} < 3.31$, $P > 0.05$, Multiple comparison of proportions, Zar 1999).

Diurnal temperature cycles. In the third experiment, mating frequency and male flight activity in colonies reared in diurnal temperature regimes, the frequency of mating in the cool weather treatment (20°C during the day to 10°C at night) was 0.038 matings per hour (mph). Mating frequency in the warm weather treatment (28°C day to 10°C night) was 0.043 mph, and in the hot weather treatment (28°Cday to 18°C night) mating frequency was 0.082 mph (Figure 4.15a). Despite a nearly two-fold difference in average matings per hour, no significant differences were detected in mating frequency between treatments ($F_{2,34} = 1.32$, $P = 0.28$, log-transformed data, Figure 4.15a). The number of male flights per hour ranged from 4.0 fph in colonies that received the cool weather diurnal treatment to 5.7 fph in the hot weather treatment. No significant differences in male flight frequency were detected between diurnal temperature treatments ($F_{2,34} = 0.24$, $P = 0.78$, log transformed data, Figure 4.15b).

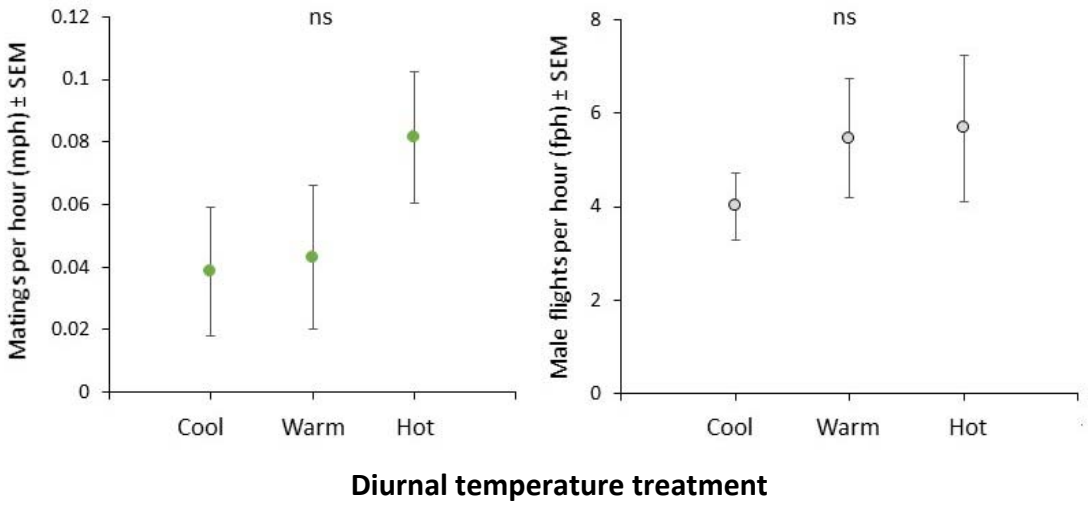


Figure 4.15 Mating frequency (a) and male flight frequency (b) of *Paralobesia viteana* recorded in colonies reared under three diurnal temperature regimes. Cool treatment colonies were reared in a temperature scheme that changed from 20°C during the day to 10°C at night. Day:night temperature cycles in the Warm and Hot treatment were 28°C:10°C and 28°C:18°C respectively. All treatments incorporated a 17:7 light: dark (day:night) cycle. "ns" denotes no significant differences were detected between treatments ($P < 0.05$).

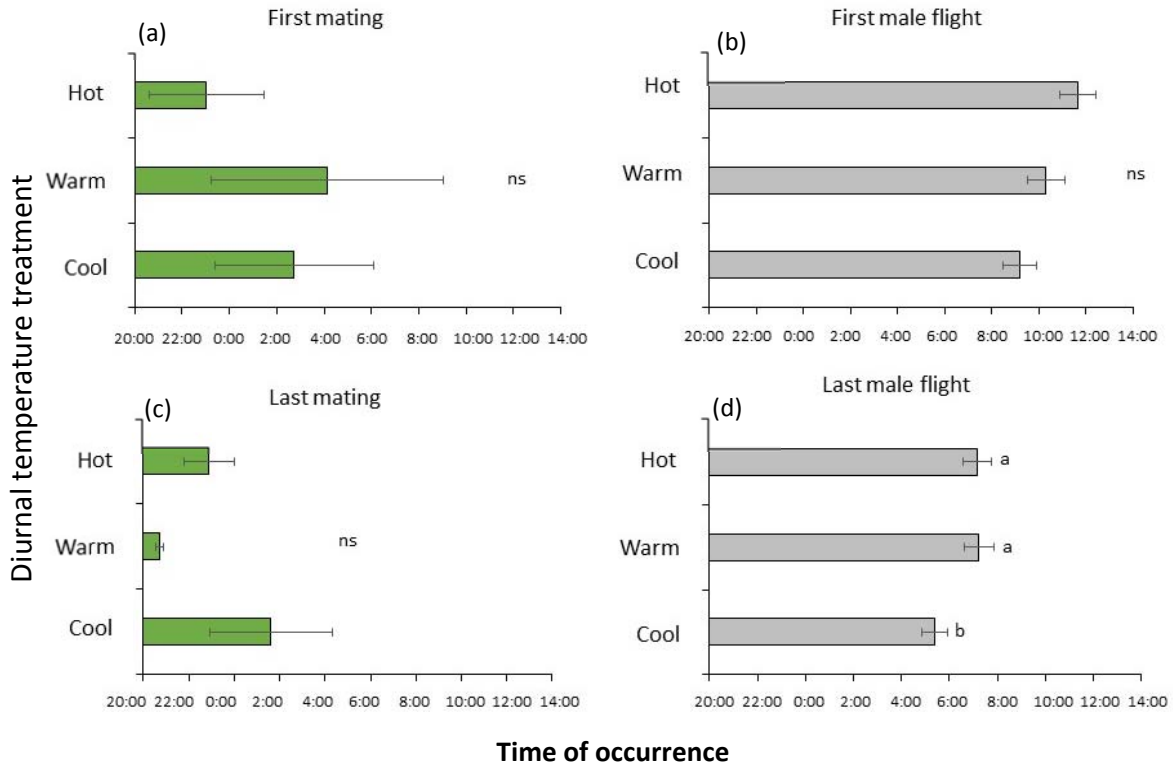


Figure 4.16. Average time of the first observation of mating pairs (a) and male flights (b), and average time of last mating pair (c) and male flight (d) in colonies reared under 3 different diurnal temperature regimes. Treatments are the same as described in Figure 4.15. "ns" denotes no significant differences were detected between treatments (P >0.05).

The average time of first mating ranged from 23:02 in the hot temperature regime to 04:07 in the warm temperature treatment, and there were no statistically significant differences among the diurnal temperature regimes in the time of first mating ($F_{2,20} = 0.32$, $P = 0.73$, log transformed data, Figure 4.16a). The average time the last mating occurred was also not significantly different between treatments ($\chi^2 = 2.97$, df =2, $P = 0.23$, Figure 4.16c), and ranged from 20:43 in the warm treatment, to 01:36 in the cool weather treatment, and the last mating in the hot weather treatment occurred at 22:53 (Figure 4.16c). The average time of the first male flight in the cool weather treatment was 09:11, while in the hot weather treatment, the average time the first flight was observed was 11:40, and the average start time for male flight was 10:18 in the warm weather treatment. These times were not significantly different among treatments

($F_{2,31} = 2.88$, $P = 0.07$, log transformed data, Figure 4.16b), but there was a significant difference in the average time of the last flight among diurnal temperature treatments. This ranged from from 05:22 in the cool weather treatment to 07:14 in the warm treatment. The last male flight occurred significantly later in the hot and warm treated colonies than in the cool weather treated colonies ($\chi^2 = 6.27$, df =2, $P = 0.046$, Figure 4.16d).

