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(*Z*,*Z*)-4,7-TRIDECADIEN-(*S*)-2-YL ACETATE: SEX PHEROMONE OF DOUGLAS-FIR CONE GALL MIDGE, *Contarinia oregonensis*

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Abstract—Our objectives were to identify and field test the sex pheromone of female Douglas-fir cone gall midge, *Contarinia oregonensis* (Diptera: Cecidomyiidae). Coupled gas chromatographic–electroantennographic detection (GC-EAD) analyses of pheromone extract revealed a single compound (**A**) that elicited responses from male antennae. Hydrogenation of pheromone extract, followed by renewed GC-EAD analysis, revealed a new EAD-active compound with chromatographic characteristics identical to those of tridecan-2-yl acetate on five fused silica columns (DB-5, DB-210, DB-23, SP-1000, and Cyclodex-B). Syntheses, chromatography, and retention index calculations of all possible tridecen-2-yl acetates suggested that the candidate pheromone **A** was a tridecadien-2-yl acetate with nonconjugated double bonds. Synthetic candidate pheromone component (*Z,Z*)-4,7-tridecadien-2-yl acetate (*Z*4*Z*7) cochromatographed with **A** on all analytical columns and elicited comparable antennal activity. In GC-EAD analyses that separated the enantiomers (*Z,Z*)-4,7-tridecadien-(*S*)-2-yl acetate (2*S*-*Z*4*Z*7) and (*Z,Z*)-4,7-tridecadien-(*R*)-2-yl acetate (2*R*-*Z*4*Z*7) with baseline resolution, only 2*S*-*Z*4*Z*7 as a component in a racemic standard or in pheromone extract elicited antennal responses. In Douglas-fir seed orchards, sticky traps baited with 2*S*-*Z*4*Z*7 captured

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male *C. oregonensis*, whereas 2*R*-*Z*4*Z*7 was behaviorally benign. Comparable catches of males in traps baited with racemic *Z*4*Z*7 (50 μg) or virgin female *C. oregonensis* suggested that synthetic pheromone baits could be developed for monitoring *C. oregonensis* populations in commercial Douglas-fir seed orchards.

Key Words—Douglas-fir cone gall midge, *Contarinia oregonensis*, Cecidomyiidae, sex pheromone, enantiomers, (*Z*,*Z*)-4,7-tridecadien-(*S*)-2-yl acetate, (*Z*,*Z*)-4,7-tridecadien-(*R*)-2-yl acetate.

INTRODUCTION

The life history of the Douglas-fir cone gall midge, *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae) is closely associated with the phenology of its host plant Douglas-fir, *Pseudotsuga menziesii*. In early spring as conelets open for pollination, female *C. oregonensis* eclose from their pupae in the duff, mate, and seek open conelets for oviposition. Females place their eggs singly or in clusters near the base of cone scales by extending their flexible ovipositor. Neonate larvae tunnel into cone scales and induce formation of, and develop within, galls near ovules. In the fall, larvae leave mature cones and overwinter in the duff as prepupae or pupae (Hedlin, 1961).

In natural forests, *C. oregonensis* is one of many insects that feed and develop on seeds. However, in coastal seed orchards of British Columbia, in Canada and Washington and Oregon, in the United States, *C. oregonensis* has become the single most important insect pest of Douglas-fir seeds. Larvae-caused gall formation results in seed fusion to the scale, preventing seed extraction at harvest or destruction of seeds at high populations. Severe infestations destroy the entire seed crop (Hedlin, 1961, 1974; Ruth, 1980).

C. oregonensis is the only coastal Douglas-fir seed orchard pest in British Columbia monitored annually. Monitoring entails tedious and time-consuming counts of egg-infested scales in samples of conelets. When a threshold number of infested scales per conelet is reached, insecticidal control of *C. oregonensis* is implemented.

Miller and Borden (1981, 1984) presented evidence for a sex attractant pheromone produced by female *C. oregonensis.* Traps baited with synthetic *C. oregonensis* pheromone would allow investigation of relationships between captures of male *C. oregonensis* and numbers of both egg-infested scales at pollination and galled scales at harvest. If reliable correlations could be established, decisions about *C. oregonensis* control could be based solely on captures of males in pheromone-baited traps. Monitoring of *C. oregonensis* would become simple and effective.

We report identification and field testing of the pheromone.

METHODS AND MATERIALS

Experimental Insects. In late August and early September 1995–1996, mature, dry Douglas-fir cones infested with larvae of *C. oregonensis* were collected into loosely woven burlap sacks. These sacks were then partially submerged in water-filled pails for 2–3 days to induce the mature larvae to leave the cones. After removing the sacks from the pails, the larvae that had exited the cones and sacks and accumulated in the bottom of pails (apparently unaffected by their temporary submergence) were obtained by pouring the water through a No. 30 standard sieve (600 μm). Sieve-restrained larvae were then transferred to 500-ml glass jars half filled with clean, damp sand and closed with fine mesh screening. To break larval developmental diapause, jars were placed in environmental chambers (18°C; 70% relative humidity.) with temperature lowered by 2°C every 5 d to 4°C. After 3.5 months of cold exposure (4°C), jars were subjected to a temperature increase from 4 to 18°C in increments of 2°C every 5 d. Emergent adults were collected several times a day and kept individually in Petri dishes. Ovipositors (with pheromone glands) of calling females and abdominal tips of males were removed and separately extracted in hexane for 5–50 min.

