

Identification of Minor Sex Pheromone Components of the Poplar Clearwing Moth *Paranthrene tabaniformis* (Lepidoptera, Sesiidae)

Raimondas Mozūraitis^{a,b,*} and Vidmantas Karalius^a

^a Institute of Ecology of Vilnius University, Akademijos 2, LT-08412 Vilnius-21, Lithuania

^b Royal Institute of Technology, Department of Chemistry, Organic Chemistry, SE-100 44 Stockholm, Sweden. E-mail: raimis@kth.se

* Author for correspondence and reprint requests

Z. Naturforsch. **62c**, 138–142 (2007); received July 6/21, 2006

A chemical analysis of the crude sex pheromone gland extracts of virgin calling *Paranthrene tabaniformis* females, obtained from the European part of Kazakhstan, revealed the presence of five compounds: (3*E*,13*Z*)-octadeca-3,13-dien-1-ol (*E*3,*Z*13-18:OH), (3*Z*,13*Z*)-octadeca-3,13-dien-1-ol (*Z*3,*Z*13-18:OH), (2*E*,13*Z*)-octadeca-2,13-dien-1-ol (*E*2,*Z*13-18:OH), (13*Z*)-octadec-13-en-1-ol (*Z*13-18:OH), and octadecan-1-ol (18:OH) at the ratios 64.0:32.4:1.4:0.9:1.3, which are structurally related to sex pheromone components of clearwing moths. Our previous field tests showed synergistic effects of *Z*3,*Z*13-18:OH and *E*2,*Z*13-18:OH to attract *P. tabaniformis* males, when these compounds were tested in binary mixtures with the known sex pheromone *E*3,*Z*13-18:OH. The three dienic alcohols should all be considered as sex pheromone components of the *P. tabaniformis* species, while the role of *Z*13-18:OH and 18:OH remained unclear.

Key words: Attraction, Synergist, Octadecadienol

Introduction

The poplar clearwing moth *Paranthrene tabaniformis* (Rottensburg, 1775) is a serious pest of poplars in the Holarctic region (Fibiger and Kristensen, 1974; Solomon, 1979; Du *et al.*, 1985; Georgiev, 2000). Caterpillars of the species bore into shoots and twigs of various *Populus* and occasionally of some *Salix* species and cause losses of planting material in tree nurseries, reduce the growing of young trees and provoke their breaks and decays. The application of conventional pesticides against this pest is limited due to the sheltered mode of life of caterpillars and the close association of the plants with urban areas. The use of sex pheromone-based control methods seems to be highly promising (Du *et al.*, 1985, 1986; Moraal *et al.*, 1993).

Investigations of the sex attractants of poplar clearwing moths were started more than two decades ago. The attractiveness of (3*E*,13*Z*)-octadeca-3,13-dien-1-ol (*E*3,*Z*13-18:OH) for males of this species was demonstrated in North America (Nielsen *et al.*, 1979; Solomon, 1979) and in Europe (Voerman, 1980). Our test, conducted in the European part of Kazakhstan, revealed that the admixture of (3*Z*,13*Z*)-octadeca-3,13-dien-1-ol (*Z*3,*Z*13-18:OH) to *E*3,*Z*13-18:OH in the ratio 1:9 improved the catches of *P. tabaniformis* males as

much as 5 times (Karalius *et al.*, 2001). (2*E*,13*Z*)-octadeca-2,13-dien-1-ol (*E*2,*Z*13-18:OH) was synergistic in binary mixtures with *E*3,*Z*13-18:OH as well. An identification of the sex pheromone of *P. tabaniformis*, performed in China (Zhang *et al.* 1986), showed that only *E*3,*Z*13-18:OH was present in female sex pheromone gland extracts. To solve this discrepancy, a chemical analysis of *P. tabaniformis* female pheromone gland extracts, obtained from insects of the European part of Kazakhstan, was undertaken and is described here.

