

Geographic Variation in Diapause Induction: The Grape Berry Moth (Lepidoptera: Tortricidae)

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ABSTRACT Diapause in insects occurs in response to environmental cues, such as changes in photoperiod, and it is a major adaptation by which insects synchronize their activity with biotic resources and environmental constraints. For multivoltine agricultural insect pests, diapause initiation is an important consideration in management decisions, particularly toward the end of the growing season. The grape berry moth, *Paralobesia viteana* (Clemens), is the main insect pest affecting viticulture, and this insect responds to postsummer solstice photoperiods to initiate diapause. Because the range of grape berry moth extends from southern Canada to the southern United States, different populations are exposed to different photoperiodic regimes. We quantified the diapause response in grape berry moth populations from Arkansas, Michigan, New York, Pennsylvania, Texas, and Virginia, and observed latitudinal variation in diapause initiation. Populations from Michigan, New York, and Pennsylvania responded significantly different than those from Arkansas, Texas, and Virginia. We also observed, as a consequence of our experiments, that the timing of our laboratory studies influenced grape berry moth's response to photoperiod, *ceteris paribus*. Experiments that were conducted when grape berry moth would be naturally in diapause resulted in a significant higher proportion of diapausing pupae at photoperiods (i.e., >15 h) that generally do not induce diapause, suggesting that attention should be paid to the timing of behavioral and physiological experiments on insects. This relationship between photoperiod and diapause induction in grape berry moth across geographic regions will provide applicable knowledge to improve pest management decisions.

KEY WORDS clinal latitudinal variation, diapause, *Paralobesia viteana*, viticulture, *Vitis*

Diapause is one of the major adaptations evolved by insects to synchronize their activity with biotic resources and environmental constraints (Lees 1955, Tauber and Tauber 1976, Denlinger 2002). Diapause occurs in response to environmental cues, primarily photoperiod, and temperature (de Wilde 1962; Tauber and Tauber 1976), but also other factors such as moisture and host quality (Hunter and McNeil 1997, Tauber et al. 1998), which influence the endocrine system before unfavorable conditions arrive (Tauber et al. 1986). Within a species there can be considerable inter- and intra-population variation in diapause responses (Bradford and Roff 1993). Studies pertaining to diapause induction are vital to insect management, particularly across large spatial scales in which individuals can be subject to variation in the primary cues for diapause induction. These seasonal adaptations underlie the interactions of a species with its physical and biological environments (Tauber and Tauber 1976).

The grape berry moth, *Paralobesia viteana* (Clemens), is a primary insect pest of cultivated grapes in the eastern United States. Larvae feed on buds or clusters, or burrow into the grape and feed, causing economic injury. It is native to North America where it feeds on native and cultivated *Vitis* (Rhamnales: Vitaceae), and *P. viteana* is distributed in North America east of the Rocky Mountains. Vineyards infested by *P. viteana* are often found in close proximity to forested areas containing wild *Vitis* spp. that provide refuge for *P. viteana* to escape pest management programs during the grape growing season. Not surprisingly, the propinquity of wild grapevines has been positively correlated with areas of moderate to severe *P. viteana* infestation of cultivated *Vitis* (Morano and Walker 1995, Botero-Garcés and Isaacs 2004).

P. viteana is multivoltine, and overwintering pupae emerge as adults in spring (Tobin et al. 2002). However, eggs and early neonates (<12 h) are the diapause-inducing stage, which responds to declining photoperiods after the summer solstice (Nagarkatti et al. 2001). Thus, depending on the photoperiod at which eggs are oviposited, a larva will either develop into an adult or enter diapause as a pupa. An accurate estimation of the photoperiods at which diapause is induced remains a critical component of *P. viteana* management because the number of generations per

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year is influenced by the degree-days (that influence development) that accumulate before diapause is obligatory (Tobin et al. 2003). A previous study (Nagarkatti et al. 2001) identified the diapause inducing cues and quantified the diapause response along a photoperiod gradient for *P. viteana* populations from the Lake Erie region. The photoperiods to which populations from this region are exposed are not relevant to southern populations that have a different photoperiod cycle (Tobin et al. 2003). Because of the importance of grape berry moth management in viticulture across eastern North America, we extend this prior study to examine the clinal latitudinal variation in diapause response using *P. viteana* populations from New York to Texas, which can be used to estimate voltinism and the consequent potential for economic injury across this broader landscape.

