Screening and Use of Sex Attractants in Monitoring of Geometrid Moths in Bulgaria*

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Four candidate sex attractants for geometrid moths have been screened individually and in mixtures. As a result, in addition to the confirmation of the known sex attractants of *Operophtera brumata* L. and *Alsophila aceraria* Denis & Schiff (= *A. quadripunctata* Esper), possible sex attractants for five other geometrid species have also been established. For *O. brumata* and *A. aceraria* an inhibitor has been found. For the same species the possibility for seasonal monitoring by means of sex pheromone traps was demonstrated.

During the last four years considerable progress has been made in the identification of female sex pheromones of a number of geometrid moth species (Lepidoptera: Geometridae). In all cases the pheromones or pheromone components were polyene hydrocarbons with 19 carbon atoms and two to four double bonds [2–8]. By field screening of these compounds, individually and in various combinations, sex attractants for some other geometrid species have also been established [2, 9, 10].

In this paper the results of two years of screening (6*Z*, 9*Z*)-6,9-nonadecadiene [Z6Z9-19Hy], a pheromone component of *Bupalus piniarius* [5], (3*Z*, 6*Z*, 9*Z*)-3,6,9-nonadecatriene [Z3Z6Z9-19Hy], a component of the pheromone complex of *Boarmia selenaria* [4], *Alsophila pometaria* [6] and *Peribatodes* (= *Boarmia*) *rhomboidaria* [8], (3*Z*, 6*Z*, 9*Z*)-1,3,6,9-nonadecatetraene [1Z3Z6Z9-19Hy], the sex pheromone of *Operophtera brumata* [2, 3], (*Z*)-6-nonadecen-9-yne [Z6Y9-19Hy], a synthesis intermediate of the nonadecadiene, and all 15 possible combinations of these are reported. Additionally, the effect of Z6Z9-19Hy admixed to the sex attractants of *O. brumata* and *A. aceraria* (= *A. quadripunctata*) was investigated and the possibilities for a seasonal monitoring of these species in Bulgaria by means of pheromone baited traps were studied.

Materials and Methods

Four C_{19}-polyene hydrocarbons, (6*Z*, 9*Z*)-6,9-nonadecadiene [Z6Z9-19Hy], (3*Z*, 6*Z*, 9*Z*)-3,6,9-nonadecatriene [Z3Z6Z9-19Hy], (3*Z*, 6*Z*, 9*Z*)-1,3,6,9-nonadecatetraene [1Z3Z6Z9-19Hy] and (*Z*)-6-nonadecen-9-yne [Z6Y9-19Hy], were selected for the screening experiments. The nonadecadiene, Z6Z9-19Hy, was obtained by transformation of linoleic acid into the corresponding aldehyde, followed by Wittig methylenation, hydroboration of the terminal double bond with 9-BBN and subsequent hydrolysis (unpublished). The triene, Z3Z6Z9-19Hy, was formed by carbonyl olefination of propanal with hexadecadienylidene phosphorane [4]. The same reaction of the phosphorane with acrolein yielded the nonadecatetraene 1Z3Z6Z9-19Hy [3]. Nonadecenyne, Z6Y9-19Hy, was a synthetic intermediate of another nonadecadiene synthesis attempt (unpublished).

Pherocon 1C traps were used with rubber penicilline vial caps to take up the bait substances. In all

Abbreviations: Z6Z9-19Hy, (6*Z*, 9*Z*)-6,9-nonadecadiene; Z3Z6Z9-19Hy, (3*Z*, 6*Z*, 9*Z*)-3,6,9-nonadecatriene; 1Z3Z6Z9-19Hy, (3*Z*, 6*Z*, 9*Z*)-1,3,6,9-nonadecatetraene; Z6Y9-19Hy, (*Z*)-6-nonadecen-9-yne.

* Pheromones, 55 [1].