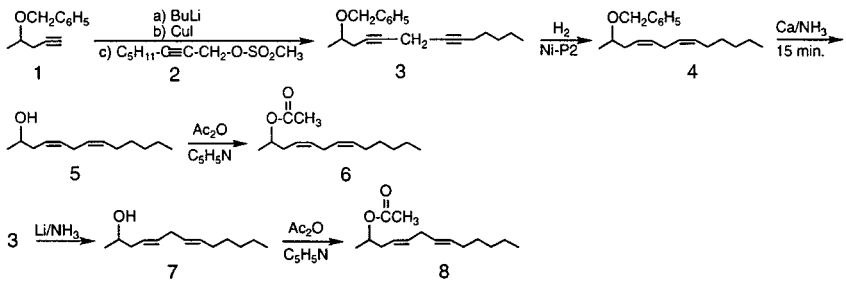
Laboratory Analyses of Pheromone Extract and General Instrumentation. Aliquots of 5–10 female and male equivalents (FE, ME) of abdominal tip extract were analyzed by gas chromatography (GC) and coupled gas chromatographic–electroantennographic detection (GC-EAD) (Arn et al., 1975), employing an Hewlett-Packard (HP) 5890 gas chromatograph fitted with a fused silica column (30 m $\times$ 0.25 or 0.32 mm ID) coated with DB-210, DB-5, DB-23, Cyclodex-B (J&W Scientific, Folsom, California, USA) or SP-1000 (Supelco, Bellefonte, Pennsylvania, USA). For GC-EAD recordings, a severed insect head (with both antennae intact) was placed into the opening of a glass capillary electrode (1.0 $\times$ 0.58 $\times$ 100 mm) (A-M Systems, Inc., Carlsborg, Washington, USA) filled with saline solution (Staddon and Everton, 1980). One antenna with its tip removed by spring microscissors (Fine Science Tools Inc., North Vancouver, British Columbia, Canada) was placed into the recording capillary electrode. Coupled GC-mass spectrometric (MS) analyses of pheromone extract (300 FE) and of synthetic standards employed a Varian II Ion Trap GC-MS fitted with the above-referenced DB-5 column.

High-performance liquid chromatography (HPLC) employed a Waters LC 626 high-performance liquid chromatograph equipped with a Waters 486 variable wavelength UV visible detector set to 210 nm, HP Chemstation software (Rev.A.07.01), and a normal phase Nova-Pak silica column (60Å, 4 μm; 3.9 $\times$ 150 mm) with 1 ml/min of hexane–ether (97:3) flow. Nuclear magnetic resonance (NMR) spectroscopy of synthetic compounds was conducted on a

Bruker AMX-400 spectrometer at 400.13 MHz for ^{1}H NMR spectra. ^{1}H chemical shifts are reported as parts per million (ppm; δ) relative to TMS. Elemental analyses were performed using a Carlo Erba model 1106 elemental analyzer. Chemicals obtained from commercial sources were used without further purification unless otherwise indicated. All moisture- and air-sensitive reactions were conducted under argon. Column chromatography refers to flash chromatography, using silica gel 60 (230–400 mesh, E. Merck, Darmstadt) (Still et al., 1978).

Syntheses

*(Z,Z)-4,7-Tridecadien-2-ol (**5**) (Scheme 1).* To a THF solution of the benzyl ether of 4-pentyn-2-ol (**1**) (4.35 g, 25 mmol), 11 ml of 2.5 M butyllithium in hexane (10% excess) was added at -70°C. The mixture was stirred under argon and then warmed to -25°C before addition of CuI (475 mg, 0.1 equiv.). After stirring for 1 hr at -25°C, a THF solution of freshly prepared 2-octyn-1-yl methanesulfonate (**2**) (30 mmol) (from 2-octyn-1-ol, methanesulfonyl chloride, triethylamine, 0°C) was added dropwise. The reaction mixture was allowed to warm to room temperature, quenched with aq. conc. NH_4Cl, and after 2 hr of stirring was extracted with hexane–ether (1:1). The desired benzyl ether of 4,7-tridecadiyn-2-ol (**3**) (40% yield) was purified (85% based on GC) by flash chromatography and reduced to the benzyl ether of (*Z*,*Z*)-4,7-tridecadien-2-ol (**4**) by catalytic hydrogenation with P-2 Ni in ethanol. Flash chromatography on $AgNO_3$-impregnated (20%) SiO_2, with a gradient of hexane–ether (99:1 to 95:5) eluent afforded (*Z*,*Z*)-diene (**4**) (76% yield, 94 % pure). Removal of the benzyl group of **4** by exposure to excess calcium (15 equiv.) in liq. ammonia (Hwu et al., 1986) for 15 min at -70°C yielded (*Z*,*Z*)-4,7-tridecadien-2-ol (**5**) (71%) and corresponding monoenes (7%), as determined by GC-MS. Therefore, **5** was repurified by flash chromatography as above to give 1.05 g (5.40 mmol) of pure **5**. Anal. calcd. for $C_{13}H_{24}O$: C 79.13; H 12.22. Found: C 79.53; H 12.32. ^{1}H NMR: $CDCl_3$, δ = 5.31-5.61 (4H, m), 3.88



SCHEME 1. Synthesis of (*Z*,*Z*)- and (*E*,*E*)-4,7-tridecadien-2-yl acetates.

