Behavioural and electrophysiological responses of grape berry moth (Lep., Tortricidae) to selected plant extracts

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Abstract: Four non-host plant extracts from Bifora radians, Arctium lappa, Humulus lupulus and Xanthium strumarium, were tested against adult grape berry moths, Paralobesia viteana, for their potential as repellents or oviposition deterrents. Responses were compared with those elicited by the major component of the P. viteana sex pheromone. Moths of both sexes exhibited varying electroantennogram (EAG) responses from 1.7 to 2.4 mV to volatile odours from plant extracts, with the greatest response to extracts of H. lupulus (2.4 mV). A multi-arm olfactometer was used to assay the behavioural response of moths to the same treatments. Male moths responded significantly to H. lupulus extract, although their strongest response was to the sex pheromone (30.0%). Female moths showed no behavioural response to the sex pheromone, attracting only 6.7% of moths, but they were attracted to extracts of H. lupulus, X. strumarium and A. lappa, with 25.0%, 21.7% and 15.0% of the released insects responding to these treatments, respectively. Choice tests were conducted to compare oviposition by P. viteana on untreated grapes and on grapes treated with one of the extracts. Despite the attraction in the olfactometer assays, H. lupulus extract significantly reduced egg laying; only 12.5% of the total deposited eggs were laid on berries treated with this extract. The extract of B. radians provided complete protection against oviposition.

Keywords: Paralobesia viteana, behaviour, deterrent, pesticide alternative, plant extract

1 Introduction

The grape berry moth, Paralobesia viteana (Clemens) (Lep., Tortricidae), is distributed across eastern North America, inhabiting vineyards and woods containing wild grapevines of the genus Vitis. It is the primary lepidopteran pest of commercial viticulture in this region (Dennehy et al. 1990), causing yield loss, contamination of grape clusters (Hoffman and Dennehy 1989; Botero-Garcés and Isaacs 2003) and rendering the fruit more susceptible to disease. Female moths lay eggs directly on grape clusters, and the larvae burrow into berries where they feed until pupation (Slingerland 1904; Johnson and Hammar 1912). Oviposition by P. viteana is highly selective for Vitis spp. plants, and all stages of the life cycle occur on this genus (Johnson and Hammar 1912; Clark and Dennehy 1988). This suggests that repellent or deterrent compounds from the many non-host plants of P. viteana may have potential for its control in vineyards by affecting the likelihood of female moths orienting to, or laying eggs on, grape clusters. Studies are underway to determine the chemicals exploited by P. viteana during host location and selection, but these are currently not known. Identification of active non-host plants would provide an alternative mode of action for reducing the economic impact of the grape berry moth in viticulture.

Paralobesia viteana is the target of a majority of the insecticides applied in vineyards in eastern North America. These insecticides are primarily broad-spectrum toxicants with the more recent addition of reduced-risk insecticides such as insect growth regulators and naturalytes (Isaacs et al. 2005). Current and anticipated restrictions on availability of broad-spectrum insecticides have increased the need for alternative approaches to pest control, based on new modes of action.

Plant extracts containing compounds such as terpenes, steroids, alkaloids, phenolics and cardiac glycosides (Duke 1990) are known to affect insect behaviour and can function as deterrents to insect pests (Blaney et al. 1988; Ge and Weston 1995; Mordue et al. 1998; Bruno et al. 2003). In the current investigation, we tested extracts of several plants known to produce such secondary compounds (Heywood et al. 1977; Katsiotis et al. 1990; Latrasse et al. 1991; Baser et al. 1998) on antennal and behavioural responses of grape berry moth. These extracts have recently been shown to have activity against two tortricid species of leafroller with a broad host range (Gökçe et al. 2005), and this study aimed to determine the response of a specialist crop pest insect to the same extracts.
The objectives of this study were to: (1) determine whether the selected plant extracts elicit antennal electroantennogram (EAG) responses in male and female grape berry moths, (2) determine whether the plant extracts elicit behavioural responses from male and female grape berry moths, using a laboratory olfactometer, and (3) determine whether plant extracts affect oviposition by female grape berry moths in choice bioassay chambers.