Materials and Methods

Insects

Pupae of *P. tabaniformis* were collected from various *Populus* species in the European part of Kazakhstan, Kandagash locality, 10 km south of the village Urda, Sajkhin district, on May 29–June 1, 2001. The characteristic plants of the Rynkum sandy-hill steppe were *Populus* sp., *Salix caspica* Pall and *Eleagnus orientalis* L., all growing in hollows. Pupae were kept in individual cages before adult emergence.

Extraction of the sex pheromone glands

In the afternoon, when the females were found calling (Būda *et al.*, 1988), their abdominal tips

containing sex pheromone glands were pushed out mechanically, excised and washed twice with 20 μ l of hexane (Merck, p.a.) for 2 min. The solution was removed and stored at -14°C . In total, the glands of 3 calling females were extracted. Before the analysis, the extract was concentrated to approx. 10 μ l under a fine nitrogen stream.

Chemical analyses

The extract was analyzed by means of a Varian 3400 gas chromatograph, connected with a Finnigan SSQ 7000 mass spectrometer. A DB-5 silica capillary column (J and W Scientific, Folsom, CA, USA; 30 m, 0.25 mm i.d., film thickness 0.25 μm) was used with the following temperature program: 60°C for 4 min, $5^{\circ}\text{C}/\text{min}$ to 160°C , then $1^{\circ}\text{C}/\text{min}$ up to 190°C , then $10^{\circ}\text{C}/\text{min}$ up to 250°C and held isothermally for 11 min. The split/splitless injector temperature was 225°C and the splitless period was 30 s. Helium was used as the carrier gas with an inlet pressure of 70 kPa. Electron ionization mass spectra were determined at 70 eV with an ion source at 150°C . Chromatograms, obtained from the sex pheromone gland extracts, were screened for compounds structurally related to the sex pheromone components, using the diagnostic ions m/z 61 (protonated acetic acid, indicating the presence of acetates), m/z 248, m/z 250 and m/z 252 ($[\text{M}^{+}-18]$, loss of water for octadecadienols, octadecenols and octadecanols, respectively, as well as $[\text{M}^{+}-60]$ loss of acetic acid for octadecadien-1-yl acetates, octadecen-1-yl acetates and octadecan-1-yl acetates, respectively). The com-

pounds selected for analyses were identified by comparison of their mass spectral data and GC retention times with the corresponding data of synthetic standards.

Results and Discussion

Five compounds, structurally related to sex pheromone components of clearwing moths, were detected from sex pheromone gland extracts of virgin females, when GC-MS data were screened by diagnostic ions.

Compounds A and B (Fig. 1) showed a general pattern of fragmentation, typical of 3,13-octadecadien-1-ols, with clear presence of the diagnostic ions m/z 266 and absence of m/z 61 (Figs. 2A, B), when they were compared with the mass spectra presented by the Mass Spectral Library, version 1.7 of the National Institute of Standard and Technology (NIST), USA. Comparison of the retention times of these natural products and synthetic standards revealed, that compound A was (3*E*,13*Z*)-octadeca-3,13-dien-1-ol (3*E*,13*Z*-18:OH) and compound B was (3*Z*,13*Z*)-octadeca-3,13-dien-1-ol (3*Z*,13*Z*-18:OH). The fragmentation patterns of the mass spectra of 3*E*,13*Z*-18:OH and 3*Z*,13*Z*-18:OH, recorded from the extracts by our GC-MS system, corresponded well to the ones of the synthetic standards.

