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Sex Pheromone of Browntail Moth, *Euproctis chrysorrhea* (L.): Synthesis and Field Deployment

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The browntail moth, *Euproctis chrysorrhea* (L.), is native to Eurasia, where periodic outbreaks result in defoliation of forest, shade, and ornamental trees. In addition to the damage caused by defoliation, human contact with larval urticating hairs often results in severe dermatitis. Hence, tools for monitoring and controlling the moth populations are desirable. The female-produced sex pheromone of the browntail moth was identified previously, but the synthesis had not been published. This paper reports the synthesis of the pheromone of the browntail moth, (7*Z*,13*Z*,16*Z*,19*Z*)-docosatetraenyl isobutyrate, using in a key step a Wittig olefination of (6*Z*)-13-(tetrahydo-2*H*-pyran-2-yloxy)tridecenal. Field trapping studies were conducted with rubber septa and string formulations of the pheromone and included dose-response, pheromone purity, and dispenser-aging trials. It was found that traps baited with 250 μg of pheromone of 91–94% isomeric purity (main impurity presumably being the 13*E* isomer) on rubber septa are suitable for monitoring moth populations during the entire flight season.

KEYWORDS: Browntail moth; *Euproctis chrysorrhea*; pheromone; synthesis; field trapping

INTRODUCTION

The browntail moth, *Euproctis chrysorrhea* (Linnaeus) (Lepidoptera: Lymantriidae), is a pest of forest and orchard trees and also a wide range of woody rosaceous plants and ornamental shrubs (*1, 2*). It is primarily found in central and southern Europe and North Africa (*2*). *E. chrysorrhea* was introduced into Somerville, MA, in the late 19th century and became abundant throughout eastern New England before declining after 1915 (*3*). Currently, the distribution of *E. chrysorrhea* in the United States is limited to coastal dunes on Cape Cod, MA, and to several coastal islands and neighboring coasts in Casco Bay, ME (*4*). Eggs hatch in late summer, and the larvae build silken webs in which they overwinter before emerging to feed in the spring (*1*). At high population levels, *E. chrysorrhea* larvae may completely defoliate hosts such as beach plum (*Prunus maritima*) and black cherry (*Prunus serotina*) in Massachusetts. Moreover, urticating hairs that are present on several life stages can cause severe dermatitis in humans (*5, 6*), making *E. chrysorrhea* a public health issue (*1, 4, 5, 7*). Efforts to control *E. chrysorrhea* have included the use of insecticides (*4, 8*), microsporidia (*5*), nucleopolyhedrosis viruses (*7*), and/or labor-intensive nest removal (*1*). Efficient tools for monitoring browntail moth populations and predicting potential outbreaks would be a great aid in managing this pest.

Surveys for *E. chrysorrhea* typically entail counting numbers of nests of this web-making lymantriid; counts can then be used in efforts to predict population levels the following year (*4, 8, 9*). Light-trap catches have also been used to monitor population dynamics and predict outbreaks of *E. chrysorrhea* (*10*). Because these methods are labor-intensive and relatively inefficient, they are generally regarded as inferior to monitoring with pheromone traps. Leonhardt et al. (*11*) identified the main component of the sex pheromone of *E. chrysorrhea* as (7*Z*,13*Z*,16*Z*,19*Z*)-docosatetraenyl isobutyrate (**1**). The authors reported on the availability of a synthetic pheromone that matched the natural compound and attracted male moths in a short field trail as efficiently as virgin females. However, the synthesis paper cited in ref *11* as being in preparation did not follow, and to our knowledge no further report on *E. chrysorrhea* pheromone has appeared in the literature.

In 1995, the Beltsville Agricultural Research Center of the USDA-ARS, received an inquiry from the Maine Forest Service and the National Park Service, U.S. Department of Interior, on the availability of a pheromone-based survey for the browntail moth, populations of which had gone up by some accounts in the Cape Cod and Casco Bay areas (*4*). We synthesized ester **1** and field-tested it in Cape Cod in 1995; we renewed our synthetic and bioassay efforts in 2005, including different

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batches of pheromone in two types of formulations. In the current paper, we present a full account of our synthesis and field trapping studies.

