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# Synthesis and Field Evaluation of Synthetic Blends of the Sex Pheromone of *Crocidosema aporema* (Lepidoptera: Tortricidae) in Soybean

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*Crocidosema* (= *Epinotia*) *aporema* (Walsingham) (Lepidoptera: Tortricidae) é uma broca das axilas que se alimenta de soja e leguminosas forrageiras. Sua importância econômica é restrita à América do Sul onde pode alternar, ao longo do ano, entre leguminosas forrageiras e grãos. O feromônio sexual das fêmeas de *C. aporema* é composto de uma mistura 15:1 de (7*Z*,9*Z*)-dodeca-7,9-dieno-1-ol e acetato de (7*Z*,9*Z*)-dodeca-7,9-dienila. Com o intuito de desenvolver uma ferramenta para monitoramento deste inseto praga, os dois componentes do feromônio foram sintetizados e avaliou-se, em um experimento de campo no Uruguai, a captura de machos em armadilhas iscadas com diferentes misturas do feromônio sintético. Os dienos conjugados foram obtidos a partir de 2-pentin-1-ol e 1,7-heptanodiol, através da oxidação do primeiro, seguido pelo acoplamento de Wittig e redução da ligação tripla catalisada com Zn. A mistura 1:1 testada no campo foi a mais eficiente na captura dos machos. As armadilhas de feromônio foram atrativas por mais de 40 dias, mesmo com pequenas quantidades dos compostos (0,1 mg) impregnadas nos septos, e com baixas densidades populacionais dos insetos no campo.

*Crocidosema* (= *Epinotia*) *aporema* (Walsingham) (Lepidoptera: Tortricidae) is a bud borer that feeds on soybean and forage legumes. Its economic importance is restricted to South America, where it can alternate throughout the year between forage and grain legumes. The sex pheromone of *C. aporema* females is composed of a 15:1 mixture of (7*Z*,9*Z*)-dodeca-7,9-dien-1-ol and (7*Z*,9*Z*)-dodeca-7,9-dienyl acetate. Aiming at the development of a monitoring tool, it was synthesized both components of the pheromone and evaluated male captures in pheromone traps baited with different blends of synthetic pheromone, in an experimental soybean field in Uruguay. The conjugated dienes were obtained from 2-pentyn-1-ol and 1,7-heptanediol, by oxidation of the former, Wittig coupling and Zn-catalyzed reduction of the triple bond. The 1:1 mixture was the most efficient in capturing males. The pheromone traps were attractive for up to 40 days, even with small septum loads (0.1 mg) and low population levels.

**Keywords**: sex pheromone synthesis, *Crocidosema (= Epinotia) aporema*, pest monitoring, pheromone traps

## Introduction

Soybean production in Uruguay has increased dramatically in the past decade,[1] resulting in an expansion of legume-specialized insects such as *Crocidosema* (= *Epinotia*) *aporema* (Walsingham) (Lepidoptera: Tortricidae). This trend correlates with a significant increase in the use of insecticides, which has risen up to three-fold between 2001 and 2011.[2]

*C. aporema* is a stem and bud borer that has become a major pest of legumes in Uruguay, Argentina, Chile and Southern Brazil.[3-6] Originally from Costa Rica, this species

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is now widely distributed across South America. The insect is active throughout the year, going through 5-6 generations *per* year concentrated between November and April.[7]

The economic importance of *C. aporema* is restricted to the Southern Cone due to the year-round availability of hosts (soybean, alfalfa, lotus, peas, flax, red clover).[8-11] Soon after hatching, the larvae bore inside leaves, stems and buds, decreasing the effectiveness of contact insecticides.[12] In soybean, the larvae feed on vegetative plant parts, affecting the normal growth of the plant. In other legumes, larval feeding can severely affect the flower and prevent the production of seeds, an important commodity in some forage legumes such as alfalfa and lotus.[8,11]

*C. aporema* populations are monitored by direct observation of the larvae, a highly inefficient method with poor predictive capacity. Chemical control is therefore prophylactic, accounting, at least in part, for the large amounts of insecticides used in legume crops in the region.

