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4 A TETRAENE ALDEHYDE AS THE MAJOR SEX PHEROMONE COMPONENT OF  
5 THE PROMETHEA MOTH (*Callosamia promethea* (Drury))

6

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4 21 **ABSTRACT** The promethea moth *Callosamia promethea* is one of three species of  
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6 22 silkmoths from the genus *Callosamia* that occur in North America. Cross attraction of  
7  
8 23 males to heterospecific calling females has been observed in the field and hybrid progeny  
9  
10 24 have been produced by pairing heterospecifics in captivity. These observations suggest  
11  
12 25 that all three species share or have considerable overlap in the sex attractant pheromones  
13  
14 26 produced by females, so that other prezygotic isolating mechanisms such as diel  
15  
16 27 differences in reproductive activity limit hybridization in the field. Coupled gas  
17  
18 28 chromatography-electroantennogram detection and gas chromatography mass-  
19  
20 29 spectrometry analyses of extracts of volatiles collected from female promethea moths  
21  
22 30 supported the identification of (4*E*,6*E*,11*Z*,13*Z*)-hexadeca-4,6,11,13-tetraenal  
23  
24 31 (4*E*,6*E*,11*Z*,13*Z*)-16:Ald as the compound in extracts that elicited the largest responses  
25  
26 32 from antennae of males. The identification was confirmed by non-selective synthesis of  
27  
28 33 several isomers as analytical standards, and stereoselective synthesis of (4*E*,6*E*,11*Z*,13*Z*)-  
29  
30 34 16:Ald for testing in field trials. Male moths were strongly attracted to synthetic  
31  
32 35 (4*E*,6*E*,11*Z*,13*Z*)-16:Ald, suggesting that this compound is the major and possibly the  
33  
34 36 only component of the sex pheromone of these large saturniid moths. Based on the cross-  
35  
36 37 attraction of heterospecifics, it is likely that this is also a major pheromone component of  
37  
38 38 the other two North American *Callosamia* species as well.  
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50 40 **KEY WORDS** *Callosamia promethea*, Saturniidae, (4*E*,6*E*,11*Z*,13*Z*)-hexadeca-  
51  
52 41 4,6,11,13-tetraenal, Sex pheromone  
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## INTRODUCTION

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45 Giant silk moths (Lepidoptera: Saturniidae) are among the largest and most  
46 attractive moths in North America. As a result, they are prized by collectors and their life  
47 histories are well known (Collins and Weast 1961; Tuskes et al. 1996). Populations of  
48 many saturniid moths are reported to have declined in the northeastern United States and  
49 several appear on state endangered species lists (Schweitzer 1988; Tuskes et al. 1996;  
50 Boettner et al. 2000). Hypotheses for the reported declines include habitat loss,  
51 disruption of mating by street lights, non-target effects of insecticides, and parasitization  
52 by the generalist parasitoid fly *Compsilura concinnata* (Schweitzer 1988; Holden 1992;  
53 Johnson et al. 1995; Tuskes et al. 1996; Boettner et al. 2000).

54 The promethea moth, *Callosamia promethea* (Drury) is one of the three species of  
55 silkmoths in the genus *Callosamia* that occur in North America (Ferguson 1972). It is the  
56 most widely distributed of the three species, occurring from Canada south to Florida and  
57 eastern Texas. In parts of its range it is sympatric and seasonally synchronic with either  
58 or both of the congeners *Callosamia angulifera* (Walker) and *Callosamia securifera*  
59 (Maassen). Although cross-attraction of male *C. angulifera* to female *C. promethea* has  
60 been observed (Skinner 1914; Toliver et al. 1979) and all of the possible male-female  
61 crosses (except ♂ *C. promethea* and ♀ *C. angulifera*) among the three species have been  
62 made in captivity (Haskins and Haskins 1958; Remington 1968; Peigler 1977, 1980),  
63 hybrids reportedly do not occur in the wild (see Peigler 1977). Differences in the diel  
64 patterns of reproductive behaviour among the three species are hypothesized to be a  
65 primary mechanism ensuring reproductive isolation in nature given that at least some

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4 66 cross-attraction has been documented. Specifically, *C. securifera* is reported to be active  
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7 67 from ~10:00-13:30 (Brown 1972, referred to as *C. carolina*), whereas *C. promethea* is  
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9 68 active from ~15:30-18:30 (Rau and Rau 1929), and *C. angulifera* is active from 19:30-  
10  
11 69 24:00 (Collins and Weast 1961).

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14 70 Because of their crucial role in mate-location and recognition, often over long  
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16 71 distances, lepidopteran sex pheromones are powerful, species-specific attractants (El-  
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18  
19 72 Sayed 2012). These characteristics make them valuable tools for sampling rare species  
20  
21 73 and low-density populations. For example, a recent study demonstrated that pheromone  
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23  
24 74 lures were an excellent tool for detecting and surveying populations of an iconic and  
25  
26 75 protected saturniid species native to Europe, the Spanish moon moth *Graellsia isabellae*  
27  
28 76 (Graells) (Millar et al. 2010). With that study as proof of concept for using pheromones  
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30  
31 77 for sampling potentially threatened lepidopteran species, the objective of the work  
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34 78 described here was to identify, synthesize, and bioassay the female-produced sex  
35  
36 79 pheromone of the promethea moth. Given the documented cross-attraction between at  
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38 80 least two of the three North American species, it is likely that the major component(s) of  
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41 81 the sex pheromone will be shared by all three species.

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## 44 45 83 METHODS AND MATERIALS

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50 85 *Insects* Pupae of *C. promethea* were collected by Craig Mitzell from Marshall, Starke,  
51  
52 86 Fulton, and Pulaski counties in northern Indiana in the winter of 2010 and shipped to the  
53  
54  
55 87 quarantine facility at the University of California, Riverside (USDA-APHIS permit #  
56  
57  
58 88 P526P-08-02964 ). Pupae were sorted by sex and placed on paper towelling inside  
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4 89 plastic boxes ( $40 \times 27 \times 16$  cm) with loose fitting lids. Paper towelling was hung from  
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6  
7 90 the sides of the inside of the boxes, and additional towelling was draped across the top to  
8  
9 91 allow emerging adults to hang upside down during wing expansion. Humidity was  
10  
11 92 provided with an open 473 ml jar of deionized water and additional moisture was added  
12  
13 93 periodically by spraying the cocoons with water. The boxes were held next to a window  
14  
15 94 to provide natural light, augmented with two 32 watt fluorescent lights on a 14:10 L:D  
16  
17 95 cycle. Room temperature was  $22^{\circ}\text{C}$  and room humidity was not controlled. Emerged  
18  
19 96 males were placed in glassine envelopes inside a plastic bag with a damp piece of  
20  
21 97 towelling in a refrigerator for storage. For all male moths used for electroantennogram  
22  
23 98 analyses, the genitalia were removed prior to removal from the quarantine facility.  
24  
25 99 Emerged females were used immediately for pheromone collection as described below,  
26  
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28  
29 100 or were refrigerated for up to 3 d in a plastic bag with a damp paper towel to accumulate  
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31  
32 101 a number of females for analysis. Chilled females were removed from refrigeration in the  
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34 102 morning of the day of use and sampled in the late afternoon (16:00 to 17:00 h). Single  
35  
36 103 adult females to be used for pheromone collections were placed in screen cages  
37  
38  
39 104 constructed of 6.3 mm mesh hardware cloth (ca. 15 cm dia.  $\times$  14 cm high) and then  
40  
41 105 placed inside a wooden double-sleeve rearing box (76 cm long  $\times$  43 cm deep  $\times$  33 cm  
42  
43 106 high in front to 50 cm high in back). The glass top of the rearing box allowed visual  
44  
45 107 confirmation of extrusion of the ovipositor, which indicated calling. Calling behavior  
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47  
48 108 was observed late in the photophase, approximately 11 h after sunrise, and only calling  
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50 109 females were used for pheromone collections.  
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58 111 *Solid Phase Micro-Extraction (SPME) of Sex Pheromone Glands* SPME collections from  
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4 112 calling females were made using similar methods to those used for *Graellsia isabellae*  
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6 113 (Millar et al. 2010). Calling females were grasped firmly by the abdomen with gentle  
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8  
9 114 pressure towards the tip of the abdomen to evert the sex pheromone gland, the scale-free  
10  
11 115 tissue just anterior to the ovipositor. The exposed gland was wiped with the SPME fiber,  
12  
13  
14 116 with all surfaces being wiped at least twice. SPME collections were made using 100  $\mu\text{m}$   
15  
16 117 polydimethylsiloxane fibers (Supelco, Bellefonte, PA). Approximately 5-7 mm of the  
17  
18 118 SPME fiber was exposed and the fiber holder was fastened to the lab bench so that both  
19  
20  
21 119 hands could be used to manipulate the insect. The loaded SPME fiber was immediately  
22  
23  
24 120 analyzed by coupled gas chromatography-electroantennogram detection (GC/EAD), or by  
25  
26 121 coupled gas chromatography-mass spectrometry (GC/MS).  
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31 123 *Solvent Extraction of Sex Pheromone Glands* Single female extracts were made from 3  
32  
33 124 calling females after their pheromone glands had been wiped with an SPME fiber. Sex  
34  
35 125 pheromone glands were removed by forcing eversion of the gland, then clamping the  
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37  
38 126 abdomen just anterior to the gland using forceps to maintain the pressure in the gland,  
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40  
41 127 then slicing the gland off with a razor blade such that the gland remained inflated in the  
42  
43 128 forceps. The gland was then soaked for 10 min in ca 50  $\mu\text{l}$  of clean pentane, taking care  
44  
45 129 not to submerge the cut end of the tissue. Three single female extracts were analyzed by  
46  
47  
48 130 coupled GC/EAD in their dilute form and subsequently combined and concentrated for  
49  
50  
51 131 GC/MS analysis.  
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55 133 *Gas Chromatography-Electroantennogram (GC-EAD) and Gas Chromatography-Mass*  
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58 134 *Spectrometry Analyses* SPME wipe samples and solvent extracts of pheromone glands  
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4 135 were analyzed first by GC-EAD. Details of the instrumentation and methods for making  
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6 136 antennal preparations have been previously described in detail (Millar et al. 2010). GC-  
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9 137 EAD analyses were conducted on a DB-5 column (30 m × 0.25 mm ID, 0.25 micron film;  
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11 138 J&W Scientific, Folsom, CA, USA) programmed from 100°C/1 min then 10°C/min to  
12  
13  
14 139 275°C for 40 min. Retention indices were calculated relative to straight chain alkanes.  
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16 140 Analyses were repeated on a DB-Wax column (30 m × 0.25 mm ID, 0.25 micron film;  
17  
18  
19 141 J&W Scientific) programmed from 100°C/1 min then 10°C per min to 250°C for 60 min.  
20  
21 142 SPME wipe samples were analyzed on an Agilent 6890N GC equipped with an HP5-MS  
22  
23 143 column (30 m × 0.25 mm ID, 0.25 micron film) and coupled to a 5975C mass selective  
24  
25  
26 144 detector (Agilent, Santa Clara, CA, USA). Analyses were run in splitless mode, with He  
27  
28  
29 145 as carrier gas, temperature programming from 40°C/1 min, 10°C/min to 280°C and hold  
30  
31 146 20 min. The injector temperature was 250°C, and loaded fibers were desorbed for 30 sec.  
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34 147  
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36 148 *Synthesis of pheromone candidates* All solvents were dried and distilled according to  
37  
38 149 standard procedures (PureSolv-EN™ Innovative Technology, Inc.). Commercially  
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41 150 available starting materials were purchased from Sigma-Aldrich Química (Madrid,  
42  
43 151 Spain). Reactions involving air- or moisture-sensitive materials were carried out under  
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45  
46 152 Ar. Unless otherwise specified, solutions of crude products were dried over anhydrous  
47  
48 153 Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. Purification by flash or vacuum flash  
49  
50  
51 154 chromatography was carried out on silica gel 60A (230-400 mesh). IR spectra were  
52  
53 155 recorded on a Nicolet Avatar 360 FT-IR spectrometer (Thermo Electron Inc., Madison,  
54  
55 156 Wisconsin, USA). NMR spectra were recorded at 400 and 500 MHz for <sup>1</sup>H and 100 and  
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57  
58 157 125 MHz for <sup>13</sup>C on a Varian Mercury 400 and Inova 500 spectrometer (Varian Inc., Palo  
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4 158 Alto, CA, USA), respectively. GC analyses were determined on a Trace<sup>TM</sup> GC 2000  
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6 159 Thermo Finnigan gas chromatograph equipped with a HP-5 column (30 m × 0.25 mm ID  
7  
8  
9 160 × 0.25 μm film) (Agilent Technologies, Santa Clara, CA, USA). Low resolution mass  
10  
11 161 spectra (MS) were obtained on a Fisons MD 800 instrument (Thermo Fisher Scientific,  
12  
13 162 Waltham, Massachusetts, USA). High resolution mass spectra (HRMS) were recorded on  
14  
15  
16 163 an UPLC Acquity (Waters) coupled to a LCT Premier XE (Waters) spectrometer.