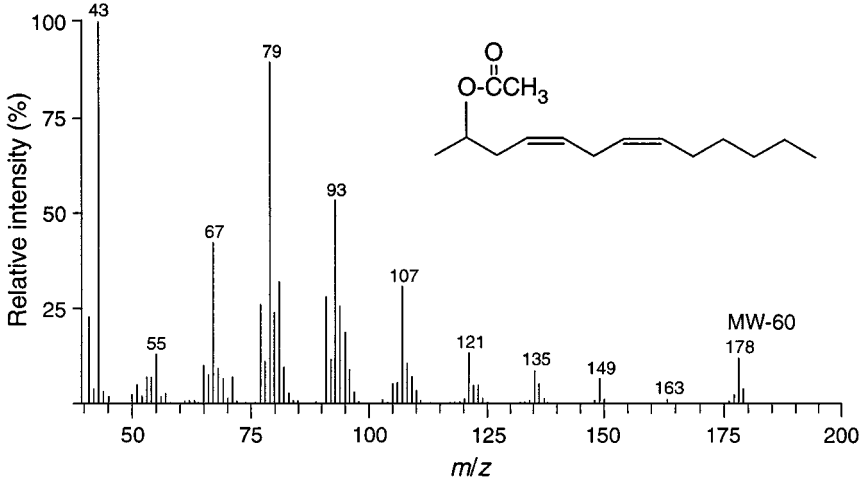


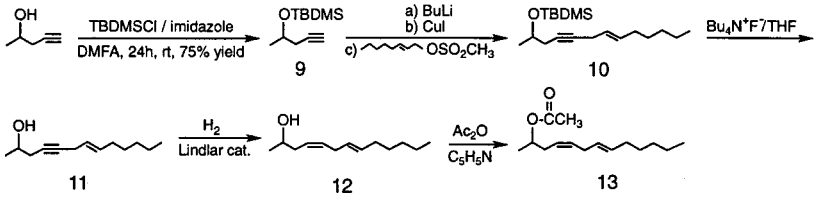
FIG. 1. Mass spectrum of synthetic (*Z*,*Z*)-4,7-tridecadien-2-yl acetate. Varian Saturn II Ion Trap GC-MS.

(1H, sextet), 2.85 (2H, t), 2.28 (2H, m), 2.08 (2H, m), 1.34 (6H, m), 1.25 (3H, d), 0.92 (3H, t).

*(Z,Z)-4,7-Tridecadien-2-yl Acetate (**6**) (Scheme 1).* Overnight reaction of **5** with acetic anhydride in pyridine afforded acetate **6** (1.20 g, 5.0 mmol) in 93% yield (20% overall yield based on **1**). Anal. calcd. for $C_{15}H_{26}O_2$: C 75.58; H 11.00. Found: C 75.80; H 10.98. ^{1}H NMR: $CDCl_3$, $\delta = 5.28$–5.54 (4H, m.), 4.92 (1H, sextet), 2.78 (2H, t), 2.31 (2H, m), 2.04 (2H, m), 2.01 (3H, s), 1.27 (6H, m), 1.22 (3H, d), 0.88 (3H, t); mass spectrum as in Figure 1.

*(E,E)-4,7-Tridecadien-2-yl Acetate (**8**) (Scheme 1).* Overnight reaction of **3** (1.13 g, 4.00 mmol) with lithium (20-fold excess) in refluxing liq. ammonia produced as the major product geometrically pure (*E*,*E*)-4,7-tridecadien-2-ol (**7**) (60% yield). It was purified by flash chromatography (see above) and acetylated as above to give (*E*,*E*)-4,7-tridecadien-2-yl acetate (**8**) (0.520 g, 2.18 mmol).

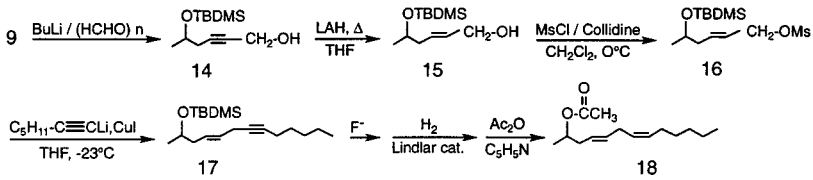
*(Z,E)-4,7-Tridecadien-2-yl Acetate (**13**) (Scheme 2).* 2-*tert*-Butyl-dimethylsilyloxy-3-pentyne (**9**) (5.14 g, 15.6 mmol) was coupled via cuprate with freshly prepared (*E*)-2-octen-1-yl methanesulfonate (15.2 mmol) (from 2-octen-1-ol, methanesulfonyl chloride, collidine at 0°C, 50% yield) in THF. The resulting crude *tert*-butyldimethylsilyl ether of (*E*)-7-tridecen-4-yn-2-ol (**10**) was de-protected with Bu_4NF in THF affording alcohol **11** (80% pure), which was hydrogenated with Lindlar catalyst in hexanes to (*Z*,*E*)-4,7-tridecadien-2-ol (**12**, 1.46 g, 7.43 mmol). Acetylation of purified **12** produced geometrically pure (*Z*,*E*)-4,7-tridecadien-2-yl acetate (**13**, 1.44 g, 6.04 mmol) in 39% overall yield.



