

# Electroantennogram Responses of Both Sexes of Grape Root Borer (Lepidoptera: Sesiidae) to Synthetic Female Sex Pheromone

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**ABSTRACT** The effect of mating status and sex on antennal response to sex pheromone components was tested in the grape root borer, *Vitacea polistiformis* (Harris). The grape root borer pheromone is a 99:1 blend of (E,Z)-2,13-octadecadien-1-ol acetate (EZ) and (Z,Z)-3,13-octadecadien-1-ol acetate (ZZ). Antennae of both virgin male and female moths exhibited similar dose-response patterns, with a threshold dosage of 1  $\mu$ g of the EZ:ZZ blend on filter paper. Four treatments were tested at a dose of 5  $\mu$ g on filter paper: EZ, ZZ, a blend of 99:1 EZ:ZZ, and the alcohol (E,Z)-2,13-octadecadien-1-ol (EZ-OH). Male antennae responded significantly to all compounds, regardless of mating status. Virgin female antennae responded significantly to all compounds, although their responses were significantly lower than the responses of male antennae. However, female antennae were relatively more responsive to EZ-OH than male antennae. Mated female antennae responded significantly to EZ and 99:1 EZ:ZZ. Pheromone detection by females has considerable implications to mating disruption strategies.

**KEY WORDS** *Vitacea polistiformis*, female behavior, pheromone, pheromone autodetection

THE GRAPE ROOT borer, *Vitacea polistiformis* (Harris), is one of the most destructive pests of grapes in North Carolina (Sorensen 1987). Chlorpyrifos treatments have provided effective control of this pest in the southern United States (All et al. 1982). However, high clay soil content in the Carolina Piedmont and different crop phenology, which affects the postspray harvest interval, make this chemical method unacceptable in North Carolina. Use of grape root borer sex pheromone for mating disruption is under consideration as an alternative management strategy.

The grape root borer pheromone is a 99:1 blend of (E,Z)-2,13-octadecadien-1-ol acetate (EZ) and (Z,Z)-3,13-octadecadien-1-ol acetate (ZZ) (Schwartz et al. 1983, Snow et al. 1987). Observations of female moths in mating disruption plots indicated that female grape root borers changed their premating behavior during exposure to their synthetic pheromone in the field by delaying female calling and increasing dispersive movements (Pearson 1992, Pearson and Meyer 1996). Additionally, the grape root borer population shifted to the upwind side of pheromone-treated vineyards, suggesting that female calling, oviposition, or both shifted to the area of least pheromone concentration (Pearson 1992). Mated grape root borer females have also been observed flying from untreated to pheromone-treated areas (Johnson et al. 1986). These observations suggest that the females' behaviors were modified by conspecific pheromone.

Electroantennogram assays were undertaken to determine if female grape root borers were capable of detecting their own pheromone and its components.

## Materials and Methods

**Insects.** Adult grape root borers were collected in an untreated vineyard on Unit 1 of the North Carolina Research Station, Raleigh (Wake County). Collection of virgin (0-d-old) moths was ensured by collecting only emerging moths with uninflated wings or newly emerged moths near fresh pupal skins at the base of vines. Mated adults were captured from pairs in copula or females in oviposition flight. Peak oviposition takes place 1 d after mating (Dutcher and All 1978). In August 1994 and 1995, 14 virgin female antennae, 6 mated female antennae, 11 mated male antennae, and 7 virgin male antennae were tested.

**Chemicals.** Synthetic compounds were supplied by J. Klun (USDA, Beltsville, MD) and dissolved in hexane to a concentration of 1  $\mu$ g/ $\mu$ l. Four treatment compounds were used: EZ; ZZ; a blend of 99:1 EZ:ZZ; and the alcohol (E,Z)-2,13-octadecadien-1-ol (EZ-OH). EZ-OH is not a component of the grape root borer pheromone, but when used in mating-disruption field trials it resulted in a significant population reduction (Pearson 1992).

