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Attraction of *Monema flavescens* males to synthetic blends of sex pheromones

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Abstract

This study was performed in Luanxian County, Hebei Province, China, from June to August of 2014 and 2015. We sought to develop a new and effective method for controlling the moth *Monema flavescens.* We synthesized the principal female sex pheromones and conducted a series of field experiments using traps baited with (E)-8-decen-1-ol (E8-10:OH), (Z)-7,9-decadien-1-ol (Z7,9-10:OH), and (Z)-9,11-dodecadien-1-ol (Z9,11-12:OH), alone or in combination. The number of males captured by traps baited with synthetic E8-10:OH increased when Z7,9-10:OH, Z9,11-12:OH, or both was/were added. Traps baited with a 10:2:1 (w/w/w) mixture of E8-10:OH, Z7,9-10:OH, and Z9,11-12:OH at a total dose of 650 µg septum^{-1} were the most efficient. Further, a delta trap hung about 1.5 m above the ground was very effective. Our work will facilitate safer and more environmentally friendly management of *M. flavescens.*

Key words: *Monema flavescens*, sex pheromone trapping, (E)-8-decen-1-ol, (Z)-7,9-decadien-1-ol, (Z)-9,11-dodecadien-1-ol.

Introduction

The oriental nettle moth, *Monema flavescens* Walker (Lepidoptera Limacodidae), is a serious defoliator of many trees across China, with the exceptions of Guizhou Province and the Tibet Autonomous Region (Ju *et al*., 2008; Li *et al*., 2010; 2013; Han *et al*., 2013). Moth larvae feed on jujube, *Lagerstroemia indica*, *Juglans regia*, *Malus pumila*, *Salix babylonica*, *Populus* spp., and 120 other plants that grow in orchards and parks (Li and Mao, 2009). The larvae create holes and incisions in the backs of leaves, and may even eat all of the leaves, seriously affecting tree growth and reducing fruit yields (Clausen, 1978). The moth is also found in Japan, Korea, and Russia (Siberia) (Lammers, 2004). The larval spines contain poisonous compounds causing serious skin irritation and inflammation; these are major hazards to gardeners and those who tend orchards. Presently, the principal control method is spraying with chemical insecticides. However, chemical control is difficult because apples, pears, and other fruits mature when the larvae cause the most harm; thus, environmental risks are a major concern. Alternative control methods are therefore required such as adoption of mating disruption. Many sex pheromones have been identified from the order Lepidoptera (Ando, 2012; El-Sayed, 2012), and several pheromone traps have been developed to monitor pest populations (Blackmer *et al*., 2008; Boddum *et al*., 2009; Cross *et al*., 2009), trap adult males to suppress pest populations (Jing *et al*., 2010; Yang *et al*., 2012), and disrupt mating (Stelinski *et al*., 2007; Vacas *et al*., 2010; Youm *et al*., 2012).

We previously used gas chromatographic electroantennographic detection and coupled gas chromatography-mass spectrometry to show that the female sex pheromones of Chinese *M. flavescens* include (E)-8-decen-1-ol (E8-10:OH), (Z)-7,9-decadien-1-ol (Z7,9-10:OH), and (Z)-9,11-dodecadien-1-ol (Z9,11-12:OH). These chemicals differ from those identified in a previous study of Japanese moths; namely, E8-10:OH and (E)-7,9-decadien-1-ol (E7,9-10:OH) (Shibasaki *et al*., 2013). However, no long-term trapping data were reported in the cited work. Here, we report a series of field experiments (performed in 2014 and 2015) that evaluated the attractiveness of all three synthetic sex pheromones to *M. flavescens* in commercial orchards located in Luanxian County, Hebei Province, China.

Materials and methods

Insects

Cocoons were collected in late April from pear trees in Luanxian County and placed in a cage to allow eclosion under natural light. Every 24 h, virgin female moths were transferred to cages (40 × 40 × 60 cm; 20 moths per cage) containing dishes filled with a 10% (w/v) sugar solution until use. Two-day-old virgin females were placed in individual cages (6 × 6 × 6 cm) as male traps.

