Inter- and Intraspecific Activities of Compounds Derived from Sex Pheromone Glands of Currant Borer, *Synanthedon tipuliformis* (Clerck) (Lepidoptera: Sesiidae)

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Gas chromatography and mass spectrometry analyses of crude sex pheromone gland extracts revealed that virgin *Synanthedon tipuliformis* (Clerck), currant borer (Lepidoptera: Sesiidae) females, produced 6 compounds, structurally related to sex pheromone components of clearwing moths. By comparison of retention times and mass spectra of natural products with corresponding properties of synthetic standards, these compounds were identified as: (2*E*,13*Z*)-octadeca-2,13-dien-1-yl acetate (*E*2,*Z*13-18:OAc), (3*E*,13*Z*)-octadeca-3,13-dien-1-yl acetate (*E*3,*Z*13-18:OAc), (13*Z*)-octadec-13-en-1-yl acetate (*Z*13-18:OAc), (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) in the ratio 100:0.7:2.7:3.2:traces:traces. The first 3 compounds were previously known to occur in the sex pheromone gland extracts of currant borers, while the last 3 chemicals are now reported for the first time. Trapping tests carried out in the black currant field revealed that *E*2,*Z*13-18:OAc, when tested separately, attracted *S. tipuliformis* males, while addition of *E*3,*Z*13-18:OAc to the main component increased the effectiveness of *E*2,*Z*13-18:OAc over seven times. The attractiveness of 6 component lures did not differ significantly from the one of the binary mixture, confirming that *E*2,*Z*13-18:OAc and *E*3,*Z*13-18:OAc in the ratio100:0.7 are essential sex pheromone components of *S. tipuliformis*. Trapping tests carried out at the dwelling place of *Synanthedon scoliaeformis* (Borkhausen) (Lepidoptera: Sesiidae) revealed that, in addition to intraspecific synergistic effect, *E*3,*Z*13-18:OAc increased the specificity of the pheromone signal of *S. tipuliformis*, acting by intraspecific mode as an attraction antagonist against *S. scoliaeformis* males. By this way, it ensured the specificity of the sex attraction signal of the currant borer. Consequently, both compounds *E*2,*Z*13-18:OAc and *E*3,*Z*13-18:OAc have to be present in pheromone formulations used for monitoring and/or control of *S. tipuliformis* to avoid effecting non-target species. Other compounds identified from the sex pheromone gland of *S. tipuliformis* did not show any significant interspecific activity for males of *S. scoliaeformis*, however, they provide a basis to achieve specificity of a pheromone signal of *S. tipuliformis* and could act as attraction antagonists against other clearwing moth species which, like *S. tipuliformis*, employ *E*2,*Z*13-18:OAc as their sex pheromone component.

Key words: Pheromone Specificity, Synergist, Attraction Antagonist

Introduction

The currant borer, *Synanthedon tipuliformis* (Clerck) (Lepidoptera: Sesiidae) is one of the most destructive pests of the cultivated currants *Ribes nigrum* L. and *R. rubrum* L., and gooseberries, *Grossularia urva-crispa* (L.), in Eurasia (Manko, 1965; Yakimova, 1968; Yonghe *et al*., 1990; Szőcs *et al*., 1991; Būda, 1993; Gottwald and Künzel, 1994; Karalius *et al*., 2003), in North America (Solomon and Dix, 1979; Szőcs *et al*., 1998), and in Australia (Scott and Harrison, 1978; Hardy, 1981). *S. tipuliformis* has one generation per year. In Lithuania, adults emerge in June. Females lay eggs on branches especially near bark wounds. About 2 weeks after hatching, caterpillars bore into the branches and develop on the starch-rich pits of currant branches. Larvae of the last stages gnaw out wide tunnels, create large pupation cells and make exit holes for emerging adults, in consequence causing the breaking of currant branches during mechanical harvesting (Hardy, 1981). In addition, microscopic fungi infest

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branches through the exit holes and cause weakening of limbs. Reduced numbers of racemes per branch, fewer flowers per raceme and worse nutrition supplies lead to significant decreases of the yields of berries (Brock *et al.*, 1964; Hardy, 1981; Vazyulya, 1982).

