Environmental Entomology Advance Access published December 26, 2016

Environmental Entomology, 2016, 1–7
doi: 10.1093/ee/nvw149
Research

OXFORD

Pest Management

Tillage Reduces Survival of Grape Berry Moth (Lepidoptera: Tortricidae), via Burial Rather Than Mechanical Injury

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Subject Editor: David Onstad

Received 4 August 2016; Editorial decision 21 October 2016

Abstract

The grape berry moth, *Paralobesia viteana* (Clemens), is a key pest of vineyards in eastern North America that overwinters as pupae in leaf litter on the vineyard floor. This presents an opportunity for tillage to disturb and bury the pupae, providing a potential nonchemical approach to control of this pest. Using a Lilleston-style rotary cultivator, we determined the distribution of pupae within the soil profile after single tillage passes, measured the type and severity of damage inflicted on pupae, and investigated how these effects on pupae influenced their survival. Survivorship of pupae recovered from the vineyard immediately after tillage and held until emergence was not significantly different from those recovered from an untilled control area, indicating little effect of mechanical damage on this pest. However, a single pass of the tillage implement buried three-quarters of pupae under at least 1 cm of soil. A laboratory experiment to recreate these conditions resulted in significant increase in mortality when pupae were buried in more than 1 cm of sand. We conclude that 1) interference with adult emergence of diapausing pupae via burial is the primary mechanism by which tillage controls grape berry moth, and 2) efforts to optimize the impact of tillage on grape berry moth populations should focus on maximizing the number of pupae buried. We discuss the potential integration of tillage into different vineyard management systems to enhance pest management.

Key words: integrated pest management, cultural control, organic viticulture

Paralobesia viteana (Clemens), the grape berry moth, is a direct pest of wild and cultivated grapes. It is multivoltine, with a potential for up to four generations per year in Michigan (Tobin et al. 2008). The moth oviposits almost exclusively on grape hosts (Isaacs et al. 2012), with egglaying on foliage and blossoms in the first generation, and on maturing fruit during subsequent generations. A single larva can feed on multiple grape berries within a cluster, and their infestation later in the season can increase the risk of disease infections of clusters (Fermaud and Le Menn 1992). Mature larvae exit clusters to form cocoons from grape leaf flaps folded over with webbing, and eggs laid after a critical photoperiod of between 14 and 15 hours will develop into diapausing pupae that remain in cocoons until the start of the next growing season (Nagarkatti et al. 2001). The diapausing pupae overwinter on the ground because leaves containing the cocoons fall from the canopy and mix with the floor litter.

Michigan is home to the second largest juice grape processing plant owned by Welch's Foods Inc., and in 2015, juice grapes accounted for ∼4,850 harvested hectares that produced an estimated 54,500 metric tons of Concord and Niagara grapes valued at US$19.3 million (USDA NASS 2016). High infestation rates of berries with grape berry moth will lead to rejection of entire truckloads, which have no other viable purchaser. Because the machine harvesting leaves little room for quality sorting, growers may be faced with leaving areas with high infestation unharvested in order to avoid the risk of rejection and the associated loss. Michigan is also home to a burgeoning wine industry, contributing US$300 million annually to the state economy through wine sales and related agrotourism (MGWIC 2015). Climate change predictions suggest that the Lake Michigan coastline will experience milder winters and warmer spring temperatures (Wang et al. 2011), which is expected to increase the number of grape berry moth generations reaching adulthood in the growing season (Tobin et al. 2008), and may increase grape berry moth pressure above economic thresholds in northern winegrape-growing regions.

