Synthesis and Field Evaluation of the Sex Pheromone of *Stenoma catenifer* (Lepidoptera: Elachistidae)

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ABSTRACT Field trials of the sex pheromone of an important and potentially invasive pest of avocados, *Persea americana* Miller (Lauraceae), *Stenoma catenifer* Walsingham (Lepidoptera: Elachistidae), were carried out in commercial avocado orchards in Guatemala. The results indicated that the pheromone consists of a single component, (9Z)-9,13-tetradecadien-11-ynal; blends of this compound with a range of ratios of the corresponding alcohol and acetate, or with (6Z,9Z)-tricosadiene, which was present in pheromone gland extracts, were equally or less attractive to male moths than the single component. A range of doses from 10 μg to 1 mg were equally attractive, and lures remained attractive for periods of several weeks. Male moth flight activity peaked between 0230 and 0430 hours. Overall, trap catches were relatively low, similar to what was reported for the congeneric *Stenoma cecropia* Meyrick, suggesting that this species may use other signals in addition to pheromones during mate location. Nevertheless, the pheromone will be useful for detection of *S. catenifer*, particularly in areas where there is a risk of the moth invading and establishing due to increased commerce in fresh avocados, and for certifying export orchards as being free of *S. catenifer*.

KEY WORDS *Stenoma catenifer*, (9Z)-9,13-tetradecadien-11-ynal, (6Z,9Z)-tricosadiene, detection, invasive species

Avocados, *Persea americana* Miller (Lauraceae) are an important fruit crop in California. The popular 'Hass' avocado accounts for ≈97% of avocado fruit produced from ≈26,500 bearing ha, and the value of the 2006–2007 harvest was ≈US$245 million (California Avocado Commission 2008). To date, no members of a guild of coevolved specialist fruit feeding insects (e.g., curculionids, tephritid flies, and lepidopterans) that are commonly associated with *P. americana* in its home range in Mexico and Central America (Hoddle 2004, 2007) have been introduced into California. Consequently, avocado production in California has few significant insect pests. This lack of destructive pests attacking the saleable product in California has resulted in fruit production with relatively little pesticide use when compared with countries where these pests are endemic.

However, the legalization of importation of fresh Hass fruit directly into California from Mexico in February 2007 has greatly increased the risk of accidental introduction of one or more important fruit feeding pests of avocados into California. One such pest, *Stenoma catenifer* Walsingham (Lepidoptera: Elachistidae), has been identified as a major biosecurity threat to avocado producers in the United States (Miller 1995, Hoddle and Hoddle 2008b). The major economic damage to avocados by *S. catenifer* is caused by larvae feeding inside fruit (Hoddle and Hoddle 2008b). Larvae also bore into young twigs and stems, and heavy attacks can result in the death of immature plants (Wolfenbarger and Colburn 1966, 1979; Cervantes Peredo et al. 1999).

Hass fruit react to larval boring and subsequent repeated clearing of tunnels that prevents wound healing by producing copious amounts of the sugar alcohol perseitol (*D*-glycero-*D*-galacto-heptitol). These exudates drip from tunnel entrances, leaving characteristic chalky white residues on the skin of infested fruit (Hoddle 2008). Hass fruit are further disfigured externally by accumulations of larval frass at tunnel entrances, which are commonly located in the middle and bottom thirds of fruit (Hoddle and Hoddle 2008b). Larvae feeding internally may cause noticeable but localized damage to fruit pulp as larvae tunnel to the seed, the preferred feeding site. Often the seed is extensively tunneled, and can be completely consumed, especially if fruit are small (<5 cm in length), or if more than one larva is developing in the seed (up to seven larvae have been recorded from individual Hass avocado seeds; Hoddle and Hoddle 2008b). Extensive pulp damage results when *S. catenifer* larvae consume only the mesocarp, leaving the avocado seed undamaged. Adult *S. catenifer* are nocturnally active and rest among leaves, weeds, and debris on the orchard floor during the day (Hoddle and Hoddle 2008c).

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Crop losses attributable to *S. catenifer* of 60 and 80% have been recorded in Brazil and Venezuela, respectively, despite intensive broad-spectrum pesticide use (Boscán de Martínez and Godoy 1982, Nava et al. 2005). In Brazil, *S. catenifer* is considered the major pest limiting avocado production (Hohmann et al. 2003). Similarly, in Mexico up to 95% of avocado fruit can be attacked, and prophylactic monthly insecticide treatments are recommended for control (Wysoki et al. 2002). In Guatemala, ≈45% of Hass fruit in orchards treated monthly with a rotating spray schedule of malathion and endosulfan can exhibit damage resulting from infestations of *S. catenifer* larvae (Hoddle and Hoddle 2008a). This level of damage to Hass fruit (≈45%) ranks this cultivar as extremely susceptible to *S. catenifer*, placing it within the three most susceptible avocado varieties for which damage data have been published (Hohmann et al. 2000).