METHODS AND MATERIALS

Gas Chromatographic Analyses. We used a Shimadzu 17A gas chromatograph (Columbia, MD) equipped with a 30 m HP-5 capillary column (0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA), flame ionization detector, Shimadzu AOC-20s autosampler, and AOC-20i autoinjector. Analyses were done in split mode using hydrogen as a carrier gas at 1.0 mL/min. Analyses of pheromone **1** were also conducted on an SPB-1 column (60 m × 0.25 mm × 0.25 μm film, Supelco, Bellefonte, PA).

Electron Impact (EI) Mass Spectra. EI-MS (70 eV) were obtained with an Agilent Technologies 5973 mass selective detector interfaced with an Agilent 6890N GC equipped with a 30 m × 0.25 mm i.d., 0.25 μm film thickness, HP-5MS capillary column. Helium was used as the carrier gas at 1.0 mL/min.

NMR Analyses. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in $CDCl_3$ with TMS as an internal standard on a Bruker QE-300 spectrometer.

Chemicals and Supplies. Unless otherwise specified, all reagents were purchased from Aldrich Chemical Co., Milwaukee, WI. Flash chromatography was carried out with 230–400 mesh silica gel (Fisher Scientific, Fair Lawn, NJ). Lindlar catalyst, 5% Pd on $CaCO_3$, lead poisoned, was purchased from Strem Chemicals, Newburyport, MA. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Methylene chloride and hexamethylphosphoramide (HMPA, highly toxic!) were distilled from calcium hydride.

Previously Described Intermediates: (3*Z*,6*Z*)-Nonadienyltriphenylphosphonium bromide (**5a**) was prepared according to a procedure of Wong et al. (*12*) with the following minor deviations. 3-Butyn-1-ol was tetrahydropyranylated in the presence of pyridinium *p*-toluenesulfonate (*13*) instead of hydrochloric acid. The resulting product was isolated by distillation (bp 50 °C/3 mmHg) in 75% yield. The tosylate of 2-pentyn-1-ol was prepared using tosyl chloride and powdered KOH in ether (*14*). The crude tosylate was used in the alkylation step without further purification. Lindlar semihydrogenation of 3,6-nonadiyn-1-ol was conducted in 94% yield in the presence of quinoline and cyclohexene in methanol (*15*) instead of using quinoline in ethanol (*12*). (3*Z*,6*Z*)-Nonadiene-1-ol of 95% purity was further purified by argentation chromatography (*16*) using 15% $AgNO_3$ on SiO_2 (hexanes/ethyl acetate/methanol, 5:1:1 to 5:3:1) to give a product of 99% purity. Phosphonium salt **5a** was prepared from (3*Z*,6*Z*)-1-bromononadiene (1.0 equiv) and triphenylphosphine (1.2 equiv) by refluxing in acetonitrile for 40 h. After evaporation of the solvent, the residue was washed with anhydrous ether, and the oily salt **5a** was used in the Wittig reaction without further purification. (3*Z*,6*Z*)-Nonadienyltriphenylphosphonium tosylate (**5b**) was prepared from (3*Z*,6*Z*)-nonadiene-1-ol by converting it to the tosylate and reacting with triphenylphosphine in refluxed acetonitrile for 13 h as described (*17*). After flash chromatography (CH_2Cl_2/MeOH, 10:1), the viscous salt **5b** was used in the olefination step without crystallization. 6-Heptyn-1-ol was prepared from 3-heptyn-1-ol (GFS Chemicals, Powell, OH) using a "zipper reaction" in the presence of lithium salt of 1,3-diaminopropane and potassium *tert*-butoxide (*18*). Tetrahydropyranyl protection of 6-bromo-1-hexanol was achieved in the presence of pyridinium *p*-toluenesulfonate (*13*).