Conventional management of grape berry moth relies predominantly on rotating applications of broad-spectrum insecticides (Isaacs et al. 2012). This style of chemical-dominated management has come under public scrutiny in recent years as a result of human health risks, deleterious impacts on the environment, and increasing levels of resistance development in pest populations (Pimentel 2005). Indeed, grape berry moth populations resistant to the broad-spectrum carbamate insecticide, carbaryl, have been discovered along Lake Erie (Nagarkatti et al. 2002). Even modern insecticides with relatively little history of field use are encountering resistant insect populations. Recently, it was discovered that *Lobesia botrana* (Denis & Schiffermüller), a totricid pest in Europe similar to the grape berry moth, is developing resistance in parts of Italy to indoxacarb, a next-generation reduced-risk insecticide registered in 2000 (USEPA 2000, Civolani et al. 2014). Rising awareness of insecticide resistance along with increased restrictions on the use of broad-spectrum insecticides has created a need to decrease reliance on chemical tactics and create more diversified and sustainable control systems.

Tillage has a long history of use for managing weeds, and its reintegration into modern weed management programs has been recommended as a means of disrupting herbicide resistance development and decreasing dependency on chemical tactics (Norsworthy et al. 2012, Vencill et al. 2012). Tillage may be similarly integrated into pest management programs for control of insect pests that spend some portion of their life at or below the soil surface (Johnson et al. 1984, Seal et al. 1992, Chu et al. 1996, Baughman et al. 2015). Extension publications from the early 1900s mention that growers in North East, PA were throwing furrows over the leaf litter beneath vine canopies in late fall or early spring to reduce grape berry moth emergence (Isely 1917). Rudimentary experiments reported in the same publication supported burial as an effective means of control. Recent work by Baughman et al. (2015) showed that burial of *Cydia pomonella* L., a totricid pest of apples, under just 1 cm of sand completely inhibited emergence after diapause. The approach presented in Baughman et al. (2015) made use of the "Swiss Sandwich" technique, in which tillage is applied to narrow strips on either side of the tree row. This style of tillage limits soil disruption and has been shown to have minimal impact on soil health (Zoppolo et al. 2011). Thus, the targeted use of tillage may provide a way for grape growers to manage grape berry moth populations while introducing diversity into vineyard pest management programs.

The overall aim of our study was to explore the potential use of tillage in reducing survivorship of diapausing grape berry moth pupae. We had four specific objectives: 1) Determine the distribution of pupae within the soil profile after tillage, 2) Characterize the type and severity of damage inflicted on pupae by a tillage implement, 3) Determine the relationship between the damage caused by tillage and survivorship, and 4) Determine how burial depth affects survivorship of this pest.

Materials and Methods

Insect Collection and Rearing

Rearing procedures were based on methodology presented by Taschenberg (1969). Plastic containers (28 W by 40 L by 16 H cm) were equipped with false bottoms made from coarse (5-mm) metal mesh. Infested grape clusters were collected from three vineyards located in Van Buren County, MI, on September 3, 2014. The grapes were placed on top of the false bottom after lining the container with 2-cm-wide strips cut from clear plastic storage bags. The strips were used to simulate leaf litter and to provide a substrate for pupation. The containers were placed in a temperature-controlled room maintained at 22°C with a photoperiod of 16:8 (L:D) h. Rearing containers were inspected biweekly for the presence of prepupae, which dropped from clusters, passed through the metal screen, and formed easily identified pupal casings in the plastic litter. The plastic around each pupal casing was cut away and the pupae were stored at 7.2 °C in 60-ml plastic portion cups, along with a piece of moistened dental wicking to maintain humidity. Wicking was remoistened as needed. Pupae were collected over an 8-wk period.

Tillage Depth

On November 6, 2014, pupae were placed on the ground beneath the canopy of grapevines in a vineyard row, and treated with a single pass of a Wonder Weeder double-gang rotary cultivator (Harris Manufacturing, Burbank, WA). Pupae were then excavated from the soil profile and the depth was noted for each recovered pupa.

Recovery of small insects from a disturbed soil profile can pose a significant challenge. To solve this, we first coated pupae with blue Luminous Powder (Bioquip, Rancho Dominguez, CA). The powder left streaks in the soil that stood out visually and aided in tracking the path and location of each pupa. A point frame with a 4 by 15 grid of 10 by 10-cm squares was used to aid in even placement of pupae and their relocation. Using this combination of techniques helped to ensure thorough excavation and provided a standardized point of reference for depth measurements. We recovered >90% of pupae from each replicate.

