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ISOLATION AND IDENTIFICATION OF SEX PHEROMONE OF *Symmetrischema tangolias* (Gyen) (LEPIDOPTERA: GELECHIIDAE)

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Abstract—The sex pheromone of the South American potato tuber moth *Symmetrischema tangolias* (syn.: *Symmetrischema plaesiosema*) was identified as a 2:1 mixture of (*E*,*Z*)-3,7-tetradecadien-1-ol acetate and (*E*)-3-tetradecen-1-ol acetate by means of dual-column GC, EAG, GC-EAD, GC-MS, NMR, and wind-tunnel bioassays. (*Z*)-5-Tetradecen-1-ol acetate and (*Z*)-7-tetradecen-1-ol acetate were also identified in the pheromone gland extract. Male *S. tangolias* were able to detect these acetates (EAG), but their addition to the two-component sex pheromone did not improve attractiveness. Field trials in Cajamarca and Cusco, Peru, showed that traps baited with the synthetic sex pheromone were able to catch large numbers of male *S. tangolias*.

Key Words—*Symmetrischema tangolias*, *Symmetrischema plaesiosema*, Gelechiidae, moth sex pheromone, potatoes, identification, (*E*)-3-tetradecen-1-ol acetate, (*E*,*Z*)-3,7-tetradecadien-1-ol acetate.

INTRODUCTION

The potato tuber moth *Symmetrischema tangolias* (Gyen) (Hodges and Os, 1990; Povolny, 1967), formerly called *Symmetrischema plaesiosema* (Turner) (Sánchez et al., 1986), is a severe pest on potatoes in the field and in storehouses in Peru. *S. tangolias* was first reported in the Montaro Valley, Peru, in 1982

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(Alcázar et al., 1982), although this insect has also been reported in Australia (Osmelak, 1987) and, since 1993, in Bolivia (Alcázar, personal communication). Its main distribution areas are the higher regions of the Peruvian Andes (CIP, 1993). In the field the larvae bore into the stems of potato plants, which causes the plants to break and die. In storehouses the larvae mine into the potato tubers making them unsuitable for human consumption. Nevertheless, the infested tubers are still planted, which causes further spread of the pest. In Peru seed potatoes are generally stored in large storehouses of cooperatives where they are sometimes literally covered with pesticides. Amounts of malathion of 1.3 g/kg potatoes have been observed. In the Peruvian Andes small farmers keep the potatoes indoors or in small open storehouses. These potatoes are not treated with pesticides and are therefore an ideal food for *S. tangolias*. Crop losses can reach up to 100% (Alcázar, personal communication). Today *S. tangolias* is considered to be an even greater pest in Peru than *Phthorimaea operculella* (Zeller), which occurs in the same regions (Ewell et al., 1990). In 1976 Persoons et al. identified the sex pheromone of *P. operculella*, which now is efficient for pest control when applied in mass-trapping (Raman and Booth, 1983). We started the identification of the sex phermone of *S. tangolias* in order to develop a pest control strategy for this moth.

METHODS AND MATERIALS

Insects. The laboratory culture of *S. tangolias* was started with pupae that were collected in a storehouse for potatoes in Cajamarca, Peru, in November 1991. The moths were reared on potato tubers (cv. Bintje) under the following conditions: 22 ± 1°C at day and 17 ± 1°C at night, 65 ± 5% relative humidity, and a 12L:12D photoperiod. The potatoes were provided with small punched holes in which the females could lay their eggs. After four days the tubers, loaded with eggs, were relocated to transparent boxes with a 1-cm layer of clean sand. About 40 days later the pupae were collected, cleaned with a 3% hypochlorite solution and separated into males and females. Moths were fed on a 10% honey solution.