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19 164 Non-stereoselective syntheses of (4*E*,6*E*,11*Z*/*E*,13*Z*)-hexadeca-4,6,11,13-  
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21 165 tetraenals are shown in Figure 1, and described in detail in the online supplement.

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26 167 *Synthesis of (4E,6E,11Z,13E)-hexadeca-4,6,11,13-tetraenal.* (Figure 2)

27  
28 168 *1-Bromo-5-chloropent-1-yne (2).* *N*-bromosuccinimide (6.25 g, 34.75 mmol) was added  
29  
30 169 in portions to a solution of 5-chloropent-1-yne (**1**) (3.24 g, 31.59 mmol) in acetone (95  
31  
32 170 ml). The mixture was stirred at room temperature until complete solution and then silver  
33  
34 171 nitrate (0.27 g, 1.58 mmol) was added. The mixture was protected from light, stirred at  
35  
36 172 room temperature for 30 min and quenched by pouring into water (150 ml). After  
37  
38 173 extraction with ether (3x100 ml), the combined organic layers were washed with brine,  
39  
40 174 dried and concentrated. The residue was purified by vacuum flash chromatography  
41  
42 175 eluting with hexane to yield 1-bromo-5-chloropent-1-yne (**2**) (5.05 g, 88%) as a yellow  
43  
44 176 oil. IR (film)  $\nu$ : 2958, 1436, 1288, 851  $\text{cm}^{-1}$ . <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  3.64 (t, *J* =  
45  
46 177 6.3 Hz, 2H), 2.42 (t, *J* = 6.8 Hz, 2H), 1.97 (tt, *J*<sub>1</sub> = *J*<sub>2</sub> = 6.6 Hz, 2H) ppm. <sup>13</sup>C NMR (100  
47  
48 178 MHz, CDCl<sub>3</sub>):  $\delta$  78.56 (CBr), 43.61 (ClCH<sub>2</sub>), 39.17 (C), 31.15 (CH<sub>2</sub>), 17.27 (CH<sub>2</sub>) ppm.  
49  
50 179 EIMS, *m/z* (%): 184 ([*M*+2]<sup>+</sup>, 35), 182 ([*M*+1]<sup>+</sup>, 69), 182 ([*M*]<sup>+</sup>, 61), 147 (41), 145 (45),  
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4 180 120 (44), 119 (56), 118 (51), 117 (58), 101 (51), 75 (30), 73 (43), 66 (52), 65 (100), 63  
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7 181 (48), 62 (33).  
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9 182  
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11 183 *9-Chloronona-3,5-diyne* (**3**). An oven-dried 3-necked flask with a dry ice condenser was  
12  
13  
14 184 charged under Ar atmosphere with CuCl (0.25 g, 2.48 mmol), NH<sub>2</sub>OH·HCl (1.38 g, 19.84  
15  
16 185 mmol) and anhydrous MeOH (140 ml). The mixture was stirred for 10 min until a  
17  
18  
19 186 homogenous solution was obtained, and then cooled to -78°C. An excess of 1-butyne (ca.  
20  
21 187 4 ml) was condensed into the mixture with the dry ice condenser, then *n*-propylamine  
22  
23 188 (3.85 ml, 46.84 mmol) was added followed by bromoalkyne **2** (5.00 g, 27.55 mmol) in  
24  
25 189 anhydrous MeOH (22 ml) via cannula over 10 min. The mixture was allowed to warm to  
26  
27  
28 190 room temperature, stirred for 2 h and quenched by pouring into water (170 ml). After  
29  
30  
31 191 filtering through a pad of Celite<sup>®</sup>, the aqueous layer was extracted with hexane (5x75 ml)  
32  
33 192 and the combined organic layers were washed with brine, dried and concentrated. The  
34  
35 193 residue was purified by vacuum flash chromatography eluting with hexane to obtain 9-  
36  
37 194 chloronona-3,5-diyne (**3**) (3.26 g, 77%) as a colorless oil. IR (film)  $\nu$ : 2915, 2879, 2840,  
38  
39 195 1455, 1429, 1315, 1290, 1063, 969, 845 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  3.64 (t, *J* =  
40  
41 196 6.3 Hz, 2H), 2.45 (t, *J* = 6.8 Hz, 2H), 2.26 (quart, *J* = 7.5 Hz, 2H), 1.97 (tt, *J*<sub>1</sub> = *J*<sub>2</sub> = 6.6  
42  
43 197 Hz, 2H), 1.15 (t, *J* = 7.5 Hz, 3H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  79.37 (C), 75.45  
44  
45 198 (C), 66.40 (C), 64.57 (C), 43.61 (ClCH<sub>2</sub>), 31.22 (CH<sub>2</sub>), 16.81 (CH<sub>2</sub>), 13.49 (CH<sub>2</sub>), 13.03  
46  
47 199 (CH<sub>3</sub>) ppm. EIMS, *m/z* (%): 157 ([M+2]<sup>+</sup>, 12), 156 ([M+1]<sup>+</sup>, 74), 155 ([M]<sup>+</sup>, 61), 154  
48  
49 200 (97), 126 (75), 119 (73), 117 (77), 115 (67), 105 (62), 104 (64), 103 (81), 92 (62), 91  
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51 201 (100), 89 (70), 79 (77), 78 (62), 77 (86), 75 (61), 65 (81), 63 (76), 51 (67).  
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4 203 (3Z,5Z)-9-Chloronona-3,5-diene (**4**). Cyclohexene (9.05 ml, 89.23 mmol) was added  
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6 204 dropwise to a solution of  $\text{BH}_3\cdot\text{SMe}_2$  (4.50 ml, 42.68 mmol) in THF (30 ml) at  $0^\circ\text{C}$ . After  
7  
8 205 the addition was complete, a further 14 ml of THF was added to the white suspension and  
9  
10 206 the mixture was stirred for 1 h at  $0^\circ\text{C}$ . Chlorodiene **3** (3.00 g, 19.40 mmol) in THF (10  
11  
12 207 ml) then was slowly added to the slurry, and the mixture was stirred and allowed to warm  
13  
14 208 to room temperature. After stirring for 5.5 h, glacial acetic acid (10 ml) was added, and  
15  
16 209 the solution was heated to  $55\text{-}60^\circ\text{C}$  for 5 h. The mixture then was cooled to room  
17  
18 210 temperature and 6M aqueous NaOH (33 ml) was added followed by careful addition of  
19  
20 211 35%  $\text{H}_2\text{O}_2$  (10 ml; caution: very exothermic!), keeping the temperature below  $40^\circ\text{C}$ .  
21  
22 212 After cooling to room temperature, the organic layer was decanted and the aqueous  
23  
24 213 residue was extracted with  $\text{Et}_2\text{O}$  (4x30 ml). The combined organic layers were dried,  
25  
26 214 concentrated, and purified by vacuum flash chromatography eluting with hexane to  
27  
28 215 provide (3Z,5Z)-9-chloronona-3,5-diene (**4**) (2.48 g, 81%) as a colorless oil. IR (film)  $\nu$ :  
29  
30 216 3037, 3003, 2963, 2931, 2871, 1446, 1306, 1067, 979, 866  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (400 MHz,  
31  
32 217  $\text{CDCl}_3$ ):  $\delta$  6.36-6.19 (m, 2H), 5.52-5.37 (m, 2H), 3.54 (t,  $J = 6.6$  Hz, 2H), 2.34 (tdd,  $J_1 =$   
33  
34 218  $J_2 = 7.4$ ,  $J_3 = 1.3$  Hz, 2H), 2.19 (ddq,  $J_1 = J_3 = 7.5$ ,  $J_2 = 1.5$  Hz, 2H), 1.87 (tt,  $J_1 = J_2 = 7.0$   
35  
36 219 Hz, 2H), 1.00 (t,  $J = 7.5$  Hz, 3H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  134.70 (CH),  
37  
38 220 129.56 (CH), 125.11 (CH), 122.76 (CH), 44.58 ( $\text{CH}_2$ ), 32.55 ( $\text{CH}_2$ ), 24.80 ( $\text{CH}_2$ ), 20.98  
39  
40 221 ( $\text{CH}_2$ ), 14.31 ( $\text{CH}_3$ ) ppm. EIMS,  $m/z$  (%): 160 ( $[\text{M}+1]^+$ , 30), 159 ( $[\text{M}]^+$ , 9), 158 (84), 116  
41  
42 222 (22), 109 (26), 96 (16), 95 (97), 93 (50), 91 (41), 82 (23), 81 (100), 80 (26), 79 (81), 77  
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44 223 (58), 68 (73), 67 (95), 65 (28), 55 (59), 53 (37), 51 (16).  
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4 225 (6Z,8Z)-Undeca-6,8-dien-1-yne (**5**). A mixture of lithium acetylide-ethylenediamine  
5  
6 226 complex (1.85 g, 20.03 mmol) and NaI (0.11 g, 0.72 mmol) in anhydrous DMSO (50 ml)  
7  
8  
9 227 was stirred in a dry flask under Ar for 5 min at room temperature, then a solution of diene  
10  
11 228 **4** (2.27 g, 14.31 mmol) in anhydrous DMSO (12 ml) was added dropwise. After 5 h, the  
12  
13  
14 229 solution was poured into water (200 ml) and extracted with pentane (5x50 ml). The  
15  
16 230 combined organic layers were dried, concentrated, and purified by flash chromatography  
17  
18  
19 231 eluting with pentane to give (6Z,8Z)-undeca-6,8-dien-1-yne (**5**) (1.35 g, 64%) as a pale  
20  
21 232 yellow oil. IR (film)  $\nu$ : 3305, 3036, 3004, 2965, 2935, 2870, 2118, 1598, 1456, 1068  $\text{cm}^{-1}$ .  
22  
23  
24 233 <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  6.33-6.20 (m, 2H), 5.50-5.39 (m, 2H), 2.30 (tdd,  $J_1 = J_2$   
25  
26 234 = 7.6,  $J_3 = 1.2$  Hz, 2H), 2.23-2.15 (m, 4H), 1.95 (t,  $J = 2.6$  Hz, 1H), 1.63 (tt,  $J_1 = J_2 = 7.2$   
27  
28 235 Hz, 2H), 1.00 (t,  $J = 7.5$  Hz, 3H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  134.34 (CH),  
29  
30 236 130.53 (CH), 124.67 (CH), 122.95 (CH), 84.49 (C), 68.55 (CH), 28.55 (CH<sub>2</sub>), 26.58  
31  
32 237 (CH<sub>2</sub>), 20.97 (CH<sub>2</sub>), 18.09 (CH<sub>2</sub>), 14.33 (CH<sub>3</sub>) ppm. EIMS,  $m/z$  (%): 148 ([M]<sup>+</sup>, 6), 133  
33  
34 238 (17), 120 (17), 119 (77), 117 (18), 108 (14), 106 (22), 105 (62), 95 (20), 93 (45), 92 (33),  
35  
36 239 91 (100), 81 (29), 80 (39), 79 (86), 78 (29), 77 (63), 68 (14), 67 (76), 66 (16), 65 (30), 55  
37  
38  
39 240 (48). HRMS: Calcd. for C<sub>11</sub>H<sub>17</sub>: 149.1330 [M+1]<sup>+</sup>; found: 149.1331.  
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44 241