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cases, except the inhibitor experiments of Tables III and IV, 1 mg of each substance was used as the bait. Thus, a one-component bait contained 1 mg, a two-component bait 2 mg, etc. After depositing the neat attractant substance/substances in the cavity of the vial caps, 1 ml of dichloromethane or hexane was added for better penetration and impregnation. In the tests with *A. aceraria* and *O. brumata,* when different ratios of two-component mixtures were used in the baits, disposable micropipettes were employed for loading the vials.

The screening of all possible 15 combinations was performed from August till the end of December 1984, and from March till midth of June 1985, in Pancharevo, a countryside near Sofia. After that point of time, only seven attractant blends consisting of the possible diene, triene and tetraene combinations and a single nonadecenyne bait was used till the end of December 1985. The distances between the traps were 10–15 m. The small-scale tests for *A. aceraria* with different ratios of Z3Z6Z9-19Hy and Z6Z9-19Hy, and for *O. brumata* with 1Z3Z6Z9-19Hy and Z6Z9-19Hy, respectively, were performed in Pancharevo and Gorna banja. A third test area for *brumata* was chosen in Bojana, both the latter locations being near Sofia also. In all cases the baits were refurnished at least monthly and checked weekly.

For the seasonal monitoring of *A. aceraria* and *O. brumata,* two traps loaded with 1Z3Z6Z9-19Hy and Z3Z6Z9-19Hy, respectively, were used in Pancharevo. Checks and replacing the sticky bottom of the traps being done weekly.

Results

In addition to the great numbers of males of *A. aceraria* and *O. brumata* captured, which will be discussed separately, male insects of five other geometrid species were also trapped (Table I). In total, 20 males of *Alcis repandata* were attracted by two two-component baits, both containing the nonadecadiene Z6Z9-19Hy as one component, but none were trapped with the diene alone. *Brephos notha* was attracted more unspecifically with three different blends (Table I). *Ecliptoperia silaceata* was attracted by two attractant mictures both having the nonadecatetraene, 1Z3Z6Z9-19Hy, in common but not to the substance alone. Three male *Eupithecia subnotata* moths were captured in a trap containing the nonadecatriene Z3Z6Z9-19Hy bait. In total, eight insects of *Ourapteryx sambucaria* were attracted by the two single compounds Z3Z6Z9-19Hy and 1Z3Z6Z9-19Hy. Beside the number of males caught, Table I also gives the time of flight observed of the respective moth species.

During the experiments, two rare species, not included in the tables, were also caught in low numbers. *Apeira syringaria* L.: 1 ♂ attracted to

Table I. Geometrid males attracted to C_{19}-polyene hydrocarbons and mixtures of them, and flight period. Test time August–December 1984, and March–December 1985, test area Pancharevo near Sofia.

Species attracted	Attractant/mixture polyene hydrocarbon C_{19}-Hy [1 mg each]	Number of males caught 1984	1985	Flight period [month]
Alcis repandata L.	[Z6Z9 + Z3Z6Z9]	0	5	VI–VIII
	[Z6Z9 + 1Z3Z6Z9]	3	12	
Brephos notha Hübner	[Z3Z6Z9 + Z6Y9]	0	1	IV
	[Z3Z6Z9 + 1Z3Z6Z9]	0	1	
	[Z3Z6Z9 + 1Z3Z6Z9 + Z6Z9 + Z6Y9]	0	1	
Ecliptoperia silaceata Denis & Schiff.	[1Z3Z6Z9 + Z6Z9]	2	0	VIII
	[1Z3Z6Z9+Z3Z6Z9+Z6Y9]	1	0	
Eupithecia subnotata Hübner (= *E. simpliciata* Haworth)	[Z3Z6Z9]	3	0	VIII
Ourapteryx sambucaria L.	[Z3Z6Z9]	0	7	VI–VIII
	[1Z3Z6Z9]	0	1	

Table II. Effect of structural analogs on the attractancy of Z3Z6Z9-19Hy and 1Z3Z6Z9-19Hy to the males of *A. aceraria* and *O. brumata* (November–December 1984, 1985; Pancharevo/Sofia).

