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Sex Attractant Pheromone of the Pecan Nut Casebearer (Lepidoptera: Pyralidae)

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Abstract—A female-produced sex pheromone for the pecan nut casebearer, *Acrobasis nuxvorella* Neunzig, has been identified from pheromone gland extracts of calling female moths. The compound (9*E*,11*Z*)-hexadecadienal [(9*E*,11*Z*)–16:Ald] was identified by coupled GC–EAD and retention time matches with a synthetic standard on four capillary GC columns of different polarities. Corroboration of the identification of (9*E*,11*Z*)–16:Ald by other analytical chemistry methods was not possible due to the minute quantities of pheromone extracted (<1 picogram/female). In field studies, gray rubber septa impregnated with 100 μg of synthetic (9*E*,11*Z*)–16:Ald were attractive to male moths, whereas higher and lower doses were less attractive. The homologous (9*E*,11*Z*)–15:Ald was also slightly attractive, while the more highly conjugated analogues, (9*E*,11*Z*,13*Z*)- and (9*E*,11*Z*,13*E*)–16:Ald, were not. Copyright © 1996 Elsevier Science Ltd

Introduction

The monophagous and multivoltine pecan nut casebearer, *A. nuxvorella* Neunzig (Lepidoptera: Pyralidae) is one of the most important pests of commercially grown pecans, *Carya illinoensis* (Wang) in the United States and Mexico. The insect is distributed throughout the southeastern United States as far west as Texas and New Mexico, and recent expansion of its range into southern New Mexico threatens commercial pecan production in this and other western states.

Overwintering casebearer larvae emerge in spring to feed on buds and shoots, while larvae of subsequent generations feed in developing pecan nuts.[1] Pecan nut casebearers of the first summer generation have the greatest potential for causing damage because a single larva will consume several nutlets, although subsequent generations can also cause significant damage. Typically, infestations of first generation larvae are controlled with a single, well-timed insecticide application. Treatment decisions and timing are based on visual inspections of nutlets for newly laid eggs.[2] A degree-day model also aids in the timing of orchard surveys for eggs.[3] However, for many producers, eggs are difficult to find and scouting for eggs is very time and labor intensive. A sensitive and selective monitoring system for adult moths would be of considerable benefit for alerting growers to the need to scout orchards for eggs, to indicate the optimum timing for insecticide treatments and, in conjunction with egg surveys, may aid in determining if insecticide treatments are warranted. A reliable monitoring tool would also be of major value in detecting and delineating new infestations.

Accurately assessing the need to apply insecticides for pecan nut casebearer is critical, because insecticide treatments can disrupt biological control of secondary pests such as the yellow pecan aphid, *Monelliopsis pecanis* Bissel, and the black-margined aphid, *Monellia caryellia* (Fitch).[4] Despite the economic importance of the pecan nut casebearer and the need for better monitoring tools, there have been no reported studies aimed at identifying sex pheromone components for this insect.

Our objectives were: (1) to identify and synthesize pecan nut casebearer sex pheromone components and (2) to develop pheromone-baited trapping methods for monitoring adult casebearer populations.

Results

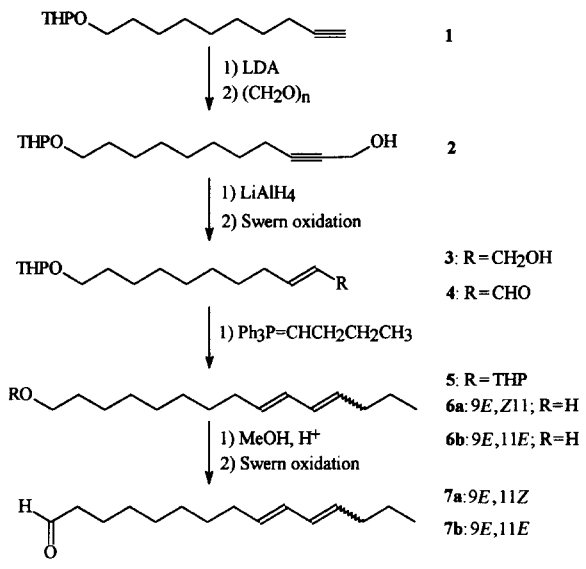
Preliminary field and laboratory studies suggested that female pecan nut casebearers produced very small amounts of pheromone, that males responded poorly

Key words: *Acrobasis nuxvorella*, (9*E*,11*Z*)-hexadecadienal, (9*E*,11*Z*)-pentadecadienal, (9*E*,11*Z*,13*Z*)-hexadecatrienal, (9*E*,11*Z*,13*E*)-hexadecatrienal.

to the pheromone or that females released pheromone infrequently. Traps baited with caged virgin females were minimally attractive, often catching no male moths. Furthermore, traps baited with pheromone gland extracts caught no moths. However, freshly prepared pheromone gland extract did elicit characteristic wing-fanning behavior by male moths in a qualitative laboratory test, suggesting the presence of one or more sex pheromone components in the extract.

Numerous initial attempts at locating potential pheromone components in pheromone gland extracts by coupled GC–EAD failed, probably due to the minute quantities of pheromone in extracts (vide infra). The first significant responses to chemical stimuli by male antennae were obtained during GC–EAD screening of synthetic compounds from a pheromone library. It was found that a trace contaminant (< 0.2%) in a sample of synthetic 10*E*,12*Z*-hexadecadienal [(10*E*,12*Z*)–16:Ald] elicited strong and reproducible antennal responses. These responses were much larger than those to (10*E*,12*Z*)–16:Ald, suggesting that the antenna was responding very selectively to specific structural features of the trace contaminant. GC–MS analysis determined that the trace contaminant had a molecular weight of 222 daltons, 14 mass units less than (10*E*,12*Z*)–16:Ald. The mass spectrum was otherwise very similar to that of (10*E*,12*Z*)–16:Ald, suggesting that the impurity was a conjugated 15 carbon aldehyde homologue of (10*E*,12*Z*)–16:Ald. The position of the conjugated diene functionality was determined from the isobutane chemical ionization (CI) mass spectrum,[5] which gave diagnostic fragment ions at *m/z* 85 (cleavage and rearrangement of a 9,10 double bond) and *m/z* 169 (cleavage and rearrangement of an 11,12 double bond), placing the diene in the 9,11 position. The assignment of the diene placement was corroborated by comparison with the CI mass spectrum of the analogous (10*E*,12*Z*)–16:Ald, which showed the same diagnostic ion at *m/z* 85, and a diagnostic fragment at *m/z* 183, one methylene unit larger than the *m/z* 169 fragment in the C_{15} compound. On the reasonable assumption that the 15 carbon compound was a chain-shortened homologue of (10*E*,12*Z*)–16:Ald, the C_{15} compound was tentatively identified as (9*E*,11*Z*)-pentadecadienal [(9*E*,11*Z*)–15:Ald]. This assignment was confirmed by synthesis (Scheme 1).

