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NEW SEX PHEROMONE COMPONENTS AND BEHAVIORAL ANTAGONISTS FOR THE PEACH TWIG BORER, *Anarsia lineatella* Zeller (LEPIDOPTERA: GELECHIIDAE)

Principal Investigators: Jocelyn G. Millar and T. C. Baker,
Dept. of Entomology, UC Riverside,

R. E. Rice, Dept. of Entomology,
UC Davis 95616.

INTRODUCTION

The peach twig borer (PTB), Anarsia lineatella Zeller, is a major pest of almonds and stone fruits in North America, Europe, and Asia (Summers et al., 1959). In California, there are as many as four generations per year. Damage is caused by the developing larvae tunnelling in fruits, buds and shoots. Damage can be so severe that in some crops, prophylactic pesticide sprays are routinely applied, even in non-bearing orchards.

Until recently, PTB infestations in California were controlled by a combination of pesticide treatments. However, potential development of resistance to registered insecticides, plus the increasingly restrictive limitations placed on the use and registration of the currently used pesticides has created an urgent need for alternative management strategies.

Two components of the PTB pheromone were identified by Roelofs et al. (1975) as E5-decenol (E5-10:OH) and E5-10:Ac. Traps baited with blends of the two components captured male moths. There were discernable differences between geographically separated populations, with a central California population responding to all blends of the two components approximately equally, while a Washington state population responded preferentially in traps baited with E5-10:OH as a single component.

Roelofs et al. (1975) also mentioned that field trials during their first two years were hampered by trace amounts of a strong behavioral antagonist in their synthetic formulations. The antagonist was not isolated and identified.

Pheromone-mediated mating disruption has been successfully applied to the control of two other important gelechiid moth pests, the pink bollworm and the tomato pinworm. Mating disruption may represent an environmentally safe strategy for control of PTB, and small-scale field trials were begun in California several years ago, culminating in 1989, when formulations from four different suppliers were evaluated. Fruit yield losses were reduced in the disrupted areas as compared to untreated control blocks, but considerable numbers of male moths were still caught in pheromone-baited traps placed throughout the disrupted blocks, indicating that male insects were still able to locate point sources of pheromone. On the basis of these observations, we established the following objectives.

OBJECTIVES

1. Reexamination of the pheromone components in the pheromone glands of PTB, with particular emphasis on identifying trace components as possible synergists.

2. Determination of the number and ratio of components released by calling female moths by aeration of individual calling females, and by mass aerations of virgin females.

3. Synthesis and field testing of potential pheromone synergists and antagonists.

4. Study of the pheromone-mediated behavior of the male moths.

MATERIALS AND METHODS

Insects and Insect Extracts. Insects were collected as pupae from cardboard bands placed around tree trunks in infested almond and peach groves near Parlier, CA. Pupae were maintained individually in 10 dram glass shell vials at approx. 25°C under a 15/9 light/dark cycle until and after eclosion.

Female pheromone gland extracts were prepared by excising the extruded pheromone glands of 2-7 day old virgin females in the first couple of hours of the scotophase, and soaking the glands in pentane (~100 µl) for 20 min. Extracts were concentrated under N_2, and analyzed by gas chromatography (GC), coupled gas chromatography-mass spectrometry (GC-MS) and coupled GC-electroantennogram detection (GC-EAD). No internal standards were added, as the extracts were found to contain hydrocarbon peaks which were used as reference points.

Extracts were initially analyzed by gas chromatography on a Hewlett-Packard 5890 GC in splitless mode, with He carrier gas. Two columns were used, a 20 m x .25 mm I.D. x 25 µ film thickness DB-5 column, and a 30 m x .25 mm x .25 l film DB-225 column (J&W Scientific, Folsom CA), using temperature programs of 80°C for 2 min, 10°/min to 250°, and 120°/15 min, 10°/min to 220°, respectively. Injector and detector temperatures were set at 250 and 275°, respectively. Extracts were also analyzed by GC-MS in splitless mode with a Hewlett-Packard 5970 mass selective detector (EI, 70 eV) coupled to a H.-P. 5890 GC. A 25 m x 0.2 mm I.D. Ultra-2 column was used (Hewlett-Packard) with He carrier gas.

Coupled GC-EAD analyses were carried out with a Varian 3700 GC with an effluent splitter (split ratio ~1:1). GC and EAD traces were recorded simultaneously on a matched pair of H.-P. 3394 integrators. Extracts were analyzed on DB-1, DB-5 and DB-225 columns (30 m x 0.25 mm I.D.; J&W Scientific) in splitless mode. Temperature programs used were 100°/2 min, 20°/min to 200° for 20 min for the DB-1 and DB-5 columns, and 80°/2 min, 15°/min to 180° for the DB-225 column.

Large-scale aerations were carried out with 20-40 virgin females placed in a cylindrical glass aeration chamber (25 cm x 8 cm I.D.). The chamber was placed in a shaded porch area, under natural daylight and temperature conditions. Charcoal-filtered compressed air was passed through the cylinder at a rate of approx. 3 l/min from dusk until 8:30 AM, with the volatiles being trapped on ~100 mg of activated charcoal (50-200 mesh). Trapped volatiles were eluted with 5 x 100 µl of CH_2Cl_2. The resulting extract was concentrated under N_2.

Effluvia from individual females were collected from extruded pheromone glands by the method of Baker et al. (1981). Trapped volatiles were eluted off the glass wool trapping material with CS_2 (ca. 200 µl), and the extract was concentrated under N_2 and analyzed by GC-EAD.

Field Tests. All field tests were conducted in almond groves near Parlier, CA, during the four PTB flight periods (June-October) as compounds become availble through synthesis. Pherocon 1C traps (Trece Inc., Salinas, CA) were used. Traps were baited with red rubber septa (Thomas Scientific, Philadelphia, PA) impregnated with heptane solutions (10 mg/ml) of the test compounds. All septa except solvent blanks were loaded with a standard blend of E5-10:Ac and E5-10:OH (81:19 blend, 1 mg total weight), obtained as a gift from Consep Membranes (Bend, OR). No antioxidants or UV stabilizers were used. Compounds to be tested were then added to the septa as

heptane solutions (1 mg/ml), in amounts of 20 or 100 μg (2 or 10% of the standard blend). Traps were set out in almond orchards in randomized block design, with each treatment replicated four times. Traps were counted approx. every second day and rerandomized. Raw data were subjected to an $(X+1/2)^{1/2}$ transformation, and the transformed data were analyzed by ANOVA. Significantly different means ($P>0.05$) were separated by Duncan's multiple range test.

Chemicals. Chemicals were obtained from the suppliers shown in Table 1, or synthesized by standard synthetic methods as described in Table 1 and as follows. Acetates were cleaved to alcohols by stirring in EtOH with catalytic amounts of NaOH. Alcohols were oxidized to aldehydes with pyridinium dichromate in methylene chloride (Corey and Schmidt, 1979). Alcohols were acetylated by treatment with acetyl chloride and pyridine in ether. E4-10:Ac was partially isomerized to Z4-10:Ac with aqueous nitrous acid (Sonnet, 1974). The geometric isomers were then separated by flash chromatography (Still et al., 1979) on silica gel impregnated with silver nitrate (15%) eluting with 6% ether in hexane. E- and Z6-10:Ac were obtained by Wittig reaction of 6-hydroxyhexyl triphenylphosphonium bromide with butyraldehyde (Ando et al., 1988), followed by acetylation of the crude alcohol products, and separation of the geometric isomers as described above. E3,E5- and Z3,E5-10:Ac were prepared in similar fashion by acetylation of the products of the Wittig reaction of hydroxypropyl triphenylphosphonium bromide with E2-heptenal. When quantitiies permitted, synthetic compounds were further purified by Kugelrohr distillation (0.2 mm Hg, oven temp ~100°C) to remove traces of chromatographic packing material and solvent. All chemicals used were >98% chemically and isomerically pure by capillary GC. The 19:81 formulated mixture of E5-10:OH and E5-10:Ac used in the basic bait mixture was a gift from Consep Membranes, Inc. (Bend, OR).

