Sex Attractants for Six Clearwing and Tineid Species (Lepidoptera, Sesiidae and Tineidae) from Kazakhstan and Lithuania

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Octadecadienols, Octadecadienyl Acetates, Daily Rhythm

Sex attractants for 3 Sesiidae and 3 Tineidae moth species in West-Kazakhstan and Lithuania were discovered by field screening tests of (3*Z*,13*Z*)-, (3*E*,13*Z*)- and (2*E*,13*Z*)-octadecadien-1-ols and their acetates as well as of some binary mixtures of these compounds. Total amount of chemicals was 0.3 mg/dispenser. Males of *Synanthedon serica* were attracted by a 5:5 mixture of 3*E*,13*Z*-18:OAc and 2*E*,13*Z*-18:OAc, *Chamaesphecia bibioniformis* by a 9:1 mixture of 3*Z*,13*Z*-18:OAc and 3*E*,13*Z*-18:OAc, *Paranthrene tabaniformis* by a 1:9 mixture of 3*Z*,13*Z*-18:OH and 3*E*,13*Z*-18:OH, *Tinea nonimella* by a 1:9 mixture of 3*E*,13*Z*-18:OH and 2*E*,13*Z*-18:OH, *Monopis monachella* by a 1:9 mixture of 3*Z*,13*Z*-18:OH and 2*E*,13*Z*-18:OH, and *Nemaxera betulinella* by a 9:1 mixture of 2*E*,13*Z*-18:OAc and the corresponding alcohol. The periods of attraction to the traps were registered for males of *S. serica* and *Ch. bibioniformis* and were found to occur at 15–18 and 15–17 o'clock, local time, respectively.

Introduction

The larvae of clearwings are borers of trees, shrubs and grass-roots, sometimes causing serious damage to agriculture and forestry. The moths of this family are good fliers, active during the light period of the day, and are not attracted by light traps at night. Consequently, they are more difficult to collect than many other moth species. Hence, the data about the chemical composition of their sex pheromones and related attractants are highly important for pest management and environmental research programmes. Some clearwing species are rare and in order to obtain data suitable for statistical analyses it is necessary to conduct the testing in those parts of their distribution areas, in which the target species are relatively abundant. West Kazakhstan and Lithuania were selected as the test sites.

The main components of known sex pheromones and attractants of sesiids are 6 closely related chemicals: (3*Z*,13*Z*)-, (3*E*,13*Z*)- and (2*E*,13*Z*)-octadecadien-1-ols and their acetates (Arn *et al.*, 2001). The field screening of these compounds and their binary mixtures conducted by us in several parts of the Palearctic (Būda *et al.*, 1993; Mozūraitis *et al.*, 1998; Karalius *et al.*, 2000), as well as similar tests done by other authors (e.g. Adler, 1983; Priesner *et al.*, 1986 and others) had proved to be effective in revealing sex attractants for clearwing species as well as for faunistic studies.

The lepidopteran fauna of the wide region situated between the lower reaches of rivers Volga and Ural is poorly investigated, but nevertheless known as being inhabited by a few endemic sesiid species (Laštuvka and Laštuvka, 1995; Špatenka *et al.*, 1999). The aim of the present study was to carry out the field screening of the above-mentioned octadecadienes both in the steppes north of the Caspian Sea and in Lithuania.

Materials and Methods

Chemicals and baits

3*Z*,13*Z*-, 3*E*,13*Z*- and 2*E*,13*Z*-18:OH and the corresponding acetates were obtained from the "Flora" company, Tartu, Estonia. The compounds were purified by preparative liquid chromatography, which was performed by means of gradient elution using an adsorbent consisting of a mixture

