Secondary Sex Pheromone Components of *Choristoneura murinana* Hb. (Lepidoptera: Tortricidae)

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The alkenyl acetates *E*9-12:Ac, Δ11-12:Ac and *Z*11-14:Ac were identified as minor components of the *C. murinana* female pheromone blend by chemical analyses of volatile pheromone gland constituents and potential pheromone precursors, electrophysiological recordings from single receptor cells, and field trapping tests. Gland washes from virgin females contained these compounds at 3%, 10% and 5%, respectively, the amount of the primary pheromone component *Z*9-12:Ac already reported. A 0.3% addition of either Δ11-12:Ac or *Z*11-14:Ac significantly raised trap captures over *Z*9-12:Ac alone and a 3–30% addition of either minor component revealed maximum captures, not increased further by including both synergists within the same blend. A functional role of the *E*9-12:Ac could not be established during this study; 3% of it when combined with the pheromonal ratio mixture of the three other components tended to increase trap captures further whereas in various other mixture combinations the *E*9-12:Ac strongly reduced captures. These inhibitory effects were more pronounced with attractant blends based on *Z*11-14:Ac rather than Δ11-12:Ac. Each blend component activated its own type of antennal receptor cell.

Male European fir budworm moths *Choristoneura murinana* Hb. (Lepidoptera: Tortricidae) respond strongly to synthetic lures containing (*Z*)-9-dodecenyl acetate and (*Z*)-11-tetradecenyl acetate in a *Z*9-12:Ac / *Z*11-14:Ac ratio of 10/1 [1, 2]. This attractant was discovered as a result of combined electrophysiological, gas chromatographic, and field trapping studies [1] and has permitted the monitoring of *C. murinana* populations in potential outbreak areas in different parts of Europe [3–5].

Analyses of hexane washes from pheromone glands of unmated *C. murinana* females, collected in 1977–1979, revealed *Z*9-12:Ac as the major component of the female sex pheromone but did not show any trace of *Z*11-14:Ac (or other tetradecenyl acetates) [1, 6]. This raised doubts as to whether this trapping synergist was a natural pheromone constituent. Receptor cells on the male antenna, sensitive to (*E*)-9-dodecenyl acetate (*E*9-12:Ac) and (*Z*)-9-tetradecenyl acetate (*Z*9-14:Ac), respectively, drew attention to these compounds as potential minor pheromone components, but these compounds rather reduced than increased trap catches [1, 2].

A shortage of insect material prevented further chemical analyses for some years, but we continued electrophysiological and field trapping studies in search for other synergist(s) that might have been missed in previous work. These studies led to the discovery of Δ11-dodecenyl acetate (Δ11-12:Ac) as a specific "receptor key compound" and potent "trapping synergist" for male *C. murinana*. Chemical analyses made in 1986 confirmed the presence of Δ11-12:Ac as well as the earlier reported synergist *Z*11-14:Ac in pheromone gland extracts.

Electrophysiology

Single unit recordings were made from sensilla trichodea on *C. murinana* male antennae using procedures applied earlier to this species [1, 2] and other Tortricinae moths [7]. The four cell types reported

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previously [1, 2], specific to *Z*9-12:Ac, *E*9-12:Ac, *Z*9-14:Ac and *Z*11-14:Ac, respectively, were regularly found again. There was no evidence in these sensilla of alcohol- or aldehyde-sensitive receptor cells, or cells tuned to a particular di-unsaturated acetate analogue. However, in some recordings, spike activity of a further unit was observed which showed only poor responses to the four "key compounds" listed above. This cell usually displayed nerve impulses with less than half the amplitude of the *Z*9-12:Ac receptor cell, and could thus be studied more closely by using sensilla in which only these two cells were active (as indicated by the lack of responses to test stimuli of the three other "key compounds"). A systematic examination of responses to synthetic chemicals showed that this unit was a specialist receptor cell for Δ11-12:Ac. Nerve impulse discharges in response to this compound usually occurred at the low stimulus amount of 10^{-2} µg, and the graded responses towards analogous chemicals [7–10] agreed closely with those observed for identified Δ11-12:Ac receptor cells in other moth species.

Field Tests, 1983–1985

Field trapping tests on potential synergistic effects of the "key compound" Δ11-12:Ac were conducted in silver-fir stands of the Vosges Mountains, eastern France, in 1983–1985. Test sites and experimental procedures were as in previous *C. murinana* studies in this area [1, 2]. All comparisons were again made at the level of 10 µg of the primary attractant component, *Z*9-12:Ac. The presence of Δ11-12:Ac in the female pheromone was unknown at that time and a broad range of different mixture ratios was therefore included in the tests.

