The Sex Pheromone of the Silver Y Moth *Chrysodeixis eriosoma* (Doubleday) in New Zealand

M. H. Benn

Chemistry Department, The University, Calgary, Alberta T2N 1N4, Canada, on leave at the Entomology Division, D.S.I.R.

R. A. Galbreath and V. A. Holt *

Entomology Division, D.S.I.R., Private Bag, Auckland, New Zealand

H. Young

Horticulture and Processing Division, D.S.I.R., Auckland, New Zealand

G. Down

Chemistry Division, D.S.I.R., Lower Hutt, New Zealand

E. Priesner

Max-Planck-Institut für Verhaltensphysiologie, D-8131 Seewiesen, German Federal Republic

Z. Naturforsch. **37 c**, 1130–1135 (1982); received August 5, 1982

Sex Pheromone, *Chrysodeixis eriosoma*, *Plusia chalcites*, Dodecenyl Acetates, Noctuidae, Olfactory Receptors

An investigation of the sex pheromone of the New Zealand Silver Y Moth *Chrysodeixis eriosoma* (Doubleday), formerly described as *Plusia chalcites* (Esper) resulted in the identification of the major active components as 7*Z*- and 9*Z*-dodecenyl acetates, in approx. 97:3 ratio. This blend was attractive to *C. eriosoma* males in the field and in a laboratory flight tunnel. 5*Z*-Dodecenyl acetate and 7*Z*-dodecenyl alcohol inhibited this response in the field. *C. eriosoma* was also attracted to the 5:1 blend of 7*Z*-dodecenyl acetate and 9*Z*-tetradecenyl acetate reported as the pheromone of *Chrysodeixis* (*Plusia*) *chalcites* in the western Palaearctic. Specialist receptor cells for all 5 compounds mentioned were found in male antennal sensilla in both species.

Introduction

The Silver Y Moth *Chrysodeixis eriosoma* (Doubleday) (Noctuidae: Plusiinae) is one of the more serious pests of horticulture in New Zealand. This species in Australia and New Zealand was formerly referred to as *Plusia chalcites* and this name is still used by some [1, 2]. However, when Kostrowicki [3] revised the Palaearctic species of the Plusiinae he reinstated the generic name *Chrysodeixis* for the latter species and divided it into two, resurrecting the name *eriosoma* Doubleday (type locality Auckland, New Zealand) which is now applied to the Indo-Australasian and Pacific Silver Y Moths [4–7] and retaining *chalcites* Esper (given as *chalcytes* by Kostrowicki; type locality Italy) for those of Africa and the western Palaearctic [4]. We undertook an examination of the sex pheromone of *C. eriosoma*, hoping to be able to utilise synthetic pheromone, along with other methods, in monitoring and controlling populations of the moth.

* Reprint requests to Dr. R. A. Galbreath

0341-0382/82/1100-1130 $ 01.30/0

Materials and Methods

Chemicals

These were from our collection. For field trials commercial samples of 7*Z*- and 9*Z*-dodecenyl acetates (7Z12:Ac, 9Z12:Ac) and 9*Z*-tetradecenyl acetate (9Z14:Ac) (Supelco Inc., Bellefonte USA) were used. All samples were > 98% pure by GLC analysis. Solvents had been purified, and redistilled.

Pheromone-extract preparation

Rearing of insects followed the procedures of Roberts [7]. They were sexed as pupae and females held at 20 °C under a reversed light cycle (16 h photophase, 8 h scotophase). At 1–2 h into scotophase, moths 2–4 days old were transferred to a cool room (4 °C) and left for about 5 min before clipping abdominal tips into pentane. The extract was filtered through a plug of glass wool (held in a Pasteur pipette) into a 1 ml Reactivial™ (Pierce Chemical Co.) and concentrated under a slow stream of nitrogen to approx. 10 µl.

Electrophysiological analyses

The electro-antennogram (EAG) responses of adult male *C. eriosoma* were determined as described by Roelofs [8]. Chemicals were tested as 1 and 10 μg amounts, applied to filter paper slips, contained in Pasteur pipettes. Single-cell responses were determined using techniques of recording, stimulation, and data analysis as in previous studies on other noctuid species [9].