Biological features and life style make this pest difficult to control. The efficacy of chemical pesticides is limited by the short period when the larvae are not protected within the canes and this period often coincides with the harvest period, when pesticide use is prohibited (Grassi *et al.*, 2002). Pheromone use as an alternative control method has been applied for monitoring and mating disruption of *S. tipuliformis* (Carde and Minks, 1995; Grassi *et al.*, 2002).

The sex pheromone of this moth has been identified as a two-component mixture, consisting of (2*E*,13*Z*)-octadeca-2,13-dien-1-yl acetate (*E*2, *Z*13-18:OAc) as a major component (Szőcs *et al.*, 1985) and (3*E*,13*Z*)-octadeca-3,13-dien-1-yl acetate (*E*3,*Z*13-18:OAc) as a minor constituent (James *et al.*, 2001; Suckling *et al.*, 2005). Our preliminary data indicated the presence of additional compounds, structurally related to sex pheromone components of the Sesiidae, in the extract obtained from pheromone glands of *S. tipuliformis* females.

The aim of this study was to determine intra- and interspecific activities of the compounds identified from sex pheromone glands of *S. tipuliformis* females.

Materials and Methods

Insects

Black currant branches containing *S. tipuliformis* pupae were collected at Vilnius University Botanical Garden, Kairėnai near Vilnius (East Lithuania) at the end of May 2000. The branches had been cut into pieces and the parts containing pupae had been transported to the laboratory and placed in glass containers. The temperature regime for the rearing was (22 ± 2) °C during the light part of the day and (18 ± 2) °C during the night, with the 17 h:7 h light/dark natural photoperiod. The glass containers were inspected every morning and emerged unmated adults were sexed, as it was known that high sex pheromone release activity of *S. tipuliformis* females started about 8 h before sunset (Būda and Karalius, 1985). Virgin females were transferred to holding containers containing a solution of 5% honey in water. In addition to moths obtained from the pupae, a number of females were collected by an entomological net in the same black currant plantation during the second half of June.

Extraction of the sex pheromone glands

When the female was found calling, her abdominal tip containing the sex pheromone gland was pushed out under mechanical pressure, excised and washed twice with 10 μl of hexane (Merck, p.a.) for 15 min. The solution was removed, concentrated to approx. 10 μl, and stored at −14 °C. In total, 12 calling females were used for this extraction.

Chemical analyses

The extract was analyzed by using the Finnigan SSQ 7000 GC-MS system, including a Varian 3400 GC instrument. Both DB-5 and DB-wax silica capillary columns (J and W Scientific, Folsom, CA, USA, 30 m, 0.25 mm i.d., film thickness 0.25 μm) were used with a temperature program of 80 °C (4 min), increased by 10 °C/min to 170 °C, then by 2 °C/min up to 210 °C and thereafter held isothermally at 210 °C for 30 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 10 psi. Electron ionization mass spectra were determined at 70 eV with the ion source at 150 °C. Mass chromatograms obtained from the sex pheromone gland extract were screened for compounds structurally related to the sex pheromone components, using diagnostic ions *m/z* 61 (protonated acetic acid, indicating presence of acetates), *m/z* 248, *m/z* 250 and *m/z* 252 ($[M^{+\cdot}-18]$, loss of water for octadecadienols, octadecenols and octadecanols, respectively, as well as $[M^{+\cdot}-60]$, loss of acetic acid for octadecadien-1-yl acetates, octadecen-1-yl acetates and octadecan-1-yl acetates, respectively). The compounds selected for analyses were identified by comparison of their mass spectral data and GC-retention times with the corresponding data from synthetic standards.

Chemicals

The synthetic compounds to be used as GC-MS standards had been obtained from Pherobank, (Wageningen, Netherlands) as well as from Flora Co. (Tartu, Estonia). The chemicals used in the

field tests had been synthesized in Tartu, Estonia, and purified by preparative liquid chromatography, as described by Mozūraitis *et al.* (1998). The isomeric and chemical purities of the compounds exceeded 99%.