A first series was set out in June of 1983. The capture rates from the two formulations containing 0.3% of Δ11-12:Ac were approx. 4-fold above those obtained with the reference lure of 10 µg *Z*9-12:Ac + 0.1 µg *Z*11-14:Ac (Table I), suggesting that the Δ11-12:Ac was equal or even superior to *Z*11-14:Ac with respect to synergizing trapping responses. The dienic analogue (*Z*)-9,11-dodecadienyl acetate (*Z*9,11-12:Ac), included in the test because of considerable activity in some sensillum recordings, did not show any synergistic effect (Table I).

Further field tests were aimed at a closer comparison of the synergists Δ11-12:Ac and *Z*11-14:Ac. Trapping results previously obtained [1, 2, 11] with different mixtures of *Z*9-12:Ac / *Z*11-14:Ac (including modifying effects of two other "key compounds" on these mixtures) are summarized in Table II. These data provided the reference for studying the new synergist, Δ11-12:Ac.

Six different mixtures of *Z*9-12:Ac / Δ11-12:Ac were compared with the optimally attractive *Z*9-12:Ac / *Z*11-14:Ac combination of 10 + 1 µg in a subsequent series. Captures were equally high for the latter combination and those of 10 µg *Z*9-12:Ac with either 0.3, 1, or 3 µg of Δ11-12:Ac; the other three *Z*9-12:Ac / Δ11-12:Ac mixtures produced significantly lower captures (Table III). This pattern compares closely with results previously obtained with *Z*9-12:Ac / *Z*11-14:Ac mixtures of these six ratios (Table II).

We then studied the detailed effects of adding to a 10 + 1 µg mixture of *Z*9-12:Ac / Δ11-12:Ac varying doses of either *Z*9-14:Ac or *E*9-12:Ac. Both com-

Table I. Captures of *C. murinana* males in tetratraps baited with *Z*9-12:Ac alone or in combinations with three potential synergists. Guebwiller, June 28 to July 4, 1983; four replicates.

Amount [µg/trap] of *Z*9-12:Ac	*Z*9,11-12:Ac	Δ11-12:Ac	*Z*11-14:Ac	Total* catch
10	0	0	0	18 bc
10	0	0.3	0	201 a
10	0	0	0.1	47 b
10	0	0.3	0.1	208 a
10	0.3	0	0	10 cd
10	0.3	0	0.1	12 cd
blank				0 d

* Numbers followed by the same letter are not significantly different (P = 0.05, Tukey's test).

Table II. Attractivity of combinations of four "receptor key compounds" for male *C. murinana*. Summary of field trapping results obtained in 1979–1983 [1, 2, 11].

Amount [µg/trap] of *Z*9-12:Ac	*Z*11-14:Ac	*E*9-12:Ac	*Z*9-14:Ac	Catch* rate
10	–	–	–	+
10	0.03	–	–	+
10	0.1	–	–	++
10	0.3	–	–	+++
10	1	–	–	+++
10	3	–	–	+++
10	10	–	–	+++
10	30	–	–	++
10	100	–	–	+
10	300	–	–	–
10	0.03	0.1	–	–
10	0.03	0.3	–	–
10	0.03	1	–	–
10	0.03	3	–	–
10	0.1	0.1	–	+
10	0.1	0.3	–	–
10	0.1	1	–	–
10	0.1	3	–	–
10	0.3	0.1	–	+++
10	0.3	0.3	–	++
10	0.3	1	–	–
10	0.3	3	–	–
10	1	0.03	–	+++
10	1	0.1	–	+++
10	1	0.3	–	+++
10	1	1	–	+
10	1	3	–	–
10	1	10	–	–
10	3	0.3	–	+++
10	3	1	–	+++
10	3	3	–	+
10	3	10	–	–
10	30	0.3	–	++
10	30	1	–	++
10	30	3	–	+
10	30	10	–	–
10	300	3	–	–
10	300	10	–	–
10	0.1	–	0.1	++
10	0.1	–	0.3	++
10	0.1	–	1	+
10	0.3	–	1	+++
10	0.3	–	3	++
10	0.3	–	30	+
10	1	–	0.1	+++
10	1	–	0.3	+++
10	1	–	1	+++
10	1	–	3	++
10	1	–	10	+
10	1	–	30	+
10	3	–	3	++
10	3	–	30	+
10	30	–	3	+
10	30	–	30	–

* Symbols +++, ++ and + denote significant captures corresponding to > 75%, 33–75% and < 33%, respectively, of standard (10 µg *Z*9-12:Ac + 1 µg *Z*11-14:Ac); – symbolizes captures not significantly different from blank.

Table III. Captures of *C. murinana* males in tetratraps baited with *Z*9-12:Ac alone or in combinations with two alternative synergists. Guebwiller, July 4 to 11, 1983; four replicates.