**Electroantennogram.** The electroantennogram apparatus was similar to that described in Roelofs (1984) and modified by Schal et al. (1992). A moth head was removed and supported in wax with its base in saline. A silver-silver chloride indifferent electrode was placed in the saline. The distal tip of the antenna was

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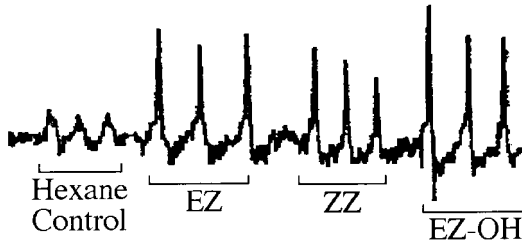


Fig. 1. Sample of EAG deflections recorded with a Chromatopac C-R3A printer. This was a recording from a virgin female grape root borer antennae, showing response to 3 pulses of each of the compounds labeled above.

clipped, removing the last 2–6 segments, and the antenna inserted into a saline-filled Pasteur pipette coupled to a silver-silver chloride-recording electrode. The preparation was placed in a continuous stream of clean, humidified air.

The solvent control and the test compounds were loaded onto 6-mm triangles of filter paper. After the solvent evaporated, the filter paper was placed in a pipette and used as stimulus 3 successive times. All pheromone dilutions and preparation of stimulus cartridges were done in a fume hood. Each stimulus consisted of a puff of 2 ml of air delivered by syringe through the pipette. A 5- $\mu$ g standard dose of 99:1 EZ:ZZ was used as a reference for the relative responsiveness of the antenna. This dose was chosen because previous work with other sesiids found that it elicited a significant change in DC potential without antennal overloading (Nielson et al. 1977). A dose-response curve was developed for males and females using the 99:1 EZ:ZZ mixture.

Electroantennogram deflections were displayed on a storage oscilloscope and recorded with a Chromatopac (Shimadzu Corp., Kyoto, Japan) C-R3A integrating printer (Fig. 1). For evaluation of EAG responses, the maximum amplitude elicited by a given stimulus and the response to the preceding hexane control were compared in a paired *t*-test (SAS Institute 1985).

### Results and Discussion

Mated female antennae were less responsive to the control hexane puffs than antennae of virgin (0-d-old) females ( $t = 2.51$ ,  $df = 17$ ,  $P = 0.02$ ). Because mated females were older than virgin females, we could not conclusively attribute their lower responses to mating status and not aging or senescence. However, mating status did not appear to have a significant effect on the responses of male antennae to control stimuli (Fig. 2). Virgin female and male antennae did not differ significantly in their responses to the hexane control, but the EAG responses of mated female and male antennae differed significantly ( $t = 3.82$ ,  $df = 15$ ,  $P = 0.0017$ ). These results suggest that the sensory acuity of mechanoreceptors that respond to wind stimuli (air puffs) might decline after mating in females, but not

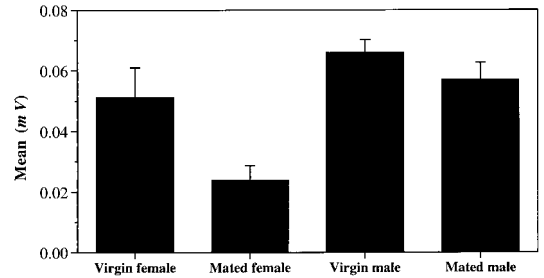


Fig. 2. Response of male and female grape root borer antennae to blank controls (5  $\mu$ l hexane on filter paper reduced to dryness).

in males. These observations fit with known grape root borer behavior; females generally mate only once, and oviposit 80% of their egg load on the 1st d after mating (Dutcher and All 1978). Males may mate more than once.

Dose-response studies showed that both virgin male and female antennae had pheromone response thresholds of  $\approx 1 \mu$ g of the 99:1 EZ:ZZ blend on filter paper (Fig. 3). However, the peak amplitude of the male EAG response was >4 times higher. Responses of virgin male antennae were significantly greater than controls for EZ ( $t = 3.74$ ,  $df = 6$ ,  $P = 0.009$ ), ZZ ( $t =$

