ATTRACTION OF MOTHS (LEPIDOPTERA: ARCTIIDAE, GEOMETRIDAE, NOCTUIDAE) TO ENANTIOMERS OF SEVERAL EPOXYDIENES

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ABSTRACT

Males of four species of moths were captured in traps baited with enantiomers of straight chain epoxydienes in north Florida. The noctuid *Renia salusalis* was captured in traps baited with (3*Z*,6*S*,7*R*,9*Z*)-6,7-epoxy-3,9-nonadecadiene, the geometrid *Probole alienaria* in traps baited with a 1:1 mixture of enantiomers of *cis*-6,7-epoxy-3,9-nonadecadiene, and the noctuid *Zale lunifera* as well as the arctiid *Halysidota tessellaris* in traps baited with (3*Z*,6*Z*,9*R*,10*S*)-9,10-epoxy-3,6-henicosadiene. These compounds may be part of the sex pheromone of females of these 4 species.

Key Words: Insecta, sex attractant, trap, bait, pheromone

RESUMEN

Fueron capturados machos de cuatro especies de polillas en trampas cebadas con enaantiómeros de epoxydienos de cadena recta en el norte de la Florida. El noctuido

Renia salusalis fue capturado en trampas cebadas con (3Z,6S,7R,9Z)-6,7-epoxy-3,9-nonadecadiene, el geométrido *Probole alienaria* en trampas cebadas con una mezcla 1:1 de enantiómeros de *cis*-6,7-epoxy-3,9-nonadecadiene, y el noctuido *Zale lunifera* así como el arctiido *Halysidota tessellaris* en trampas cebadas con (3Z,6Z,9R,10S)-9,10-epoxy-3,6-henicosadiene. Estos compuestos pueden ser parte de la feromona sexual de las hembras de estas 4 especies.

A number of triene hydrocarbon compounds and their monoepoxide derivatives are female sex pheromones, and/or function as sex attractants, for a number of Geometridae, Noctuidae, and Arctiidae. Many species of moths are known to be attracted to these compounds through screening tests designed to document the capture of attracted moths in baited traps. For example, Wong et al. (1985) captured 17 species of Geometridae and 9 species of Noctuidae in traps baited with mixtures of C_{18} to C_{22} (3*Z*,6*Z*,9*Z*)-triene hydrocarbons and corresponding epoxydienes derived from these compounds. Subsequent studies by Millar et al. (1990a,b,c), which expanded on the studies of Wong et al. (1985), clearly demonstrated the importance of optical isomers on the sexual attractiveness of particular epoxydienes and extended the list of species responding to these compounds and the triene hydrocarbons to over 80 in central Canada alone. Efforts by others in Europe have revealed similar usage of triene hydrocarbons and their monoepoxydiene derivatives as moth sex attractants, primarily within the Geometridae (i.e., Tóth et al. 1991,1992, 1995).

Undoubtedly, comprehensive testing of these compounds in other geographic areas and throughout the seasons of moth activity would reveal a more complete picture of the patterns of activity of these compounds as sex attractants. As a contribution towards this goal, we conducted a screening program to evaluate the attractiveness of a set of epoxydiene derivatives of C17, C19, and C21 triene hydrocarbons.

We report here the capture of four species of moths in traps baited with these compounds in screening trials conducted in north Florida. Enantiomers and 1:1 combinations of enantiomers of 6 compounds were tested in a forest in north-central Florida from early spring to late autumn of 1994.

Materials and Methods

The enantiomers of (3*Z*,9*Z*)-cis-6,7-epoxy-3,9-nonadecadiene were synthesized by Mori & Brevet (1991). Other chemicals were synthesized and purified as reported in Szöcs et al. (1993). All compounds were tested in dosages of 100 micrograms per enantiomer, applied in hexane to 0.5 cm long pieces of 1 cm wide rubber tubing (Taurus, Budapest, Hungary, No., MSZ 9691/6). Prior to usage tubing was extracted 3 times in boiling ethanol for 10 min, then also 3 times overnight in methylene chloride (Tóth et al. 1995). Racemates produced as 1:1 mixtures of enantiomers consisted of 200 ug total material per lure. Lures were wrapped in aluminum foil for shipping and storage and were kept at -10°C until used in field tests.