As it is the case for many tortricid moths, population monitoring with pheromone traps would result in a more informed use of insecticides, reducing both environmental damage and production costs for soybean and other legumes. To develop a pheromone-based monitoring tool for the management of *C. aporema* in soybean, our group has previously studied the reproductive behavior of virgin females[13] and characterized (7*Z*,9*Z*)-dodeca-7,9-dien-1-ol and (7*Z*,9*Z*)-dodeca-7,9-dienyl acetate (hereafter referred to as *Z*7,*Z*9-12:OH and *Z*7,*Z*9-12:Ac) as the sex pheromone components in female gland extracts.[14] Since field studies are an essential step to develop a monitoring strategy, it is now reported the synthesis of *Z*7,*Z*9-12:OH and *Z*7,*Z*9-12:Ac in preparative scale, as well as male captures in a soybean field with four synthetic pheromone blends.

## Experimental

### Synthesis

#### (*Z*)-Dodec-7-en-9-ynyl acetate (**4**)

An amount of 1.16 g (9.8 mmol) of freshly prepared $MnO_2$ was added to a solution of 100 mg (1.19 mmol) of 2-pentyn-1-ol (**1**) in dry $CH_2Cl_2$ under nitrogen atmosphere. The mixture was stirred at room temperature during 24 h. The course of the reaction was monitored by gas chromatography: $R_t$ (**1**) = 5.7 min; $R_t$ (**2**) = 3.4 min. Crude reaction was filtered using silica-gel and washed with $CH_2Cl_2$. The solvent was distilled under reduced pressure and the resulting crude was used in the following reaction without further purification. Under nitrogen atmosphere, 0.5 g (3.6 mmol) of $K_2CO_3$ and 18-crown-6 in catalytic amount were added to a solution of 0.4 g (0.8 mmol) of the phosphonium salt **3** in 15 mL of dry toluene. The reaction mixture was refluxed for 2 h when aldehyde **2** was added. After 72 h, the reaction suspension was filtered using silica-gel and the solvent removed under reduced pressure. The residue was purified by column chromatography (hexane:AcOEt 8:2). Overall yield: 92%, *Z*/*E* ratio 85:15.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$/ppm 1.17 (t, 3H, *J* 7.5 Hz, 7*E*); 1.19 (t, 3H, *J* 7.5 Hz, 7*Z*); 1.30-1.47 (m, 6H, 7*Z* + 6H, 7*E*); 1.59-1.69 (m, 2H, 7*Z* + 2H, 7*E*); 2.06 (s, 3H, 7*E* + 3H, 7*Z*); 2.27-2.33 (m, 2H, 7*Z*, + 2H, 7*E*); 2.37 (dq, $J_1$ 7.5 Hz, $J_2$ 2.1 Hz, 2H, 7*Z* + 2H, 7*E*); 4.06 (t, 2H, *J* 6.7 Hz, 7*E*); 4.07 (t, 2H, *J* 6.7 Hz, 7*Z*); 5.43-5.49 (m, 6H, 7*E* + 6H, 7*Z*); 5.80 (dt, 1H, $J_1$ 10.7 Hz, $J_2$ 7.3 Hz, 7*Z*); 6.05 (dt, 1H, $J_1$ 15.8 Hz, $J_2$ 7.1 Hz, 7*E*); $^{13}$C NMR (400 MHz, $CDCl_3$): $\delta$/ppm 13.2; 14.1; 21.1; 25.7; 28.5; 28.7; 28.8; 29.7; 29.8; 64.5; 64.6; 109.5; 109.9; 142.3; 143.1; 171.3; EI-MS (70 eV) *m/z* (%) 222 (2.5), 162 (5.5), 161 (2.2), 147 (6.1), 135 (3.5), 134 (13.9), 133 (28.4), 123 (2.3), 121 (9.1), 120 (10.7), 119 (31.2), 117 (3.9), 108 (4.2), 107 (11.1), 106 (16.3), 105 (44.9), 103 (2.7), 96 (2.3), 95 (9.5), 94 (13.4), 93 (25.8), 92 (22.3), 91 (100.0), 83 (2.8), 82 (8.3), 81 (12.3), 80 (23.2), 79 (79.4), 78 (20.0), 77 (37.1), 69 (8.1), 68 (2.9), 67 (26.0), 66 (5.3), 65 (12.8), 63 (2.2), 61 (4.0), 56 (2.0), 55 (14.6), 54 (3.2), 53 (7.0), 52 (3.5), 51 (4.9), 43 (62.8), 42 (2.9), 41 (24.9) (Figures S1-S3 in Supplementary Information (SI) section).