13-(Tetrahydo-2 *H*-pyran-2-yloxy)-6-tridecyn-1-ol (2). A solution of methyl lithium in ether (0.176 mol; 110 mL of 1.6 M) was slowly added under N_2 atmosphere to a mechanically stirred solution of 6-heptyn-1-ol (9.88 g, 0.088 mol) in dry THF (90 mL) at ~5 °C. The suspension was stirred for about 20 min and cooled to −40 °C before dry hexamethylphosphoramide (225 mL) was added. 2-(6-Bromohexyloxy)tetrahydro-2*H*-pyran (21.83 g, 0.082 mol) was added, and the mixture was slowly warmed to ~10 °C within 1.5 h. The mixture was poured into ice–water and extracted with hexanes/ether (1:1), and the combined organic extract was washed with a solution of NH_4Cl and dried with Na_2SO_4. Evaporation of the solvent and subsequent distillation afforded **2** (16.18 g, 67%): bp 170 °C/0.04 mmHg; ^{1}H NMR δ 1.00–1.90 (m, 20 H), 2.13 (m, H-5, H-8), 3.31–3.90 (m, 6H), 4.56 (m,1H); ^{13}C NMR δ 18.6, 18.7, 19.7, 25.0, 25.5, 25.8, 28.6, 28.9, 29.0. 29.6, 30.8, 32.3; 62.3, 62.8, 67.6, 79.9, 80.4, 98.8. Anal. Calcd for $C_{18}H_{32}O_3$: C, 72.91; H, 10.90. Found: C, 72.48; H, 10.84.

(6*Z*)-13-(Tetrahydo-2*H*-pyran-2-yloxy)tridecen-1-ol (3). Alcohol **2** (15.68 g, 53 mmol) in hexanes (200 mL) was agitated in the presence of Lindlar catalyst (1.57 g) in H_2 atmosphere for ~4 h or until GC analysis showed no starting material present. The catalyst was removed by filtration through Celite 521, and the filtrate was concentrated to furnish **3** (15.37 g) of 94% purity. A part of this product (1.95 g) was chromatographed on 20% $AgNO_3$−SiO_2 with hexanes/ethyl acetate, 1:1, to give alcohol **3** (1.16 g) of >99% purity: ^{1}H NMR δ 1.00–1.90 (m, 20 H), 2.01 (m, H-5, H-8), 3.31–3.53 (m, 2H), 3.61 (t, H-1, J = 6.5 Hz), 3.66–3.90 (m, 2H), 4.56 (m, 1H), 5.33 (m, H-6, H-7); ^{13}C NMR δ 19.7, 25.4, 25.5, 26.1, 27.1 (two carbons), 29.1, 29.5, 29.6, 29.7, 30.8, 32.7; 62.3, 62.9, 67.6, 98.8, 129.6, 130.0. Anal. Calcd for $C_{18}H_{34}O_3$: C, 72.42; H, 11.50. Found: C, 72.11; H, 11.38.

(6*Z*)-13-(Tetrahydo-2*H*-pyran-2-yloxy)tridecenal (4). Alcohol **3** (1.75 g, 5.87 mmol) was oxidized with pyridinium chlorochromate (PCC; 2.53 g, 11.72 mmol) in the presence of anhydrous sodium acetate (1.59 g) in dry CH_2Cl_2 at 0−25 °C using a procedure of Corey and Suggs (*19*). Conventional workup followed by subsequent flash chromatography afforded aldehyde **4** (1.19 g, 68%): ^{1}H NMR δ 1.00–1.90 (m, 18H), 2.05 (m, H-5, H-8), 2.40 (m, 2H), 3.40 (m, 1H), 3.52 (m, 1H), 3.73 (m, 1H), 3.87 (m, 1H), 4.60 (m, H-2′), 5.35 (m, H-6, H-7), 9.78 (m, H-1). Anal. Calcd for $C_{18}H_{32}O_3$: C, 72.91; H, 10.90. Found: C, 72.64; H, 10.79.