Compound C (Fig. 1) showed a mass spectrum that was very similar to those of A and B. The only important difference in the mass spectrum of C from those of A and B was a clear presence of the diagnostic ion m/z 248 instead of m/z 266 of A and

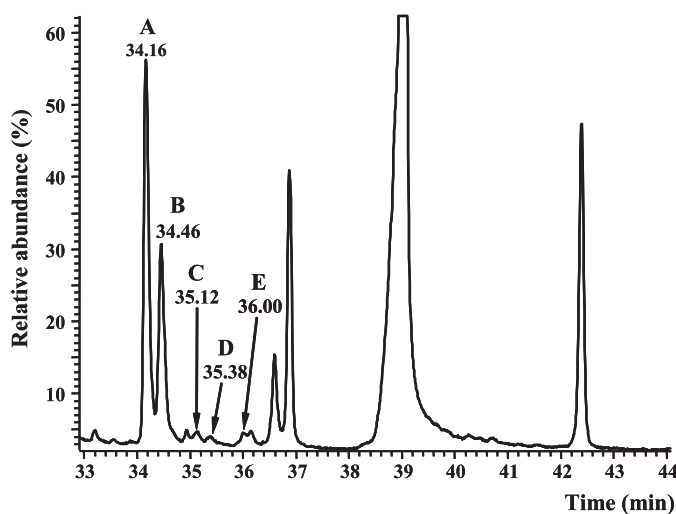


Fig. 1. Total ion chromatogram of extracts obtained from 3 calling virgin *Paranthrene tabaniformis* females in the range m/z 30–400; DB-5 fused silica capillary column (30 m \times 0.25 mm i.d., 0.25 μm film thickness); A, 3*E*,13*Z*-18:OH; B, 3*Z*,13*Z*-18:OH; C, 3*E*,13*E*-18:OH; D, 3*Z*,13*E*-18:OH; E, 18:OH.