Field tests

The synthetic sex pheromone components were dissolved in hexane (Merck, p.a.) and soaked from the inside into the walls of red rubber tube dispensers (8 × 15 mm). The compounds were applied either alone or in mixtures, as indicated in Tables I and II. Each lure was placed in an opaque white delta trap (trapping window sides 10 cm × 11 cm × 10 cm and trap length 18 cm), which had an exchangeable bottom (11 cm × 18 cm), coated with sticky material. ("Atracon A" traps and Pestifix glue were obtained from Flora Co., Tartu, Estonia.) Tests of the attractiveness of synthetic compounds, identified from sex pheromone gland extracts of *S. tipuliformis* females, to conspecific males were carried out in the black currant plantation at Vilnius University Botanical Garden, Kairėnai near Vilnius (East Lithuania) from June 1 to 29, 2001. Each trap was fixed on a black currant branch at ¾ of the shrub height, which is the optimal position for achieving the most abundant catches (Būda and Karalius, 1993), and was inspected and moved to the next trap location (within each replication) every 3 d. The distance between the traps was at least 15 m. Five replicates of each compound and mixture listed in Table I were used.

Bioactivity tests of synthetic compounds identified from sex pheromone gland extracts of *S. tipuliformis* females toward *S. scoliaeformis* males were conducted at the edge of a deciduous forest with birch trees dominant in Visoriai and Žemaitėliai near Vilnius (East Lithuania). Traps were fixed on branches of the shrubs growing close to birch trees about 2 m above ground and were inspected and moved to the next trap location (within each replication) every 3 d. The distance between the traps was at least 15 m. Five replicates of each compound and mixture listed in Table II were used.

Identification of moth species

The moths captured were identified through their external morphological characters. Representative specimens were deposited in the insect collection at the Institute of Ecology, Vilnius, Lithuania.

Statistical analyses

Data from the field tests were analyzed by nonparametric Kruskal-Wallis (Sokal and Rohlf, 1995) analyses of variance, followed by Mann-Whitney U-test (Sokal and Rohlf, 1995) and significantly different catches were marked with different letters at $P < 0.05$.

Results

Chemical analysis of the sex pheromone glands extract

Six 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 II and IV (Fig. 1A) showed a clear presence of the diagnostic ions *m/z* 248 and *m/z* 61, typical of octadecadien-1-yl acetates (Fig. 1B). Comparison of the retention times of natural products and synthetic standards on two capillary columns of different polarities revealed compound II as (3*E*,13*Z*)-octadeca-3,13-dien-1-yl acetate (*E*3, *Z*13-18:OAc) and compound IV as (2*E*,13*Z*)-octadeca-2,13-dien-1-yl acetate (*E*2,*Z*13-18:OAc). Fragmentation patterns of mass spectra of *E*3,*Z*13-18:OAc and *E*2,*Z*13-18:OAc, recorded from the extracts, corresponded well to the ones of synthetic standards.

Compound VI showed a mass spectrum that was very similar to those of II and IV. The complete absence of diagnostic ions at *m/z* 61 (Fig. 1B) suggested that compound VI was octadecadienol. Synthetic (2*E*,13*Z*)-octadeca-2,13-dien-1-ol (*E*2,*Z*13-18:OH) had the same retention time as compound VI and the two compounds showed identical mass spectra.

The occurrence of diagnostic ions at *m/z* 250 in mass spectra obtained from compounds I and V as well as the presence of *m/z* 61 only in the mass spectrum of compound I indicated that the natural products I and V were octadecen-1-yl acetate and octadecenol, respectively (Fig. 1B). The stereochemistry and the position of the double bond in both compounds were determined as *Z*13-, by comparison of retention times of natural products and corresponding characteristics of synthetic