Gas chromatography (GC) and GC-mass spectrometry (GC-MS)

Preparative GC of female tip extract was performed with a Varian 1700 instrument which had been modified by the introduction of a variable-ratio effluent splitter (Scientific Glass Engineering Pty) which was housed in the detector oven; this allowed part of the effluent from the GC column to reach a flame ionisation detector (FID) while the rest was vented along a stainless steel tube (0.75 mm ID, 1.6 mm OD) through a heated exit port. A split ratio of approx. 9:1 (exit: FID) was normally used. Samples were collected by attaching the tip of a long-nosed (approx. 10 cm × 1.6 mm OD) Pasteur pipette to the mouth of the exit tube using a short length of Teflon™ tubing so that a snug glass-to-metal contact was achieved. During the collections the Pasteur pipette was cooled in a tray containing powdered solid carbon dioxide.

The GC column was a stainless steel tube (2 m × 2.1 mm ID) packed with 3% polydimethylsiloxane OV™-1, on Chromosorb™ W AW-DMCS (80/100 mesh). Nitrogen was used as a carrier gas (approx. 15 ml min^{-1}) and a temperature programme was selected to give about a 4 min separation of dodecenyl, tetradecenyl, and hexadecenyl acetates (U12:Ac, U14:Ac, U16:Ac).

Analytical GLC was performed using capillary columns; A: polymethylphenylsiloxane (50% phenyl) OV-17 (SCOT, 25 m × 0.2 mm ID glass); B: polydimethylsiloxane, OV-101 (WCOT, 50 m × 0.2 mm ID, fused silica); and C: polyoxyethylene glycol, Carbowax™ 20 M (SCOT, 50 m × 0.25 mm ID). Helium was used as the carrier gas (approx. 20 cm min^{-1}). The gas chromatograph was interfaced via a membrane separator with a MS-30 mass-spectrometer (MS) operating in the electron-impact ionisation mode at 20 eV, and set up for selective ion monitoring (SIM) for up to 4 selected masses.

Field trapping

Chemicals in pentane solution were applied to 5 mm sleeve-type rubber caps (1780-B10, Arthur H. Thomas Co., USA). Pherocon 1 C traps (Zoecon Corporation, USA) baited with the caps were hung 1.5 m above ground 15 m or more apart in garden or crop areas infested with *C. eriosoma*. Trap positions were initially randomised and rotated each time the traps were checked, normally 3 times weekly. Differences between mean catches were tested for significance by the SNK test on transformed ($\sqrt{n+1/2}$) data [10].

Behavioural observations

Behavioural responses of male *C. eriosoma* to airborne scent of female tip extract and synthetic mixtures were observed in a 1.2 × 0.7 × 0.5 m flight tunnel based on that of Miller and Roelofs [11]. Male moths 1 – 3 h into scotophase were placed in an open ended gauze container held 18 cm above the floor at the end of the tunnel. Test chemicals were applied (in pentane solution) to 1 cm squares of filter paper. When the moths had settled, a test paper was placed edge on to the air flow in a wire clip at the upstream end of the tunnel so that the scent plume passed directly through the container of insects, and the behaviour of the moths recorded. Air flow through the tunnel was at 20 cm/s and the illumination measured at the tunnel floor was 2 lux.

Results and Discussion

When we commenced our work we were aware that traps set out to monitor populations of another Noctuid pest, the Greasy Cutworm, *Agrotis ipsilon* (Hufn.) were catching male *C. eriosoma* also. The lure in these traps was the 3:1 blend of 7*Z*12:Ac and 9*Z*14:Ac reported for *A. ipsilon* [12].

Among the Plusiinae, 7*Z*12:Ac is a component of most previously reported sex attractants [13] or sex pheromones [14 – 17] so we were not surprised to find that in our initial EAG screening of *C. eriosoma* to U12:Ac, U14:Ac and U16:Ac standards, the greatest response of the male antenna was to 7*Z*12:Ac (followed by 5*Z*12:Ac).