#### (7*Z*,9*Z*)-Dodeca-7,9-dienyl acetate (*Z*7,*Z*9-12:Ac)

30 mg (0.15 mmol) of **4** and TMSCl (0.15 mL) were added to a suspension of Zn in MeOH:$H_2O$ 1:1 (Zn was previously activated by stirring for 30 min in HCl 10%). The reaction was stirred 24 h at room temperature, and monitored by gas chromatography. The crude was filtered with silica-gel and washed with methanol, which was removed afterwards under reduced pressure. The residue was re-suspended in AcOEt, washed with brine (3 × 10 mL) and dried with $Na_2SO_4$, then filtered and concentrated under vacuum. The residue was purified by column chromatography using hex:AcOEt (1:1). Yield: 66% (oil). *Z*/*E* ratio 85:15.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$/ppm 1.01 (t, 3H, *J* 7.6, 7*E*,9*Z* isomer); 1.02 (t, 3H, *J* 7.6, 7*Z*,9*Z* isomer); 1.32-1.46 (m, 4H, 7*Z*,9*Z* isomer + 4H, 7*E*,9*Z* isomer); 1.59-1.67 (m, 4H, 7*Z*,9*Z* isomer + 4H, 7*E*,9*Z* isomer); 2.07 (s, 3H, 7*Z*,9*Z* isomer + 3H, 7*E*,9*Z* isomer); 2.16-2.24 (m, 4H, 7*Z*,9*Z* isomer + 4H, 7*E*,9*Z* isomer); 4.07 (t, 2H, *J* 6, 9 Hz, 7*Z*,9*Z* isomer + 2H, 7*E*,9*Z* isomer); 5.29-5.36 (m, 1H, 7*E*,9*Z* isomer ); 5.42-5.50 (m, 2H, 7*Z*,9*Z* isomer); 5.63-5.70 (m, 1H, 7*E*,9*Z* isomer); 5.90-5.96 (m, 1H, 7*E*,9*Z* isomer); 6.19-6.36 (m, 2H, 7*Z*,9*Z* isomer + 1H, 7*E*,9*Z* isomer); $^{13}$C NMR (400 MHz, $CDCl_3$): $\delta$/ppm 14.2; 14.3; 21.0; 21.1; 25.8;

27.3; 28.6; 28.9; 29.3; 29.5; 32.8; 64.6; 122.9; 123.6; 125.6; 127.9; 131.8; 133.5; 133.8; 134.4; 171.3. EI-MS (70 eV) *m/z* (%) 224 (8.6), 165 (3.6), 164 (25.7), 136 (6.7), 135 (24.6), 123 (2.6), 122 (9.3), 121 (31.4), 110 (2.2), 109 (5.8), 108 (15.7), 107 (19.2), 105 (2.1), 97 (3.9), 96 (20.8), 95 (34.7), 94 (22.5), 93 (41.2), 92 (2.7), 91 (10.2), 83 (7.6), 82 (47.6), 81 (36.6), 80 (24.4), 79 (73.6), 78 (7.6), 77 (11.7), 69 (8.9), 68 (26.1), 67 (100.0), 66 (9.0), 65 (5.9), 61 (2.5), 57 (3.6), 55 (28.3), 54 (8.2), 53 (9.6), 43 (57.7), 42 (4.0), 41 (31.0), 40 (2.1) (Figures S4-S6, in the SI section).

(7*Z*,9*Z*)-Dodeca7,9-dien-1-ol (*Z*7,*Z*9-12:OH)

30 mg of Lipase B from *Candida antarctica* (CaL B, Novozym 435) were added to a solution of *Z*7,*Z*9-12:Ac (0.2 g, 1 mmol) in 2 mL of methanol. The mixture was stirred for 2 h in an orbital shaker at 30 ºC. The enzyme was filtered, the solvent was distilled under reduced pressure and the crude was purified by column chromatography using hex:AcOEt 1:1. Yield 98% (oil). *Z*/*E* ratio 85:15.