Four point frames were placed on one side of the grape row, one for each replicate, spaced 5 m apart, and oriented so that the long edges were parallel to the grape row. Each pupa was placed in the center of a grid cell. Not all cells in the frame were used: the frames were filled row-wise, beginning with the cells closest to grape row. After placement, the locations of the point frames were marked with flags, and then the frames were removed. After the tillage pass, the point frames were returned to their original positions. Each cell was carefully excavated with small spade trowels to recover the buried pupae. The distance of a recovered pupa from the center of the plane of the cell above it was noted, along with the distance from each edge of the cell to the soil surface. Burial depth was calculated as the difference between the distance of the pupa and the average distance of the cell edges. This was done to account for the uneven substrate pattern created during tillage. Any pupa recovered from the surface was assigned a burial depth of zero. Some pupae were recovered outside of the original placement area, in which case the point frame was shifted to recover depth information. Each recovered pupa was placed in a separate container, returned to the lab, and stored at room temperature with a piece of moistened dental wicking. A control treatment, which did not receive a tillage pass, was established in an identical fashion on the opposite side of the row. Forty pupae were assigned to each control replicate and 50 pupae to each tillage replicate. The additional pupae in the tillage treatment were added to account for the likelihood of incomplete recovery.

Tillage Damage and Survival

Every pupa recovered from the tillage depth experiment was inspected and assigned a score for damage from one of four categories: tears in the plastic wrapping, peeling of the plastic wrapping, punctures and lacerations of the silken cocoon, or crushing and laceration of the pupa body (Table 1). A single investigator performed all of the scoring to ensure consistency. After assessing the damage resulting from tillage, pupae were placed in a growth chamber to simulate overwintering, beginning on November 24, 2014, with a photoperiod of 12:12 (L:D) h and 80% RH. Temperature was initially set to 20 °C, lowered weekly by 5 °C, and then maintained at 5 °C until February 3, 2015. The pupae were then warmed back to 20 °C using the same schedule. Pupae were checked weekly for

Table 1. Criteria for qualitative damage assessment scores of grape berry moth pupae that were subjected to tillage with a rotary cultivator

	Score			
	0	1	2	3
Torn wrapping	Not present	A single pin-sized puncture	Multiple pin-sized punctures or a single large puncture	>50% torn
Peeled wrapping	Not present	<33% opened	33–66% opened	>66% opened
Cocoon	Not present	A single pin-sized puncture	Multiple pin-sized punctures or a single large puncture	>50% torn
Pupa body	Not present	Present	–	–

Table 2. Comparing the occurrence of various damage types in grape berry moth pupae subjected to a tillage pass with a rotary cultivator to an uncultivated control group

Group	Pupae counted by damage class							
	Torn wrapping				Peeled wrapping			
	0	1	2	3	0	1	2	3
Tillage	173 (94.5%)	7 (3.8%)	3 (1.6%)	0	122 (65.2%)	23 (12.3%)	12 (6.4%)	26 (13.9%)
Control	152 (96.8%)	5 (3.2%)	0	0	96 (61.1%)	32 (20.4%)	9 (5.7%)	20 (12.7%)
	Chi-square	**df**	**Sample size**	***P* value**	**Chi-square**	**df**	**Sample size**	***P* value**
Test stats	2.7179	2	340	0.2569	3.8189	3	340	0.2855
Group	**Puncture/tear of cocoon**				**Pupae crushing and laceration**			
	0	**1**	**2**	**3**	**Not present**		**Present**	
Tillage	177 (96.7%)	4 (2.2%)	1 (0.05%)	1 (0.05%)	181 (98.9%)		2 (1.1%)	
Control	152 (96.8%)	5 (3.2%)	0	0	157 (100%)		0	
	Chi-square	**df**	**Sample size**	***P* value**	**Chi-square**	**df**	**Sample size**	***P* value**
Test stats	2.0345	3	340	0.9239	1.717	1	340	0.5017

Test statistics are reported for Fischer's Exact Test.

emergence after the start of the warming period, and until there were two consecutive weeks without emergence.