The average duration of the mating period ranged from 36 minutes in the warm weather treatment to 158 minutes in the hot weather regime, and mating period duration in the cool weather treatment was 112 minutes (Figure 4.17a). There were no significant differences in mating period duration between diurnal temperature treatments ($F_{2,20} = 1.72$, $P = 0.21$, log transformed data, Fig 4.17a). The average duration of the male flight period ranged from 1169 minutes to 1255 minutes, indicating males were active for most of the 24 hour cycle. There were no significant differences in the duration of the male flight period, which shows that the male

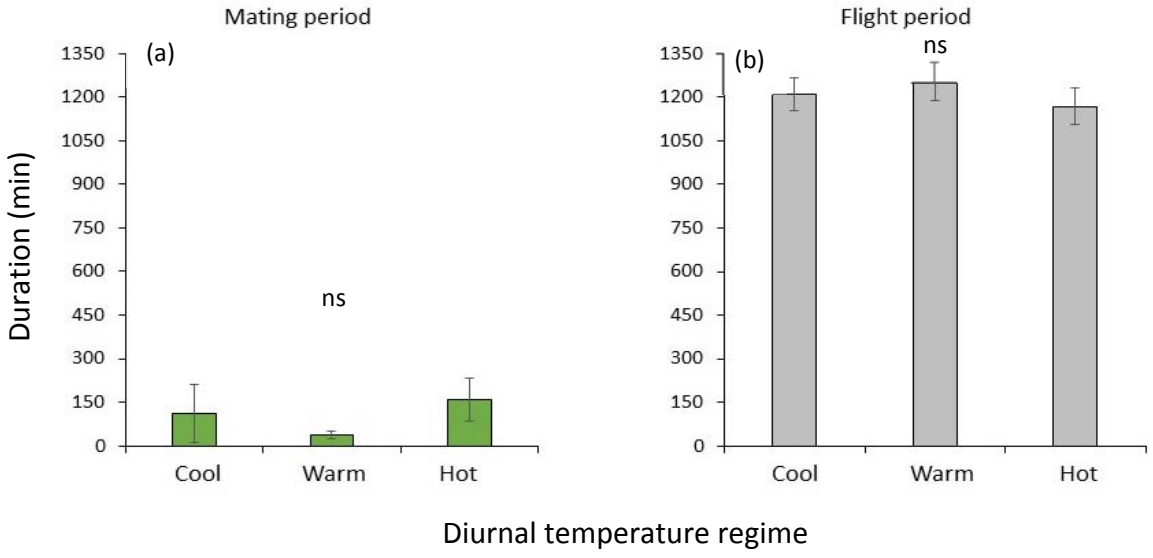


Figure 4.17. Mating period duration (a) and flight period duration (b), in colonies of *P. viteana* reared under different diurnal temperature regimes. Treatments are the same as described in Figure 4.16. "ns" denotes no significant differences were detected between treatments ($P > 0.05$).

flight period is not strongly influenced by these different temperature regimes ($F_{2,31} = 0.33$, $P = 0.72$, log trans-formed data, Fig 4.17b). In colonies reared in the cool weather treatment, the duration of the flight period was 1211 minutes and this was significantly longer than the mating period of 112 minutes ($\chi^2 = 13.8$, df =1, $P = 0.0002$). Similarly, in the warm weather treatment the male flight period was 1255 minutes, and this was significantly longer than the length of the mating period of 526 minutes ($\chi^2 = 14.2$, df =1, $P = 0.0002$). This indicates regime, in that all of the diurnal temperature treatments, males were flying when females were longer than the mating period of 36 minutes ($\chi^2 = 10.7$, df =1, $P = 0.0011$). In the hot weather not receptive

The proportion of male flights that occurred outside of the mating period was highest in colonies that experienced the cool weather treatment (20°C:10°C day:night cycle). Under these conditions almost three quarters (73%) of male flights occurred when mated pairs were not observed, and this was significantly higher than colonies held under the Hot weather treatment (28°C:18°C day:night cycle, Figure 4.18). In colonies reared under the Warm weather treatment (28°C:10°C day:night cycle), two thirds (67%) of male flights occurred during periods when no mating pairs were observed, and this was similar to the proportion of male flights in colonies held in the cool weather treatment. The lowest proportion of male flights that occurred outside of the mating period (22%) was recorded in colonies that were reared in the hot weather treatment, and this value was significantly lower than that for the cool or warm weather treatments. Thus, under cooler conditions, higher proportions of male flights occurred when mating pairs were not observed compared to colonies under warmer conditions (Table 4.5).

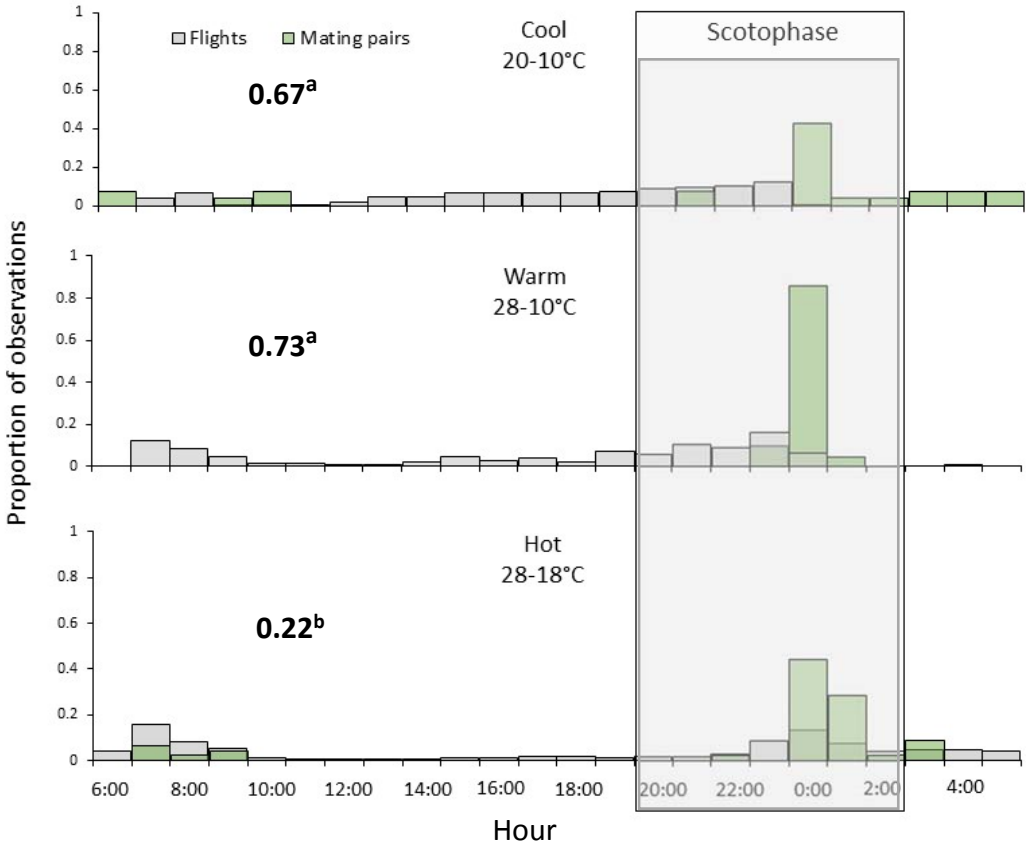


Figure 4.18 Proportion of mated pairs and male flights of *P. viteana* observed each hour in colonies reared under diurnal temperature treatments and constant day length 17:7 and 60 % RH. The shaded rectangle surrounding the time from 20:00 to 03:00 shows the scotophase. Proportions of male flights that occurred when mating was not observed are given in bold on the left hand side of the graphs. Proportions with the same letter are not statistically different ($Q_{0.05, \infty, 3} > 3.31$, $P < 0.05$, Multiple comparison of proportions, Zar 1999).

Table 4.5. Summary of mating frequency and male flight measurements for colonies held under diurnal temperature regimes. Values in a column values with different letters are significantly different ($P < 0.05$).

Diurnal Treat-ment	Proportion male flights outside mating period	mph*	# times behavior observed	Hours observed	fph**	# times behavior observed	Hours observed
Cool	0.67 a	0.04	26	635	4.0	2444	635
Warm	0.73 a	0.04	21	470	5.5	2454	470
Hot	0.22 b	0.08	45	493	5.7	3351	493

* mph = Average number of mating pairs per hour averaged across all hours.
** fph = Average number of male flights per hour averaged across all hours.