2 Materials and Methods

2.1 Insects

Moths were selected from a colony of *P. viteana* maintained at Michigan State University, reared using methods modified from Nagarkatti et al. (2000). Table grapes in the colony were purchased from a local store, surface sterilized by immersion in 1% bleach solution for 5 min, rinsed thoroughly in deionized water, dried and presented to *P. viteana* moths. New clusters were added to the colony three times weekly and infested grapes were removed and stored in plastic containers at room temperature (23 ± 3°C). Under these conditions, eggs eclosed in approximately 4–6 days, yielding a steady supply of neonates that were transferred to the meridic diet described by Nagarkatti et al. (2000).

2.2 Plant extracts

Four plant extracts were prepared according to the procedure described by Gökçe et al. (2005). The plants (table 1) were all collected during spring and summer of 2002. Samples were dried at room temperature and subsequently ground. Fifty-gram samples of dried plants were treated with 500 ml of methanol (Aldrich Chemical Company, Milwaukee, WI, USA) for 24 h and the suspension was filtered through two layers of cheese cloth before excess methanol was evaporated in a rotary evaporator (RV 05 Basic 1B; IKA Group, Wilmington, NC, USA) at 32 ± 2°C. The resulting residues were eluted with sufficient acetone to yield a 2% (w/w) plant suspension.

2.3 Electroantennograms

The EAG system and test protocols are described by Stelinski et al. (2003a)). The EAG system used was a data acquisition interface board (Type IDAC-02) and universal single-ended probe (Type PRS-1) from Syntech (Hilversum, The Netherlands). The recording and indifferent electrodes consisted of silver wire in 10 μl glass micropipettes filled with 0.5 M KCl. Data were recorded onto a Gateway 2000 (P-75) computer equipped with an interface card and software (PC-EAG version 2.4) from Syntech. The reference electrode was inserted into the head near the base of the antenna. For each chemical assayed, EAGs were recorded from 10 moths of each sex. Control stimulations (using filter paper impregnated with 20 μl of acetone solvent) were delivered before and after each stimulus presentation. Two puffs of each volatile treatment and control spaced 12 s apart were applied to the antenna to yield duplicate depolarization amplitudes for each replicate moth. The experiment was conducted in a randomized complete block design with chemical and moth sex as factors.

2.4 Olfactometer study

The olfactometer system and test protocols have been described by Gökçe et al. (2005). Male or female grape berry moths used in this study were 1–3 days old. Fifty-five-millimetre-diameter discs were cut from the sticky liners of pheromone traps (LPD Scinturon Guardpost; Suterra, Bend, OR, USA) used for catching Lepidoptera. Prior to insertion in the olfactometer, a 20-mm-diameter Whatman Number 1 filter paper disc, was placed centrally on top of each 55-mm sticky disc, and then transferred into a sterile 90-mm disposable Petri dish. Twenty-five microlitres of each plant extract, diluted in acetone (2% w/w), was applied to the central filter paper disc. In the control treatment, 25 μl of acetone was applied to the disc. In addition to these control discs, the grape berry moth pheromone was also used as a standard because this is an olfactory stimulus known to elicit behaviour in male *P. viteana*. Septa were loaded with 0.5 mg (Z)-9-dodecenyl acetate, lot no. 90441 (Shin Etsu Tokyo, Japan). The reference discs (four plant extracts and the control) were left to dry in a fume hood for 15 min prior to assays. The treated discs were left to dry in a fume hood for 15 min prior to assays. The discs (four plant extracts and the control) and rubber septa with pheromone were transferred to an eight-arm olfactometer (Gökçe et al. 2005) using clean forceps, while the remaining two arms of the olfactometer were left empty. The wheel olfactometer was connected to a vacuum pump set at 100 mmHg, which suctioned clean air into the olfactometer through a hydrocarbon filter. For each replicate, 10 unmated male or 10 unmated female grape berry moth were released into the central release point of the olfactometer. Each replicate was conducted at 24°C under a 16 : 8 h light : dark (L : D) photoperiod. Counts of grape berry moth in each olfactometer arm were made after 24 h. The experiment was repeated on six different days and for statistical analysis, the data were blocked by day.

2.5 Oviposition bioassay

Grapes were prepared by rinsing with a 0.1% bleach solution in deionized water to remove any pesticide and dust residue, then left to dry in a fume hood for 60 min.

