Use of Synthetic Sex Attractant of Larch Bud Moth *Zeiraphera diniana* (Gn.) in Monitoring Traps under Different Conditions, and Antagonistic Action of *cis*-Isomere[1]

Low concentrations of synthetic *trans*-11-tetradecenyl acetate (TTA) are highly attractive to males of the larch bud moth *Zeiraphera diniana* in field tests[2]. All evidence available speaks in favour of the hypothesis that the substance is identical with the natural sex attractant emitted by the females of this species[2,3]. Both, synthetic TTA and the natural pheromone, have no aphrodisiac activity in the males, but are specific attractants with action on distance only[3]. Neither the *cis*-isomere of the substance (CTA) nor the alcohols of the two isomeres proved to be attractive to *Z. diniana* in field tests[2]. However, CTA and its alcohol were attractive to males of *Zygaena transalpina*[4] (Lepidoptera: Zygaenidae).

Field traps with fresh rubber stoppers treated with 10 µg or 1 ng of TTA proved to be most attractive to males of *Z. diniana*, whereas a large amount of 5 mg of TTA per stopper never attracted any males and 2 mg per stopper only after 40 days had elapsed. However, 100 µg of TTA, which initially was less attractive than virgin females, became as attractive or better within a month of exposure in a trap[2]. The results indicate that in the course of a month the rate of evaporation of TTA from rubber stoppers is reduced to about 10% of the initial value.

Experiments were made in order to find out whether or not the addition of oil as an adjuvant would reduce the rate of evaporation of TTA from rubber stoppers and thus perhaps allow both a steadier and prolonged release of the attractant. Instead of diluting TTA with acetone and then applying it to rubber stoppers, the substance was diluted with olive oil or paraffin oil. The presence of oil evidently reduced the rate of TTA evaporation. Field tests conducted in 1971 in a forest with a moderately high population density of *Z. diniana* in the upper Engadin (1900 m above sea level) showed that the optimum concentration of TTA could be 10 times elevated without loss of attractiveness (Table I). Olive oil and paraffin oil gave the same results at this altitude. However, parallel tests conducted in a larch forest with a very low population density near Trimmis (750 m above sea level) showed that paraffin oil is the better adjuvant under these warmer climatic conditions. Within 6 days, 3 traps containing virgin females cought 35 males, whereas the same number of traps with 10 µg of TTA in paraffin or olive oil caught 40 and 17 males respectively. Practically identical results for the first two types of traps were obtained in 1972 (Table II, July 8–19).

The results of tests with different concentrations of TTA in paraffin oil conducted in 1972 in Trimmis (4 traps per concentration) are computed in Table II. The results show that a rubber stopper treated with 1 µl of paraffin oil containing 100 µg of TTA attracts more males than virgin females for at least 70 days. At the end of the season when one female trap caught 23 males per 29 days, 8 traps furnished with freshly treated rubber stoppers caught 192 or an average of 24 males/trap. Form this it

Table I. Male captures in traps in the Engadine baited with either 2 virgin females of *Z. diniana* (control) or rubber stoppers treated with 1 µl of acetone or olive oil containing different concentrations of TTA

Dose of TTA/stopper (mg)	Diluted with Acetone	Olive oil
1	46	23
10^{-1}	46	216
10^{-2}	236	239
10^{-3}	184	41
Control (2♀♀)	93	

The females were replaced each fortnight. For the combination of 1 mg of TTA and oil, 2 µl of a 50% solution of TTA in oil were applied.

Table II. Male captures in Trimmis during different time intervals in traps baited with different concentrations of TTA in paraffin oil or virgin females of *Z. diniana* (see Table I)

Dose of TTA/stopper (mg)	8–19 July	Period next 31 days	next 40 days
10^{-1}	—	125	432
10^{-2}	40	53	—
10^{-3}	8	8	—
10^{-4}	2	—	—
Control (2♀♀)	34	77	316

4 traps per variant; dashes = not tested.

Table III. Comparison of attractivity of traps containing virgin females only (control) or females and a rubber stopper with different concentrations of CTA, indicating antagonistic effect of CTA

Trap with 2♀♀ and	Period (1972) 30.8.–6.9.	next 22 days
Control	42	154
1 µg CTA	66	—
10 µg CTA	2	22
100 µg CTA	0	9

3 traps per variant.

Table IV. Antagosnism of CTA to TTA for 2 periods

Trap with 100 µg of	Period (1972) 6–28 September	next 29 days
TTA	51.3 (3)	23 (1)
TTA + CTA	2.3 (3)	0.25 (4)
empty trap	—	1 (4)

Figures indicate males/trap; figures in brackets = number of traps.

[1] Contribution Nr. 48 of the research team for the investigation of the population dynamics of the larch bud moth. The research was aided by a grant of the Swiss National Founds for Scientific Research.

[2] W. L. Roelofs, R. Cardé, G. Benz and G. von Salis, Experientia *27*, 1438 (1971).

[3] G. Benz, Experientia *29*, 553 (1973).

[4] We thank Professor W. Sauter of our Institute for the determination of the species.

may be deduced that at low population density, under the climatic conditions of Trimmis, TTA traps attract a maximum number of males of *Z. diniana* for a whole season. Virgin females on the other hand attract a smaller number of males and have to be replaced each fortnight.