Response to pheromone lures at different times of the season

Traps baited with lures that contained pheromone caught more males than the control traps that were baited with a blank lure (Figure 4.19). Captures of male moths declined as the pheromone quantity increased when data were summed over all sampling periods ($F_{5,30} = 92.2$; $P < 0.0001$, log transformed data, Figure 4.19). Traps loaded with a 15 µg lure caught significantly more males than traps baited with a lure containing the standard amount of pheromone in commercial lures (100 µg). Male captures in traps containing the 15 µg lure were significantly higher than captures in traps with the 125 µg lure. The number of males captured in traps loaded with either the 60 µg lure or the 75 µg lure were not statistically different than captures in any of the other

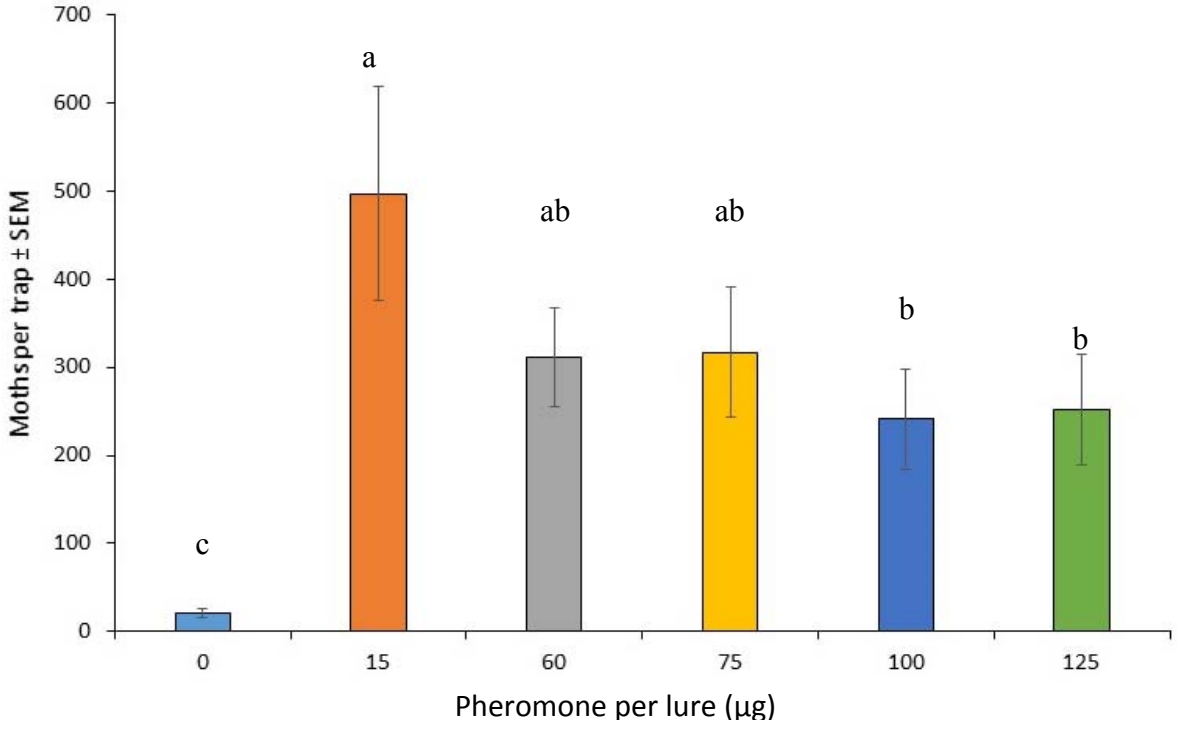


Figure 4.19 Total male moth captures of *P. viteana* for the entire season in traps loaded with different amounts of pheromone per lure. Data are averages from seven replicate vineyards in southwest Michigan. Columns labeled with different letters are significantly different ($P < 0.05$).

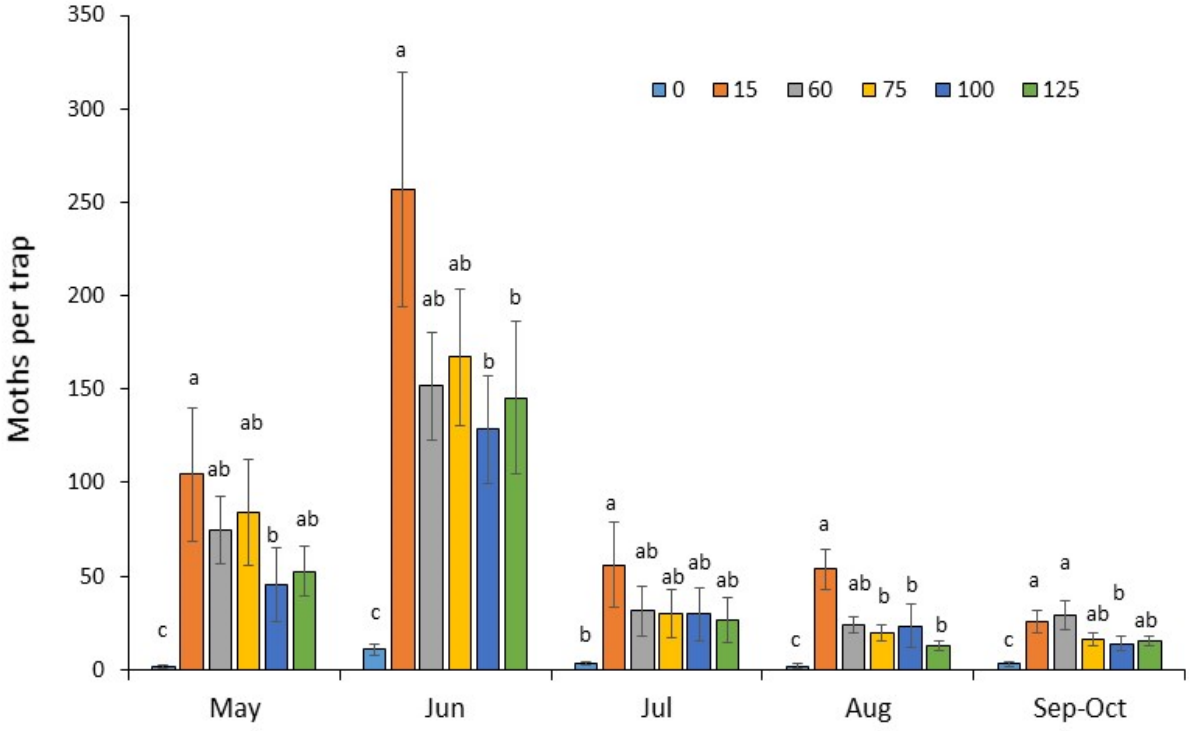


Figure 4.20. Captures of male *P. viteana* in traps baited with lures containing different amounts of pheromone in different sampling periods. The legend gives the amount of pheromone per lure in µg. In any sampling period, columns with different letters are significantly different ($P < 0.05$).

pheromone loaded traps (Figure 4.19). Male captures varied significantly among lure treatments for the five sampling periods (May: $F_{5,30} = 56.8$, $P < 0.0001$; June: $F_{5,30} = 38.4$, $P < 0.0001$; July: $F_{5,30} = 12.2$, $P < 0.0001$; Aug: $F_{5,30} = 22.0$, $P < 0.0001$; Sep-Oct: $F_{5,30} = 18.5$, $P < 0.0001$, all data log transformed, Figure 4.20). The lowest average daily temperature (16.8°C) and the lowest average temperature during the scotophase from 20:00 to 5:00 when males are likely to fly (12.3°C) occurred during the May sampling period. During this period, the captures in all traps with pheromone lures were significantly higher than in the blank lure control traps, and captures in the traps containing 15µg lures were significantly higher than in traps baited with 100 µg lures. In the June sampling period, average daily temperature was 21.8°C and average night time temperature was 18.2°C. The highest average male captures occurred during June and ranged

from 10.7 ± 3.1 in the blank lure traps to 257 ± 62.5 males in the 15µg traps. Significantly more males were captured in the 15µg traps than in the 100µg or 125µg traps (128.6 ± 28.9 and 145.4 ± 41.0 males, respectively). In July, average daily temperature increased to 22.7°C and scotophase temperatures averaged 19.6ºC, and male captures dropped off considerably. Male captures ranged from 3.1 ± 1.0 males in the blank lure traps to 56.1 ± 22.6 males in the 15µg traps, and this was the only significant difference in captures detected during this period. In August the average daily temperature cooled to 19.9°C, and during the scotophase temperature averaged 17.1°C. Male captures ranged from 2.0 ± 1.0 males in the blank lure traps to 53.7 ± 10.7 males in the 15µg traps. In August, captures in the 15µg traps were significantly higher than captures in all other traps except for traps with the 60 µg lure. In the final trapping period that ran from September through October, average daily temperature fell to 17.8°C and during the scotophase average temperature was 13.8°C. All traps with lures containing pheromone were significantly higher than the traps baited with blank lures. Captures in the 15µg traps were similar to captures in the 60µg traps, and these were both significantly higher than the traps with the 100µg lures.