Species-specific sex pheromones have been developed for many insects and are used in traps for detection and monitoring insect distributions, population phenologies, and pest densities. Phenology and density data can assist with pest control decisions in commercial agricultural settings, and with certifying export areas as being pest free. Distribution data can be used for delineating lower density leading edges of expanding pest populations, and for proactive early detection monitoring of potentially invasive species not yet found in areas considered vulnerable to incursion (Ridgway et al. 1990). Use of the sex pheromone of *S. catenifer* as a trap bait would provide a sensitive and highly species-specific tool for monitoring pest populations in areas where this insect is endemic and that have commercial avocado orchards from which fresh fruit are exported. Pheromone-baited traps also would provide a sensitive method of detecting incursions of this insect into California and other areas that receive imports of fresh avocados from countries with endemic *S. catenifer* populations.

As a proactive measure to counteract the invasion threat posed by *S. catenifer* to the California avocado industry, we recently identified the sex pheromone of *S. catenifer* as (9Z)-9,13-tetradecadien-11-ynal, the first representative of a new class of natural products (Millar et al. 2008). The dienyne structure of the *S. catenifer* pheromone is remarkable both in terms of the presence of the alkyne, which is a very unusual functional group in insect semiochemicals, and in terms of the overall high degree of unsaturation (Millar et al. 2008).

After the identification of the *S. catenifer* sex pheromone and verification of its attractiveness to male moths (Millar et al. 2008), large-scale field testing in commercial Hass avocado orchards with endemic pest populations managed with insecticides and subjected to high levels of parasitism was necessary to determine operational parameters for practical use of the pheromone. These included determination of the optimal dose of the aldehyde, and the possible effects of other components found in pheromone gland extracts, including (9Z)-9,13-tetradecadien-11-yn-1-ol, (9Z)-9,13-tetradecadien-11-yn-1-yl acetate, and a diene hydrocarbon, (6Z,9Z)-tricosadiene. Additionally, studies were conducted to determine the longevity of pheromone lures under field conditions, and the periodicity of male arrival to traps at night. Here, we present the results of these studies.

Materials and Methods

Field Site for Pheromone Evaluation Studies. Studies were conducted in a ≈5-ha commercial Hass avocado orchard consisting of ≈800–900 trees in San Miguel Dueñas, Sacatepéquez, Guatemala (14° 31.461 N, 90° 46.579 W; elevation, 1,500 m). This study site was selected based on results of a broader survey of avocados in Guatemala for fruit-feeding Lepidoptera (Hoddle and Hoddle 2008c) because of the relatively high levels of fruit damage caused by *S. catenifer*, and because this study site was a Hass orchard, the same cultivar grown commercially in California. Trees in the orchard were ≈7 to 8 yr old, ≈6–8 m in height, and canopies were separated by ≈5–7 m of clear ground, allowing full sun exposure. The orchard was treated monthly with a rotating schedule of malathion and endosulfan and ≈30% of trees were under varying stages of heavy pruning. Approximately 43–45% of fruit exhibited *S. catenifer* damage, parasitism of *S. catenifer* larvae inside fruit ranged from 37 to 53%, and larval to adult emergence rates were ≈38% (Hoddle and Hoddle 2008a,b).

Pheromone Synthesis. (6Z,9Z)-Tricosadiene was synthesized from linoleic acid by the method of Conner et al. (1983). (9Z)-9,13-Tetradecadien-11-yn-1-ol and (9Z)-9,13-tetradecadien-11-yn-1-al were synthesized as shown in Fig. 1. The corresponding acetate was prepared from the alcohol as described previously (Millar et al. 2008).

General. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under argon atmosphere. ^{1}H and ^{13}C NMR spectra were recorded with an INOVA-400 (400 and 100.5 MHz, respectively) spectrometer (Varian, Inc., Palo Alto, CA), as $CDCl_3$ solutions. Mass spectra were obtained with a Hewlett–Packard (H-P) 5890 gas chromatograph (GC) interfaced to an H-P 5970 mass selective detector, in electron ionization mode (70 eV) with helium carrier gas. The GC was equipped with a DB5-MS column (25 m by 0.20 mm i.d. × 0.33-μm film; J&W Scientific, Folsom, CA). High-resolution mass spectrometry (HRMS) analysis was performed on a 6210 LC-TOF instrument (Agilent Technologies, Santa Clara, CA) by using electrospray ionization or atmospheric pressure chemical ionization (ESI/APCI). Infrared (IR) spectra were recorded on a Spectrum One FT-IR spectrometer (PerkinElmer Life and Analytical Sciences, Boston, MA) by using the ATR accessory. Products were purified by flash or vacuum flash chromatography using silica gel (230–400 mesh; EMD Chemicals, Inc., Gibbstown, NJ). Unless specified otherwise, solutions were dried over anhydrous Na_2SO_4 and concentrated by rotary evaporation under reduced pressure.