Commercial pheromone lures and mating disruption dispensers. Samples of pheromone trap lures and mating disruption dispensers were obtained from the following companies: BASF (Ludwigshafen, West Germany), white plastic double reservoir, combination mating disruption dispenser for PTB and oriental fruit moth; Consep Membranes Inc. (Bend, OR) Biolure^{R} (Lot #73021392) permeable membrane lure and rubber septa (Lot #P.W3L8150189); Trece Inc. (Salinas, CA) rubber septa (Lot #9680669, 1989, and #10690990, 1990). Volatiles were collected from the pheromone trap lures by aeration of the devices for 24 hr with clean air (activated charcoal filter) at a flow rate of approx. 1 li/min, trapping the volatiles on a 5mm bed of precleaned activated charcoal (thermally desorbed at 120°C under He atmosphere, 2 hr) between 2 glass wool plugs in a Pasteur pipette. The trapped volatiles were extracted from the charcoal trap by rinsing with glass distilled CH_2Cl_2, and analyzed by GC and GC-MS as described above.

RESULTS

Analysis of pheromone gland extracts. A number of minor components were identified in pheromone gland extracts (Table 2) in addition to the previously reported major components, E5-10:OH and E5-10:Ac (Roelofs et al. 1975). These trace components included decyl acetate, E3-10:Ac, Z3,E5-10:Ac, and E3,E5-10:Ac. These minor components were present in trace amounts (>2% of the major component), and consequently were identified by retention time matches versus synthetic standards on three different capillary GC columns, and by comparison of their mass spectra with those of synthetic standards. Synthetic standards of the Z and E isomers of

decenols and decenyl acetates with the double bond in the 3,4,5,6, or 7 position were available for comparison (See table 1). The positional isomers of the decenols and decenyl acetates had characteristic and distinguishable mass spectra, and in most cases different retention times, so that double bond positions could be reliably assigned. The E3,E5- and Z3,E5-10:Ac were identified by comparison of retention times (on three GC columns) and mass spectra with synthetic standards. Further confirmation of the double bond geometry assignments was obtained by scrambling the double bond geometries of 3E,5E-10:Ac by exposing an ether solution of the dienyl acetate and a catalytic amount of iodine to sunlight for several hours. GC analysis (DB-225 column) of the resulting mixture of isomers showed four well-resolved peaks, indicating that all four geometric isomers were readily separable.

There was no evidence detected of any longer or shorter chain homologs of the pheromone compounds.

GC-EAD Analyses of Pheromone Gland Extracts. Pheromone gland extracts were analyzed by GC-EAD on three capillary columns of different polarity (DB-1, DB-5, DB-225), using excised male antennae. Consistent antennal responses were seen at the retention times of the major components, E5-10:OH (9/10 runs) and E5-10:Ac (10/10 runs). In 3 out of 10 runs, antennal responses corresponding to E3- or Z3-10:Ac were seen.

Analysis of large scale aerations of virgin females. Only E5-10:OH and E5-10:Ac were consistently identified in large scale aerations. In GC-MS analyses of some of the aerations, there was a trace peak seen at the retention times of 10:Ac, but the mass spectra were too fragmentary to confirm the presence of these compounds. In addition, the ratios of E5-10:OH and E5-10:Ac in different aerations were not reproducible. There was usually a preponderance of E5-10:OH, which is the lesser of the two major components in gland extracts.

GC Analysis of Effluvia from Single females. Volatiles were collected from the extruded pheromone glands of a number of calling virgin female moths, and analyzed by GC on a DB-225 column. E5-10:OH, E5-10:Ac, 10:Ac, and E3-10:Ac were tentatively identified in the extracts by comparison of retention times with those of standards. The ratio of the components was approx. 18:100:1.6:<1 respectively. Neither of the decadienyl acetates which had been found in the pheromone gland extracts were detected in the female effluvia. The identities of E5-10:Ac, E5-10:OH and 10:Ac were confirmed by GC-MS with selected ion monitoring (5 ion matches versus 500 pg standards).

Field testing of pheromone blends. A complete series of ratios of the two major components of the pheromone had been exhaustively tested previously (Roelofs et al., 1975), so we concentrated on testing the minor components of pheromone gland extracts as possible synergists. A variety of isomers and analogs of the known pheromone components were also tested for possible synergistic or antagonistic activity. Compounds were added to a basic lure blend of E5-10:OH and E5-10:Ac (19:81, a commercial formulation), in amounts equal to 2 or 10 % of the basic lure. Of all the compounds tested (Table 1), only the lures with 10% Z5-10:Al resulted in significant increases in trap captures versus the basic bait (Totals from four replicates counted four times, 484 versus 344 moths caught respectively, Table 3). This compound was not a component of any of the insect-produced volatiles.

Two compounds were found to significantly suppress trap captures, Table 3. E6-10:Ac significantly decreased trap captures at the 2% (1207 moths caught) and 10% levels (496 moths caught) versus the standard blend (7235 moths). When added at the 2% level, E7-10:Ac lowered the overall trap captures, although the decrease in capture was not statistically significant. At the 10% level, E7-10:Ac decreased trap captures by an order of magnitude (26 moths caught versus 344 moths attracted to the standard lure).

Analysis of commercial lures and dispensers. The volatiles recovered from commercial pheromone trap lures and the contents of mating disruption dispensers varied in composition, both in the number of compounds present, and in their ratios (Table 4). Generally, the pheromone lures contained formulations of high purity, with the impurities comprising less than 2% of the volatile pheromone. However, the 1989 Trece septa contained considerable quantities of E4-and E6-10:Ac. This finding was significant, as these septa had performed very poorly in field trials versus the 1990 septa from the same company (trap catch totals for 4 reps counted 4 times: 11 vs. 334 moths; second trial, 4 reps counted 3 times: 29 vs. 753 moths).

The BASF mating disruption devices contained considerable quantities of Z5-10:OH and Z5-10:Ac, the geometric isomers of the pheromone components. There was no significant difference in the ratio of the components in devices aged for two months in the field and unused devices.

DISCUSSION

Our research was conducted on a limited number of hand-collected specimens of PTB. In consequence, it was not possible to isolate sufficient quantities of the minor components of the pheromone for full spectroscopic and chemical identification. However, it was still possible to conclusively identify the trace components in the pheromone blend and in the synthetic pheromone formulations by comparison of retention and mass spectral properties with those of an extensive set of synthetic standards. It was found that the decenol and decenyl acetate positional isomers were distinguishable by careful comparison of their mass spectra with those of standards, backed up by retention time comparisons on several capillary GC columns of different polarities. Similar methods have been used for the identification of the homologous dodecenyl (Horiike and Hirano, 1982; Lanne et al., 1985) and tetradecenyl acetates (Horiike et al., 1981). Having ascertained the position of the double bond, the geometry of the double bond was readily determined by comparison of retention times with those of standards, especially on the polar cyanopropyl DB-225 column, where the geometric isomers were separated by a minimum of 15 sec (Oven temp. 120^{0}C isothermal).

The identification of the decadienyl acetate isomers was more straightforward, as the mass spectra of the positional isomers were more readily distinguishable than those of the monoene analogs. In particular, the mass spectra (electron impact, 70 eV) of the 3,5-decadienyl acetates found in the PTB extracts were characterized by the lack of a molecular ion due to the facile loss of acetic acid to form a conjugated triene fragment, and a base peak at *m/z* 79, from the further facile fragmentation of the triene fragment to give a $C_6H_7^+$ ion. A similar method for the facile identification of the positional isomers of the dodecadienyl acetate analogs by EI GC-MS has been previously reported (Ando et al., 1985). Geometric isomers were readily distinguishable on the basis of retention times.