To illustrate the relationship between male captures and pheromone release at different times of the season, moth capture was regressed on pheromone release for each sampling period. There was a consistent negative relationship between moth captures and pheromone release with the strongest effect of increasing pheromone release in May and August (Figure 4.21).

Analysis of covariance was used to determine if the relationship between moth capture and pheromone release is consistent at different times of the season (Table 4.6), revealing significant effects of sampling period ($P = 0.00001$) and pheromone release/day ($P = 0.00009$) on captures, and we also detected a significant interactive effect of sampling period and pheromone release on male captures ($P = 0.043$).

Table 4.6. Analysis of covariance to determine if the relationship between male captures of *P. viteana* and pheromone release differs between samping periods.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	9	61.01	6.78	17.44	<0.0001
Error	15	5.83	0.39		
Total	24	66.84			

Comparison of the least square means from this analysis showed that the relationship between captures and pheromone loss was similar in the May and August sampling periods, but different than the effect of pheromone release on captures in the June, July and September-October sampling periods (Table 4.7). That is, increasing pheromone release caused a larger reduction in captures in May and August than in the other sampling periods.

Table 4.7. Comparison of least squares means for captures of *P. viteana* in different sample periods (Tukey's HSD). Means with the same letter are not significantly different.

Sample period	Least square means for captures
May	4.61 a
August	3.41 a
June	1.59 b
Sep-Oct	1.58 b
July	0.91 b

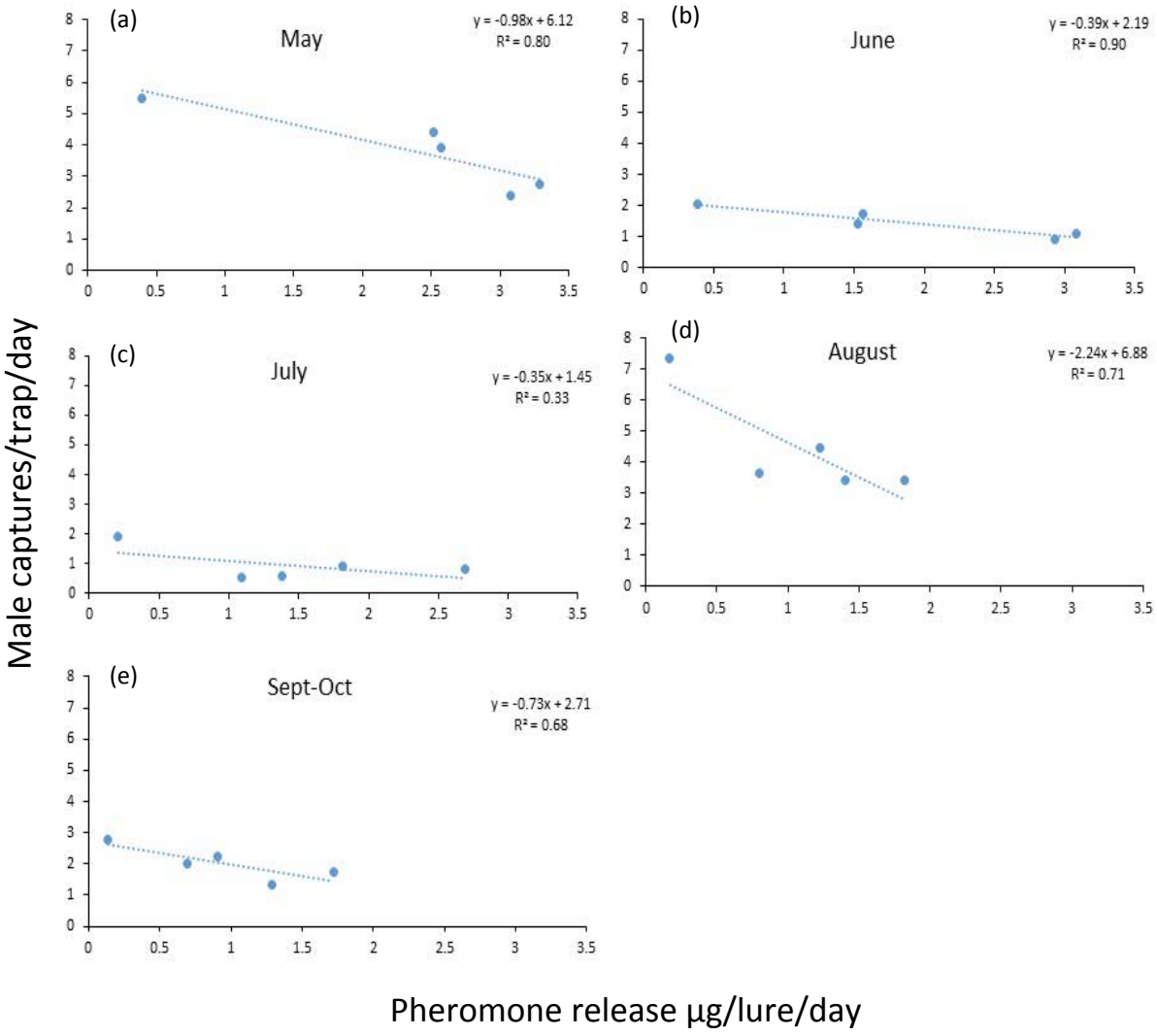


Figure 4.21 Relationship between captures of *Paralobesia viteana* and pheromone release for different sampling periods in 2017. Each point represents the average male capture and pheromone release for each lure load (15, 60, 75, 100, 125 µg/lure) at 7 southwest Michigan vineyards. Blank lures were not included in this analysis because they did not contain pheromone.

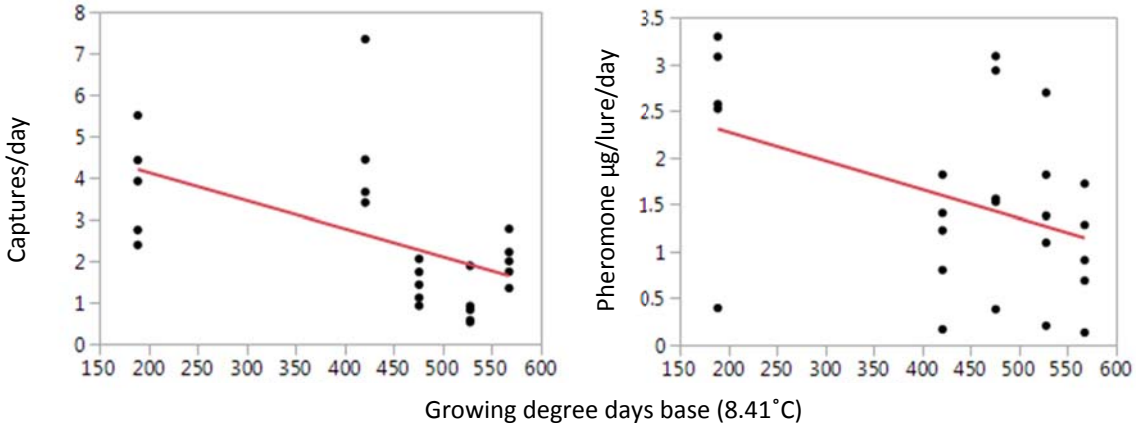


Figure 4.22 Regression of *P. viteana* captures (a) and pheromone release (b) on accumulated heat units ($GDD_{8.41}$). Residuals from (a) were regressed on the residuals in (b) to test the effect of pheromone release on captures with the effect of temperature removed (Table 4.8).