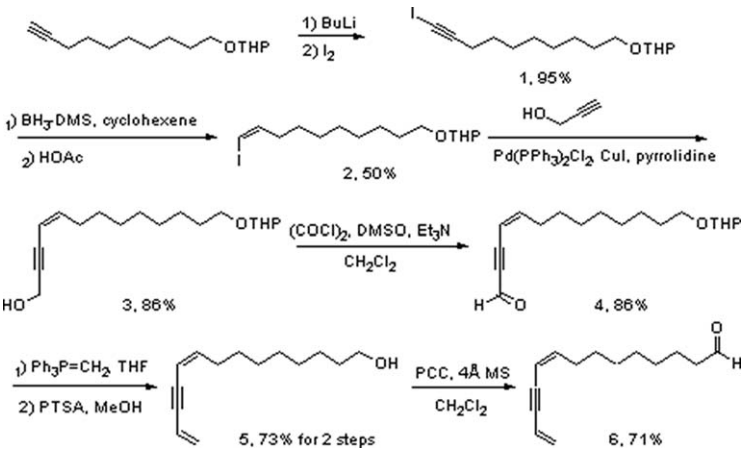

Fig. 1. Synthesis of (9Z)-9,13-tetradecadien-11-ynal.

2-(10-Iodo-dec-9-ynyloxy)-tetrahydro-2H-pyran (1). *n*-Butyllithium (2.58 M in hexane, 0.85 ml, 2.2 mmol) was added dropwise to a solution of 2-(dec-9-ynyloxy)-tetrahydro-2*H*-pyran (0.48 g, 2.0 mmol) in THF (3 ml) under Ar and cooled at −78°C. After 30 min, a solution of I_2 (0.61 g, 2.4 mmol) in THF (2 ml) was slowly added. At the end of the addition, a red color persisted. The mixture was warmed to room temperature and poured into saturated aqueous $Na_2S_2O_3$. The mixture was extracted with hexanes, and the hexane layer was washed with H_2O and brine, dried, and concentrated. The crude product was purified by vacuum flash chromatography (hexanes/EtOAc = 95/5) to give 0.69 g (95%) of **1** as a light yellow oil. ^{1}H NMR: δ 4.56 (m, 1H), 3.86 (m, 1H), 3.72 (m, 1H), 3.49 (m, 1H), 3.37 (m, 1H), 2.34 (t, J = 7.2 Hz, 2H), 1.22–1.89 (m, 18H); ^{13}C NMR: δ 99.0 (CH), 95.0 (C), 67.8 (CH_2), 62.5 (CH_2), 31.0 (CH_2), 29.9 (CH_2), 29.4 (CH_2), 29.1 (CH_2), 28.8 (CH_2), 28.6 (CH_2), 26.3 (CH_2), 25.7 (CH_2), 20.9 (CH_2), 19.9 (CH_2), −7.5 (C). The ^{1}H NMR spectrum was in agreement with that reported in the literature (Michelot 1983).

2-[(9Z)-10-Iodo-dec-9-enyloxy]-tetrahydro-2H-pyran (2). Cyclohexene (2.4 ml, 24 mmol) was added to a solution of borane-dimethylsulfide complex (1.2 ml, 12 mmol) in THF (15 ml) under Ar and cooled at 0°C. The mixture was stirred for 2 h while allowing the temperature to increase to room temperature. A white precipitate formed a few minutes after the addition. The reaction mixture then was cooled to 0°C again and alkyne **1** (3.64 g, 10 mmol) in THF (10 ml) was added. The reaction mixture was stirred at 0°C for 30 min, and then at room temperature for 1 h, during which the white slurry became a clear solution. The reaction mixture then was cooled to 0°C again and AcOH (5.2 ml, 90 mmol) was slowly added. The cooling bath was removed after the addition and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with hexanes, washed with H_2O, saturated aqueous $NaHCO_3$, and brine, then dried and concentrated. The crude product was purified by flash chromatography (hexanes/Et_2O = 95/5) to give 1.84 g (50%) of **2** as a colorless oil. ^{1}H NMR: δ 6.16 (m, 2H), 4.56 (m, 1H), 3.86 (m, 1H), 3.72 (m, 1H), 3.49 (m, 1H), 3.37 (m, 1H), 2.12 (m, 2H), 1.86–1.26 (m, 18H). ^{13}C NMR: δ 141.6 (CH), 99.0 (CH), 82.3 (CH), 67.8 (CH_2), 62.5 (CH_2), 34.8 (CH_2), 30.9 (CH_2), 29.9 (CH_2), 29.5 ($2CH_2$), 29.2 (CH_2), 28.1 (CH_2), 26.3 (CH_2), 25.6 (CH_2), 19.8 (CH_2). The ^{1}H NMR spectrum was in agreement with that reported in the literature (Michelot 1983).