Pheromone Extracts. Pheromone glands from 3-day-old virgin females 7–10 hr into the scotophase were collected. Female moths had been observed to call in this time span. A little more than just the tip of the abdomen was cut off as reported in Attygalle et al. (1987) and kept in redistilled and degassed hexane. The extracts were stored under nitrogen at −20°C. Bioassays were carried out by offering a piece of filter paper (6 × 0.8 cm, Schleicher & Schuell 589[2]) loaded with 2FE (female equivalents) of extact to male moths. Bioactive fractions caused intensive "flutter" responses and attempts to copulate with the filter paper.

Electroantennograms (EAGs). Antennae from 3-day-old males were used for EAG measurements. The antenna was cut off at the base. About four segments of the tip were cut off to enable electrical contact with the recording electrodes. The tip and basal part were connected to glass electrodes filled with a 0.1 M KCl solution. All tetradecen-1-ol acetate isomers used (>99% purity), were obtained from the IPO-DLO pheromone bank (Voerman, 1988). Test cartridges were prepared by applying 100 μl of a 5 PPM solution of the acetate in hexane on a piece of filter paper (6 × 0.8 cm, Schleicher & Schuell 589^2). After evaporation of the solvent, the paper was put into a Pasteur pipet. A Pasteur pipet filled with a filter paper containing 25 μl of a 1% solution (v/v) of (Z)-3-hexen-1-ol acetate (Carl Roth, Karlsruhe, Germany) in paraffin oil (Merck, Uvasol) served as reference stimulus. Silver wires connected the electrodes to the recording instruments: Grass HIP16A input probe, Grass P16D DC-amplifier (rise time: 30 ms), Philips PM3302 oscilloscope, Krenz TRC 4010 transient-recorder and Estate PC AT386. For details on the recording and calculation of EAG data, see Visser and Piron (1995).

Coupled Gas Chromatography–Electroantennographic Detection (GC-EAD). GC-EAD measurements were carried out at Syntech Laboratories, Hilversum, The Netherlands. The GC was a Chrompack 9000 equipped with a split/splitless injection system. Injections were done in splitless mode only. The column was a J&W 12-m DB-5 (5% phenyl methyl polysiloxane), 0.20-mm ID and 0.33-μm film thickness. The sample was equally split between a flame ionization detector (FID) and the EAG detector. Conditions were: carrier gas, helium; inlet pressure, 150 kPA; temperature programming, 100°C (0 min hold) to 220°C (0 min hold) at 10°C/min; injector and detector temperature, 250°C. The EAG recorder, software, IDAC (Intelligent Data Acquisition Controller) interface board for the AT486 PC and other peripheral equipment were manufactured by Syntech Laboratories.

Dual-Column GC. Gas chromatographic analyses were performed on a Hewlett Packard (HP) 5890A equipped with a split/splitless injection system, a 1 : 1 inlet splitter, and two fused silica columns each equipped with an FID. The two columns were a J&W 60-m DB-1 (100% methyl polysiloxane), and a J&W 60-m DB-Wax (polyethylene glycol), both having 0.25-mm ID and 0.25-μm film thickness. Conditions were: carrier gas, hydrogen; inlet pressure, 20 psi; linear velocity, 35 cm/sec; temperature programming, 50°C (0 min hold) to 238°C (8 min hold) at 4°C/min; injector temperature 220°C; detector temperature 260°C. Retention Indices (RI) were calculated according to Kovats (1964); the real retention times instead of the adjusted retention times were used for RI calculations. The RI were calculated by comparing the retention times of the components of interest with those of the C_7–C_{23} alkanes. For the GC spiking experiments, a just-detectable amount of a reference compound was added to the gland extract. Both the extract and the extract combined with the reference

compound were examined by GC. Compounds were considered identified when on both columns the peaks of interest would rise by the same amount.