Further experiments were conducted to clarify whether (9*E*,11*Z*)–15:Ald was indeed a pheromone component, or whether it simply possessed structural features mimicking those of a casebearer pheromone component. Several GC–EAD experiments were run to determine the importance of various structural features. First, when (10*E*,12*Z*)-hexadecatriene alcohol, aldehyde or acetate were tested, male antenna only gave significant responses to (10*E*,12*Z*)–16:Ald, indicating the importance of the aldehyde function. However, an aldehyde functionality alone was not sufficient for the strong activity, because minimal responses were obtained to a series of saturated straight-chain aldehydes (dodecanal to hexadecanal),



Scheme 1.

indicating that the diene functionality was also crucial. Antennal responses to a variety of unsaturated aldehydes [(9*Z*)–14:Ald, (10*Z*)– and (10*E*)–15:Ald, (11*E*)–15:Ald, (9*Z*,11*E*)–14:Ald] were also minimal, and synthetic (9*E*,11*Z*)–15:Ald elicited antennal responses an order of magnitude larger than those elicited by (9*E*,11*E*)–15:Ald, corroborating the high degree of specificity of the antennal receptors for the (9*E*,11*Z*)-diene placement and geometry.

It was still not clear whether the 15 carbon chain length was correct or whether (9*E*,11*Z*)–15:Ald was a homologue of a 14 or 16 carbon compound. Even numbered chain lengths are much more common for lepidopteran pheromones than odd numbered ones, but 15 carbon compounds are not without precedent. In particular, 15 carbon compounds have been reported as pheromone components or sex attractants for other pyralid species [e.g., *Chilo auricilius* Dudgeon, (10*Z*)–15:Ac],[6] including two species in the *Acrobasis* genus [*A. rufilimbalis* Wileman, (9*Z*)–15:Ac; *A. vaccinii* Riley, (8*E*,10*Z*)–15:Ac + (9*E*)–15:Ac].[7a,b] Consequently, (9*E*,11*Z*)–15:Ald was field tested. Seven traps baited with 0.1 mg doses of (9*E*,11*Z*)–15:Ald on rubber septa caught only three moths over a period of several days, suggesting that the (9*E*,11*Z*)–15:Ald structure was not quite correct.

Further efforts to obtain GC–EAD evidence from female pheromone gland extracts were finally successful, producing a single reproducible antennal response (Fig. 1). The compound eliciting the response eluted at a retention time consistent with that of a conjugated 16 carbon aldehyde. The quantity of the compound in the extract was minute (vide infra), being well below the limit of detection of the flame ionization detector on the GC–EAD (detection limit < 0.1 ng).

From the fact that (9*E*,11*Z*)–15:Ald gave much larger antennal responses than the homologous (10*E*,12*Z*)–16:Ald in GC–EAD tests, the correct pheromone

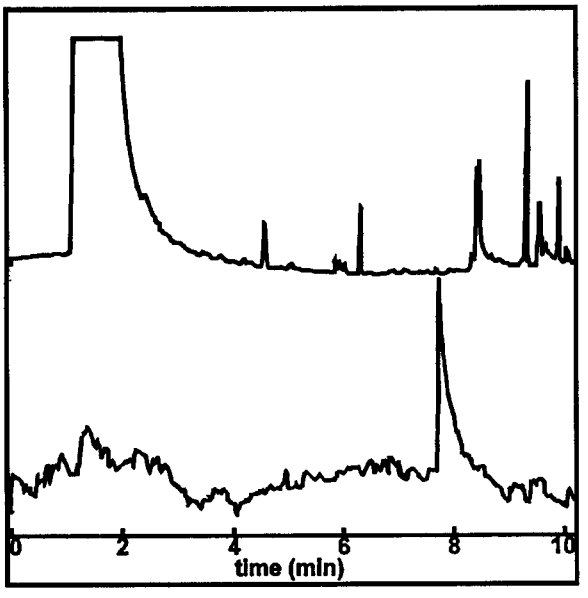
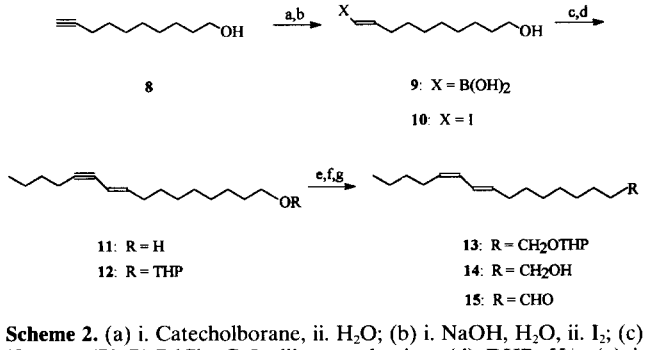


Figure 1. Coupled GC–EAD traces for a pecan nut casebearer male moth antenna responding to a combined pheromone gland extract from 34 female moths. Top trace = gas chromatograph detector response; bottom trace = antennal response. The antennal response occurred at a retention time a few seconds later than the small GC peak at 7.71 min, in an area where there was no detectable GC peak.

structure was tentatively assigned as (9*E*,11*Z*)–16:Ald. This structure was confirmed by unambiguous synthesis (Scheme 2). Synthetic (9*E*,11*Z*)–16:Ald had retention times identical to the compound in the pheromone extract on four capillary GC columns with differing polarities [DB-5, Kovats retention index (KI) 1853; DB-210, KI 2251; DB-23, KI 2473; DB-WAX], using male moth antennae as the detectors. Further attempts at obtaining analytical data in support of the structure were stymied by the minute quantities of pheromone in extracts.

The amount of pheromone extracted from females was estimated by calibration of male antennal responses with synthetic (9*E*,11*Z*)–16:Ald. Three of six male antennae tested gave useable calibration lines, resulting in estimates of 7 ($r^2=0.80$), 53 ($r^2=0.98$), and 162 ($r^2=0.83$) femtograms per female equivalent from three extracts.