Attractant C_{19}-Hy [1 mg each]	Total number of males caught *A. aceraria* 1984	1985	*O. brumata* 1984	1985
[Z3Z6Z9]	25	86	–	–
[Z3Z6Z9 + Z6Y9]	9	nt	–	–
[Z3Z6Z9 + Z6Z9]	11	6	–	–
[Z3Z6Z9 + 1Z3Z6Z9]	12	15	68	257
[1Z3Z6Z9]	–	–	101	253
[1Z3Z6Z9 + Z6Y9]	–	–	118	nt
[1Z3Z6Z9 + Z6Z9]	–	–	26	155

nt = not tested.

Z3Z6Z9-19Hy; *Crocalis tusciaria* Borhausen: 1 ♂ attracted to a mixture of Z3Z6Z9-19Hy and 1Z3Z6Z9-19Hy. In addition to these, males of the common geometrid *Epirhoe alternata* Müller were attracted to three baits: 2 ♂ to Z3Z6Z9-19Hy, 1 ♂ to 1Z3Z6Z9-19Hy, and 2 ♂ to a mixture of these compounds.

With the screening experiments, the effect of structural analogs on the attractancy of the sex attractants Z3Z6Z9-19Hy and 1Z3Z6Z9-19Hy of the corresponding species, *A. aceraria* and *O. brumata,* respectively, was also studied. In both years, 1984 and 1985, the traps baited with the two-component mixture 1Z3Z6Z9-19Hy and Z6Z9-19Hy caught a less number of *O. brumata* males compared with the traps containing only the pheromone, 1Z3Z6Z9-19Hy. A similar inhibitory effect of Z6Z9-19Hy on the attractancy of Z3Z6Z9-19Hy to *A. aceraria* males was established (Table II). Mixing the two sex attractants, 1Z3Z6Z9-19Hy and Z3Z6Z9-19Hy together, seemed not to effect the attractivity of each other for the corresponding species significantly. The smaller number of *A. aceraria* males caught with the two-component blend may be due to the competition of *O. brumata* for the same lure and the latter being more successful in filling the traps.

The special trial performed in 1985, when the effects of a more graded addition of the inhibitor Z6Z9-19Hy to the attractants of *A. aceraria* and *O. brumata* was studied, showed that the diene at least in a ratio of 1:1 acted as an inhibitor. Adding smaller amounts only (10 or 50 percents) of the nonadecadiene, Z6Z9-19Hy, to the corresponding sex attractant, did not result in a significant decrease of capture rates (Table III and IV).

Table III. Effect of Z6Z9-19Hy on the attractiveness of Z3Z6Z9-19Hy to *A. aceraria* males (test period November 26 – December 9, 1985, two weeks; test area Pancharevo and Gorna banja).

Amount of Z6Z9-19Hy [µg] added to 1000 µg of Z3Z6Z9-19Hy	Total number of males caught Pancharevo week I	II	Gorna banja week I	II
0	27	32	17	0
100	32	17	6	0
500	24	5	7	0
1000	7	2	2	0

The flight period of *O. brumata,* as it was registered by means of pheromone traps, began in both years at the beginning of November and lasted almost till the end of December. The males of *A. aceraria* appeared somewhat later, but the end of their flight period coincided with that of *O. brumata* (Fig. 1).

Table IV. Effect of Z6Z9-19Hy on the attractancy of 1Z3Z6Z9-19Hy to *O. brumata* males (November 11–17, 1985; three test locations, Pancharevo, Gorna banja and Bojana, near Sofia).

Amount of Z6Z9-19Hy [µg] added to 1000 µg of 1Z3Z6Z9-19Hy	Total number of males caught Pancharevo	Gorna banja	Bojana
0	101	18	71
100	89	19	37
500	63	1	11
1000	*	9	28

* Trap found fallen.