Ten-carbon compounds have been identified as attractants or pheromones for comparitively few lepidopteran species, primarily in the genera *Coleophora* (Coleophoridae), *Euxoa* (Noctuidae), *Agrotis* (Noctuidae), and *Agonopterix* (Oecophoridae) (Compilation to 1986, Arn et al.). Of the moths attracted by ten-carbon compounds, PTB is the only species known to use a monounsaturated compound with a trans double bond. In addition, to our knowledge, this is the first identification of decadienyl components in a lepidopteran pheromone gland.

There was considerable variability in the amount of pheromone components from effluvia collected from the PTB females, with approximately half of the moths producing no detectable pheromone, despite all the moths being of approximately the same age (2-5 days old) and aerations being carried out at the approximately the same time (ifrst 2-3 hours of scotophase). However, at least part of this variation may be attributable to the difficulty in working with these small moths, particularly with the apparatus used, whereby the pheromone gland had to be forcibly extruded through a small hole into the airstream.

The mass aerations of female PTB were also only partially successful, giving variable ratios of the two major components in relatively small quantities. It is thought that the acetate component may have been degraded by contact with the large amount of loose moth scales on the inside of the aeration chamber and at the effluent trap inlet. Furthermore, the small amount of material recovered suggested that the pheromone components were being adsorbed onto the moths and the loose scales, as well as onto the walls of the container. This notion was supported by the fact that no pheromone was recovered from fresh charcoal traps when the aerations were continued for a second or third night without cleaning out the aeration chamber.

A number of minor components were identified in pheromone gland extracts and in effluvia collected from live female PTB. However, no biological activity as measured by changes in trap captures versus the standard blend, either as attractants or behavioral antagonists, was found for the minor components in field tests. These compounds also caused no significant or consistent electroantennogram activity when male antennae were challenged with the pheromone gland extract in coupled GC-EAD trials. These compounds may represent redundant components of the pheromone blend, as has been reported with some of the pheromone gland components of other moths such as the cabbage looper (Linn and Roelofs, 1989). Alternatively, they may have some role in close range courtship or copulation, which would not necessarily be detected by the relatively crude bioassay criterion of altered trap captures. Work is currently underway to establish laboratory cultures of PTB for more detailed wind tunnel studies, where the behavioral effects of the minor components of the blend can be studied in detail.

Of the variety of isomers and analogs of the pheromone components which were field tested (Table 1), Z4-10:Al, E7-10:Ac, and E6-10:Ac were found to be biologically active. For unknown reasons, Z4-10:Al significantly increased the attractiveness of the standard blend. This increase was inexplicable, as this compound was not found in the pheromone gland extracts or the female PTB effluvia.

E7-10:Ac and E6-10:Ac drastically suppressed trap captures, indicating that they were strong behavioral antagonists. At this point, the ecological significance of the antagonistic effects is unknown, as these compounds are not known pheromone components for any other lepidopteran species, that is, they have no known role in preventing interspecific attraction. Behavioral antagonism of male PTB by a

suspected contaminant in a synthetic pheromone component had been previously reported (Roelofs et al., 1975), but the inhibitory compound was not isolated and identified (W. Roelofs, pers. comm.).

The pheromone lure formulations were generally of high purity, with the exception of the 1989 Trece septa which were found to contain significant quantities of two isomers, E4- and E6-10:Ac. The strong behavioral antagonism exhibited by the latter compound in field trials suggests a reason for the very poor performance of these lures versus the 1990 lures from the same company.

The mating disruption dispensers from BASF were found to contain considerable quantities of the Z double bond isomers of the pheromone components. Although these compounds exerted no significant effect on the attractiveness of pheromone lures, their effects in mating disruption have not been determined.

The findings described above underscore the need for rigourous quality control in the preparation and testing of pheromone formulations. In contrast to pesticides, where impurities in the technical formulation almost invariably have little or no effect on the efficacy and lethality of the active ingredient, even trace impurities in pheromone formulations may drastically alter the biological activity and the consequent efficacy of the pheromone product. At this relatively early stage in the widespread use and acceptance of pheromone technology and mating disruption techniques in particular, one well-publicized failure of a pheromone product due to an impure formulation may set back the adoption by growers of pheromone-based strategies as an integral part of pest management by several years. Given the increasing public awareness of and dissatisfaction with large-scale continued use of toxic pesticides, and the consequent increasingly restrictive limitations placed on pesticide usage, it is imperative that researchers, and companies providing pheromone products, do their utmost to prevent these types of failures.

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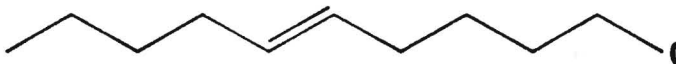
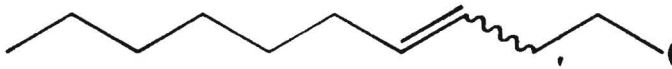
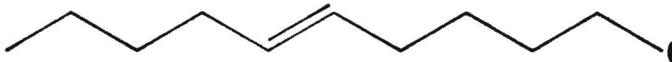
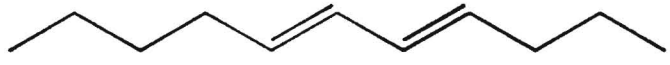
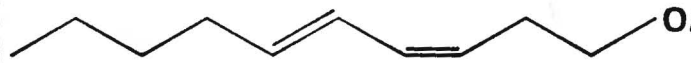
Table 1. Sources of chemicals tested in peach twig borer field bioassays.

Chemical	Abbreviation	Source[a]	Synthetic method
Decanol	10:OH	Polyscience	
Decanoldehyde	10:Al	Aldrich	
Decyl acetate	10:Ac	Sigma	
E3-decenyl acetate	E3-10:Ac	Ref. std.	
Z3-decenyl acetate	Z3-10:Ac	Ref. std.	
E4-decenyl acetate	E4-10:Ac	-	Isomerize Z4-10:Ac
Z4-decenyl acetate	Z4-10:Ac	Lancaster	
E5-decenyl acetate	E5-10:Ac	Sigma[b]	
Z5-decenyl acetate	Z5-10:Ac	Sigma	
E6-decenyl acetate	E6-10:Ac	-	Wittig reaction; acetylation
Z6-decenyl acetate	Z6-10:Ac	-	Wittig reaction; acetylation
E7-decenyl acetate	E7-10:Ac	Ref. std.	
Z7-decenyl acetate	Z7-10:Ac	Ref. std.	
E5-decenol	E5-10:OH	-[b]	Hydrolysis of E5-10:Ac
Z5-decenol	Z5-10:OH	-	Hydrolysis of Z5-10:Ac
E5-decenaldehyde	E5-10:Al	-	Oxidation of E5-10:OH
Z5-decenaldehyde	Z5-10:Al	-	Oxidation of Z5-10:OH
E7-dodecenyl acetate	E7-12:Ac	Ref. std.	
Z7-dodecenyl acetate	Z7-12:Ac	Ref. std.	
E3,E5-decadienol	E3,E5-10:OH	-	Wittig reaction
Z3,E5-decadienol	Z3,E5-10:OH	-	Wittig reaction
E3,E5-decadienyl acetate	E3,E5-10:Ac	-	Acetylation of E3,E5-10:OH
Z3,E5-decadienyl acetate	Z3,E5-10:Ac	-	Acetylation of Z3,E5-10:OH

[a]Sources are as follows: Polyscience Corporation; Sigma Chemical Co.; ; Aldrich Chemical Co. Ref. std. = reference stadard from pheromone collection of T.C.B., originally obtained from ; Lancaster = Lancaster Synthesis.