Scheme 2. (a) i. Catecholborane, ii. H_2O; (b) i. NaOH, H_2O, ii. I_2; (c) Hexyne, $(Ph_3P)_2PdCl_2$, CuI, diisopropylamine; (d) DHP, H^+; (e) i. Dicyclohexylborane, ii. AcOH; (f) MeOH, H^+; (g) Swern oxidation.

Attempts to quantify the amount of pheromone actually released by collection and GC–EAD analyses of volatiles produced by batches of calling female moths were not successful; no antennal responses were obtained from any of the five extracts tested.

In the first field trials of (9*E*,11*Z*)–16:Ald, male pecan nut casebearer moths were attracted to the synthetic compound; in a nine day period (1–10 Sept. 1993), five Delta traps baited with 100 μg of (9*E*,11*Z*)–16:Ald caught 194 male moths. During the same time period, eight traps baited with virgin females (replaced as often as required) caught only a single male moth.

In two subsequent experiments testing pheromone dose, traps baited with 100 μg doses captured the most moths, with significantly lower trap catches obtained with lower doses (Table 1). Reduced attraction to the 1000 μg dose suggested that male moths were repelled or arrested by higher doses.

Results of season-long trapping in 1994 defined four major flight periods in El Paso, Texas (Fig. 2),

Table 1. Numbers of male pecan nut casebearer caught in traps baited with different doses of (9*E*,11*Z*)-hexadecadienal [(9*E*,11*Z*)–16:Ald]

Dose (μg)	Mean (±SEM) trap catch per sample date[a]	
	Trial 1[b]	Trial 2[b]
1000	0.57 ± 0.24^b	0.47 ± 0.21^a
100	1.23 ± 0.29^c	1.03 ± 0.24^b
10	0.20 ± 0.09^b	0.37 ± 0.18^a
1	0.03 ± 0.03^a	0
0.1	0	0
Blank	0	0

[a]Numbers followed by the same letter are not significantly different ($\alpha=0.05$, Fisher's LSD test).
[b]Trial 1: 9–19 May 1994; six replicates of each dose, Navarro Co., Texas. Trial 2: 3–14 July 1994; six replicates of each dose, Collins Co., Texas.

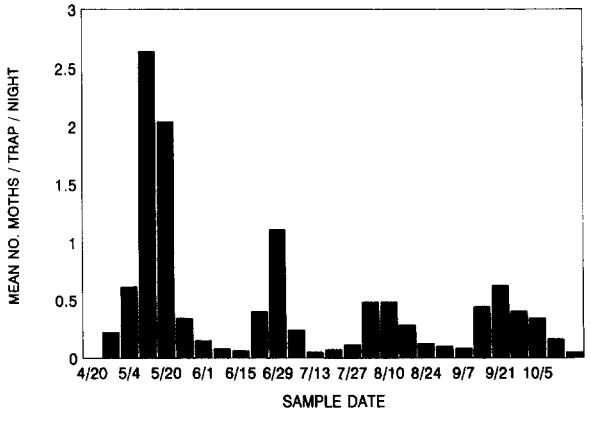


Figure 2. Seasonal flight activity of male pecan nut casebearer moths in El Paso, Texas, as monitored with Delta sticky traps baited with (9*E*,11*Z*)-hexadecadienal (100 μg/trap). Traps were counted weekly and lures were replaced every 3 weeks.

consistent with previous observations that pecan nut casebearer completes 4–5 generations per year with ca. 42 days between generations.[1]

At the end of the 1994 field season, trap catches were lower than expected (1–2 moths/trap/night). The relatively low trap catches, coupled with the very small quantities of (9*E*,11*Z*)–16:Ald found in the pheromone gland extracts, led us to hypothesize that (9*E*,11*Z*)–16:Ald might be only a minor component of the pheromone blend and that the major component had escaped detection during GC–EAD analyses due to thermal instability. Unstable conjugated triene aldehyde pheromone components, in combination with the analogous dienes and monoenes, recently have been identified from several moth species [e.g. *Ectomyelois ceratoniae*, (9*Z*,11*E*,13)–14:Ald;[8a,b] *Manduca sexta*, (10*E*,12*E*,14*Z*)–16:Ald[9]]. Consequently, the more highly conjugated analogs of (9*E*,11*Z*)–16:Ald [i.e. (9*E*,11*Z*,13*Z*)- and (9*E*,11*Z*,13*E*)–16:Ald] were synthesized (Scheme 3) and field tested. Traps baited with either of the two compounds (50 μg doses, four replicates) caught no moths during a 1 week period (9–16 May 1995). During the same period, four control traps baited with (9*E*,11*Z*)–16:Ald (50 μg/trap) caught a total of 24 moths.

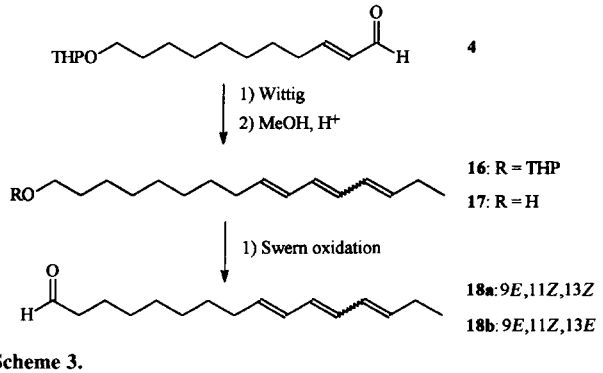
Discussion

The successful identification of (9*E*,11*Z*)–16:Ald as a pheromone component for the pecan nut casebearer hinged upon the results of a series of GC–EAD bioassays using male moth antennae as detectors. The females of this species appear to produce unusually small amounts of pheromone, with subpicogram levels being detected in pheromone gland extracts and with undetectable levels being collected from calling female moths. The fact that virgin females or extracts prepared from virgin females are ineffective as trap lures provides further circumstantial evidence of a very low pheromone titer per female.