Amount [µg/trap] of *Z*9-12:Ac	*Δ*11-12:Ac	*Z*11-14:Ac	Total* catch
10	0	0	2 c
10	0.03	0	12 bc
10	0.1	0	66 ab
10	0.3	0	138 a
10	1	0	117 ab
10	3	0	143 a
10	10	0	90 ab
10	0	0.1	182 a
10	0	1	124 a
blank			1 c

* Numbers followed by the same letter are not different (P = 0.05).

pounds are perceived *via* separate specialist receptor cells [2] and were earlier found to reduce *C. murinana* trap catches when added in more than a few percent to synergistic *Z*9-12:Ac / *Z*11-14:Ac blends [1, 2]. The *Z*9-14:Ac at 3 µg or 10 µg significantly lowered responses to the 10 + 1 µg *Z*9-12:Ac / *Δ*11-12:Ac attractant blend; a 0.3 µg of *E*9-12:Ac had no obvious effect whereas 3 µg abolished captures (Table IV). Similar reducing effects of the two compounds have previously been observed with respect to the 10 + 1 µg mixture of *Z*9-12:Ac / *Z*11-14:Ac (Table II).

However, in these third-component tests, distinct differences between the synergists *Δ*11-12:Ac and *Z*11-14:Ac became apparent when using attractant mixtures other than 10 + 1 µg. Thus, with 10 + 0.1 µg or 10 + 0.3 µg of *Z*9-12:Ac / *Δ*11-12:Ac, the addition of up to 0.3 µg of *E*9-12:Ac had only a weak, if any, lowering effect on captures (Table V A). In contrast, 0.3 µg *E*9-12:Ac when added to the 10 + 0.1 µg mixture of *Z*9-12:Ac / *Z*11-14:Ac totally prevented captures (Table II). Another test series (Table V B) showed that *E*9-12:Ac doses as high as 10 µg did not markedly reduce responses to 10 +

Table V. Captures of *C. murinana* males in tetratraps baited with 10 µg of *Z*9-12:Ac and varying amounts of synergist (*Δ*11-12:Ac) and inhibitor (*E*9-12:Ac), in two test series (A, B). Guebwiller, July 4 to August 7, 1984; each series four replicates.

	Amount [µg/trap] of *Z*9-12:Ac	*Δ*11-12:Ac	*E*9-12:Ac	Total* catch
A	10	0	0	98 c
	10	0.1	0	333 b
	10	0.1	0.1	363 b
	10	0.1	0.3	151 bc
	10	0.1	1	100 c
	10	0.3	0	599 a
	10	0.3	0.3	465 ab
	10	0.3	1	499 ab
	blank			2 d
B	10	0	0	66 c
	10	3	0	390 a
	10	3	1	475 a
	10	3	3	404 a
	10	3	10	174 bc
	10	30	1	258 ab
	10	30	3	384 a
	10	30	10	367 a
	blank			0 d

* Numbers within the same column followed by the same letter are not different (P = 0.05).

Table IV. Captures of *C. murinana* males in tetratraps baited with 10 µg *Z*9-12:Ac + 1 µg *Δ*11-12:Ac as the basic lure and varying quantities of *E*9-12:Ac or *Z*9-14:Ac. Guebwiller, July 11 to 20, 1983; four replicates.

Amount [µg/trap] of *Z*9-12:Ac	*Δ*11-12:Ac	*E*9-12:Ac	*Z*9-14:Ac	Total* catch
10	1	0	0	290 a
10	1	0.3	0	354 a
10	1	1	0	244 a
10	1	3	0	5 c
10	1	0	3	133 ab
10	1	0	10	38 b
blank				2 c

* Numbers followed by the same letter are not different (P = 0.05).

Table VI. Captures of *C. murinana* males in tetratraps baited with 10 µg of *Z*9-12:Ac and varying amounts of synergist (*Δ*11-12:Ac or *Z*11-14:Ac) and inhibitor (*E*9-12:Ac). Guebwiller, July 8 to 17, 1985; four replicates.

Amount [µg/trap] of *Z*9-12:Ac	*Δ*11-12:Ac	*Z*11-14:Ac	*E*9-12:Ac	Total* catch
10	0	0	0	30 c
10	3	0	0	242 a
10	3	0	3	196 ab
10	0	3	0	235 a
10	0	3	3	12 c
10	30	0	0	111 b
10	30	0	10	18 c
10	0	30	0	125 ab
10	0	30	10	16 c
blank				1 d

* Numbers followed by the same letter are not different (P = 0.05).

3 µg or 10 + 30 µg of *Z*9-12:Ac / *Δ*11-12:Ac. This is again in contrast to results obtained on *Z*9-12:Ac / *Z*11-14:Ac mixtures of these same ratios (Table II). Additional tests were conducted in the 1985 flight season, now including ternary *Z*9-12:Ac / *Δ*11-12:Ac / *E*9-12:Ac and *Z*9-12:Ac / *Z*11-14:Ac / *E*9-12:Ac mixtures in the same component ratios within the same test series. The results were fully consistent with those presented here.

