Supplementary Data on the Sex Attractant System of *Panolis flammea*

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Pheromonal synergism and inhibition in *P. flammea* was further studied through electrophysiological and field trapping tests. *Z*11-tetradecenyl acetate and *Z*11-hexadecenyl acetate, each acting upon a separate type of male sensory cell, were equally effective in synergizing attraction responses to the major pheromone component, *Z*9-tetradecenyl acetate. Addition of *Z*7-dodecenyl acetate to these lures reduced captures. Male attraction specificity markedly varied with local moth density.

Baker *et al.* reported in 1982 [1] the identification of the female sex pheromone of *Panolis flammea* Schiff. (Lepidoptera: Noctuidae; "pine beauty moth", "Kieferneule") as a combination of three mono-unsaturated acetates, *viz.* (*Z*)-9- and (*Z*)-11-tetradecenyl acetates and (*Z*)-11-hexadecenyl acetate, in a ratio of *Z*9-14:Ac + *Z*11-14:Ac + *Z*11-16:Ac of 100/1/5. Preceeding this chemical pheromone elucidation, the electrophysiological analysis of male receptor types in conjunction with field trapping studies had permitted the establishment of a highly potent synthetic lure for males of this moth species [2]. It consisted of a 100/5 mixture of *Z*9-14:Ac + *Z*11-14:Ac, thus comprising two of the later-identified pheromone components but in a ratio different from that found in the female moth. This "standard attractant" has served over five years for baiting survey traps in monitoring programs [2–7].

On continuing electrophysiological studies of the receptor system of the *Sensilla trichodea* on *P. flammea* male antennae it became evident that these sensilla contained a total of four different cell types. Two of these were specialist receptors for *Z*9-14:Ac and *Z*11-14:Ac, as reported in 1978 [2]. The remaining cells (types C and D) were also studied for their chemical specificities, using experimental procedures as in previous work on other noctuid moths [8]. The test stimuli included known components of noctuid sex pheromones and some 100 structural analogues. In these recordings, the C cells revealed a response spectrum closely resembling those reported from *Z*11-16:Ac receptors in other noctuid species [9–11]. The respective data for the D cells proved that (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac) was the appropriate ("key") stimulant for these cells.

The *Z*11-16:Ac is, in fact, a minor component found in the *P. flammea* pheromone secretion, as mentioned above, whereas the *Z*7-12:Ac does not appear to be produced by the female moth [1]. For neither compound have behavioural effects on *P. flammea* males been reported. We present here field trapping data aimed at elucidating possible behavioural functions of the two chemicals, *Z*11-16:Ac and *Z*7-12:Ac, through captures of male moths attracted to sticky traps.

A preliminary test series was conducted in forests of Lodgepole pine (*Pinus contorta*) at Bareagle, Scotland, in spring of 1980 [12]. The pheromonal combination of *Z*9-14:Ac + *Z*11-14:Ac + *Z*11-16:Ac of 100/1/5 was unknown at that time, and is also missing from this test series, which included four other mixtures with *Z*11-16:Ac (Table I). Over the two weeks test period, high captures were likewise obtained by four different test formulations: the 100/5 "standard attractant"; the 100/25 combination of *Z*9-14:Ac+*Z*11-16: Ac; and the two ternary mixtures (ratios 100/5/5 and 100/5/25). Compared to these, captures by the 100/5 binary combination of *Z*9-14:Ac + *Z*11-16:Ac were the lower (Table I), with all four test replications. It should be added

Table I. Captures of *Panolis flammea* males in traps baited with identified pheromone components. Bareagle, Scotland, April 1980; four replicates [12].

Amount of chemical [µg/trap]			$\bar{X}$ males/trap
*Z*9-14:Ac	*Z*11-14:Ac	*Z*11-16:Ac	
100	0	0	5.0
100	5	0	79.5
100	0	5	44.25
100	0	25	82.0
100	5	5	74.75
100	5	25	74.25

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that this experiment was conducted on a high local moth population, causing rapid exhaustion of the "holding capacities" of the sticky traps [13], which might have possibly levelled capture differences among the more effective lures. It was therefore decided to use lower-density experimental sites for continuing these studies.

