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(3*E*,8*Z*,11*Z*)-3,8,11-Tetradecatrienyl Acetate, Major Sex Pheromone Component of the Tomato Pest *Scrobipalpuloides absoluta* (Lepidoptera: Gelechiidae)

Athula B. Attygalle,[a*] Gulab N. Jham,[a†] Aleš Svatoš,[a‡] Rosa T. S. Frighetto,[a§] Fernando A. Ferrara,[b] Evaldo F. Vilela,[b] Manoel A. Uchôa-Fernandes[b] and Jerrold Meinwald[a]

[a] *Baker Laboratory, Department of Chemistry, Cornell University, Ithaca, NY 14853, U.S.A.*

[b] *Department of Animal Biology, Universidade Federal de Viçosa, Viçosa, MG 36571, Brazil*

Abstract—The major sex attractant emitted by *Scrobipalpuloides absoluta* females is shown to be (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate by a novel strategy involving the random reduction of double bonds, followed by methylthiolation of the reduced products. Each female sex gland contains ca. 1–5 ng of this pheromone. This triene ester, synthesized by a stereospecific procedure, shows spectral and gas chromatographic properties identical to those of the natural substance. In field tests and wind tunnel bioassays, the synthetic ester was found to be highly attractive to conspecific males. The male response to this pheromone, however, is restricted to the same early-morning time window during which females exhibit calling behavior. Copyright © 1996 Elsevier Science Ltd

Introduction

Tomato (*Lycopersicon esculentum*) is an economically important plant cultivated extensively in most countries. Tomato plants are nevertheless extremely susceptible to insect attack; one of the most devastating pests on tomato in Brazil and in many other South American countries is *S. absoluta* (Lepidoptera: Gelechiidae: Gelechiinae). The insect causes severe damage,[1] and losses up to 100% have been reported. There is currently no environmentally acceptable method of control for this pest. Blanket spraying of conventional insecticides, the approach currently in use, is rather ineffective since the larvae burrow into leaves, stems and fruits of the tomato plants. In addition, continuous use of pesticides can lead to the emergence of insect strains which show resistance to the chemicals being applied. Finally, some pesticide residues are known to move along the foodweb, exposing many nontarget organisms, including humans, to these hazardous chemicals. Clearly, a new approach to the control of this pest, based on a knowledge of its chemical ecology is highly desirable.

Females of *S. absoluta* were shown recently to release a potent sex pheromone attractive to conspecific males.[2,3] In a preliminary communication,[4] we recently reported that the major component of this pheromone is (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate. Here, we present the full details of the structure elucidation, synthesis and field test results of this novel insect pheromone.

Key words: pheromone, Lepidoptera, moth, tomato pest, (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate.

†Permanent address: Department of Chemistry, Universidade Federal de Viçosa, Viçosa, MG 36571, Brazil.

‡Permanent address: Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic.

§Permanent address: Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Rodovia SP 340, Caixa Postal 69, Jaguariuna SP, Brazil.

Results

Calling behavior

Preliminary studies of the mating behavior of *S. absoluta* showed that the females attract males even on the first day after emergence. Over 50% of females ($n = 34$) in a laboratory colony showed maximum calling activity from 5.30 to 7.30 a.m. Bioassays conducted with caged calling females in a wind-tunnel showed that the males respond and fly immediately to females only during this short period of time when the females release their pheromones. During the calling period, the females extrude the ovipositor and expose an intersegmental glandular membrane at the tip of their abdomen. This intersegmental membrane ('pheromone gland') was excised and extracted with solvent in order to obtain a sample of pheromone for chemical analysis.

Pheromone identification

The extract obtained in this way, when tested in wind-tunnel bioassays, was found to be highly attractive to males. Such behaviorally active extracts, examined by GC–MS [Fig. 1(a)], using a capillary column coated with a polar stationary phase (FFAP) showed one

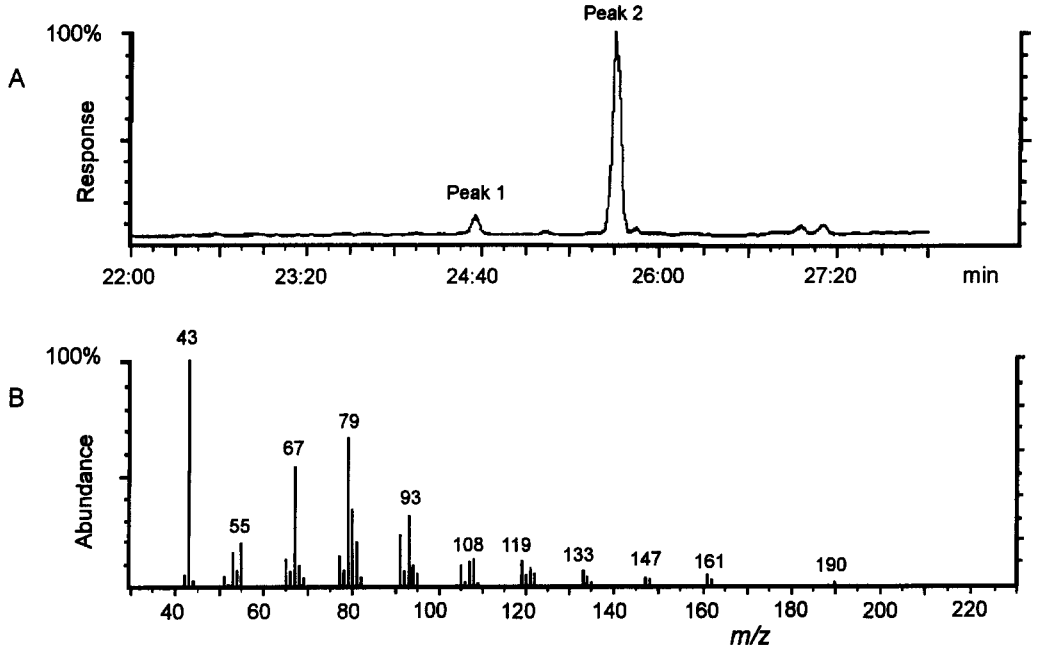


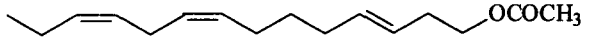
Figure 1. (a) Section of the reconstructed gas chromatogram obtained from the volatiles present in a female pheromone gland extract of *S. absoluta* [30 m × 0.22 mm fused silica capillary column coated with FFAP stationary phase; temperature program: 40 °C for 4 min, 6 °C/min to 190 °C]. (b) Electron-ionization mass spectrum (70 eV) of the major female sex pheromone component of *S. absoluta*.

major peak (>90%) in the region where chromatographic peaks for lepidopteran pheromones usually appear. The mass spectrum corresponding to this peak [Fig. 1(b)] showed a base peak at *m/z* 43, indicating that it is an acetate ester. The peak at *m/z* 190, resulting from a loss of acetic acid from the (undetected) parent ion, suggested that the compound is a tetradecatrienyl acetate. Based on comparisons of the integrated GC peak area of this major component with that of an external standard, we determined the amount of pheromone obtainable from each female gland to be only 1–5 ng. This observation motivated us to seek a micro-method to determine the position and configuration of the three double bonds in this pheromone.