Fisher's Exact Tests were performed to determine whether there were differences in the severity of damage for each observed damage type, and also to determine whether survivorship was different between the tillage and control groups. All analyses were performed in SAS 9.4 using PROC FREQ (SAS Institute Inc 2015, Cary NC).

Burial Depth and Survival

Pupae allocated to this experiment were combined into a single container and maintained at room temperature until November 24, 2014, at which time they were transferred to the same growth chamber as the pupae used in the experiment described above, and managed identically. Immediately after warming, pupae were assigned to one of five burial treatments: 0, 1, 3, 5, and 15 cm depth burial. Each treatment was replicated five times and each replicate utilized 18 pupae. Experimental arenas consisted of a Schedule 40 PVC tube with a 15.3 cm internal diameter mounted to a piece of plywood with 0.158-cm holes drilled into the capped bottom for drainage. A piece of landscape fabric lined the bottom and 8 cm of sand was placed on top of the fabric. The sand used in the experiment was play sand (Home Depot Inc.). The sand was rinsed with three times its volume of distilled water to remove any salts and allowed to air dry to aid in ease of handling. After drying, the sand was autoclaved for 8 h, maintained at room temperature inside the autoclaved container, and then autoclaved again for an additional 8 h to destroy any germinating spores. The pupae were evenly distributed on the surface of the sand, and then covered with an additional layer of sand whose thickness corresponded to the particular burial treatment. The sand was then slowly wetted with distilled water until runoff was observed from the drainage holes. A fine, see-through mesh was placed over the top of the arenas and held in place with a rubber band. The arenas were allowed to drain and then transferred to a growth chamber at 28°C with 50% RH and a photoperiod of 16:8 (L:D) h. The arenas were checked daily for emerged adult grape berry moth, which were counted and then removed. Arenas were checked until no emergence was observed for two consecutive weeks.

Survivorship was expressed in terms of the proportion of pupae in each experimental unit that emerged over the course of the experiment. Survivorship values were arcsine transformed to achieve a normal distribution and homogeneity of variance was confirmed using Levene's Test at $\alpha = 0.05$. Treatment effects were tested using a one-way ANOVA and multiple comparisons were performed using Fisher's Protected LSD at $\alpha = 0.05$. A contrast statement was used to test the overall significance of burial compared with the control group. All analyses were performed in SAS 9.4 using PROC Mixed (SAS Institute Inc 2015, Cary, NC).

Results and Discussion

Mechanical Effects on Pupae

We found examples of damage fitting each of the four categories and did not encounter any damage that was not classifiable within those categories. The vast majority (>94%) of pupae in both treatments were undamaged with regard to tears, punctures, or lacerations. Approximately 40% of the pupae exhibited some degree of peeling of the plastic wrapping. However, damage to pupae was not significantly different between the tillage and control groups in any of the categories (Table 2), suggesting that most of this damage occurred as a result of handling. Furthermore, survivorship of pupae recovered from the field immediately after a tillage pass was not