From the various trapping results we concluded that *Δ*11-12:Ac and *Z*11-14:Ac were equally effective with respect to enhancing attraction responses of male *C. murinana* to its primary pheromone component *Z*9-12:Ac. However, mixtures based on *Δ*11-12:Ac appeared to be less affected by the addition of third components, compared to mixtures based on *Z*11-14:Ac.

With the latter compound combination it had earlier been found that increasing additions of *E*9-12:Ac could be "compensated for" by raising the portion of *Z*11-14:Ac in the mixture. For example, whereas 1 µg of *E*9-12:Ac was sufficient to nullify responses to *Z*9-12:Ac / *Z*11-14:Ac mixtures such as 10 + 0.3 µg or 10 + 1 µg, a 10 µg of it was required to obtain the same effect on a 10 + 3 µg or a 10 + 30 µg mixture (Table II). An analogous relationship is not apparent for the *Z*9-12:Ac / *Δ*11-12:Ac combinations studied (Tables IV, V), due to the far lower overall reduction effect of the *E*9-12:Ac on these mixtures.

Chemical Analyses

In the pheromone gland washes analyzed in 1977–1979 [6] (obtained from females collected as pupae in a Polish outbreak area), only the major component (*Z*9-12:Ac) could be identified. The Silar 10 C glass capillary column used in these early analyses separated the *Z*9-12:Ac from all positional isomers except *Δ*11-12:Ac, which thus might have been present [12]. The *Z*11-14:Ac was absent from these washes at a detection limit of 0.2% of the *Z*9-12:Ac [1, 6].

In 1986 a limited number of female *C. murinana* collected as pupae near Sion, western Switzerland, were available for gas chromatographic analysis. Two extracts of 6 and 17 pheromone glands, respectively, were prepared in redistilled hexane from 2–3 day old insects 2 h into the 7 h dark period. The hexane samples were analyzed by GC after a few min of extraction. The 17 glands were subsequently subjected to total lipid extraction with a 2:1 mixture of chloroform : methanol (v/v). The fatty acid moieties were then converted to methyl esters by base methanolysis as described in ref. [13].

The samples were injected splitless (split-valve opened 1 min after injection) on Hewlett Packard model 5830 and 5880 gas chromatographs equipped with flame ionization detectors. The injector temperature was 250 °C and hydrogen carrier gas was supplied at 40 cm/s linear flow. A medium polar polyethylene glycol capillary column (DB-wax, 30 m × 0.25 mm i.d.) and a non-polar methyl silicone column (DB-1, 30 m × 0.25 mm i.d.) were employed for the GC analysis. Both columns were manufactured by J & W Scientific Inc., Rancho Cordova, Ca. Separation of the pheromone samples on the DB-wax column (Fig. 1 A) revealed a major peak with the same retention

time as synthetic *Z*9-12:Ac and *Δ*11-12:Ac, which coelute on this type of stationary phase. Other peaks matched the retention times of synthetic 12:Ac, *E*9-12:Ac and *Z*11-14:Ac. Analysis on the non-polar column (Fig. 1B) gave a major peak with the retention time of *Z*9-12:Ac and minor peaks with the retention times of *Δ*11-12:Ac, 12:Ac and *Z*11-14:Ac. A leading shoulder on the major peak had the same retention time as synthetic *E*9-12:Ac. Thus based on analysis of the hexane extracts on the two columns of different polarity we concluded that female *C. murinana* moths produce *Z*9-12:Ac, *Δ*11-12:Ac, *E*9-12:Ac, 12:Ac, and *Z*11-14:Ac in the approximate proportions 100/10/3/6/3. The absolute amount of *Z*9-12:Ac per female gland was about 0.5 ng.

The occurrence of *E*9-12:Ac and *Z*11-14:Ac in the extracts was quite contrary to earlier results [1, 6, 12], but both compounds could be expected as minor gland constituents from postulated biosynthetic routes to *Z*9-12:Ac and *Δ*11-12:Ac (Fig. 2). Moth