(4Z)-13-(Tetrahydro-2H-pyran-2-yloxy)tridec-4-en-2-yn-1-ol (3). A two-neck flask under Ar was charged with $(Ph_3P)_2Pd$ (II) dichloride (0.053 g, 0.075 mmol), CuI (0.029 g, 0.15 mmol), and pyrrolidine (4 ml), and the mixture was stirred and cooled at 0°C. Iodide **2** (1.10 g, 3.0 mmol) in pyrrolidine (2 ml) was added dropwise, followed by dropwise addition of propargyl alcohol (0.21 ml, 3.6 mmol). The reaction mixture was stirred for 3 h while the temperature gradually increased to room temperature and then poured into ice-cold 1 M KH_2PO_4 solution and extracted with hexanes. The hexane extract was washed with H_2O and brine, dried, and concentrated. The crude product was purified by vacuum flash chromatography (hexanes/EtOAc = 9/1–3/1) to give 0.76 g (86%) of **3** as a light yellow oil. ^{1}H NMR δ 5.91 (dt, J = 10.0, 7.6 Hz, 1H), 5.47 (dt, J = 10.8, 1.6 Hz, 1H), 4.56 (m, 1H), 4.41 (dd, J = 5.6, 1.6 Hz, 1H), 3.86 (m, 1H), 3.72 (m, 1H), 3.49 (m, 1H), 3.37 (m, 1H), 2.28 (m, 2H), 1.86–1.26 (m, 18H). ^{13}C NMR δ 144.7 (CH), 108.3 (CH), 98.9 (CH), 91.3 (C), 82.5 (C), 67.7 (CH_2), 62.4 (CH_2), 51.6 (CH_2), 30.7 (CH_2), 30.1 (CH_2), 29.6 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 28.6 (CH_2), 26.1 (CH_2), 25.5 (CH_2), 19.7 (CH_2). IR: (neat) 3427, 2926, 2854, 1454, 1441, 1353, 1135, 1118, 1076, 1021 cm^{-1}. HRMS (ESI/APCI) calcd. for $C_{18}H_{30}O_3Na$ $[M + Na]^+$: 317.2093, found 317.2087.

(4Z)-13-(Tetrahydro-2H-pyran-2-yloxy)tridec-4-en-2-ynal (4). Dimethyl sulfoxide (0.43 ml, 6.0 mmol) in CH_2Cl_2 (5 ml) was added dropwise to a solution of oxalyl chloride (0.26 ml, 3.0 mmol) in CH_2Cl_2 (10 ml) under Ar and cooled at −78°C. The reaction mixture was stirred for 30 min, and then a solution of alcohol **3** (0.74 g, 2.5 mmol) in CH_2Cl_2 (10 ml) was added dropwise. After 1 h, Et_3N (1.75 ml, 12.6 mmol) was added and the reaction mixture was allowed to warm

to room temperature. The mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with H_2O and brine, dried, and concentrated. The crude product was purified by vacuum flash chromatography (hexanes/EtOAc = 95/5) to give 0.63 g (86%) of **4** as a colorless oil. ^{1}H NMR: δ 9.34 (d, J = 1.6 Hz, 1H), 6.34 (dt, J = 10.8, 7.6 Hz, 1H), 5.64 (d, J = 11.2 Hz, 1H), 4.56 (m, 1H), 3.86 (m, 1H), 3.72 (m, 1H), 3.49 (m, 1H), 3.37 (m, 1H), 2.38 (m, 2H), 1.86–1.26 (m, 18H). ^{13}C NMR: δ 176.9 (CH), 153.2 (CH), 106.8 (CH), 99.0 (CH), 92.8 (C), 92.5 (C), 67.8 (CH_2), 62.5 (CH_2), 31.3 (CH_2), 30.9 (CH_2), 29.9 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 28.7 (CH_2), 26.3 (CH_2), 25.7 (CH_2), 19.9 (CH_2). IR (neat): 2927, 2855, 2174, 1662, 1605, 1455, 1441, 1385, 1352, 1200, 1135, 1119, 1077, 1030 cm^{-1}. HRMS (ESI/APCI) calcd. for $C_{18}H_{28}O_3Na$ $[M + Na]^+$: 315.1936, found 315.1928.

*(9Z)-Tetradeca-9,13-dien-11-yn-1-ol (**5**).* A three-neck flask under Ar was charged with methyltriphenylphosphonium bromide (0.79 g, 2.2 mmol) and THF (12 ml), and cooled to 0°C. Sodium hexamethyldisilazide (NaHMDS, 1 M in THF, 2.3 ml, 2.3 mmol) was added dropwise, the mixture was stirred at 0°C for 1 h, and then cooled to −78°C, and aldehyde **4** (0.43 g, 1.47 mmol) in THF (3 ml) was added dropwise. After 1.5 h, the cold bath was removed and the reaction mixture was stirred for 1.5 h at room temperature. The reaction was quenched with saturated aqueous NH_4Cl and extracted with ether. The combined organic layer was washed with brine, dried, and concentrated. The crude product was purified by vacuum flash chromatography (hexanes/EtOAc = 95/5) to give 0.38 g (88%) of 2-[(9Z)-tetradeca-9,13-dien-11-yn-1-yloxy)tetrahydro-2*H*-pyran as a light yellow oil, which was dissolved in MeOH (7 ml) and stirred with PTSA (0.012 g, 0.065 mmol) for 2.5 h. The mixture was concentrated and the residue was dissolved in hexanes. The solution was washed with saturated aqueous $NaHCO_3$ and brine, dried and concentrated, and the crude product was purified by vacuum flash chromatography (hexanes/EtOAc = 9/1–5/1) to give 0.23 g (81%, 73% over two steps) of alcohol **5** as a colorless oil. ^{1}H NMR: δ 5.92 (m, 2H), 5.60 (dd, J = 16.8, 2.2 Hz, 1H), 5.55 (dd, J = 10.8, 1.6 Hz, 1H), 5.44 (dd, J = 10.8, 1.8 Hz, 1H), 3.62 (t, J = 6.4 Hz, 2H), 2.30 (m, 2H), 1.62–1.22 (m, 12H). ^{13}C NMR: δ 144.5 (CH), 126.2 (CH_2), 117.6 (CH), 109.0 (CH), 92.3 (C), 87.2 (C), 63.2 (CH_2), 32.9 (CH_2), 30.4 (CH_2), 29.5 ($2CH_2$), 29.2 (CH_2), 28.9 (CH_2), 25.8 (CH_2). IR (neat): 3328, 3019, 2926, 2855, 1599, 1464, 1414, 1164, 1055, 970, 913, 734 cm^{-1}. HRMS (ESI/APCI) calcd. for $C_{14}H_{23}O$ $[M + H]^+$: 207.1749, found 207.1750.