Captures and pheromone release both decline with the heat units accumulated during the sampling period ($GDD_{8.41}$) (Figure 4.22). Regression analysis using the residuals from the linear regressions of captures and pheromone release on $GDD_{8.41°C}$ showed the reduction in captures due to pheromone release with the effect of temperature removed (Table 4.8). This shows that lures that release larger quantities of pheromone have lower captures of male *P. viteana*.

Table 4.8. Analysis of variance and parameter estimates from linear regression of residuals from the analyses in Figure 4.22 to evaluate the effect of pheromone release on captures of male *P. viteana* after removing the effect of temperature

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	1	22.15	22.15	20.77	0.0001
Error	23	24.53	1.07		
Total	24	46.68			

DISCUSSION

This research highlights temperature as a critical factor affecting many behaviors and physiological processes associated with mating by *P. viteana,* and how these effects determine the observed phenology of this pest in Michigan vineyards. With increasing temperature, longevity decreases, but mating, male flight frequency and oviposition, all increase. The proportion of males that fly outside of the mating period (i.e., when females are not receptive to mating) is highest during cool temperatures and decreases with increasing temperature. This provides support for the model outlined in Figure 4.1 that predicts cool temperatures reduce the duration of the female calling period relative to the duration of the period when males respond to pheromone. While, during periods of high temperatures, like those that occur during the mid-summer *P. viteana* generation, the duration of the mating period is similar in length to the flight period. This results in fewer male flights that occur when females are not receptive to mating, compared to the first generation. These relationships are likely to contribute to higher male captures in traps in cool conditions because males are flying (receptive to pheromone) and searching for females, but no calling behavior is occurring. In this situation, pheromone-baited traps would not be in competition with calling females, and males would be more likely to be attracted to and captured in pheromone-baited traps.

High temperature reduced adult male and female *P. viteana* longevity, and this decrease was similar between the sexes. There was a six-fold decrease in longevity between 10 and 28°C and a two-fold decrease between 18 and 28°C. This is consistent with other studies that show decreased longevity in response to constant experimental temperatures in several insect orders including; Lepidoptera, Hymenoptera, Coleoptera, and Hemiptera (Graham et al. 1967, Emana 2007, Gómez et al. 2009, Régnière et al. 2012). Several studies have reported longevity for tortricid moths held at different constant temperatures, and these results are similar to my results

for *P. viteana*. Semi-field bioassays using caged wild moths showed that longevity in successive generations of the Nantucket pine tip moth decreased significantly as temperature increased through the season (Asaro and Berisford 2001). In constant temperature experiments in the same study, male longevity was reduced by 8 to 10-fold over the range from 5 to 40°C. Although the authors did not compare longevity between sexes, it appears females live almost twice as long as males (Asaro and Berisford 2001), and decreased male longevity caused by high ambient temperatures in the second and third generations was a contributing factor to low captures of males in the later generations when ambient temperatures were high (30-35°C). Codling moth adults held in constant temperatures survived approximately 4 times longer at 14°C than those held at 33°C (Aghdam et al. 2009). The increased longevity of male moths at low temperatures may contribute to high first generation *P. viteana* male captures. If males live longer in low nonlethal temperatures, they may have an increased probability of being caught in traps, assuming that for a portion of their lives temperatures are high enough for flight. It is also likely that decreased male longevity at high temperatures contributes to the reduction in *P. viteana* male captures in the second and third generation during high temperatures. Increased female longevity at low temperatures may allow first generation females to wait until warm conditions to begin laying eggs.

The temperature required for flight in male and female *P. viteana* was found to be 15°C and this is similar to the lower temperature thresholds for flight in other tortricids. Among the Tortricidae, the minimum temperature for flight is typically in the range of 12-15°C. Reported temperatures include 14°C for the eastern spruce bud worm, *Choristoneura fumiferana* (Greenbank et al. 1980); 15°C for oriental fruit moth, *Grapholita molesta* (Rothschild and Minks 1974) and 12.7°C for codling moth, *Cydia pomonella* (Batiste et al. 1973). The minimum temperature for flight in the light brown apple moth, *Epiphysis postvittana* was considerably

lower than other tortricids and ranged from 8 to 11°C (Danthanarayana 1976). However, this study used suction traps to collect moths during natural temperature variation, and it is possible this trapping method may have underestimated the minimum threshold by collecting moths that were near the trap, but not flying. My finding of this lower threshold for *P. viteana* could be integrated into the degree-day model that is currently used for this pest to better predict when egglaying will start in the spring and through the rest of the growing season.

Oviposition in *P. viteana* is highly dependent on temperature, and I found more eggs on grapes that were attached to the oviposition cage floor than on grapes suspended in the cages. This likely reflects that walking and flying females could reach the grapes more easily in these cages compared to when the grapes were suspended, and only climbing or flying moths could reach the grapes. These results agree with egg laying rates in previous research with some overlap in the range of temperatures that were used. In pink bollworm, oviposition increases 10-fold between females held at 15.5°C and those reared at temperatures ranging from 18.3 to 26.7°C (Graham et al. 1967). Særthre and Hofsvang (2002) showed codling moth were able to lay eggs at temperatures as low as 12.3°C, and the amount of oviposition doubled when temperatures increased to 17°C and reached a maximum at 25°C, which was the highest temperature tested. In tests of Oriental fruit moth oviposition temperature preference, egg laying was highest in the range 22 to 30°C, and drops off rapidly at 33°C with no egg laying at 35°C (Notter-Hausmann and Dorn 2010). This study did not measure oviposition at temperatures below 22°C, so I cannot directly compare the lower temperature limits of *P. viteana* to that for Oriental fruit moth. However, these studies show that, in *P. viteana* and other species, oviposition is positively related to temperature, and oviposition over the range of temperatures that insects are active can increase 10 to 100-fold from the lowest to highest temperatures. This

provides strong support for temperature being a major factor determining oviposition rates and for the phenology of egg laying in *P. viteana* (Chapter 1, Figure 1.3).

In *P. viteana* colonies reared at different constant temperatures, I observed strong positive relationships between temperature and the frequency of mating (mph) and the frequency of male flights (fph). Mating frequency increased exponentially with temperature over the range of 10 to 28°C (from 0.01 to 0.52 mph), but mating was reduced at 35°C indicating that between 28 and 35°C there is a critical maximum for this behavior. The frequency of *P. viteana* male flights was also strongly linked to experimental temperature in a positive exponential relationship. Both mating and flight frequency show characteristic thermal performance curves (Huey and Kingsolver 1989) that increase through a range temperatures above a critical low value, but decline rapidly at or above a critical high temperature (Lachenicht et al. 2010).

The time of first mating was not significantly different between colonies reared at different constant temperatures, in contrast to other work that shows female calling begins earlier at lower temperatures (Cardé et al. 1975b, Cardé and Minks 1995). In my experiments, all of the average start times for mating were within the scotophase of the photoperiod, and this is similar to reports of many other tortricids that mate during the dark phase (Cardé, et al. 1975a, Baker and Cardé 1979a, Delisle and McNeil 1987, Webster 1988). The time of last mating was significantly later in colonies held at 28°C compared to colonies reared at 15 or 23°C. There were significant differences in the duration of the mating period, and this period was longer at 28°C than at either 15 or 18°C indicating the period females are receptive to mating is longer at higher temperatures. This is similar to previous studies that show female calling period starts and ends later at higher temperatures, and results in a longer period where females are calling and receptive to mating (Cardé et al. 1975b, Baker and Cardé 1979a, Webster 1988).