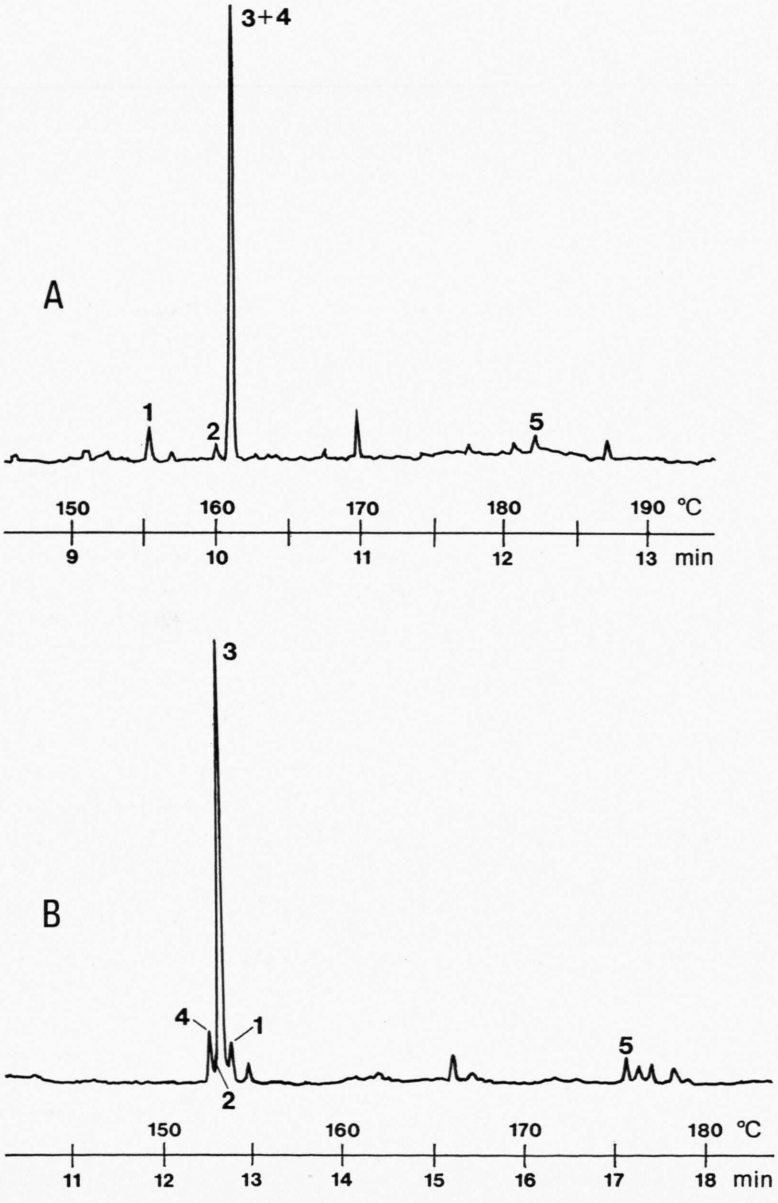

Fig. 1. Gas chromatographic analyses of hexane extracts of pheromone glands from female *C. murinana* on a medium polar DB-wax column (A) and on a non-polar DB-1 column (B). The samples injected amounts to 20% of an extract containing 17 female equivalents (FE) and half of an extract containing 6 FE, respectively. 1 = dodecyl acetate (12:Ac); 2 = (*E*)-9-dodecenyl acetate (*E*9-12:Ac); 3 = (*Z*)-9-dodecenyl acetate (*Z*9-12:Ac); 4 = *Δ*11-dodecenyl acetate (*Δ*11-12:Ac); 5 = (*Z*)-11-tetradecenyl acetate (*Z*11-14:Ac).

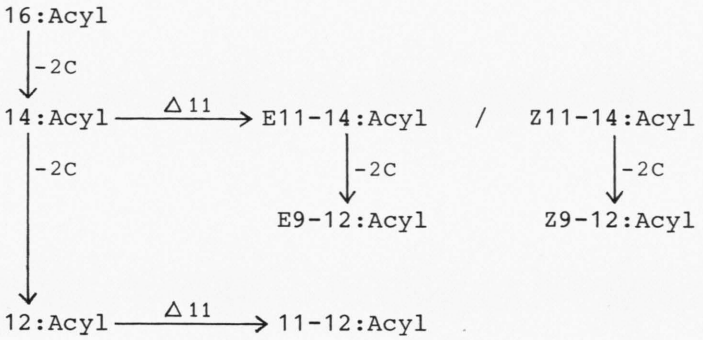

Fig. 2. Postulated biosynthetic routes to the acetates found in female *C. murinana* pheromone gland extracts, *via* chain shortening and delta 11-desaturation. The fatty acids so produced are finally reduced to alcohols and acetylated.

pheromones in most cases seem to be biosynthetically derived from palmitic acid by a combination of chain shortening and delta 11-desaturation [14, 15]. Analysis of fatty acids in the *C. murinana* pheromone glands (Table VII) confirmed the presence of the suggested precursors and supported the proposed pathways, which corroborates the assignments of the minor components.