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of 10% $AgNO_3$ and 90% silica gel (Merck 60, 230–400 mesh, 0.040–0.063 mm), dry packed in a 15 mm i.d. glass column. Distilled hexane was used as solvent. It was delivered to the column from an open vessel by a metering pump at a rate of 30 ml/min. Gradient elution was accomplished by consecutively adding 40 ml solutions of each mixture of hexane and ethyl acetate as a polar solvent (in proportions 1.25, 2.5, 5.0, 7.5, 10, 15, 20, 30, 40, 50, 60, 70, 80 and 100% of the polar solvent) to a dropping funnel, the stem of which reached the surface of the stirred liquid in the vessel originally containing pure hexane. All fractions were checked by TLC on silica gel (Merck 60, precoated aluminium foil), elution with 20% ethyl acetate in hexane, and development with vanillin and sulfuric acid in ethanol. The isomeric and chemical purity of each compound was checked by gas chromatography. The purity of the compounds used in the tests exceeded 99%.

Field tests

The compounds were tested under field conditions both alone and in binary mixtures in the three ratios 9:1, 5:5 and 1:9. Total amount of chemicals was 0.3 mg/dispenser. Each compound was dissolved in hexane and the mixtures listed in Table I were prepared. The single compounds and their binary mixtures were impregnated into a red rubber tube septum (8 × 15 mm). Each bait was fixed into an opaque white delta trap Atracon A which had an exchangeable bottom coated with Pestifix glue (for a detailed description of the trap see Mozūraitis *et al.*, 1999). A trap not supplied with a dispenser served as a control.

The field tests were carried out in Kazakhstan and Lithuania within the following localities and periods:

I. The European part of Kazakhstan: Kandagash locality, 15 km south-east of the village Urda, June 5–16, 1999. Rynkum sandy-hill steppe with *Populus alba* L., *Salix* sp. and plantations of *Pinus sylvestris* L. growing in hollows.

II. The European part of Kazakhstan: Dzhanibek research station of the Institute of Forestry of the Russian Academy of Science, Dzhanibek, June 18–27, 1999. Steppe with field-protecting forest plantations.

III. Lithuania: Žemaitėliai village, 20 km north of Vilnius, June 17- July 20, 2000. Edges of mixed forest, bordering with meadows and arable land.

Three replications of each compound and mixture listed in Table I were tested at each location. The traps were fixed 1 to 2 m above the ground to branches of trees or shrubs, and were inspected twice a week. The distance between the traps was at least 10 m. In order to diminish the impact of biotopic variation on the trapping results, all of the traps of one replication set were moved to the location of the traps of an another replication set once a week.

Identification of the species

The moths captured were identified by their external morphology, colouring pattern and genitalia (Laštuvka and Laštuvka, 1995; Špatenka *et al.*, 1999; Zagulajev, 1981). When both body and wings were covered by sticky material, the moth was rinsed in hexane before identification. Representative specimens were placed in an insect collection at the Institute of Ecology, Vilnius, Lithuania.

Statistical analysis

The data from the field tests were transformed according to the formula $(x+1)^{0.5}$, where x was the number of moths captured in the trap. The values obtained were analysed by Duncan's multiple range test and significantly different values at $p \leq 0.05$ were marked with different letters (Sokal and Rohlf, 1995), as presented in Table I.

Results and Discussion

Sex attractants for 3 clearwing (Sesiidae) and 3 tineid (Tineidae) moth species were found during the field tests.

Sesiidae

Synanthedon serica Alpheraky. One hundred and six males were trapped in the Kandagash locality (Table I). The mixture of 3*E*,13*Z*-18:OAc with its positional isomer 2*E*,13*Z*-18:OAc in the ratio 5:5 was found to be the most attractive lure for males of this species. The binary blend consisting of the same compounds but in the ratio 9:1 was attractive as well, but significantly less effi-

Table I. Attraction of clearwing and tineid males to 2,13- and 3,13-octadecadienols, corresponding acetates and some of their binary mixtures under field tests.