Tests with the *cis*-isomere (CTA) conducted at Trimmis in 1972 indicate that this substance is a strong antagonist of the natural pheromone and synthetic TTA. Male captures of traps furnished with 2 virgin females (controls) or 2 virgin females and a rubber stopper treated with 1 µl of paraffin oil and different concentrations of CTA are computed in Table III. These results indicate that 100 µg of CTA in paraffin oil completely or almost completely antagonize the action of the natural pheromone of 2 virgin females for about 4 weeks. The 9 males trapped during the period September 6–28 may have been caught accidentally, as indicated by the results of Table IV (last column). Antagonism of CTA and TTA is shown by the results of Table IV. The findings further strengthen the hypothesis that TTA is the natural pheromone of *Z. diniana*.

The results presented in this paper show that 1 µl of a 10% solution of TTA in paraffin oil applied to a rubber stopper presents a powerful lure for males of *Z. diniana*. Because of its long lasting superior effect it can more than only replace virgin females in monitoring traps. CTA at a relatively low concentration has proved to be such a powerful antagonist of the female pheromone of *Z. diniana* that it may be called a true synthetic antipheromone. It is hoped that the substance can be used to prevent pheromone guided meetings of the sexes of the larch bud moth when the population density of the species is low enough to prevent chance meeting[3]. It may thus become a tool for the control of *Z. diniana*. Theoretically much smaller quantities of CTA should be needed to reach this effect than if TTA was used for male confusion.

Zusammenfassung. Gummizapfen mit 1 µl 10%iger Lösung von *trans*-11-Tetradecenylacetat in Paraffinöl sind während 70–100 Tagen attraktiver für Männchen des Lärchenwicklers als Fallen mit je 2 virginen Weibchen der Art, die zudem alle 2 Wochen ersetzt werden müssen. Das *cis*-Isomer der Verbindung ist ein spezifischer Lockstoff für Männchen des Widderchens *Zygaena transalpina* und ein starker Antagonist sowohl des natürlichen Weibchenpheromons des Lärchenwicklers wie des synthetischen Lockstoffs.

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Biodegradation of Microbial Lipopolysaccharides

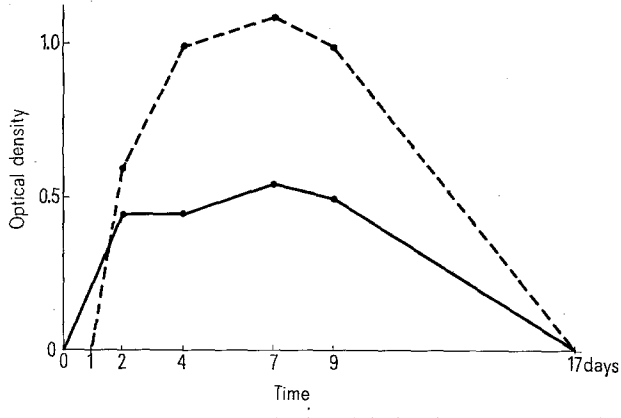
In a foregoing paper, VOETS and BEYAERT[1] described the isolation of a *Bacillus* species growing on a mineral medium with 0.2% lipopolysaccharides (LPS) extracted from a *Salmonella minnesota* R-250 strain.

In further investigations, the LPS fractions of the following bacterial strains were used: *Salmonella typhimurium* type W, *Salmonella minnesota* R-2051 and *Escherichia coli*. The impure LPS of *S. typhimurium* were obtained from Difco and purified by the hot phenol method of WESTPHAL et al.[2]. For the isolation of the LPS from *S. minnesota* R-2051, the strain was grown in batch cultures on the following medium: beef-extract 10 g; peptone 10 g; NaCl 5 g; distilled water 1 l (pH = 7.3). After 24 h of growth at 37 °C, the cells were centrifuged and acetone-dried. The LPS were extracted according to the method of GALANOS et al.[3]. Acetone-dried cells of *E. coli* were obtained from several batch cultures in nutrient broth and extracted according to the method of WESTPHAL et al.[2] as modified by O'NEILL and TODD[4].

Microbial strains growing on bacterial LPS were isolated on the following mineral medium, to which 0.2% LPS were added as sole source of carbon: $(NH_4)_2SO_4$ 1 g; K_2HPO_4 1 g; $NaH_2PO_4 \cdot 2\,H_2O$ 1.25 g; $MgSO_4 \cdot 7\,H_2O$ 0.5 g; $CaCl_2$ 0.01 g; $FeSO_4 \cdot 7\,H_2O$ 0.01 g; distilled water 1 l (pH = 7.0). On the purified LPS of *Salmonella typhimurium* type W, a few bacterial strains were isolated from a soil sample. Transfer of these strains to a liquid medium with the same composition resulted in weak growth. However, when glucose was supplied as sole source of carbon to the culture medium, abundant growth was obtained. According to Bergey's Manual of Determinative Bacteriology[5], a *Bacillus macerans* strain could be isolated in this way.

As a result of this phenomenon, further experiments were carried out with this species. The strain was inoculated into the mineral medium, containing LPS as sole source of carbon and in function of the incubation time – at 37 °C – the growth of the microorganism and the degradation pattern of the LPS were followed.

Intact LPS and its chemically degraded polysaccharide fraction (prepared by mild acid hydrolysis) could be separated easily on a Sephadex G 100 column. Small con-



---- = Liberation of free fatty acids (FFA) during the growth of *Bacillus macerans* on LPS from *Salmonella minnesota* R-2051.
—— = Liberation of free fatty acids (FFA) during the growth of *Micrococcus* sp. on purified LPS from *Salmonella typhimurium* type W.

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