In most Central European survey areas the *P. flammea* populations are presently at a low, latency stage. However, both pupal counts and pheromone trap captures indicated an increase in population density from 1979 towards 1982 on various localities [14]. For example, in Scots pine (*Pinus sylvestris*) plantations near Seligenstadt, southeastern Hessen (25 km east of Frankfurt), traps baited with the "standard attractant" revealed a mean catch of 4 *P. flammea* males/trap for 1981 but >10 males/trap for 1982. This area was thus chosen for continuing trapping experiments on the effects of the two "key compounds", *Z*11-16:Ac and *Z*7-12:Ac. As in 1978 [2], tetratraps with rubber-cup odour dispensers were used, and tests were again conducted as series comprising 6 or 7 traps, each baited with a different chemical formulation. Traps were set on pine branches at eye level, spaced 40 m within series and 100 m or more between replications.

The first series included four of the formulations already tested in Scotland, supplemented by the 100/1/5 pheromonal mixture. The captures obtained over the 8 weeks test period were in the order of 20 males/trap for the 100/5 "standard attractant" and the 100/1/5 synthetic pheromone, not differing significantly between these two lures (Table II). Of the binary mixtures with *Z*11-16:Ac, the 100/5 combination likewise revealed high captures, whereas the results were poor for the 100/25 combination of these compounds, as for the 100/5/25 ternary mixture (Table II). These data were in obvious contrast to results earlier obtained on these same lures in Scotland, mentioned above.

A successive series, conducted in spring of 1983, further investigated the synergistic properties of *Z*11-16:Ac in binary combinations with the primary pheromone component, *Z*9-14:Ac. Four different mixtures of the two compounds were offered in comparison to pure *Z*9-14:Ac alone and the *Z*9-14:Ac + *Z*11-14:Ac 100/5 "standard" (Table III). The *Z*9-14:Ac + *Z*11-16:Ac mixtures of 100/1 and 100/5 revealed captures of the same magnitude as

Table II. Captures of *Panolis flammea* males in tetratraps baited with synthetic pheromone components. Seligenstadt, March 16 to May 11, 1982; 12 replicates.

Amount of chemical [μg/trap]			$\bar{X}$ males/trap
*Z*9-14:Ac	*Z*11-14:Ac	*Z*11-16:Ac	
100	5	0	20.4
100	0	5	14.6
100	0	25	2.3
100	5	25	3.6
100	1	5	19.3

Table III. Captures of *Panolis flammea* males in tetratraps baited with synthetic pheromone components. Seligenstadt, March 27 to May 31, 1983; four replicates.

Amount of chemical [μg/trap]			$\bar{X}$ males/trap
*Z*9-14:Ac	*Z*11-14:Ac	*Z*11-16:Ac	
100	0	0	0.25
100	0	1	10.5
100	0	5	15.75
100	0	25	1.0
100	0	100	1.25
100	5	0	11.0

the "standard", whereas in the higher ratios of 100/25 and 100/100 the *Z*11-16:Ac caused only weak if any synergism over pure *Z*9-14:Ac alone (Table III). This is in full agreement with the respective data obtained in 1982 (see *Z*9-14:Ac + *Z*11-16:Ac combinations 100/5 *vs.* 100/25 in Table II).

Binary mixtures with *Z*11-14:Ac had already been studied at Celle (Lüneburger Heide) in 1978 [2]. In these earlier trials the *P. flammea* males had clearly preferred the mixture ratio of 100/5 over ratios of these compounds of 100/1 or 100/25, in all test replications [2]. Thus, although *Z*11-14:Ac has been isolated from the pheromone secretion of virgin *P. flammea* females at levels of only 1% of the major constituent (*Z*9-14:Ac) [1], in binary combinations a 5% relative amount of this compound is the more synergistic.