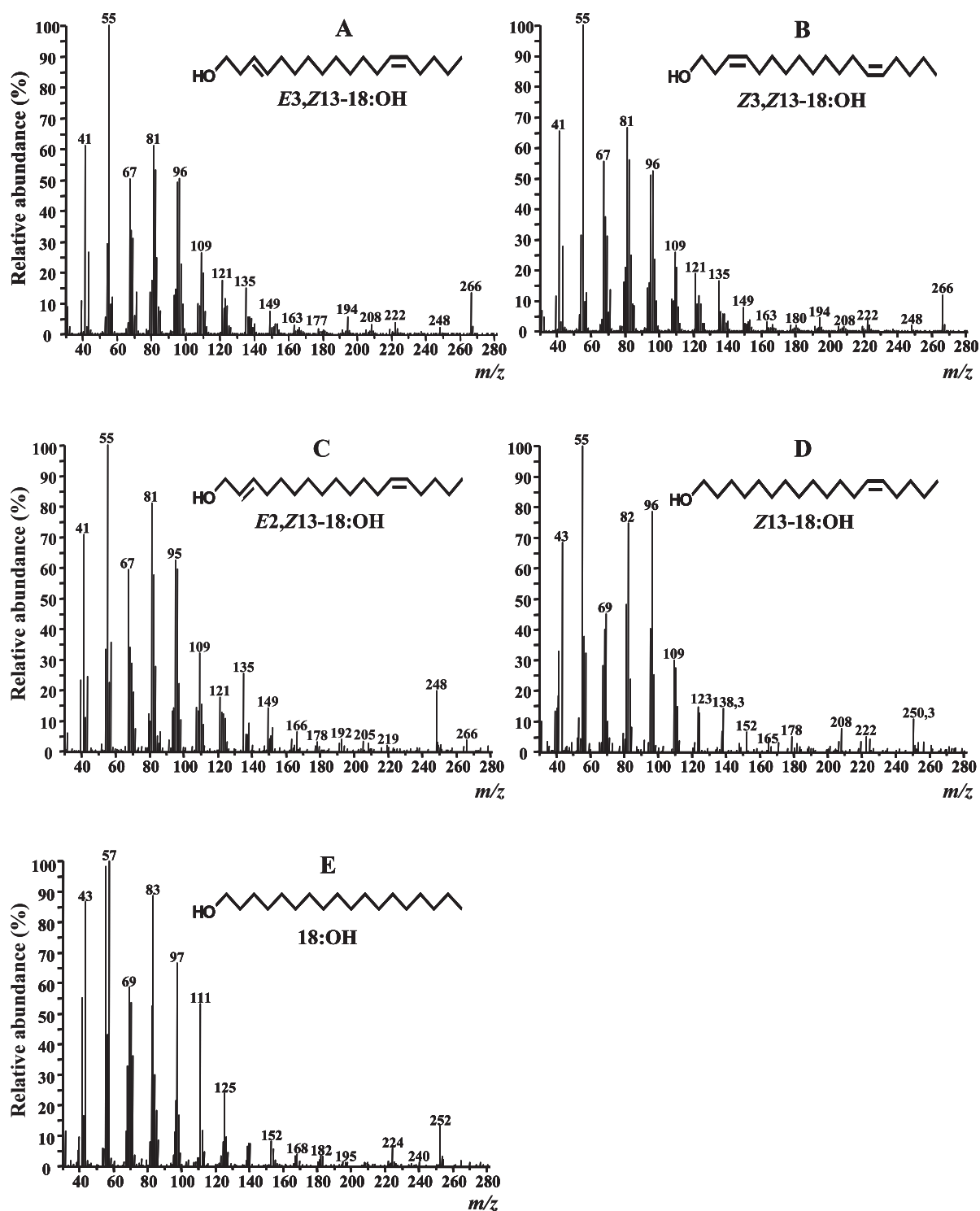


Fig. 2. Mass spectra of five compounds, structurally related to sex pheromone components of clearwing moths, obtained from sex pheromone gland extracts of 3 calling virgin *Paranthrene tabaniformis* females.

B. In addition, the absence of m/z 61 in C as well as in A and B indicated that compound C was 2,13-octadecadien-1-ol (Fig. 2C). A comparison of the retention times of the natural product and the synthetic standards revealed that compound C was (2*E*,13*Z*)-octadeca-2,13-dien-1-ol (*E*2,*Z*13-18:OH). The fragmentation pattern of the mass spectrum of *E*2,*Z*13-18:OH, recorded from the extracts, corresponded well to the one of the synthetic standard.

The general pattern of the fragmentation and occurrence of the diagnostic ion at m/z 250 as well as the absence of m/z 61 in the mass spectrum obtained from compound D (Fig. 1) indicated that the natural product was octadecen-1-ol (Fig. 2D). The stereochemistry and the position of the double bond were determined as *Z*13 by comparison of the retention times of the natural product and the corresponding characteristics of the synthetic standard, indicating that compound D was (13*Z*)-octadec-13-en-1-ol (*Z*13-18:OH).

Our natural product E (Fig. 1) showed a mass spectrum that was very similar to that of octadecan-1-ol (18:OH) presented in the NIST library. Comparisons of the mass spectra and retention times of the natural product and the ones of the synthetic standard confirmed that compound E was octadecan-1-ol.

In conclusion, the percentages of *E*3,*Z*13-18:OH; *Z*3,*Z*13-18:OH; *E*2,*Z*13-18:OH; *Z*13-18:OH and 18:OH from the sex pheromone gland extracts of 3 females were found to be 64.0:32.4:1.4:0.9:1.3.

Sex pheromone identification data confirmed our previous field experiments, in which both *Z*3,*Z*13-18:OH and *E*2,*Z*13-18:OH showed synergistic effects in attraction of *P. tabaniformis* males when these compounds were tested in binary mixtures with the known sex pheromone *E*3,*Z*13-18:OH (Karalius *et al.*, 2001). The three alcohols *Z*3,*Z*13-18:OH, *E*2,*Z*13-18:OH and *E*3,*Z*13-18:OH should be considered as essential sex pheromone components of *P. tabaniformis* species, because they were found in sex pheromone gland extracts of virgin females and demonstrated biological activity towards conspecific males in field tests.