The strategy developed to solve this problem should prove applicable to similar problems in and beyond the realm of pheromone chemistry. Our first step was to carry out a partial reduction of the material (by the diimide procedure[5,6]) in the hope of obtaining a product mixture containing all possible monoene acetates, since excellent techniques are available for the determination of the position of a double bond in monoenes.[7–9] The exact conditions required to carry out this procedure using less than 100 ng of the starting material were worked out using (4*Z*,7*Z*,10*Z*)-4,7,10-tetradecatrienyl acetate[10] as a model. The three expected tetradecenyl acetates were readily characterized by GC–MS by comparing retention times and mass spectra of the products with those obtained from authentic standards. The identifications were confirmed by converting the tetradecenyl acetates to their dimethyl disulfide adducts and recording the mass spectra of these adducts.[7,8]

Once the optimal conditions for the necessary micromanipulations were established, the natural *S. absoluta* pheromone extract was subjected to a partial diimide reduction. GC–MS analysis of the resulting product mixture showed the presence of the three anticipated tetradecenyl acetates, along with doubly unsaturated and saturated acetates. In order to identify these three tetradecenyl acetates precisely, we measured the mass spectra as well as the GC retention times of all 23 possible tetradecenyl acetates (excluding the two enol acetates) on two different gas chromatographic stationary phases (DBWax and DB-23) and compared these data to those obtained for the three tetradecenyl acetates that resulted from partial reduction of the natural pheromone. In this way, two of the monoenes derived from the natural pheromone were identified immediately and unambiguously as (*E*)-3-tetradecenyl acetate and (*Z*)-8-tetradecenyl acetate. However, retention data alone did not establish the identity of the third isomer, since the retention times of (*Z*)-11-tetradecenyl acetate and the terminally unsaturated 13-tetradecenyl acetate were very similar on both GC phases. To establish the identity of the third tetradecenyl acetate, the mixture was converted into a mixture of the corresponding dimethyl disulfide (DMDS) adducts.[7–9] GC–MS analysis of this mixture showed the expected presence of the DMDS adduct of an 8-tetradecenyl acetate [*m/z* (%), 348 (M^+, 10), 217 (40), 131 (23)] and that of an 11-tetradecenyl acetate [*m/z* (%), 348 (M^+, 15), 259 (95), 89 (38)], which identifies the only ambiguous monoene as (*Z*)-11-tetradecenyl acetate. Interestingly, the anticipated peak corresponding to a DMDS adduct of a (*E*)-3-tetradecenyl acetate could not be found. However, since this monoene had already been identified, this information

was not crucial to the structure determination. Finally, the retention times, obtained on a DB-1 capillary column, of the two adducts were identical to those obtained from the DMDS derivatives of authentic (Z)-8-tetradecenyl acetate and (Z)-11-tetradecenyl acetate. Based on these results, the triply unsaturated compound could be recognized as a trienic acetate with a 3E, an 8Z and an 11Z double bond, leading to the unambiguous identification of the pheromone as (3E,8Z,11Z)-3,8,11-tetradecatrien-1-yl acetate (**1**).

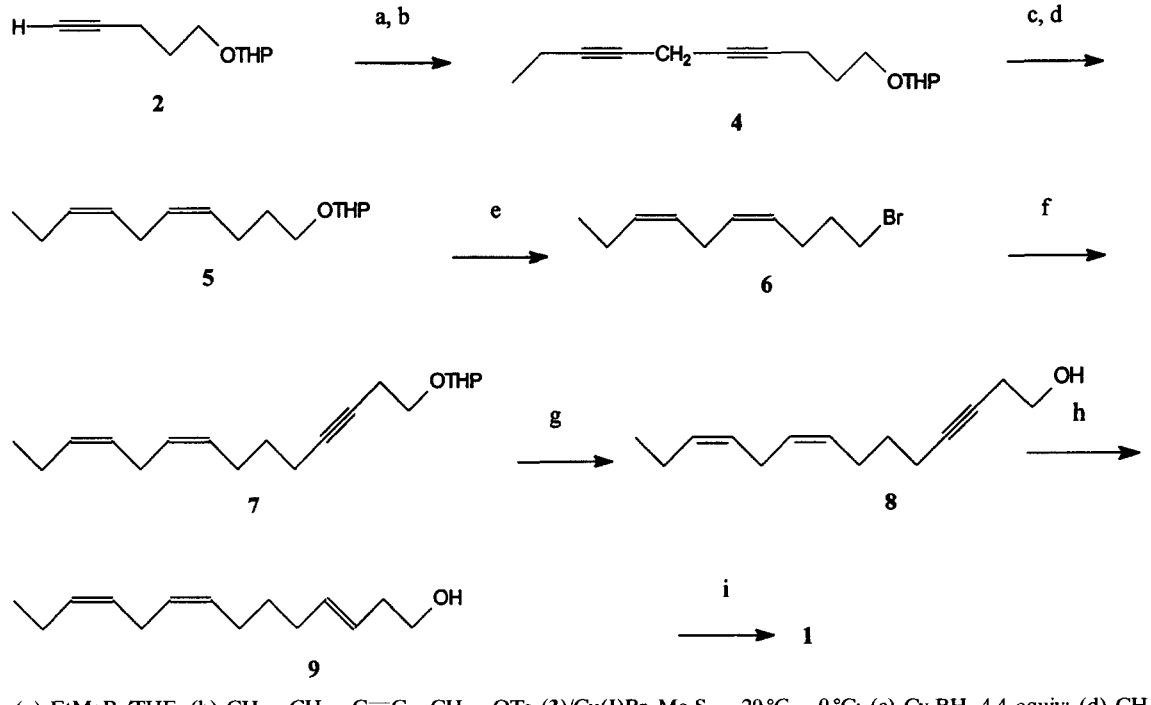


(**1**)

Pheromone synthesis

To confirm this structural assignment, and to provide samples of suitable size for laboratory and field bioassays, we have carried out a stereospecific synthesis of this pheromone. This synthesis of (3E,8Z,11Z)-3,8,11-tetradecatrienyl acetate **1** , which is summarized in Scheme 1, started from protected pentynol **2**, which was converted to an acetylenic Grignard reagent by heating with ethylmagnesium bromide.