Materials and Methods

Study Sites. During the 2005 growing season, two grape berry moth colonies were initiated at the Lake Erie Regional Grape Research and Extension Center, North East, PA (42.22° N, 79.83° W), using larvae from on-site and neighboring vineyards, and larvae from wild grapes collected from Presque Isle State Park, PA (42.16° N, 80.11° W). In 2006, grape berry moth larvae were collected from three vineyards in the Finger Lakes region of New York (Geneva, 42.87° N, 76.98° W; Ovid, 42.68° N, 76.82° W; and Dresden, 42.68° N, 76.96° W) and from two vineyards in Michigan (Paw Paw, 42.22° N, 85.89° W; and Lawton, 42.17° N, 85.85° W). Colonies were maintained in growth chambers (VWR Signature Diurnal Growth Chamber, model 2015, West Chester, PA) under laboratory conditions (25 ± 0.5°C, 45–55% RH) to induce morphogenesis. In-house colonies from Altus, AR (35.44° N, 93.75° W), Cat Spring, TX (29.85° N, 96.33° W), and Stuarts Draft, VA (38.03° N, 79.03° W) were established in 2007 and 2008 under the same laboratory conditions. Collection sites of all colonies is shown in Fig. 1 with their respective photoperiodicity. Each colony was bolstered with the seasonal additions of wild moths from their respective location. Store-bought Thompson red-seedless grapes were hung in each of the colonies to obtain cohorts of eggs (≤24 h postoviposition) for the diapause experiments. Live pupae that did not emerge after 6 wk, which considerably exceeds the mean time of ≈11 d (at 26°C) required for pupal development (Tobin et al. 2001), were considered to be in diapause. Dead pupae were ascertained through observation of obvious desiccation.

Suitable host plants for grape berry moth are present at all collection sites; however, native *Vitis* spp. were different across sites (Galet 1979, U.S. Department of Agriculture Plants Database 2010). In Michigan, New York, and Pennsylvania, six *Vitis* species are common: *V. aestivalis* Michaux, *V. labrusca* L., *V. riparia* Michaux, *V. vulpina* L., *V. cinerea* Engelm., and *V. novae-angliae*, with *V. riparia* being the most prevalent species (Seaman et al. 1990). It typically blooms and ripens 1–2 wk earlier than the cultivated

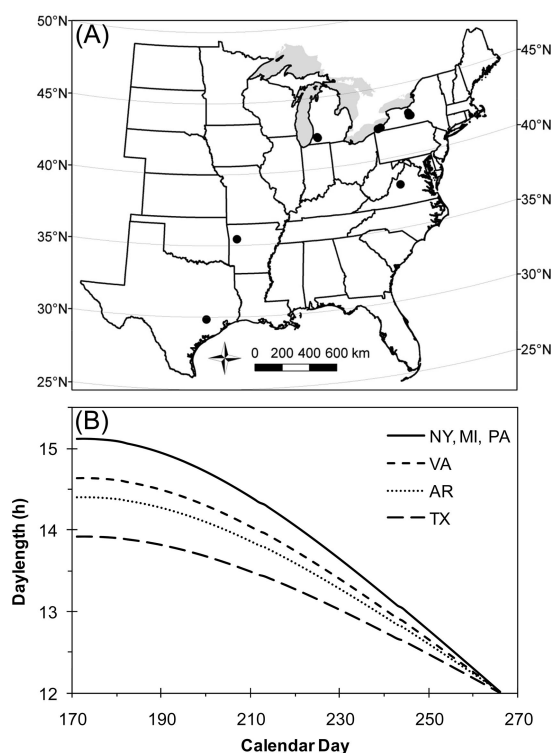


Fig. 1. (A) Locations of grape berry moth populations used in this study. (B) Daily photoperiods from the summer solstice to the autumn equinox of the grape berry moth populations used in the study.