The status and role of the other two compounds, (13*Z*)-octadec-13-en-1-ol (*Z*13-18:OH) and octadecan-1-ol (18:OH), identified from sex pheromone gland extracts of *P. tabaniformis* remained unclear. *Z*13-18:OH was detected in pheromone gland extracts of two other clearwing moth species: *Melittia cucurbitae* (Harris) (Klun *et al.*, 1990) and *Synanthedon tipuliformis* (Clerck) (Mozūraitis *et al.*, 2006). For none of the species, however, any behavioural activities of these compounds were confirmed. As far as we know, an attractiveness of *Z*13-18:OH in a mixture with *E*3,*Z*13-18:Ac at the ratio 1:1 was reported for only one clearwing moth, *Synanthedon affinis* (Staudinger) (Buda *et al.*, 1993). Octadecan-1-ol was found in sex pheromone gland extracts of only one clearwing moth species, *S. tipuliformis*, and its biological function remained unknown (Mozūraitis *et al.*, 2006).

*E*3,*Z*13-18:OH as a single sex pheromone component of *P. tabaniformis* was identified from sex pheromone gland extracts in China (Zhang *et al.*, 1986), and it is most probable, that the minor compounds of the female pheromone remained below the detection level due to the limited sensitivity of the technique that was available at that time.

On the other hand, *P. tabaniformis* is a Holarctic species with a very wide distribution area and the existence of polymorphism or “dialects” in the sex pheromone communication between populations similar to those reported for *Agrotis segetum* (Denis and Schiffermüller) (Löfstedt *et al.*, 1986), *Ostrinia nubilalis* (Hubner.) (Sorenson *et al.*, 2005), *Choristoneura rosaceana* (Harris) (El-Sayed *et al.*, 2003), *Cnaphalocrocis medinalis* (Guenee) (Kawazu *et al.*, 2000) and *Hemileuca eglanterina* (Boisduval) (McElfresh and Millar, 2001) is possible and remains to be investigated.

Acknowledgements

We thank Dr. G. Aulin-Erdtman for valuable discussions on this manuscript, Mr. Jan Miatleuski for help in organizing and conducting the expedition in the European part of Kazakhstan as well as Dr. Marina Lvovna Sizemskaja and Dr. Mamaj Kazievich Sapanov for sheltering our stay there. This study was supported by state grants to the Laboratory of Chemical and Behavioural Ecology at the Institute of Ecology, Vilnius University, Lithuania.