$^1$H NMR (400 MHz, $CDCl_3$): $\delta$/ppm 1.01 (t, 3H, *J* 7.1 Hz, 7*E*,9*Z* isomer); 1.02 (t, 3H, *J* 7.1 Hz, 7*Z*,9*Z* isomer); 1.34-1.46 (m, 2H, 7*Z*,9*Z* isomer + 2H, 7*E*,9*Z* isomer); 1.56-1.65 (m, 2H, 7*Z*,9*Z* isomer + 2H, 7*E*,9*Z* isomer); 2.16-2.25 (m, 2H, 7*Z*,9*Z* isomer + 2H, 7*E*,9*Z* isomer); 3.66 (t, 2H, *J* 5.4 Hz 7*Z*,9*Z* isomer + 2H, 7*E*,9*Z* isomer); 5.28-5.36 (m, 1H, 7*E*,9*Z* isomer); 5.43-5.50 (m, 2H, 7*Z*,9*Z* isomer); 5.64-5.71 (m, 1H, 7*E*,9*Z* isomer); 5.90-5.96 (m, 1H, 7*E*,9*Z* isomer); 6.20-6.36 (m, 2H, 7*Z*,9*Z* isomer + 1H, 7*E*,9*Z* isomer); $^{13}$C NMR (400 MHz, $CDCl_3$): $\delta$/ppm 14.2; 14.3; 21.0; 21.1; 25.6; 27.4; 29.0; 29.3; 29.6; 32.7; 32.8; 60.44; 63.0; 122.9; 123.6; 125.6; 127.9; 131.8; 131.9; 133.8; 134.5. EI-MS (70 eV) *m/z* (%) 182 (6.1), 164 (6.3), 135 (6.7), 122 (4.0), 121 (11.3), 111 (3.6), 109 (5.2), 108 (6.0), 107 (8.2), 98 (6.7), 97 (5.2), 96 (12.7), 95 (27.3), 94 (9.2), 93 (25.7), 91 (8.4), 84 (3.0), 83 (7.7), 82 (47.3), 81 (31.8), 80 (15.6), 79 (47.0), 78 (4.7), 77 (11.6), 71 (2.9), 70 (3.8), 69 (9.1), 68 (27.3), 67 (100.0), 66 (6.2), 65 (6.4), 57 (4.4), 56 (2.5), 55 (27.7), 54 (9.8), 53 (9.9), 51 (2.0), 43 (6.8), 42 (2.4), 41 (32.2), 40 (2.0) (Figures S7-S9, in the SI section).

## General experimental procedures

Solvents were purified and dried by conventional methods. Commercial reactants were purchased from Sigma-Aldrich Inc. (Germany) Lipase Novozym 435 (*C. antarctica* B, CaL B) was obtained from Novozymes (USA). The phosphonium salt **3** [(7-acetoxyheptyl)triphenylphosphonium bromide] was prepared according to published procedures.[15]

The degree of advance of the reactions and the purity of reactants were preliminary monitored using analytical (thin layer chromatograph) on silica gel (Kieselgel HF254 from Macherey-Nagel, Germany) and visualized with UV light (254 nm) and/or *p*-anisaldehyde in acidic ethanolic solution. Further analyses were performed by gas chromatography (GC) in a Shimadzu 2014 equipment (Japan), with FID detector and a Carbowax 20M MEGA column (30 m × 0.25 mm × 0.25 μm). The temperature program was 80-15 ºC *per* min, 120 ºC (5 min). $T_{SPLIT}$: 220 ºC, $T_{FID}$: 250 ºC.

GC-MS (GC-mass chromatograph) analyses were done using a QP-2010 Shimadzu GC-MS (Japan), equipped with a AT-5 MS column (Alltech) (30 m × 0.25 mm, 0.25 μm), operated with a constant carrier flow of 1 mL *per* min (He). The temperature program was 70 ºC (1 min), 7 ºC *per* min, 250 ºC. The injector temperature, 250 ºC; the interphase temperature, 300 ºC. Injection (1 μL) was in the split mode. EI-MS (electron ionization-MS) were obtained in scan mode from *m/z* 40 to 350.

Column chromatography was performed using silica gel flash (Kieselgel 60, EM reagent, 230-240 mesh.) from Macherey-Nagel.