Preparative GC. Preparative GC was performed on an HP 5890 series II with an HP 7673 automatic sampler. The temperature controlled injection port (controller model 504) and trap unit (controller model 580) were obtained from Gerstel Mulheim, Germany. The column was a 60-m J&W DB-Wax, 0.53-mm ID and 1.0-μm film thickness. Conditions were: carrier gas, hydrogen; inlet pressure, 150 kPa; temperature programming, 150°C (1 min hold) to 230°C (1 min hold) at 5°C/min; injector temperature, 50°C (1 min hold) to 240°C (1 min hold) at 15°C/sec; detector temperature 260°C.

Dimethyl Disulfide (DMDS) Derivatization. Approximately 10 μl of freshly distilled DMDS and 5 μl of 5% iodine in diethylether were added to 20 FE of extract from which the solvent had been evaporated by a gentle stream of nitrogen. The mixture was heated for 16 hr at 60°C in a small airtight flask. The reaction was then stopped by addition of a drop of saturated $Na_2S_2O_3$ solution. The organic phase was separated and concentrated to approximately 10 μl by evaporating the solvent at 40°C in a nitrogen atmosphere (Vincenti et al., 1987; Schulz, 1987).

Coupled Gas Chromatography–Mass Spectrometry (GC-MS). GC-MS was performed on a Finnigan MAT 95 mass spectrometer (70 eV), coupled to a Varian GC equipped with a split/splitless injection system. Injections were done in splitless mode only. The column was a J&W 25-m DB-5 fused silica column, 0.25-mm ID and 0.25-μm film thickness. Conditions were: carrier gas, helium; temperature programming, 50°C (2 min hold) to 100°C (0 min hold) at 25°C/min and then to 280°C (8 min hold) at 4°C/min; injector and transfer-line temperature, 250°C.

NMR. NMR spectra were recorded on a Bruker AC-E 200 at 200 MHz in $CDCl_3$ (100.0% D, Janssen Chimica). Chemical shifts are reported in parts per million (δ) relative to TMS. As internal reference $CHCl_3$ ($\delta = 7.24$) was used.

Synthesis. All chemicals used were of Pro Analyse (PA) quality. 3-Decyn-1-ol was purchased from ABCR Chemie, Karlsruhe, Germany. The *cis* reduction with the P-2 nickel catalyst in ethanol was carried out according to Brown and Ahuja (1973). More than 99% isomeric purity of the synthesized products was achieved through purification on a 200-cm × 0.8-cm Lewatit SP 1080/Ag^+ column (Voerman and Rothschild, 1977). Products were stored under nitrogen at −20°C with 0.1% 2,6-di-*tert*-butyl-4-methylphenol (BHT) as antioxidant (Goto et al., 1974).

Wind-Tunnel Bioassays. Male moths were given a dual-choice situation in the 3.0 (l) × 1.3 (w) × 0.8 (h) m flight section of a wind tunnel, which was entirely constructed from glass and stainless steel (Visser, unpublished data). Two black-painted delta traps (IPO-DLO, Wageningen, Netherlands) were placed at the same height 30 cm apart at the upwind side. Inside each trap a

horizontal strip of filter paper (6 × 0.8 cm, Schleicher & Schuell 589[2]) was suspended. These filter papers were supplied with 100 μl of a 1 ppm solution of a (mixture of) acetate(s) in double-distilled hexane. Conditions were: temperature 17 ± 1°C, 65 ± 5 relative humidity, 12 cm/sec wind speed, and ≤4 mW/m^2 red light. The tests started 2–3 hr into the scotophase with the release of 10–50 male moths at the downwind side. The males were left for 6 hr. The caught males were counted and subjected to the Wilcoxon matched-pairs, signed-rank test (Siegel, 1956). NS means $P > 0.05$ (two-tailed).