Chemicals	Ratio*	*S. serica*	*Ch. bibioniformis*	*P. tabaniformis*	*T. nonimella*	*M. monachella*	*N. betulinella*
3*Z*,13*Z*-18:OAc		·	·	·	·	·	·
3*Z*,13*Z*-18:OAc + 3*Z*,13*Z*-18:OH	5:5	·	·	·	·	·	·
3*Z*,13*Z*-18:OAc + 3*Z*,13*Z*-18:OH	9:1	·	·	·	·	·	·
3*Z*,13*Z*-18:OAc + 3*Z*,13*Z*-18:OH	1:9	·	·	·	·	·	·
3*Z*,13*Z*-18:OAc + 3*E*,13*Z*-18:OAc	5:5	·	·	·	·	·	·
3*Z*,13*Z*-18:OAc + 3*E*,13*Z*-18:OAc	9:1	·	35c	·	·	·	·
3*Z*,13*Z*-18:OAc + 3*E*,13*Z*-18:OAc	1:9	·	·	·	·	·	·
3*Z*,13*Z*-18:OAc + 3*E*,13*Z*-18:OH	5:5	·	10b	·	·	·	·
3*Z*,13*Z*-18:OAc + 3*E*,13*Z*-18:OH	9:1	·	·	·	·	·	·
3*Z*,13*Z*-18:OAc + 3*E*,13*Z*-18:OH	1:9	·	·	1ab	·	·	·
3*Z*,13*Z*-18:OAc + 2*E*,13*Z*-18:OAc	5:5	·	·	·	·	·	·
3*Z*,13*Z*-18:OAc + 2*E*,13*Z*-18:OAc	9:1	·	·	·	·	·	·
3*Z*,13*Z*-18:OAc + 2*E*,13*Z*-18:OAc	1:9	·	·	·	·	·	·
3*Z*,13*Z*-18:OAc + 2*E*,13*Z*-18:OH	5:5	·	·	·	·	3ab	·
3*Z*,13*Z*-18:OAc + 2*E*,13*Z*-18:OH	9:1	·	·	·	·	·	·
3*Z*,13*Z*-18:OAc + 2*E*,13*Z*-18:OH	1:9	·	·	·	·	6b	·
3*Z*,13*Z*-18:OH		·	·	·	·	·	·
3*Z*,13*Z*-18:OH + 3*E*,13*Z*-18:OAc	5:5	·	·	·	·	·	·
3*Z*,13*Z*-18:OH + 3*E*,13*Z*-18:OAc	9:1	·	·	·	·	·	·
3*Z*,13*Z*-18:OH + 3*E*,13*Z*-18:OAc	1:9	·	·	·	·	·	·
3*Z*,13*Z*-18:OH + 3*E*,13*Z*-18:OH	5:5	·	·	3ab	·	·	·
3*Z*,13*Z*-18:OH + 3*E*,13*Z*-18:OH	9:1	·	·	1ab	·	·	·
3*Z*,13*Z*-18:OH + 3*E*,13*Z*-18:OH	1:9	·	·	29d	·	·	·
3*Z*,13*Z*-18:OH + 2*E*,13*Z*-18:OAc	5:5	·	·	·	·	·	·
3*Z*,13*Z*-18:OH + 2*E*,13*Z*-18:OAc	9:1	·	·	·	·	·	·
3*Z*,13*Z*-18:OH + 2*E*,13*Z*-18:OAc	1:9	·	·	·	·	·	·
3*Z*,13*Z*-18:OH + 2*E*,13*Z*-18:OH	5:5	·	·	·	·	22c	·
3*Z*,13*Z*-18:OH + 2*E*,13*Z*-18:OH	9:1	·	·	·	·	·	·
3*Z*,13*Z*-18:OH + 2*E*,13*Z*-18:OH	1:9	·	·	·	·	98d	·
3*E*,13*Z*-18:OAc		·	2a	·	·	·	·
3*E*,13*Z*-18:OAc + 3*E*,13*Z*-18:OH	5:5	·	·	·	·	·	·
3*E*,13*Z*-18:OAc + 3*E*,13*Z*-18:OH	9:1	·	·	·	·	·	·
3*E*,13*Z*-18:OAc + 3*E*,13*Z*-18:OH	1:9	·	·	2ab	·	·	·
3*E*,13*Z*-18:OAc + 2*E*,13*Z*-18:OAc	5:5	95c	·	·	·	·	·
3*E*,13*Z*-18:OAc + 2*E*,13*Z*-18:OAc	9:1	11b	·	·	·	·	·
3*E*,13*Z*-18:OAc + 2*E*,13*Z*-18:OAc	1:9	·	·	·	·	·	·
3*E*,13*Z*-18:OAc + 2*E*,13*Z*-18:OH	5:5	·	·	·	·	1ab	·
3*E*,13*Z*-18:OAc + 2*E*,13*Z*-18:OH	9:1	·	·	·	·	·	·
3*E*,13*Z*-18:OAc + 2*E*,13*Z*-18:OH	1:9	·	·	·	·	5ab	·
3*E*,13*Z*-18:OH		·	·	5b	·	·	·
3*E*,13*Z*-18:OH + 2*E*,13*Z*-18:OAc	5:5	·	·	1ab	·	·	·
3*E*,13*Z*-18:OH + 2*E*,13*Z*-18:OAc	9:1	·	·	1ab	·	·	·
3*E*,13*Z*-18:OH + 2*E*,13*Z*-18:OAc	1:9	·	·	·	·	·	·
3*E*,13*Z*-18:OH + 2*E*,13*Z*-18:OH	5:5	·	·	5b	·	·	·
3*E*,13*Z*-18:OH + 2*E*,13*Z*-18:OH	9:1	·	·	12c	·	·	·
3*E*,13*Z*-18:OH + 2*E*,13*Z*-18:OH	1:9	·	·	·	351b	·	·
2*E*,13*Z*-18:OAc		·	·	·	·	·	8bc
2*E*,13*Z*-18:OAc + 2*E*,13*Z*-18:OH	5:5	·	·	·	·	·	12c
2*E*,13*Z*-18:OAc + 2*E*,13*Z*-18:OH	9:1	·	·	·	·	·	125d
2*E*,13*Z*-18:OAc + 2*E*,13*Z*-18:OH	1:9	·	·	·	·	·	2ab
2*E*,13*Z*-18:OH		·	·	·	·	2ab	·
Control		·	·	·	·	·	·
Total		106	47	60	351	137	147