In 1987 two batch extracts were prepared from French insects (Mont Ventoux) and two from Swiss (Sion), containing 13, 11, 11, and 14 female equivalents, respectively. The average relative amounts of *Z*9-12:Ac, *Δ*11-12:Ac, *E*9-12:Ac, 12:Ac, and *Z*11-14:Ac (ranges within parentheses) were 100 (by definition) / 10 (8–13) / 2.7 (2.2–2.9) / 9.3 (7.1–11) / 7.2 (3.9–13), respectively. These amounts confirmed our results from the year before. After the analysis the remaining portions of the four extracts were pooled in one extract, which was subjected to GC-MS analysis on a Hewlett Packard 5970B mass spectrometer equipped with a 30 m × 0.25 mm i.d. DB-wax capillary column. Electron impact mass spectra were recorded by scanning from *m/z* 40 to 300. The peaks earlier identified as 12 and 14 carbon acetates based on their retention times had mass spectra that agreed with those of the respective synthetic reference and all had *m/z* 43 as the base peak. The peak assigned 12:Ac contained the diagnostic fragments (relative abundance) *m/z* 61 (39) and 168 (6); *E*9-12:Ac contained *m/z* 61 (15) and 166 (9); the peak presumably containing coeluting *Z*9-12:Ac and *Δ*11-12:Ac had *m/z* 61 (9) and 166 (8); and finally the peak assigned *Z*11-14:Ac was characterized by *m/z* 61 (10) and 194 (10).

Table VII. Fatty acids identified from pheromone glands of female *C. murinana*, based on the correspondance of their retention times with those of synthetic reference compounds on two capillary GC columns of different polarity (see text).

Fatty acid	Amount relative to *Z*9-12:acyl
12:Acyl	52
*E*9-12:Acyl	6
*Z*9-12:Acyl	100
Δ11-12:Acyl	6
14:Acyl	191
*E*11-14:Acyl	173
*Z*11-14:Acyl	370

Field Tests, 1987/88

The 1983–1985 field tests were conducted not knowing the identity and relative amounts of minor components present in the *C. murinana* female pheromone blend. Although the five "receptor key compounds" included in these tests were, with the exception of *Z*9-14:Ac, later found to be present in the gland washes, most mixture ratios field tested in 1983–1985 were far from the pheromonal one. Thus, a 100/10/5 mixture of *Z*9-12:Ac / *Δ*11-12:Ac / *Z*11-14:Ac (as found in the female secretion) was missing from the test, and potential enhancing effects on trap captures of low amounts of *E*9-12:Ac were not studied in detail.

New test series were therefore set out in 1987 with emphasis on *Z*9-12:Ac / *Δ*11-12:Ac / *Z*11-14:Ac / *E*9-12:Ac combinations close to the pheromonal one. In these tests the 100/10/3/3 mixture of all four compounds (as identified from the female secretion) revealed the highest trap catch among all test formulations though the difference against the respective 100/10/3 ternary mixture missing the *E*9-12:Ac was not statistically significant (Table VIII). A similarly

Table VIII. Captures of *C. murinana* males in tetratraps baited with 10 µg of *Z*9-12:Ac and varying amounts of three minor pheromone components, in two test series (A, B). Guebwiller and Ehrenstetten, July 2 to 16, 1987; each series six replicates.

	Amount [µg/trap] of *Z*9-12:Ac	*Δ*11-12:Ac	*Z*11-14:Ac	*E*9-12:Ac	Total* catch
A	10	0	0	0	34 c
	10	0.03	0	0	122 bc
	10	0	0.03	0	26 c
	10	0.03	0.03	0	147 bc
	10	0.1	0	0	321 a
	10	0.1	0.03	0	330 a
	10	0.1	0.03	0.3	150 b
B	10	0	0	0	75 c
	10	1	0	0	316 ab
	10	0	0.3	0	247 ab
	10	1	0.3	0	348 ab
	10	1	0.3	0.3	479 a**
	10	1	0	3	182 bc
	10	1	0.3	3	154 bc

* Numbers within the same column followed by the same letter are not different (P = 0.05).
** Pheromonal component ratio.

low enhancing effect of a 3% *E*9-12:Ac addition, previously observed for the 100/10 *Z*9-12:Ac / *Δ*11-12:Ac binary attractant combination, was neither statistically significant (Table IV).

The present tests also show that the inclusion of both synergists (*Δ*11-12:Ac and *Z*11-14:Ac) within an attractant blend does not appear to enhance captures in comparison to the respective binary blend containing the *Δ*11-12:Ac alone; this holds for the 100/10/3 (pheromonal) *Z*9-12:Ac / *Δ*11-12:Ac / *Z*11-14:Ac attractant combination as well as combinations of these compounds such as 100/1/0.3 or 100/0.3/0.3 (Table VIII). Also, as shown from results presented in this Table, the 30% overdose addition of the *E*9-12:Ac similarly reduces responses to the 100/10 binary as to the 100/10/3 ternary attractant. Finally, the results from these tests further illustrate that a 3% of the *E*9-12:Ac (such as found in the gland washes) has a lowering rather than enhancing effect on captures when combined with attractive mixtures containing the other minor components in a lower than the pheromonal amount.