RESULTS AND DISCUSSION

Identification. From about 2000 virgin female moths the sex pheromone glands were collected and kept in 10 ml hexane. When exposed to male moths, 2-FE of this extract caused intensive "flutter" responses. The extract was concentrated to approximately 1.5 ml under a gentle stream of nitrogen and fractionated on a 6-ml Baker 10 SPE column packed with 500 mg, 45 μm, 60 Å silicagel. The column was successively eluted with 1.5-ml portions of 0, 2, 5, 10, 20, 50, and 100% of *tert*-butylmethylether in hexane. The column was sucked dry between each fraction. Only the 5% fraction generated a behavioral response. The EAG responses of this 5% fraction and the total extract were not significantly different. From GC-EAD measurements of this fraction, it was concluded that EAG-active peaks eluted during a short period of the entire GC run. The resolution of the GC-EAD equipment made it impossible to discriminate between the four peaks in the gas chromatogram of the active fraction on DB-1 (Figure 1). By comparison of the calculated RI on both the DB-1 and DB-Wax columns with those calculated for reference components from IPO pheromone bank (Voerman, 1988), it became obvious that peaks **2, 3,** and **4** were tetradecen-1-ol acetate isomers. The mass spectra of these peaks were in agreement with this. Peak **1** could be tentatively identified as a nonconjugated tetradecadien-1-ol acetate isomer because of its typical extra difference in RI on the DB-1 and DB-Wax columns. The mass spectrum of **1** (Figure 2) supported this hypothesis. Compounds **2, 3,** and **4** were identified, respectively, as (*Z*)-5-, (*Z*)-7-, and (*E*)-3-tetradecen-1-ol acetate by comparing the MS and the RI with those obtained from reference compounds. GC spiking experiments provided additional evidence (Figure 3). About 10 μg of the two main peaks, **1** and **4,** were isolated with preparative GC, which proved sufficient for NMR analysis. The NMR spectrum of **4** was identical to that of synthetic (*E*)-3-tetradecen-1-ol acetate. The NMR spectrum of **1** showed the same signal at 2.29 PPM as the NMR spectrum of (*E*)-3-tetradecen-1-ol acetate, which is characteristic for a double bond at the 3-position (Figure 4). Comparison of the olefinic part of the NMR spectrum of **1** with those of synthetic (*Z*)-3- and (*E*)-3-tetradecen-1-ol

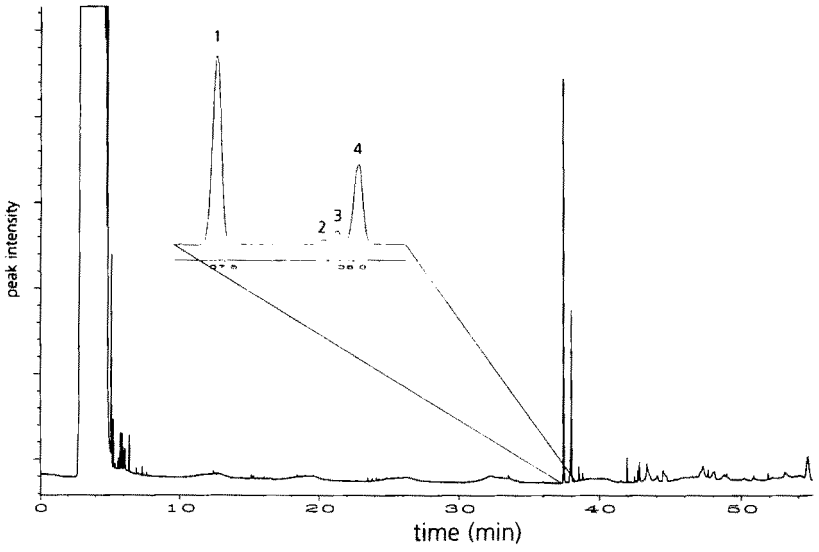


FIG. 1. Gas chromatogram of the biologically active fraction "5%" (splitless 2 μl, on the 60-m DB-1 column). The inset displays the time interval where EAG activity has been observed. For explanation of peak numbers 1–4, see text.