45  
46 242 2-(1E,6Z,8Z)-Undeca-1,6,8-trienyl-1,3-dioxaborinane (**6**). A solution of 1M  
47  
48 243 HBBBr<sub>2</sub>·SMe<sub>2</sub> in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (3.84 ml, 3.84 mmol) was added dropwise to a flask  
49  
50 244 charged with (6Z,8Z)-undeca-6,8-dien-1-yne (**5**) (0.57 g, 3.84 mmol) in anhydrous  
51  
52 245 CH<sub>2</sub>Cl<sub>2</sub> (28 ml) at 0°C under Ar. After stirring for 15 min at 0°C and 15 h at room  
53  
54 246 temperature, 1M aqueous NaOH (8.5 ml) was added and the mixture was stirred for 15  
55  
56  
57 247 min. The organic layer was then decanted, dried, and concentrated. The crude product  
58  
59  
60  
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4 248 was taken up in pentane (20 ml) and 1,3-propanediol (0.30 ml, 4.18 mmol) was added.  
5  
6 249 The mixture was stirred for 3 h at room temperature, then crystalline anhydrous Na<sub>2</sub>SO<sub>4</sub>  
7  
8  
9 250 was added. After stirring 1 h, the slurry was filtered and the filtrate was concentrated.  
10  
11 251 The residue was purified by vacuum flash chromatography on silica gel eluting with  
12  
13 252 mixtures of hexane: Et<sub>2</sub>O (1:0 to 1:1) to obtain compound **6** (0.55 g, 61%) as a yellow oil.  
14  
15 253 <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 6.50 (dt, *J* = 17.7, 6.5 Hz, 1H), 6.28-6.17 (m, 2H), 5.47-  
16  
17 254 5.39 (m, 2H), 5.32 (dt, *J* = 17.7, 1.5 Hz, 1H), 4.02 (t, *J* = 5.5 Hz, 4H), 2.22-2.11 (m, 6H),  
18  
19 255 1.96 (quint, *J* = 5.5 Hz, 2H), 1.50 (tt, *J*<sub>1</sub> = *J*<sub>2</sub> = 7.5 Hz, 2H), 0.99 (t, *J* = 7.5 Hz, 3H) ppm.  
20  
21 256 <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 151.20 (CH), 133.89 (CH), 131.72 (CH), 127.56 (CH-B),  
22  
23 257 123.92 (CH), 123.16 (CH), 61.85 (2CH<sub>2</sub>O), 35.25 (CH<sub>2</sub>), 28.60 (CH<sub>2</sub>), 27.57 (CH<sub>2</sub>),  
24  
25 258 27.18 (CH<sub>2</sub>), 20.94 (CH<sub>2</sub>), 14.35 (CH<sub>3</sub>) ppm. HRMS: Calcd. for C<sub>14</sub>H<sub>24</sub>BO<sub>2</sub>: 235.1869  
26  
27 259 [M+1]<sup>+</sup>; found: 235.1863.  
28  
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34 260  
35  
36 261 *1-tert-Butyldimethylsilyloxy*pent-4-*yne* (**7**). Imidazole (4.86 g, 71.33 mmol) and *tert*-  
37  
38 262 butyldimethylsilyl chloride (8.60 g, 57.06 mmol) were added to a solution of 4-pentyn-1-  
39  
40 263 ol (4.00 g, 47.55 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (120 ml) and the mixture was stirred for 3 h  
41  
42 264 at room temperature. Saturated aqueous NaHCO<sub>3</sub> (200 ml) was then added, and after  
43  
44 265 stirring, the organic layer was separated and the aqueous layer was extracted with EtOAc  
45  
46 266 (2x120 ml). The combined organic layers were washed with saturated aqueous NH<sub>4</sub>Cl  
47  
48 267 (200 ml) and brine (300 ml), dried, and concentrated. The residue was purified by  
49  
50 268 vacuum flash chromatography eluting with hexane to yield *1-tert*-  
51  
52 269 butyldimethylsilyloxy)pent-4-*yne* (**7**) (9.12 g, 97%) as a colorless oil. <sup>1</sup>H NMR (500  
53  
54 270 MHz, CDCl<sub>3</sub>): δ 3.70 (t, *J* = 6.0 Hz, 2H), 2.28 (td, *J* = 7.1, 2.7 Hz, 2H), 1.93 (t, *J* = 2.7