To our knowledge, this is the first report of (9*E*,11*Z*)–16:Ald as an insect pheromone component, although this compound was synthesized as a potential pheromone candidate in 1984.[10] In fact, conjugated 16 carbon dienes with the diene moiety in the 9,11 position are comparatively unknown as lepidopteran pheromone components; we are aware of only one other report of a similar compound being identified as an insect pheromone [the pyralid species *Diatraea saccharalis* (Fabricius); (9*Z*,11*E*)–16:Ald].[11]

A single reproducible GC–EAD response has been obtained from pheromone gland extracts to date. However, it is possible that other as yet undetected compounds may comprise part of the pheromone blend. Although the complete lack of attraction to the triene analogues of (9*E*,11*Z*)–16:Ald [i.e. (9*E*,11*Z*,13*Z*)- and (9*E*,11*Z*,13*E*)–16:Ald] suggests that these particular compounds are probably not part of the pheromone blend, it is entirely possible that other trace components were destroyed or irreversibly adsorbed on the GC columns during analyses or that the responses of male antennae to other blend components were weak and below the limits of detection of our apparatus. Furthermore, the peak obtained from an antennal response is comparatively broad (Fig. 1), so that compounds with retention times similar to that of (9*E*,11*Z*)–16:Ald, such as the other 9,11–16:Ald isomers, may go undetected.

Relatively low pheromone trap catches have been reported for several other pyralid moth species which use conjugated diene aldehydes as sex pheromones, even though the synthetic pheromones being used as trap baits appear to closely mimic the natural pheromone blends. For example, the carob moth, *Ectomyelois ceratoniae* (Zeller), was less attracted to traps baited with synthetic pheromone blend than to traps baited with virgin females.[8b] Combinations of live females and synthetic pheromone were slightly more attractive than virgin females alone, indicating that the poorer performance of the synthetic pheromone relative to virgin females was not due to the presence of inhibitory components in the synthetic blend. The pheromone of the navel orangeworm, *Amyelois transitella* (Walker), a key pest of several nut crops, was identified more than 15 years ago,[12] but it has not found use in monitoring or mating disruption, apparently due to unsatisfactory performance in the field. Results of detailed studies with the rice stem borer, *Chilo suppressalis* (Walker) suggest that the efficacy of synthetic pheromone as a trap bait decreases as populations of female moths build up and provide competition for the pheromone lures.[13a,b]



Scheme 3.

The synthetic (9*E*,11*Z*)–16:Ald used to make trap baits was of greater than 97% chemical purity and greater than 98.5% isomeric purity. It is conceivable, and certainly not without precedent, that one or more of the trace impurities from synthesis or from degradation of the pheromone under field conditions, may have hindered male moth attraction to traps in 1993 and 1994 field tests. However, ongoing field tests suggest that the relatively low trap catches in field tests during 1993 and 1994 were due at least in part to suboptimal trap design and placement. Trap designs currently under investigation routinely produce mean trap catches of >10 moths/trap/night during peak

moth flight periods, with individual traps catching >30 moths/night. Results from these trials will be reported in due course.

In summary, analytical data and field trapping results provide strong evidence that (9*E*,11*Z*)-16:Ald is a pheromone component of the pecan nut casebearer. This compound was identified from subpicogram quantities in extracts prepared from calling female moths and was the only consistently GC-EAD active compound in the extracts. Ongoing field trials have demonstrated that traps baited with (9*E*,11*Z*)-16:Ald can be used as a simple and rapid monitoring method for pecan nut casebearer, with many hundred traps currently in use. Further work is planned or in progress to optimize trap design and to clarify the role of potential synergists and antagonists in the sex pheromone chemistry of this pest insect.

Experimental

Insects

Methods for maintaining the pecan nut casebearer in laboratory culture have not been reported, although field-collected larvae have been reared to adults on artificial diet.[14] Adult pecan nut casebearers used in this study were obtained from pupae or larvae taken from infested nuts collected in north central Texas. Larvae were reared to the pupal stage on an artificial diet (Knutson, unpublished). Pupae were shipped to the quarantine facility at the University of California, Riverside, and maintained at 25 °C and ~25% RH until emergence under a natural photoperiod (~12:12 L:D). Emerging adult moths were collected daily and sexed, and segregated in individual 150 mL plastic vials. Virgin females were held for 1–3 days, then used to prepare pheromone gland extracts. Emerging male moths were used within 2 days of emergence, to provide antennae for coupled GC-EAD studies as described below.

Preparation and analysis of pheromone gland extracts

Pheromone glands were harvested from 1–3 day old calling female moths during the middle of their calling period (approx. 1 a.m.). The pheromone glands were everted by gentle pressure on the abdomen and clipped off with iris scissors. Individual excised glands were soaked in 20 μL of pentane for 5 min and the extracts from several glands were then combined in one conical-bottomed vial and concentrated by passive evaporation.

Aliquots of extracts were analyzed by GC-EAD on four columns (DB-5, 30 m × 0.32 mm i.d., temperature program 100 °C/0 min, 15 °C/min to 275 °C/10 min; DB-WAX, 30 m × 0.32 m ID, 100 °C/0 min, 15 °C/min to 240 °C/30 min; DB-23, 30 m × 0.32 mm, temperature program 100 °C/1 min, 10 °C/min to 200 °C for 20 min; DB-210, 30 m × 0.32 mm, temperature program 100 °C/1 min, 10 °C/min to 220 °C/20 min; J&W Scientific, Folsom, California). Analyses were run using a Hewlett Packard (HP, Avondale, Pennsylvania) 5880 or 5890 Series II GC in splitless mode, with Helium carrier gas. For runs with the first two columns, the column effluent was split equally between two 0.25 mm i.d. deactivated fused silica capillaries, with one capillary routed to the GC flame ionization detector (FID) and the other routed through a heated transfer line (250 °C) to a small, septum sealed hole in the glass sample delivery tube. For the latter two columns, the entire column effluent was directed into the sample delivery tube. The delivery tube consisted of a 1 cm ID glass tube, with the distal end terminating in a hose nipple and the proximal end being slightly flattened and flared to accommodate the insect antenna. Air flow rate through the sample delivery tube was 400 mL/min, with the air being humidified by passage over a pad of water-saturated glass wool on the bottom of the tube.

Male antennae were removed by gently grasping the antennal scape and pulling the antenna free from the head. The terminal end of the antenna was cut off with a razor blade and the antenna was suspended between two saline-filled glass capillary electrodes fitted over chloridized silver wires, with the wires running down the centers of the electrodes to within 2 mm of the suspended antenna. The preparation was moved as close as possible to the end of the stimulus delivery tube. Antennal reponses were amplified with a custom-built multi-stage amplifier and the amplified signal was recorded on an HP 3394 integrator. For those analyses where the GC effluent was split between the antennal preparation and the FID, the FID response was simultaneously recorded on a second HP 3394 integrator.

