Identification of 4 Sex Pheromone Components Isolated from Calling Females of *Mamestra brassicae*

D. L. Struble

Agriculture Canada, Research Station, Lethbridge, Alberta TIJ 4B1, Canada, on transfer of work (1977–78) at Eidg. Forschungsanstalt für Obst-, Wein- und Gartenbau, CH-8820 Wädenswil

H. Arn, H. R. Buser, E. Städler,

Eidg. Forschungsanstalt für Obst-, Wein- und Gartenbau, CH-8820 Wädenswil

and J. Freuler

Station Féderale de Recherches Agronomiques de Changins, CH-1260 Nyon

Z. Naturforsch. **35 c,** 45–48 (1980); received October 9, 1979

Sex Pheromone, (*Z*)-11-Hexadecenyl Acetate, Pheromone Inhibitors, *Mamestra brassicae*, Calling Females

Evidence obtained by glass capillary gas chromatography coupled to an electroantennographic detector or a mass spectrometer confirmed that *Z*-11-hexadecenyl acetate is the major component in the pheromone gland washes of calling *Mamestra brassicae* female moths. Three other components were identified, tetradecanyl acetate, hexadecanyl acetate and *E*-11-hexadecenyl acetate; but none of these had obvious synergistic effects in attracting males in field tests. The attraction of males to *Z*-11-hexadecenyl acetate was inhibited by 0.1% *Z*-11-hexadecenol or 1% *Z*-9-tetradecenyl acetate.

The main sex pheromone component of the cabbage armyworm, *Mamestra brassicae* Linné (Lepidoptera: Noctuidae) has been identified as *Z*-11-hexadecenyl acetate (*Z*11–16Ac) [1–4]. We have analyzed a biologically active methylene chloride wash of the pheromone glands of calling female moths, without any cleanup, directly by gas chromatography coupled to an electroantennographic detector (GC-EAD) and by gas chromatography-mass spectrometry (GC-MS). This was done to confirm the presence of *Z*11–16Ac and to search for other minor pheromone components. Field tests with highly purified *Z*11–16Ac and with combinations of the identified sex pheromone components are reported.

M. brassicae were reared on a artificial diet [5] and the moths were fed a dilute sugar solution. The female moths were maintained on a reverse diurnal cycle with 8 h of dark phase at 20 °C and 90% RH. A background light of about 4 lx was used during the dark phase. Females that were 3 to 9 days old were observed in a calling position from 3 to 7 h from the beginning of the dark phase (Fig. 1).

To confirm that the females were releasing pheromone when they adopted this position, they were placed in the air intake of a tube-type olfactometer that contained receptive males (Fig. 1). The males exhibited the usual strong sexual responses including extending their claspers and attempting to copulate with other males. The extended abdomen tip of each calling female was washed with a few drops of methylene chloride so that a minimum of fatty material was collected. The pheromone wash was considered to be biologically active when 0.1 female equivalents (FE) of solution elicited sexual responses from the male moths in the olfactometer similar to those elicited by the calling females. The pheromone washes were filtered, reduced to an appropriate volume and analyzed without further cleanup. By using this technique, the minor pheromone components were not lost nor was the pheromone contaminated by trace amounts of other chemicals, which may occur during the usual extensive liquid chromatography fractionation of pheromone extracts [3].

Gas chromatography of the female washes was done on a Silar 10C, 20-m, glass capillary column. The effluent gas was split in a 10 : 1 ratio between the flame ionization (FID) and the electroantennographic detector (EAD) [6]. The EAD recordings consistently showed 1 peak which coincided with a peak in the FID trace, which co-chromatographed with an authentic sample of *Z*11–16Ac. No

Reprint requests to Dr. D. L. Struble.
0341-0382/80/0100-0045 $ 01.00/0

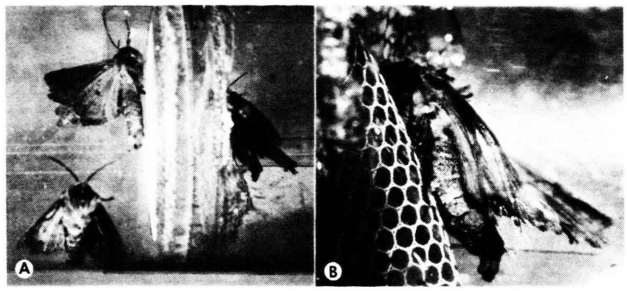

Fig. 1. Typical response of *M. brassicae* males (A) to a calling female (B) in an olfactometer.