This Grignard reagent was used to carry out a Cu(I)-catalyzed nucleophilic displacement on the tosylate of 2-pentyn-1-ol (**3**), giving diyne **4** in a quantitative yield and 98% purity. We found the $CuBr \cdot Me_2S$ complex to be a more efficient catalyst for the preparation of skipped di- and triynes in this way (Svatoš, A., Attygalle, A. B., and Meinwald, J., unpublished results) than the usual cuprous iodide.[11] The rather unstable diyne **4** was also prepared with equal success (experimental details are not given) according to the alternative coupling procedure described by Lapitskaya et al.[12] Twofold hydroboration of **4** was accomplished using dicyclohexylborane, formed from the borane–methyl sulfide complex and cyclohexene.[13] After hydrolysis of the resulting alkadienyldiborane with acetic acid, followed by careful oxidation of the residual borane species with H_2O_2, a mixture of the deprotected (4Z,7Z)-4,7-decadien-1-ol and its THP protected derivative **5** (1:1) was isolated. Fortunately, this mixture could be conveniently converted directly to bromide **6** by treating with triphenylphosphine dibromide[14] in 32% overall yield based on compound **2**. This bromination method proved superior to a published procedure[15] using an excess of triphenylphosphine and tetrabromomethane, which was sluggish in our hands and required several days for a good conversion. The stereoisomeric purity of bromide **6** was 97% as determined by GC. Bromide **6** was coupled with the lithium salt of 1-(tetrahydropyran-2-yloxy)-3-butyne[16] in a mixture of THF/DMPU[17] at 0 °C to give compound **7** in 55–82% yield. Although THP-protected alcohols are usually stable to basic and reductive conditions, our attempts to reduce the carbon–carbon triple bond in **7** to a 3E double bond with sodium in liquid ammonia were unsuccessful. Instead of the expected THP derivative of trienol **9**, a nonpolar material characterized (GC–MS and ^{13}C NMR) as a complex mixture of polyunsaturated hydrocarbons was obtained. It appears that under these conditions the homopropargyl and/or homoallyl THPO group is eliminated. This homopropargyl system also proved to be sensitive to strong acids. For



Scheme 1. (a) EtMgBr/THF; (b) $CH_3—CH_2—C\equiv C—CH_2—OTs$ (**3**)/Cu(I)Br·Me_2S, −20 °C −0 °C; (c) Cy_2BH, 4.4 equiv; (d) CH_3CO_2H; (e) PPh_3/Br_2 1.5 equiv, CH_2Cl_2; (f) Li—≡—⟨ OTHP, DMPU/THF 0 °C; (g) Dowex/MeOH; (h) $LiAlH_4$/diglyme, 120–140 °C; (i) Ac_2O/pyridine.

example, *p*-toluenesulfonic acid in methanol caused elimination of the OH group during the prolonged reaction time required to remove the THP group. However, the deprotection of **7** to alcohol **8** could be accomplished efficiently by stirring with ion-exchange resin (Dowex 50WX8) in methanol. Alcohol **8** was reduced under Rossi conditions[18] with $LiAlH_4$ in dry diglyme at 120–140 °C for 2–5 h. Here also we noted that heating at higher temperatures and/or longer reaction times affords a product contaminated with over-reduced material and isomerized polyenols (GC–MS analyses). Finally, the alcohol **9** was acetylated to the desired (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate in 7% overall yield and 97% purity (GC analyses on SE-54 and Carbowax capillary columns).

The mass spectrum of synthetic **1** and that of the major component of the *S. absoluta* sex pheromone, obtained using an ion-trap detector under identical conditions, were indistinguishable from each other. In addition, gas chromatographic retention times of both compounds are the same. To support this spectral and gas chromatographic evidence, the biological activity of the synthetic compound was evaluated by wind tunnel and field bioassays.

Wind-tunnel bioassays

Wind-tunnel bioassays were conducted using an instrument in which the flight path was 1-m long. The males placed on the 'take-off' platform showed significant behavioral responses at all concentrations of the pheromone that were tested (Fig. 2). For example, males showed induced wing fanning, oriented flight and landing on the source of the synthetic pheromone, even at the 10 ng level, the lowest amount tested. As expected, the control dispensers loaded only with hexane did not induce any significant behavioral responses. Highest responses were observed at the 1000 ng level. At this concentration, 100% of the test insects ($n = 30$) showed wing fanning and 87% initiated an oriented flight toward the dispenser with 83% landing on the dispenser (Fig. 2). The corresponding responses obtained by using nine calling females as the pheromone source were 38, 66 and 61%, respectively. From the results presented in Figure 2 it is evident that a dispenser loaded with 1000 ng of the synthetic **1** is thoroughly competitive with the attractivity of a group of nine calling females.

Field tests

An initial series of experiments was carried out to evaluate the efficacy of different trap designs. Five trap designs were evaluated. While all traps caught a large number of *S. absoluta* males, the designs D and E, which were open from all directions and used water containing a little detergent as the restraining agent, were much more efficient than traps A, B and C (Fig. 3). The most efficient design was trap E; a total number of 12,166 males was caught in one night in 15 of these traps baited with dispensers loaded with 1 μg each of synthetic **1**.

In the second series of field tests, the attractivity of dispensers loaded with 1, 10 and 100 μg of (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate was evaluated. As the results presented in Figure 4 show, all baited traps attracted large numbers of *S. absoluta* males, proving further that (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate (**1**) is a potent attractant to these