To estimate the amount of pheromone per gland, the responses of male antennae were calibrated by sequential injection of log doses of synthetic pheromone (1×10^{-7} to 1 ng), from lowest to highest dose. In the middle of the calibration sequence a solvent blank was injected, followed by 2–2.5 female equivalent of pheromone gland extract. Calibration lines were prepared by plotting the height of the peaks generated by the antennal response versus the log of the injected dose.

Volatiles were collected from calling female moths using cylindrical glass aeration chambers (3 cm i.d. × 20 cm long). Five batches of female moths (4–12 moths, 1–5 days old) were placed in the chamber and charcoal-filtered air was passed through the chamber (300 mL/min), collecting the volatiles on a 5 mm i.d. × 1 cm bed of activated charcoal (50–200 mesh). Aerations were carried out from approx. 6.00 p.m. to 8.00 a.m. the following morning, under natural light conditions. The charcoal traps were eluted with pentane (250 μL) into conical-bottomed vials and concentrated to <5 μL by passive evaporation.

Synthesis

Proton NMR spectra were taken on a General Electric QE-300 instrument (300 MHz), in $CDCl_3$ solution.

EI–MS (70 eV) were taken with an HP 5970B mass selective detector interfaced to an HP 5890 gas chromatograph fitted with a DB-5MS column (20 m × 0.2 mm i.d., J&W Scientific). Mass spectra are reported as *m/z* (abundance). High resolution exact mass measurements were made with a VG 7070E double focusing magnetic sector instrument (EI, 50 eV), using a direct insertion probe. All synthetic operations were carried out in oven-dried glassware. THF was purified by distillation from sodium benzophenone ketyl under nitrogen. Unless otherwise specified, worked up reaction mixtures were dried with anhydrous Na_2SO_4 and concentrated by rotary evaporation under reduced pressure. Flash chromatography was carried out with 230–400 mesh silica gel (grade 60).

Synthesis of (9*E*,11*Z*)- and (9*E*,11*E*)-pentadecadienals (7a and 7b, Scheme 1)

(2*E*)-11-[(Tetrahydro-2*H*-pyran-2-yl)oxy]-undec-2-en-1-ol (3). LDA (1.5 M, 18.7 mL; 28 mmol) was added dropwise to an ice-cold solution of protected alkynol **1**[15] (6.08 g, 25.5 mmol) in THF (100 mL). After stirring 1 h, paraformaldehyde (1.5 g, 50 mmol) was added in one portion and the mixture was stirred at room temperature overnight. The mixture was then partitioned between water and hexane, and the hexane layer was washed with brine, dried and concentrated. The crude alkyne was taken up in ether and added dropwise to a slurry of $LiAlH_4$ in ether (50 mL). After stirring 16 h at room temp, the mixture was quenched by careful dropwise addition of water (2 mL), 20% aq NaOH (1.5 mL) and water (7.0 mL), while cooling in an ice-bath. The resulting mixture was stirred 20 min, then filtered, washed with brine, dried and concentrated. The residue was flash chromatographed (25% EtOAc in hexanes), yielding 5.8 g of the known alkenol **3**.[16] NMR: δ 5.67 (m, 2H), 4.57 (br t, 1H, J=3.5 Hz), 4.09 (d, 2H, J=4.8 Hz), 3.87 (m, 1H), 3.73 (dt, 1H, J=9.5, 6.9 Hz), 3.5 (m, 1H), 3.38 (dt, 1H, J=9.5, 6.6 Hz), 2.04 (br quart, 2H, J=6.5 Hz), 1.9–1.65 (m, 2H), 1.65–1.45 (m, 7H), 1.45–1.22 (m, 10H).

(2*E*)-11-[(Tetrahydro-2*H*-pyran-2-yl)oxy]-undec-2-en-1-al (4). DMSO (2.13 mL, 30 mmol) in CH_2Cl_2 (5 mL) was added dropwise over 15 min to a cooled (−78 °C) solution of oxalyl chloride (1.33 mL, 15 mmol) in CH_2Cl_2 (40 mL).[19] The mixture was stirred 15 min, followed by dropwise addition of alcohol **3** (2.0 g, 7.4 mmol). After stirring 45 min at −78 °C, Et_3N (9 mL, 65 mmol) was added over 5 min, the mixture was warmed to room temperature and poured into water and the known aldehyde **4**[16] was extracted with hexane. The hexane layer was washed with 1 M HCl, water and brine, and concentrated to 1.6 g (81%) of yellow oil. Crude **4**, which gave one spot on TLC, was used immediately without further purification. NMR: δ 9.50 (d, 1H, J=7.9 Hz), 6.86 (dt, 1H, J=15.0, 6.8 Hz), 6.12 (ddt, 1H, J=15.5, 7.9, 1.2 Hz), 4.57 (m, 1H), 3.87 (m, 1H), 3.72 (dt, 1H, J=9.5, 6.8 Hz), 3.50 (m, 1H), 3.38 (dt, 1H, J=9.5, 6.6 Hz), 2.33 (m, 2H), 1.9–1.65 (m, 2H), 1.65–1.45 (m, 8H), 1.45–1.25 (m, 8H). MS: 268 (M^+, trace), 101 (43), 85 (100), 67 (23), 55 (51), 41 (60).

(9*E*,11*Z*)- and (9*E*,11*E*)-Pentadecadienols (6a and 6b). BuLi (2.5 M in hexanes, 1.4 mL, 3.5 mmol) was added dropwise to a slurry of butyltriphenylphosphonium bromide (1.2 g, 3 mmol) in THF (10 mL) at −30 °C. The mixture was allowed to warm to room temperature, then cooled again to −30 °C, and aldehyde **9** (0.536 g, 2 mmol) was added dropwise. The mixture was allowed to warm to room temperature, then poured into water and extracted with hexane. The hexane extract was washed with brine, dried and concentrated. The residue was taken up in MeOH (25 mL) and a few crystals of PTSA were added. After stirring overnight at room temperature, solid $NaHCO_3$ (1 g) was added and the mixture was concentrated. The residue was partitioned between water and hexane, and the hexane layer was washed with water and brine, dried and concentrated. The residue was flash chromatographed (20% EtOAc in hexanes), yielding 240 mg of (9*E*,11*Z*)- and (9*E*,11*E*)-pentadecadienols **6a** and **6b** (7:3). An aliquot (70 mg) was separated into the individual isomers by passage through a 1.2 m × 6 mm i.d. column of macroporous ion exchange resin (Lewatit SP 1080, Ag^+ form, E. Merck),[17] eluting with MeOH at 1.5 mL/min and monitoring the effluent with a Dynamax RI-2 refractive index detector (Rainin Instruments). (9*E*,11*Z*)-isomer **6a**. NMR: δ 6.31 (dd, 1H, J=15.0, 11.3 Hz), 5.95 (br t, 1H, J=10.9 Hz), 5.66 (dt, 1H, J=15.0, 7.0 Hz), 5.32 (dt, 1H, J=10.6, 7.6 Hz), 3.64 (t, 2H, J=6.6 Hz), 2.12 (m, 4H), 1.63–1.50 (m, 4H), 1.48–1.20 (m, 11H), 0.92 (t, 3H, J=7.2 Hz). MS: 224 (M^+, 13), 135 (6), 121 (10), 109 (12), 96 (26), 81 (60), 67 (100), 55 (39), 41 (49). HRMS: calcd for $C_{15}H_{28}O$: 224.2140. Found: 224.2138.