Single-cell studies revealed that the olfactory hair sensilla (*S. trichodea*) on male *C. eriosoma* antennae had four different types of acetate receptor cells, and one cell type responsive to alcohols. A closer exam-

ination of the response spectra of these cells showed that the maximally effective ('key stimulus') compounds were 5*Z*12:Ac, 7*Z*12:Ac, 9*Z*12:Ac, 9*Z*14:Ac and 7*Z*12:OH, respectively.

We then turned to an examination of female *C. eriosoma*. An extract of the abdominal tips of some 70 virgin female moths was fractionated by GLC. Under the conditions used (130 – 190 °, at 2 ° min^{-1}) the R_Ts of standards were as follows: 7*Z*12:OH 3 min 30 sec, 3*E*12:Ac 5 min 5 sec, 12*E*14:Ac 10 min 30 sec, and 7*Z*16:Ac 15 min 30 sec. The FID trace of the *C. eriosoma* extract revealed signals at 5 min 35 sec, 12 min 50 sec, and 17 min 45 sec. Timed, one- min collections were made, and the two tubes corresponding to the 4 – 6 min collection were rinsed out with pentane, and the combined washings were concentrated and reanalysed by capillary GLC (column C at 140 °). SIM was carried out for ions of mass *m/z* 168, 166 and 164 amu, corresponding to major fragment ions (M-60) of dodecyl acetate (12:Ac), dodecenyl acetate (U12:Ac), and dodecadienyl acetate (DU12:Ac). No signal was seen on the DU12:Ac monitor, but a very strong signal was observed on the 166-monitor at the correct retention time for a U12:Ac, preceded by a weak signal on the 168 trace. The component responsible for this last signal co-chromatographed with authentic 12:Ac. The retention time relative to 12:Ac internal standard (R_T^{rel}) of the major U12:Ac was 1.130 and, of all the U12:Ac standards examined under the same GLC conditions, only 7*Z*12:Ac matched this with R_T^{rel} 1.128. (In all cases the error on R_T^{rel} was ±0.003.) Besides the major U12:Ac signal there was a minor one, R_T^{rel} 1.196, uniquely matched by 9*Z*12:Ac with R_T^{rel} 1.194. The ratio of these two components was approx. 97:3.

In another experiment, an abdominal tip extract from some 20 *C. eriosoma* females was fractionated by GLC under the same conditions as before, but making a single collection from 2 – 16 min *i.e.* so as to include along with U12:Acs any U12:OH and U14:Ac that might be present. The pentane washings of this collection-tube were then analysed by chromatography on the Carbowax 20 M capillary column, with SIM for ions with *m/z* 164, 166, 168 and 194 amu. No signals were seen on the 194-monitor corresponding to U14:Ac, or on the 166-monitor corresponding to U12:OH, or on the 164 trace for DU12:Ac (or OH). As before there were an intense and a weak signal on the 166-monitor corresponding to 7*Z*12:Ac and 9*Z*12:Ac respectively, while 12:Ac was also seen on the 168 monitor. In no case did we detect any 5*Z*12:Ac.

We thus surmised that the sex pheromone of *C. eriosoma* was most probably a blend of 7*Z*12:Ac and 9*Z*12:Ac, perhaps with 12:Ac. In fact 50 µg of 7*Z*12:Ac and 9*Z*12:Ac in 50:1 ratio proved to be a good lure for *C. eriosoma* males when used in traps in the field (Table I) and, in a separate experiment attracted on average the same number (15/trap/night) as 1 – 2 day old females caged singly in similar traps on the same nights. It was noticeable however from regular observations of trap catches during the night that the female moths appeared to be more attractive in the first half hour after sunset and ceased to attract males after about 4 hours, presumably reflecting the pattern of calling behaviour, while the synthetic mixture continued to attract males at a gradually declining rate through the night (Fig. 1). Thus despite comparable nightly catches, the synthetic mixture was probably not as attractive as the pheromone released by the female.