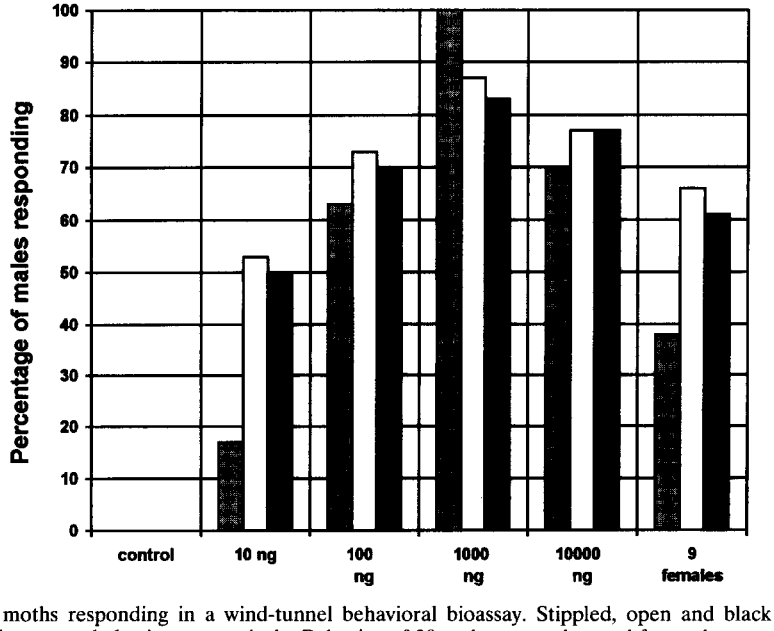


Figure 2. Percentage of male moths responding in a wind-tunnel behavioral bioassay. Stippled, open and black bars represent wing fanning, oriented flight and landing-at-the-source behavior, respectively. Behavior of 30 males were observed for each test stimulus. Hexane (100 μL), and different amounts (10 ng–10 μg) of (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate applied to rubber septa and nine calling females were used as stimuli.

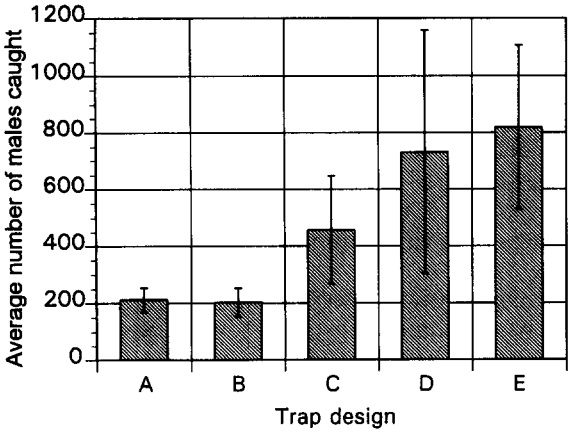


Figure 3. Average number of males caught per night per trap ($n = 15$). Five different trap designs (A–E) were used. Vertical bars represent standard deviations. See Experimental for details of trap designs. Each trap was baited with 1 μg of synthetic (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate on a rubber dispenser.

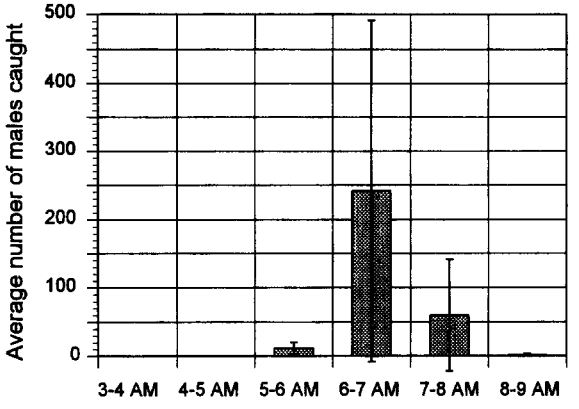


Figure 5. Average number of males caught per trap ($n = 8$) per h by traps baited with 1 μg of synthetic (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate on a rubber dispenser. Vertical bars represent standard deviations.

insects. The number of males caught per trap gradually increased as the amount of pheromone loaded on the dispensers increased (Fig. 4). The traps baited with 100 μg of **1** caught on average 1200 males per trap per night, while those baited with one 1-day-old virgin female caught only 201 males. Even the traps baited with 1 μg of **1**, which caught ca. 535 males per trap per night, were observed to compete well with virgin females in terms of attracting males in the field. It is apparent that these tests were conducted in a highly infested area, since even the control traps having dispensers loaded only with hexane also caught some males (ca. 20 per trap).

A third series of field tests was carried out to investigate the possible temporal dependence of male responses. From the observed results, it is evident that the males of this species have a very distinct time period in which they will actively search for calling females (Fig. 5). Appropriately baited traps caught an average of 240 males/trap during the time interval 6.00 to 7.00 a.m. Many fewer males were caught from 7.00 to 8.00 a.m., and the numbers caught became negligible after 8.00 a.m. Since no males were caught before 5 a.m. or after 10 a.m., the data for the rest of the 24 h time period are not presented in Figure 5.

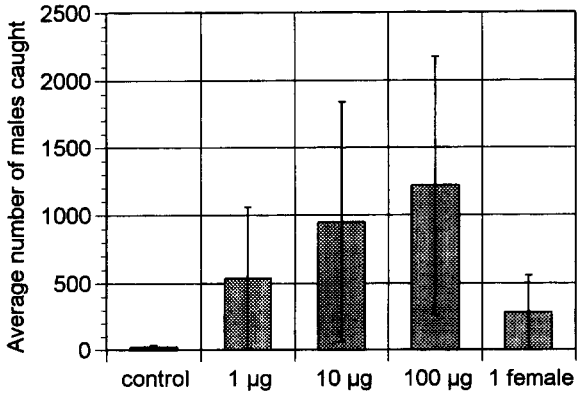


Figure 4. Average number of male moths caught per trap ($n = 20$) per night by traps baited with 1, 10, and 100 μg of synthetic (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate on a rubber dispenser and with one 1-day-old virgin female. Control baits were treated with 100 μL of hexane. Vertical bars represent standard deviations.

Among lepidopteran female sex pheromones, (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate (**1**) is not only a new compound but also one which is closely related structurally to only a few previously known examples.[19,20] In many polyunsaturated hydrocarbons and related epoxides identified as female sex attractants of moths, mostly belonging to the superfamily Geometridae, the ω-3, ω-6 structural pattern is found. However, this moiety is rare in acetate type sex pheromones.[19,20] A somewhat related compound, (4*E*,7*Z*,10*Z*)-4,7,10-tridecatrienyl acetate, together with the corresponding diene, (4*E*,7*Z*)-4,7-tridecadienyl acetate, has been characterized as the sex attractant of another gelechiide, *Phthorimaea operculella*.[21] Therefore, it is not entirely surprising that our traps baited with female *S. absoluta* also attracted a few *P. operculella* males. It is interesting to note that (*E*)-3-decen-1-yl acetate has been identified as a constituent of the sex pheromone of a related moth, *Scrobipalpopsis solanivora* (Povolny).[22] Related moths of the genus *Scrobipalpa* which use compounds with 3*E* unsaturation include *Scrobipalpa heliopa* (Lower), which produces (3*E*)-3-tridecen-1-yl acetate[23] and *Scrobipalpa ocellatella* (Boyd), which utilizes a lower homologue, (3*E*)-3-dodecen-1-yl acetate.[24]

Procedures that will help us to reduce our dependence on hard pesticides as the most efficient means of insect control are avidly sought by researchers throughout the world. Biorational methods such as those based on pheromonal intervention are not only more gentle to the environment, but also help in conservation by sparing nontargeted species, including natural predators and pollinators. There are at least two promising ways of using pheromones in pest control. In the first

method, called mating disruption, synthetic pheromones are used to permeate the atmosphere so as to prevent communication between the sexes and hence subsequent mating. The second technique employs traps baited with synthetic pheromones to monitor pest populations. In this way blanket spraying of pesticides can be limited and farmers can be instructed to spray selectively.