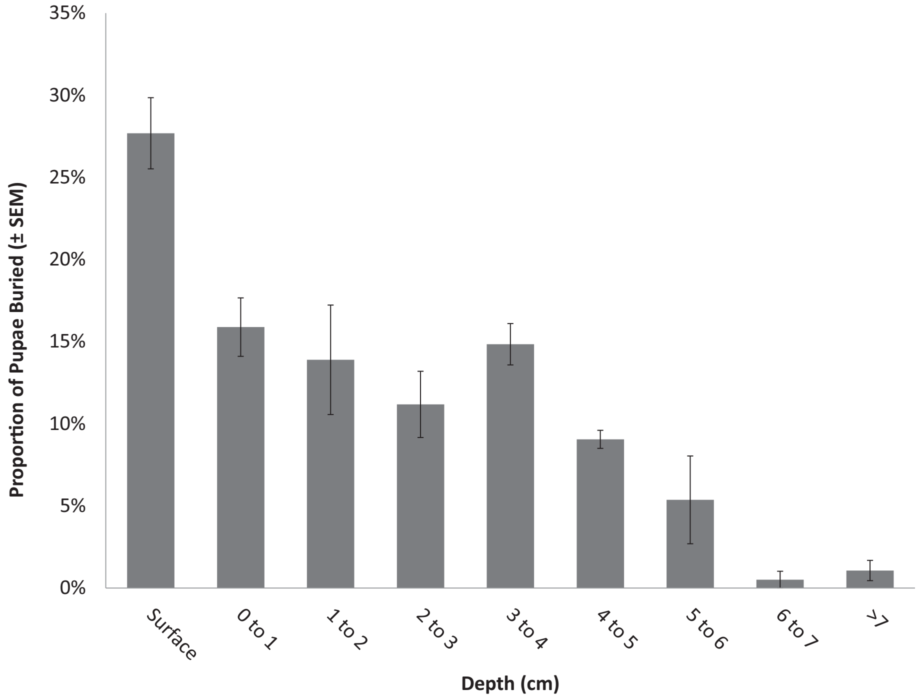

Fig. 1. Distribution of grape berry moth pupae within the soil profile after a tillage pass with a rotary cultivator.

significantly different than those recovered from an untilled control group ($\chi^2 = 2.25$; df = 1; $P = 0.148$). These results are consistent with other research on tillage of surface-dwelling pests, where little mechanical damage was inflicted in spite of a considerable alteration in the distribution of the organisms within the soil profile (Stinner and House 1990, Baughman et al. 2015). Thus, a tillage implement is unlikely to impart levels of damage that will lead to reduced survival of this pest and reduced crop infestation.

Burial Effects on Pupae

A single pass of a rotary tillage implement buried three-quarters of pupae under at least 1 cm of soil (Fig. 1). Less than 3% of pupae were recovered from a depth >6 cm, suggesting that this is the practical depth limit for this style of tillage. The laboratory burial study revealed that covering pupae by at least 1 cm of sand in otherwise idealized conditions resulted in a significant increase in mortality ($F = 87.86$; df = 1,20; $P < 0.0001$) compared with the unburied control. No significant differences were found in mortality between the 1, 3, and 5 cm depth treatments (Fig. 2). From these results, it can be concluded that interference with adult emergence of the diapausing cohort via burial is the primary mechanism by which tillage controls grape berry moth. We also conclude that burial depth is not a critical factor affecting adult emergence; thus, we suggest that efforts to optimize the impact of tillage on grape berry moth populations should focus on maximizing the proportion of pupae buried, regardless of depth.

The precise manner in which burial interferes with adult emergence is still unclear. Two plausible explanations are—1) the weight of substrate above a buried pupa pins the organism in place, and 2) the substrate abrades the organism as it moves, resulting in physical impairment and lethal loss of fluids. The pupae in this study were placed in the substrate just prior to their expected exit from diapause, which minimized the amount of time that the pupae had to move about in the substrate and inflict abrasive damage. Therefore, it would be expected that the degree of abrasive damage would be related to the passage of an adult through the substrate profile. If this was the case, then a greater number of adults should have been observed in the 1-cm treatment than any of the other burial treatments. We saw no difference between burial treatments, which suggests that the former hypothesis is most likely in this case. Of course, in the field where pupae are buried for an entire winter, abrasion in the loamy sand substrates of southwest Michigan could contribute considerably to reducing winter survivorship.

Incorporating Tillage Into Vineyard Management

It is important to acknowledge that the identification of an effective mechanism for reducing a pest population is only one part of a viable pest-control strategy. It is also necessary to understand how an effective technique can be implemented into existing cultural practices and adapted to the realities of a particular agroecosystem before it can be regarded as a practical solution to a pest problem. The efficacy of tillage is highly dependent on identifying exploitable windows of vulnerability within pest life cycles and behaviors, and determining if it is practical to apply tillage within a cropping system during those windows (Willson and Eisley 1992, Seal et al. 1992, Chu et al. 1996, Baughman et al. 2015). The nature of these points and methods for their exploitation varies considerably between pest–crop complexes. For grape berry moth, this means considering the seasonal and spatial dynamics of vineyard infestation, and how tillage, a resource intensive operation, can be applied in a cost-effective manner.