(9*E*,11*E*)-isomer **6b**. NMR: δ 6.01 (m, 2H), 5.57 (m, 2H), 3.63 (t, 2H, J=6.6 Hz), 2.05 (m, 4H), 1.53 (m, 3H), 1.45–1.20 (m, 12H), 0.90 (t, 3H, J=6.8 Hz). The EI mass spectrum was very similar to that of the (9*E*,11*Z*) isomer.

(9*E*,11*Z*)- and (9*E*,11*E*)-Pentadecadienals (7a and 7b). Alcohol **6a** (35 mg, 0.15 mmol) was stirred overnight with a slurry of pyridinium dichromate (400 mg, 1.06 mmol) in CH_2Cl_2 (4 mL). The mixture was diluted with hexane and filtered through a short plug of Celite®. The filtrate was concentrated and flash chromatographed (5% ether in hexane), yielding 25 mg (72%) of (9*E*,11*Z*)-pentadecadienal (**7a**). The aldehyde was stored at −20 °C as a dilute solution in hexane, with butylated hydroxytoluene (BHT) added as antioxidant. NMR: δ 9.77 (t, 1H, J=1.5 Hz), 6.31 (br dd, 1H, J=13.7, 11 Hz), 5.97 (t, 1H, J=10.9 Hz), 5.65 (dt, 1H, J=15.1, 7.0 Hz), 5.32 (dt, 1H, J=10.7, 7.6 Hz), 2.43 (td, 2H, J=7.3, 1.5 Hz), 2.12 (m, 4H), 1.63 (m, 2H), 1.50–1.25 (m, 10H), 0.92 (t, 3H, J=7.3 Hz). MS: 222 (M^+, 9), 179 (1), 123 (3), 109 (11), 95 (22), 81 (44), 67 (100), 55 (29), 41 (54). HRMS: calcd for $C_{15}H_{26}O$: 222.1984. Found: 222.1984.

(9*E*,11*E*)-pentadecadienal (**7b**) was prepared in a similar fashion from **6b**. NMR: δ 9.77 (t, 1H, $J=7.5$ Hz), 6.0 (m, 2H), 5.57 (m, 2H), 2.42 (td, 2H, $J=7.2$, 1.5 Hz), 2.04 (m, 4H), 1.7–1.25 (m, 12H), 0.90 (t, 3H, $J=7.3$ Hz). MS: 222 (M^+, 9), 123 (7), 109 (17), 95 (26), 81 (43), 67 (100), 55 (33), 41 (53).

Synthesis of (9*E*,11*Z*)-hexadecadienal (15) (Scheme 2)

(9*E*)-10-Iodo-9-decen-1-ol (10). Catecholborane (18 mL, 170 mmol) was added dropwise to a solution of 9-decyn-1-ol (**8**) (11.2 g, 73 mmol) in THF (12 mL). After stirring for 16 h at 70 °C under Ar, the solution was cooled and quenched by slow addition of crushed ice until foaming (liberation of H_2) ceased. Water (500 mL) was added and the mixture was stirred for 4 h at room temperature, then cooled to 0 °C and filtered. The solids were resuspended in 200 mL ice water and filtered again, rinsing the solid cake on the filter several times with ice water. The amorphous white solid was air dried, yielding 12.9 g of (1*E*)-10-hydroxy-1-decenylboronic acid (**9**), which was used without further purification.

Crude **9** (12.74 g, 64.3 mmol) was dissolved in ether (200 mL), 1.5 M NaOH (300 mL) was added and the mixture was stirred vigourously for 1.5 h. It was then cooled to 0 °C and a solution of iodine (17.8 g, 70 mmol) in ether (200 mL) was added dropwise over 1 h. After stirring a further 1 h at 0 °C, the layers were separated and the aqueous layer was extracted twice with ether. The combined ether layers were washed with $Na_2S_2O_3$ solution and brine, and concentrated. The residue was taken up in hexane and passed through a short column of silica gel (10 × 5 cm i.d.), eluting with 30% EtOAc in hexane, yielding 9.8 g (54%) of iodide **10** as a semicrystalline oil. The proton NMR and IR spectra matched lit. values.[18] MS: 264 (M^+-18, trace), 180 (50), 167 (32), 154 (6), 137 (9), 109 (8), 95 (76), 81 (85), 69 (34), 67 (78), 55 (100).

(9*E*)-Hexadecen-11-yn-1-ol (11). Bis(triphenylphosphine)palladium(II) chloride (0.5 g), CuI (0.5 g), iodide **10** (9.0 g, 32 mmol) and 1-hexyne (5.25 g, 64 mmol) were added sequentially to 150 mL of THF under Ar. The mixture was cooled to −10 °C and dry diisopropylamine (14 mL) was added dropwise. The mixture was warmed to room temperature and stirred for 1 h, then an equal volume of hexane was added and the mixture was filtered and concentrated. The residue was taken up in hexane, washed with 1 M HCl, twice with satd aq NH_4Cl and once with brine. The hexane solution was dried and concentrated, and crude **11** was used in the next step without further purification. The NMR spectrum of crude **11** was a satisfactory match with literature data.[10]

(9*E*,11*Z*)-Hexadecadien-1-ol (14). An ether solution of crude **11**, dihydropyran (4.0 g, 48 mmol) and a few crystals of PTSA were stirred overnight. The resulting solution was washed twice with 1 M $NaHCO_3$, once with brine, dried and concentrated, yielding protected alcohol **12**.