From our wind-tunnel and field-test results it is evident that the lures baited with the synthetic pheromone can be equally or more attractive than calling virgin females. Although the release rates of the natural pheromone by calling females has not yet been determined, it must be of the magnitude of a few nanogram per day, since each female gland contains only ca. 1–5 ng. In the wind-tunnel bioassay rubber dispensers loaded with 10 ng of synthetic **1** released male behavioral responses comparable to those evoked by nine calling females. Therefore, it is apparent that the release rate of acetate **1** from rubber dispensers is approximately of the same order of magnitude as that of virgin females. Interestingly, wind-tunnel experiments showed that male behavioral responses evoked by dispensers loaded with higher amounts of **1** were somewhat less than those elicited by lower amounts (Fig. 2). The number of males induced by 10 μg of **1** to make an oriented flight and land at the source was slightly smaller than that evoked by 1 μg. Based on these observations, **1** could be expected to act as an efficient mating disruptant. Furthermore, field tests showed that traps baited with lures loaded with 1 μg of **1** caught nearly double the number of males caught by traps containing a virgin female. Since less than 1 μg of **1** is required for an efficient trap, it might be economically feasible to use **1** even in mass trapping programs in order to reduce populations of *S. absoluta*.

Our one-component synthetic pheromone lures were highly attractive to *S. absoluta* males, although a minor component is also present in the natural pheromone mixture. Since it is well established that some Lepidoptera are highly sensitive to small qualitative or even quantitative changes in the composition of pheromone blends, it will be interesting to see whether or not a binary mixture which includes the minor component of the females secretion is more effective than **1** alone. Although it is premature to draw a conclusion from the limited amount of data available, it appears that moths using structurally less complicated chemicals as pheromones, such as (*Z*)-11-tetradecenyl acetate (a simple ester shared by many species of Lepidoptera as a component of pheromone blends), are more sensitive to qualitative and quantitative variations in composition than are those using less common, more complicated structures.

Experimental

S. absoluta larvae were collected from tomato plantations near the Federal University of Viçosa and reared in the laboratory on a diet of fresh tomato leaves (*L. esculentum*).[25] Pupae were collected every 3 days and sexed.[26] Five specimens of each sex were placed in glass tubes (8.0 × 2.5 cm) and insects of each sex were kept in a different room. All the insects were maintained at a temperature of 23 ± 2 °C, under a 14:10 h light–dark cycle and a relative humidity of 75 ± 5%. After emergence, the females were observed during the scotophase and the beginning of the photophase to recognize their calling time and behavior.

Hydrazine hydrate (100%) and hydrogen peroxide (30% wt % solution in water) were purchased from Mallinckrodt (Chesterfield, Missouri) and Fisher Chemical Co. (Fair Lawn, New Jersey), respectively. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl prior to use. 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU, Aldrich Chemical Co., Milwaukee, Wisconsin) was dried over molecular sieves. Dichloromethane was dried over calcium chloride, distilled and filtered thorough activated alumina (Brockmann I) prior to use. Diglyme was distilled from $LiAlH_4$ under nitrogen. Butyllithium (1.6 M and 2.5 M solutions in hexane), 2-(3-butynyloxy)tetrahydro-2*H*-pyran [1-(tetrahydropyran-2-yloxy)-3-butyne], $LiAlH_4$, $NaBH_4$, borane–methylsulfide complex, $Cu(I)Br \cdot Me_2S$ complex and *p*-toluenesulfonyl chloride from Aldrich, and ethylenediamine, bromine, triphenylphosphine and nickel(II) acetate from Fluka Chemical Co. (Ronkonkoma, New York) were used as purchased.

Mass spectra were obtained using an HP 5890 gas chromatograph linked to a Finnigan ion-trap detector (ITD 800). Analyses of natural samples were carried out using fused silica capillary columns (0.22 mm × 30 m) coated with FFAP or DB-5 stationary phases (J&W Scientific, Folsom, California). FABMS, (in a glycerin matrix) were obtained on a ZAB-Q (VG) instrument.

NMR spectra were recorded as $CDCl_3$ solutions at room temperature using a Varian Unity-200 (^1H, 200 MHz, Varian) or a Unity-400 (^1H NMR, 400 MHz, ^{13}C NMR, 100.6 MHz, Varian) instrument. Chemical shifts, given in ppm, are expressed as δ values measured from the residual $CHCl_3$ signal (7.26 ppm).

Vapor phase IR spectra were recorded using a Hewlett Packard (HP) 5965A IRD coupled to a HP 5890 GC. Gas chromatographic analyses of synthetic samples (hexane, ca. 1 mg/mL) were carried out using split mode and a temperature program of 60 °C for 4 min, 10 °C/min to 270 °C and for 20 min. Column chromatography was run on silica gel and reactions were monitored by TLC, using Baker-flex Silica gel IB2-F plates (J.T. Baker). Silver nitrate column chromatography was performed on silica gel impregnated with $AgNO_3$ (20% of $AgNO_3$ on silica gel Merck H 60).

Pheromone characterization

An ethanol extract (ca. 100 μL) containing 125 female sex glands was reduced to a few μL under a gentle stream of N_2. To this concentrate, a solution of hydra-

zine (10 μL, 10% in ethanol) and hydrogen peroxide (10 μL, 0.6% in ethanol) were added. The mixture was heated at 60 °C for 2.5 h. After the mixture cooled to room temperature, 20 μL of dil HCl (10%) was added and extracted with hexane (3 × 15 μL). The combined hexane layers were reduced to 2–3 μL and reconstituted to 10 μL with hexane. One μL of this extract, together with hexadecane and tetracosane as internal standards, was examined by GC-MS. The rest of the extract was derivatized with dimethyl disulfide as previously described.[7]

Chemical synthesis

2-Pentyn-1-yl *p*-toluenesulfonate (3).