[b]Also obtained as a blend, E5-10:OH + E5-10:Ac (19:81) from Consep Membranes, Bend, OR.

Table 2. PTB Pheromone Gland Components

Compound	Structure	Ret. time	Ratio in① gland	Ratio in② Effluvia
E5-10:OH		12.36	4.1	18
E-or Z3-10:Ac		14.21	0.4	0.3?
E5-10:Ac		14.34	100	100
10:Ac		14.46	1.3	1.7
E3,E5-10:Ac		14.83	trace	-
Z3,E5-10:Ac		15.07	0.9	-

①Mean of 3 extracts
②Mean of 2 aerations

Table 3. Effects of test chemicals on pheromone trap catches of peach twig borer

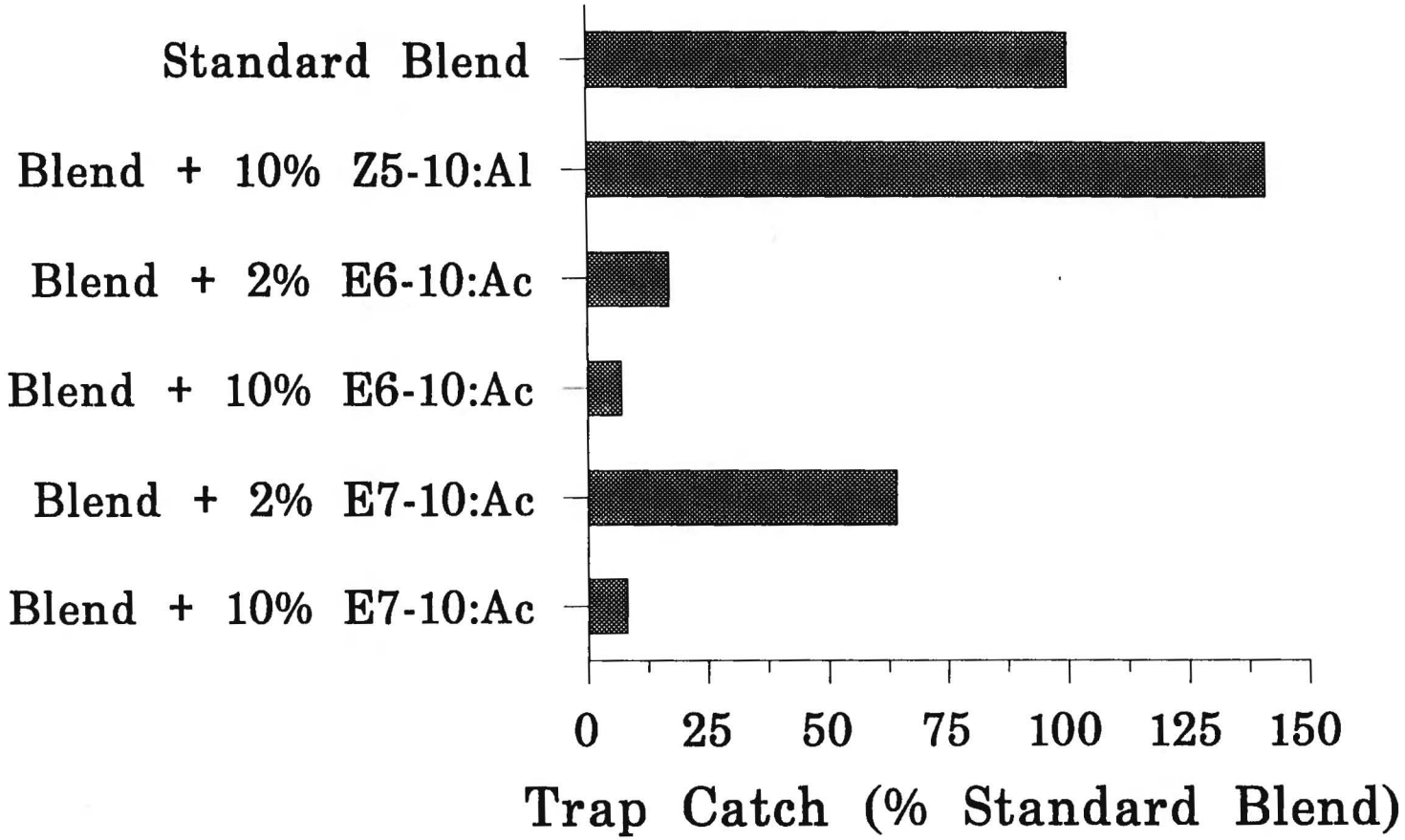


Table 4. Analysis of commercial peach twig borer pheromone lures and mating disruption dispenser on DB-225 capillary column[a].

Product	Composition, as percentage of E5-10:Ac content					
	E5-10:OH	E5-10:Ac	Z5-10:OH	Z5-10:Ac	E4-10:Ac	E6-10:Ac
BASF, unused[b]	21	100	5.8	26.5	-	-
BASF, field aged[b] 2 months	24	100	6.0	24.7	-	-
Consep Biolure[c]	23.6	100	0.2	1.2	-	-
Consep, membrane[b]	23	100	Trace	1.2	-	-
Consep septum[c]	30	100	-	Trace	-	-
Trécé septum, ~~1989~~ 1990[c]	18	100	-	-	-	-
Trécé septum, ~~1990~~ 1989[c]	27	100	-	0.9	5.2	3.3

[a]Temp. program 120°/15 min, 10°/min 220°.

[b]Contents of mating disruption dispenser removed by syringe, dilute with hexone.

[c]Lures aerated, with volatiles collected on activated charcoal.

UNIVERSITY OF CALIFORNIA, RIVERSIDE

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COLLEGE OF NATURAL AND
AGRICULTURAL SCIENCES
DEPARTMENT OF ENTOMOLOGY
FAX: (714) 787-3086

RIVERSIDE, CALIFORNIA 92521

May 15, 1991.

To: Susan McCloud, Research Director, Almond Board

From: Jocelyn Millar, Asst. Professor, Entomology, UC Riverside

Re: Spring Progress Report

When our 1991 proposal was prepared, we had planned on synthesizing three compounds in multigram amounts for mating disruption trials. However, a subsequent more rigourous statistical analysis of the data suggested that the slightly increased attraction to standard lures caused by one of the compounds may not be significant. Because of this, and because we were granted approximately 10% less funds thatn we had requested, we have delayed large scale synthesis of this compound (which would take several weeks to make) until further small-scale field trials are conducted.

Approx. 25 grams of each of the other two compounds have been synthesized. However, field testing has been delayed because we only just received the release devices required, despite verbal and written assurances from the supplier (BASF product via Pacific Biocontrol) that we would have them by the end of March. These devices will be loaded and deployed in time for the second PTB flight in July.

We have received the first shipments of peach twig borer (PTB) for analyzing pheromone from different field populations. Arrangements have been made for further shipments to follow shortly. The analytical methodology for analyzing pheromone from individual insects has been optimized (See accompanying manuscript) and the method will shortly be transferred to a gas chromatograph with an automatic sampler, so that we can increase throughput.

PTB are notoriously difficult to rear in the lab. We have developed new rearing techniques and have successfully established a colony, and our culture of PTB is currently being augmented with field-collected specimens to broaden the genetic base. We have received several inquiries about supplying PTB to other research groups, and have trained a student from Kent Dana's group in rearing techniques. A paper describing optimized rearing conditions is in preparation.

Further testing of multicomponent blends as trap lures will be conducted over the summer, as we were only able to test two-component blends last year.

The behavioral studies with male PTB moths will not be started until late summer or fall, once the bulk of the field work is completed.

I enclose a copy of a manuscript submitted to the Journal of Economic Entomology, describing our 1990 PTB work which was funded by the ALmond Board.