(7*Z*,13*Z*,16*Z*,19*Z*)-Docosatetraenyl Isobutyrate (1). Sodium bis(trimethylsilyl)amide (4.1 mL, 1.0 M in THF, 4.1 mmol) was added slowly under N_2 atmosphere to a stirred suspension of **5b** (2.45 g, 4.4 mmol) in dry THF (60 mL) at −70 °C. The mixture was stirred at −75 °C for 3 h, and then a solution of aldehyde **4** (580 mg, 2.0 mmol) in THF (3 mL) was introduced dropwise. The resulting mixture was stirred at this temperature for 1 h, slowly warmed to 20 °C within 3 h, and then quenched with a saturated NH_4Cl solution. The mixture was extracted with hexanes/ether, 3:1, and the combined organic extracts were dried (Na_2SO_4), concentrated, and flash chromatographed with hexanes/ethyl acetate, 25:1, to provide the Wittig condensation product (680 mg). This was deprotected by refluxing for 4 h with pyridinium tosylate (PPTS, 10 mg) in methanol (15 mL). After evaporation of methanol, the resulting alcohol was taken into ether/hexanes, 1:1, and the solution was washed with water, dried with Na_2SO_4, and concentrated. The crude alcohol was acylated with isobutyryl chloride (266 μL) and pyridine (210 μL) in dry toluene (15 mL) at 0−25 °C for 2 h. Water was added, and the mixture was extracted with hexanes/ether, 1:1, and the organic extract was washed with 5% HCl and water and then dried with Na_2SO_4. Evaporation of the solvent and flash chromatography (hexanes/ethyl acetate, 40:1) provided ester **1** (625 mg, 81%): ^{1}H NMR δ 0.97 (t, 3H, J = 7.5 Hz), 1.15 (d, 6H, J = 6.8 Hz), 1.35 (m, 10H), 1.62 (m, 2H, H-2), 1.90–2.20 (m, 8H), 2.53 (qq, J = 6.8, 7.2 Hz, 1H), 2.80 (dd, J ~ 5.7 Hz, H-15, H-18), 4.05 (t, J = 6.9 Hz, H-1), 5.36 (m, 8H); ^{13}C NMR δ 14.25, 19.00 (two carbons), 20.54, 25.53, 25.63, 25.84, 27.11 (two carbons), 27.14, 28.63, 28.88, 29.27, 29.37, 29.60, 34.05, 64.35, 127.11, 127.77, 128.24, 128.30, 129.78, 129.84, 130.19, 131.96, 177.27; GC-MS (EI), m/z (%) 388 (M^+, 2), 359 (1), 345 (2), 319 (3), 306 (2), 231 (2), 217 (6), 203 (6), 189 (4), 175 (5), 161 (10), 147 (14), 135 (22), 121 (32), 108 (46), 95 (67), 79 (100), 71 (40), 67 (91), 55 (56). Mass spectral data matched those of the natural pheromone (*11*). Ester **1** was 92% pure by GC on HP-5 and SPB-1 columns. The main impurity (4%) that eluted after the pheromone peak had a mass spectrum nearly identical to that of **1**. Two other impurities (total >3%) also had mass spectral profiles similar to that of ester **1**.

The reaction was also conducted with 4.0 mmol of aldehyde **4** and 10.0 mmol of salt **5a** with other components increased proportionally. The yield of olefination was 90%, and the GC purity of ester **1** was 88%. The amount of the main impurity that eluted after the pheromone peak was 7%; two other impurities having mass spectra similar to that of ester **1** amounted to ~2%, and unidentified byproducts totaled 3%. A part of this material (200 mg) was further purified by flash

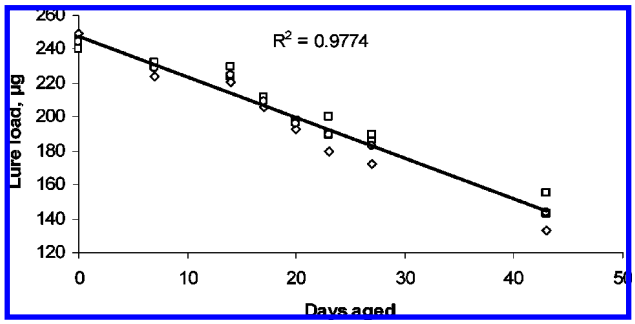


Figure 1. Residual amounts of browntail moth pheromone **1** (93% isomeric purity) on rubber septa exposed to field conditions (initial loading, 250 μg/lure; three replicates).

chromatography on 15% $AgNO_3-SiO_2$ with hexanes/ethyl acetate, 10:1 to 1:2, to provide pheromone **1** (102 mg) of 94% chemical and isomeric purities.

Pheromone Dispensers and Release Rates. Hexane solutions of **1** (10 mg/mL) containing 1.0% butylated hydroxytoluene (BHT) and 1.0% Tinuvin 328 UV light absorber (Ciba Specialty Chemicals, Basel, Switzerland) were applied via a syringe into caps of gray (halobutyl) rubber septa (The West Company, Kearney, NE) washed with acetone/hexane, 1:1, in a Soxhlet extractor prior to use. Poly(vinyl chloride) (PVC)-coated string dispensers were prepared from 94% pheromone batch stabilized with 1.0% BHT and 1.0% Tinuvin 328 as described by Leonhardt et al. (*20*). The loading of the pheromone was 73 μg/cm.

