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SHORT COMMUNICATIONS

C. J. Persoons[1], C. van der Kraan[2], W. J. Nooijen[1], F. J. Ritter[1], S. Voerman[2] and T. C. Baker[3]: *Sex pheromone of the beet armyworm,* Spodoptera exigua: *isolation, identification and preliminary field evaluation.*

In 1976 the beet armyworm, *Spodoptera exigua,* (Hübner) (Lepidoptera: Noctuidae) was accidentally imported into the Netherlands. The insect found its way into greenhouses, and immediately became a serious pest, mainly for chrysanthemums and gerberae, and to a lesser extent for egg-plants.

Intensive application of synthetic pyrethroids initially gave good control, but very soon proved to be insufficient. An urgent search for other control methods was therefore undertaken. Pheromones seemed to be a possible alternative, and research on the pheromone of the moth was begun. Brady & Ganyard (1972) identified (Z, E)-9, 12-tetradecadienyl acetate (1) as a sex pheromone of the moth. However, trapping experiments using (1), either alone or in combination with (Z)-9-tetradecenyl acetate (2) as a bait, were unsuccessful (Campion, 1975; Mitchell & Doolittle, 1976). These findings prompted us to reinvestigate the chemistry of the pheromone. The insects were reared on an artificial medium and the following materials were analysed:

— abdominal tips of virgin ♀♀ aged 2—3 days;
— excised glands of virgin ♀♀;
— air volatiles trapped on glass beads (Weatherston *et al.*, 1981);
— excised glands injected directly into the gas chromatograph by means of the capsule injection method (MS 41, Perkin & Elmer), (Décoins & Gallois, 1979).

An extract of abdominal tips was subsequently fractionated on silica gel and analysed by gas chromatography. Electroantennography (EAG) indicated that only the fraction containing the $\Delta^{9,12}$ C14-acetates elicited antennal responses.

Of a total of about 150 potential pheromones tested by the EAG technique, only (1) proved to be active. Compounds tested included most of the mono-unsaturated C12-, C13-, C14-, C15-, and C16-acetates, the corresponding alcohols of the C12- and C14-acetates, the 4 geometrical isomers of $\Delta^{9,12}$ C14-acetate and some aldehydes. Tests were repeated 4 times. Only two of the compounds tested [(Z) 7:12 Ac and (Z) 9:14 OH] reached the 50% level of (1). Purity of the compounds was at least 98%. We therefore had to rely on a behavioural bioassay using a flight apparatus (C. van der Kraan, *in litt.*) consisting essentially of four small identical wind tunnels, with funnels at the upwind ends, and a common chamber downwind. The samples were placed in the funnels and the male moths released halfway along the tunnels. The behaviour of the moths was observed and the numbers trapped in the funnels were taken as a measure of the attractiveness of the samples. The crude extract was fractionated on silica gel by eluting successively with hexane, 10% ether in hexane, 50% ether in hexane and ether. The behavioural test indicated the activity to be confined to the fraction containing the C14-acetates (10% ether in hexane). Combining this fraction with the other fractions collected, did not increase the activity of the former. Identification of the C14-acetates (saturated, mono- and di-unsaturated) in this fraction was then undertaken and their biological activity tested afterwards.

Analysis based on mass spectrometry, ozonolysis, and retention indices revealed the presence of (1), (2), (ZZ)-9, 12-tetradecadienyl acetate (3), (Z)-11-tetradecenyl acetate (4), and tetradecanyl acetate (5). The same five compounds were isolated from carefully excised glands free from abdominal fragments and briefly rinsed with hexane. The ratio in which the five compounds occurred in abdominal tip extracts and in excised glands was rather different. The respective ratios of (1) : (2) : (3) : (4) : (5) are 1.7 : 100 : 0.1 : 13.6 : 1.7 and 113.6 : 100 : 5.7 : 15.9 : 1.7. Analysis of air volatiles and direct injection of about 60 excised

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glands by means of the capsule injection method (MS 41, Perkin & Elmer) both revealed the presence of at least three C14-acetates, viz. (1), (2) and (3). The ratios of (1) : (2) : (3), as found in air volatiles and by the capsule injection method are 100 : 15 : 17 and 100 : 58 : 4.6, respectively. For eliciting upwind search behaviour, both (1) and (2) appeared to be necessary, while (3) is important for short range courtship behaviour, like hair pencil display and copulation attempts.

Field experiments were carried out near Riverside (California) using Pherocon-2 sticky traps and polyethylene caps as dispensers. In a first field experiment, carried out from 13—18 October, 1980 (4 trapping nights, 3 replicates per sample), a mixture containing 450 µg of (1), 450 µg of (2), 22.5 µg of (3) and 45 µg of (4) captured an average of 8.5 ♂♂ per trap per night, whereas traps baited with two virgin females caught 8.0 ♂♂ per night per trap.

A second field experiment was carried out from May 11—20, 1981 (9 trapping nights, 4 replicates per sample). The overall outcome of this experiment confirmed our previous findings. Unfortunately, no virgin ♀♀ were tested in the second experiment. The average catch per night per trap was considerably lower than in the first experiment. This might be due to the fact that the second experiment was carried out during the beginning of the flight season of this moth.

Surprisingly, a mixture of (1) and (2) (600 and 300 µg, respectively) appeared to be about as attractive as two virgin ♀♀, viz. 7.8 ♂♂ per trap per night. Mitchell & Doolittle (1976) tested a wide range of mixtures of (1) and (2), including the ratio 2 : 1, but none of them showed any field attractancy. This failure may have been due to impurities in the compounds, or to the high amounts used (10 mg), or to both.

Field experiments are now being carried out in different parts of the world to determine the optimal ratio of the various compounds.

The presence of (1), (2) and (3) in all materials analysed, the behavioural responses of ♂♂ towards them, and the field trapping results clearly indicate that the three compounds are genuine pheromone components. The function of the other two, (4) and (5), found in the extracts derived from abdominal tips and excised glands, is not yet clear, but may be of minor importance.

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Note added in proof

While this article was in press, a publication on the same subject appeared (J. H. Tumlinson, E. R. Mitchell & P. E. Sonnet. Sex pheromone components of the beet armyworm, *Spodoptera exigua*. *J. Environ. Sci. Health A16* (2), 189—200, 1981). In this article the authors claim that apart from (Z,E)9,12 : 14 Ac, (Z)9 : 14OH is an essential part of the pheromone. So far we have not been able to confirm these findings. To obtain attraction comparable to that of living ♀♀, the authors used excessive amounts of material (about 45 mg/polyethylene vial).