Please contact me at (714) 787-5821 if you require any further information.

Jocelyn A. Millar

For: Journal of Econ. Entomology

Address correspondence to:

Jocelyn G. Millar
Department of Entomology
University of California
Riverside CA 92521

Reexamination of the Female Sex Pheromone of the Peach Twig Borer, *Anarsia lineatella* Zeller. Identification of Minor Components from the Pheromone Gland and Field Screening of Pheromone Analogs

JOCELYN G. MILLAR and RICHARD E. RICE[1]

Department of Entomology, University of California

Riverside California 92521

ABSTRACT Several minor components in extracts of female peach twig borer (*Anarsia lineatella* Zeller; Lepidoptera: Gelechiidae) pheromone glands have been identified as decanyl acetate, (*E*)- and (*Z*)-4-decenyl acetate, and (*E*,*E*)- and (*Z*,*E*)-decadienyl acetate. Decenyl acetate and (*E*)-4-decenyl acetate were also identified in effluvia from live female moths. None of the identified compounds enhanced the attractiveness of the standard blend of (*E*)-5-decenol and (*E*)-5-decenyl acetate (19:81) in field tests. However, the analogous compounds (*E*)-6-decenyl acetate and (*E*)-7-decenyl acetate were identified as behavioral antagonists, and strongly suppressed trap captures. The contents of a variety of commercial pheromone lures and mating disruption devices were analyzed, and one batch of lures, which had performed very poorly in field tests, was found to contain the inhibitory compound (*E*)-6-decenyl acetate.

KEY WORDS: Insecta, pheromone, behavioral antagonist, mating disruption.

THE PEACH TWIG BORER (PTB), *Anarsia lineatella* Zeller, is a major pest of almonds and stone fruits in North America, Europe, and Asia (Bailey 1948, Summers et al. 1959). In California, there are as many as four generations per year. Damage is caused by the developing larvae tunnelling in fruits, buds and shoots (Hathaway et al. 1985). Damage can be so severe that in most crops, prophylactic pesticide sprays are routinely applied, even in non-bearing orchards (Summers 1955).

Until recently, PTB infestations in California have been fairly well controlled by a combination of pesticide treatments. However, development of resistance to insecticides, reported as long ago as 1959 (Summers et al. 1959) plus the increasingly restrictive limitations placed on the use and registration of the currently used pesticides has created an urgent need for alternative management strategies.

Two components of the PTB female sex pheromone were identified by Roelofs et al. (1975) as (*E*)-5-decenol (*E*5-10:OH) and (*E*)-5-decenyl acetate (*E*5-10:Ac), and traps baited with blends of the two components captured male moths. There were discernable differences between geographically separated populations, with a central California population responding to all blends of the two components approximately equally, while a Washington state population was attracted preferentially to traps baited with *E*5-10:OH as a single component.

Roelofs et al. (1975) also mentioned that field trials during their first two years were hampered by trace amounts of a strong behavioral antagonist in their synthetic formulations. The antagonist was not identified.

Pheromone-mediated mating disruption has been successfully applied for the control of two other important gelechiid moth pests, the pink bollworm (Baker et al. 1990) and the tomato pinworm (Jenkins et al. 1990). Mating disruption may represent an environmentally safe strategy for control of PTB, and small-scale field trials were begun in California several years ago, culminating in 1989, when formulations from four different suppliers were evaluated. Fruit yield losses were reduced in the disrupted areas as compared to untreated control blocks, but considerable numbers of male moths were still caught in pheromone-baited traps placed throughout the disrupted blocks, indicating that male insects were still able to locate point sources of pheromone (R.E. Rice, personal observation). On the basis of these observations, the following objectives were established:

1. To reexamine the pheromone components in the pheromone glands of PTB, with particular emphasis on identifying trace components to improve the attractancy of the blend.

2. To determine the number and ratio of volatile compounds released by calling female moths by aeration of virgin calling females.

3. To synthesize and field test potential minor pheromone components, and possible antagonists.

4. To analyze the contents of commercially produced PTB pheromone lures and mating disruption dispensers. This objective was formulated after beginning the study, because we received reports of a batch of pheromone lures which were not attractive to male moths, suggesting the presence of a behavioral antagonist generated during the manufacturing process.

Materials And Methods

Insects and insect extracts. Insects were collected as pupae from cardboard bands placed around tree trunks in infested almond and peach groves in Fresno and Tulare counties near Parlier, CA, and shipped by courier to UC Riverside. Pupae were collected in April, late June, and early August, corresponding to the first three generations. Once received, pupae were maintained individually in 10-dram glass shell vials at approx. 25°C under a 15/9 light/dark cycle until and after eclosion.

Female pheromone gland extracts were prepared by excising the extruded pheromone glands of 2-7 day old virgin females (calling behavior is initiated on the second day after emergence, S. McElfresh, pers. communication) in the first couple of hours of the scotophase, and soaking the glands in pentane (~100 μl) for 20 min. Extracts were concentrated under N_2, and analyzed by gas chromatography (GC), coupled gas chromatography-mass spectrometry (GC-MS) and coupled GC-electroantennogram detection (GC-EAD) as previously described (Arn et al. 1975, Baker et al. 1991). No internal standards were added, as the extracts were found to contain decyl acetate which was used as a reference.

Extracts were initially analyzed by gas chromatography on a Hewlett-Packard 5890 GC (Avondale, PA) in splitless mode, coupled to an H.-P. 5970 mass selective detector operated in electron impact mode (70 eV). Two columns were used: 1) a 20 m x .25 mm I.D. x .25μ film thickness Ultra 2 column (Hewlett-Packard), temperature program 60°C/1 min, 20°/min to 110°C, then 3°/min to 150°C, then 20°/min to 250°C for 10 min, head pressure 90 kPa He,

and 2) 30 m x .25 mm x .25μ film DB-225 column (J&W Scientific, Folsom, CA), program 60°C/1 min, 20°/min to 120°C, then 4°/min to 200°C, 104 kPa He. Injector and transfer line temperatures were set at 250°C.

Coupled GC-EAD analyses were carried out with a Varian 3700 GC with an effluent splitter (split ratio ~1:1), as described in detail elsewhere (Baker et al. 1991). GC and EAD traces were recorded simultaneously on a matched pair of H.-P. 3394 integrators. Extracts were analyzed on DB-1, DB-5 and DB-225 columns (30 m x 0.25 mm I.D.; J&W Scientific) in splitless mode. Temperature programs used were 100°/2 min, 20°/min to 200° for 20 min for the DB-1 and DB-5 columns, and 80°/2 min, 15°/min to 180° for 20 min for the DB-225 column.

Effluvia from individual virgin females were collected from extruded pheromone glands by the method of Baker et al. (1981), during the first four hours of the scotophase. Extruded glands were aerated for periods of 20-30 min, the trapped volatiles were eluted off the glass wool trapping material with CS_2 (ca. 200 μl), the extract was concentrated under N_2, and analyzed by GC-EAD and/or GC-MS.

Field tests. All field tests were conducted in almond groves near Parlier, CA, during the four PTB flight periods (June-October) as compounds become available through synthesis. Pherocon 1C traps (Trece Inc., Salinas, CA) were used. Traps were baited with red rubber septa (Thomas Scientific, Philadelphia, PA) impregnated with heptane solutions (10 mg/ml) of the test compounds. All septa except solvent blanks were loaded with a standard blend of *E*5-10:Ac and *E*5-10:OH (81:19 blend, 1 mg total weight), obtained as a gift from Consep

Membranes, Inc. (Bend, OR). No antioxidants or UV stabilizers were used. Compounds to be tested were then added to the septa as heptane solutions (1 mg/ml), in amounts of 20 or 100 μg (2 or 10% of the standard blend). Traps were set out in almond orchards in randomized complete block design, with each treatment replicated four times. Traps were counted approx. every second day, and moved one position at each count. Data were analyzed with the SAS PROCGLM program, version 6.03 (SAS Institute, Carey, NC). Raw data were subjected to an $(X+1/2)^{1/2}$ transformation, and the transformed data were analyzed by 2-way ANOVA (with treatment and block as the classification variables), followed by Ryan's modified Q test (Day & Quinn 1989) to separate significantly different treatment means. Treatments with zero catches overall were not included in the ANOVA, as the lack of variance would violate an assumption of the ANOVA treatment.