In the wind tunnel, preliminary experiments with female tip-extract showed that between 0.05 and 0.5 female tips elicited a characteristic behavioural sequence in male moths: raising of the antennae, wing vibration, flight with lateral casting across the plume, then more direct upwind flight to the pheromone source on the filter paper square. There the moth either hovered briefly within a few cm of the paper before flying out of the plume, or landed on the paper. Many of those that landed everted their scent brushes and made apparent copulatory attempts, especially if other moths were in the vicinity. The behavioural sequence elicited by female tip ex-

Table I. Captures of *Chrysodeixis eriosoma* males in traps baited with mixtures of 7*Z*12:Ac and 9*Z*12:Ac, at Auckland, April 13 to May 13, 1981.

7*Z*12:Ac	9*Z*12:Ac	Catch/trap/week
50	0	1.7 a
50	0.25	7.3 ab
50	0.5	7.7 ab
50	1.0	25.0 b
50	2.5	10.0 ab
50	5.0	11.7 ab
0	50	0.0 a

3 Replicates; bait compositions in µg.
Catches followed by the same letter are not significantly different at $P = 0.05$.

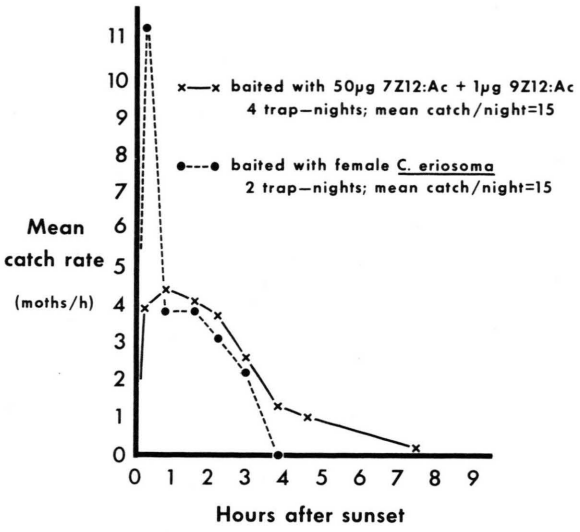

Fig. 1. Variation in rate of catch of male *C. eriosoma* during the night in traps baited with virgin female moths or 7Z12:Ac+9Z12:Ac (50:1).

tract was also elicited by 7Z12:Ac alone (at 0.1 µg) and by 7Z12:Ac+9Z12:Ac (50:1, 0.1 µg total). With the same mixture at 10:1 ratio, upwind flights were initiated but did not reach the filter paper. There was no response to 9Z12:Ac alone.

At this stage we became aware of the results of Dunkelblum *et al.* [17], and Descoins [16], who had studied the sex pheromone of Silver Y Moths in Israel and France respectively, *i.e. C. chalcites.*

Both had concluded that the sex pheromone of these moths was 7Z12:Ac plus 9Z14:Ac in approx. 5:1 ratio. These two compounds had been detected in female tip-extracts together with 12:Ac, 9Z12:Ac, 14:Ac, and 16:Ac. Dunkelblum *et al.* quoted the approximate percentage abundance of these compounds in the tip-extract as 67, 14, 1.5, 0.5, 1.0, and 16, respectively [17]. They found a 5:1 mixture of 7Z12:Ac and 9Z14:Ac was attractive to male *C. chalcites* in the field.

The major differences between these results and our own are the presence of 9Z14:Ac and 16:Ac in major amount. Given the close relationship of the Western Palaearctic and Australasian Silver Y Moths we decided to reinvestigate the New Zealand moths: in particular searching for 9Z14:Ac, and 16:Ac (the latter compound would have been missed in our previous analyses).

This time we analysed *C. eriosoma* tip-extracts, without prior GLC fractionation. On column A, programmed from 150–190 ° at 4 ° min^{-1}, with SIM for ions of mass 166, 194, 196, and 224 amu, strong signals were seen on the 166-trace with the correct R_T, 9 min 35 sec for 7Z12:Ac, and on the 224-trace at R_T 24 min 20 sec, corresponding to 16:Ac. The relative amounts of these two components was approx. 3.5:1. The amount of 7Z12:Ac was estimated at 250 ng per female moth. There was a very weak signal on the 194-monitor at the time expected for 9Z14:Ac, R_T 14 min 38 sec, but the amount relative to 7Z12:Ac was no more than approx. 1:150.