Application of Tillage in Conventional Vineyards

One efficient way of incorporating tillage into conventional systems is to optimize the timing of other tillage-based vineyard practices to coincide with the susceptibility window of the grape berry moth overwintering population. Two overlapping periods of opportunity to use tillage occur in typical vineyard management: late fall and early spring. In late fall, growers may use tillage to incorporate

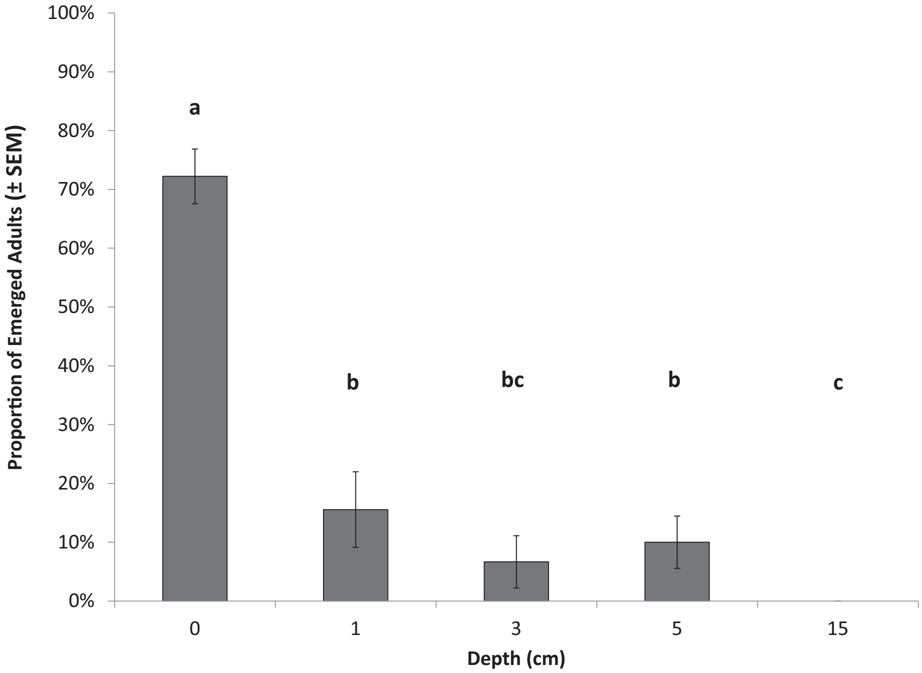

Fig 2. Proportion of diapausing grape berry moth pupae emerging as adults after burial under different depths of sand. Multiple comparisons were performed using Fisher's Protected LSD at $\alpha = 0.05$. Values sharing a letter are not statistically different.

slow-release amendments into soil or form a berm under the vine to protect cold-sensitive graft unions (Weigle and Carroll 2015). Growers in the Southeast mound soil in the summer to control grape root borer; the mounds have to be taken down in the fall (Bergh 2012). Inverting the soil in the mound as it is being spread could also be used to cover grape berry moth pupae. In the early spring, cultivation is recommended as a means of reducing vineyard pathogen loads by burying potentially inoculated debris, and controlling weeds as they begin to emerge. For many conventional growers, this may still involve a significant overhaul of other management strategies (weed and soil fertility management, in particular) in order to justify the purchase of the required tillage equipment. However, as seen with insect pests and insecticides (Nagarkatti et al. 2002), continued applications of broad-spectrum herbicides has led to the development of resistance in major weed species across the majority of herbicide chemistries (Heap 2016). Therefore, it may be cost effective over the long run for growers to diversify both their weed and pest management strategies by including tillage.