(a) 2-Pentyn-1-ol. Propargyl alcohol (Aldrich, 11.8 g, 200 mmol) was added dropwise (30 min) to a suspension of lithium amide in liquid ammonia (600 mL), formed from lithium wire (2.8 g, 0.4 mol), followed by ethyl bromide (21.8 g, 200 mmol), which was added during a period of 45 min. The reaction mixture was stirred for 1 h in boiling ammonia. Usual work up and distillation under reduced pressure afforded 2-pentyn-1-ol (14.2 g, 85% yield) of bp 63–66 °C/23 torr. ^{1}H NMR (200 MHz): δ 4.22 (bs, 2H, CH_2O-1), 2.21 (tq, J=2.2, 2.2, 2.2, 7.5, 7.5 Hz, 2H, CH_2-4), 1.80 (bs, 1H, OH), 1.12 (t, J = 7.5, 7.5 Hz, 3H, CH_3).

(b) 2-Pentyn-1-yl *p*-toluenesulfonate (3). A solution of 2-pentyn-1-ol (8.4 g, 100 mmol) in THF (60 mL) was treated with *p*-toluenesulfonyl chloride (23.9 g, 125 mmol) at −10 °C, followed by pulverized KOH (11.3 g, 200 mmol), without allowing the temperature to exceed −5 °C. After 1.5 h of stirring at −10 °C, 50 mL of saturated brine was added and the product extracted into CH_2Cl_2. The product, 2-pentyn-1-yl *p*-toluenesulfonate (**3**), was isolated by flash chromatography (18.2 g, 76% yield). ^{1}H NMR (200 MHz): δ 7.81 (d, J=8.4 Hz, 2H, arom. CH), 7.33 (d, J=7.9 Hz, 2H, arom. CH), 4.68 (t, J = 2.2, 2.2 Hz, 2H, CH_2O-1), 2.44 (s, 3H, arom. CH_3), 2.13 (tq, J=2.2, 2.2, 2.2, 7.5, 7.5 Hz, 2H, CH_2-4), 1.00 (t, J=7.5, 7.5 Hz, 3H, CH_3-5).

1-(Tetrahydropyran-2-yloxy)-4,7-decadiyne (4). 1-(Tetrahydropyran-2-yloxy)-4-pentyne (**2**) was prepared from the corresponding alcohol according to the procedure of Robertson.[27] A solution of **2** (8.40 g, 50 mmol) in dry THF (30 mL) was treated with C_2H_5MgBr in THF (30 mL), which was freshly prepared from ethyl bromide (6.0 g, 55 mmol) and magnesium (1.58 g, 66 mmol). After intensive evolution of ethane decreased, the mixture was refluxed at 60 °C for 1 h. The solution was transferred to a suspension of Cu(I)Br·Me_2S complex (0.51 g, 5 mol %) in dry THF (50 mL). The solid dissolved gradually and formed a dark solution which was stirred at room temperature for 20 min. The mixture was cooled on an ice-bath and a solution of **3** (8.4 g, 35 mmol) in dry THF (10 mL) was added dropwise during 30 min. After an additional 1 h on the ice-bath, the mixture was stirred at room temperature for 12 h. The reaction was quenched with a mixture (50 mL) of concentrated aq ammonia and saturated NH_4Cl solution (1:2, v/v). The usual work up afforded a crude product (11.6 g), containing 98% of the desired product (**4**) and 2% of the starting tosylate. ^{1}H NMR (200 MHz): δ 4.60 (m, 1H, CH-2′), 3.82 (m, 2H, CH_2-6′ and 1), 3.45 (m, 2H, CH_2-6′ and 1), 3.10 (dt, J=4 × 2.3 Hz, 2H, =CH—CH_2—CH=), 2.28 (m, 2H, CH_2-3), 2.16 (tq, J=2.4, 2.3, 2.4, 7.6, 7.6 Hz, 2H, CH_2-9), 1.8–1.4 (m, 8H, CH_2-2, -3′, -4′, and -5′), 1.11 (t, J=7.6, 7.6 Hz, 3H, CH_3-10). ^{13}C NMR (100.6 MHz): δ 98.7 (C-2′), 81.8 (C-7), 79.7 (C-5), 74.7 (C-8), 68.4 (C-4), 66.6 (C-1), 62.1 (C-6′), 30.7 (C-3′), 28.9 (C-2), 25.5 (C-4′), 19.5 (C-6), 15.7 (C-5′), 13.9 (C-3), 12.4 (C-9), 9.7 (C-10).

(4Z,7Z)-1-(Tetrahydropyran-2-yloxy)-4,7-decadiene (5). An ice-cooled solution of cyclohexene (24 mL, 240 mmol) in dry THF (50 mL) was treated with a borane–methylsulfide complex solution in toluene (60 mL, 120 mmol) for 20 min. After an additional 20 min of stirring on an ice-bath, the resulting suspension was stirred at room temperature for 2 h. This dicyclohexylborane slurry was treated with a solution of **4** (11.6 g, 49.8 mmol) in dry THF (50 mL) at 0 °C. After 12 h at room temperature the mixture was treated with glacial acetic acid (40 mL) at 0 °C, and stirred for 7 h. The mixture was made basic with 5 M NaOH and carefully oxidized with H_2O_2 (30%, 40 mL) for 1 h. The reaction products were extracted into ether and cyclohexanol was distilled off using a Vigreux column under reduced pressure. Finally, 12.4 g of a crude oil was isolated, which was shown by GC analysis to consists of a 1:1 mixture of **5** and (4Z,7Z)-4,7-decadien-1-ol. **5**: ^{1}H NMR (200 MHz): δ 5.38 (m, 4H, CH=CH -4, -5, -7 and -8), 4.57 (m, 1H, CH -2′), 3.72 (m, 2H, CH_2-6′ and 1), 3.41 (m, 2H, CH_2-6′ and 1), 2.77 (dd, J=5.6, 2.8 Hz, 2H, =CH—CH_2—CH=), 2.13 (m, 4H, CH_2-3, 9), 1.8–1.4 (m, 8H, CH_2-2, -3′, -4′ and -5′), 0.96 (t, J=7.5, 7.5 Hz, 3H, CH_3-10). MS [EI, *m/z* (%)]: 169 (5), 153 (7), 135 (5), 123 (5), 109 (7), 95 (10), 85 (100), 67 (18). MS (FAB, *m/z*): 237 ($M^+ - 1$).