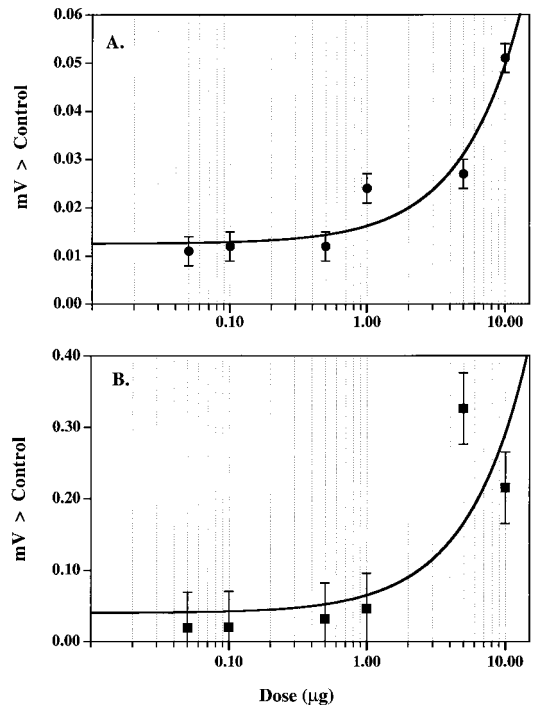


Fig. 3. Dose-response curve for the grape root borer to EZ:ZZ pheromone blend. Responses to control stimuli were subtracted from responses to pheromone. (A) Response of virgin female antennae. (B) Response of virgin male antennae.

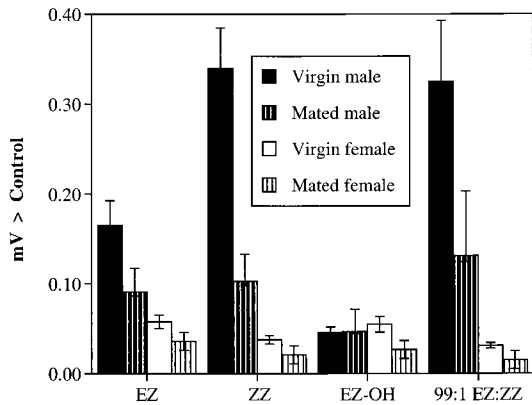


Fig. 4. Electroantennogram responses of antennae of male and female grape root borer to 5  $\mu$ l of test compounds and blends applied to filter paper. Responses to control stimuli were subtracted from responses to pheromone.

6.74,  $df = 6$ ,  $P = 0.0005$ ), EZ-OH ( $t = 4.77$ ,  $df = 6$ ,  $P = 0.003$ ), and for the EZ:ZZ blend ( $t = 6.02$ ,  $df = 6$ ,  $P = 0.002$ ) (Fig. 4). Antennae of mated males also responded significantly to the same compounds ( $t = 8.78$ ,  $df = 10$ ,  $P = 0.0001$ ;  $t = 8.29$ ,  $df = 10$ ,  $P = 0.0001$ ;  $t = 3.55$ ,  $df = 10$ ,  $P = 0.005$ ; and  $t = 3.15$ ,  $df = 6$ ,  $P = 0.025$ , respectively). However, for all compounds and combinations except EZ-OH, the responses of virgin male antennae were significantly greater than responses of mated males. In some lepidopterans (Seabrook et al. 1987) and cockroaches (Liang and Schal 1990), the peripheral olfactory system of males requires a day or more to mature. In grape root borers, both male and female moths usually mate within 4–6 h of emergence from the pupa (Dutcher and All 1978, Pearson and Meyer 1996), therefore 0-d-old virgin males might be expected to have high sensory acuity. The lower antennal responsiveness of mated males may thus be related to mated status, age, or both.

Pheromone component overlap in the Sesiidae is well documented, along with cross-species attraction (Nielson and Balserton 1974, Dutcher and All 1978, Snow et al. 1989, Snow et al. 1991). Virgin males exhibited greater EAG responses to ZZ, the minor component of the female sex pheromone, than to the main component, EZ (Fig. 4). The pheromone overlap of sympatric species may mean that the ZZ component functions as a species recognition mechanism in the grape root borer, signaling that the male is approaching a member of the proper species. Snow et al. (1987) proposed that the ZZ component of the grape root borer pheromone functions as a short-range attractant.