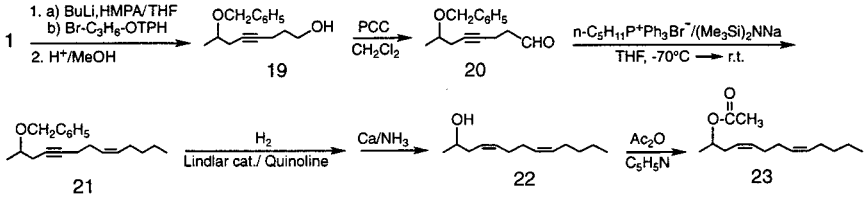
SCHEME 2. Synthesis of (*Z*,*E*)-4,7-tridecadien-2-yl acetate.

*(E,Z)-4,7-Tridecadien-2-yl Acetate (**18**) (Scheme 3).* Compound **9** in THF (5.70 g, 28.8 mmol) (Scheme 1) was extended by one carbon (BuLi at −70°C, then paraformaldehyde, warm to room temperature, and stirring for 24 hr) to the mono-protected diol **14** (95% yield). Reduction of **14** (3.1 g, 13.6 mmol) with lithium aluminum hydride (LAH, 7 molar excess) in refluxing THF produced allylic alcohol **15** (1.90 g, 8.26 mmol) in 60% yield. Metylation of **15** with methanesulfonyl chloride in dichloromethane (0°C) in the presence of 2,4,6-collidine gave 5-(*tert*-butyldimethylsilyloxy)-2-hexenyl methanesulfonate (**16**). Up to 50% formation of the unwanted allylic chloride side product occurred. Reaction of sulfonate **16** with heptynyllithium in the presence of CuI (0.1 equiv.) gave the *tert*-butyldimethylsilyl ether of (*E*)-4-tridecen-7-yn-2-ol (**17**). Removal of the protective group from **17**, hydrogenation, and acetylation (as described for **10** in Scheme 2) afforded after purification (*E*,*Z*)-4,7-tridecadien-2-yl acetate (**18**, 0.18 g, 7.56 mmol) in 9% yield based on **15**.

*(Z,Z)-4,8-Tridecadien-2-ol (**23**) (Scheme 4).* Benzyl ether **1** (4.35 g, 25 mmol) was coupled at −70°C via the lithium salt with 1-*O*-tetrahydropyranyl-3-bromopropanol (1.3 equiv.) [−70°C to room temperature, 24 hr, THF–HMPA (1:1)]. Subsequent removal of the THP group in methanol in the presence of trifluoroacetic acid afforded 7-benzyloxy-4-octyn-1-ol (**19**) (1.32 g, 5.71 mmol). Oxidation of alcohol **19** with PCC in dichloromethane at room temperature, and immediate coupling of the crude aldehyde **20** with *n*-pentylidene triphenylphosphorane in THF yielded the benzyl ether of (*Z*)-8-tridecen-4-yn-2-ol (**21**). Compound **21** partially purified by flash chromatograhy was hydrogenated (4 hr) with Lindlar catalyst in hexanes in the presence of quinoline, and the resulting diene-ether was



SCHEME 3. Synthesis of (*E*,*Z*)-4,7-tridecadien-2-yl acetate.

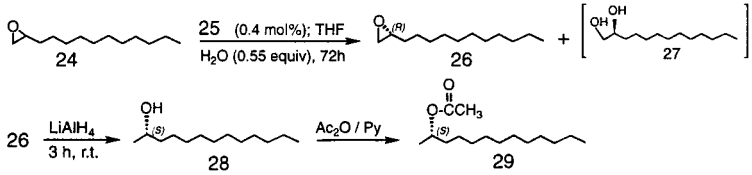


SCHEME 4. Synthesis of (*Z*,*Z*)-4,8-tridecadien-2-yl acetate.

treated with 10 equivalents of calcium in liq. ammonia for 45 min. Usual work-up and purification of the diunsaturated alcohol **22**, followed by overnight acetylation with acetic anhydride in pyridine, yielded (*Z*,*Z*)-4,8-tridecadien-2-yl acetate (**23**, 0.265 g, 1.10 mmol) in 4.5% yield based on **1**.

*(S)- and (R)-2-Tridecanyl Acetate (**29, 34**) (Scheme 5).* Hydrolytic kinetic resolution of racemic 1,2-epoxytridecane (**24**) (1.75 g, 8.80 mmol) with 25 mg of (*R*,*R*)-(−)-salen complex of Co(II) [Jacobsen's catalyst; (*R*,*R*)-(−)-N,N′-bis (3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II)] (**25**) (Sigma-Aldrich, Oakville, Ontario, Canada) and 90 μl of water (Tokunaga et al., 1997; Schaus et al., 1998; Gries et al., 2000) yielded after 3 d at room temperature (*R*)-1,2-epoxytridecane (**26**) (0.88 g, 4.44 mmol) which was separated from (*S*)-1,2-tridecanediol (**27**) by flash chromatography (hexanes–ether, 10:1). Ring opening of **26** with LAH in ether afforded (*S*)-2-tridecanol (**28**) (0.84 g, 4.24 mmol). Acetylation of **28** gave (*S*)-2-tridecanyl acetate (**29**) (Kamezawa et al., 1994, Sharma et al., 1996) in 47% overall yield and with an enantiomeric excess (ee) of 91.9%, as determined by GC analysis on a Cyclodex-B column. Corresponding hydrokinetic resolution of **24** with the (*S*,*S*)-(+)-salen complex of Co(II), and corresponding follow-up reactions (see above) afforded (*S*)-1,2-epoxytridecane, (*R*)-2-tridecanol, and finally (*R*)-2-tridecanyl acetate (47% overall yield, 91.3% ee).