(4Z,7Z)-4,7-Decadien-1-ol. ^{1}H NMR (200 MHz): δ 5.37 (m, 4H, CH=CH -4, 5, 7, 8), 3.64 (m, 2H, CH_2-1), 2.77 (dd, J=5.1, 5.7 Hz, 2H, =CH—CH_2—CH=), 2.10 (m, 4H, CH_2-3 and -9), 1.64 (tt, J=6.5, 6.6, 7.0, 7.5 Hz, 2H, CH_2-2), 0.96 (t, J=7.5, 7.5 Hz, 3H, CH_3). ^{13}C NMR (100.6 MHz): δ 131.9 (C-8), 129.1 (C-5), 128.8 (C-7), 127.1 (C-4), 62.5 (C-1), 32.7 (C-2), 25.5 (C-6), 23.5 (C-3), 20.5 (C-9), 14.3 (C-10).

(4Z,7Z)-1-Bromo-4,7-decadiene (6). A solution of triphenylphosphine (20.9 g, 80 mmol) in CH_2Cl_2 (100 mL) was treated with Br_2 (7.8 g, 49 mmol) in CH_2Cl_2 (15 mL, 0 °C) and the mixture was stirred at room temperature for 20 min. Into the slightly yellow suspension that formed, the crude mixture of **5** and (4Z,7Z)-4,7-decadien-1-ol (12.4 g) in CH_2Cl_2 (15 mL) was added dropwise. After stirring for 1 h, a solution of satd $NaHCO_3$ (50 mL) and water (50 mL) were added. The mixture was extracted with pentane and combined

extracts were stored overnight in a freezer. In this way triphenylphosphine oxide could be crystallized and removed by filtration. Column chromatography followed by distillation at reduced pressure afforded **6** (3.48 g, 32% yield based on **2**) of bp 80–85 °C/2.5 torr. ^{1}H NMR (200 MHz): δ 5.37 (m, 4H, CH=CH), 3.41 (t, J=6.6, 6.6 Hz, 2H, CH_2—Br), 2.80 (dd, J=6.4, 6.5 Hz, 2H, =CH—CH_2—CH=), 2.21 (dt, J=7.0, 6.9, 6.8, 6.9 Hz, 2H, CH_2-3), 2.07 (dq, J=7.4 Hz, CH_2-9), 1.91 (tt, J=6.7, 6.6, 7.0, 7.0, 2H, CH_2-2), 0.97 (t, J=7.5, 7.5 Hz, 3H, CH_3). ^{13}C NMR (100.6 MHz): δ 132.1 (C-8), 129.8 (C-5), 127.6 (C-7), 127.0 (C-4), 33.4 (C-1), 32.5 (C-2), 25.6 (C-3), 25.5 (C-6), 20.6 (C-9), 14.3 (C-10). MS [EI, *m/z* (%)]: 218 (M^+, 15), 216 (M^+, 17), 137 (6), 109 (13), 107 (18), 96 (14), 95 (78), 82 (18), 81 (74), 79 (22), 68 (27), 67 (100), 55 (17), 53 (15), 42 (11), 41 (38), 40 (40).

(8*Z*,11*Z*)-1-(Tetrahydropyran-2-yloxy)-8,11-tetradecadien-3-yne (7). A solution of 1-(tetrahydropyran-2-yloxy)-3-butyne (2.25 g, 14 mmol) in dry THF (15 mL) was treated at −50 °C with *n*-butyllithium hexane solution (11 mL, 17.5 mmol). After 15 min at −30 °C, the mixture was kept at room temperature for 30 min. The mixture was then treated at 0 °C with **6** (3.0 g, 13.8 mmol) dissolved in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidine (DMPU) (15 mL) during 30 min. The reaction mixture was stirred for 12 h at room temperature and quenched with saturated NH_4Cl solution (30 ml). The product (**7**) was removed with ether (3 × 50 mL) and purified by silica gel flash chromatography (2.2 g, 55% yield). ^{1}H NMR (200 MHz): δ 5.36 (m, 4H, CH=CH), 4.64 (m, 1H, CH-2′), 3.81 (m, 2H, CH_2-6′ and -1), 3.54 (m, 2H, CH_2-6 and 1), 2.78 (dd, J=6.0, 6.0 Hz, 2H, =CH—CH_2—CH=), 2.47 (m, 4H, CH_2-2 and -5), 2.15 (m, 4H, CH_2-7 and -13), 1.8–1.4 (m, 8H, CH_2-6, -3′, -4′, and -5′), 0.96 (t, J=7.5, 7.6 Hz, 3H, CH_3). ^{13}C NMR (100.6 MHz): δ 131.8 (C-12), 128.9 (C-9, C-11), 127.2 (C-8), 98.7 (C-2′), 81.0 (C-3), 76.6 (C-4), 66.2 (C-1), 62.2 (C-6′), 33.4 (C-2), 30.6 (C-3′), 28.9 (C-7), 26.2 (C-6), 25.5 (C10), 25.4 (C-4′), 20.5 (C-5), 20.2 (C-13), 18.3 (C-5′), 14.3 (C-14). MS [EI, *m/z* (%)]: 159 (7), 131 (9), 119 (8), 117 (9), 105 (8), 93 (11), 91 (16), 86 (12), 85 (100), 79 (18), 77 (11), 67 (36), 57 (16), 55 (15), 43 (29), 42 (16), 41 (51), 40 (24).

(8*Z*,11*Z*)-8,11-Tetradecadien-3-yn-1-ol (8). A solution of **7** (2.2 g, 7.5 mmol) in methanol (50 mL) was stirred with Dowex 50WX8 ion-exchange resin (2 g) and the formation of **8** was monitored by TLC. The resin was removed by filtration and washed with methanol (3 × 20 mL). The alcohol **8** was purified by flash chromatography (1.3 g, 83% yield). ^{1}H NMR (200 MHz) δ: 5.36 (m, 4H, CH=CH), 3.66 (t, J= 6.1, 6.4 Hz, 2H, CH_2-1), 2.78 (t, J=6.4, 6.7 Hz, 2H, =CH—CH_2—CH=), 2.42 (tt, J=2.1, 2.4. 6.1, 6.4 Hz, 2H, CH_2-2), 2.16 (tt, J=2.1, 2.4. 7.0, 7.3 Hz, 2H, CH_2-5), 2.15 (m, 2H, CH_2-7), 2.06 (dq, J=7.3, 7.0, 7.3, 7.6 Hz, 2H, CH_2-13), 1.55 (tt, J=7.1, 7.3, 7.3, 7.3 Hz, 2H, CH_2-6), 0.96 (t, J=7.3, 7.6 Hz, 3H, CH_3). ^{13}C NMR (100.6 MHz): δ 131.9 (C-12), 129.0 (C-11), 128.8 (C-9), 127.2 (C-8), 82.3 (C-3), 76.6 (C-4), 61.3 (C-1), 28.8 (C-7), 26.2 (C-6), 25.5 (C-10), 23.1 (C-5), 20.5 (C-2), 18.2 (C-13), 14.2 (C-14). MS [EI, *m/z* (%)]: 161 (15), 159 (20), 145 (30), 133 (25), 131 (27), 119 (34), 117 (42), 105 (42), 95 (17), 93 (27), 91 (83), 79 (76), 77 (37), 69 (22), 67 (78), 65 (29), 55 (46), 53 (39), 44 (29), 43 (24), 42 (34), 41 (100), 40 (61).