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3  
4 271 Hz, 1H), 1.73 (m, 2H), 0.90 (s, 9H), 0.06 (s, 6H) ppm.  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$   
5  
6 272 99.9 (C), 84.4 (CH), 61.6 ( $\text{CH}_2\text{O}$ ), 31.7 ( $\text{CH}_2$ ), 26.1 ( $\text{C}(\text{CH}_3)_3$ ), 18.5 ( $\text{C}(\text{CH}_3)_3$ ), 15.0  
7  
8  
9 273 ( $\text{CH}_2$ ), -5.1 ( $\text{CH}_3\text{SiCH}_3$ ) ppm. HRMS: Calcd. for  $\text{C}_{11}\text{H}_{23}\text{OSi}$ : 199.1518  $[\text{M}+1]^+$ ; found:  
10  
11 274 119.1519. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra agreed with those previously reported (Sparling  
12  
13  
14 275 et al. 2009).  
15  
16 276  
17  
18  
19 277 (*4E*)-1-*tert*-Butyldimethylsilyloxy-5-iodopent-4-ene (**8**). An oven-dried flask was charged  
20  
21 278 with bis(cyclopentadienyl)zirconium hydridochloride (1.39 g, 5.40 mmol), and after  
22  
23 279 flushing thoroughly with Ar, dry THF was added (15 ml). The suspension was chilled to  
24  
25 280  $0^\circ\text{C}$  and stirred for 15 min. A solution of pentyne **7** (0.98 g, 4.90 mmol) in dry THF (5  
26  
27 281 ml) was then added, the flask was shielded from light, and the mixture was stirred at  $0^\circ\text{C}$   
28  
29 282 for 30 min. After warming to room temperature, the reaction mixture was stirred for 3.5  
30  
31 283 h more. The resulting solution was cooled again to  $0^\circ\text{C}$ , and a solution of iodine (1.37 g,  
32  
33 284 5.40 mmol) in dry THF (7 ml) was added dropwise, and stirring was continued for 45  
34  
35 285 min. The mixture was warmed to room temperature, quenched by pouring into saturated  
36  
37 286 aqueous  $\text{Na}_2\text{S}_2\text{O}_3$  (40 ml), and the slurry was filtered through a pad of Celite<sup>®</sup> eluting  
38  
39 287 with hexane (200 ml). The organic solution was washed with saturated aqueous  $\text{Na}_2\text{S}_2\text{O}_3$   
40  
41 288 (100 ml) and brine (150 ml), dried, and concentrated. The residue was purified by flash  
42  
43 289 chromatography on silica gel eluting with mixtures of hexane:EtOAc (1:0 to 95:5) to  
44  
45 290 provide (*4E*)-1-*tert*-butyldimethylsilyloxy-5-iodopent-4-ene (**8**) (1.25 g, 78%) as a yellow  
46  
47 291 oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.52 (dt,  $J = 14.0, 6.8$  Hz, 1H), 6.00 (dt,  $J = 14.0, 1.4$   
48  
49 292 Hz, 1H), 3.60 (t,  $J = 6.8$  Hz, 2H), 2.13 (td,  $J = 6.8, 1.4$  Hz, 2H), 1.60 (quint,  $J = 6.8$  Hz,  
50  
51 293 2H), 0.89 (s, 9H), 0.04 (s, 6H) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  146.15 (CH), 74.63  
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4 294 (CH), 61.94 (CH<sub>2</sub>O), 32.44 (CH<sub>2</sub>), 31.34 (C(CH<sub>3</sub>)<sub>3</sub>), 25.91 (C(CH<sub>3</sub>)<sub>3</sub>), 18.28 (CH<sub>2</sub>), -5.34  
5  
6  
7 295 (CH<sub>3</sub>SiCH<sub>3</sub>) ppm. HRMS: Calcd. for C<sub>11</sub>H<sub>24</sub>OSi: 327.0641 [M+1]<sup>+</sup>; found: 327.0652.  
8  
9 296 The <sup>1</sup>H and <sup>13</sup>C NMR spectra agreed with those previously reported (Mukai et al. 1998).  
10  
11 297  
12  
13  
14 298 (*4E,6E,11Z,13Z*)-Hexadeca-4,6,11,13-tetraenol (**9**). A solution of pentene **8** (0.58 g, 1.78  
15  
16 299 mmol) in THF (4 ml) was added to a solution of borinane **6** (0.38 g, 1.62 mmol) in THF  
17  
18 300 (4 ml), followed by Pd(PPh<sub>3</sub>)<sub>4</sub> (0.09 g, 0.08 mmol) and 1M aqueous KOH (3.4 ml, 3.40  
19  
20 301 mmol). The mixture was stirred under nitrogen at 60°C for 4.5 h, cooled to room  
21  
22 302 temperature and then anhydrous Na<sub>2</sub>SO<sub>4</sub> and hexane (40 ml) were added. The slurry was  
23  
24 303 filtered through a pad of Celite<sup>®</sup>, the filtrate was concentrated, and the residue was  
25  
26 304 filtered through a pad of silica gel eluting with a mixture of hexane: EtOAc 9:1. The  
27  
28 305 resulting product was concentrated, diluted with THF (10 ml), and 1M  
29  
30 306 tetrabutylammonium fluoride in THF (1.8 ml, 1.80 mmol) was added. After stirring at  
31  
32 307 room temperature for 15 h, the mixture was diluted with saturated aqueous NH<sub>4</sub>Cl (20  
33  
34 308 ml), the organic layer was separated, and the aqueous layer was extracted with EtOAc  
35  
36 309 (4x15 ml). The combined organic layers were dried, concentrated, and purified by flash  
37  
38 310 chromatography eluting with mixtures of hexane:EtOAc (9:1 to 2:5) to provide  
39  
40 311 (*4E,6E,11Z,13Z*)-hexadeca-4,6,11,13-tetraenol (**9**) (0.24 g, 63% over 2 steps) as a yellow  
41  
42 312 oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 6.30-6.15 (m, 2H), 6.08-5.96 (m, 2H), 5.62-5.52 (m,  
43  
44 313 2H), 5.48-5.40 (m, 2H), 3.66 (t, *J* = 6.5 Hz, 2H), 2.23-2.13 (m, 6H), 2.08 (dt, , *J*<sub>1</sub> = *J*<sub>2</sub> =  
45  
46 314 7.2 Hz, 2H), 1.66 (tt, *J*<sub>1</sub> = *J*<sub>2</sub> = 7.0 Hz, 2H), 1.47 (tt, *J*<sub>1</sub> = *J*<sub>2</sub> = 7.5 Hz, 2H), 1.40 (bs, OH),  
47  
48 315 1.00 (t, *J* = 7.5 Hz, 3H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 133.94 (CH), 132.51 (CH),  
49  
50 316 131.69 (CH), 131.46 (CH), 131.12 (CH), 130.64 (CH), 123.95 (CH), 123.14 (CH), 62.66  
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4 317 (CH<sub>2</sub>O), 32.45 (CH<sub>2</sub>), 32.27 (CH<sub>2</sub>), 29.42 (CH<sub>2</sub>), 29.05 (CH<sub>2</sub>), 27.11 (CH<sub>2</sub>), 20.95 (CH<sub>2</sub>),  
5  
6 318 14.35 (CH<sub>3</sub>) ppm. EIMS, *m/z* (%): 234 ([M]<sup>+</sup>, 4), 205 (4), 190 (19), 175 (32), 147 (33),  
7  
8 319 136 (17), 135 (77), 134 (31), 133 (32), 131 (37), 123 (15), 122 (63), 121 (55), 120 (23),  
9  
10 320 119 (81), 109 (21), 108 (42), 107 (75), 106 (30), 105 (78), 95 (55), 94 (40), 93 (92), 92  
11  
12 321 (46), 91 (91), 81 (72), 80 (67), 79 (100), 78 (36), 77 (76), 69 (37), 68 (21), 67 (89), 66  
13  
14 322 (31), 65 (41), 55 (77), 53 (49), 43 (30), 41 (76). HRMS: Calcd. for C<sub>16</sub>H<sub>27</sub>O: 235.2062  
15  
16 323 [M+1]<sup>+</sup>; found: 235.2071.  
17  
18  
19 324  
20  
21 325 (*4E,6E,11Z,13Z*)-Hexadeca-4,6,11,13-tetraenal (**10**). A mixture of dry DMSO (0.16 ml)  
22  
23 and dry CH<sub>2</sub>Cl<sub>2</sub> (0.7 ml) was added dropwise under Ar to a cooled (-78°C) solution of  
24  
25 326 oxalyl chloride (0.09 ml, 1.02 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (2 ml). A solution of tetraenol **9**  
26  
27 327 (0.16 g, 0.68 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (0.7 ml) was then added and the mixture was stirred at  
28  
29 328 -78°C for 1.5 h. Triethylamine (0.7 ml, 5.03 mmol) was added and the mixture was  
30  
31 329 stirred for 30 min more at -78°C. The reaction was then warmed to room temperature  
32  
33 330 and quenched with water (30 ml). The organic layer was separated and the aqueous layer  
34  
35 331 was extracted with hexane (4x10 ml). The combined organic layers were dried,  
36  
37 332 concentrated, and the residue was purified by flash chromatography on silica gel eluting  
38  
39 333 with hexane:EtOAc 95:5 to give (*4E,6E,11Z,13Z*)-hexadeca-4,6,11,13-tetraenal (**10**)  
40  
41 334 (0.05 g, 32%, ≥98% isomeric purity) as a pale yellow oil. The aldehyde was diluted  
42  
43 335 immediately with hexane to a 0.03% solution, BHT (6.5 mg) was added as stabilizer, and  
44  
45 336 the solution was sealed in a glass ampoule flushed with Ar to minimize degradation.  
46  
47 337 NMR spectra were run in CDCl<sub>3</sub>, previously filtered through a pad of solid NaHCO<sub>3</sub> to  
48  
49 338 minimize possible acid-catalyzed trimerization of the aldehyde. <sup>1</sup>H NMR (400 MHz,  
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51 339  
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4 340 CDCl<sub>3</sub>): δ 9.77 (t,  $J = 1.6$  Hz, 1H), 6.30-6.15 (m, 2H), 6.08-5.95 (m, 2H), 5.63-5.51 (m,  
5  
6  
7 341 2H), 5.48-5.39 (m, 2H), 2.53 (dt,  $J = 7.4, 1.6$  Hz, 2H), 2.40 (dt,  $J_1 = J_2 = 7.0$  Hz, 2H),  
8  
9 342 2.23-2.14 (m, 4H), 2.08 (dt,  $J_1 = J_2 = 7.3$  Hz, 2H), 1.47 (tt,  $J_1 = J_2 = 7.4$  Hz, 2H), 1.00 (t,  
10  
11  $J = 7.5$  Hz, 3H) ppm. <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 202.11 (CHO), 133.95 (CH),  
12  
13 343 133.32 (CH), 131.77 (CH), 131.61 (CH), 130.30 (CH), 129.41 (CH), 123.98 (CH),  
14  
15 344 123.11 (CH), 43.48 (CH<sub>2</sub>), 32.24 (CH<sub>2</sub>), 29.34 (CH<sub>2</sub>), 27.08 (CH<sub>2</sub>), 25.25 (CH<sub>2</sub>), 20.94  
16  
17 345 (CH<sub>2</sub>), 14.34 (CH<sub>3</sub>) ppm. HRMS: Calcd. for C<sub>16</sub>H<sub>25</sub>O: 233.1905 [M+1]<sup>+</sup>; found:  
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19 346 233.1901.  
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21 347  
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25  
26 349 *Field Trials* Five mm diam. rubber septa (Thomas Scientific, Swedesboro, NJ, USA)  
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28 350 were extracted twice with hexane, allowed to dry, then loaded with 50 μg of  
29  
30 351 (4*E*,6*E*,11*Z*,13*Z*)-16:Ald in 50 μl hexane. Septa were stored in a -20°C freezer until  
31  
32 352 needed (replaced at ca. 14 d intervals in the field). Initial observations were made of  
33  
34 353 moths responding to a septum atop a 1m tall PVC post near a suburban home (GPS  
35  
36 354 38.026205, -84.569868). Subsequent observations of moths responding to and/or being  
37  
38 355 trapped in a 53 × 53 × 53 cm cube-shaped trap constructed of PVC pipe and fiberglass  
39  
40 356 screen were made at the same location. Each vertical side of the trap had a central cone-  
41  
42 357 shape surface that narrowed from 25 to a 12 cm diam. opening towards the inside of the  
43  
44 358 trap (see Figure 5A) to limit egress. Between 15 May and 24 May 2012, moths were  
45  
46 359 captured with a sweep net or trap at this location as they approached a septum.  
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52  
53 360 Between 24 May 2012 and 13 June 2012 a cube trap baited with 50 μg of  
54  
55 361 (4*E*,6*E*,11*Z*,13*Z*)-16:Ald and an identical control trap baited with a septum dosed with 50  
56  
57 362 μl hexane were hung from tulip poplar trees (*Liriodendron tulipifera* L.) at the University  
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4 363 of Kentucky Arboretum (GPS 38.016382,-84.502477). Tulip poplar is a reported host for  
5  
6 364 both *C. angulifera* and *C. promethea* (Ferguson 1972; Tuskes et al. 1996). The openings  
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8  
9 365 of the traps were ca. 1 m above ground level. Treatment and control traps were separated  
10  
11 366 by about 10 m. Positions of baited and control traps were switched 6 times over 20 d.

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13  
14 367 Between 17 July 2012 and 13 August 2012 baited and control traps were hung  
15  
16 368 from trees at the University of Kentucky's North Farm (GPS 38.127451,-84.512887).  
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18  
19 369 Treatment and control traps were separated by at least 50 m, with 2000 m between pairs  
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21 370 of traps. The surrounding farm areas had fence rows that included black cherry (*Prunus*  
22  
23 371 *serotina*) and other reported host plants for *C. promethea*. Within a treatment-control pair  
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25  
26 372 of traps, positions were switched 7 times over 27 d. A voucher specimen, netted while  
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28  
29 373 responding to a pheromone lure, has been deposited in the insect collection of the  
30  
31 374 Department of Entomology, University of Kentucky, Specimen Record-H11025.