2-Tridecenyl acetates with *E* and *Z* unsaturation at C-3 to C-12 were synthesized by oxidation (PCC in dichloromethane) of the dodecenols (available in our laboratory from previous studies) and by immediate reaction of the crude aldehydes



SCHEME 5. Synthesis of (*S*)-2-tridecanyl acetate.

with methylmagnesium bromide in ether followed by acetylation (Ac_2O/Py) of the resulting 2-tridecenols.

Absolute Configuration of Pheromone. The absolute configuration of the pheromone was determined in five steps: (1) acetyl lactate derivatization (Slessor et al., 1985) of racemic (*Z*,*Z*)-4,7-tridecadien-2-ol (**5** in Scheme 1); (2) separation of diasteromeric derivatives by HPLC (conditions described above); (3) removal of the acetyl lactate functionality and acetylation of the resulting enantiospecific alcohols; (4) GC-EAD analyses (Figure 3 below) and field testing (Figure 4 below) of (*Z*,*Z*)-4,7-tridecadien-(*S*)-2-yl acetate (2*S*-*Z*4*Z*7) and (*Z*,*Z*)-4,7-tridecadien-(*R*)-2-yl acetate (2*R*-*Z*4*Z*7); and (5) comparison of retention times (Cyclodex-B column) of the saturated equivalents of 2*S*-*Z*4*Z*7 [(*S*)-2-tridecanyl acetate] and 2*R*-*Z*4*Z*7 [(*R*)-2-tridecanyl acetate] with those of enantioselectively synthesized (*S*)- and (*R*)-2-tridecanyl acetate (Scheme 5).

Field Experiments. Field experiments were conducted in Nootka, Mount Newton, and Saanich Douglas-fir seed orchards, and the Douglas-fir seed orchard at the Saanichton Forestry Centre, all near Saanichton, Vancouver Island, British Columbia. Trees were 11–25 years old with spacings within and between rows ranging from 3.8×6.1 m to 4×10 m. At 15–20-m intervals, self-made sticky 2-l milk carton traps (Gray et al., 1984) were suspended from trees 1.5 m above ground in complete randomized blocks. Traps were baited with a gray sleeve stopper (The West Company, Lionville, Pennsylvania, USA) impregnated with test chemicals in HPLC-grade hexane. All test chemicals were >93% chemically and geometrically pure. Enantiomeric excess of 2*S*-*Z*4*Z*7 and 2*R*-*Z*4*Z*7 was 95.5% and 97.8%, respectively, based on GC analyses on a Cyclodex-B column.

Experiment 1 tested candidate pheromone *Z*4*Z*7 at doses of 1, 10, 100, and 1,000 μg. Experiments 2 and 3 tested *S* and *R* enantiomers of *Z*4*Z*7 singly and in combination at doses of 10 and 2 μg, respectively. Experiment 4 tested attractiveness of racemic *Z*4*Z*7 at 50 μg versus that of caged female *C. oregonensis*.

Despite transformation, trap catch data were not normally distributed and were, thus, analyzed by nonparametric analyses of variance (Friedman's test) followed by comparison of means by Bonferroni (Dunn) t test (Zar, 1984; SAS/STAT, 1988). In all analyses, $\alpha = 0.05$.

RESULTS AND DISCUSSION

Pheromone Analyses. Comparison of GC traces of abdominal tip extracts of male and female *C. oregonensis* failed to disclose sex-specific volatiles. Coupled GC-EAD analyses of female pheromone extract revealed a single EAD-active compound (**A** in Figure 2) that occurred below detection threshold of the flame ionization detector of the GC. Retention indices (RI) (Van den Dool and Kratz, 1963) of **A** (DB-5: 1605; DB-210: 1872; DB-23: 1932; SP-1000: 1927;

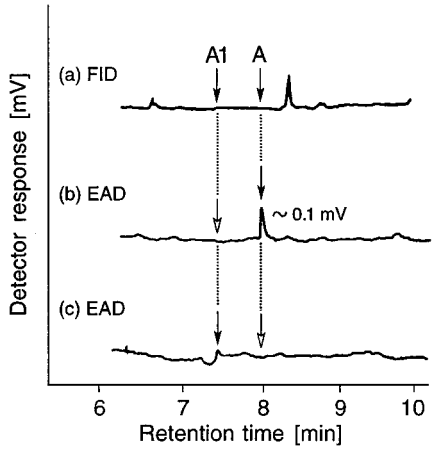


FIG. 2. Representative GC-EAD recording (8 replicates) of pheromone gland extract of female *C. oregonensis*; (**a**) flame ionization detector (FID) chromatograph of 10 female equivalents (10 FE) of pheromone gland extract; (**b, c**) corresponding recording of electroantennographic detector (EAD: male *C. oregonensis* antenna) responses to 10 FE of (**b**) untreated and (**c**) hydrogenated pheromone extract. Chromatography: Hewlett Packard 5890A gas chromatograph; splitless injection; injector temperature: 220°C; detector temperature: 250°C; DB-23 column; temperature program: 100°C (1 min) 10°C per min to 200°C. A = (*Z*,*Z*)-4,7-tridecadien-2-yl acetate; A1 = tridecan-2-yl acetate.