The antennae of virgin females also responded significantly to all compounds (Fig. 4). Responses were significantly higher than to controls for EZ ( $t = 7.02$ ,  $df = 13$ ,  $P = 0.0001$ ), ZZ ( $t = 5.02$ ,  $df = 13$ ,  $P = 0.0002$ ), EZ-OH ( $t = 6.30$ ,  $df = 13$ ,  $P = 0.0001$ ), and to the 99:1 EZ:ZZ blend ( $t = 4.92$ ,  $df = 12$ ,  $P = 0.0005$ ). Mated female antennae responded significantly only to EZ ( $t = 8.13$ ,  $df = 5$ ,  $P = 0.0005$ ) and the EZ:ZZ blend ( $t = 4.71$ ,  $df = 5$ ,  $P = 0.009$ ) (Fig. 4).

Whether sesiid females directly respond to the pheromone information received by their antennae is unclear, but field observations of virgin female grape root borers suggest that this is likely (Pearson 1992). Females exposed to synthetic pheromone treatments called at different heights, moved less before call initiation and moved more after call initiation than control females. Pheromone gland dragging and wing fanning also increased significantly during pheromone treatments. By monitoring the major *V. polistiformis* pheromone component, females would gain information about intrasexual competition. Changes in calling initiation times and reduced movements before calling in pheromone treatments might be explained as grape root borer females waiting out intraspecific signaling competition.

Although autodetection of pheromone has been reported in several lepidopterans, primarily noctuids, tortricids, and arctiids (see review by Schneider et al. 1998), few studies have documented behavioral responses by females to their own pheromones. Several moth species have been shown to alter calling and oviposition patterns in the presence of conspecific female pheromone (Noguchi and Tamaki 1985, Palaniswamy and Seabrook 1985, Weissling and Knight 1996). Female pheromone is known to act as a dispersal trigger for female spruce budworm moths (Palaniswamy and Seabrook 1978, Sanders 1987) and *Ephestia kuehniella* (Zeller), a stored product pest (Trematerra and Battaini 1987). Saad and Scott (1981) found female pheromone acted as a repellent in 2 *Heliothis* species. Low-dose pheromone treatments for monitoring of *Melittia cucurbitae* Harris (Lepidoptera: Sesiidae), resulted in increased infestations, suggesting that the female pheromone might function as a female attractant (Pearson 1995).

Further investigation is warranted into the effects of pheromones on dispersal of conspecific females that perceive them, including the grape root borer. Any environmental pressure that reduces mating success, such as inter- or intraspecific pheromone competition, creates the potential for selection of behaviors that increase individuals' reproductive potential (Lundberg and Löfstedt 1987). There may be a risk of females moving out of an area of high pheromone concentration created by a mating disruption treatment, mating, and then returning to the treated area to oviposit.

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#### References Cited

- All, J. N., M. C. Saunders, and J. P. Dutcher. 1982. Soil treatment with chlorpyrifos for control of first instar grape borers. *Insectic. Acaric. Tests* 7: 37.