Figures are numbers of moths trapped; numbers followed by the same letter within each column do not differ significantly by Duncan's multiple range test ($p > 0.05$); dot: catch corresponds to 0a. *Total amount of chemicals was 0.3 mg/dispenser.

cient than the 5:5 blend. When tested separately, none of the compounds was attractive for the males of this species. Thus, the binary blend of 3*E*,13*Z*-18:OAc and 2*E*,13*Z*-18:OAc in the ratio 5:5 should be considered as a sex attractant for *S. serica* males. Sex attractant for *S. serica* was not known before.

Direct observation of the male flights to the baits revealed that the males of this clearwing species were sexually active at 15–18 o'clock, local time.

S. serica had been known from Central Asia only (Špatenka *et al.*, 1999). We found it in Europe for the first time. We registered this species at June 5–16, 1999 in the European part of Kazakhstan, 15 km south-east of the village Urda, in the Kandagash locality (132 males) and additionally at May 27, 2000 in Ujaly, about 100 km east of the Urda locality (5 males).

It is noteworthy that *S. serica* is morphologically very closely related to *S. formicaeformis* (Esper) (Špatenka *et al.*, 1999). Males of both species prefer sex attractants, composed of the same compounds in the same ratio (present work and Priesner *et al.*, 1986). Our preliminary observations show, that the periods of sexual activity during the day also overlap. According to the distribution data available (Špatenka *et al.*, 1999) these species are of allopatric origin. Consequently, *S. serica* and *S. formicaeformis* may serve as model objects of further investigations about the sex pheromone signal specificity in closely related but geographically isolated clearwing moth species.