grapes (mostly Concord variety, *Vitis x labruscana* Bailey) in these areas. In Virginia, *V. aestivalis* Michaux, *V. labrusca* L., and *V. riparia*, which ripen from August to October, are the most common wild grapes. A wide diversity of genetically unique grape species have been identified as native to Texas with over ten native *Vitis* species. The dominate species are *V. candicans* Engelm. (mustang grape) and *V. rotundifolia* Michaux (muscadine grape). Both of these species ripen in late August and early September. Of the five species of *Vitis* identified in Arkansas, *V. rotundifolia* Michaux is the most prevalent of the wild grape species.

Population-Specific Diapause Response. We analyzed the main effects of population (i.e., Arkansas, Michigan, New York, Presque Isle PA, Texas, Virginia) and photoperiod, and their interaction, to develop population-specific diapause response relationships. Even though the Presque Isle population originated from Pennsylvania, and was collected roughly 31 km from cultivated grapes in North East, PA, we maintained this population separately because it represented a wild, native population of grape berry moth feeding primarily on wild, native *Vitis* spp. as opposed to cultivated grape. Eggs, which are the diapause sensitive stage (Nagarkatti et al. 2001) from each population were subjected to photoperiods from 12 to 16 h and maintained under laboratory conditions. We then

Table 1. Number of pupae developing from eggs subjected to different photoperiods that were used to develop pop-specific diapause responses (see Fig. 2)

Photoperiod (h)	Number of pupae	
	Arkansas, Texas, and Virginia	Michigan, New York, and Pennsylvania
12.0	42	58
12.5	23	768
13.0	—	123
14.0	72	279
14.5	129	328
15.0	—	185
15.5	122	308
16.0	360	2,016

Data are from in-season experiments only.

recorded the number of pupae that subsequently emerged as adults. We considered the number of pupae that entered diapause relative to the initial number of pupae at each photoperiod-population combination as our response variable. Significance of effects was based on G^2 for Type 3 analysis (PROC GENMOD, SAS Institute 1999). We had previously observed statistical differences in the proportion of pupae that entered diapause when exposed to photoperiods ≥ 15 h and when the experiment was conducted “off season” (explained below); thus, we excluded data obtained from experiments conducted off-season at photoperiods ≥ 15 h. The proportion of diapausing pupae (P_d) over photoperiod (h) was fit using an exponential model according to,

$$P_d = \exp(-\exp(r \times h - b)), \quad [1]$$

in which r and b are parameters estimating the rate of increase and lag, respectively. Nonlinear convergence was based on the Marquardt algorithm in PROC NONLIN (SAS Institute 1999). Total sample sizes by population and photoperiod ranged from 16 to 854 pupae, and the sample sizes when pooling data from nonsignificant populations (see Results) ranged from 23 to 2,016 pupae for each photoperiod (Table 1).

Photoperiod and the Timing of Experiments. Based upon preliminary laboratory observations, we examined the interacting effect of photoperiod and the time of the year at which experiments were conducted. Because of limitations in the availability of environmental growth chambers, we conducted our experiments throughout the year, from 2006 to 2009. We divided the timing of experiments into two groups: (1) “in season,” which were experiments initiated from 9 March to 19 September, which represents a period during which grape berry moth could be in a nondiapaused state, and (2) “off season,” which were experiments that were initiated from 12 October to 10 February of the following year, which represents a period during which grape berry moth would be in diapause (Nagarkatti et al. 2001). We considered the number of pupae that entered diapause relative to the initial number of pupae at each photoperiod-season combination as our response variable. Significance of the interaction of photoperiod and season (in or off) was