NMR (nuclear magnetic resonance) spectra ($^1$H and $^{13}$C) were carried out in a Bruker Avance DPX 400 MHz (Germany) equipment. All experiments were taken at 30 ºC, $CDCl_3$ was used as solvent and TMS as internal standard was purchased to SIgma-Aldrich Inc. (USA).

## Field experiments

Pheromone blends were evaluated in red rubber septa placed in the center of plastic Delta traps (15 × 15 × 25 cm). The traps were distributed within an experimental soybean field (2 Ha) situated in an agricultural experimental station (Estación Experimental Mario A. Cassinoni, Facultad de Agronomía, 32.5° South, 58.0° West). The experiment was conducted for six weeks, during March and April of 2009.

The treatments were blends (15:1, 1:1, 1:0, 0:1) of the synthetic pheromone (*Z*7,*Z*9-12:OH : *Z*7,*Z*9-12:Ac), in all cases with a total septum load of 0.1 mg in 10 μL of hexane. Septa loaded with hexane were used as controls. The experiment was arranged in a randomized block design, with 8 blocks separated by at least 30 m, and 15 m trap separation within a block. The traps were hang just above the height of the foliage, and rotated randomly twice a week upon recording male captures. During the experiment, *C. aporema* larvae were sampled from 10 random plants within each experimental block.

Missing data resulting from traps that fell to the ground between measurements were estimated by an iterative method.[16] Male captures, after log (x + 1) transformation, were analyzed using a factorial ANOVA (analysis of variance) to compare means among treatments, with blocks

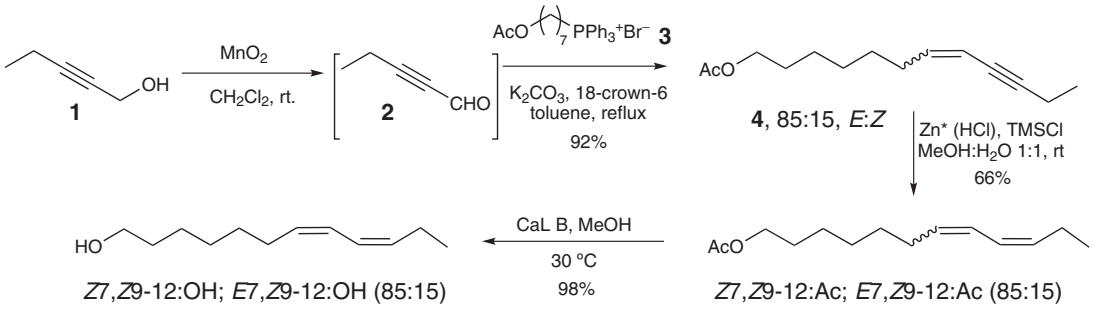


**Figure 1.** Synthetic route to *Z*7,*Z*9-12:OH and *Z*7,*Z*9-12:Ac, components of the sex pheromone of *Crocidosema aporema*.

and time as factors, using the Tukey's test for pairwise comparisons among treatments ($p < 0.05$).

## Results and Discussion

Both pheromone components were prepared through a concise chemoenzymatic synthetic design based on a Wittig olefination and stereoselective Z alkyne reduction. This approach differs from previous reports starting from diynols[17] since it avoids the use of protecting groups and does not afford complex mixtures of stereoisomers. Using 2-pentynol (**1**) as starting material, it was developed a novel oxidation-olefination strategy to afford compound **4** in a rapid and efficient manner (Figure 1). A screening of oxidants and bases was performed, and the best results were obtained using activated $MnO_2$ as the oxidating agent, and a Wittig-Boden protocol[18,19] with phosphonium salt **3**, to yield **4** in an excellent yield (92%) and a good *Z*:*E* stereoselectivity (85:15). The oxidation and alkylation steps may also be achieved *in situ*, as previously shown.[20,21] Phosphonium salt **3** was prepared from readily available 1,7-heptanediol as described elsewhere.[22] The presence of a *J* coupling of 10.7 Hz for the olefinic protons in the $^1$H NMR of the major isomer of **4** confirmed the *Z*-stereochemical assignment, in agreement with previous reports.[17,23]