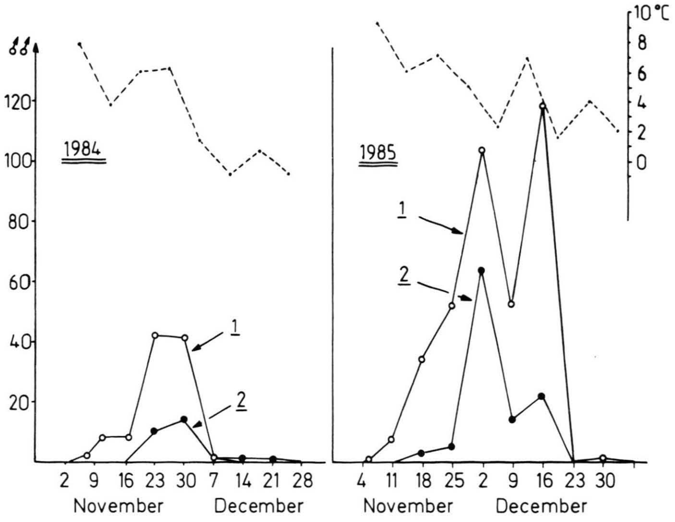

Fig. 1. Flight period of *O. brumata* (**1**, ○——○) and *A. aceraria* (**2**, ●——●) in 1984 and 1985, monitored with one pheromone trap per species (Pancharevo/Sofia), and mean week temperature (– – –).

Discussion

Our results confirm the finding of Bogenschütz *et al.* [9], that *A. repandata* males are well attracted by both mixtures, Z6Z9-19Hy + Z3Z6Z9-19Hy and Z6Z9-19Hy + 1Z3Z6Z9-19Hy. *Brephos notha* is a rare species in Bulgaria and the presence of Z3Z6Z9-19Hy in all attractive baits could show that this compound has some attractancy towards conspecific males. In the case of *E. silaceata* (not a very common species), 1Z3Z6Z9-19Hy could be accepted as a similar attractant. This compound was also the common constituent of three different lures that attracted the males of this species (= *Diactrinia siliceata*) in Germany [9], the catch rate (total of 9 ♂♂) was slightly greater.

The attractancy of Z3Z6Z9-19Hy to the males of *O. sambucaria,* a common species in Bulgaria, and to the males of the rare species, *E. subnotata* (= *E. simplicata*) was significantly more. Bogenschütz *et al.* [9] reported the trapping of one male moth of *O. sambucaria* in traps baited with two parts of Z3Z6Z9-19Hy and one part of 1Z3Z6Z9-19Hy. The German authors also reported the capture of *E. alternata,* – 4 males, one each attracted by four differently baited traps, each containing 1Z3Z6Z9-19Hy. However, taking into account, that this species is quite common in Bulgaria, we are inclined to regard these captures as rather occasional and not specific.

Szöcs *et al.* reported a two-component sex attractant for *A. quadripunctata* (= *A. aceraria*) found in Hungary [10] in which Z6Z9-19Hy has shown to possess a well defined synergistic effect to the main attractant of this species, Z3Z6Z9-19Hy. Our results, however, failed to confirm any synergistic activity of Z6Z9-19Hy (*cf.* Table III). On the contrary, the compound, at least when admixed in a ratio of 1:1 to Z3Z6Z9-19Hy, clearly inhibited its attractancy to this species. When a smaller amount of Z6Z9-19Hy was admixed to Z3Z6Z9-19Hy, this effect was not evident anymore. This contradiction could be explained by the existence of a geographical variation in the pheromone systems between the populations of the species in the two countries, and further experiments are needed to support this speculation.

Knauf *et al.* have proved the high potency of 1Z3Z6Z9-19Hy as a sex attractant for *O. brumata* in field trials in Western Germany [11]. In addition, they have concluded, that none of the structural analogs (including Z6Z9-19Hy) tested by them, has significant inhibitory effect on the *brumata* attractant. However, in our experiments a somewhat smaller number of *O. brumata* males was caught with the mixture of 1Z3Z6Z9-19Hy and Z6Z9-19Hy. But again, this inhibition could be observed significantly only, when 50–100 percent of the diene had been added to the tetraene sex attractant.

At least for Bulgaria, a seasonal monitoring of the geometrids, *O. brumata* and *A. aceraria* by means of pheromone traps has been carried out for the first time. Although we have not done any comparison with other methods of monitoring, the good captures in both cases show that pheromone traps are a promising tool for seasonal monitoring of these species.

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