During the 1995 field test, the release of the pheromone from rubber septa was measured by residual analyses of dispensers (250 μg, 93% isomerically pure) that were aged outdoors in Pherocon 1C traps (Trécé Inc., Adair, OK) at the Otis laboratory on Cape Cod. After aging for a specified time (**Figure 1**) three septa per age were individually Soxhlet-extracted with 15 mL of hexane, and the solutions were quantitatively analyzed by GC in a splitless mode using external standards. Aging of septa was concurrent with the field bioassay.

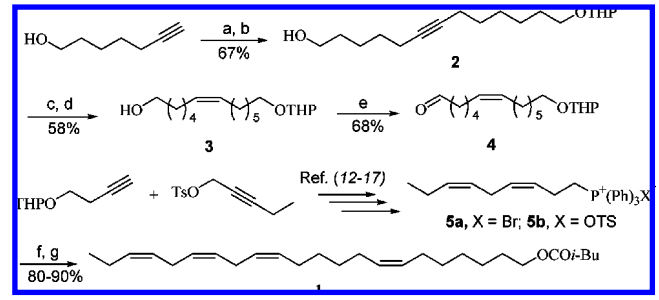
Field Bioassay. In 1995, we tested pheromone of 93% isomeric purity on rubber septa in four doses, 25, 50, 100, and 250 μg/septum. The test was conducted in the Pilgrim Heights area of Truro, MA, within the boundaries of the Cape Cod National Seashore, from July 17 to August 1. Pherocon 1C sticky traps were assembled with spacer tubes (provided) in place, resulting in a 1.5−2 cm gap between the top and bottom sections of the trap. Septa were attached to the underside of the top of the trap. Traps were hung from metal stakes at ~1 m above the ground with a minimum intertrap spacing of 40 m. The test design was a randomized complete block with five replicates. Captured moths were counted 10 times (every 1–2 days) during the test; at each check, positions of bait treatments within each block were rerandomized.

In 2005, the field test was again conducted in Pilgrim Heights using methods similar to those described above. Traps were checked and rerandomized within each of the five blocks six times (every 2–3 days) from July 15 to July 29. Lures included rubber septa and string formulations loaded with pheromone **1** from a batch of 94% isomeric purity. Treatments on rubber septa contained 0, 25, 100, 250, and 1000 μg/lure; those on string dispensers contained 250 and 1000 μg/lure. Septa and string lures containing 1000 μg were also aged outdoors in white cardboard delta traps for 2 or 4 weeks immediately prior to the field trial. In addition, we tested rubber septa that contained 250 and 1000 μg of pheromone **1** from a second batch of 91% isomeric purity.

RESULTS AND DISCUSSION

Synthesis. The key step in the assembly of a skipped triene moiety of the pheromone **1** (**Scheme 1**) was the Wittig olefination of the unsaturated aldehyde **4** with a phosphorane generated from the salt **5**. Both bromide **5a** (*12*) and tosylate **5b** (*17*) have previously been described and used in similar olefinations. The unsaturated aldehyde **4** was prepared using a conventional acetylenic chemistry, particularly employing an efficient alkylation of lithium acetylides in THF/HMPA (*21*).

Scheme 1. Synthesis of (7*Z*,13*Z*,16*Z*,19*Z*)-Docosatetraenyl Isobutyrate Sex Pheromone of Browntail Moth, *Euproctis chrysorrhea* (L.)[a]



[a] Reagent and conditions: (a) 2MeLi/ether-THF; (b) $Br(CH_2)_6OTHP$/HMPA; (c) H_2/Pd-$CaCO_3$; (d) $AgNO_3-SiO_2$; (e) PCC/AcONa; (f) 1, $[(CH_3)_3Si]_2NNa$/THF, −70 °C; 2, **4**, −75 °C; (g) 1, PPTS/MeOH; 2, $(CH_3)_2CHCOCl$/py.