acetate suggested that the double bond at the 3-position of **1** has the *trans* configuration. The extract was derivatized with DMDS to get information about the position of the second double bond. The mass spectrum of the DMDS-derivative of **1** is shown in Figure 5. The fragmentation pattern suggested **1** to be a 3,7-tetradecadien-1-ol acetate isomer. To determine the *cis/trans* configuration of the double bonds of **1**, all tetradecen-1-ol acetate isomers were tested with EAG (Figure 6). The difference in response between the (*E*)-7- and the (*Z*)-7- isomer suggested the (*E*,*Z*)-3,7- stereochemistry for **1**. This was proven

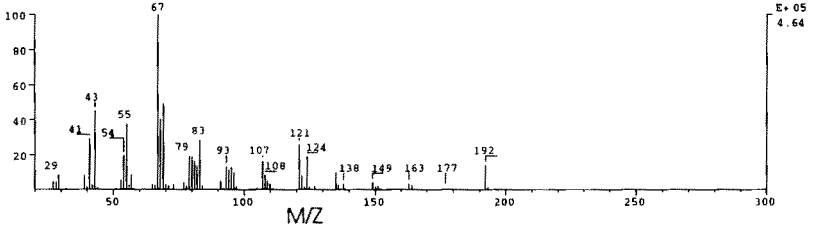


FIG. 2. Mass spectrum of compound **1**. The molecular ion (M^+) could not be detected.

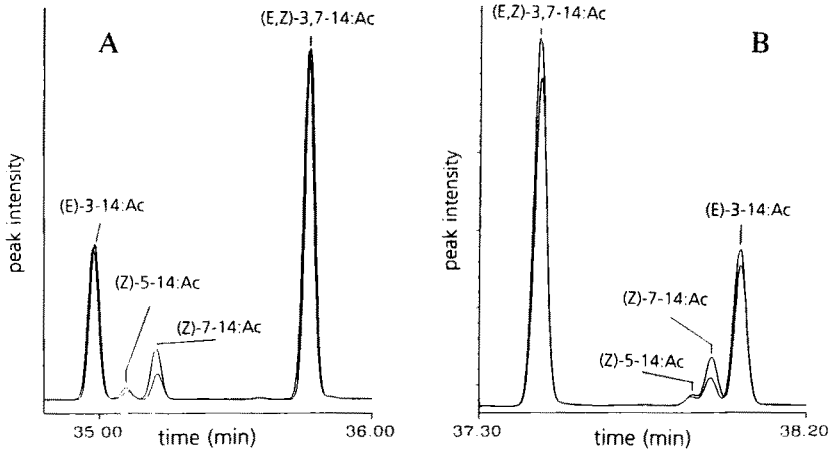


FIG. 3. GC spiking chromatograms of the sex pheromone extract with and without additional (Z)-7-tetradecen-1-ol acetate; (A) on the DB-Wax column, (B) on the DB-1 column.

by synthesis. (*E*,*Z*)-3,7-Tetradecadien-1-ol acetate was synthesized according to Scheme 1 in an isomeric purity of more than 99% (GC). ^{1}H NMR: δ 0.86 (br t, 3H, J = 6.5 Hz; H-14), 1.26 (br m, 8H; H-10 to H-13), 2.03 (s, 3H; O_2CMe), 2.03 (br m, 6H; H-5, H-6 and H-9), 2.29 (dt, 2H, J = 6.9, 6.9 Hz; H-2), 4.04 (t, 2H, J = 6.9 Hz; H-1), 5.39 (m, 4H; H-3, H-4, H-7 and H-8)