*(9Z)-Tetradeca-9,13-dien-11-ynal (**6**).* Alcohol **5** (0.17 g, 0.80 mmol) in CH_2Cl_2 (1 ml) was added to a suspension of pyridinium chlorochromate (0.35 g, 1.60 mmol) and powdered 4-Å molecular sieve (0.40 g) in anhydrous CH_2Cl_2 (3 ml) under Ar, and the mixture was vigorously stirred for 2 h. The reaction mixture was diluted with Et_2O (12 ml) and filtered through a pad of Celite and silica gel (Celite on the bottom), rinsing well with Et_2O. The filtrate was concentrated and the crude product was purified by vacuum flash chromatography (hexanes/Et_2O = 95/5) to give 0.12 g (71%) of **6** as a colorless oil. ^{1}H NMR: δ 9.75 (t, J = 2.0 Hz, 1H), 5.92 (m, 2H), 5.60 (dd, J = 17.6, 2.4 Hz, 1H), 5.56 (dd, J = 11.2, 1.6 Hz, 1H), 5.45 (dd, J = 11.2, 2.0 Hz, 1H), 2.41 (td, J = 7.4, 2.0 Hz, 2H), 2.30 (qd, J = 7.3, 1.6 Hz, 2H), 1.62 (m, 2H), 1.44–1.28 (m, 8H). ^{13}C NMR δ 203.0 (CH), 144.4 (CH), 126.2 (CH_2), 117.6 (CH), 109.1 (CH), 92.4 (C), 87.2 (C), 44.1 (CH_2), 30.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 22.2 (CH_2). IR (neat) 3018, 2928, 2856, 2719, 1724, 1599, 1464, 1414, 1392, 1164, 971, 917, 738, 674 cm^{-1}. HRMS (ESI/APCI) calcd. for $C_{14}H_{21}O$ $[M + H]^+$: 205.1592, found 205.1589.

Compounds and Pheromone Blends Tested, Timing of Field Tests, and Trap Deployment Protocols. Test compounds were loaded on 11 mm gray silicon rubber septa (West Pharmaceutical Services Inc., Lionville PA) as hexane solutions. Lures were placed in Pherocon 1C wing traps (Trécé Incorporated, Adair, OK), which were hung inside the canopy of Hass avocado trees at head height (≈1.75 m).

In preliminary field trials conducted when (9Z)-9,13-tetradecadien-11-ynal was first identified in pheromone gland extracts, we had demonstrated that this compound was attractive to male moths as a single component (Millar et al. 2008). The possible effects of the analogs (9Z)-9,13-tetradecadien-11-yn-1-ol and (9Z)-9,13-tetradecadien-11-yn-1-yl acetate, the first of which had been identified as a minor component in the pheromone gland extracts, were tested in two field trials. In addition, (6Z,9Z)-tricosadiene, a compound present in pheromone gland extracts that elicited responses from antennae of male moths in coupled gas chromatography-electroantennogram detection (GC-EAD) analyses (Millar et al. 2008) also was tested as a lure component. Field tests of (9Z)-9,13-tetradecadien-11-ynal (100 μg) as a single component and in combination with (9Z)-9,13-tetradecadien-11-yn-1-ol (0.1–100 μg), (9Z)-9,13-tetradecadien-11-yn-1-yl acetate (0.1–100 μg), or (6Z,9Z)-tricosadiene (10–1,000 μg) were conducted over two different time periods, from 13 November to 21 December 2007 and from 13 March to 1 May 2008. Blend ratios tested are shown in the figures and figure captions. Treatments were deployed in replicated blocks (minimum of five blocks). Treatments (range, one to eight treatments in a block) were randomly assigned to trees within blocks. Each treatment in a block was separated by at least two avocado trees (≈8 m), and blocks were staggered and separated by a minimum of two rows of trees (≈10–12 m). Spacing of any one block and treatment within a block sometimes varied due to the orchard shape. Captures of male moths per treatment were recorded at 4-d intervals after deployment, and traps in each block were rerandomized and redeployed for a further 4 d before being inspected again. This 4-d inspection, rerandomization, and redeployment protocol for blocks of experimental treatments was repeated at least four to five times for a 16–20-d test period.