Chamaesphecia bibioniformis (Esper). Forty-seven males were trapped at Kandagash, in the European part of Kazakhstan (Table I). Two baits, both containing 3*Z*,13*Z*-18:OAc were attractive. The binary mixture of 3*Z*,13*Z*-18:OAc and 3*E*,13*Z*-18:OAc in the ratio 9:1 attracted 35 males and was the most effective one, differing significantly from all the other lures tested. The attractivity of 3*Z*,13*Z*-18:OAc mixed with 3*E*,13*Z*-18:OH in the ratio 5:5 was significantly lower than that of the 9:1 mixture of the two acetates, but still significantly higher than that of the control. When the compounds were tested separately, 2 males were indeed caught in the traps baited with 3*E*,13*Z*-18:OAc, but the difference between this catch and that of the control was not significant. In conclusion, the binary mixture 3*Z*,13*Z*-18:OAc/3*E*,13*Z*-18:OAc in the ratio 9:1 should be considered as the sex attractant for *Ch. bibioniformis* males. Neither this nor any other sex attractant for *Ch. bibioniformis* was known before.

The catch of *Ch. bibioniformis* males by the traps baited with 3*Z*,13*Z*-18:OAc/3*E*,13*Z*-18:OAc was recorded between 15 and 17 o'clock, local time.

By using this attractant to lure *Ch. bibioniformis* males we got new data about the distribution area of this species. At May 14–16, 2000 we trapped 4 *Ch. bibioniformis* males in West Kazakhstan, in the Ushtagan area (120 km east of Aktau), on the Mangishlak peninsula. This locality is situated about 300 km south of the known southern border the distribution area of *Ch. bibioniformis*, which lies between the northern parts of the Caspian Sea and the Aral Sea (Špatenka *et al.*, 1999).

Paranthrene tabaniformis (Rottemburg), dusky clearwing. In total, 60 males were trapped in locations I and II, in the European part of Kazakhstan (Table I). The attractiveness of 3*E*,13*Z*-18:OH alone and of the binary mixtures of 3*Z*,13*Z*-18:OH and 3*E*,13*Z*-18:OH in the ratio 1:9 as well as those of 3*E*,13*Z*-18:OH and 2*E*,13*Z*-18:OH in the ratios 5:5 and 9:1 differed significantly from those of the control. The binary mixture of 3*Z*,13*Z*-18:OH and 3*E*,13*Z*-18:OH in the ratio 1:9 was the most effective one, differing significantly from all of the other lures tested. Furthermore, the mixture of 3*E*,13*Z*-18:OH and 2*E*,13*Z*-18:OH in the ratio 9:1 attracted significantly more males than 3*E*,13*Z*-18:OH when used alone. Thus, our field experiments demonstrated that both 3*Z*,13*Z*-18:OH and 2*E*,13*Z*-18:OH showed synergistic effects when 10% of them were added to 3*E*,13*Z*-18:OH. In conclusion, the binary mixture containing 3*Z*,13*Z*-18:OH and 3*E*,13*Z*-18:OH in the ratio 1:9 should be considered as the most effective sex attractant for *P. tabaniformis* males known at present. Three-component mixture tests including 3*E*,13*Z*-18:OH, 3*Z*,13*Z*-18:OH and 2*E*,13*Z*-18:OH would be a perspective for further improvement of the composition of the sex attractant for the dusky clearwing moth.

Investigations of sex attractants for the dusky clearwing, known as an important pest in tree nurseries, were started more than two decades ago. The attractiveness of 3*E*,13*Z*-18:OH for males of this species was shown both in North America

(Nielsen *et al.*, 1979; Solomon, 1979) and in Europe (Voerman, 1980). In the last publication preliminary data indicated, that an admixture of 3*Z*,13*Z*-18:OH to 3*E*,13*Z*-18:OH improved the catches of *P. tabaniformis* males to some extent. We proved this synergistic effect.