A slurry of dicyclohexylborane was prepared by dropwise addition of cyclohexene (9.1 mL, 90 mmol) to a cooled solution (0 °C) of borane–dimethylsulfide complex (10 M, 4.5 mL, 90 mmol) in dry THF (50 mL) under Ar. The mixture was warmed to 20 °C and stirred for 1.5 h, then cooled to 0 °C and protected alcohol **12** (11.1 g, ~30 mmol) was added dropwise. The slurry was warmed to 20 °C and stirred until all the starting material had been consumed. The mixture was then cooled to 0 °C, acetic acid (18 mL) was added dropwise and the mixture was warmed to room temperature overnight. The solution was then cooled to 0 °C and 5 M NaOH (70 mL) was added, followed by dropwise addition of 30% H_2O_2 (18 mL; **CAUTION**: very exothermic!). The mixture was then diluted with water and extracted with hexane. The hexane extract was washed with brine, dried, concentrated and pumped under vacuum (0.1 mm Hg) overnight. The THP protecting group was removed as described for **6a** and **6b** above. The residue was purified in two batches by flash chromatography (25 × 5 cm i.d. column), eluting with 20% EtOAc in hexane, yielding 5.65 g of dienol **14**. The NMR spectrum matched literature values.[10] MS: 238 (M^+, 14), 135 (7), 121 (11), 109 (13), 96 (25), 95 (31), 93 (17), 82 (37), 81 (64), 79 (41), 68 (32), 67 (100), 55 (38).

(9*E*,11*Z*)-Hexadecadienal (15). Oxalyl chloride (884 μL, 10 mmol) was added to 40 mL of dry CH_2Cl_2, and the mixture was cooled to −78 °C. DMSO (1.42 mL, 20 mmol) was added dropwise, the mixture was stirred 10 min and alcohol **14** (1 g, 4.24 mmol) in 5 mL CH_2Cl_2 was added dropwise. The mixture was stirred 30 min, then triethylamine (6 mL) was added and the mixture was warmed to room temperature. The mixture was poured into water and extracted three times with hexane. The hexane extracts were washed with water and brine, dried and concentrated. The residue was purified by flash chromatography (22 × 2.5 cm i.d.), eluting with 5% ether in hexane. The purified compound was then Kugelrohr distilled (oven temperature 130 °C, 0.05 mm Hg; lit. bp,[10] 135–142 °C/0.02 mm Hg) to remove traces of silica, giving 722 mg of aldehyde **15**. The NMR spectrum closely matched lit. data.[10] MS: 236 (M^+, 14), 151 (2), 148 (2), 137 (3), 123 (6), 109 (10), 98 (13), 96 (16), 95 (24), 82 (23), 81 (59), 79 (33), 67 (100), 55 (28).

Synthesis of 9,11,13-hexadecatrienals (Scheme 3)

9,11,13-Hexadecatrienols (17). (2*Z*)-Penten-1-yl triphenylphosphonium bromide was prepared from (2*Z*)-bromopentene (7.45 g, 50 mmol) and triphenylphosphine (13.1 g, 50 mmol) in dry benzene (30 mL). The mixture was allowed to stand for 60 h at room temperature, during which time the phosphonium salt precipitated. The mixture was filtered under N_2, rinsing the solids with several aliquots of benzene. The

crystalline salt was then dried over P_2O_5 under vacuum overnight.

LDA (1.5 M) was added dropwise to a slurry of the phosphonium salt (1.23 g, 3 mmol) in ether at 0 °C until a red–orange color persisted. A further 2.0 mL (3.0 mmol) of LDA was then added and the mixture was stirred 1 h at 0 °C, resulting in a slightly cloudy deep orange–red solution. Aldehyde **4** (0.536 g, 2 mmol) in ether (5 mL) was added dropwise, resulting in rapid formation of a voluminous precipitate. After stirring 1 h, the mixture was worked up and the THP protecting group was hydrolyzed as described above for dienols **6a** and **6b**. The residue was flash chromatographed (25% EtOAc in hexanes), yielding 300 mg of a mixture of the *EZZ* and *EEZ* alcohols. The isomers were separated in five batches on the Ag^+ cation exchange resin as described above. The fractions containing the purified isomers were combined, concentrated, taken up in hexane and washed with brine to remove traces of silver nitrate bled off the column. The purified compounds were stored in dilute hexane solution as described above. (9*E*,11*Z*,13*Z*)-isomer. NMR: δ 6.51 (br dd, 1H, J=15, 12 Hz), 6.41 (br t, 1H, J=11.2 Hz), 6.14 (t, 1H, J=11.1 Hz), 5.97 (t, 1H, J=11.0 Hz), 5.74 (dt, 1H, J=15.0, 7.1 Hz), 5.49 (m, 1H), 3.65 (br t, 2H, $J\simeq$7 Hz), 2.22 (m, 2H), 2.13 (m, 2H), 1.57 (m, 2H), 1.47–1.2 (m, 11H), 1.01 (t, 3H, J=7.3 Hz). MS: 236 (33), 135 (7), 121 (13), 107 (18), 105 (25), 93 (58), 91 (85), 79 (100), 77 (48), 67 (46), 55 (40), 43 (30), 41 (54). HRMS: calcd for $C_{16}H_{28}O$: 236.2140. Found: 236.2131.

(9*E*,11*E*,13*Z*)-isomer. NMR: δ 6.39 (dd, 1H, J=13.0, 11.8 Hz), 6.14 (m, 2H), 5.97 (t, 1H, J=10.9 Hz), 5.71 (m, 1H), 5.41 (dt, 1H, J=10.6, 7.5 Hz), 3.63 (t, 2H, J=6.5 Hz), 2.21 (m, 2H), 2.10 (m, 2H), 1.7–1.2 (m, 13 H), 1.00 (t, 3H, J=7.4 Hz). MS: 236 (32), 135 (6), 121 (18), 107 (24), 105 (19), 93 (48), 91 (51), 79 (100), 77 (27), 67 (21), 55 (44), 43 (22), 41 (42).