The effect of adding increasing amounts of *Z*11-16:Ac to the binary "standard attractant", *Z*9-14:Ac + *Z*11-14:Ac in ratio 100/5, is shown in Table IV. At the lower doses the *Z*11-16:Ac had no apparent modifying effect (ratios 100/5/1 and 100/5/5) whereas the addition of higher amounts of this compound abolished captures (ratios 100/5/25 and 100/5/100 in Table IV). The analogous effect of adding higher doses of *Z*11-14:Ac to the 100/5 combination of *Z*9-14:Ac + *Z*11-16:Ac is seen from Table V. Thus, in the various test series conducted in 1982 and 1983, the two "synergists" appeared to be mutually "inhibitory" on each other when used as third components in an overdose amount.

The lack of responses to the 100/25 combination of *Z*9-14:Ac + *Z*11-14:Ac in the tests in Hessen, 1983, is in obvious disagreement to results obtained with this mixture on a comparatively higher moth population at Celle in 1978. These earlier studies included four different combinations of *Z*9-14:Ac + *Z*11-14:Ac. Among these the 100/5 mixture ratio was the more effective in all test replications but the 100/25 ratio was also considerably attractive, capturing approx. 50 fold more males as compared to the same amount of pure *Z*9-14:Ac alone [2]. In test on even higher *P. flammea* populations in Scotland, 1979/81, combinations of *Z*9-14:Ac + *Z*11-14:Ac of the different ratios of 100/5, 100/25 and 100/100 all gave high catches [13]. Analogously, on studying binary combinations of *Z*9-14:Ac + *Z*11-16:Ac, the 100/25 mixture ratio was highly attractive in Scotland in 1980 but almost unattractive in Hessen in 1983, as mentioned above (see Tables I *vs.* III); and as a third component in addition to the "standard attractant" (*Z*9-14:Ac + *Z*11-14:Ac, 100/5) this same amount of *Z*11-16:Ac did not markedly reduce captures in the tests in Scotland whereas it was strongly "inhibitory" in Hessen in 1983 (see 100/5/25 in Tables I *vs.* IV). The test areas mentioned represented different levels of local moth populations, ranging from low (Hessen) to high-density sites (Scotland). The above results thus do not necessarily implicate geographic differences in pheromonal responses within this moth species – the results could also indicate density-dependent actions of the two "synergists", *Z*11-14:Ac and *Z*11-16:Ac, on male orientation responses as reflected by trap captures. According to this hypothesis, a preference for the ternary pheromonal mixture, not observed during the present study, might possible emerge from further tests on even lower moth populations.

Table IV. Captures of *Panolis flammea* males in tetratraps baited with synthetic pheromone components. Seligenstadt, March 27 to May 31, 1983; four replicates.

Amount of chemical [μg/trap]			$\bar{X}$ males/trap
*Z*9-14:Ac	*Z*11-14:Ac	*Z*11-16:Ac	
100	0	0	1.0
100	5	1	10.0
100	5	5	8.5
100	5	25	0.5
100	5	100	0.25
100	5	0	6.25

Table V. Captures of *Panolis flammea* males in tetratraps baited with synthetic pheromone components. Seligenstadt, April 20 to May 31, 1983; four replicates.

Amount of chemical [μg/trap]			$\bar{X}$ males/trap
*Z*9-14:Ac	*Z*11-14:Ac	*Z*11-16:Ac	
100	0	5	11.0
100	5	5	7.5
100	25	5	0.5
100	100	5	0
100	25	0	1.0
100	100	0	0.25