Cyclodex B: 1690), and RI intercolumn differentials, suggested that it had an ester (acetate) functionality. GC-EAD analysis of hydrogenated pheromone extract revealed a new EAD-active compound (**A1** in Figure 2), suggesting that hydrogenation of **A** had resulted in a saturated volatile (**A1**) with different chromatographic characteristics [RIs: 1631 (DB-5); 1890 (DB-210); 1879 (DB-23); and 1870 (SP-1000)]. Compound **A1** was determined to be 2-tridecanyl acetate [an aggregation pheromone component of *Drosophila mulleri* and *D. busckii* (Kamezawa et al. 1994)], which cochromatographed with **A1** on all above-referenced columns and had comparable EAD activity as **A1**. Similar chromatographic characteristics of **A** and **A1** suggested that the former was a tridecen-2-yl acetate. However, syntheses and chromatography of all possible tridecen-2-yl acetates determined that neither co-chromatographed with **A**, nor elicited antennal responses indicative of a potential double bond position.

Hypothesizing then that **A** was a nonconjugated tridecadien-2-yl acetate, candidate tridecadien-2-yl acetates were approximated (Gries et al., 1993) by (1) determining retention index differentials between tridecan-2-yl acetate and each of the tridecen-2-yl acetates (Table 1), and (2) adding these differentials to, or subtracting them from, the respective tridecen-2-yl acetate. For example, the RI 1604

TABLE 1. RETENTION INDICES AND DIFFERENTIALS (BOLD FACE TYPE) BETWEEN SYNTHETIC TRIDECEN-2-YL ACETATES AND SYNTHETIC TRIDECAN-2-YL ACETATE (SAT) ON GC COLUMNS COATED WITH DB-5, DB-210, DB-23, OR SP-1000

dbp[a]	DB-5	DB-210	DB-23	SP-1000
sat	1631	1890	1879	1870
E 3	1620: −**11**	1864: −**26**	1890:+11	1900: +**30**
E 4	1616: −**15**	1876: −**14**	1885:+**6**	1884: +**14**
E 5	1614: −**17**	1859: −**31**	1885:+**6**	1891: +**21**
E 6	1614: −**17**	1871: −**19**	1891:+**12**	1894: +**24**
E 7	1618: −**13**	1881: −**9**	1897: +**18**	1894: +**24**
E 8	1619: −**12**	1875: −**15**	1899:+**20**	1900: +**30**
E 9	1621: −**10**	1881: −**9**	1901:+**22**	1900: +**30**
E 10	1627: −**4**	1889: −**1**	1910:+**31**	1910: +**40**
E 11	1635: +**4**	1895: +**5**	1931:+**52**	1931: +**61**
Δ12	1623: −**8**	1904: +**14**	1931: +**52**	1920: +**50**
Z 3	1589: −**42**	1796: −**94**	1830: −**49**	1844: −**26**
Z 4	1617: −**14**	1871: −**19**	1898: +**19**	1894: +**24**
Z 5	1604: −**27**	1871: −**19**	1888: +**9**	1880: +**10**
Z 6	1609: −**22**	1873: −**17**	1896: +**17**	1890: +**20**
Z 7	1610: −**21**	1881: −**9**	1903: +**24**	1894: +**24**
Z 8	1618: −**13**	1890: ±**0**	1913: +**34**	1903: +**33**
Z 9	1624: −**8**	1898: +**8**	1920: +**41**	1911: +**41**
Z 10	1630: −**1**	1903: +**13**	1929: +**50**	1920: +**50**
Z 11	1648: +**17**	1925: +**35**	1956: +**77**	1951: +**81**

[a] dbp = double bond position.

of (*E*,*E*)-4,8-tridecadien-2-yl acetate on DB-5 was predicted by subtracting the RI differential between tridecan-2-yl acetate and (*E*)-8-tridecen-2-yl acetate (=12; Table 1) from the RI of (*E*)-4-tridecen-2-yl acetate (1616 − 12 = 1604). Taking the data for all five columns into account, (*Z*,*Z*)-4,8-tridecadien-2-yl acetate (*Z*4*Z*8) was predicted to be the most probable diene acetate to match chromatographic characteristics of **A**. However, synthetic *Z*4*Z*8 (Scheme 4) had RIs 1-3 RI units different from those of the target **A** on all five columns.

Strong EAD activity of *Z*4*Z*8 suggested that its molecular structure was similar to that of the candidate pheromone **A**. An allylic double bond in C-3, instead of C-4, would have greatly decreased the RI of the molecule (RG + GG, unpublished data) and was discounted. Hypothesizing then that the geometry and position of the double bond in C-8 was incorrect, (*Z*,*Z*)-4,7-tridecadien-2-yl acetate (*Z*4*Z*7) was synthesized (Scheme 1) as an alternative structure for the candidate pheromone. This compound, but none of its three isomers (*ZE*, *EZ*, *EE*) (Schemes 1–3), cochromatographed with **A** on all columns, and elicited comparable antennal responses.