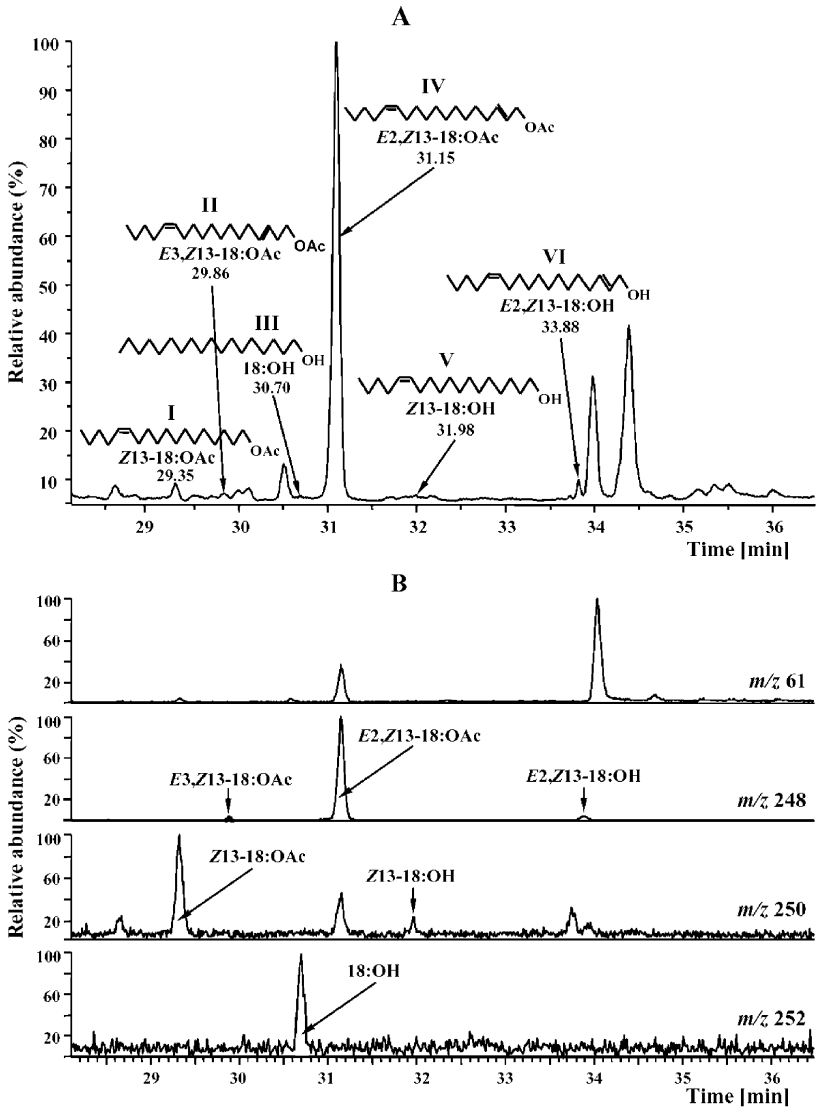

Fig. 1. Total and selected ion chromatogram records of extracts obtained from 6 virgin calling *Synanthedon tipuliformis* females. (A) Total ion chromatogram in the range *m/z* 30–400; polar DB-Wax fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). (B) Selected ion chromatograms, with *m/z* 61 ion fragments indicating the presence of acetates; *m/z* 248, *m/z* 250 and *m/z* 252 are the molecular ions characteristic of octadecadienols, octadecenols, octadecanols and their acetates, respectively.

standards, indicating that compounds I and V were (13*Z*)-octadec-13-en-1-yl acetate (*Z*13-18:OAc) and (13*Z*)-octadec-13-en-1-ol (*Z*13-18:OH), respectively.

Compound III showed a mass spectrum that was very similar to that of octadecan-1-ol (18:OH), presented by the Mass Spectral Library, version 1.7 of National Institute of Standard and Technology, USA. Comparisons of the mass spectra and retention times of the natural product and the ones of the synthetic standard confirmed that compound III was octadecan-1-ol.

In conclusion, *E*2,*Z*13-18:OAc, *E*3,*Z*13-18:OAc, *Z*13-18:OAc, *E*2,*Z*13-18:OH, *Z*13-18:OH and 18:OH in the ratio 100:0.7:2.7:3.2:traces:traces were determined from the sex pheromone gland extracts of 6 females.

Field trapping

Bioassay tests of synthetic compounds, identified from sex pheromone gland extracts of virgin *S. tipuliformis* females, revealed that only the main component *E*2,*Z*13-18:OAc was attractive to conspecific males, when two sex pheromone components were tested alone under field conditions (Table I). Addition of *E*3,*Z*13-18:OAc to the main component increased the effectiveness of *E*2,*Z*13-

Table I. Attraction of *Synanthedon tipuliformis* and *S. scoliaeformis* males in a black currant plantation to synthetic compounds identified from sex pheromone gland extracts of *S. tipuliformis* females.