The time of the first male flight differed significantly between colonies reared at 18°C and those reared at 23°C and 35°C, but more importantly all first flights were observed during the photophase of the photoperiod. This shows that male flights occur before the scotophase, when females are not yet receptive to mating. There were no significant differences in the average time of the last male flight, but these times were all outside of the scotophase showing that male flight activity starts and ends beyond the period when females are receptive to mating.

For all constant temperature treatments except 15°C, the male flight period was also significantly longer than the mating period (Figure 4.9a and b). This is strong evidence that males are actively seeking mates or mating sites when females are not receptive to mating, and this has been shown in other Lepidoptera such as *Antherea polyphemus* (L.) (Kochansky et al. 1977). This is expected to contribute to higher male captures in traps if flying males are receptive to pheromone and searching for females, but few receptive (calling) females are present. This decreased competition between traps and calling females at different times of the season has been noted in other moth species (Palanisawamy and Seabrook 1978, Noguchi and Tamaki 1985, Sanders 1987). This increases the likelihood that males will find pheromone-baited monitoring traps, because the traps release pheromone throughout the day and night, and thus would be the only sources of pheromone during the day as females only call at night.

My analysis of the proportion of male flights that occur outside of the mating period clearly demonstrated that more male activity occurs outside of the mating period in colonies held in low temperatures compared to colonies reared at high temperatures. A significantly higher proportion of male flights occurred outside of the mating period in colonies held at 15, 18 or 35°C than in colonies reared at 28°C. This supports the hypothesis that there would be higher male captures in traps during cool temperature conditions, such as in the spring in Michigan

when the first generation of *P. viteana* is active, than during the hot part of the growing season (mid-summer) when the second and third generations are active. It also fits the observed pattern of moth capture in Chapter 1, Figure 1.3.

The role of day length in determining the timing and intensity of mating behaviors is less clear than for temperature. However, I observed some significant effects of day length on some mating parameters that align with observed phenology patterns. Mating frequency and male flight frequency were significantly higher in colonies held under the longest day length (17h) compared to colonies reared under either the moderate (14h) or short (12h) day length treatment. In the field, the longest day lengths occur during midsummer during Generation 2, and this is when there are substantial increases in egg laying and fruit damage in vineyards (Chapter 1, Figure 1.3) (Hoffman et al. 1992, Teixeira et al. 2009). In addition, the duration of the mating period was longer in the moderate and long day length treatments, and this agrees with observations in the field, because periods of highest egg laying are seen when day lengths are moderate or long. This shows that increasing day length could also play a role in the high levels of oviposition in the second and third grape berry moth generations. For all day length treatments tested, some male flights always occur outside of the time when females are receptive to mating. Even though the flight period is longer than the mating period for all treatments, day length did not significantly affect the proportion of male flights that occurred outside of the mating period. In addition, the proportions of male flights that occurred outside of the mating period in the day length treatments were much lower than observed in the constant temperature treatment (Figures 4.10 and 4.14). Overall, these results suggest that the effect of day length on mating behaviors is much less important than the effect of temperature, and seasonal changes in day length are not likely to be primary factors responsible for the lower captures of male *P. viteana* in the middle and late parts of the season.

Diurnal temperature fluctuations in colonies had little effect on mating or male flight frequency or timing, except that the time of the last male flight was significantly earlier in the Cool season treatment. This contrasts with previous research in different species that showed female calling and male responsiveness to pheromone start and end earlier in cool weather (Cardé et al. 1975a, b, Webster 1988). This may reflect the different methods that were used in the current study compared to the previous experiments. Previous studies directly measured female calling, male antennal responses and/or male precopulatory and mating behaviors in response to changes in temperature (Cardé et al. 1975a, b, Webster 1988). I measured the frequency of mating as a proxy for female receptivity, and male responsiveness was inferred from the frequency of flights. Although these are vastly different methodologies, the past and present research show that temperature is a key factor in determining the patterns of mating behaviors.

The frequency of mating in colonies under a diurnal temperature regime was generally lower than in the constant temperature and day length experiments, but the frequency of male flights was more similar in all three data sets (Figures 4.10, 4.14 and 4.18). Overall, the mating period in the diurnal temperature experiment was shorter than in the constant temperature or day length experiments. It is likely that the decreased temperatures in these treatments during the scotophase reduced the frequency of mating. The proportion of male flights that occurred outside of the mating period was significantly lower for the hot weather treatment than in the cool or warm weather treatments. This result also supports my interpretation of how temperature affects mating and observed phenology. That is, if flying males are receptive to pheromone, and they are searching for females, but no females are calling, males will be more likely to be attracted to the pheromone lures in monitoring traps. If this is occurring in the field, then I would expect to catch more males in the cooler part of the growing season, as observed across the temperate, northern

portion of eastern North America. Additional studies that compare pheromone trap and temperature data across the latitudinal range of *P. viteana* from Ontario to Texas would certainly help to test this prediction, especially if there is not geographic variation in male response to pheromone (see Chapter 2).

In the field experiment to determine the response of male *P. viteana* to different rates of pheromone release, traps loaded with lures that contained (and released) higher amounts of pheromone caught significantly fewer moths. Pheromone release is dependent on temperature and the quantity of pheromone in a lure (McDonough et al. 1989). High pheromone release rates in high temperatures may be inhibitory to male *P. viteana* and contribute to lower captures in pheromone traps as has been shown in other species (Baker et al. 1980, Allen et al. 1986, McDonough et al. 1989, Charlton et al. 1993). In the present study, analysis of covariance showed that in two of the cooler sampling periods, May and August, pheromone release from high load lures had a stronger effect on reducing male captures than was observed in the June, July or September-October sampling periods. These results suggest that during the warmer periods in June and July the evaporative release of pheromone from any lure was high enough to suppress male captures in a trap. Surprisingly there was not a strong effect of pheromone loss on captures in the September-October sampling period, because these were cool periods compared to June and July. It is likely that the effect of pheromone release on capture was diminished because the sampling period was twice as long as the other periods in this experiment. Still, these data do suggest high rates of pheromone release from lures is a contributing factor that helps explain low male moth captures in mid- and late-summer when temperatures are high.

My field experiment shows that temperature and pheromone release contribute to the observed *P. viteana* phenology that is evident from monitoring traps. The consistent high captures in the traps baited with 15µg lures and the consistent low captures in traps baited with

the standard quantity of pheromone (100µg) suggests seasonal temperature changes affect male captures and higher load lures can suppress male captures in the hottest parts of the season. However, even in traps baited with 15µg lures, male captures declined through successive sampling periods. Whether this is a suppressive effect resulting from high pheromone release or is caused by pheromone loss resulting in low pheromone quantity and reduced attraction to traps during high temperature is not clear. Overall, low load lures appear to release an appropriate pheromone dose and could result in improved capture of male *P. viteana,* not only in the hottest part of the season, but also during the first generation that occurs in the spring. This highlights the need for future testing of lures with pheromone loads of 15µg and lower.

The relationships between temperature, mating and male flight activity in *P. viteana* described in my research provide new insights to explain the pattern of moth activity and oviposition phenology observed in Michigan vineyards. In the spring, cool temperatures prevail which increases the longevity of male *P. viteana*, and because they are living longer, there is a higher probability that males will encounter and be caught in traps. At these low temperatures, a higher proportion of male flights occur when females are not calling and therefore not receptive to mating. This could also lead to high male captures because lures in traps constantly release pheromone, and this would attract flying males. Furthermore during the spring, daytime temperatures are above the threshold for flight, but often temperatures are below this threshold during the night. My data show that less mating occurs in the daytime and during low temperatures, so females are presumably not calling during cool spring nights. So it is probable that males flying in the spring are more likely to encounter pheromone from a trap than from a calling female. Low temperatures also reduce oviposition, and this combined with the lower flight and mating activity of the pest is why there are few eggs and larvae seen on clusters in the first generation. In the second generation that occurs in mid-summer, temperatures are usually at

their seasonal maximum. Mating increases with temperature, so more females are calling and receptive to mating. This leads to a lower proportion of male flights that occur when mating is not occurring, so fewer males are caught in traps because they are attracted to the calling females. More eggs are also observed during this time because the high mid-summer temperatures increase mating and increase the rate of the physiological processes associated with oviposition.