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33 375

## 36 376 RESULTS AND DISCUSSION

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41 378 *Identification of the Major Pheromone Component* Analysis of SPME wipe samples of  
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43 379 pheromone glands by GC-EAD showed one major antennal response to a small peak with  
44  
45 380 a retention index of 1926 on a DB-5 GC column, and several minor responses (Figure 3).  
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47  
48 381 Subsequent GC-MS analysis of two other SPME wipe samples showed that the  
49  
50 382 compound eliciting the largest response exhibited a small molecular ion at  $m/z$  232 with  
51  
52 383 losses of 18 (loss of water) and 44 mass units (loss of  $C_2H_4O$ ) from the molecular ion  
53  
54  
55 384 (Figure 4). This molecular weight suggested a molecular formula of  $C_{16}H_{24}O$ , for a  
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57  
58 385 possible tetra-unsubstituted aldehyde. This was supported by the fact that the retention  
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4 386 index of the unknown was noticeably larger than that of (4*E*,6*E*,11*Z*)-hexadeca-4,6,11-  
5  
6 387 trienal (1861; Millar et al. 2010). Furthermore, the increase of approximately 50  
7  
8 388 retention units for a conjugated diene on DB-5 (e.g., retention index of (11*Z*,13*Z*)-  
9  
10 389 hexadecan-11,13-dienal: 1875)) versus hexadecanal (1821) on the DB-5 column  
11  
12 390 suggested that the unknown might have two isolated conjugated diene systems.  
13  
14 391 Additional retention index data from standards suggested that the unknown probably did  
15  
16 392 not have a monoene and a conjugated triene because this would result in an increase of  
17  
18 393 approximately 130-140 retention units versus hexadecanal. That is, (10*E*,12*E*,14*E*)- and  
19  
20 394 (10*E*,12*E*,14*Z*)-hexadeca-10,12,14-trienals had retention indices of 1961 and 1967  
21  
22 395 respectively (~145 units more than hexadecanal), and (*Z*)-11-hexadecenal had a retention  
23  
24 396 index of 1810 on this column (11 units less than hexadecanal). With this data suggesting  
25  
26 397 a C16 tetraenal with two conjugated diene systems, we then turned to the literature on  
27  
28 398 pheromones identified from saturniid species. In particular, the pheromone of *Antheraea*  
29  
30 399 *polyphemus* Cramer had been reported as (6*E*,11*Z*)-hexadeca-6,11-dienal (Kochansky et  
31  
32 400 al. 1975), whereas the pheromones of *Philosamia cynthia ricini* Boisduval (Bestmann et  
33  
34 401 al. 1989) and *Graellsia isabellae* Graells had been identified as (4*E*,6*E*,11*Z*)-hexadeca-  
35  
36 402 4,6,11-trienal (Millar et al. 2010). This suggested likely structures as being (4,6,9,11)- or  
37  
38 403 (4,6,11,13)-hexadecatetraenals, each with 16 possible stereoisomers, and with the latter  
39  
40 404 structure assessed as being easier to synthesize. We further assumed that based on these  
41  
42 405 precedents, the diene closest to the aldehyde function might be in the 4,6 position, with  
43  
44 406 (4*E*,6*E*)-stereochemistry. Close examination of the mass spectrum showed an even-mass  
45  
46 407 ion at  $m/z$  122 (14%) from possible cleavage between allylic carbon 8 and carbon 9, with  
47  
48 408 a hydrogen transfer, providing a small fragment of evidence in support of a 4,6- dienal  
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4 409 structure. The remainder of the mass spectrum was dominated by ions which, from their  
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6 410 masses, could only contain carbon and hydrogen, with one to five unsaturations. We  
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9 411 were not able to glean any further solid information about the possible positions of the  
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11 412 double bonds from the mass spectrum.  
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14 413 Further evidence for the (4*E*,6*E*) and the (Z11) stereochemistry was obtained by  
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16 414 GC-EAD analysis of a mixed standard of (4*E*,6*E*,11*Z*)- and (4*Z*,6*E*,11*Z*)-hexadeca-4,6-  
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19 415 11-trienals, available from work with *G. isabellae* (Millar et al. 2010). The (4*E*,6*E*,11*Z*)-  
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21 416 isomer elicited large responses from antennae, whereas the responses to the (4*Z*,6*E*,11*Z*)-  
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23 417 isomer were much smaller.  
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26 418 Thus, we first focused our attention on developing non-selective syntheses that  
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28 419 would provide all four of the (4*E*,6*E*,11*Z*/*E*,13*Z*/*E*)-isomers as pairs of isomers (Figure 1,  
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30 420 and supporting online information). The four isomers were differentiable on the DB-5  
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32 421 column (retention indices: (4*E*,6*E*,11*Z*,13*Z*) 1926; (4*E*,6*E*,11*E*,13*Z*) 1920;  
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34 422 (4*E*,6*E*,11*Z*,13*E*) and (4*E*,6*E*,11*E*,13*E*) pair, 1906 and 1936), with the retention index and  
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36 423 mass spectrum of the (4*E*,6*E*,11*Z*,13*Z*)-isomer matching that of the unknown. In sum, the  
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38 424 available evidence all supported the pheromone structure as being (4*E*,6*E*,11*Z*,13*Z*)-  
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41 425 hexadeca- 4,6,11,13-tetraenal, and so a stereoselective synthesis of this compound was  
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43 426 developed to provide material for field testing.  
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50 428 *Synthesis of (4E,6E,11Z,13Z)-hexadeca-4,6,11,13-tetraenal (10)* (Figure 2). We required  
51  
52 429 a short and efficient synthesis that could generate a conjugated (Z,Z)-diene and a second  
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54 430 conjugated (E,E)-diene with high stereoselectivity. We envisioned that the former could  
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57 431 be derived from Z-selective reduction of a conjugated diyne, whereas the latter could be  
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4 432 made from a palladium catalyzed Suzuki-type coupling of an *E*-vinyl halide and an *E*-  
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6 433 vinyl boronate. Thus, bromination of chloroalkyne **1** with *N*-bromosuccinimide and  
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9 434 AgNO<sub>3</sub> catalysis in acetone (Jurberg et al. 2010) provided bromoalkyne **2** in 88% yield.  
10  
11 435 Then Cadiot-Chodkiewicz coupling (Bayer and Maier 2004) of bromoalkyne **2** with 1-  
12  
13 436 butyne in the presence of CuCl, hydroxylamine hydrochloride, and *n*-propylamine in  
14  
15 437 MeOH gave diyne **3** (77%) which was stereoselectively reduced to the corresponding  
16  
17 438 diene (*Z,Z*)-**4** with dicyclohexylborane, prepared *in situ* (Brown et al. 1977).  
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19 439 Acetylenation of **4** with lithium acetylide-ethylene diamine complex catalyzed with NaI  
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21 440 in DMSO (Sonnet and Heath 1980) provided dienyne **5** in 64% yield. With this  
22  
23 441 intermediate in hand, we initially tried to convert **5** into the corresponding iodoalkene by  
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25 442 reaction with bis(cyclopentadienyl)zirconium hydridochloride (Schwartz's reagent)  
26  
27 443 followed by iodine (Ribe et al. 2000), but the reaction failed, possibly because of  
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29 444 coordination of Zr with the conjugated double bond system. Thus, as an alternative,  
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31 445 dienyne **5** was transformed into the corresponding borinane **6** by treatment with  
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33 446 dibromoborane-dimethyl sulfide complex followed by hydrolysis and reaction with 1,3-  
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35 447 propanediol (Organ and Ghasemi 2004) (61% overall yield from **5**), completing the first  
36  
37 448 key intermediate. The second key intermediate, protected (*E*)-iodoalkenol **8**, was obtained  
38  
39 449 by hydrido-zirconation of alkyne **7** with Schwartz's reagent followed by iodination to  
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41 450 provide *tert*-butyldimethylsilyl-protected (*E*)-iodoalkenol **8** in 78% yield and high  
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43 451 stereoselectivity. Suzuki reaction of borinane **6** with compound **8**, catalyzed by  
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45 452 tetrakis(triphenylphosphine) palladium(0) and aqueous KOH, coupled the two  
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47 453 intermediates to complete the tetraene framework. Tetrabutylammonium fluoride-  
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49 454 induced deprotection then gave tetraenol **9** in 63% yield from **6** (2 steps). Finally, Swern  
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4 455 oxidation of alcohol **9** gave tetraenal **10**, which was immediately diluted in hexane,  
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6 456 treated with BHT antioxidant and sealed in an ampoule to minimize degradation until the  
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9 457 compound could be used in field trials.  
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14 459 *Field Trials* All direct observations of moth activity were made at the suburban home of  
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16 460 one of the authors (KH). Direct observations were made of the responses of several male  
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19 461 *C. promethea* responding to a tetraenal lure during late afternoon to early evening (17:20  
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21 462 and 19:00 h). Males exhibited slow, zigzagging flight with nearly continuous upwind  
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24 463 progress towards the lure, followed by contact with the pheromone septum while  
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26 464 continuing to fan their wings. After several seconds on the lure two males were observed  
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29 465 to drop ca. 5 m downwind with an abrupt flight, only to reorient to the lure again. Two  
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31 466 other moths were netted as they reoriented to the lure. One of these was pinned and has  
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33 467 been deposited at the University of Kentucky's Insect Collection as a voucher specimen  
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36 468 (Specimen Record-H11025). Male moths also were observed as they approached and  
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39 469 entered a cube trap, and then contacted the pheromone lure (Figure 5A,B). No moths  
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41 470 were observed to stop short and veer away from the trap while in flight, but sometimes  
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43 471 they contacted the external screen sides before entering through the trap cone. Within the  
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46 472 trap, a male would repeatedly contact the lure and often remained active for many  
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48 473 minutes before coming to rest at the top or sides of the trap (Figure 5C). On one occasion  
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51 474 a moth was observed to exit the trap after contacting the lure by flying directly downwind  
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53 475 only to reorient and enter the trap a second time. At least two moths that assumed resting  
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55 476 positions within the trap eventually escaped before morning. Thus, this trap design does  
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58 477 not appear to limit the entrance of moths, but it also does not retain all moths. Nine  
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4 478 moths were caught in traps or netted in 10 d at this location. No control traps were used  
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6 479 at this location.  
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9 480 At the UK Arboretum, 7 male *C. promethea* were caught in baited traps over 20 d.  
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11 481 No moths were caught in control traps (binomial probability,  $P=0.008$ ). No moths were  
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13 482 caught between 1 June and 13 June 2012, suggesting that the first of the expected two  
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15 483 seasonal flight periods was complete by early June. During mid-July to mid-August  
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17 484 2012, 9 male *C. promethea* were trapped in two baited traps and no moths were caught in  
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19 485 two control traps over 27 days ( $P=0.002$ ). The entire trapping effort in both locations  
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21 486 (not including the suburban area) included 74 trapping days with 16 males captured in  
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23 487 traps baited with lures (0.22 males per baited trap per day) versus no males being caught  
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25 488 in control traps (16 vs. 0; binomial probability;  $P=0.00002$ ).  
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31 489 We have no information on the natural density of these insects, or the  
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33 490 effectiveness of the pheromone relative to a female. The 50  $\mu\text{g}$  loading rate was arbitrary  
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35 491 based on the initial success at the suburban area, and on the rather limited amounts of  
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37 492 synthetic pheromone available. A higher loading rate very likely would have captured  
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39 493 more males. To further place these results in perspective, Waldbauer and Sternburg  
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41 494 (1985) used caged females to trap male *promethea* moths in late June in Michigan and  
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43 495 reported the capture of 1.28 and 1.19 males per trap per day in 1983 and 1984,  
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45 496 respectively. For a non-pest species reported to have experienced population declines  
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47 497 (see Boettner et al. 2000), it may not be surprising for trap captures to be low when host  
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49 498 resources are dispersed over wide areas.  
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55 499 Thus, our analyses of a limited number of pheromone gland extracts and the  
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57 500 results of field trials with a synthetic compound support the hypothesis that the novel  
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4 501 structure (4*E*,6*E*,11*Z*,13*Z*)-hexadeca- 4,6,11,13-tetraenal is a major pheromone  
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6 502 component of the promethea moth. It is possible that one or more of the minor  
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9 503 components of pheromone gland extracts that elicited responses from the antennae of  
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11 504 male moths may also be components of the full pheromone blend. Nevertheless, the  
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14 505 tetraenal alone provides a useful tool for detection and monitoring of threatened and  
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16 506 declining *Callosamia* moth populations.  
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20  
21 508 *Acknowledgments* - We are indebted to the Ministry of Science and Innovation for a FPI  
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23 509 fellowship to RG. We also thank Shelby Stamper for assistance with the field trials at the  
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28 511 AgCenter, to KFH by Kentucky Agricultural Experiment Station, to AG by CICYT  
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30  
31 512 through project AGL2009-13452-C02-01, and to JGM through Hatch project CA-R\*-  
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33 513 5181-H.  
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38 515 SUPPORTING ONLINE INFORMATION  
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43 517 Supporting information includes descriptions of the syntheses of isomeric mixtures of  
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45 518 (4*E*,6*E*,11*Z*/*E*,13*E*)-hexadeca-4,6,11,13-tetraenal (**20a**), (4*E*,6*E*,11*E*/*Z*,13*Z*)-hexadeca-  
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48 519 4,6,11,13-tetraenal (**20b**) and (4*E*,6*E*,11*E*,13*Z*/*E*)- hexadeca-4,6,11,13-tetraen-1-ol (**23**),  
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50 520 and a video showing the responses of male moths to pheromone lures.  
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4 **597 Figure Captions**

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7 598 Figure 1 Nonstereoselective syntheses of (4*E*,6*E*,11*Z*/*E*,13*Z*)-hexadeca- 4,6,11,13-  
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9 599 tetraenals and (4*E*,6*E*,11*E*,13*Z*/*E*)-hexadeca- 4,6,11,13-tetraenals. Details of the  
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11 600 syntheses are described in the online Supplementary Information.