We purified the intermediates in early stages of the synthesis by argentation chromatography (*16*) to ensure 99% geometric purity of both olefination components. Sodium bis(trimethylsilyl)amide (*22*) was used as a base to give 90% yield of the Wittig olefination from bromide **5a**. On the basis of GC and GC-MS data, as well as the literature on the stereoselectivity of the Witting reaction (*22*), we concluded that the main impurity (7%) that eluted after the pheromone peak on both GC columns was the 13*E* isomer of **1**. Two additional impurities (total 2%) with comparable retention times and mass spectra were apparently other isomers of **1**. Thus, the chemical purity of the pheromone was 88% and the isomeric purity 91%. This product was further purified by argentation chromatography to furnish a pheromone of 94% chemical and isomeric purities (containing 4% of 13*E* isomer). From the tosylate **5b** we obtained the final product of 92% chemical and 93% isomeric purity in 81% yield. Thus, both bromide **5a** and tosylate **5b** offer high yields and comparable stereochemical outcomes in the olefination step. However, the tosylate route may seem advantageous because the starting salt is easier to prepare (13 vs 40 h of reaction time in the case of bromide).

Field Bioassay. In both years' field trials, numbers of male *E. chrysorrhea* captured in sticky traps increased with increasing doses of pheromone **1** on the lures. In 1995, the 25 μg/septum dosage captured significantly fewer moths than did any of the higher dosages, and traps baited with 50 μg septa caught significantly fewer than those baited with 250 μg. Capture with 100 μg septa was intermediate to, but not significantly different from, capture with 50 and 250 μg septa. Leonhardt et al. (*11*) reported that traps baited with 5–50 μg of the synthetic pheromone **1** dispensed from rubber septa gave male moth captures that were comparable to those of traps baited with three virgin females. However, their 250 μg/septum dose was not significantly different from a 25 μg dose in the same field trial (*11*). We measured the release rate of the pheromone from 250 μg dispensers under field conditions (**Figure 1**) and found a good linear regression with an average release of ~2.4 μg/day that represents both emitted pheromone and possible decomposition products. About 59% of material remained after 43 days of exposure, thus indicating that this loading may persist during entire *E. chrysorrhea* flight season, which lasts several weeks.

In 2005, in addition to rubber septa, we incorporated in our field test string formulations that were successfully used in field trapping of the gypsy moth, *Lymantria dispar* (*20*), and the pink gypsy moth, *Lymantria mathura* (*15*). We also conducted a dose–response experiment, as well as an aging test and a pheromone purity trial. The results of the dose–response field test presented in **Table 2** were consistent with those obtained

Table 1. Capture of Male Browntail Moths in Traps Baited with Different Amounts of (7*Z*,13*Z*,16*Z*,19*Z*)-Docosatetraenyl Isobutyrate (**1**, 93% Isomeric Purity) on Rubber Septa (Cape Cod National Seashore, from July 17 to August 1, 1995)

attractant per septum (μg)	total male moths per trap[a] ($\bar{x} \pm$ SE)
25	83 ± 10 a
50	127 ± 21 b
100	164 ± 35 bc
250	200 ± 37 c

[a] Means followed by the same letter are not significantly different as determined by Tukey's HSD test ($\alpha = 0.05$). Traps were checked daily, but data for each of six traps per treatment were summed across days and transformed to $\ln(n + 1)$ prior to ANOVA ($F = 15.9$; df = 3, 15; $P < 0.0001$).

Table 2. Capture of Male Browntail Moths in Traps Baited with Different Amounts of (7*Z*,13*Z*,16*Z*,19*Z*)-Docosatetraenyl Isobutyrate (**1**, 94% Isomeric Purity) on Rubber Septa (Pilgrim Heights Area of Truro, MA, Cape Cod National Seashore Park, July 2005)

attractant per septum (μg)	male moths per trap per week[a] ($\bar{x} \pm$ SE)
0	0.4 ± 0.2
25	7.1 ± 1.4 a
100	16.7 ± 2.4 b
250	29.8 ± 4.2 bc
1000	36.2 ± 5.1 c

[a] Means that are not followed by the same letter are significantly different as determined by Tukey's HSD test ($\alpha = 0.05$). Data for each trap were summed within each of 2 weeks and transformed to $\ln(n + 1)$ prior to ANOVA ($F = 13.96$; df = 3, 31; $P < 0.0001$; 4 treatments, 5 blocks, 2 weeks). Data for 0 μg lures were not included in the analysis. Interactions between main effects were not significant and were not included in the final model.