In traps in the field there was no difference in attractiveness to male *C. eriosoma* between the 5:1 mixture of 7Z12:Ac and 9Z14:Ac reported by Dunkelblum *et al.* [17] for *C. chalcites*, and the 50:1 mixture of 7Z12:Ac and 9Z12:Ac reported here for *C. eriosoma* (Table II). Evidently cross attraction between the two species could be expected.

Addition of 9Z14:Ac and 16:Ac to the 50:1 mixture of 7Z12:Ac and 9Z12:Ac in proportions approximating those found in the *C. eriosoma* tip extract had no significant effect on trap catch (Table II). However addition of 5Z12:Ac, or the alcohol

Table II. Captures of *Chrysodeixis eriosoma* males in traps baited with synthetic mixtures, at Auckland, February 2 to 16, 1982.

Compound: 5Z12:Ac	7Z12:Ac	9Z12:Ac	7Z12:OH	9Z14:Ac	16:Ac	Catch/trap/week
	50					0.7 a
	50	1				10.3 b
	50			10		9.2 b
5	50	1				0.3 a
	50	1	1			0.0 a
	50	1		0.25	10	7.5 b

6 Replicates; bait compositions in µg.
Catches followed by the same letter are not significantly different at $P=0.05$.

7Z12:OH, even in small amounts, suppressed trap catches (Table II). Dunkelblum *et al.* [19] reported similar effects in *C. chalcites*: addition of 16:Ac had no effect but 7Z12:OH inhibited attraction. 7Z12:OH has also been reported to inhibit attraction of *Trichoplusia ni* to 7Z12:Ac [21].

Further electrophysiological studies were made on *C. chalcites* obtained from southern France and North Africa which revealed essentially the same five types of receptor cells as in *C. eriosoma*.

As for the efficacy of the *A. ipsilon* lure (7Z12:Ac and 9Z14:Ac in 3:1 ratio) in attracting *C. eriosoma*, we had first thought that the 9Z14:Ac might have contained 9Z12:Ac as an impurity. However, GLC analyses revealed the 9Z14:Ac to be free of any detectable amount of U12:Ac (< 0.1%). But given that *C. eriosoma* is attracted to the *C. chalcites* lure (the same compounds in 5:1 ratio) it is not surprising that it is also attracted to the *A. ipsilon* lure.

Conclusions

We find that the abdominal tips of female *C. eriosoma* moths in New Zealand contain 7Z12:Ac, 9Z12:Ac, 12:Ac and 16:Ac, possibly accompanied by traces of 9Z14:Ac (Table III). A 50:1 blend of 7Z12:Ac and 9Z12:Ac attracted male *C. eriosoma* moths both in the flight tunnel and in field traps. We conclude that these are the major components of the natural sex pheromone.

Table III. Relative composition of female abdominal tip extracts of *Chrysodeixis eriosoma* and *C. chalcites*. (Results for *C. chalcites* from Dunkelblum *et al.* [17].)

Compound	*C. eriosoma*	*C. chalcites*
12:Ac	1.5	1.5
7Z12:Ac	75	67
9Z12:Ac	2.5	0.5
14:Ac	not detected	1.0
9Z14:Ac	< 0.5	14
16:Ac	21	16

Comparing *C. eriosoma* and *C. chalcites*, the sex pheromones produced by the female moths, as indicated by abdominal tip extracts, contain essentially the same compounds in different proportions: that of *C. eriosoma* contains more 9Z12:Ac and much less 9Z14:Ac. However the males of both species have the same set of receptor cells, responsive to 7Z12:Ac (the major pheromone component in both), 9Z12:Ac (secondary component in *eriosoma*), 9Z14:Ac (secondary component in *chalcites*), 5Z12:Ac (inhibitor for *eriosoma*, effect on *chalcites* not known) and 7Z12:OH (inhibitor for both). Furthermore, *C. eriosoma* males were attracted to the *chalcites* pheromone.