In GC-EAD analyses that separated the enantiomers of *Z*4*Z*7 with baseline resolution (Figure 3, a), antennal responses were obtained only to the early

eluting enantiomer in a racemic standard (Figure 3, b) or in pheromone extract (Figure 3, c). This enantiomer was determined to have the *S* configuration, because its hydrogenated equivalent had a retention time on Cyclodex-B that was identical with that of enantiospecifically synthesized (*S*)-2-tridecanyl acetate (**29**) (Scheme 5). (*R*)-2-Tridecanyl acetate, in contrast, eluted 0.35 min later. Lack of response to later eluting 2*R*-*Z*4*Z*7 could not be attributed to a refractory period of the antenna, because two sequential injections (at 0.5 min intervals) of isothermally chromatographed 2*S*-*Z*4*Z*7 elicited two very similar antennal responses (Figure 3, d). When 2*S*- and 2*R*-*Z*4*Z*7 were analyzed individually, only the *S* enantiomer was EAD-active, supporting contention that female *C. oregonensis* produce 2*S*-*Z*4*Z*7 for pheromonal attraction of mates.

Field Experiments. In experiment 1, a lure dose of 100 μg of racemic Z4Z7 was more effective than lower or higher lure doses in attracting male *C. oregonensis* (Figure 4, experiment 1), indicating that proper lure loadings are critical for optimal attractiveness. Captures of male *C. oregonensis* in traps baited with 2*S*-*Z*4*Z*7 (Figure 4, experiments 2 and 3) were consistent with EAD-activity only of the *S* enantiomer (Figure 3, b). The few males captured in traps baited with 2*R*-*Z*4*Z*7 (Figure 4, experiment 2) were probably attracted to trace amounts of the *S* enantiomer in the bait. The antipode 2*R*-*Z*4*Z*7 is not part of the *C. oregonensis* pheromone because it elicited neither EAD-activity (Figure 3, b) nor behavioral activity when tested alone at a low dose (Figure 4, experiment 3) or in combination with pheromonal 2*S*-Z4Z7 (Figure 4, experiments 2 and 3). Similar numbers of males captured in traps baited with synthetic *Z*4*Z*7 (50 μg) or virgin female *C. oregonensis* (Figure 4, experiment 4) indicate competitive attractiveness of natural and synthetic pheromone.

Pheromones in Cecidomyiids and Practical Implications. Evidence for female-produced sex pheromones has been reported for 10 cecidomyiids (reviewed by Harris and Foster, 1999) but only four pheromones have been identified. In pioneering studies, (*E*)-10-tridecen-(*S*)-2-yl acetate was determined to be a sex pheromone component of the Hessian fly, *Mayetiola destructor* (Foster et al., 1991; Harris and Foster, 1991; Millar et al., 1991). Attractiveness of this compound to male *M. destructor* in wind-tunnel but not field experiments suggested that female *M. destructor* deploy additional pheromone components that have not yet been identified (Harris and Foster, 1999). Meticulous work by Hillbur et al. (1999, 2000, 2001) deciphered the three-component pheromone of the pea midge, *Contarinia pisi*. Synthetic (2*S*,11*S*)-diacetoxytridecane, (2*S*,12*S*)-diacetoxytridecane, and 2-acetoxytridecane at a blend ratio as found in pheromone gland extracts attracted male *C. pisi* in both wind-tunnel and field experiments. Concurrent work by Gries et al. (2000) revealed the pheromone of the orange wheat blossom midge, *Sitodiplosis mosellana*, to be (2*S*,7*S*)-nonanediyl dibutyrate, which attracted male *S. mosellana* in field experiments in Saskatchewan. While *SR*, *RS*,