Seasonal Survey

Lures were placed in Pherocon 1C traps, suspended by wire from the trap roof to within 3 cm of the sticky trap liner. Traps were hung from vines, shrubs and tree branches at about 2 m above ground. Two linear blocks of traps were set up in late

February of 1994, with each block comprised of one trap for each of the nine treatments. These were 1) (3*R*,4*S*,6*Z*,9*Z*)-3,4-epoxy-6,9-heptadecadiene (*Z*6*Z*9-3*R*4*S*-epo-17Hy), 2) (3*S*,4*R*,6*Z*,9*Z*)-3,4-epoxy-6,9-heptadecadiene (*Z*6*Z*9-3*S*4*R*-epo-17Hy), 3)a 1:1 mixture of enantiomers of (6*Z*,9*Z*)-*cis*-3,4-epoxy-6,9-heptadecadiene (*Z*6*Z*9-3,4-epo-17Hy) 4) (3*Z*,6*R*,7*S*,9*Z*)-6,7-epoxy-3,9-nonadecadiene (*Z*3*Z*9-6*R*7*S*-epo-19Hy), 5) 3*Z*,6*S*,7*R*,9*Z*)-6,7-epoxy-3,9-nonadecadiene (*Z*3*Z*9-6*S*7*R*-epo-19Hy) 6) a racemic mixture of enantiomers of (3*Z*,9*Z*)-*cis*-6,7-epoxy-3,9-nonadecadiene (*Z*3*Z*9-6,7-epo-19Hy), 7) (3*Z*,6*Z*,9*R*,10*S*)-9,10-epoxy-3,6-henicosadiene (*Z*3*Z*6-9*R*10*S*-epo-21Hy), 8) (3*Z*,6*Z*,9*S*,10*R*)-9,10-epoxy-3,6-henicosadiene (*Z*3*Z*6-9*S*10*R*-epo-21Hy), and 9) a 1:1 mixture of enantiomers of (3*Z*,6*Z*-*cis*-9,10-epoxy-3,6-henicosadiene (*Z*3*Z*6-9,10-epo-21Hy). Within a block, traps were placed 50 m apart. The two trapping blocks were located in two forested locations in Alachua County, Florida. Plot No. 1 was in an area comprised primarily of second growth hardwood trees. The area of the second plot was primarily planted slash pine (*Pinus elliottii* Engelman). Traps were set up initially in late February and were maintained until November. Traps were checked twice per week and were rotated one position in the plot each week. Trap liners were changed when needed and lures were replaced monthly.

Follow-up Treatment Comparisons

Additional traps were set up at other locations to evaluate further the attractiveness of moths to 3 treatments that showed some activity in the seasonal survey. In these cases, Pherocon 1C traps baited with a treatment were directly compared with unbaited traps, for statistical purposes. Seven pairs of traps were set up in 1994 and 3 pairs of traps were set up in 1995 to further evaluate the attraction of *Probole alienaria* Herrich-Schaeffer to *Z*3*Z*9-6,7-epo-19Hy. These traps were maintained for 1 week during 1994 and for 3 weeks in 1995. Similarly, twelve pairs of traps were set up in 1994 and 3 pair of traps were set up in 1995 to assess the attraction of *Renia salusalis* Walker to *Z*6*Z*9-6*S*7*R*-epo-19Hy. These traps were maintained for 3 days in 1994 and 3 weeks in 1995. Traps in 1995 were checked every 3 days. Three pairs of traps were set up in 1994 and 3 pairs in 1995 to evaluate the attractiveness of (3Z,6Z,9R,10S)-9,10-epoxy-3,6-heneicosadiene to *Halysidota tessellaris* Smith. These traps were monitored for 3 days in 1994, and 3 weeks in 1995 and were checked for captured moths every 3 days. Treatment and control positions for traps were alternated every 3 days.

Mean trap captures for direct comparisons between treatments and unbaited control traps were evaluated by Student's T-test to determine significant differences between treatments. Voucher specimens of *P. alienaria, R. salusalis, H. tessellaris*, and *Zale lunifera* Hübner are in the collection of the first author.

RESULTS

Four species of moths were captured in traps baited with the listed epoxydienes during 1994 and 1995. These were *R. salusalis*, *P. alienaria*, *Z. lunifera*, and *H. tessellaris*.