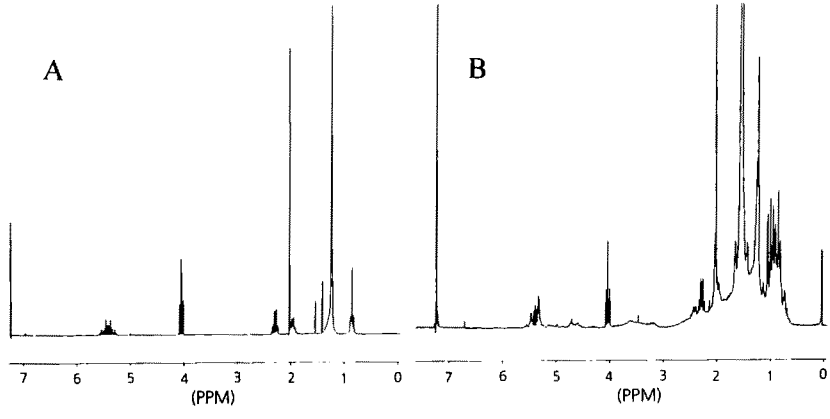


FIG. 4. (A) NMR spectrum of (synthetic) (*E*)-3-tetradecen-1-ol acetate; (B) NMR spectrum of compound **1**.

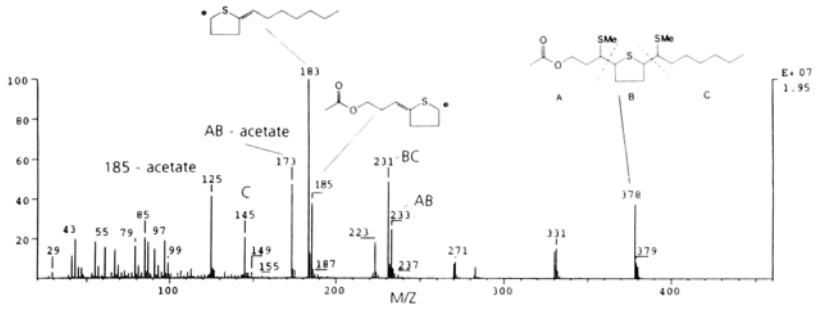


FIG. 5. Mass spectrum of the DMDS derivative of compound **1.** Molecular ion (M^+) at *m/z* 378.

^{13}C NMR ($CDCl_3$, 125 MHz); δ 14.0 (q), 20.9 (q), 22.6 (t), 27.0 (t), 27.2 (t), 28.9 (t), 29.6 (t), 31.5 (t), 31.9 (t), 32.6 (t), 64.0 (t), 125.3 (d), 128.7 (d), 130.4 (d), 132.8 (d), 171.0 (s). Both MS and ^1H NMR spectra of the synthetic (*E*,*Z*)-3,7-tetradecadien-1-ol acetate were identical to the spectra of **1.** The overall yield of the synthetic route was only a few percent, mainly due to a side reaction in step iii, where 1-bromo-(*Z*)-3-decene predominantly eliminates HBr

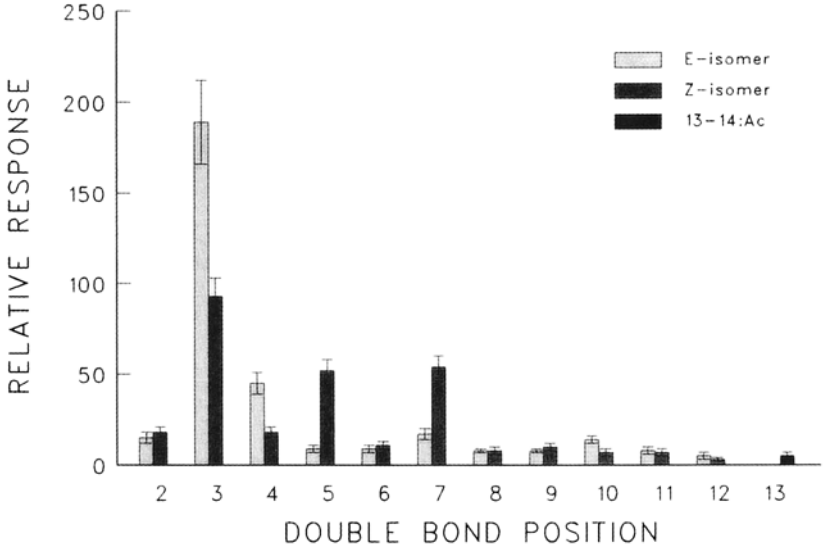
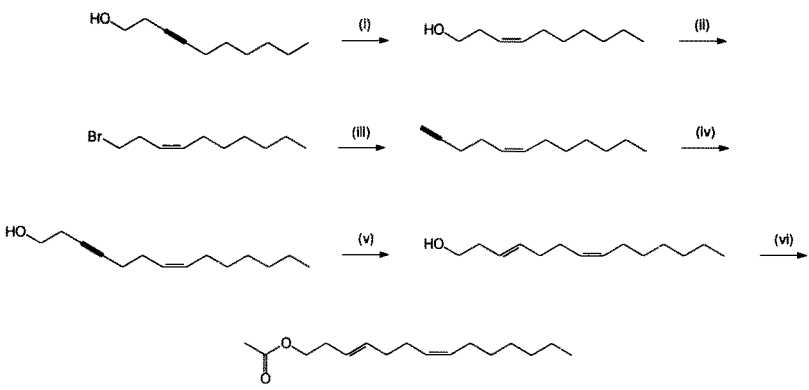