The *EZE* and *EEE* isomers were prepared in similar yield, substituting (2*E*)-for (2*Z*)-penten-1-yl triphenylphosphonium bromide [from (2*E*)-bromopentene and triphenylphosphine] in the Wittig reaction. The resulting mixture of triene alcohols did not separate well on the silver ion column. However, the *EEE* isomer crystallized out of a 10% solution of the isomers in MeOH held at −20 °C for 2 weeks, giving the *EEE* isomer in high (>99.5%) purity, with the liquor enriched (~80%) in the *EZE* isomer. Residual *EEE* isomer was removed from an aliquot (230 mg, 1 mmol) of the enriched sample by selective Diels–Alder reaction with the powerful dienophile tetracyanoethylene (38 mg, 0.3 mmol) in ether (5 mL) at room temperature for 3 h. The resulting mixture was concentrated and triturated with hexane and the hexane soluble portion was purified by flash chromatography (25% EtOAc in hexane), yielding 180 mg of (9*E*,11*Z*,13*E*)-hexadecatrienol. This isomer, unlike the *EEE* isomer, partially decomposed/rearranged on GC and GCMS. (9*E*,11*Z*,13*E*)-isomer. NMR: δ 6.48 (m, 2H), 5.86 (m, 2H), 5.72 (m, 2H), 3.64 (m, 2H), 2.14 (m, 4H), 1.57 (m, 2H), 1.47–1.20 (m, 11H), 1.03 (t, 3H, J=7.3 Hz). HRMS: calcd for $C_{16}H_{28}O$: 236.2140. Found: 236.2144. (9*E*,11*E*,13*E*)-isomer. NMR: δ 6.15–5.98 (m, 4H), 5.77–5.60 (m, 2H), 3.65 (m, 2H), 2.10 (m, 4H), 1.58 (m, 2H), 1.45–1.19 (m, 11H), 1.02 (t, 3H, J=7.4 Hz). MS: 236 (28), 135 (4), 121 (19), 107 (24), 105 (17), 93 (53), 91 (54), 79 (100), 77 (27), 67 (28), 55 (42), 43 (26), 41 (46).

The olefin regions of the proton NMR spectra of the four isomers were a reasonable match to those of the previously reported 8,10,12-hexadecatrienyl acetate isomers in both chemical shifts and coupling patterns.[20]

(9*E*,11*Z*,13*Z*)- and (9*E*,11*Z*,13*E*)-Hexadecatrienals (18a and 18b). (9*E*,11*Z*,13*Z*)- and (9*E*,11*Z*,13*E*)-Hexadecatrienols were oxidized using the Swern conditions as described above, yielding aldehydes **18a** (73%) and **18b** (81%) after flash chromatographic purification (3% EtOAc in hexanes). Hexane solutions of the purified materials were gravity filtered through filter paper to remove traces of silica gel, then stored as dilute solutions in hexane as described above for dienal **15**. (9*E*,11*Z*,13*Z*)-isomer **18a**. NMR: δ 9.77 (t, 1H, J=1.6 Hz), 6.50 (br dd, 1H, J = 14.7, 11.2 Hz), 6.41 (t, 1H, J=11.2 Hz), 6.15 (t, 1H, J=11.4 Hz), 5.97 (t, 1H, J=11.0 Hz), 5.73 (dt, 1H, J=14.9, 11.1 Hz), 5.49 (m, 1H), 2.43 (td, 2H, J=7.4, 1.6 Hz), 2.21 (br t, 2H, J=7.0 Hz), 2.13 (quart, 2H, J=7.1 Hz), 1.63 (m, 2H), 1.45–1.25 (m, 8H), 1.01 (t, 3H, J=7.5 Hz). MS: 234 (M^+, 31), 190 (2), 149 (4), 135 (7), 131 (9), 121 (13), 107 (27), 105 (20), 93 (57), 91 (63), 79 (100), 77 (40), 67 (38), 55 (30), 43 (29), 41 (61). HRMS: calcd for $C_{16}H_{26}O$: 234.1984. Found: 234.1981. (9*E*,11*Z*,13*E*)-isomer **18b**. NMR: δ 9.78 (t, 1H, J=1.6 Hz), 6.49 (m, 2H), 5.86 (m, 2H), 5.71 (m, 2H), 2.43 (td, 2H, J=7.3, 1.6 Hz), 2.15 (m, 4H), 1.64 (m, 2H), 1.5–1.24 (m, 8H), 1.04 (t, 3H, J=7.4 Hz). This isomer degraded on attempted GCMS. HRMS: calcd for $C_{16}H_{26}O$: 234.1984. Found: 234.1987.

Field tests

Lures consisted of 11 mm grey rubber septa (The West Co., Lititz, Pennsylvania) loaded with heptane solutions of test compounds, with butylated hydroxytoluene (BHT) antioxidant added at a rate equivalent to 10% of the test compound. Candidate pheromone lures were placed in orange Delta traps (Scentry Inc., Salinas, California) at several field sites in Texas. In field experiments, traps were positioned in pecan trees 2.4–4.6 m above the ground on branch terminals bearing pecan nuts. Captured moths were not removed from traps during the experiment. Identification of moths as pecan nut casebearers was confirmed by Edward G. Riley, Department of Entomology, Texas A&M University, College Station, Texas.

The relationship between pheromone dose and attraction of male pecan nut casebearers was tested in two experiments. The first trial was conducted in a

commercial pecan orchard in Navarro County, Texas. A single Delta trap was placed in individual trees planted on a 10.7×10.7 m spacing. Traps were placed in every third tree, with each row containing one replication of each dose, using doses of 0, 0.1, 1, 10, 100 or 1000 μg of (9*E*,11*Z*)-16:Ald per septum. Each dose was replicated six times. Treatments within a replicate were randomized and trap position within replications was rotated to the next trap location on each sampling date. The number of male pecan nut casebearers caught was tabulated every 2–3 days during the flight of the overwintering generation (9–19 May 1994).

The second dose response trial was conducted in Collin County, Texas, in large native pecan trees. Each replicate consisted of six trees in a circular array, with each tree containing a trap baited with one of the six doses spaced around the outside of the canopy. Trees were spaced a minimum of 100 m apart. Traps were randomized within replicates and rotated one position at each sampling date. Trap catch was tabulated every 2–3 days for the period 3–4 July 1994.

The raw trap catch data (counts/sample date) were transformed $[\ln(x+1)]$ and the transformed data were analyzed by SuperANOVA® version 1.1,[21] and differences between means were determined using Fischer's LSD test.[21]

The attractiveness of the synthetic pheromone component throughout the pecan nut casebearer flight period was determined in an urban area of El Paso, Texas. The 48 Delta traps were placed in pecan trees located in a grid pattern spaced ca. 400 m apart, and traps were counted weekly from 24 March–7 November 1994. Lures (100 μg on grey rubber septa) were replaced every 3 weeks.

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