The moths are clearly very closely related. Possibly, as suggested to us by Prof. Descoins [18], *C. eriosoma* is the Australasian ecotype of the Silver Y, having evolved a slightly different sex pheromone from the western Palaearctic ecotype, but still capable of responding to the latter. Intraspecific variation of sex pheromones with geographical location has been demonstrated in several moth species including a noctuid [20–23]. Perhaps the currently accepted taxonomic interpretation, that *C. eriosoma* and *chalcites* are vicariant species [4] (*i.e.* geographical splitting has proceeded to the extent that they are distinct [24]), should be re-examined.

Acknowledgements

We thank Professor C. Descoins for his courtesy in describing the results of his group's study of the sex-pheromone of *C. chalcites* (Esper).

[1] D. E. Gaskin, Butterflies and Common Moths of New Zealand, p. 116, Whitcombe and Tombs, Christchurch 1966.
[2] C.S.I.R.O., The Insects of Australia, p. 865, Melbourne Univ. Press, Carlton, Victoria 1970.
[3] A. Kostrowicki, Acta Zoologica Cracoviensia **6,** 367 (1961).
[4] C. Dufay, Bull. Soc. Entomol. France **80,** 157 (1975).
[5] J. S. Dugdale, Silver Y Moth, *Chrysodeixis eriosoma* Doubleday, Life Cycle, D.S.I.R. Information Series No. 105/20, Wellington 1976.
[6] J. D. Holloway, The Lepidoptera of Norfolk Island, Their Biogeography and Ecology, p. 82, Dr. W. Junk B. V., The Hague 1977.
[7] L. I. N. Roberts, N. Z. Entomologist **7,** 52 (1979)
[8] W. L. Roelofs, Crop Protection Agents (N. R. McFarlane, ed.), p. 147, Academic Press, New York 1977 and references therein.
[9] E. Priesner, Z. Naturforsch. **35c,** 990 (1980) and references therein.
[10] R. R. Sokal and F. J. Rohlf, Biometry, p. 239, W. H. Freeman, San Francisco 1969.
[11] J. R. Miller and W. L. Roelofs, J. Chem. Ecol. **4,** 187 (1978).
[12] A. S. Hill, R. W. Rings, S. R. Swier, and W. L. Roelofs, J. Chem. Ecol. **5,** 439 (1979).
[13] W. Steck, E. W. Underhill, and M. D. Chisholm, J Chem. Ecol. **8,** 731 (1982).
[14] R. S. Berger, Ann. Entomol. Soc. Am. **59,** 767 (1966).
[15] J. H. Tumlinson, E. R. Mitchell, S. M. Browner, and D. A. Lindquist, Envir. Entomol. **1,** 466 (1972).
[16] W. F. Steck, E. W. Underhill, M. D. Chisholm, and H. S. Gerber, Envir. Entomol. **8,** 373 (1979).
[17] E. Dunkelblum, S. Gothilf, and M. Kehat, J. Chem. Ecol. **7,** 1081 (1981).
[18] C. Descoins, pers. comm.
[19] E. Dunkelblum, S. Gothilf, and M. Kehat, Les Mediateurs Chimiques (Colloques de l'INRA 7), p. 271, INRA Publ. 1981.
[20] D. C. Campion, P. Hunter-Jones, L. J. McVeigh, and A. Dignan, Bull. ent. Res. **70,** 673 (1980).
[21] J. H. Tumlinson, E. R. Mitchell, S. M. Browner, M. S. Mayer, N. Green, R. Hines, and D. A. Lindquist, Envir. Entomol. **1,** 354 (1971).
[22] J. A. Klun and G. A. Junk, J. Chem. Ecol. **3,** 447 (1977) and references therein.
[23] A. S. Hill, B. G. Kovalev, L. N. Kovaleva, and W. L. Roelofs, J. Chem. Ecol. **8,** 383 (1982).
[24] L. Croizat, G. Nelson, and D. E. Rosen, Systematic Zool. **23,** 265 (1974).