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**Table 1.** Plant species and tissue types used in assays measuring the response of grape berry moth

<table>
<thead>
<tr>
<th>Family name</th>
<th>Scientific name</th>
<th>Tissue used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apiaceae</td>
<td>Bifora radians</td>
<td>Leaves</td>
</tr>
<tr>
<td>Asteraceae</td>
<td>Arctium lappa</td>
<td>Leaves</td>
</tr>
<tr>
<td>Asteraceae</td>
<td>Xanthium strumarium</td>
<td>Fruit</td>
</tr>
<tr>
<td>Cannabaceae</td>
<td>Humulus lupulus</td>
<td>Inflorescences</td>
</tr>
</tbody>
</table>
Grapes were subsequently treated by dipping them individually into 20-ml plant-extract suspensions (2% w/w) for 10 s using forceps and drying them in a fume hood for 20 min. Berries from each treatment were transferred into 90-mm-diameter Petri dishes loaded with sterile Whatman filter paper until their use in assays. In the control treatment, a grape was dipped into acetone for 10 s and transferred into Petri dishes.

Bioassays were conducted using plastic 16-oz. bioassay cups 8 cm in height and 11 cm in diameter. Two grapes, one treated with a plant extract and the other from the control treatment, were glued at the bottom of each cup using small drops of hot glue. Ten 1–3-day-old grape berry moth adults (five female : five male) were transferred to each cup and the top of cups were covered with mesh. The cups were incubated at 28 ± 2°C and 16 : 8 h L : D photoperiod for 72 h. The number of individual eggs was counted on each berry and berries were replaced with new ones every 24 h for 3 days. A randomized block design was used in this study, with each block consisting of four treatment–control cups and one control–control cup. The whole experiment was replicated seven times.

2.6 Statistical analyses

Analysis of variance (ANOVA) was conducted on EAG data and differences in pairs of mean values between treatments were separated using Tukey’s multiple comparisons test (SAS Institute 2000). In the olfactometer tests, the number of male or female insects arriving at each treatment arm was expressed as a percentage of the total number of insects tested in each replicate. The resulting preference values for the treatments totalled 100%. The data were arcsine-transformed (Zar 1999) and analysed using ANOVA (Minitab Release 14; McKenzie and Goldman 2005), with a critical P-value of 0.05. Two-sample t-tests (Minitab Release 14; McKenzie and Goldman 2005) were performed to determine whether moth response to plant extracts and pheromone varied significantly between the sexes. For the oviposition choice test, the number of eggs counted on each treatment was presented as a percentage. Within replicates, the cumulative number of eggs laid on each treatment was divided by the total number of eggs laid. The resulting preference values for the treatments totaled 100%. The data were arcsine-transformed (Zar 1999) and subjected to paired t-tests (P = 0.05) (Minitab Release 14; McKenzie and Goldman 2005).

3 Results

3.1 Electroantennograms

The EAG responses of male grape berry moths to pheromone were significantly (F = 14.5, d.f. = 1, 108, P < 0.01) higher than those of females. However, females also showed a significantly higher response to pheromone compared with the control (P < 0.01). The EAG responses of both sexes of grape berry moth to all of the plant extracts tested were significantly higher than control responses (F-values = 9.7, 10.3, d.f. = 5, 108, P < 0.01) (table 2). There were no significant differences between male and female responses to any of the plant extracts tested (F = 1.1 d.f. = 1, 108 P = 0.1). The highest responses, from both males and females, were to extracts of H. lupulus (table 2).

### Table 2. Magnitude of EAG responses by male and female grape berry moths to plant extracts or to pheromone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EAG responses (mV ± SE) upon stimulation with 1 ml of air through stimulus cartridge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>Control</td>
<td>0.42 ± 0.14 d NS</td>
</tr>
<tr>
<td>Pheromone</td>
<td>4.45 ± 0.75 a*</td>
</tr>
<tr>
<td>B. radians</td>
<td>1.74 ± 0.25 a NS</td>
</tr>
<tr>
<td>X. strumarium</td>
<td>1.15 ± 0.24 c NS</td>
</tr>
<tr>
<td>H. lupulus</td>
<td>2.37 ± 0.31 b NS</td>
</tr>
<tr>
<td>A. lappa</td>
<td>1.51 ± 0.24 c NS</td>
</tr>
</tbody>
</table>

*Values within columns followed by the same letter are not significantly different (P < 0.05, Tukey's multiple comparisons test). Paired values within rows marked with an asterisk are significantly different (P < 0.05) and NS indicates lack of significance.