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14 601 Figure 2 Stereoselective synthesis of (4*E*,6*E*,11*Z*,13*Z*)-hexadeca- 4,6,11,13-tetraenal  
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16 602 (10).

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19 603 Figure 3 Representative coupled gas chromatography-electroantennogram analysis on a  
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21 604 DB-5 column of a SPME wipe sample of the extruded pheromone gland of a  
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23 605 female *Callosamia promethea* moth Upper trace is the gas chromatogram, lower  
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25 606 trace is the antennal response.

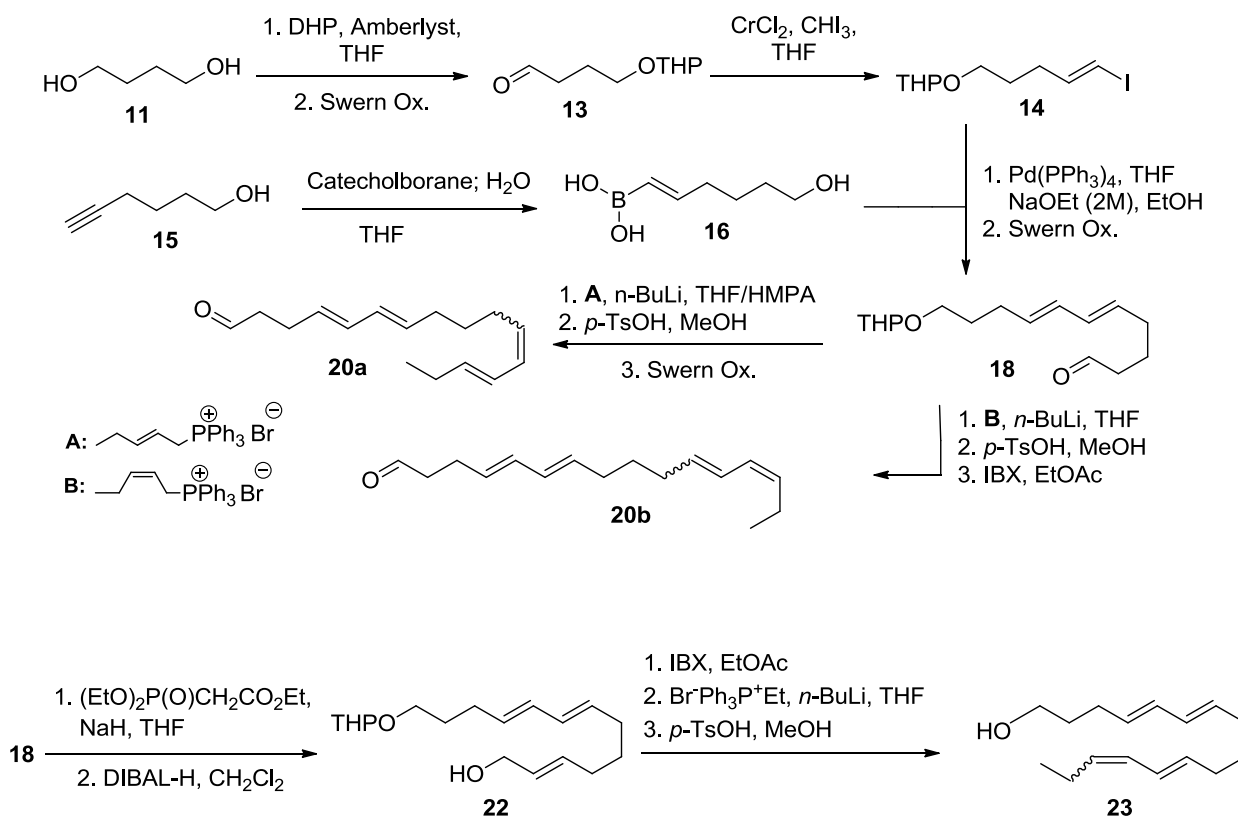
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28 607 Figure 4 EI mass spectra of the component in the insect extract that elicited the largest  
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30 608 response from male antennae in GC-EAD analyses (top) and synthetic  
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32 609 (4*E*,6*E*,11*Z*,13*Z*)-hexadeca- 4,6,11,13-tetraenal (bottom).

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36 610 Figure 5 A) Cube trap built for field bioassays; B) Male *Callosamia promethea* on a  
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38 611 pheromone-baited rubber septum inside the cube trap; C) A male *Callosamia*  
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40 612 *promethea* moth that was netted at a pheromone lure.

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614 Figure 1

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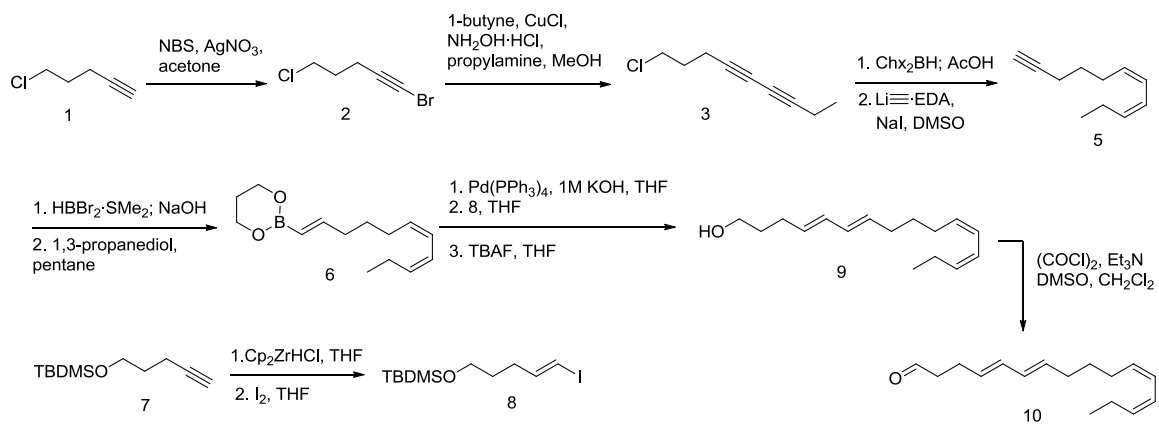
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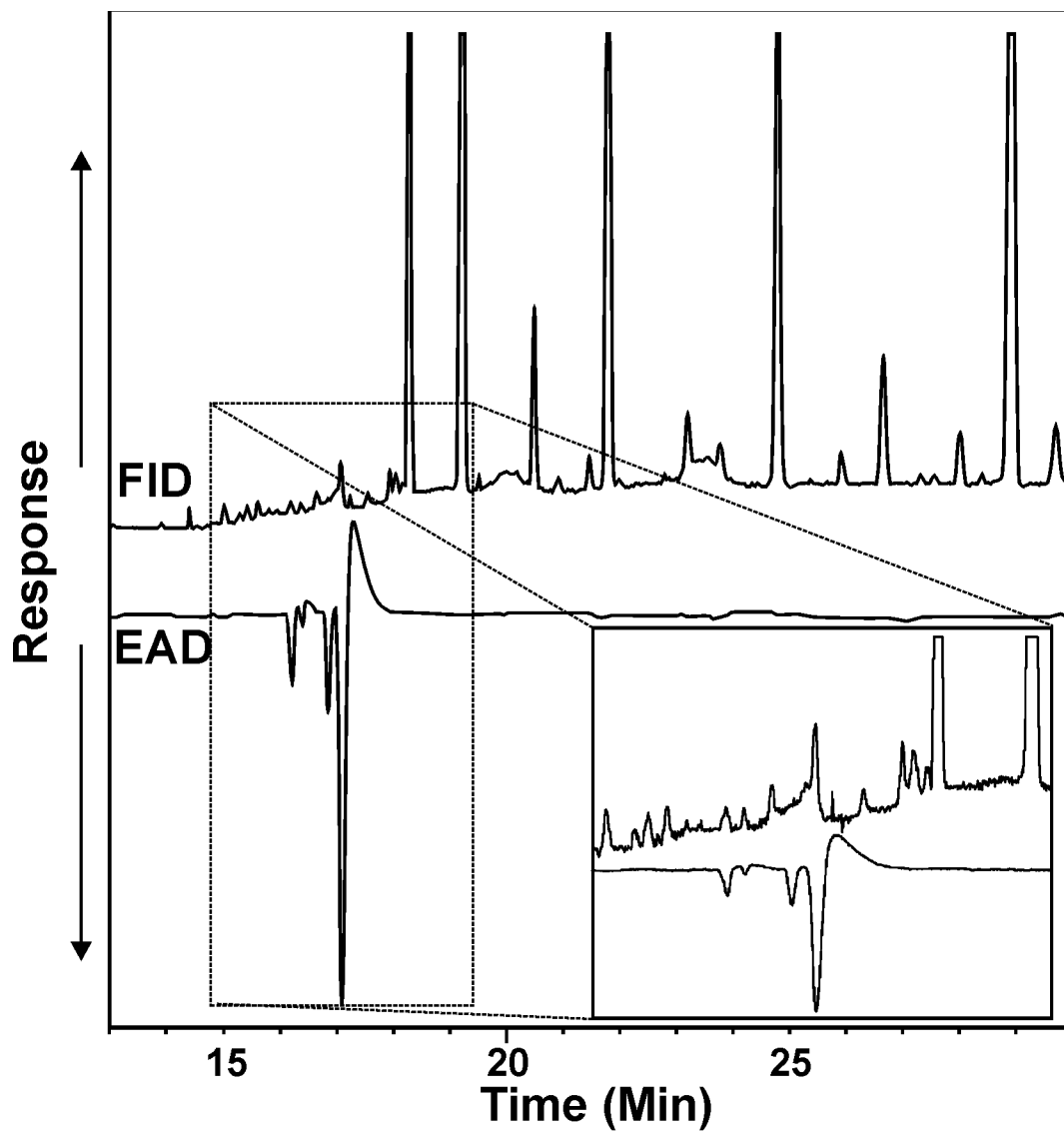
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628 Figure 3