Chemicals. Chemicals were obtained from commercial sources, or synthesized by standard synthetic methods as described in Table 1 and as follows. Acetates were cleaved to alcohols by stirring in EtOH with catalytic amounts of NaOH. Alcohols were oxidized to aldehydes with pyridinium dichromate in methylene chloride (Corey &Schmidt, 1979). Alcohols were acetylated by treatment with acetyl chloride and pyridine in ether. *Z*4-10:Ac was partially isomerized to *E*4-10:Ac with aqueous nitrous acid (Sonnet 1974). The geometric isomers were then separated by flash chromatography (Still et al. 1978) on silica gel impregnated with silver nitrate (15%) eluting with 6% ether in hexane. *E*- and *Z*6-10:Ac were obtained by Wittig reaction of 6-hydroxyhexyl triphenylphosphonium bromide with butyraldehyde (Ando et al. 1988), followed

by acetylation of the crude alcohol products, and separation of the geometric isomers as described above. *E*3,*E*5- and *Z*3,*E*5-10:Ac were prepared in similar fashion by acetylation of the products of the Wittig reaction of hydroxypropyl triphenylphosphonium bromide with *E*2-heptenal. When quantities permitted, synthetic compounds were further purified by Kugelrohr distillation (0.2 mm Hg, oven temp ~100°C) to remove traces of chromatographic packing material and solvent. All chemicals used were >98% chemically and isomerically pure by capillary GC. The 19:81 formulated mixture of *E*5-10:OH and *E*5-10:Ac used in the basic bait mixture was a gift from Consep Membranes, Inc. (Bend, OR).

Commercial pheromone lures and mating disruption dispensers. Samples of pheromone trap lures and mating disruption dispensers were obtained from the following companies: BASF (Ludwigshafen, West Germany), white plastic double reservoir, combination mating disruption dispenser for PTB and oriental fruit moth; Consep Membranes Inc. (Bend, OR) Biolure® (Lot #73021392) permeable membrane lure and rubber septa (Lot #P.W3L8150189); Trece Inc. (Salinas, CA) rubber septa (Lot #9680669, 1989, and #10690990, 1990). Volatiles were collected from the pheromone trap lures by aeration of the devices for 24 hr with clean air (activated charcoal filtered) at a flow rate of approx. 1 l/min, trapping the volatiles on a 5mm bed of activated charcoal (precleaned by thermal desorbtion at 220°C under He atmosphere, 2 hr) between 2 glass wool plugs in a Pasteur pipette. The trapped volatiles were extracted from the charcoal trap by elution with glass distilled CH_2Cl_2, and analyzed by GC and GC-MS as described above.

Results

Analysis of pheromone gland extracts. A number of previously unidentified compounds were identified in pheromone gland extracts (Table 2) in addition to the previously reported major components, *E*5-10:OH and *E*5-10:Ac (Roelofs et al. 1975). These trace components included decyl acetate (10:Ac), *Z*4- and *E*4-decenyl acetate (*Z*4- and *E*4-10:Ac), *Z*3,*E*5-decadienyl acetate (*Z*3,*E*5-10:Ac), and *E*3,*E*5-decadienyl acetate (*E*3,*E*5-10:Ac). These compounds were present in trace amounts (<2% of the major component), and consequently were identified by retention time matches versus synthetic standards on polar and nonpolar capillary GC columns (Table 2). Synthetic standards of the *Z* and *E* isomers decenyl acetates with the double bond in the 3,4,5,6, or 7 position were available for comparison (See Table 1). Comparison of retention times on the two capillary columns was crucial to the identification, because the standards were not completely resolved on either one of the two columns. However, in all cases, compounds which overlapped on one column were resolved on the second column, and vice versa. Thus, for example, *E*4-10:Ac was not resolved from the front edge of the large *E*5-10:Ac peak in the insect extract on the Ultra 2 column, but it was cleanly resolved on the DB-225 column.

The identifications from retention time matches were corroborated by comparison of mass spectra of insect-produced compounds with those of standards. The positional isomers of the decenyl acetates had similar, but characteristic and distinguishable mass spectra so that double bond positions could be confirmed. The mass spectra of the *E* and *Z* isomers of a particular positional

isomer were virtually indistinguishable, but the geometry of the double bonds could be reliably assigned from the retention time comparisons.

The *E*3,*E*5- and *Z*3,*E*5-10:Ac were identified by comparison of retention times (on three GC columns) and mass spectra with synthetic standards. Further confirmation of the double bond geometry assignments was obtained by scrambling the double bond geometries of *E*3,*E*5-10:Ac by exposing an ether solution of the dienyl acetate and a catalytic amount of iodine to sunlight for several hours. GC analysis (DB-225 column) of the resulting mixture of isomers showed four well-resolved peaks, indicating that all four geometric isomers were separable.

There was no evidence of any longer or shorter chain homologs of the pheromone compounds.

GC-EAD Analyses of Pheromone Gland Extracts. Pheromone gland extracts were analyzed by GC-EAD on three capillary columns of different polarity (DB-1, DB-5, DB-225), using excised male antennae. Consistent antennal responses were seen at the retention times of the major components, *E*5-10:OH (9/10 runs) and *E*5-10:Ac (10/10 runs). Antennal responses were not seen consistently to any other compounds in the insect extract.

Analysis of large scale aerations of virgin females. Only *E*5-10:OH and *E*5-10:Ac were consistently identified from aerations of groups of virgin females. In GC-MS analyses of some of the aerations, there was a trace peak seen at the retention time of 10:Ac, but the mass spectra were too fragmentary to confirm the presence of this compound. In addition, the ratios of *E*5-10:OH and *E*5-10:Ac in different aerations were not reproducible. There was usually a

preponderance of *E*5-10:OH, which is the lesser of the two major components in gland extracts.

GC analysis of effluvia from single females. Volatiles were collected from the extruded pheromone glands of calling virgin female moths as previously described (Baker et al. 1981), and analyzed by GC on a DB-225 column. *E*5-10:OH, *E*5-10:Ac, 10:Ac, and *E*4-10:Ac were tentatively identified in the extracts by comparison of retention times with those of standards. The ratio of the components was approx. 18:100:1.6:<1, respectively (N=3). Neither of the decadienyl acetates which had been found in the pheromone gland extracts were detected in the female effluvia. The identities of *E*5-10:Ac, *E*5-10:OH and 10:Ac were confirmed by GC-MS with selected ion monitoring (ion ratios matches with 5 ions versus 500 pg of standards).

Field testing of blends. A complete series of ratios of the two major components of the pheromone had been exhaustively tested previously (Roelofs et al. 1975), so we concentrated on testing the minor components of pheromone gland extracts as possible synergists. A variety of isomers and analogs of the known pheromone components were also tested for possible synergistic or antagonistic activity. When added to the basic lure blend of *E*5-10:OH and *E*5-10:Ac (19:81) in amounts equal to 2 or 10 % of the basic lure, none of the trial compounds, resulted in significantly increased trap captures versus the basic bait (Tables 3 & 4).

Two compounds were found to significantly suppress trap captures. *E*6-10:Ac drastically decreased trap captures to levels not significantly different from

solvent controls when added to the basic lure blend at 2% and 10% levels (Table 4). When added at the 2% level (Table 3), *E*7-10:Ac lowered the overall trap captures, although the decrease in capture was not statistically significant at the 95% probability confidence interval. However, at the 10% level, *E*7-10:Ac decreased trap captures by an order of magnitude.