For the reduction of the conjugated triple bond, the traditional Lindlar hydrogenation protocol[24,25] was not stereoselective, resulting in a mixture of geometrical isomers. Using instead activated Zn as the reducing agent[26] afforded the *Z*7,*Z*9-12:Ac isomer with complete stereoselectivity and 66% yield. Our own previous attempts to obtain the *Z*7,*Z*9-12 carbon skeleton were based on a "one-pot" oxidation-olefination procedure of (*Z*)-2-penten-1-ol (prepared from 2-pentyn-1-ol by Lindlar's reduction). According to previous reports,[27] this procedure would minimize the isomerization of (*Z*)-2-pentenal to the thermodynamically more stable (*E*)- isomer, prior to the olefination reaction with phosphonium salt **3**. Nevertheless, complete isomerization took place, and the decomposition of the Wittig product to a terminal alkene was also detected.

Further hydrolysis of the mixture of *Z*7,*Z*9-12:Ac and *E*7,*Z*9-12:Ac was performed through enzymatic transesterification with lipase B from *Candida antarctica* (CaL B) as biocatalyst, and methanol as nucleophile-solvent, yielding *Z*7,*Z*9-12:OH in 98% yield. This biocatalytic hydrolysis is an efficient and clean strategy for acetate removal, requiring virtually no workup other than filtering the immobilized enzyme.

Overall, the desired pheromone components were prepared in a stereoselective fashion, using a concise 3 step synthesis with a 60% overall yield, therefore constituting a more efficient approach than those previously reported.[28-32]

Traps loaded with hexane (control) and the proportion 0:1 (OH:Ac) did not catch any males and were therefore not included in the statistical analysis. All the remaining treatments captured males, showing a population peak toward the end of the experiment (Figure 2A). Male captures were significantly affected by the three factors: time $F_{10,140} = 8.78$,

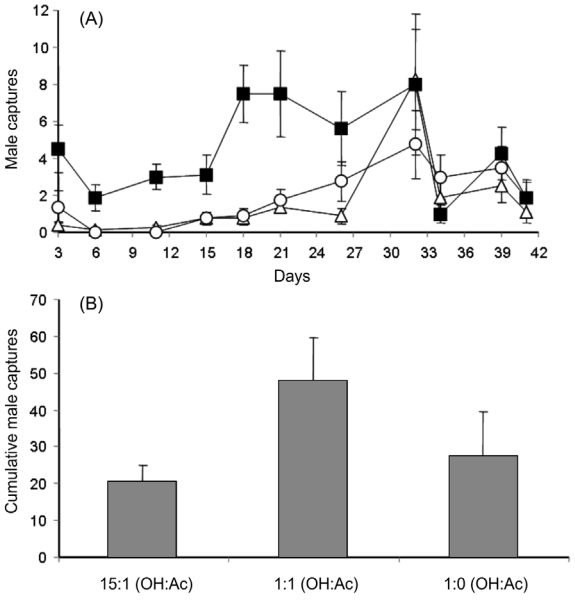


**Figure 2.** Mean (A) and cumulative (B) captures of *Crocidosema aporema* males with different blends of *Z*7,*Z*9-12:OH and *Z*7,*Z*9-12:Ac. The 1:1 blend (black squares) captured significantly more males than the major compound (1:0, open triangles) and the blend in female glands (15:1, open circles) (Tukey's test, $p < 0.05$). Error bars represent SE.

blocks $F_{7,140} = 6.63$, and treatments $F_{2,140} = 30.86$; $p < 0.0001$. Pairwise comparisons among treatments showed that the 1:1 mixture (OH:Ac) was more attractive than either the alcohol alone or the proportion in the gland extracts (15:1) (Tukey's test, $p < 0.05$) (Figure 2). Cumulative captures showed a clear difference between the 1:1 mixture and the other treatments, trapping an average of 48 ± 12 males during the whole experiment (mean ± SE; n = 8 blocks; 11 capture recordings throughout 41 days) (Figure 2B).

The field experiment shows that a small amount of pheromone (0.1 mg initial load *per* septum) is enough for capturing males for several weeks, and that the presence of the *E*,*Z* (about 15%) isomers is not inhibitory for male attraction. Moreover, the synthetic pheromone is attractive enough to capture males even in low population densities. Indeed, only one *C. aporema* larva was found in the 2900 buds monitored during the experiment, which made it impossible to correlate male captures with larval population density. The low population of *C. aporema* during this experiment is probably explained by climatic effects, and contrasts with the 2007/2008 season, during which more than 650 males were captured in a preliminary field experiment with the 1:1 pheromone blend (0.1 mg *per* septa, 6 weeks, data not shown). Such oscillation in population levels among seasons underlines the importance of an efficient monitoring tool for determining the need of chemical treatments against *C. aporema*.