Fifty-eight male *R. salusalis* were captured in Pherocon 1C traps baited with *Z*3*Z*9-6*S*7*R*-epo-19Hy during the 1994 season-long survey, primarily in March (Fig. 1). No *R. salusalis* were captured in traps baited with any other treatment. Fifty-four of the 58 captured were from the first plot, dominated by hardwood trees, with 4 in the second plot dominated by pine trees. In a direct comparison of unbaited traps and traps baited with *Z*3*Z*9-6*S*7*R*-epo-19-Hy, 4.92 ± 0.98 ($\bar{x} \pm$ SE) male *R. salusalis* were captured per baited trap per 3 days, and no moths were captured in unbaited traps

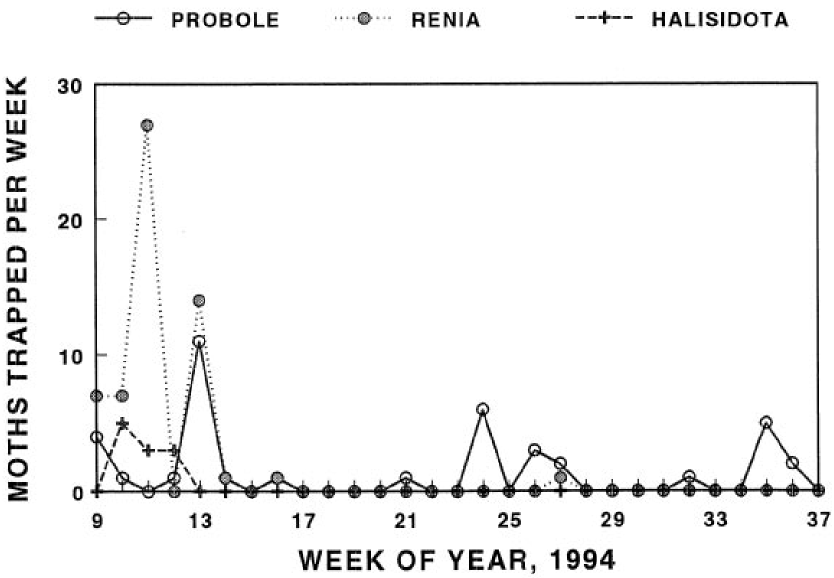

Fig. 1. Numbers of moths captured per week in blocks 1 & 2 combined throughout the 1993 season. Solid line with open circles is for *Probole alienaria*; broken line with plus signs is for *Halysidota tessellaris*; and the dotted line with closed circles is for *Renia salusalis*.

(t=5.0, df=46, p=0.9 x 10^{-5}). Nine additional *R. salusalis* were captured in a Universal moth trap (Unitrap) baited with *Z*3*Z*9-6*S*7*R*-epo-19Hy placed near the first plot during the third week of March in order to obtain voucher specimens.

During the 1994 season-long survey, forty male *P. alienaria* were captured in traps baited with the racemic mixture of *Z*3*Z*9-6,7-epo-19Hy. Fourteen of these moths were captured in plot 1 and 26 were captured in plot 2. This species was captured intermittently throughout the season, with 3 possible generations or flight periods (Fig. 1). None were captured in traps baited with any other treatment. In a test which directly compared numbers of *P. alienaria* in traps baited with a 1:1 mixture of enantiomers of *Z*3*Z*9-6,7-epo-19Hy and unbaited traps, 2.5 ± 0.6 ($\bar{x}$ ± SE) males were captured per treated trap per week compared to 0 moths captured in unbaited traps (t=4.2, df=46, p=0.0001).

Eleven *H. tessellaris* males were captured in traps during the 1994 season-long survey, all in traps baited with *Z*3*Z*6-9*R*10*S*-epo-21Hy, and all were captured in March (Fig. 1). During the subsequent 1995 test which compared captures of *H. tessellaris* in unbaited traps to traps baited with *Z*3*Z*6-9*R*10*S*-epo-21Hy, only 1 *H. tessellaris* male was caught. However, 12 male *Z. lunifera* were caught in this test, all in baited traps and all during March 1994 (t = 2.69, df = 18, p = 0.015). One male Z. *lunifera* was captured during the 1994 season-long survey. This was in March in a trap baited with *Z*3*Z*6-9*R*10*S*-epo-21Hy.