FIG. 6. Electroantennogram of all tetradecen-1-ol acetate isomers. Bars indicate 95% confidence intervals. $N = 15$.



SCHEME. 1. Synthetic route to (*E*,*Z*)-3,7-tetradecadien-1-ol acetate (**1**). Reagents: (i) P-2 Ni/ethanol; (ii) $CBr_4/Ph_3P/THF$; (iii) lithium acetylide–ethylene–diamine complex/THF/HMPA; (iv) ethyleneoxide/*n*-BuLi/HMPA; (v) $LiAlH_4$/THF/diglyme; (vi) Ac_2O/HOAc.

under formation of the diene rather than to couple with the lithium acetylide. More efficient routes for the synthesis of **1** are under investigation.

Behavioral Bioassays. Different mixtures of identified components were tested in the wind tunnel. Although the ratio of the four identified compounds in the gland extract was: (*E*,*Z*)-3,7-(*E*)-3-:(*Z*)-7-:(*Z*)-5 = 62:31:6:1, best results were obtained with a mixture containing two components, namely (*E*,*Z*)-3,7-tetradecadien-1-ol acetate and (*E*)-3-tetradecen-1-ol acetate. No significant difference in attractiveness could be determined between 2:1 and 1:1 mixtures of these two components. Addition of the two minor compounds (*Z*)-7-tetradecen-1-ol acetate and (*Z*)-5-tetradecen-1-ol acetate in the same ratio that had been found in the gland extract did not influence the attractiveness of the mixture. The addition of 10% of (*Z*)-5-tetradecen-1-ol acetate decreased the attractiveness of the mixture. The function of the (*Z*)-5- and (*Z*)-7-tetradecen-1-ol acetates, which were detected in the gland extracts, therefore remains unclear. It may be that the trap catches in the wind tunnel bioassays were not sensitive enough to reveal behavioral differences.

The 2:1 mixture of (*E*,*Z*)-3,7-tetradecadien-1-ol acetate and (*E*)-3-tetradecen-1-ol acetate was tested in potato fields and storehouses in Peru. Catches up to 120 and 450 individuals per night per trap for field and storehouse experiments, respectively, were achieved. The field catches emphasized the great attractiveness of the synthetic pheromone blend since no *S. tangolias* could be detected by us in an extensive search of the potato field and the surrounding areas. The catches in the storehouses dropped considerably after the first couple of days. Replacement of the traps by new ones did not improve the catches.

This means that traps indoors are able to diminish pest populations quite rapidly and may be efficient for pest control in storehouses through mass-trapping of pest insects. Large-scale field experiments will be carried out in Peru in the near future in order to determine the efficiency of the synthetic pheromone in the control of *S. tangolias*.

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