3.2 Olfactometer study

The olfactometer study results support the hypothesis that the plant extracts elicit behavioural effects on male grape berry moth given that there were significant differences in attractiveness among the extracts tested (F = 10.5, d.f. = 5, 25, P < 0.01). Not surprisingly, the highest percentage of male moths was captured by the pheromone treatment (30.0%); this response was similar to that recorded for the H. lupulus extract (25.0%) and there was no significant difference between these two treatments. The X. strumarium extract captured 15.0% of the male moths tested, B. radians and A. lappa extracts captured 11.7% and 13.3% of males, respectively, and the response to these three treatments was not significantly different from that to the control (table 3). The response of female grape berry moth adults to certain plant extracts was also statistically significant (F = 10.2, d.f. = 5, 25, P < 0.01). H. lupulus was the most attractive extract capturing an average 25.0% of the released insects. X. strumarium and A. lappa extracts captured 21.7% and

### Table 3. Percentage of male and female grape berry moths responding to plant extracts or pheromone in an olfactometer

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of moths responding (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>Control</td>
<td>1.67 (0.00–5.36)</td>
</tr>
<tr>
<td>Pheromone</td>
<td>30.00 (14.88–47.07)</td>
</tr>
<tr>
<td>B. radians</td>
<td>1.67 (2.71–25.16)</td>
</tr>
<tr>
<td>X. strumarium</td>
<td>15.00 (4.28–28.98)</td>
</tr>
<tr>
<td>H. lupulus</td>
<td>25.00 (11.22–41.67)</td>
</tr>
<tr>
<td>A. lappa</td>
<td>13.33 (1.40–21.22)</td>
</tr>
</tbody>
</table>

*Untransformed mean values from six replicates.

95% confidence intervals (original data transformed arcsin and subjected to ANOVA, untransformed percentages are presented). Mean values followed by the same letter within columns are not significantly different (P > 0.05). Paired values within rows marked with an asterisk are significantly different (P < 0.01) whereas those marked NS are not.
15.0% of the released moths, respectively. There was no significant difference in percentage of attraction among *H. lupulus*, *X. strumarium* and *A. lappa* (table 3). Compared with the control, there was no significant increase in capture for the sex pheromone or *B. radians* treatments (table 3).

The behavioural responses of female and male grape berry moths to the tested stimuli were similar except for that to the pheromone treatment and the *B. radians* extract (table 3). Significantly more males were attracted to the pheromone treatment than females (*t* = 3.82, d.f. = 10, *P* < 0.05). Significantly fewer female moths (0.2%) were captured in the *B. radians* treatment than males (11.5%) (*t* = 4.98, d.f. = 10, *P* < 0.05). Both sexes showed similar behavioural responses in the olfactometer test to *H. lupulus, A. lappa* and the control, and there was no significant difference between sexes in their preference for these treatments.

### 3.3 Oviposition assay

All the tested plant extracts reduced the number of eggs laid on grapes by female grape berry moth (fig. 1). The most pronounced effect was observed for *B. radians* given that only 0.08% of eggs were laid on berries treated with this extract; this was significantly lower than that on the untreated grapes (99.9%) (*t* = −26.3, d.f. = 6, *P* < 0.01). Grape berry moth females deposited significantly fewer eggs on grapes treated with *H. lupulus* extract (12.5%) than on untreated grapes (87.5%) (*t* = −3.05, d.f. = 6, *P* < 0.01). Although there was also a reduction in the percentage of eggs deposited on *X. strumarium* (20.7%) and *A. lappa* (27.8%), these treatments were not significantly different from the untreated control (fig. 1).