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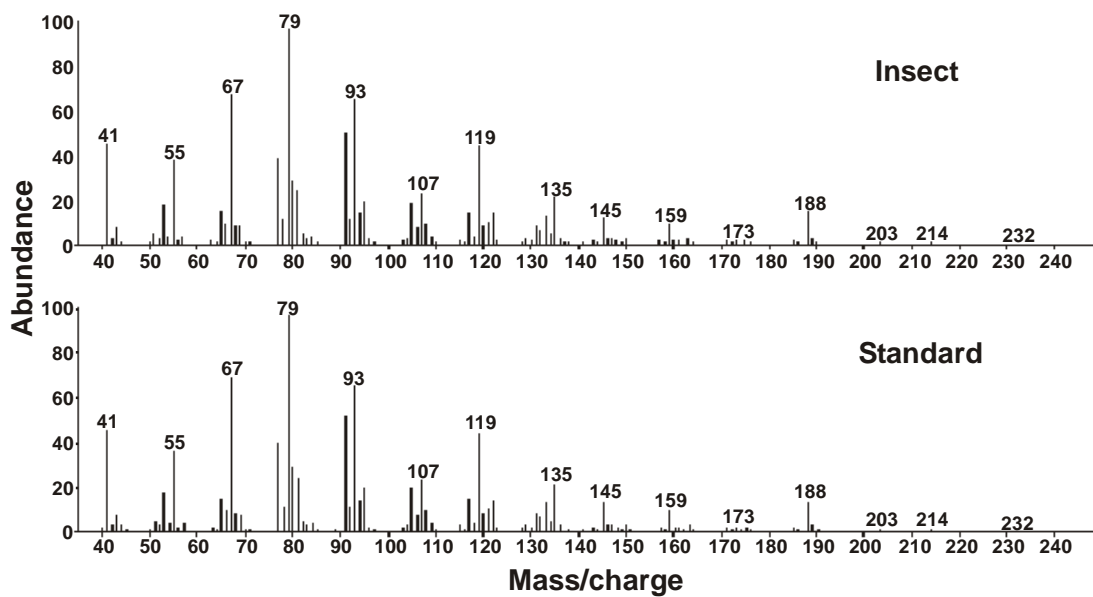
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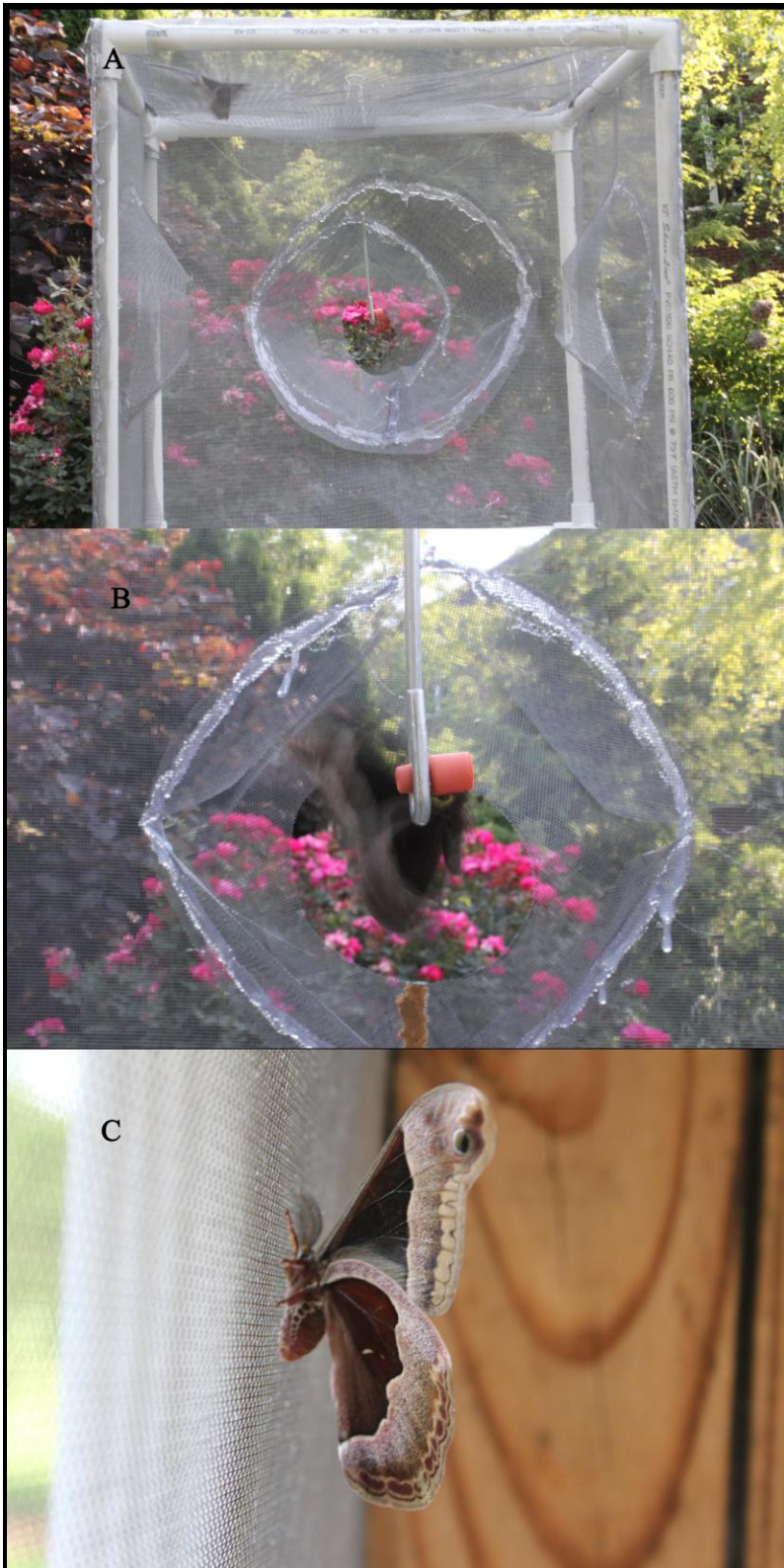


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639 Figure 5



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Figure 1

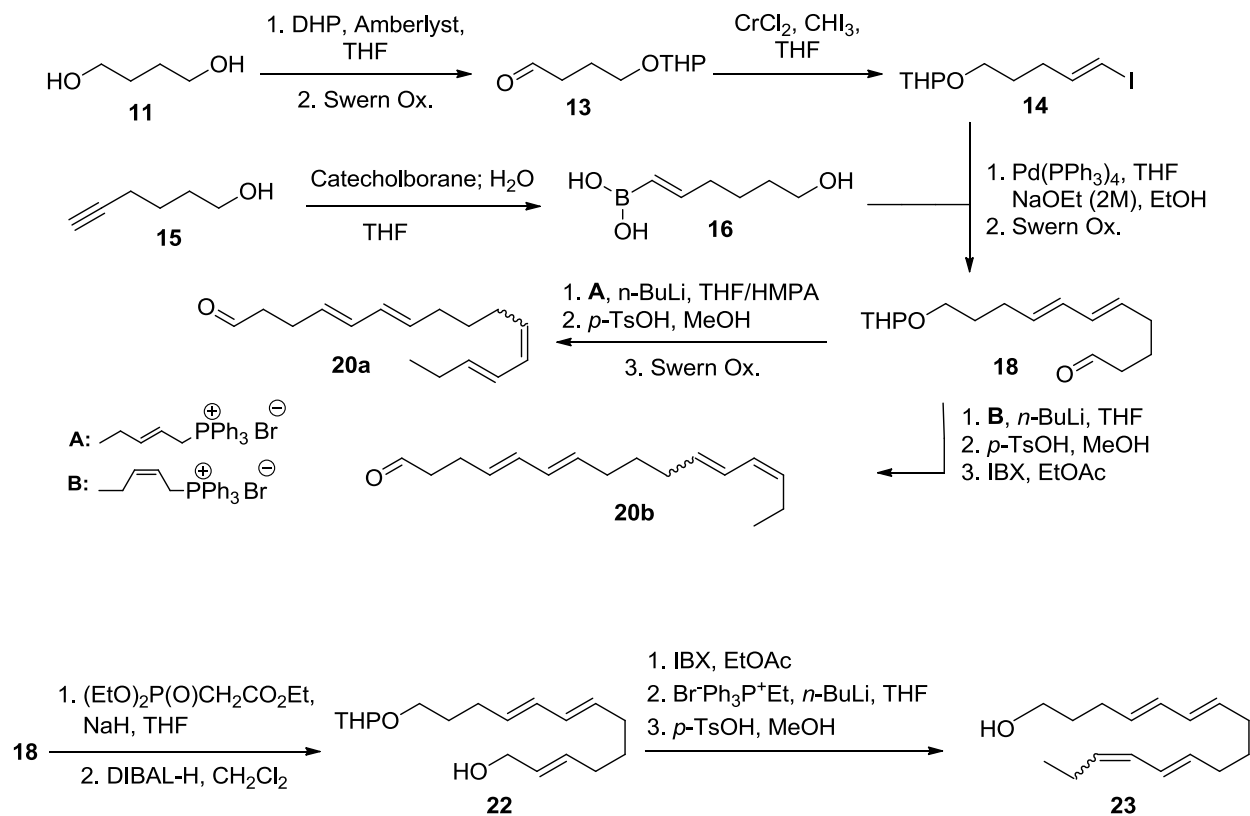


Figure 2.