The above tests were carried out with traps fixed, at eye level, to the trunks of host trees, the standard arrangement used in most *C. murinana* studies [1–4]. Preliminary trap placement experiments, conducted in 1979 [1], have pointed to a somewhat closer discrimination among test formulations when these were suspended within host crown (the usual site of mating flight of the species), rather than on tree trunks. This kind of set-up was accordingly used for conducting a final test series upon potential enhancing effects of the minor component *E*9-12:Ac on male attraction. The four formulations included were: the 10/1/0.3/0.3 (pheromonal ratio) combination of *Z*9-12:Ac / *Δ*11-12:Ac / *Z*11-14:Ac / *E*9-12:Ac; the 10/1/3/0.3 combination of these same compounds (using a 10-fold higher relative amount of *Z*11-14:Ac to account for its lower release rate [16, 17] from dispensers); and the two respective ternary mixtures missing the *E*9-12:Ac. Dispensers and traps were as used previously. The test sites were stands of *A. alba* near Freiburg in the Black Forest, southwestern Germany, moderately infested by *C. murinana* as indicated by monitor trapping. The traps were each suspended within a separate tree crown, at heights of 15–23 m and distances of 15–25 m from each other. They were inspected every 1–4 d thereby rotating their positions and replacing sticky layers when necessary. Lures were replaced twice during this experiment, which took place from June 21 to July 27, 1988.

Only the capture sums over the entire trapping periods for all 6 replicates are presented (Table IX).

Table IX. Captures of *C. murinana* males in tetratraps baited with four different combinations of pheromone components *Z*9-12:Ac, *Δ*11-12:Ac, *Z*11-14:Ac and *E*9-12:Ac, placed within host tree crowns at a height of 15–23 m. Freiburg, June 21 to July 27, 1988; 6 replicates, 18 readings each.

Amount [μg/trap] of *Z*9-12:Ac	*Δ*11-12:Ac	*Z*11-14:Ac	*E*9-12:Ac	Total* catch
10	1	0.3	0	1852
10	1	0.3	0.3	1997**
10	1	3	0	1763
10	1	3	0.3	1967

* The four capture sums are not significantly different (P = 0.05).
** Pheromonal component ratio.

They show a slight increase for the two formulations containing 0.3 μg *E*9-12:Ac; for the 10/1/0.3 (pheromonal ratio) combination of the 3 other components the increase is 7.8%, and for the 10/1/3 combination it is 11.6%. However, due to considerable variation among values (not specified here) of individual readings, this increase in overall catch proved statistically insignificant (Tukey's test, P = 0.05). Thus, in this experiment again, a synergistic effect of the minor component *E*9-12:Ac could not be fully established.

Discussion

The present study provides a partial chemical and functional characterization of the long sought-for minor constituents of the *C. murinana* female sex pheromone blend. Although both synergists chemically identified, *Δ*11-12:Ac and *Z*11-14:Ac, could be expected as minor pheromone components from their behavioural effectiveness in field tests, an earlier investigation of the *C. murinana* pheromone secretion [1, 6] had failed to find any trace of *Z*11-14:Ac. This difference with respect to our present results might be due to differences in the analytical procedures used or to actual pheromone differences between the populations studied. The respective samples stem from western Switzerland and the French Alps on the one hand and central Poland on the other. A possible geographic shift in pheromone composition should be kept in mind considering the disjunct distribution of this species in Europe [18, 19].

The *Δ*11-12:Ac is a reported component of the sex pheromones of several species of Noctuidae [20–26]. In the Tortricidae it has been identified from four species; the tea tortrix *Homona magnanima* (Diak.) uses a pheromone blend consisting of *Z*11-14:Ac / *Z*9-12:Ac / *Δ*11-12:Ac in a 30/3/1 ratio [27], whereas *Homona spargotis* (Meyr.), *Argyrotaenia velutinana* (Walk.) and *Lobesia botrana* (Den. & Schiff.) females produce pheromones that include trace amounts of *Δ*11-12:Ac of unknown function [24, 28–30]. The *E*9-12:Ac and *Z*11-14:Ac are common components of tortricid sex pheromones identified from a variety of species [26].

In the electrophysiological recordings, *Δ*11-12:Ac and *Z*11-14:Ac were each found to activate a particular type of sensory receptor cell, located within separate hair sensilla on the *C. murinana* male antenna. The cells showed the usual high response specificities [7–10], the mutual activation by the other compound requiring an increase in stimulus amount of approx. 100-fold. Thus, the "synergistic" effects produced by low amounts of either *Δ*11-12:Ac or *Z*11-14:Ac in the field trapping tests cannot be due to activation of a common type of sensory cell. The results rather indicate that there are two different "attractive cell type combinations" in *C. murinana,* each capable of inducing oriented flight towards an attractant source.