Analysis of commercial lures and dispensers. The volatiles recovered from commercial pheromone trap lures and the contents of mating disruption dispensers varied in composition, both in the number of compounds present, and in their ratios (Table 5). Generally, the pheromone lures contained formulations of high purity, with the impurities comprising less than 2% of the volatile pheromone. However, the 1989 Trece septa contained considerable quantities of *E*4- and *E*6-10:Ac. This finding was significant, as our experiments had shown *E*6-10:Ac to be antagonistic, and these septa had performed very poorly in field trials versus the 1990 septa from the same company (Trece septa vs standard blend, trap catch totals for 4 reps. counted 4 times: 11 vs. 334 moths; second trial, 4 reps counted 3 times: 29 vs. 753 moths).

The mating disruption devices from BASF contained considerable quantities of *Z*5-10:OH and *Z*5-10:Ac, the geometric isomers of the pheromone components. There was no difference in the ratio of the components in devices aged for two months in the field and unused devices.

Discussion

Our research was conducted on a limited number of hand-collected specimens of PTB. In consequence, it was not possible to isolate sufficient

quantities of the minor components of the pheromone gland extracts for full spectroscopic and chemical analyses. However, it was still possible to conclusively identify most of the minor compounds from gland extracts and in the synthetic pheromone formulations by comparison of retention times and mass spectral properties with those of synthetic standards. The decenol and decenyl acetate positional and geometric isomers were distinguishable by comparisons of retention times on two capillary columns of differing polarity, backed up by comparisons of mass spectra with those of standards (Table 2). Similar methods have been used for the identification of the homologous dodecenyl (Horiike & Hirano 1982, Lanne et al. 1985) and tetradecenyl acetates (Horiike et al. 1981).

The identification of the decadienyl acetate isomers was more straightforward, as the mass spectra of the positional isomers were more readily distinguishable than those of the monoene analogs. In particular, the mass spectra (electron impact, 70 eV) of the 3,5-decadienyl acetates found in the PTB extracts were characterized by the lack of a molecular ion, probably due to the facile loss of acetic acid to form a conjugated triene fragment, and a base peak at *m/z* 79, from the further facile fragmentation of the triene fragment to give a $C_6H_7^+$ ion. A similar method for the identification of the positional isomers of the dodecadienyl acetate analogs by EI GC-MS has been previously reported (Ando et al. 1985). Geometric isomers were readily distinguishable on the basis of retention times.

Ten-carbon compounds have been identified as attractants or pheromones for comparatively few lepidopteran species, primarily in the genera *Coleophora* (Coleophoridae), *Euxoa* (Noctuidae), *Agrotis* (Noctuidae), and *Agonopterix*

(Oecophoridae) (Compilation to 1986, Arn et al.). Of the moths using ten-carbon compounds in their pheromones, PTB is the only species known to use a monounsaturated compound with a trans double bond. In addition, to our knowledge, this is the first identification of decadienyl compounds from a lepidopteran pheromone gland.

There was considerable variability in the amount of pheromone components from effluvia collected from PTB females, with approximately half of the moths producing no detectable pheromone, despite all the moths being of approximately the same age (2-5 days old) and aerations being carried out at approximately the same time (first 2-3 hours of scotophase). However, at least part of this variation may be attributable to the difficulty in working with these small moths, particularly with the apparatus used, whereby the pheromone gland was forcibly extruded through a small hole into the airstream (for a full description of aeration apparatus and technique, see Baker et al. 1981).

Mass aerations of female PTB were also only partially successful, giving variable ratios of the two major components in relatively small quantities. It is thought that the acetate component may have been degraded or adsorbed on contact with the large amount of loose moth scales on the inside of the aeration chamber and at the effluent trap inlet. Furthermore, the small amount of material recovered suggested that the pheromone components were being adsorbed onto the moths and the loose scales, as well as onto the walls of the container.

Trace amounts of several volatile compounds were identified in pheromone gland extracts and in effluvia collected from live female PTB. However, no biological activity as measured by changes in trap captures versus the standard

blend, either as attractants or behavioral antagonists, was found for the minor components in field tests. These compounds also resulted in no significant or consistent electroantennogram activity when male antennae were challenged with pheromone gland extract in coupled GC-EAD trials. These compounds may represent redundant components of the pheromone blend, as has been reported with some pheromone gland components of other moths such as the cabbage looper (Linn & Roelofs 1989). Alternatively, they may have some role in close range courtship or copulation, which would not necessarily be detected by the relatively crude bioassay criterion of altered trap captures.

Of the variety of isomers and analogs of the pheromone components which were field tested (Tables 3 & 4), only *E*7-10:Ac and *E*6-10:Ac were found to affect trap captures, being strong behavioral antagonists. The possible significance of the antagonistic effects is unknown, as to our knowledge, these compounds have not been reported as pheromone components for any other lepidopteran species, that is, they have no known role in preventing interspecific attraction. Behavioral antagonism of male PTB by a suspected contaminant in a synthetic pheromone component had been previously reported (Roelofs et al. 1975), but the inhibitory compound was not isolated and identified (W. Roelofs, pers. comm.).

The commercial pheromone lure formulations were generally of high purity, with the exception of the 1989 Trece septa which were found to contain significant quantities of two isomers, *E*4- and *E*6-10:Ac. The strong behavioral antagonism exhibited by the latter compound in field trials suggests a reason for the poor performance of these lures versus the 1990 lures from the same

company. These findings underscore the need for rigorous quality control in the preparation and testing of pheromone formulations.

The mating disruption dispensers from BASF were found to contain considerable quantities of the *Z* double bond isomers of the pheromone components. Although these compounds exerted no significant effect on the attractiveness of pheromone lures, their effects in mating disruption have not to our knowledge been determined.

Acknowledgments

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Footnotes

[1]Department of Entomology, University of California, Davis CA 95616.

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Table 1. Sources of chemicals tested in peach twig borer field bioassays.

Chemical	Abbreviation	Source[a]	Synthetic method
Decanol	10:OH	Polyscience	
Decanaldehyde	10:Al	Aldrich	
Decyl acetate	10:Ac	Sigma	
*E*3-decenyl acetate	*E*3-10:Ac	Ref. std.	
*Z*3-decenyl acetate	*Z*3-10:Ac	Ref. std.	
*E*4-decenyl acetate	*E*4-10:Ac	-	Isomerize *Z*4-10:Ac
*Z*4-decenyl acetate	*Z*4-10:Ac	Lancaster	
*E*5-decenyl acetate	*E*5-10:Ac	Sigma[b]	
*Z*5-decenyl acetate	*Z*5-10:Ac	Sigma	
*E*6-decenyl acetate	*E*6-10:Ac	-	Wittig reaction; acetylation
*Z*6-decenyl acetate	*Z*6-10:Ac	-	Wittig reaction; acetylation
*E*7-decenyl acetate	*E*7-10:Ac	Ref. std.	
*Z*7-decenyl acetate	*Z*7-10:Ac	Ref. std.	
*E*5-decenol	*E*5-10:OH	-[b]	Hydrolysis of *E*5-10:Ac
*Z*5-decenol	*Z*5-10:OH	-	Hydrolysis of *Z*5-10:Ac
*E*5-decenaldehyde	*E*5-10:Al	-	Oxidation of *E*5-10:OH
*Z*5-decenaldehyde	*Z*5-10:Al	-	Oxidation of *Z*5-10:OH
*E*7-dodecenyl acetate	*E*7-12:Ac	Ref. std.	
*Z*7-dodecenyl acetate	*Z*7-12:Ac	Ref. std.	
*E*3,*E*5-decadienol	*E*3,*E*5-10:OH	-	Wittig reaction
*Z*3,*E*5-decadienol	*Z*3,*E*5-10:OH	-	Wittig reaction
*E*3,*E*5-decadienyl acetate	*E*3,*E*5-10:Ac	-	Acetylation of *E*3,*E*5-10:OH
*Z*3,*E*5-decadienyl acetate	*Z*3,*E*5-10:Ac	-	Acetylation of *Z*3,*E*5-10:OH

[a]Sources are as follows: Alltech Assoc., Deerfield, IL; Sigma Chemical Co., St. Louis, MO; Aldrich Chemical Co., Milwaukee, WI; Lancaster Synthesis, Windham, N.H. Ref. std. = reference standard from pheromone collection of T.C.Baker.