Tillage could also be used to further advance recent efforts to focus grape berry moth spray programs on protecting border rows instead of entire vineyards. Multiple trapping studies have shown that infestation of conventional vineyards by grape berry moth is an annual cycle, in which a small portion of a standing population present in surrounding wood lots migrates into the vineyard, moving from border rows into the vineyard interior as the season progresses (Hoffman 1987, Johnson et al. 1988, Biever and Hostetter 1989, Trimble et al. 1991, Botero-Garcés and Isaacs 2004). Border-focused pest control works by knocking down the interior pest population early in the season with a single, vineyard-wide insecticide application, and then preventing pest migration from woodlots by making repeated applications of targeted insecticides to border rows. This style of control reduces overall application volumes, making the use of high-specificity insecticides an affordable option and decreasing the likelihood of detecting insecticide residues in harvested fruit. Mason et al. (2016) conducted the first field-scale evaluation of border-focused grape berry moth management. They found no significant differences in grape berry moth damage within vineyard interiors between vineyards managed with the new IPM program and those sprayed across the entire vineyard. Tillage could potentially replace the start of season, whole vineyard, pesticide application in a border-focused IPM program.

Application of Tillage in Organic Vineyards

Organically managed vineyards are well-suited to adopt tillage-based grape berry moth management strategies. Herbicides compliant with the USDA National Organic Program are either prohibitively expensive or lack efficacy to reliably manage ground cover (Liebman and Davis 2009). Therefore, organic-certified growers rely heavily on tillage and cultivation when suppression of ground cover competition is required. This means that organic operations are more likely to possess the equipment and training necessary to cultivate within and between vine rows. It also means that organic growers are already making multiple passes through the vineyard each season.

Organic viticulturalists are also limited in their ability to control pest outbreaks once they have exceeded economic thresholds because of the restrictions placed on using synthetic compounds. The 2015 Cornell Production Guide for Organic Grapes specifically states that "pesticides should not be relied on as a primary method of pest control"(Weigle and Carroll 2015). Chemical methods of controlling grape berry moth in organic vineyards are based on biologically derived compounds like Bt and spinosad, and pheromone mating disruption (Martinson 1995, Teixeira et al. 2010, Weigle and Carroll 2015). Biopesticides tend to have short half-lives due to photolysis, persisting for only a few days on fruit and foliage (Kollman 2002, Sanahuja et al. 2011, Hung et al. 2016). Mating disruption is not directly lethal, and therefore, has no persisting toxicological impact on grape berry moth. The infestation cycle of grape berry moth in organic vineyards is likely quite different than that of conventional vineyards. The nature of organic pest management is such that the vineyard is free of the top-down chemical pressures associated with continued use of synthetic insecticides and therefore may be suitable for sustaining a standing population of grape berry moth. The dynamics of grape berry moth populations and infestation cycles in organic vineyards has yet to be investigated. However, if organic vineyards do support standing grape berry moth populations, then vineyard-wide tillage to disrupt the overwintering grape berry moth cohort could have a considerable impact on grape berry moth pest pressure in these systems.

In conclusion, we have shown that burial under even minimal amounts of sand interferes with the successful emergence of diapausing grape berry moth. We also demonstrated that tillage is not effective at directly imparting damage to pupae in the field, which is consistent with previous studies. Organic grape growers are well positioned for adoption of tillage for management of grape berry moth, requiring only small changes to existing cultural practices. Conventional growers are likely to encounter short-term barriers to adoption related to the purchase of specialized tillage equipment and adjustment of management strategies. However, conventional growers are also in a position to incorporate tillage into next-generation IPM programs based on border-applied, high-specificity insecticides. A more thorough understanding of the relationship between vineyard management styles and grape berry moth population dynamics will assist in the development of diversified and sustainable pest control strategies.

Acknowledgments

Thanks to Mark Gregory of Gregory Farms (Lawton, MI) for providing a research site and assisting with field operations. Funding for this project was provided through the USDA-NIFA Pest Management Alternatives Program, Grant 2013-34381-21204 to RI and MG.

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