Compound	Amount	Males per trap (mean ± SD)	
	[μg]	*S. tipuliformis*	*S. scoliaeformis*
*E*2,*Z*13-18:OAc	280	2.8 ± 1.5 b	1.6 ± 1.1 a
*E*3,*Z*13-18:OAc	2.3	0 ± 0	0 ± 0
*E*2,*Z*13-18:OAc + *E*3,*Z*13-18:OAc	280 + 2.3	20.8 ± 7.3 a	0.6 ± 0.9 ab
Identified mixture	300	19.6 ± 5.5 a	0 ± 0
Control	–	0 ± 0	0 ± 0

Identified mixture corresponds to *E*2,*Z*13-18:OAc 280 μg + *E*2,*Z*13-18:OH 9 μg + *Z*13-18:OAc 8.5 μg + *E*3,*Z*13-18:OAc 2.3 μg + *Z*13-18:OH 0.1 μg + 18:OH 0.1 μg; control represents dispensers loaded with solvent only; SD denotes standard deviation; 5 replicates; the differences between numbers followed by different letters are statistically significant at $P < 0.05$ according to nonparametric Kruskal-Wallis analyses of variance, followed by Mann-Whitney U-test.

Table II. Attraction of *Synanthedon scoliaeformis* males under field bioassay to synthetic compounds identified from sex pheromone gland extracts of *S. tipuliformis* females.

Compound	Amount	Males per trap (mean ± SD)
	[μg]	*S. scoliaeformis*
*E*2,*Z*13-18:OAc	280	3.4 ± 1.9 a
*E*2,*Z*13-18:OAc + *E*2,*Z*13-18:OH	280 + 9	5.0 ± 1.5 a
*E*2,*Z*13-18:OAc + *E*3,*Z*13-18:OAc	280 + 2.3	0 ± 0
*E*2,*Z*13-18:OAc + *Z*13-18:OAc	280 + 8.5	4.2 ± 1.8 a
*E*2,*Z*13-18:OAc + *Z*13-18:OH	280 + 0.1	5.1 ± 2.1 a
*E*2,*Z*13-18:OAc + 18:OH	280 + 0.1	4.7 ± 2.2 a
Identified mixture	300	0 ± 0
Control	–	0 ± 0

Identified mixture corresponds to 2 *E*2,*Z*13-18:OAc 280 μg + *E*2,*Z*13-18:OH 9 μg + *Z*13-18:OAc 8.5 μg + *E*3,*Z*13-18:OAc 2.3 μg + *Z*13-18:OH 0.1 μg + 18:OH 0.1 μg; control represents dispensers loaded with solvent only; SD denotes standard deviation; 5 replicates; the differences between numbers followed by different letters are statistically significant at $P < 0.05$ according to nonparametric Kruskal-Wallis analyses of variance, followed by Mann-Whitney U-test.

18:OAc over seven times. The attractiveness of six component lures did not differ significantly from the one of the binary lures. In conclusion, *E*3,*Z*13-18:OAc was the synergist of *E*2,*Z*13-18:OAc and these two acetates in the ratio 0.7:100 were essential sex pheromone components of *S. tipuliformis.*

*E*2,*Z*13-18:OAc, tested alone, was also attractive to the males of another *Synanthedon* species, *S. scoliaeformis.* The presence of *E*3,*Z*13-18:OAc in the binary mixture with *E*2,*Z*13-18:OAc suppressed the attraction of *S. scoliaeformis* males 2.5 times. However, due to small catches of moths in the black currant field, the antagonistic effect was not statistically significant (Table I).

The trapping tests, carried out at the dwelling place of *S. scoliaeformis*, revealed that *E*3,*Z*13-18:OAc, when present in the binary mixture with *E*2,*Z*13-18:OAc, suppressed the attractiveness of the latter acetate entirely (Table II). Other compounds identified from sex pheromone glands of *S. tipuliformis* did not have a significant effect on the attraction of *S. scoliaeformis* males.

Thus, in addition to the intraspecific synergistic effect, *E*3,*Z*13-18:OAc increased the specificity of the pheromone signal for *S. tipuliformis*, when acting by interspecific way as an attraction antagonist to *S. scoliaeformis* males.

Discussion

GC-MS analyses of crude pheromone gland extracts demonstrated that virgin *S. tipuliformis* females produced *E*2,*Z*13-18:OAc, *E*3,*Z*13-18:OAc, *Z*13-18:OAc, *E*2,*Z*13-18:OH, *Z*13-18:OH and 18:OH in the ratio 100:0.7:2.7:3.2:traces:traces. The first 3 compounds were previously known to

occur in the sex pheromone gland extracts, while the last 3 chemicals were reported for the first time.