[b]Also obtained as a blend, *E*5-10:OH + *E*5-10:Ac (19:81) from Consep Membranes, Bend, OR.

Table 2. Retention times of peach twig borer pheromone gland components and analogs on Ultra 2 and DB-225 capillary columns.

	Ultra 2[a]		DB-225[a]		Amount per
	Relative retention time[b]		Relative retention time[b]		gland (ng)
Compound	Standard	Insect Extr.[d]	Standard	Insect Extr.	(Mean + S.D.)[c]
*E*5-10:OH	0.6951	+	0.9710	+	10.0 ± 6.1
10:Ac	1.0000	+	1.0000	+	4.0 ± 2.1
*E*3-10:Ac	0.9649	-	1.0065	-	
*Z*3-10:Ac	0.9592	-	1.0168	-	
*E*4-10:Ac	0.9721	?	1.0206	+	1. [c]
*Z*4-10:Ac	0.9538	+	1.0280	+	1. [c]
*E*5-10:Ac	0.9758	+	1.0336	+	273.4 ± 69.8
*Z*5-10:Ac	0.9726	-	1.0514	?	
*E*6-10:Ac	0.9816	?	1.0402	?	
*Z*6-10:Ac	0.9823	-	1.0626	-	
*E*7-10:Ac	0.9897	-	1.0486	-	
*Z*7-10:Ac	0.9982	?	1.0766	-	
*E*3,*E*5-10:Ac	1.0999	+	1.2542	+	1.7 ± 0.6
*Z*3,*E*5-10:Ac	1.0566	+	1.2159	+	0.9 ± 0.3

[a]Ultra 2 column, 20 m x 0.2 mm, 60°C/1 min, 20°/min to 110°C, then 3°/min to 150°C, then 20°/min to 250°C for 10 min. DB-225 column, 25 m x 0.25 mm.

[b]Retention times relative to 10:Ac.

[c]Mean of ten replicates, except for *E*4- and *Z*4-10:Ac, where a 10-female equivalent extract was used, and the quantity estimated by comparison with the *E*5-10:Ac peak.

[d]+ Indicates present in extract, - indicates absent, ? indicates that peak would be obscured by one of major components present.

Table 3. Moths captured in traps containing lures with the standard PTB pheromone blend[a] plus 2% or 10% additions of test compounds.

		Compound	2% Addition (Mean ± S.E.)	10% Addition (Mean ± S.E.)
Expt. 1[b]	1.	Solvent control	0.1 ± 0.1 a	0
	2.	Std. blend	11.7 ± 0.8 b	20.9 ± 1.8 ab
	3.	Std. blend + decanol	11.6 ± 1.8 b	27.2 ± 4.1 a
	4.	decanaldehyde	12.9 ± 1.7 b	21.6 ± 2.3 ab
	5.	decyl acetate	12.9 ± 2.1 b	23.2 ± 1.7 a
	6.	*Z*5-10:Ac	8.1 ± 1.5 b	12.6 ± 1.7 b
	7.	*Z*5-10:Al	9.9 ± 1.5 b	22.9 ± 2.4 a
	8.	*Z*5-10:OH	10.4 ± 1.1 b	23.6 ± 3.7 a
	9.	*E*5-10:Al	13.4 ± 1.4 b	25.4 ± 2.4 a
	10.	*E*3-10:Ac	10.8 ± 1.1 b	22.6 ± 2.4 a
	11.	*Z*3-10:Ac	12.8 ± 1.6 b	26.1 ± 3.1 a
	12.	*E*7-10:Ac	7.5 ± 0.9 b	1.6 ± 0.4 c
	13.	*Z*7-10:Ac	11.6 ± 1.9 b	27.4 ± 3.0 a
	14.	*E*7-12:Ac	10.4 ± 0.9 b	22.3 ± 2.3 a
	15.	*Z*7-12:Ac	11.7 ± 1.4 b	24.0 ± 2.5 a

[a]Standard blend = 1 mg of *E*5-10:OH + *E*5-10:Ac (19:81).

[b]Bioassays with 2% additions carried out 6/14-6/23/90; bioassays with 10% additions carried out 6/30-7/6/90. All traps counted 4 times at 2-day intervals. Numbers followed by the same letter are statistically equivalent ($P \geq 0.05$).

Table 4. Moths captured in traps containing lures with the standard PTB pheromone blend[a] plus 2% or 10% additions of test compounds.

	Blend (% test compound)	Trap catch (Mean ± S.E.)
Expt. 3[b]	Std. blend	43.8 ± 12.1 a
	Std. blend + *E*3,*E*5-10:OH (2)	45.0 ± 12.5 a
	+ *Z*3,*E*5-10:OH (2)	44.4 ± 13.7 a
	+ *E*3,*E*5-10:Ac (2)	41.8 ± 13.9 a
	+ *E*3,*E*5-10:Ac (10)	43.9 ± 13.4 a
Expt. 4[b]	Std. blend	31.4 ± 4.1 a
	Std. blend + *E*4-10:Ac (2)	29.4 ± 2.8 a
	+ *E*4-10:Ac (10)	33.5 ± 4.5 a
	+ *Z*4-10:Ac (2)	35.8 ± 6.1 a
	+ *Z*4-10:Ac (10)	35.2 ± 4.1 a
Expt. 5[b]	Std. blend	226.1 ± 27.2 a
	Std. blend + *E*6-10:Ac (2)	37.7 ± 13.7 b
	+ *E*6-10:Ac (10)	15.5 ± 6.2 b
	+ *Z*6-10:Ac (2)	209.4 ± 24.5 a
	+ *Z*6-10:Ac (10)	190.5 ± 26.0 a
	Solvent blank	3.0 ± 1.3 b

[a]Standard blend = 1 mg of *E*5-10:OH + *E*5-10:Ac (19:81).

[b]Expt. 3: 7/21-7/27/90, counted 3 times; Expt. 4: 8/8-8/20/90, counted 5 times; Expt. 5: 10/3-10/22/90, counted 8 times.

Table 5. Analysis of commercial peach twig borer pheromone lures and mating disruption dispensers on a DB-225 capillary column[a].

	Composition, as percentage of *E*5-10:Ac content					
Product	*E*5-10:OH	*E*5-10:Ac	*Z*5-10:OH	*Z*5-10:Ac	*E*4-10:Ac	*E*6-10:Ac
BASF, unused[b]	21	100	5.8	26.5	-	-
BASF, field aged[b] 2 months	24	100	6.0	24.7	-	-
Consep Biolure[c]	23.6	100	0.2	1.2	-	-
Consep, membrane[b]	23	100	Trace	1.2	-	-
Consep septum[c]	30	100	-	Trace	-	-
Trécé septum, 1990[c]	18	100	-	-	-	-
Trécé septum, 1989[c]	27	100	-	0.9	5.2	3.3

[a]Temp. program 120°/15 min, 10°/min 220°.

[b]Contents of mating disruption dispenser removed by syringe, diluted with hexane.

[c]Lures aerated, with volatiles collected on activated charcoal.

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