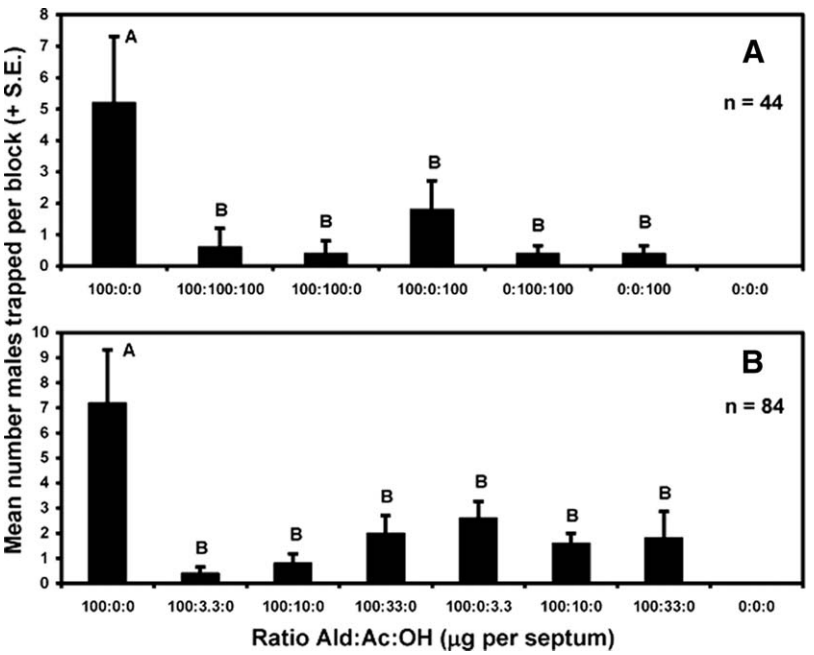

Fig. 2. Numbers of male *S. catenifer* caught in traps baited with blends of (9Z)-9,13-tetradecadien-11-ynal with (9Z)-9,13-tetradecadien-11-yn-1-ol and (9Z)-9,13-tetradecadien-11-yn-1-yl acetate. (A) All three components, all blends of two components, and single components (100 μg per compound). (B) (9Z)-9,13-Tetradecadien-11-ynal (100 μg) blended with variable amounts of (9Z)-9,13-tetradecadien-11-yn-1-ol and (9Z)-9,13-tetradecadien-11-yn-1-yl acetate. Treatments with different letters are significantly different (two-way ANOVA followed by Student–Newman–Keuls tests). For A, treatment effect $F = 6.80$: df = 5, 29; $P = 0.0007$ and block effect: $F = 4.39$; df = 4, 29; $P = 0.0104$. For B, treatment effect: $F = 5.54$; df = 7, 39; $P = 0.0004$ and block effect: $F = 0.29$; df = 4, 39; $P = 0.88$.

Evaluation of (9Z)-9,13-Tetradecadien-11-ynal Doses for Efficacy and Assessment of Lure Longevity. Trial results indicated that (9Z)-9,13-tetradecadien-11-ynal as a single component was the best attractant for male *S. catenifer* (see below). To determine the optimal dose of (9Z)-9,13-tetradecadien-11-ynal to use in rubber septum lures, doses from 1 to 1,000 μg in logarithmic steps were field tested. In addition, aldehyde-only lures (100-μg doses) that had been aged for 0, 1, 2, 3, and 4 wk in a greenhouse in Riverside, CA, from 5 February to 5 March, 2008 (temperature range, ≈4–30°C; average, ≈13°C) were simultaneously field tested to assess lure longevity. All lures were deployed in traps at the study site on 25 March 2008, and captures were recorded on 1 and 8 April 2008, with traps rerandomized after the first count.

Determining Periodicity of Male Response to Pheromone Lures. Nocturnal flight times of male *S. catenifer* to aldehyde-only lures were conducted on two consecutive nights (21 and 22 March 2008) by inspecting 85 traps (65 traps were hung at 1–1.75 m inside the tree canopy; 20 traps were hung on plastic poles either 0 m [$n = 10$] or 2.0 m [$n = 10$] above the ground and between tree rows) baited with gray rubber septum lures containing 100 μg of (9Z)-9,13-tetradecadien-11-ynal. Traps were checked hourly for arrival of male moths from 1 h after sunset to ≈30 min before sunrise. The numbers of males caught in traps per inspection time were recorded. During these trials, air temperatures were recorded in the orchard with a Hobo data logger (Onsett Corp., Bourne, MA) at 60-min intervals.