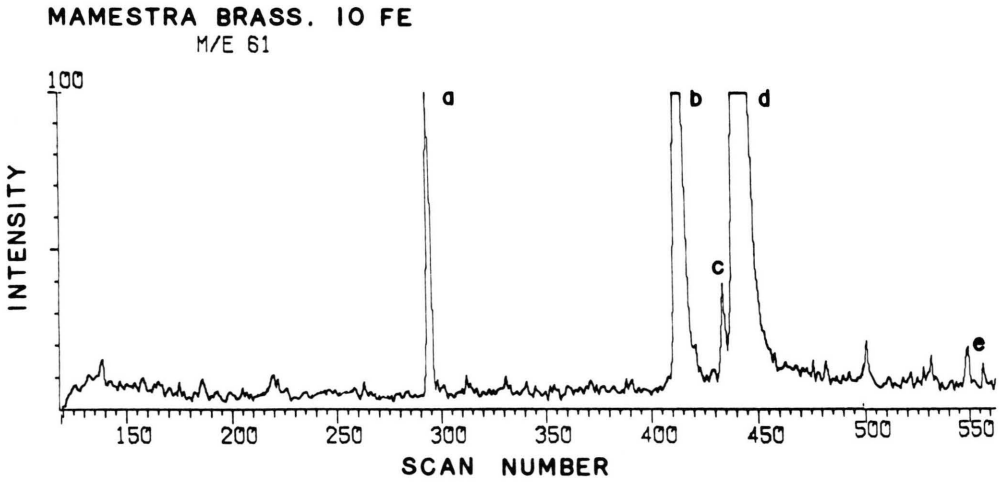

Fig. 2. Mass chromatogram (*m/e* 61, electron impact ionization 70 eV) of *M. brassicae* pheromone wash. Ten female equivalents were injected on Silar 10C glass capillary column, 50 m × 0.35 mm i. d., splitless injection 2 min 100 °C, 2 °C/min to 180 °C, 1 scan/sec (*m/e* 35–400). The components were: (a) 14Ac, (b) 16Ac, (c) *E*11–16Ac, (d) *Z*11–16Ac, (e) an octadecenyl acetate.

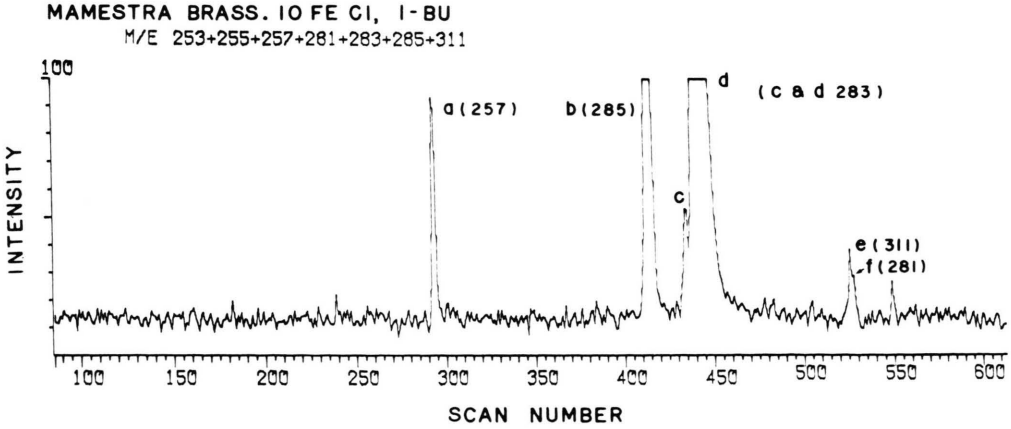

Fig. 3. Mass chromatogram (chemical ionization isobutane) showing a summation of 7 selected ions and the 6 compounds detected in a *M. brassicae* pheromone wash. Components (a) to (e) and the conditions are the same as in Fig. 2, but component (f) may be a hexadecadienyl acetate.

other EAD peaks were ever detected with the pheromone washes within the temperature program range of C_{10} to C_{18} carbon compounds.