The attractiveness of *E*2,*Z*13-18:OAc to *S. tipuliformis* males was discovered by Voerman *et al.* (1984). Two years later, Priesner *et al.* (1986) reported, that the attractiveness of *E*2,*Z*13-18:OAc was significantly enhanced by the positional isomer *E*3,*Z*13-18:OAc, when they were tested together at the ratios 100:3 and 100:10. Later research on synergistic effects of *E*3,*Z*13-18:OAc revealed that two strains of currant borers exist according to the response of males to a two-component attractant. Males of the first strain occurring only in Tasmania were clearly attracted to *E*2,*Z*13-18:OAc as a single compound (Szőcs *et al.*, 1990; Suckling *et al.*, 2005), whereas males of the second strain significantly preferred a two-component blend, consisting of *E*2,*Z*13-18:OAc and 3% *E*3,*Z*13-18:OAc. The second strain was found in Europe (Voerman *et al.*, 1984; Szőcs *et al.*, 1990, 1991), New Zealand (Szőcs *et al.*, 1990) and North America (Szőcs *et al.*, 1998). Our trapping data confirmed the synergistic effect of *E*3,*Z*13-18:OAc on *E*2,*Z*13-18:OAc which was expected to occur for the European population of currant borers.

Shortly thereafter the sex attractant was reported: two compounds, *E*2,*Z*13-18:OAc and *Z*13-18:OAc in the ratio 97:3, were identified from sex pheromone gland extracts by Szőcs *et al.* (1985) and only the dienic acetate was confirmed as the sex pheromone. A minor sex pheromone component, *E*3,*Z*13-18:OAc, in trace amounts, was detected by means of the GC-EAG method from sex pheromone gland extracts of single females (James *et al.*, 2001). Suckling *et al.* (2005) have demonstrated that in New Zealand sex pheromone components occurred at the ratio 97:3. We have found that in sex pheromone gland extracts, obtained from currant borer females of Lithuanian population, *E*2,*Z*13-18:OAc and *E*3,*Z*13-18:OAc were present in the ratio 100:0.7 which is somewhat higher than 100:3 that was used in the optimized lures reported by Szőcs *et al.* (1990) and identified from sex pheromone gland extracts of New Zealand population (Suckling *et al.*, 2005).

The compositions of sex pheromones and attractants among Sesiidae are highly conserved as, to date, the *E*,*Z*- and *Z*,*Z*-isomers of 3,13- and 2,13-octadecadienols, the corresponding acetates and (2*E*,13*Z*)-octadeca-2,13-dienal are used in sexual communication by clearwing species [http://www.pherobase.com, an updated website based on the book of Arn *et al.* (1992)]. Consequently, numbers of Sesiidae species use the same compound as the main sex attractant component, and the specificity of a sex attraction signal could be achieved due to minor components with inter- or/and intraspecific activity. Būda *et al.* (1993) demonstrated that two related *Synanthedon* species, *S. tipuliformis* and *S. scoliaeformis*, used *E*2,*Z*13-18:OAc as their main attractant component. Our data revealed that *E*3,*Z*13-18:OAc showed a dual behavioural activity, by synergising attractiveness of *E*2,*Z*13-18:OAc to *S. tipuliformis* males, and acting as an attraction antagonist to males of the *S. scoliaeformis* species, by this way ensuring the specificity of the sex attraction signal of the currant borer.

In addition to the sex pheromone compounds of *S. tipuliformis*, another 4 chemicals *E*2,*Z*13-18:OH, *Z*13-18:OAc, *Z*13-18:OH and 18:OH, identified from the sex pheromone glands, did not show any biological activity, neither to *S. tipuliformis* nor to *S. scoliaeformis* males. However, they provide a basis to achieve specificity of a pheromone signal of *S. tipuliformis* and could act as attraction antagonists against other clearwing moth species which, like *S. tipuliformis*, employ *E*2,*Z*13-18:OAc as their sex pheromone component. Some of these compounds could be intermediates or side products in biosynthesis of a sex pheromone as well.

It is known that moths from one and seven species of the families Cossidae and Sesiidae, respectively, were attracted to single *E*2,*Z*13-18:OAc (based on http://www.pherobase.com). Consequently, the two compounds *E*2,*Z*13-18:OAc and *E*3,*Z*13-18:OAc have to be present in pheromone formulations used either for monitoring or for control of *S. tipuliformis* to avoid effects on non-target species.

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