A sex pheromone of *P. tabaniformis* was identifed in China (Zhang *et al.*, 1985). Contrary to our data, only 3*E*,13*Z*-18:OH was detected in the female extract investigated. It is possible, that the minor compounds of the female pheromone due to limited sensitivity of the technique might have occurred in quantities below the detection level and had been overlooked. On the other hand, *P. tabaniformis* is a holarctic species with a very wide distribution area and an existence of "dialects" in the chemical communication between populations, similar to those reported for *Synanthedon tipuliformis* (Szöcs *et al.*, 1990), is possible and remains to be investigated.

Below, we present our trapping data of another two clearwing species, although we consider those data as preliminary due to the low number of specimens trapped.

Chamaesphecia crassicornis Bartel. Four males were trapped at Dzhanibek in the European part of Kazakhstan. Three of them were attracted to the mixture of 3*Z*,13*Z*-18:OAc and 3*E*,13*Z*-18:OAc in the ratio 1:9. One male was caught by the traps baited with 3*Z*,13*Z*-18:OAc and 3*E*,13*Z*-18:OH in the ratio 1:9. When tested separately, none of the three compounds was attractive.

Chamaesphecia astatiformis (Herrich-Schäffer). Six males were trapped at the Dzhanibek research station in the European part of Kazakhstan, by four different binary mixtures, all of them containing 3*Z*,13*Z*-18:OAc. This compound attracted none when tested singly. Mixtures of this acetate and 3*Z*,13*Z*-18:OH lured 3 males, and mixtures with 3*E*,13*Z*-18:OH attracted 2 males. These results were similar to those, which we obtained in Altai (Karalius *et al.*, 2000), where *Ch. astatiformis* males were attracted to binary mixtures of 3*Z*,13*Z*-18:OAc and either 3*E*,13*Z*-18:OH or 2*E*,13*Z*-18:OH or 3*Z*,13*Z*-18:OH. In no cases the catches of the males differ significantly from those of the control lures. An optimal attractant composition for *Ch. astatiformis* males was obviously lacking among the baits tested.

Tineidae

Tinea nonimella (Zagulajev). In total, 351 males were trapped at Kandagash and Dzhanibek in the European part of Kazakhstan (Table I). All of them were caught by traps baited with 3*E*,13*Z*-18:OH and 2*E*,13*Z*-18:OH in the ratio 1:9, demonstrating a rather narrow reaction spectrum. Thus, this mixture should be considered as the sex attractant for *T. nonimella* males. A sex attractant for *T. nonimella* was not known before.

Monopis monachella (Hübner). One hundred and thirty-seven males were trapped in Lithuania (Table I). Three bait compositions were attractive. The most effective one, the mixture of 3*Z*,13*Z*-18:OH and 2*E*,13*Z*-18:OH in the ratio 1:9, differed significantly in efficiency from the other blends. The same compounds in the ratio 5:5 were also attractive, although significantly less than the first blend. The 9:1 mixture was ineffective. When tested separately, 2*E*,13*Z*-18:OH lured 2 males only, and the difference between this catch and that of the control was not statistically significant. Thus, the binary blend containing 3*Z*,13*Z*-18:OH and 2*E*,13*Z*-18:OH in the ratio 1:9 should be considered as the sex attractant for *M. monachella*. A sex attractant for *M. monachella* was not known before.

Nemaxera betulinella (Paykull). One hundred and forty-seven males were trapped in Žemaiteliai, Lithuania (Table I). 2*E*,13*Z*-18:OAc was attractive alone as well as in binary mixtures with 2*E*,13*Z*-18:OH. The highest catch (125 males), differing significantly from that of other baits, was recorded for the mixture in the ratio 9:1. Thus, the binary mixture of 2*E*,13*Z*-18:OAc and 2*E*,13*Z*-18:OH in the ratio 9:1 should be considered as the sex attractant for *N. betulinella* males.

The attraction of *N. betulinella* males to 2*E*,13*Z*-18:OAc had been reported earlier (Būda *et al.*, 1993), but binary mixtures with 2*E*,13*Z*-18:OH had not been tested.

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