It is well established that, in pheromone systems of Lepidoptera, different minor components of the female-produced blend might substitute for one another, a phenomenon characterized as "redundancy of the chemical signal" [23, 24]. Bjostad *et al.* [22] found that female cabbage loopers *Trichoplusia ni* (Hbn.) release a six-component acetate blend; in subtraction tests all five-component blends, with the exception of the one lacking the primary pheromone component *Z*7-12:Ac, and several four-component blends elicited similar peak levels of behavioural ac-

tivity to that observed with the six-component blend. Female pine beauty moths *Panolis flammea* (Schiff.) release a ternary blend of *Z*9-14:Ac / *Z*11-14:Ac / *Z*11-16:Ac in a 100/1/5 ratio [31, 32]; each compound activated its corresponding type of male sensory cell in electrophysiological recordings, and binary combinations of the primary component with either synergist produced the full rate of attraction responses in wind tunnel and field trapping tests [32–34]. A similar pattern appears to apply to *C. murinana* considering the present finding that attractant formulations containing only one minor component, either *Δ*11-12:Ac or *Z*11-14:Ac, produced full capture rates.

Our field tests of various attractant mixtures did not reveal any marked difference between these two minor components concerning their synergistic effectiveness. Either compound as a 0.3 to 300% addition enhanced the response of male *C. murinana* to *Z*9-12:Ac sources, with maximum attraction occurring at additions ranging from 3 to 100%. We do not know of similar attraction patterns observed in any other moth species. Usually with sex-attractant systems of Lepitoptera, synergistic effects of secondary pheromone components on trap captures are limited to a narrow range of relative amount, with higher amounts reducing rather than increasing captures. In *C. murinana* the two synergistic minor pheromone components (*Δ*11-12:Ac, *Z*11-14:Ac) did not show inhibitory properties even at a 10-fold overdose over the primary component.

With respect to this unusual attraction pattern, it should be kept in mind that a trap capture study may not necessarily sort out the maximally effective attractant formulation for a test species, especially when using an experimental set-up in which suboptimal formulations are likely to lead responding males to the attractant source. Possibly this could have occurred in those of our *C. murinana* tests in which traps were fixed, at eye level, to the trunks of host trees at sites of relatively high moth density. Yet, even with traps suspended within tree crowns (an arrangement that appeared to produce more specific attraction responses in some earlier tests [1]), the pheromonal four-component combination was not significantly preferred over several related (quaternary or ternary) ones. Further field trapping at sites of even lower moth density (requiring upwind orientation over a greater distance), and the detailed observation of male flight behaviour in the wind tunnel and/or the natural habitat, may reveal differences between attractant formulations which in the present study have shown up equally effectively.

Although a functional role of the *E*9-12:Ac could not be established during the present study, it is noteworthy that a 3% of this compound (such as found in the washes) when added to the 100/10/3 (pheromonal) combination of *Z*9-12:Ac / *Δ*11-12:Ac / *Z*11-14:Ac tended to increase trap captures further. Such a tendency was not apparent for *E*9-12:Ac additions lower or higher than 3%, or for mixture ratios of the three other compounds other than 100/10/3. This could point to a potential functional role of the *E*9-12:Ac within the context of a proper component ratio of all pheromone constituents, to be clarified in further behavioural studies.

Another phenomenon calling for a detailed analysis of male flight behaviour is the striking antagonism between components *Z*11-14:Ac and *E*9-12:Ac as apparent from the trapping data presented for various ternary *Z*9-12:Ac / *Z*11-14:Ac / *E*9-12:Ac mixtures. In these tests the *E*9-12:Ac consistently acted "inhibitorily" when present in an amount equal or higher than the *Z*11-14:Ac but not (with few exceptions) in a lower relative amount. Accordingly, the reducing effect on captures displayed by a given amount of *E*9-12:Ac could be "compensated for" by raising the portion of *Z*11-14:Ac in the test mixture (Table II). This could suggest some "stabilization", by the overdose amount of one minor component (*Z*11-14:Ac), against distortion of male orientation flight by the other (*E*9-12:Ac). We are not aware of similar phenomena having been observed in other moth species.

Compared to the *C. murinana* female pheromone reported here, the standard lure composed of 100 µg *Z*9-12:Ac + 10 µg *Z*11-14:Ac [1] is an incomplete representation of the female-produced blend, missing the minor components *Δ*11-12:Ac and *E*9-12:Ac. However, this binary attractant has performed adequately at various test sites [3–5] and will be maintained the standard trap bait in future population monitoring.

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