During the latter part of summer, *P. viteana* damage is at the highest levels of the season even though temperatures are generally not as high as in the mid-summer. Mating and egg laying occur at a rapid pace during this period, and the resulting high levels of damage observed at this time of year are likely due in part to increased moth longevity. In the typical range of temperatures that occur during this time of the year, the proportion of male flights when mating is not occurring is higher than in mid-summer, but lower than in the spring, so there is a slight increase in the number of males in traps compared to the previous generation, but fewer males are trapped than in the first generation.

Temperature also affects the main monitoring tool for tracking *P. viteana* phenology, the pheromone-baited trap. The rate of release of pheromone from a lure increases with temperature, and *P. viteana* male response is influenced by the amount of pheromone released (Roelofs et al. 1971, Witzgall et al. 2000). High rates of pheromone release from lures can reduce the attractiveness of traps, and result in reduced captures. This is most likely to occur during the middle and late summer generations of *P. viteana*, contributing to the reduced captures that occur in the middle and late summer. If males in summer generations could be trapped consistently this could refine predictions of when egg laying will begin in the middle and late summer generations. This would potentially improve timing of insecticide applications and provide better control of this key vineyard pest.

Currently *P. viteana* management involves using a degree-day model to time insecticide applications that target the eggs and newly emerged larvae of the second (middle summer) and third (late summer) generations (Teixeira et al. 2009, 2011). The MSU Enviroweather model predicts when egg laying is likely to begin based solely on temperature accruals using wild grape (*Vitis riparia* L) bloom as a biofix. This, along with the addition of new insecticide chemistries, has improved *P. viteana* management over calendar-based conventional insecticide approaches (Teixeira 2009). However, there is still room for improvement as many Michigan vineyards can have 75 to 100% of clusters infested with *P. viteana* in areas of high pest pressure. In Michigan, wild grape bloom occurs in late May or early June and this is typically over a month before second generation oviposition begins. Testing whether traps baited with pheromone lures that contain lower pheromone quantities than the standard 100µg/lure load can be used to detect the start of the second flight, and then using this as a mid-season biofix would be a useful first step to try and improve control of the summer generations. In addition, the results of my research could be used to refine the current model and inform future predictive models, by incorporating factors that take into account the effect temperature has on moth longevity, oviposition and mating activity. This would potentially allow for the formulation of action thresholds based on male captures, and better control.

An additional point that my research brings to light is that increasing global temperature is likely to increase the risk of *P. viteana* infestation for Michigan grape growers. Average daily temperatures during the growing season are generally in the range of 15 to 23°C. If those averages are increased by 5°C, an increase in mating and oviposition during the middle and late summer would lead to higher levels of damage in vineyards. In addition, higher temperatures would lead to increases in the rate of *P. viteana* larval development and the number of *P. viteana*

generations per season could potentially increase (Tobin et al. 2008, Lamichhane et al. 2015). All of these factors could make *P. viteana* management even more challenging than it is today.

CHAPTER 5

SYNTHESIS AND FUTURE RESEARCH NEEDS

We are taught early on in school that science is an intellectual journey designed to help explain phenomena that we see in the natural world. We make observations and formulate hypotheses, and then test them by asking questions that we can answer through inductive reasoning and experimentation[1]. Typically these questions are designed to be answered using whatever experimental means are necessary, appropriate or available. However, the dynamic nature of science, the logistical challenges of our experiments, or in some cases human limitations dictate that the path of the investigation is altered along the way. This highlights one of the hidden benefits of scientific research; that most questions are actually open-ended, and because of this answering them is bound to uncover other ideas, and in turn other questions are revealed whether or not the original query is satisfactorily explained. I would argue that while the original question is important, and every reasonable effort should be made to fully answer it, the other questions that occur to us along the way are just as critical to scientific progress as the initial query. As Sir Francis Bacon wrote in *Of the Advancement of Learning* in 1605, "Every act of discovery advances the art of discovery," (Bacon 1857). So in fact, we leave two important things for those who follow us down this path of science; we leave what we do know, and what we do not. With that I pass on this collection of ideas and questions that have been unearthed, but that remain largely unanswered.

The main objective of my research reported in Chapter 2 was to determine if the regional variation in *P. viteana* male captures in Michigan vineyards is due to differences in the male response to pheromone. Regional variation of male response and pheromone composition has

[1] For these ideas we all are indebted to Sir Francis Bacon, who laid the groundwork for modern science in *Novum Organum* (Bacon 1904).

been described for some Lepidoptera including the Tortricidae (Foster et al. 1986, El-Sayed et al. 2003, Stelinski et al. 2007b), Noctuidae (Hansson et al. 1986, Löfstedt et al. 1986, Tòth et al. 1992) and Crambidae (Kawazu et al. 2009). My results from laboratory and field studies suggest that male response does not vary between populations in different regions in Michigan. I have reached this conclusion based in part on the results of choice tests using the Y-tube olfactometer, a methodology that has not been used to a large extent to study moth behavior. Although male moths did walk or fly through the apparatus and some fanned their wings, I did not observe the other pre-copulatory behaviors such as abdominal curling or attempted copulation with the top of the sample chamber adjacent to the pheromone source. This suggests that the methods used were adequate for movement behaviors, but did not provide an opportunity to observe behaviors later in the sequence before mating as observed in other related species (Cardé et al. 1975a, Baker and Cardé 1979b, Curkovic et al. 2006).

A wind tunnel may be more suitable for choice tests between pheromone sources, and my conclusions certainly would be strengthened if comparable results were obtained in wind tunnel tests and Y-tube assessments. Wind tunnels have been used to determine the response of *P. viteana* response to leaf, shoot and cluster volatiles for lure development (Cha et al. 2008 a,b) and my original research plan did include testing male responses in the wind tunnel. However, previous researchers have found it difficult to get *P. viteana* to fly reliably in a wind tunnel (Greg Loeb, pers. comm.), and my efforts were hampered by logistical constraints including limited moth availability and insufficient time to work out wind tunnel methods. Thus I will leave this additional work for a future researcher.

My original research plan also included collecting, analyzing and comparing sex pheromone composition in female *P. viteana* from different regions. This was attempted by excising the terminal abdominal segments that contain the pheromone gland and associated

structures, and placing groups of 10-20 glands in hexane to extract the pheromone. Despite combining moth samples this way from over 600 glands, analysis with GC-MS did not show distinct peaks that would allow me to determine whether pheromone composition varies between regions. I suspect that too many other compounds were present in the excised abdominal tips and these obscured the presence of pheromone. This also reduced the number of moths that were available for other experiments, and development of new extraction or analysis methods were beyond the scope of my research. Furthermore, the preliminary results from Y-tube bioassays and field trials with pheromone lures and captive females showed that males did not respond preferentially to females from the same region. These facts, along with the advice of my research committee, led me to invoke my researcher's prerogative and leave the analysis of pheromone composition for future investigations.

My results do provide sufficient evidence to postulate that the low captures in the northwest grape growing region are due to lower population size than that in the southwest of Michigan. Investigating how *P. viteana* population size and native host plant abundance affects the number of males captured in pheromone traps in vineyards and natural areas across Michigan grape production regions would be an excellent graduate student research project. I encourage future students to consider comparing the captures in woodland in grape growing areas of Southwest and Northwest Michigan to captures in vineyards in those regions (see Chapter 2 discussion). It would also be important to characterize habitat surrounding those vineyards, as the amount of wild grape present in this habitat is an important factor in determining risk of *P. viteana* infestation (Hoffman et al. 1992, Fergusson-Kolmes and Dennehy 1993, Botero-Garcés and Isaacs 2003, 2004a, Isaacs et al. 2012b).