Discussion

The capture of males of these four species of moths, *P. alienaria, R. salusalis*, *Z. lunifera* and *H. tessellaris*, in traps baited with a 1:1 mixture of enantiomers of *Z*3*Z*9-

6,7-epo-19Hy, with *Z*3*Z*9-6*S*7*R*-epo-19Hy and *Z*3*Z*6-9*R*10*S*-epo-21Hy, respectively, indicates that these compounds may function as sex attractants for these species. This is further supported by the failure to capture moths in traps baited with other compounds. In the cases of *R. salusalis*, *P. alienaria* and *Z. lunifera*, sexual attraction was subsequently demonstrated with significant captures of males in baited traps compared to unbaited traps. These compounds may be female-produced sex pheromone components for these species, or they may be structurally related and may mimic the natural pheromones.

There are no previous demonstrations of sexual attraction or reported sex pheromones for *P. alienaria*, which we captured in traps baited with a 1:1 mixture of enantiomers of (3*Z*,9*Z*)-*cis*-6,7-epoxy-3,9-nonadecadiene. A closely related species, *Probole amicaria* Herrich-Schaffer, is attracted to racemic (6*Z*,9*Z*)-*cis*-3,4-epoxy-6,9-nonadecadiene, which is enhanced in attracting *P. amicaria* by the addition of racemic (3*Z*,9*Z*)-*cis*-6,7-epoxy-3,9-nonadecadiene (Millar et al. 1990b). These two species may share at least this one component in their respective sex pheromones. It is not known if the 3,4-epoxynonadecadiene has any effect on sex attraction of *P. alienaria*, but *P. amicaria* did not respond to the 6,7-epoxynonadecadiene when presented alone in field tests (Millar et al. 1990b). The genus *Probole* is placed in the geometrid subfamily Ennominae. A number of other species in this group are attracted to combinations of (3*Z*,6*Z*,9*Z*)-nonadecatriene and structurally-related epoxynonadecadienes (i.e., *Prochoerodes transversata* (Drury) and *Epirrhoe sperryi* (Herbulot) Wong et al. 1985), *Erannis defoliaria* Clerck (Hansson et al. 1990), and *Boarmia selenaria* Schiffermuller (Becker et al. 1983). Also, the female pheromone of *Peribatodes rhomboidaria* (Schiffermuller) is a combination of (Z,Z)-6,9-nonadecadien-3-one and (Z,Z,Z)-3,6,9-nonadecatriene (Buser et al. 1985).

There are no pheromone identifications and no prior demonstrations of sexual attraction of species of the herminiine noctuid genus *Renia*, which responded to (3*Z*,6*S*,7*R*,9*Z*)-6,7-epoxy-3,9-nonadecadiene. However, other species in the Herminiinae are attracted to similar compounds. *Rivula propinquialis* (Guenee) is attracted to the same enantiomer of *Z*3*Z*9-6,7-epo-19Hy as well as to the related triene (Millar et al. 1990). *Bleptina caradrinalis* (Guenee), *Idia americalis* (Guenee), and *Idia aemula* (Hübner) are attracted to (*Z*3,*Z*6,*Z*9)-heneicosatriene (Wong et al. 1985).

This report is the second documentation of a species of *Zale* responding to an epoxide derivative of a triene hydrocarbon. While we trapped *Z. lunifera* with *Z*3*Z*6-9R10*S*-epo-21Hy, Wong et al. (1985) reported the capture of *Zale duplicata* (Bethune) in traps baited with this same compound.

Males of *H. tessellaris* were also caught in traps baited with *Z*3*Z*6-9*R*10*S*-21Hy, and other arctiids are known to be attracted to similar compounds. The salt marsh caterpillar female, *Estigmene acrea* (Drury) produces a sex pheromone comprised in part of (3*Z*,6*Z*,9*S*,10*R*)-9,10-epoxy-3,6-henicosadiene (Hill & Roelofs 1981). A mixture of epoxydienes and epoxytrienes have been identified as possible pheromone components in the glands of female fall webworm adults, *Hyphantria cunea* (Drury) (Tóth et al. 1989; Hill et al. 1982). Another genus of Arctiidae, *Creatonotos*, uses 21 carbon trienes and an epoxy derivative as a sex pheromone (Bell & Meinwald 1986).

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