(3*E*,8*Z*,11*Z*)-3,8,11-Tetradecatrien-1-yl acetate (1). A solution of **8** (0.4 g, 1.94 mmol) in dry diglyme (6 mL) was added slowly to a suspension of $LiAlH_4$ (0.23 g, 5.7 mmol) in dry diglyme (4 mL) at room temperature. The mixture was refluxed at 120–140 °C for 5 h and then cooled in an ice-bath. Ethyl acetate (5 mL) was carefully added and the mixture was poured into an ice and concd HCl mixture (10 mL). Extraction with ether followed by flash chromatography afforded the alcohol **9** (0.23 g), which was treated with acetic anhydride (1 mL) and pyridine (3 mL) at room temperature for 1 h. Flash chromatography and purification on 20% $AgNO_3$-impregnated silica gel afforded the desired product (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate (0.24 g, 50% yield based on (8*Z*,11*Z*)-8,11-tetradecadien-3-yn-1-ol). ^{1}H NMR (400 MHz): δ 5.50 (dtt, J= 5.2, 6.4, 6.4, 2.3, 2.4 Hz, 1H, *trans* =CH-4), 5.36 (m, 5H, CH=CH), 4.06 (t, J=6.7, 7.0 Hz, 2H, CH_2-1), 2.77 (dd, J=5.2, 6.8 Hz, 2H, =CH—CH_2—CH=), 2.31 (dt, J = 2.3, 6.8, 7.0 Hz, 2H, CH_2-2), 2.06 (m, 6H, CH_2-5, -7 and -13), 2.04 (s, 3H, $COCH_3$), 1.42 (tt, J=7.3, 7.3, 7.6., 7.6 Hz, 2H, CH_2-6), 0.97 (t, J=7.3, 7.3 Hz, 3H, CH_3). ^{13}C NMR (100.6 MHz): δ 171.1 (C=O), 133.2 (C-4), 131.8 (C-12), 129.7 (C-9), 128.3 (C-11), 127.3 (C-8), 125.4 (C-3), 64.1 (C-1), 32.1 (C-2), 31.9 (C-2), 29.3 (C-6), 26.6 (C-7), 25.6 (C-10), 21.0 ($\underline{C}H_3$), 20.5 (C-13), 14.3 (C-14) MS [EI, *m/z* (%)]: 190 (M^+-60, 4), 161 (9), 133 (13), 119 (9), 108 (24), 107 (15), 105 (10), 93 (54), 91 (22), 80 (43), 79 (91), 67 (68), 55 (29), 43 (100), 41 (45). IR (gas phase, cm^{-1}): 3017 (*cis* =C—H str), 2936 (CH_2), 1761 (C=O), 1231, 1037 (C—O—), 967 (*trans* =C—H wag).

Wind-tunnel experiments

Synthetic samples of acetate **1** were applied as pentane solutions to rubber septa (cleaned by washing with CH_2Cl_2 for 20 h in a Soxhlet apparatus), which were used as baits in the wind-tunnel. The attractivity of each bait, loaded with 10, 100, 1000 and 10,000 ng of acetate **1**, was compared with that of 9 calling virgin females (1- to 4-day-old) in a cage. The control baits were treated with 100 µL of hexane. The wind-tunnel (3.8 × 0.50 m) was operated at a flux speed of 30 cm/s. The landing platform was 1 m away from the 'take-off' platform. For each experiment, 3 males (1- to 3-day-old)[28] were placed on the 'take-off' platform and their behavioral responses were observed for 5 min and categorized as no response, wing fanning, none-oriented flight oriented flight, and landing on the source. The test was repeated 10 times for each concentration.

Field tests

These experiments were carried out at a 20 ha tomato (Santa Clara variety) plantation in Araguari (State of Minas Gerais, Brazil) from 1 April 1994 to 11 April 1994. The traps were placed at a height of 1.20 m and at 30 m intervals as suggested by Uchôa-Fernandes and Vilela.[2,3]

The first series of field experiments were conducted from 1 April to 4 April, using five different trap designs (A–E). Trap A was made from white cylindrical PVC tubing (20 diameter × 25 cm). Trap B was similar to Trap A, however, the cylinder was split into two halves and the two parts were connected with four metal wires to form two parallel 5 × 25 cm openings on both sides. Trap C was a delta type with a maximum vertical gap of 7 cm at the triangular opening and a base area of 29 × 30 cm. The bottoms of traps A, B and C were lined with removable sticky paper to catch insects. The trap D was made from a plastic tray (24 × 37 × 6 cm), to which a V-shape hood was attached with four metal rods. This house-shape trap was open from all four sides with a 8 cm opening at the minimum and 15 cm gap at the maximum opening in the middle. Trap E was made from two 20 and 32 cm diameter black plastic plates with 2 cm vertical sides, joined at three points with strong metal wire to form a cage. Both D and E traps contained water in the bottom plate with a few drops of a neutral detergent to catch insects. Rubber septa loaded with 1 μg of acetate **1** were hung inside all traps as baits and the number of insects caught in each trap ($n = 15$) was counted each day.

In the second series of field experiments (20 replications), rubber baits loaded with 1, 10 and 100 μg of acetate **1**, or 100 μL of hexane, were used in E-type traps. These tests were carried out between 8 April 1994 and 11 April 1994. For comparison, a 1-day-old virgin female in a cylindrical cage (4 × 4 cm) with the open ends covered with nylon mesh, was hung inside an E type trap. The control traps were baited with 100 μL of distilled hexane. The number of males caught per trap was counted each morning.

In the third series of experiments, two C type delta traps with sticky bottoms (27 × 20 cm) were baited with 1 μg of acetate **1.** For a period of 24 h the number of males caught per trap at the end of each 1 h period was counted and the sticky cards were replaced when necessary. The test was repeated for four 24 h periods ($n = 8$).

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