- Dutcher, J. D., and J. N. All. 1978. Reproductive behavior of *Vitacea polistiformis* Harris. *J. Ga. Entomol. Soc.* 13: 59–63.
- Johnson, D. T., J. R. Meyer, and R. L. Mayes. 1986. Evaluation of Hercon laminated dispensers baited with Z,Z-3,13-octadecadien-1-ol acetate for suppression of the grape root borer, *Vitacea polistiformis* (Harris) (Lepidoptera: Sesiidae), populations in grapes. *J. Entomol. Sci.* 21: 231–236.
- Liang, D., and C. Schal. 1990. Circadian rhythmicity and development of the behavioural response to sex pheromone in male brown-banded cockroaches. *Physiol. Entomol.* 15: 355–361.
- Lundberg, S., and C. Löfstedt. 1987. Intra-specific competition in the sex communication channel: a selective force in the evolution of moth pheromones? *J. Theor. Biol.* 125: 15–24.
- Nielson, D. G., and C. P. Balesrston. 1974. Evidence for intergeneric sex attraction among aegeriids. *Ann. Entomol. Soc. Am.* 66: 227–228.
- Nielson, D. G., F. F. Purrington, and G. F. Shambaugh. 1977. EAG and field responses of sesiid males to sex pheromones and related compounds, pp. 11–26. *In* Pheromones of the Sesiidae. U.S. Dep. Agric. Res. Serv. 6.
- Noguchi, H., and Y. Tamaki. 1985. Conspecific female sex pheromone delays calling behavior of *Adoxopyes* sp. and *Homona magnaima* (Lepidoptera: Tortricidae). *Jpn. J. Appl. Entomol. Zool.* 29: 113–118.
- Palanaswamy, P., and W. D. Seabrook. 1978. Behavioral responses of the female eastern spruce budworm *Choristoneura fumiferana* (Lepidoptera, Tortricidae) to the sex pheromone of her own species. *J. Chem. Ecol.* 4: 649–655.
- Palaniswamy, P., and W. D. Seabrook. 1985. The alteration of calling behavior by female *Choristoneura fumiferana* when exposed to synthetic sex pheromone. *Entomol. Exp. Appl.* 37: 13–16.
- Pearson, G. 1992. Pheromone effects on mating success and female behavior in the grape root borer. Ph.D. dissertation, North Carolina State University, Raleigh.
- Pearson, G. 1995. Sesiid pheromone increases squash vine borer (Lepidoptera: Sesiidae) infestation. *Environ. Entomol.* 26: 1627–1632.
- Pearson, G. A., and J. R. Meyer. 1996. Female grape root borer (Lepidoptera: Sesiidae) mating success under synthetic sesiid sex pheromone treatment. *J. Entomol. Sci.* 31: 323–330.
- Roelofs, W. R. 1984. Electroantennogram assays: rapid and convenient screening procedures for pheromones, pp. 131–159. *In* H. E. Hummel and T. A. Miller [eds.], *Techniques in pheromone research*. Springer, New York.
- Saad, D., and D. R. Scott. 1981. Repellency of pheromones released by females *Heliothis armigera* and *H. zea* to females of the species. *Entomol. Exp. Appl.* 30: 123–127.
- Sanders, C. J. 1987. Flight and copulation of female spruce budworm in pheromone-treated air. *J. Chem. Ecol.* 13: 1749–1758.
- SAS Institute. 1985. SAS user's guide: statistics, version 5.0. SAS Institute, Cary, NC.
- Schal C., D. Liang, L. K. Hazarika, R. E. Charlton, and W. L. Roelofs. 1992. Site of pheromone production in female *Supella longipalpa* (F.) (Diptera: Blattellidae): Behavioral, electrophysiological and morphological evidence. *Ann. Entomol. Soc. Am.* 85: 605–611.
- Schneider, D., S. Schulz, E. Priesner, J. Ziesmann, and W. Francke. 1998. Autodetection and chemistry of female and male pheromone in both sexes of the tiger moth *Panaxia quadripunctaria*. *J. Comp. Physiol. A* 182: 153–161.
- Schwartz, M., J. A. Klun, B. A. Leonhardt, and D. T. Johnson. 1983. (E,Z)-2,13-octadecadien-1-ol acetate: a new pheromone structure for moths. *Tetrahedron Lett.* 24: 1007–1010.
- Seabrook, W. D., C. E. Linn, L. J. Dyer, and H. H. Shorey. 1987. Comparison of electroantennograms from female and male cabbage looper moths (*Trichoplusia ni*) of different ages and for various pheromone concentrations. *J. Chem. Ecol.* 13: 1443–1453.
- Sorensen, K. 1987. Insects reported on grapes in North Carolina. N.C. Agric. Ext. Serv. Fruit Note G-2.
- Snow, W. J., M. Schwartz, and J. A. Klun. 1987. The attraction of the grape root borer, *Vitacea polistiformis* (Harris) (Lepidoptera: Sesiidae) to (E,Z)-2,13 octadecadienyl acetate and the effects of related isomers on attraction. *J. Entomol. Sci.* 4: 371–374.
- Snow, J. W., M. Schwarz, and T. D. Eichlin. 1989. Captures of clearwing moths (Lepidoptera: Sesiidae). *Environ. Entomol.* 18: 216–220.
- Snow, W. J., D. T. Johnson, and J. R. Meyer. 1991. The seasonal occurrence of the grape root borer (Lepidoptera: Sesiidae) in the eastern United States. *J. Entomol. Sci.* 26: 157–168.
- Trematerra, P., and F. Battaini. 1987. Control of *Ephestia kuehniella* Zeller by mass trapping. *J. Appl. Entomol.* 104: 336–340.
- Weissling, T. J., and A. L. Knight. 1996. Oviposition and calling behavior of codling moth (Lepidoptera: Tortricidae) in the presence of codlemone. *Ann. Entomol. Soc. Am.* 89: 142–147.

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