Table 3. Capture of Male Browntail Moths in Traps Baited with (7*Z*,13*Z*,16*Z*,19*Z*)-Docosatetraenyl Isobutyrate of 91 and 94% Isomeric Purities on Rubber Septa (Pilgrim Heights Area of Truro, MA, Cape Cod National Seashore Park, July 2005)

attractant per septum (μg)	isomeric purity (%)	male moths per trap per week[a] ($\bar{x} \pm$ SE)
250	94	29.8 ± 4.2
1000	94	36.2 ± 5.1
250	91	25.6 ± 4.5
1000	91	41.9 ± 7.0

[a] Data for each trap were summed within each of 2 weeks and transformed to $\ln(n + 1)$ prior to ANOVA. Dose and purity were treated as separate effects (2 levels each; 5 blocks, 2 weeks). Dose–effects were significant ($F = 4.57$; df = 1, 32; $P = 0.040$); purity was not ($F = 0.002$; df = 1, 32; $P = 0.97$). Interactions between main effects were not significant and were not included in the final model.

in the 1995 (**Table 1**). The 100 and 250 μg treatments were significantly better than a 25 μg dose but not significantly different from each other. The highest dose, 1000 μg/lure, did not capture significantly higher numbers of male *E. chrysorrhea* than the 250 μg treatment. At any rate, the 250 μg/septum dose was both efficient and economical from the two field trials. The performances of traps baited with pheromones of 91 and 94% isomeric purities at two doses were not significantly different (**Table 3**) thus rendering unnecessary a costly argentation chromatography in the last step.

The results of aging and dispenser type studies are presented in **Table 4**. It is noteworthy that all four treatments on rubber septa in both week 1 and week 2 trappings were not significantly different from each other. Thus, aging of the 1000 μg lure for 4 weeks under field conditions before the trial did not affect the lure attractiveness. In contrast, a string dispenser with the 1000 μg pheromone loading completely lost its attractiveness

Table 4. Capture of Male Browntail Moths in Traps Baited with 250 or 1000 μg of (7*Z*,13*Z*,16*Z*,19*Z*)-Docosatetraenyl Isobutyrate (**1**, 94% Isomeric Purity) on Rubber Septa or PVC-Plasticized String Formulations, Aged 0, 2, or 4 Weeks (Pilgrim Heights Area of Truro, MA, Cape Cod National Seashore Park, July 2005)

			male moths per trap per week ($\bar{x} \pm$ SE)[a]	
dispenser	loading (μg)	age (weeks)	week 1	week 2
septum	250	0	19.4 ± 3.6 b	40.2 ± 3.7 cd
	1000	0	29.2 ± 7.0 b	43.2 ± 6.5 cd
	1000	2	27.0 ± 5.1 b	60.4 ± 8.2 d
	1000	4	24.0 ± 3.4 b	49.0 ± 8.5 d
string	250	0	19.0 ± 3.6 b	11.2 ± 7.5 b
	1000	0	18.6 ± 3.6 b	14.8 ± 5.1 bc
	1000	2	13.2 ± 2.5 b	0.4 ± 0.2 a
	1000	4	1.2 ± 0.6 a	0.0 ± 0.0 a

[a] Within a column, means that are not followed by the same letter are significantly different as determined by Tukey's HSD test ($\alpha = 0.05$). Data for each trap were summed within each of 2 weeks and transformed to $\ln(n + 1)$ prior to ANOVA. In initial analyses, there was a significant interaction between week and treatment ($F = 9.56$; df = 7, 28; $P < 0.0001$), so data for each week were analyzed individually. The treatment effect (incorporating dispenser, loading, and aging) was highly significant for both week 1 ($F = 13.45$; df = 7, 28; $P < 0.0001$) and week 2 ($F = 32.5$; df = 7, 28; $P < 0.0001$).

after 4 weeks of aging (**Table 4**, last row), and the string dispenser with the same loading aged for 2 weeks retained the attractiveness only in the week 1 test but was entirely inactive in week 2. Similarly, a fresh 250 μg string dispenser was as active as rubber septa formulations in week 1 but caught a significantly fewer number of moths in week 2. We did not measure release rates in 2005, but it seems that the string dispensers (unlike rubber septa) became depleted of pheromone lure in 2−3 weeks under field condition.

In summary, we presented the first synthesis of the sex pheromone of the browntail moth, *E. chrysorrhea*, (7*Z*,13*Z*,-16*Z*,19*Z*)-docosatetraenyl isobutyrate. Rubber septa formulations of the pheromone of 91–94% isomeric purity (presumably containing 4–7% of the 13*E* isomer) can be efficiently used for monitoring the moth populations during the entire flight season.

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