Statistical Analyses of Trap Captures. For statistical analyses of field trial results, possible day effects were eliminated by pooling counts for a particular treatment within a block. This sum was then transformed ($\sqrt{x} + 0.5$ or, if necessary, $\log_{10}x + 1$), before conducting a two-way analysis of variance (ANOVA), followed by Student–Newman–Keuls tests for separation of means (SigmaStat 1.0, Jandel Scientific Corporation, 1992–1994). Any treatments or blocks that failed to attract males were not included in the statistical analysis to avoid violating assumptions of ANOVA (i.e., zero values have no variance).

Results

Efficacy of Pheromone Blends. The first field trial (13 November–21 December 2007) tested the blend of three components, the three possible blends of two components, and the aldehyde and alcohol as single components. The aldehyde alone was significantly more attractive than any of the blends, and the alcohol as a single component was minimally attractive (Fig. 2A). These results were corroborated in a second trial (conducted from 13 March to 1 May 2008), in which the aldehyde was tested in blends with 3.3, 10, or 33% of the alcohol or acetate, respectively. All blends were

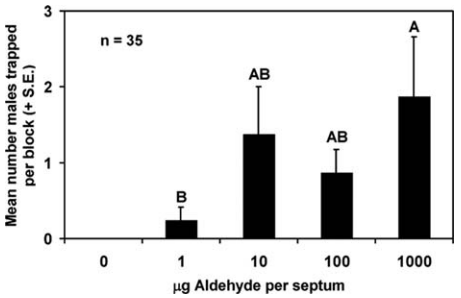

Fig. 3. Numbers of male *S. catenifer* caught in traps baited with different doses of (9Z)-9,13-tetradecadien-11-ynal. Treatments with different letters are significantly different (two-way ANOVA followed by Student–Newman–Keuls test). Treatment effect: $F = 3.10$; df = 3, 31; $P = 0.049$ and block effect: $F = 3.60$; df = 7, 31; $P = 0.011$.

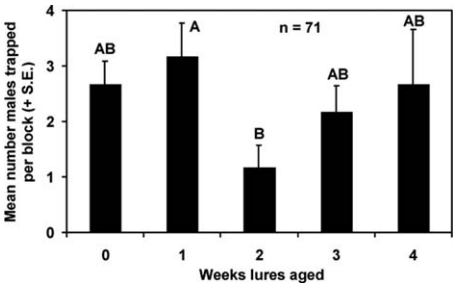

Fig. 4. Effect of pheromone lure age on attraction of male *S. catenifer*. Treatments with different letters are significantly different (two-way ANOVA followed by Student–Newman–Keuls test). Treatment effect: $F = 2.88$; df = 4, 29; $P = 0.049$ and block effect: $F = 3.93$; df = 5, 29; $P = 0.012$.

less attractive than the aldehyde as a single component (Fig. 2B).

Field bioassays testing various blends of (9Z)-9,13-tetradecadien-11-ynal with (6Z,9Z)-tricosadiene showed there were no significant differences between the numbers of moths attracted to the aldehyde as a single component and to any of the two component blends (data not shown; two-way ANOVA for treatment effect: $F = 1.61$; df = 5, 29; $P = 0.20$; for block effect: $F = 0.27$; df = 4, 29; $P = 0.90$; total moths captured = 90). Thus, the diene does not seem to be a pheromone component.

Determination of the Optimal Dose and Longevity of Attractiveness of (9Z)-9,13-Tetradecadien-11-ynal. The highest dose of the aldehyde (1,000 μg) attracted significantly more moths than the lowest dose (1 μg), with the responses to the 10- and 100-μg doses being intermediate (Fig. 3). Further differences between the doses may have been obscured by the relatively low number of moths caught in this trial overall (35 moths total). Furthermore, combination of data from two separate field trials using semilog doses from 10 to 1,000 μg indicated there were no significant differences between any of the doses in the numbers of moths caught (data not shown).

There were no clear differences between fresh lures and lures that had been aged on gray rubber septa under field conditions for periods of 1–4 wk (Fig. 4). Traps baited with lures that had been aged for 1 wk before deployment were statistically better than lures aged for 2 wk, but this difference may be biologically meaningless because lures aged for three and 4 wk were no different than the lures aged for either 1 or 2 wk.

Periodicity of Male Response to Pheromone Traps. The pattern of flight activity of male moths arriving at pheromone traps over a two-night period indicated that males first arrived in traps at 2:30 a.m. (mean temperature, 15.5°C), with captures peaking at 4:30 a.m. (15.3°C), before dropping abruptly at 5:30 a.m., ≈15–20 min before sunrise (Fig. 5).

Discussion

This study demonstrated the feasibility of "proactive chemical ecology," that is, the identification of pheromones of invasive pest species in advance of their introduction into new areas of the world. Some of the logistical problems that had to be overcome included the limited number of insects that were available for study because our importation permits did not allow the establishment of a colony of *S. catenifer* in California, and the very small amount of pheromone produced by the insects (<1 ng per female in pheromone gland extracts). Furthermore, the requirement that all insect collection, insect rearing, and field bioassays had to be carried out in Guatemala required considerable logistical planning and financial support for long-term deployment of two personnel (four visits over a 2-yr period with each stay ranging from 2.5 to 5 mo), the location of suitable study sites, and modification of experiments to fit available supplies and local conditions.