It is worth to note that 25 days into the experiment there was an increase in male captures in the 1:0 and 15:1 treatments, reaching the capture levels of the 1:1 blend (Figure 2A). This probably responds to a peak in the adult population, and shows that the alcohol alone is sufficient for attracting males. This increase in male captures also indicates that a small initial pheromone load (0.1 mg *per* septa) does not evaporate completely for at least one month, highlighting the monitoring power of the pheromone.

Differing from the wind tunnel experiments, in which more males responded to the pheromone blend found in female glands (15:1; OH:Ac),[14] more males were captured in the field with the 1:1 mixture. This result shows the importance of conducting behavioral experiments both in the laboratory and the field. Wind tunnel and field experiments with semiochemicals are indeed not redundant, inasmuch as they differ both in their spatial and temporal scales. Considering the later, wind tunnel experiments are conducted with freshly-loaded dispensers, which emit the pheromone blend only during the duration of a given replicate (usually 10 min), and under controlled temperature conditions. To the contrary, dispensers in the field emit pheromone for several days or weeks, depending on the rate of septum replacement, and under more variable field conditions. When taking into account the spatial component of both experiments, it is also clear that different results may be obtained from them. The behavior of the male in the wind tunnel is studied in close proximity to the dispenser, about 1 or 2 m depending on the tunnel dimensions. In the field, however, male catches depend on several other factors, among them, the long-range attraction of the males. A blend that performs better in close proximity to the trap may not be the best for long-range attraction because the relative proportions of the blend vary with the distance from the trap, depending on the relative volatility of the compounds. It is therefore likely that changes in the pheromone blend in time and space explain the difference between wind tunnel and field experiments. In our experiment, it is proposed that the blend becomes slowly enriched in the alcohol, given that it is less volatile than the acetate[33,34] (Vp = 4.37E-4 torr and Vp = 6.35E-4 torr, respectively),[35] and it may therefore be concluded that soon after the experiment begun, the 15:1 (OH:Ac) initial mixture approximated to the 1:0, decreasing hence its attractivity, and that the 1:1 was enriched in the alcohol, becoming more similar to the blend that was most active in the wind tunnel. In fact, the 15:1 and 1:0 initial mixtures performed similarly throughout the experiment, and so did the 1:1 initial mixture towards the end (Figure 2), possibly due to a complete evaporation of the acetate. Similar results have been found for other moth species such as *Eucosma notanthes* (Lepidoptera: Tortricidae), whose pheromone is composed of *Z*8-12:Ac and *Z*8-12:OH,[36] and for *Dioryctria abietivorella* (Lepidoptera: Pyralidae), which produces a mixture of *Z*9,*E*11-14:Ac and *Z*3,*Z*6,*Z*9,*Z*12,*Z*15-25:Hy.[37]

## Conclusions

The *Z*,*Z* conjugated dienes that compose the sex pheromone of *C. aporema* can be synthesized in milligram quantities from readily available 2-pentynol and 1,7-heptanediol, using a "one-pot" oxidation-olefination strategy. Sub-milligram amounts of synthetic pheromone are sufficient to load rubber septa, which under field conditions remain attractive to *C. aporema* males for several weeks, even with a small proportion of the *E*,*Z* stereoisomer stemming from the Wittig reaction. Further work is needed in order to finally develop a monitoring strategy for massive use. Indeed, parameters such as septum load, septum duration, trap height and color, as well as threshold capture levels, need to be optimized for a final monitoring product. It was shown, however, that the two key factors are possible to achieve, namely, a convenient and scalable synthetic route, and the capture of males in field traps baited with synthetic pheromone. Therefore, population monitoring of *C. aporema* in legume crops is practical and feasible, and may result in

a significant reduction in insecticide use when chemical control is employed preventively.

## Supplementary Information

Supplementary information (Figures S1-S9) is available free of charge at http://jbcs.sbq.org.br as a PDF file.

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