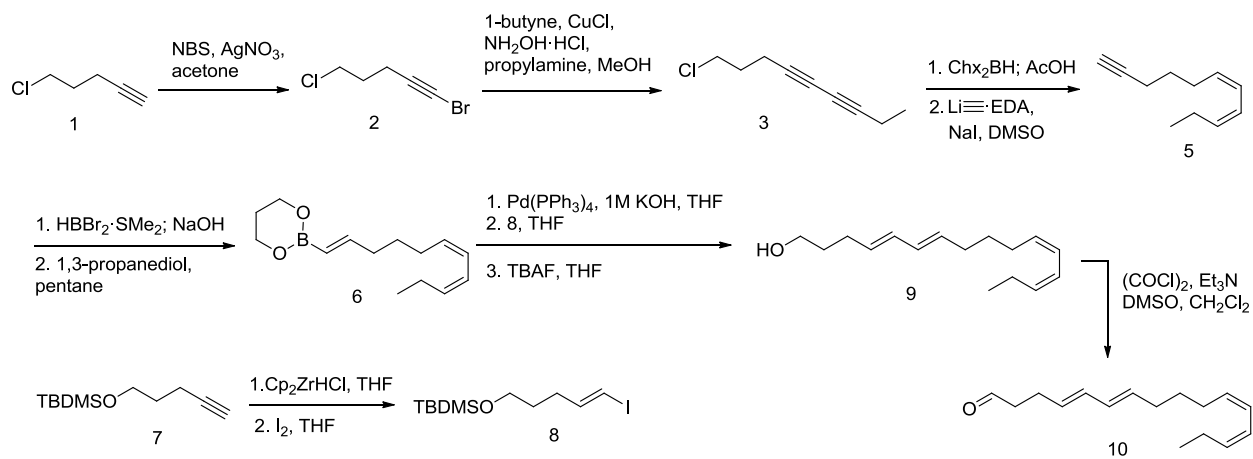


Figure 3  
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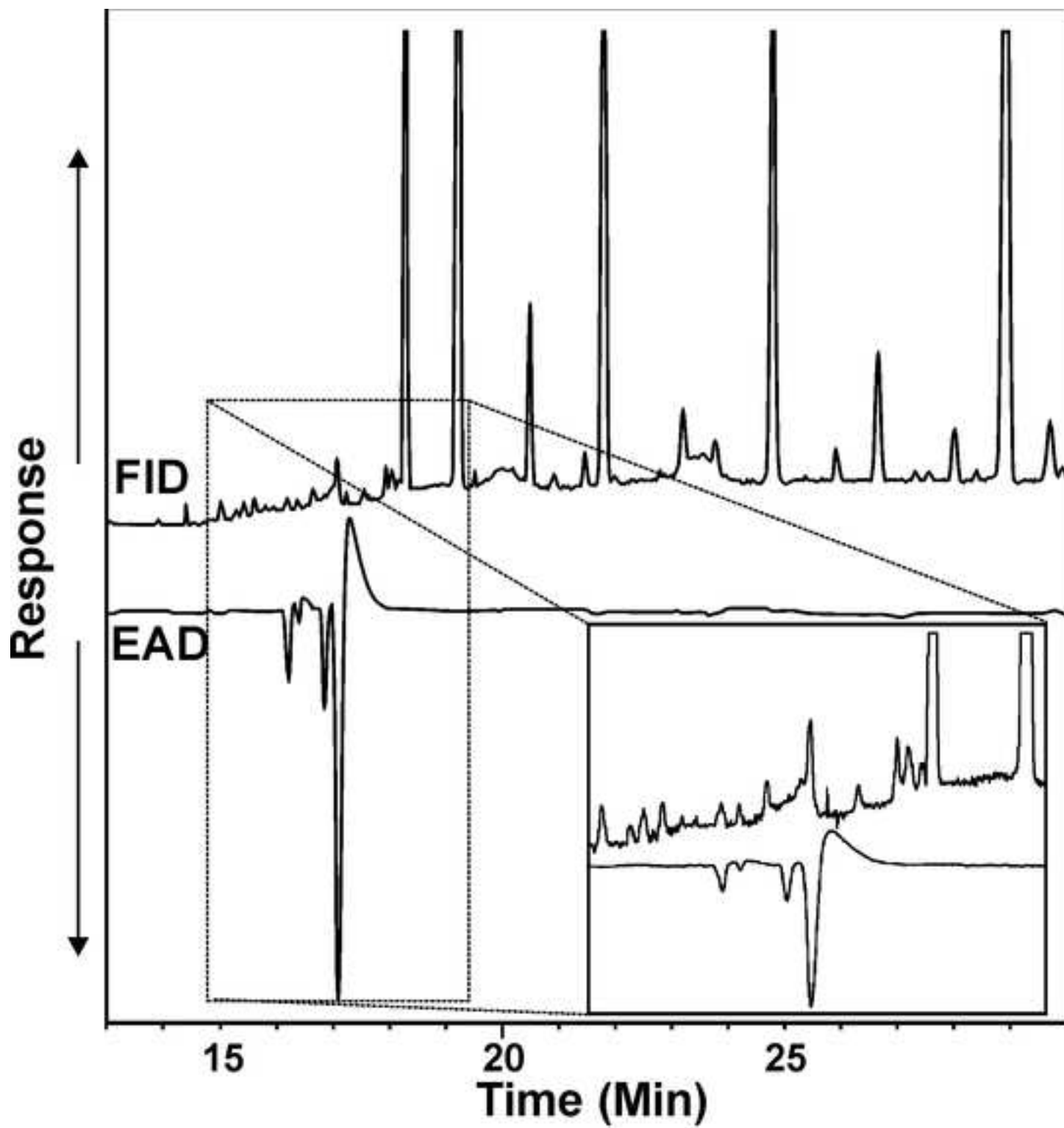


Figure 4  
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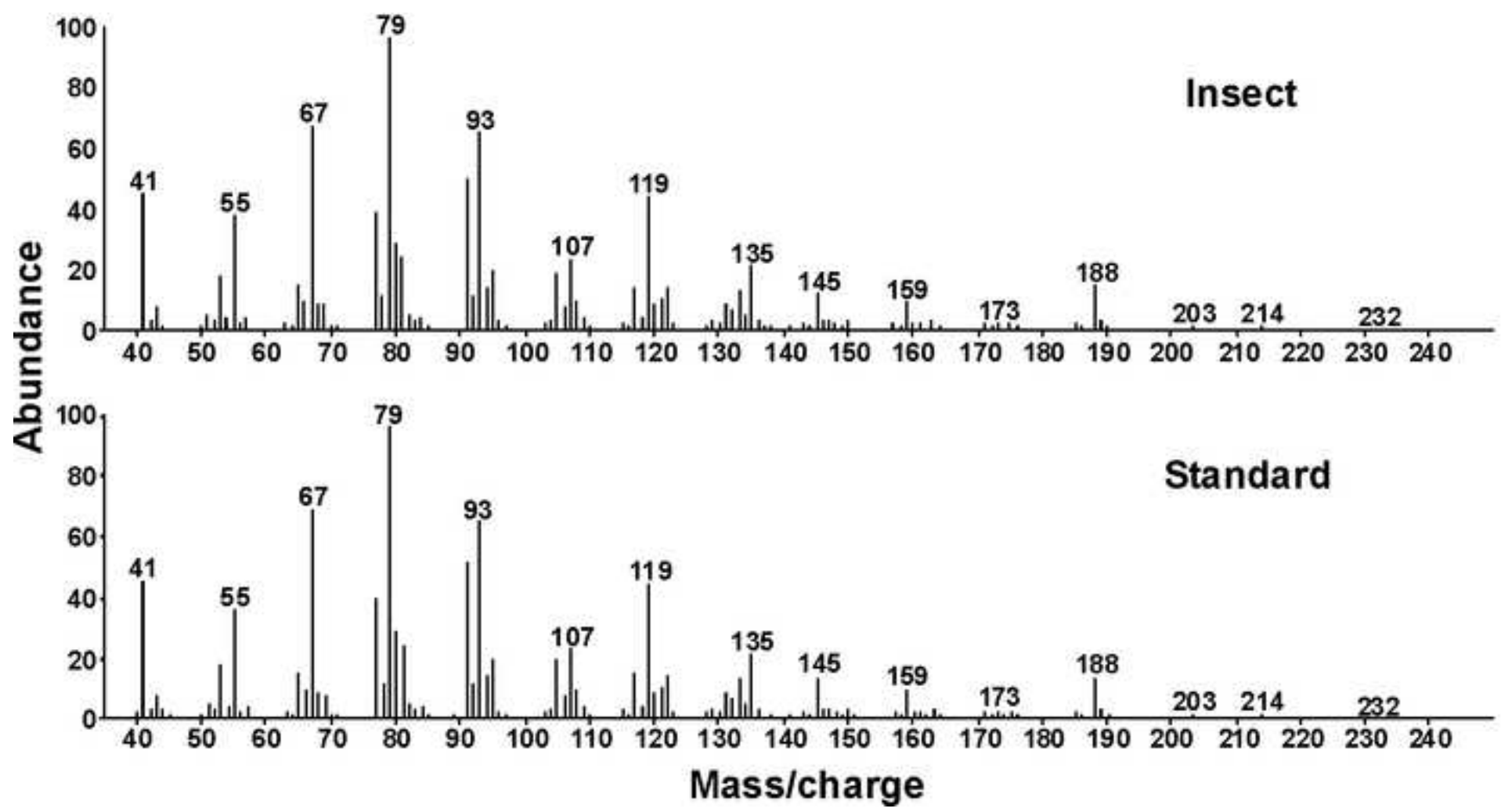
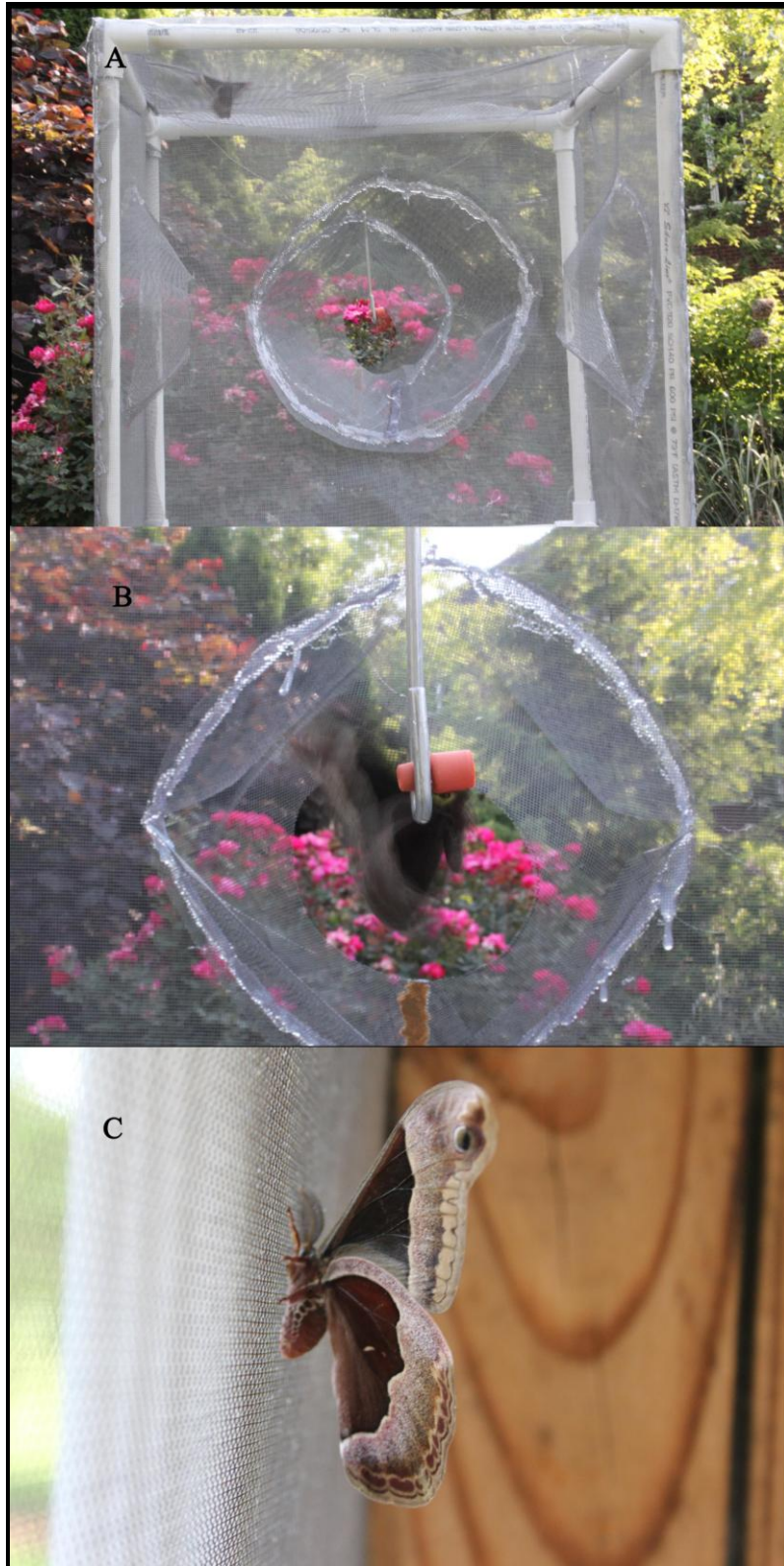




Figure 5  
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Supplementary Material

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