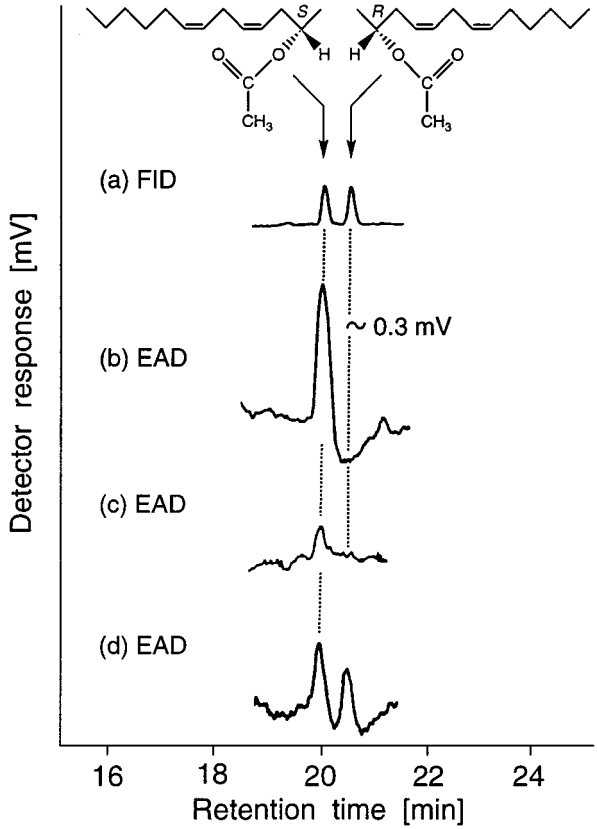


FIG. 3. Representative GC-EAD recording (5 replicates) of synthetic standards and pheromone gland extract of female *C. oregonensis*; (**a**) flame ionization detector (FID) chromatogram of synthetic (*Z*,*Z*)-4,7-tridecadien-2-yl acetate (*Z*4*Z*7) with baseline resolution of the enantiomers (*Z*,*Z*)-4,7-tridecadien-(*S*)-2-yl acetate (2*S*-*Z*4*Z*7; left) and (*Z*,*Z*)-4,7-tridecadien-(*R*)-2-yl acetate (2*R*-*Z*4*Z*7; right); (**b–d**) corresponding recordings of electroantennographic detector (EAD: male *C. oregonensis* antenna) responses to (**b**) synthetic *Z*4*Z*7, (**c**) 10 female equivalents of pheromone gland extract and (**d**) two sequential injections of 2*S*-*Z*4*Z*7. Note: first and second injection of 2*S*-*Z*4*Z*7 were separated by 0.5 min., equivalent to the 0.5 min separation of 2*S*-*Z*4*Z*7 and 2*R*-*Z*4*Z*7 in the racemic standard (**a**); lack of response to later eluting 2*R*-*Z*4*Z*7 (**b**) could not be attributed to a refractory period of the antenna, because two sequential injections (at 0.5-min intervals) of isothermally chromatographed 2*S*-*Z*4*Z*7 elicited two very similar antennal responses (**d**). Chromatography: Hewlett-Packard 5890A gas chromatograph equipped with a chiral Cyclodex-B column; temperature program: 150°C isothermal; split injection of 25 ng per compound with 0.5 ng reaching the EAD detector.

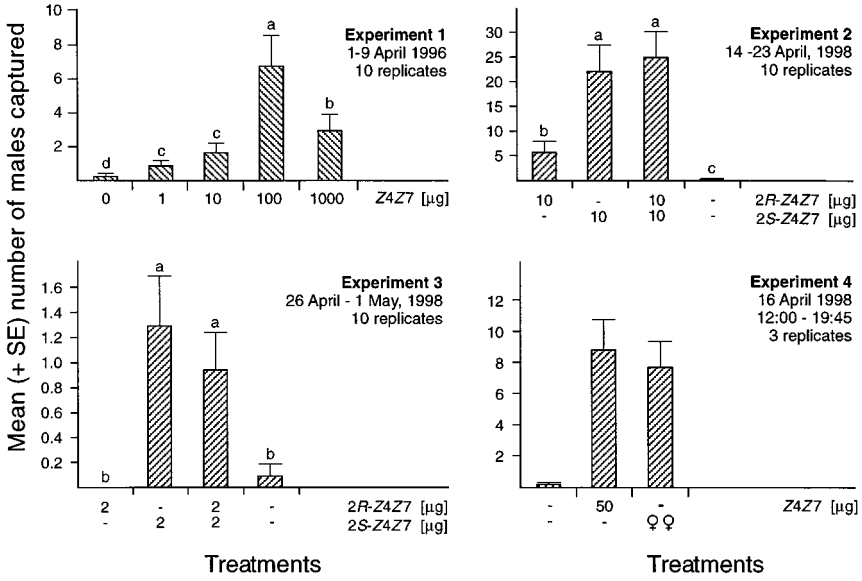


FIG. 4. Mean (+SE) number of male *C. oregonensis* captured during experimental periods in sticky Delta milk carton traps baited with various doses of racemic (*Z*,*Z*)-4,7-tridecadien-2-yl acetate (*Z*4*Z*7) (experiment 1), (*Z*,*Z*)-4,7-tridecadien-(*S*)-2-yl acetate (2*S*-*Z*4*Z*7), and (*Z*,*Z*)-4,7-tridecadien-(*R*)-2-yl acetate (2*R*-*Z*4*Z*7) singly and in combination (experiments 2, 3), or baited with racemic *Z*4*Z*7 or caged virgin female *C. oregonensis* (experiment 4). Douglas-fir seed orchards near Saanichton, Vancouver Island, British Columbia. Bars in each experiment with different letter superscripts are significantly different; nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means [Bonferroni (Dunn) *t* test, $P < 0.05$] (SAS/STAT, 1988).

and *RR* stereoisomers of the pheromone had no adverse effects on attraction of male *S. mosellana*, attraction of male *C. pisi* to the synthetic *C. pisi* pheromone blend (see above) was strongly inhibited by the presence (20%) of the 2*S*,11*R*- and 2*R*,11*S*-stereoisomers of diacetoxytridecane (Hillbur et al., 2001). Male *C. oregonensis*, in contrast, did not perceive the antipode of pheromonal 2*S*-*Z*4*Z*7 (Figure 3, b) and were not inhibited by it (Figure 4, experiments 2 and 3).

The identification of the *C. oregonensis* pheromone should lead to improved monitoring of *C. oregonensis* populations and management decisions in commercial seed orchards. In both 2000 and 2001, numbers of male *C. oregonensis* captured in pheromone-baited traps in seed orchards in Washington and Oregon were correlated with numbers of both egg-infested scales in conelets and damaged seed in cones (Morewood et al., 2002). Trap captures of male *C. oregonensis* are, thus, predictive of seed damage at harvest, and should help orchard managers decide whether insecticidal control of *C. oregonensis* populations is warranted.

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