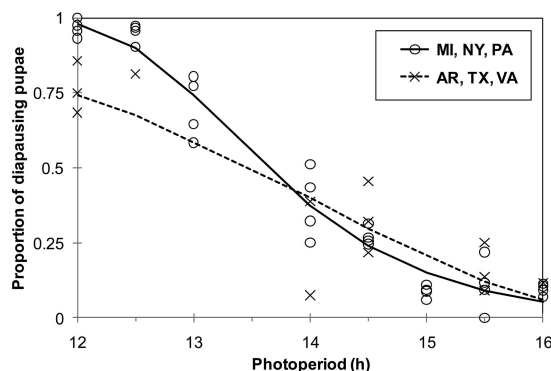


Fig. 2. Population-specific diapause responses to the photoperiod at which eggs are exposed. The groupings represent nonsignificant pairings. Model fits: Michigan, New York, Pennsylvania: proportion in diapause = $\exp(-\exp(1.06 [SE = 0.07] \times h - 14.10 [SE = 0.96]))$; Arkansas, Texas, Virginia: proportion in diapause = $\exp(-\exp(0.56 [SE = 0.11] \times h - 7.96 [SE = 1.61]))$.

based on G^2 for Type 3 analysis, and odds ratios and associated confidence intervals were estimated based on the Wald χ^2 (PROC LOGISTIC, SAS Institute 1999). We conducted this analysis using data collected from all sites. Total sample sizes at each photoperiod ranged from 87 to 2,376 pupae.

Results

Population-Specific Diapause Response. We observed a significant interaction effect between population and photoperiod ($G^2 = 43.6$; $df = 6$; $P < 0.01$), suggesting that populations were differentially affected by photoperiods in diapause induction. There was no main effect of population ($G^2 = 2.7$; $df = 3$; $P = 0.44$), or interacting effect of population and photoperiod ($G^2 = 2.8$; $df = 3$; $P = 0.43$) among the Michigan, New York, and Pennsylvania (including Presque Isle) populations. Populations from Arkansas, Texas, and Virginia also showed no significant effect of population ($G^2 = 0.2$; $df = 2$; $P = 0.91$), or interacting effect of population and photoperiod ($G^2 = 0.5$; $df = 2$; $P = 0.78$). When considering both of these pooled groups, we observed a significant effect of population ($G^2 = 67.5$; $df = 1$; $P < 0.01$), and interacting effect of population and photoperiod ($G^2 = 63.0$; $df = 1$; $P < 0.01$). This suggests that grape berry moth populations from Michigan, New York, and Pennsylvania respond differently to photoperiod than those populations from Arkansas, Texas, and Virginia in initiating diapause (Fig. 2).

Photoperiod and the Timing of Experiments. We observed a significant interaction between season and photoperiod ($G^2 = 23.8$; $df = 1$; $P < 0.01$). The difference was because of diapause response at photoperiods ≥ 15 h (Fig. 3), which generally do not induce diapause. At photoperiods < 15 h, there was no significant interaction between season and photoperiod ($G^2 = 1.9$; $df = 1$; $P < 0.17$). When eggs were subjected to photoperiods ≥ 15 h during the off-season months,

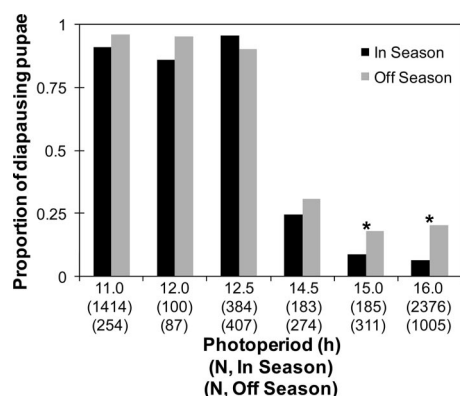


Fig. 3. Proportion of diapausing pupae by the photoperiod at which eggs were exposed, and whether the experiment was conducted "In Season" (i.e., months during which grape berry moth is naturally nondiapausing) and "Off Season" (i.e., months during which grape berry moth is naturally diapausing). Significant differences are denoted by asterisks. Sample sizes for each season and photoperiod are listed along the x-axis.

they were 3.4 times more likely (95% confidence interval [CI] = 2.8, 4.2) to develop into diapausing pupae than those subjected to the same photoperiods but during the in-season months. We did not detect a significant interaction effect among photoperiod, season, and population ($G^2 = 7.8$; $df = 6$; $P = 0.25$), suggesting that this phenomenon was not driven by latitude but rather by the season in which the experiments were initiated.