In the preliminary report of the identification of (9Z)-9,13-tetradecadien-11-ynal as a pheromone component of *S. catenifer*, analyses of pheromone gland extracts by GC-EAD showed that two other compounds in the extracts besides the aldehyde elicited consistent responses from antennae of male moths (Millar et al. 2008). These were identified as (9Z)-9,13-tetradecadien-11-yn-1-ol and (6Z,9Z)-tricosadiene. However, in field experiments, neither of these compounds seemed to enhance attraction, over a wide range of blend ratios. (9Z)-9,13-Tetradecadien-11-yn-1-yl acetate, which was tested because of its structural similarity to (9Z)-9,13-tetradecadien-11-ynal and (9Z)-9,13-tetradecadien-11-yn-1-ol, also did not increase the attractiveness of lures. It is possible that (9Z)-9,13-tetradecadien-11-yn-1-ol was present in the gland extracts only as a precursor to (9Z)-9,13-tetradecadien-11-ynal, and that it elicited antennal responses because of its structural similarity to the aldehyde. However, the presence of comparatively large quantities of (6Z,9Z)-tricosadiene in the extracts, and the fact that it elicited consistent responses from antennae of male moths in GC-EAD analyses, is inexplicable, given that there is no indication from

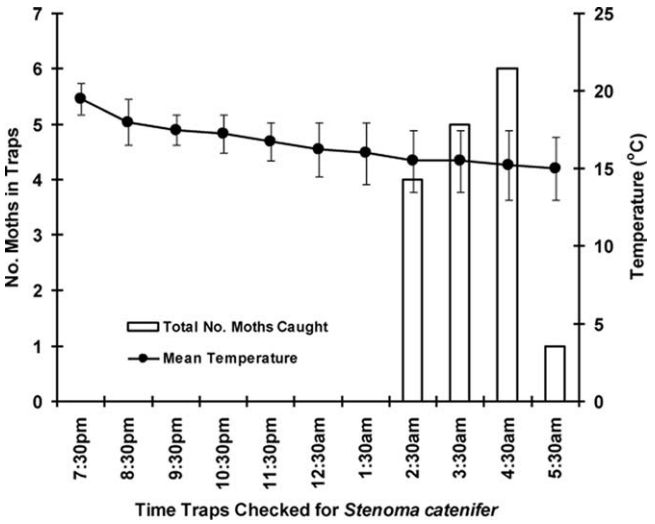

Fig. 5. Total numbers of male *S. catenifer* caught hourly in pheromone traps with corresponding mean hourly temperature (±SE) in a commercial Hass avocado orchard in Guatemala.

bioassays that it forms part of the active pheromone blend. The antennal responses elicited by this compound were also quite specific because none of the other hydrocarbons or lipids present in the crude extract of the pheromone glands elicited responses from antennae of males. Furthermore, this compound has been implicated in the pheromone chemistry of several other moth species (arctiids, Schneider et al. 1998; geometrids, Wei et al. 2003), so detailed behavioral studies may reveal some role for this compound as a semiochemical.

Throughout the course of the experiments described here, the overall number of moths trapped was relatively low. Although this might be an indication of missing pheromone components, suboptimal dose, or antagonism by trace impurities in the synthetic pheromone, we do not believe that this is the case, for several reasons. First, as described, we field tested a broad array of blend ratios of the three components in pheromone gland extracts that elicited antennal responses from male moths. Second, we tested a wide range of pheromone doses, from 1 μg to 1 mg, and there was no indication of inhibition of responses by possibly excessive doses. Third, in the report of the identification of the pheromone for *S. cecropia* (Zagatti et al. 1996), the only other *Stenoma* species for which a pheromone has been identified, the authors reported relatively low attraction of male moths in a wind tunnel to crushed female pheromone glands or to the pheromone blend reconstructed from synthesized compounds. In contrast, these authors reported that in field trials, large numbers of male moths were caught in traps baited with virgin female moths, whereas traps baited with synthetic pheromone caught relatively few moths. However, even traps baited with virgin females caught relatively few moths when female populations in the field were abundant (Zagatti et al. 1996). Together, trap catch data presented here and from Zagatti et al. (1996) suggest that sex attractant pheromones may constitute only one part of the mate location system for moths in the genus *Stenoma*, and that other factors possibly are involved in mate location. This possibility will only be resolved by detailed observations (preferably under field conditions) of the mating behavior of these moths, to determine whether other types of signals (e.g., acoustic stimuli) also may be used in mate location and other reproductive interactions.

Nevertheless, the identification of an attractant pheromone for *S. catenifer* provides a tool for detecting and monitoring this important avocado pest, particularly in situations where competition from female moths is low. Thus, the pheromone is anticipated to be particularly useful for detection of low-level populations, as would occur during the initial stages of invasion of new geographic areas, and for certifying commercially managed orchards in areas with endemic populations of *S. catenifer* that are exporting avocados as being free of this pest.

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