Pheromone washes containing the largest quantities of *Z*11−16Ac were analyzed on a Finnigan GC-MS equipped with a data system using electron impact ionization (EI) and chemical ionization (CI) with isobutane. A Silar 10C, 50-m, glass capillary column was used for the GC-MS analysis. After data acquisition, the mass chromatograms were searched by the data system for selected ions of acetates (EI, M^+-60, *m/e* 61; CI, M^++1, $(M^++1)-60$), alcohols (EI, M^+-18; CI, M^++1, $(M^++1)-18$), and aldehydes (EI, M^+-1, M^+-18; CI, M^++1, M^+-1, $(M^++1)-18$) of saturated, monoenes, and dienes of C_{10} to C_{18} carbon compounds.

There were 5 acetates detected by EI GC-MS and Fig. 2 is a mass chromatogram, *m/e* 61, of these. The complete mass spectra of compounds (a) to (d) and their retention times were consistent with authentic samples of a, 14Ac; b, 16Ac; c, *E*11−16Ac; d, *Z*11−16Ac. Component (e) was present in trace amounts and its major ions were indicative of an octadecenyl acetate.

The CI (isobutane) GC-MS revealed the presence of the same compounds and Fig. 3 is a mass chromatogram showing the summation of 7 selected ions. Again, component (e) had the correct major ions for an octadecenyl acetate; however, another component (f) (M+1, 281), indicative of a hexadecadienyl acetate appeared to be unresolved from this component. Quantities of these components were insufficient to permit further identification. Fig. 3 also shows that ions *m/e* 255 and 253, the M+1 ions for tetradecenyl and tetradecadienyl acetates, respectively, were not detectable in the pheromone washes. The *Z*9−14Ac, *m/e* 255 (M^++1), has been reported [7] as an essential minor component in the attractant blend for *Mamestra configurata*, but it was not detectable in these *M. brassicae* pheromone washes.

No other compounds of the C_{10} to C_{18} carbon series of acetates, alcohols, or aldehydes were detected in the *M. brassicae* pheromone washes. The isomer assignments of *Z*- and *E*11−16Ac were based on their retention times on Silar 10C under various temperature conditions by co-chromatography with authentic samples. The *E*11−16Ac was not fully resolved from *Z*9−16Ac, therefore there could be trace amounts of *Z*9−16Ac in the pheromone and for this reason it was included in the field tests.

The GC and GC-MS analyses of 12 pheromone washes of 10 to 30 females per wash contained about 10 ng of *Z*11−16Ac/FE, and the 4 components were of the order of: 14Ac, 2%; 16Ac, 7%; *E*11−16Ac, 1%, and *Z*11−16Ac, 90%.

Field tests were done in 7 locations throughout Switzerland in 1977 and 1978. The tests were based upon 1000 µg of *Z*11−16Ac alone and in several combinations dispensed on polyethylene caps or rubber serum bottle caps (Tellergummikappen, No. 90 142, Auer Bittman Soulié, AG, Zürich) in Tetra traps [8]. The chemicals tested were found by analytical GC using glass capillary columns to be at least 99% pure. The unsaturated compounds were all purified by argentation high pressure liquid chromatography (Ag^+-HPLC). One batch of *Z*11−16Ac was further purified by liquid chromatography on silica gel by eluting with pentane-diethyl ether to remove any possible traces of *Z*11−16OH (Table I).

During the initial test, August 4 to September 15, 1977, single traps baited with *Z*11−16Ac (Ag^+-HPLC) on polyethylene caps captured a total of 34 males at 2 locations (Wädenswil and Bassins). In another test in 1978, *Z*11−16Ac and *Z*11−16Ac (1000 µg) plus *Z*9−14Ac at 200 µg and 100 µg or plus *Z*11−16OH at 5 µg did not attract any males. The *Z*9−14Ac and *Z*11−16OH appeared to inhibit

Table I. *Mamestra brassicae* males captured in Tetra traps baited with *Z*-11-hexadecenyl acetate (1000 µg per polyethylene cap) alone and with other chemicals at 7 locations in Switzerland from August 16 to September 19, 1978.