Coincidentally, there are new vineyard plantings in 'The Tip of the Mitt', Michigan's newest AVA (American Viticultural Area) in the northcentral portion of Michigan's lower

peninsula. This provides a unique opportunity to monitor the population of this major grape pest as the grape and wine industry expands into a heavily wooded area. This is appealing from an agro-ecosystem perspective, because early efforts to monitor the expansion of this native insect could be used to help prevent populations from reaching economically damaging levels. Additionally, because *P. viteana* populations are presumably at low levels in these areas, IPM tactics such as mating disruption (Dennehy et al. 1989, Dennehy 1991, Jenkins and Isaacs 2008, Crowder et al. 2010, Isaacs et al. 2012a), biocontrol (Nagarkatti et al. 2002a, Jenkins and Isaacs 2007b) or cultural controls such as native host removal (Prokopy 2003, Jenkins and Isaacs 2007a) or tilling (Johnson and Hammar 1912, Isley 1917, Pettit 1932, Matlock et al. 2016) may be more effective than they have proved to be in areas with high *P. viteana* populations. If these tactics could be incorporated into an area-wide system of deployment, and if reduced-risk insecticides were used for insect management, the industry would likely benefit from better control, and allow the buildup of natural enemies through conservation biological control. This could reduce the need for chemical controls and increase vineyard sustainability. This presents an interesting opportunity for a research/extension project that would involve trapping and monitoring experiments, integrated pest management trials and building relationships with growers and other grape industry members (Kroma 2006, Ohmart 2008, Shaw et al. 2011).

One important point to take from the results of Chapter 2 is that there is an immediate need to educate grape growers and vineyard managers in Northwest Michigan about the potential for rapid increases in *P. viteana* populations in this region. This could involve setting up a network of cooperating growers that will use sex pheromone monitoring traps to get baseline data to characterize the current size of local *P. viteana* populations. It would also be important to monitor traps from year to year to determine if populations are growing. Given that currently few moths are caught in pheromone traps in this region, scouting programs should be developed that

help grape producers recognize the characteristic signs of infestation on the clusters. These programs should also include training workshops that focus on using the MSU Enviroweather model (Isaacs 2017) to predict when egg laying is likely to occur and when treatments should be applied.

The results of the cluster removal experiments in Chapter 3 provide a strong case for refuting my hypothesis that the presence of grape clusters reduces male moth captures in pheromone traps in the middle and late parts of the growing season. Similarly the canopy manipulation experiment in the same chapter shows that the dense juice grape canopy does not reduce male captures. In fact there were more males caught in traps in plots with unaltered canopies than in traps with an open canopy. My interpretation of the results of these two experiments is that grape shoots and leaves and the volatiles they produce are more important for mate (and trap) finding behaviors than the fruit. This agrees with the results of previous research that shows the relative importance of shoot and leaf volatiles compared with volatiles from fruit (Cha et al. 2008a,b).

Male moths may first orient to the grapevine using leaf volatile cues, and then find females (or traps) by short hopping flights on grape shoots or walking along the shoot until a pheromone source is detected. Exploration of these topics could be accomplished by video recording male behavior around tethered or captive *P. viteana* females either in small observation chambers as described in Chapter 4, or in larger field cages set up over a single vine and seeded with male moths. Traps could also be modified by attaching natural or artificial shoots that provide walkways into the traps to determine if males use shoots to locate females or traps. The importance of shoot and leaf volatiles in trap finding could be explored further by comparing male captures in modified traps with artificial shoots that contain leaf volatiles, and traps with untreated artificial shoots. These experiments could provide some insight into how

males orient to traps and this could be used to improve male trapping consistency, and improved efficacy for better prediction of the activity and abundance of this pest. It is possible that new trap designs with artificial shoots could be used for future mass trapping or lure and kill experiments.

It is widely known that temperature is an important factor in many aspects of insect physiology. Hence temperature influences the reproductive biology and mating behaviors in most insects. In Chapter 4, I measured the effects of temperature on several aspects of the reproductive biology of *P. viteana.* The positive relationships between temperature and longevity, flight ability, oviposition, mating frequency, male flight frequency, and the duration of mating period and male flight period, as well as the duration of the period of male activity relative to the period that females are receptive to mating were used to explain the observed phenology of male captures in pheromone traps and the large increases in oviposition that occur from the middle of the summer through the period of harvest in the autumn.

My data reveal a strong link between temperature, mating and phenology. A logical next step for improving *P. viteana* management would be to incorporate some of these findings into the MSU Enviroweather model (Isaacs 2017) that predicts when grape berry moth oviposition is likely to occur. For example, given that mating and oviposition decrease with temperature and neither sex can fly below 14.5ºC, mathematical modeling could be used to estimate the expected level of infestation based on cumulative weather conditions using the data I have collected. This could inform grower management decisions for each *P. viteana* generation.

A potential caveat lies in the limitations of using laboratory colonies to make inferences about what is happening in the vineyard. It is possible that this may have influenced some of myexperiments, and I think this is most evident in testing the effect of day length. For all of my experiments moths were reared in set conditions (28°C, 17:7 light:dark, 60% RH), and we cannot

rule out that immatures or adults were not irreversibly entrained to behave in specific ways that were molded by rearing conditions. This may have occurred despite adding new *P. viteana* stock to the colonies each year and allowing experimental colonies to acclimate to new treatment conditions for 3 to 5 days before taking data. For example, the length of the observed mating periods in the day length experiments were longer than we found in the constant temperature or diurnal temperature experiments (Figures 4.9, 4.13 and 4.17). Previous research with other tortricids has shown that moth mating periodicity can be entrained to a new temperature or day length regime in 24-48 hours (Cardé et al. 1975b, Castrovillo and Cardé 1979, Schal and Cardé 1986). In addition, and related to the length of the mating period, the proportion of male flights outside of the mating period was considerably lower in the day length experiments compared to the constant temperature or diurnal temperature tests. This is particularly surprising given that the same conditions (28°C, 17L:7D, 60% RH) were included in the constant temperature and day length experiments. Why this occurred is not evident from my data, but a factorial experiment that varies day length and temperature conditions may help to explain these inconsistencies.

Another important avenue of further research is found in the results of my field study to test male response to different quantities of pheromone, and different rates of pheromone release. I found in 4 out of 5 sampling periods that traps baited with lures containing 15µg of the standard pheromone blend caught significantly more male moths than traps baited with a 100µg lure that is similar to most commercial and experimental lures (Roelofs et al. 1971, Taschenberg et al. 1974, Jordan et al. 2013). The sampling periods encompassed the entire growing season and included a range of average daily temperatures from 16.8 to 22.7ºC. The response to the 15µg lure suggests that a reduced rate lure may provide more consistent male captures and allow for more precise monitoring during the entire season. It is likely that if male captures could be used as the biofix for the MSU Enviroweather model in the middle and late season, then the start

of the corresponding *P. viteana* egg laying periods could be more precisely estimated, allowing for improved insecticide application timing.

My data show there is decreased attraction of lures with large quantities of pheromone during the middle and late season, and this effect appears to be linked to the high temperatures that occur during this part of the season. My data also suggest there is a rate effect and the reduction in captures is greater for lures with higher quantities of pheromone. Because pheromone release increases and lure attraction decrease with increasing temperature, this likely contributes to reduced male captures in the middle and late summer when conditions are hot or warm (Sanders 1981, McDonough et al. 1989, Charlton et al. 1993, Cork et al. 2003, Cork 2016, Cardé et al. 2018). This could be tested empirically using a wind tunnel set up in a walk-in environmental chamber with adjustable temperature settings. The proportion of moths that fly, approach or contact different load lures at different temperatures could be compared across the lure loads and temperatures. This may also be possible in the field using temperature monitors and surveillance cameras set up to observe lures of different loads throughout the season and across a range of temperatures.

My research has answered several questions, and I have pointed to how this new knowledge will inform *P. viteana* management. However, I have at the same time, created a number of additional lines of potential research into important facets of *P. viteana* mating biology and phenology, and these remain open for additional exploration.

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2018-04

Author and Title of thesis:
Keith Scott Mason.
FACTORS AFFECTING MATING, MONITORING AND PHENOLOGY OF GRAPE BERRY MOTH, *PARALOBESIA VITEANA*, IN MICHIGAN VINEYARDS.

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Tortricidae	*Paralobesia viteana*	adult	10	pinned

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