### 4 Discussion

Both sexes of grape berry moth responded to the non-host plant extracts in the EAG assays and in the olfactometer, exhibiting attraction to many of the extracts. *H. lupulus* was the most attractive plant extract to both sexes in the olfactometer. Flavonoids, alpha- and beta-acids, and essential oils have been isolated from *H. lupulus* (Hermans-Lokkerbol et al. 1997; Stevens et al. 1997; De Cooman et al. 1998) and some of these chemicals elicit behavioural responses in other arthropods (Jones et al. 1996, 2003; Gökte et al. 2005). In addition, some plant volatiles are perceived in a synergistic manner, and employed by insects to find mates and increase reproduction (Reddy and Guerrero 2004) and interactions between pheromones and plant volatiles have been observed in the laboratory and field (Dickens et al. 1993; Light et al. 1993; Reddy and Guerrero 2000). *H. lupulus* extract alone or as a mixture with grape berry moth pheromone may improve monitoring tactics of this destructive grape pest as part of an integrated pest management (IPM) strategy, particularly within vineyards treated with sexpheromone mating disruption (Knight and Light 2005; Dickens 2006). The antennae of both sexes of grape berry moth responding by EAG to each of the plant extracts tested, suggesting that although this moth is a specialist on *Vitis* spp. vines, these non-hosts may produce similar or common volatiles. Given that an EAG records a summed response from activated sensillae across the entire antenna, an absolute correlation between an EAG and strength of behavioural response should not be expected. Moreover, it is unknown whether this antennal response was an indicator of stimulatory or inhibitory effects on behaviour. It is interesting that the behavioural response of male grape berry moths to pheromone and *H. lupulus* was equivalent in the olfactometer, despite a higher EAG response to the former compound. This is probably indicative of a lower antennal detection threshold for pheromone relative to the *H. lupulus* extract. This difference in antennal detection threshold between these two treatments was not observed in the context of the multi-choice olfactometer. Perhaps the concentration of pheromone tested in this behavioural assay was higher than optimally attractive relative to the concentrations of plant extracts tested. There was no evidence of sexual dimorphism in antennal response to plant

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**Fig. 1.** Percentage of eggs oviposited by female grape berry moth, *Paralobesia viteana*, on various plant extracts (black bars) and solvent controls (white bars) in choice tests. Paired bars with an asterisk are significantly different (*P* < 0.05; paired *t*-test)
extracts in our study, suggesting that perception of non-hosts is important for both sexes. Tortricids are known to respond electrophysiologically and behaviourally to both green leaf and host-fruit volatiles (Stelinski et al. 2003b; Ansebo et al. 2004). Thus, it is possible that green-leaf volatile constituents of the plant extracts tested in the current study were responsible for eliciting behavioural and antennal activity. Future studies are planned to identify the active volatile constituent(s) of the plant extracts tested in the current study.

In the current oviposition assays, *B. radians* extracts almost completely inhibited egg laying by female grape berry moths. Similar effects of this plant extract were observed on the obliquebanded leafroller, *Choristoneura rosaceana* (Harris), females as they also did not lay eggs on *B. radians*-treated wax paper (Gökçe et al. 2005). *H. lupulus* deterred oviposition but was attractive to females in the olfactometer, suggesting that gustatory detection is required before *P. viteana* is deterred by this extract. Alternatively, it is possible that the behavioural responses of grape berry moth to *H. lupulus* were context-dependent and changed as a function of mating status given that unmated moths were tested in the olfactometer while mated moths were tested in the oviposition assays.

In contrast to *H. lupulus*, *B. radians* was not attractive to female grape berry moths in the olfactometer and it also deterred oviposition. *B. radians* produces and releases many secondary plant volatiles, giving it a distinctive odour (Latrasse et al. 1991; Baser et al. 1998) and is associated with fruit orchard systems deterring many mammals from feeding on its leaves including cows and sheep, and arthropods (A. Gökçe pers. obs.). It is also toxic to important insect pest species (Pons 2004; Gökçe et al. 2006). Given the results described here, extracts of this plant could be useful for ‘push-pull’ IPM strategies (Miller and Cowles 1990). Specifically, *B. radians* extract applied to *Vitis* vines may have potential as an oviposition deterrent for female grape berry moth.

There remains a significant need for effective and economic strategies to reduce the pest status of grape berry moth in North America. Recurrent use of neurotoxic insecticides has led to the development of resistance in many arthropods (Zhao et al. 2000; Nauen and Denholm 2005), and recent reports have shown that grape berry moth has developed varying levels of resistance to carbaryl in Pennsylvania vineyards (Nagarkatti et al. 2002). Delaying or preventing resistance depends on the availability of control tactics that operate through different modes of action. In this study, behavioural response was examined in two ways, and the results indicate that although non-host extracts may be attractive to adult moths (table 3), these same extracts can disrupt oviposition by this specialist herbivore (fig. 1). If deployed under field conditions, prevention of oviposition is expected to decrease the likelihood of crop infestation. Both deterring female grape berry moths from oviposition on grape clusters and developing attractive kairomonal lures for both sexes (Knight and Light 2005) would exploit behavioural, rather than neurological, targets.

Reduction of egglaying on vines would be expected to have a further benefit of minimizing the disease and yield losses associated with infestations. Field trials are planned to determine the degree of: (1) vine protection from ovipositing females and (2) attractiveness of extracts as monitoring lures afforded by the most promising candidate non-host extracts identified in this study.

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**References**