Chemicals (µg added to *Z*11-16Ac)	Total males captured at 7 locations [a]
None (*Z*11-16Ac, Ag^+-HPLC)	15
None [b] (*Z*11-16Ac, Ag^+-HPLC, silica gel)	14
14Ac (20), (100)	10, 13
16Ac (50), (200)	12, 7
*E*11-16Ac (20), (100)	8, 16
*Z*9-16Ac (20), (100)	11, 2
*E*9-16Ac (10)	14
14Ac (20), 16Ac (50), *E*11-16Ac (20)	8
16Ac (50), *E*11-16Ac (20)	15
16Ac (50), *Z*9-16Ac (20)	10
*Z*9-14Ac (10)	7, 0
*Z*11-16OH (1), (5)	2, 1

[a] Insufficient males were captured to warrant statistical analysis and the population of males was assumed to be light.
[b] This purity (99.6%) *Z*11-16Ac was used in all listed combinations.

the attraction of males and $E11-16$Ac had no obvious effect. In a similar test during this period, $Z11-16$Ac (1000 µg, Ag^+-HPLC) was dispensed on polyethylene caps and rubber serum bottle caps and 15 and 3 males were captured, respectively. This was repeated in a second test where 24 and 8 males were captured. The polyethylene caps seem to be more suitable as dispensers for this trapping system.

The most highly purified $Z11-16$Ac (Ag^+-HPLC and silica gel) and $Z11-16$Ac (Ag^+-HPLC) were tested in the field from August 16 to September 19, 1978 at 7 locations (Table I). Several combinations of chemicals with the most highly purified $Z11-16$Ac were also included in this test. The 2 samples of $Z11-16$Ac attracted similar numbers of males. Although the numbers captured were small, none of the other chemicals at the quantities tested had any obvious synergistic effects. The most outstanding results were the reduction in trap catches when the $Z11-16$Ac contained $Z11-16$OH or $Z9-14$Ac percentages as low as 0.1% and 1%, respectively. These clearly illustrate the importance of using only highly purified chemicals in field tests.

Our results have confirmed that $Z11-16$Ac is the major pheromone component of *M. brassicae* and, although 14Ac, 16Ac and $E11-16$Ac were also identified in the pheromone washes, our preliminary field tests indicated that they do not have any strong synergistic effects on the attraction of males. The $Z11-16$Ac must be essentially free (less than 0.1%) of $Z11-16$OH which presents new challenges in purification and analysis. It appears that $Z11-16$Ac alone may be all that is required for the attraction of males in the field.

Small numbers of males of *Ochropleura plecta* and *Discestra trifolii* were captured in traps baited with $Z11-16$Ac alone and with $Z11-16$Ac containing $Z11-16$OH.

Acknowledgements

The technical help of Fritz Gfeller and Max Hächler is gratefully acknowledged.

[1] Y. Hirai, H. Kimura, K. Kawasaki, and Y. Tamaki, Appl. Entomol. Zool. **13,** 136 (1978).
[2] C. Descoins, E. Priesner, M. Gallois, H. Arn, and G. Martin, C. R. Acad. Sci. Paris **286,** D, 77 (1978).
[3] H. J. Bestmann, O. Vostrowsky, K. H. Koschatzky, H. Platz, and A. Szymanska, Tetrahedron Lett. **1978,** 608.
[4] S. F. Nedopjekina, 4th Int. Congr. on Pesticide Chemistry (IUPAC), Zurich 1978.
[5] T. C. Reese, L. M. English, T. R. Yonke, and M. L. Fairchild, J. Econ. Entomol. **65,** 1047 (1972).
[6] H. Arn, E. Städler, and S. Rauscher, Z. Naturforsch. **36 c,** 722 (1975).
[7] E. W. Underhill, W. F. Steck, and M. D. Chisholm, Can. Entomol. **109,** 1335 (1977).
[8] H. Arn, S. Rauscher, and A. Schmid, Mitt. Schweiz. Entomol. Ges. **52,** 49 (1979).