- Būda V., Karalius V., and Tatjanskaitė L. (1988), Behaviour of dusky clearwing females *Paranthrene tabaniformis* Rott. (Lepidoptera, Sesiidae) during pheromone release and localization of pheromone gland. *Khemoretseptsyia nasekomykh.* **10**, 5–14 (in Russian).
- Būda V., Maeorg U., Karalius V., Rothschild G. H. L., Kolonistova S., Ivinskis P., and Mozuraitis R. (1993), C₁₈ dienes as attractants for 18 clearwing (Sesiidae), tineid (Tineidae), and choreutid (Choreutidae) moth species. *J. Chem. Ecol.* **19**, 799–813.
- Du J. W., Xu S. F., Dai X. J., and Zhang X. (1985), Strategies for control of poplar clearwing moth *Paranthrene tabaniformis* Rott. by mass trapping. *Contrib. Shanghai Inst. Entomol.* **4**, 19–24.
- Du J. W., Xu S. F., Dai X. J., and Zhang X. (1986), Field test on controlling poplar clearwing moth *Paranthrene tabaniformis* Rott. by mass trapping. *Contrib. Shanghai Inst. Entomol.* **5**, 53–58.
- El-Sayed A. M., Delisle J., De Lury N., Gut L. J., Judd G. J. R., Legrand S., Reissig W. H., Roelofs W. L., Unelius C. R., and Trimble R. M. (2003), Geographic variation in pheromone chemistry, antennal electrophysiology, and pheromone-mediated trap catch of North American populations of the obliquebanded leafroller. *Environ. Entomol.* **32**, 470–476.
- Fibiger M. and Kristensen, N. P. (1974), The Sesiidae (Lepidoptera) of Fenoscandia and Denmark. *Fauna Entomol. Scand.* **2**, 1–91.
- Georgiev G. (2000), Studies on larval parasitoids of *Paranthrene tabaniformis* (Rott.) (Lepidoptera: Sesiidae) on urban poplars (*Populus* spp.) in Sofia, Bulgaria. *Ann. For. Sci.* **57**, 181–186.
- Karalius V., Mozūraitis R., Miatleuski J., Būda V., and Ivinskis P. (2001), Sex attractants for six clearwing and tineid species (Lepidoptera, Sesiidae and Tineidae) from Kazakhstan and Lithuania. *Z. Naturforsch.* **56c**, 1120–1125.
- Kawazu K., Hasegawa J., Honda H., Ishikawa Y., Wakamura S., Sugie H., Kamiwada H., Kamimuro T., Yoshiyasu Y., and Tatsuki S. (2000), Geographical variation in female sex pheromones of the rice leafroller moth, *Cnaphalocrocis medinalis*: identification of pheromone components in Japan. *Entomol. Exp. Appl.* **96**, 103–109.
- Klun J. A., Schwarz M., Leonhardt B. A., and Cantelo W. W. (1990), Sex pheromone of the female squash vine borer (Lepidoptera: Sesiidae). *J. Entomol. Sci.* **25**, 64–72.
- Löfstedt C., Löfqvist J., Lanne B. S., Van der Pers J. N. C., and Hansson B. S. (1986), Pheromone dialects in European turnip moths *Agrotis segetum*. *Oikos* **46**, 250–257.
- McElfresh J. S., and Millar J. G. (2001), Geographic variation in the pheromone system of the saturniid moth *Hemileuca eglanterina*. *Ecology* **82**, 3505–3518.
- Moraal L. G., Van der Kraan C., and Van der Voet H. (1993), Studies on the efficacy of the sex attractant of *Paranthrene tabaniformis* Rott. (Lep., Sesiidae). *J. Appl. Entomol.* **116**, 364–370.
- Mozūraitis R., Karalius V., Būda V., and Borg-Karlson A.-K. (2006), Inter- and intra-specific activities of compounds derived from sex pheromone glands of the currant borer, *Synanthedon tipuliformis* (Clerck) (Lepidoptera: Sesiidae). *Z. Naturforsch.* **61c**, 278–284.
- Nielsen D. G., Purrington F. F., and Shambaugh G. F. (1979), EAG and field responses of sesiid males to sex pheromones and related compounds. US Department of Agriculture, Science and Education Administration. *Agricultural Research Results ARR-NE.* **6**, 11–26.
- Solomon J. D. (1979), Trapping and biology of *Podosesia* and *Paranthrene* borers. US Department of Agriculture, Science and Education Administration. *Agricultural Research Results ARR-NE.* **6**, 47–54.
- Sorenson C. E., Kennedy G. G., Schal C., and Walgenbach J. F. (2005), Geographical variation in pheromone response of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae), in North Carolina: A 20-Y perspective. *Environ. Entomol.* **34**, 1057–1062.
- Voerman S. (1980), Field experience with a sex attractant for the dusky clearwing moth, *Paranthrene tabaniformis* (Rottemburg) (Lepidoptera, Sesiidae). *Les Colloques de L'INRA 7. Les Pheromones sexuelles et les Mediateurs Chimiques chez les Insectes: Utilisation en Lutie Integree.* Colmar, 25–27 Nov. 1980, pp. 87–90.
- Zhang X. H., Guo G. Z., Lin G. Q., Wu Y. W., Wu P. H., Li Z. Y., and Wei K. N. (1986), Studies on identification and syntheses of insect pheromones. XXII. Sex pheromone of poplar twig clearwing moth *Paranthrene tabaniformis* Rott. – structure and synthesis. *J. Chem. Ecol.* **12**, 1263–1271.