The remaining "key compound", *Z*7-12:Ac, was field tested in the Hessen area only, setting up a preliminary series towards the end of the 1982 flight season. In this series two different amounts of *Z*7-12:Ac were combined to the 100/5 "standard attractant" and the 100/1/5 pheromonal mixture (Table VI). The addition of 10 μg of *Z*7-12:Ac had no apparent modifying effect, whereas the 100 μg dosis strongly reduced captures, for both attractants (Table VI). In 1983, five different amounts of *Z*7-12:Ac were tested against these two attractants and the 100/5 *Z*9-14:Ac + *Z*11-16:Ac binary mixture, in three concurrent series (Tables VII – IX). A lowering effect is now apparent even for the 1 μg dosis of *Z*7-12:Ac, and in all three series the captures can be noted to decrease gradually on further raising the amount of this chemical, although a few moths still responded to the mixtures containing 100 μg of *Z*7-12:Ac (Tables VII – IX). Based on these data the *Z*7-12:Ac may be classified as an "attraction inhibitor" in *P. flammea*, the *Z*7-12:Ac receptor providing a further example of an "inhibitory cell", as already documented for other species of this moth family (see [9 – 11]).

The combination of the same four types of receptor cells (specialized for *Z*11-14:Ac, *Z*9-14:Ac, *Z*11-16:Ac, and *Z*7-12:Ac, respectively) has already been reported from another Noctuidae: Hadeninae species, the broom moth *Polia pisi* (L.) [8]. Virgin

Table VI. Captures of *Panolis flammea* males in tetratraps baited with three pheromone components and the non-pheromonal compound Z7-12:Ac. Seligenstadt, April 16 to May 11, 1982; four replicates.

Amount of chemical [μg/trap]				$\bar{X}$ males/trap
Z9-14:Ac	Z11-14:Ac	Z11-16:Ac	Z7-12:Ac	
100	5	0	0	9.5
100	1	5	0	7.0
100	5	0	10	9.5
100	5	0	100	0
100	1	5	10	5.25
100	1	5	100	1.25

Table VII. Captures of *Panolis flammea* males in tetratraps baited with two pheromone components and the "inhibitor" Z7-12:Ac. Seligenstadt, March 27 to May 31, 1983; four replicates.

Amount of chemical [μg/trap]			$\bar{X}$ males/trap
Z9-14:Ac	Z11-14:Ac	Z7-12:Ac	
100	5	0	9.25
100	5	1	6.25
100	5	3	1.0
100	5	10	1.75
100	5	30	0
100	5	100	0.25

Table VIII. Captures of *Panolis flammea* males in tetratraps baited with two pheromone components and the "inhibitor" Z7-12:Ac. Seligenstadt, March 27 to May 31, 1983; four replicates.

Amount of chemical [μg/trap]			$\bar{X}$ males/trap
Z9-14:Ac	Z11-16:Ac	Z7-12:Ac	
100	5	0	15
100	5	1	12.5
100	5	3	2.75
100	5	10	4.25
100	5	30	1.0
100	5	100	0.75

Table IX. Captures of *Panolis flammea* males in tetratraps baited with synthetic female pheromone and the "inhibitor" Z7-12:Ac. Seligenstadt, March 27 to May 31, 1983; four replicates.

Amount of chemical [μg/trap]				$\bar{X}$ males/trap
Z9-14:Ac	Z11-14:Ac	Z11-16:Ac	Z7-12:Ac	
100	1	5	0	17.25
100	1	5	1	5.5
100	1	5	3	3.75
100	1	5	10	3.0
100	1	5	30	1.5
100	1	5	100	1.0

P. pisi females produce as their sex pheromone a binary mixture of Z9-14:Ac + Z11-14:Ac [15], with no traces of C_{12} or C_{16} acetates found in the pheromone gland effluents. In field trapping tests, *P. pisi* males were selectively attracted to binary combinations of Z9-14:Ac + Z11-14:Ac in a ratio of close to 100/100; Z7-12:Ac or Z11-16:Ac did not show synergistic properties in this species, at any combinations, but rather reduced captures when added to the above (100/100) attractant as third components [8]. This is different from the pattern of responses reported here for *P. flammea*. The two noctuid species thus provide a further case of moth species that maintain their species-specific patterns of behavioural responses to pheromonal stimuli through using a common set of male receptor types (see [9–11]).

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