Discussion

Like many other insects, the critical photoperiod for diapause induction in grape berry moth varies by latitude. At the latitude of the grape producing regions of the Great Lakes states of Michigan, New York, and Pennsylvania, grape berry moth diapause initiation relies on the same photoperiodic cues, which occur at the same calendar date across this region. Populations from the southern United States differed significantly from the Great Lakes states in their response to photoperiod, but were not significantly different from one another. This difference was not unexpected, given that the longest daylength at the Texas site (Fig. 1) is shorter than the daylength that triggers 50% diapause in the Great Lakes states (Fig. 2; Nagarkatti et al. 2001). This suggests that grape berry moth in the southern United States is less likely to initiate diapause at photoperiods <14 h. Additionally, at 12 h of daylight, which in populations from the Great Lakes sites results in nearly 100% diapause initiation (Fig. 2; Nagarkatti et al. 2001), only 75% of individuals from the southern populations entered diapause (Fig. 2). Latitudinal differences in the rates of diapause initiation across an arthropod's range have been previously reported. For example, Suwa and Gotoh (2006) demonstrated that the critical photoperiod for *Tetranychus pueraricola* Ehara and Gotoh (Acari: Tet-

ranychidae) decreased by ≈ 1 h for each 5° of latitude to the south. At the southern end of its range, *T. pueraricola* never entered diapause. Tauber and Tauber (1972) showed that New York populations of *Chrysopa carnea* Stephen (Neuroptera: Chrysopidae) entered diapause at longer day lengths than Arizona populations of *C. carnea*. This study concluded that this difference allowed the New York populations to enter diapause earlier in the season as an adaptation to long summer day lengths and early harsh winters.

The observed diapause induction behavior in southern populations of grape berry moth does not appear to contradict these other studies. Indeed, given the late summer-to-early fall ripening times for many species of wild southern grape varieties, the mildness of the winter weather relative to more northern latitudes, and the potential presence of non-*Vitis* host plants for a large portion of the year, this diapausing strategy seems reasonable. There also appears to be the possibility that some proportion of the populations of southern grape berry moth is nondiapausing (Fig. 2). Future studies that subject southern populations to photoperiods <12 h could determine the existence of nondiapausing grape berry moth in these regions.

Awareness of the timing of important phenological events in a pest insect's life history is important in the development of effective pest management strategies. For the management of grape berry moth, the ability to anticipate the onset of diapause is key for decision support. At the shared latitude of the grape growing regions of Michigan, the Lake Erie shore in Pennsylvania and New York, and the Finger Lakes region of New York, grape berry moth responds to the same photoperiodic cues for initiating diapause. This homogeneity will enhance researchers' ability to develop common pest management protocols for growers in these regions. For example, in a warmer-than-average summer at this latitude, the eggs of the midsummer generation will likely be oviposited well before the daylength at which diapause is induced (Nagarkatti et al. 2001, Tobin et al. 2003). Under these conditions, grape growers should expect a larger than expected third generation of grape berry moth late in the growing season, while the inverse (i.e., cooler-than-average year) would suggest a lesser potential for economic injury from a third generation.

One surprising result of this study was the observation that this insect's response to photoperiod varied with the time of year at which we conducted our studies. Even though these studies took place in laboratory growth chambers shielded from other environmental cues, insects tested between mid October and early March evinced different responses to photoperiod than those insects tested when they would not normally be in diapause (Fig. 3). This ability to perceive the time of the year and differentially respond to external cues suggests careful consideration should be paid to the timing of behavioral and physiological experiments on insects.

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