Chemicals

We synthesized E8-10:OH using the scheme of Shibasaki *et al*. (2013) (figure 1). MS: m/z 156 (1) [M^+], 138 (4) [M^+-H_2O], 110 (6) [M^+-C_2H_5OH], 109 (15), 96 (19), 95 (25), 82 (42), 81 (44), 69 (20), 68 (73), 67 (73), 57 (13), 56 (14), 55 (100), 54 (35), 53 (14), 41 (57), 31 (12) [CH_2OH^+]. We prepared Z7,9-10:OH, E7,9-10:OH, and Z9,11-12:OH as described in a previous study of *Parasa lepida lepida* Cramer pheromones (Islam *et al*., 2009) (figures 2 and 3, respectively). MS: Z7,9-10:OH: m/z 154 (1) [M^+], 136 (12) [M^+-H_2O], 121 (11), 111 (6), 108 (7) [M^+-C_2H_5OH], 107 (13), 98 (9), 95 (15) [$C_7H_{11}^+$], 93 (20), 82 (20), 80 (68), 79 (85) [$C_6H_7^+$], 77 (16), 69 (15), 68 (38), 67 (100), 66 (14), 65 (17), 57 (11), 55 (23), 54 (60) , 53 (18), 41 (71) [CH_2=$CHCH_2^+$], 31 (21) [CH_2OH^+]; E7,9-10:OH: m/z 154 (8) [M^+], 136

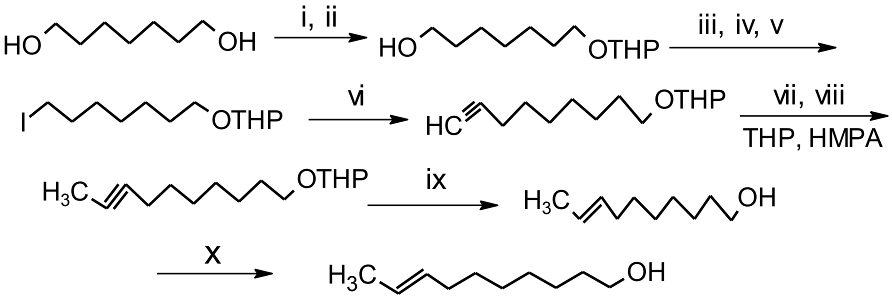

Figure 1. Synthetic pathway to (E)-8-decen-1-ol (E8-10:OH). i, 3,4-dihydro-2H-pyran; ii, HCl; iii, triphenylphosphine; iv, imidazole; v, I_2; vi, lithium acetylide-ethylenediamine complex/ DMSO; vii, iodomethane; viii, butyllithium; ix, Birch reduction with Li in ethylamine ; x, p-TsOH/EtOH/H_2O.

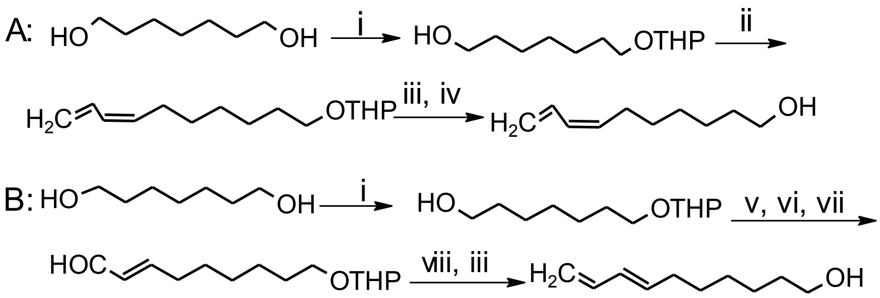

Figure 2. Synthetic pathways to (Z)-7,9-Decadien-1-ol (Z7,9-10:OH, A) and (E)-7,9-Decadien-1-ol (E7,9-10:OH, B). i, 3,4-dihydro-2H-pyran; ii, $CH_2=CHCH=PPh_3$/THF; iii, p-TsOH/EtOH; iv, $(CN)_2C=C(CN)_2$/benzene; v, $CH_3OCOCH=PPh_3$/benzene; vi, $LiAlH_2(OEt)_2$/ether; vii, $(COCl)_2$, DMSO, Et_3N/CH_2Cl_2; viii, $CH_2= PPh_3$/THF.

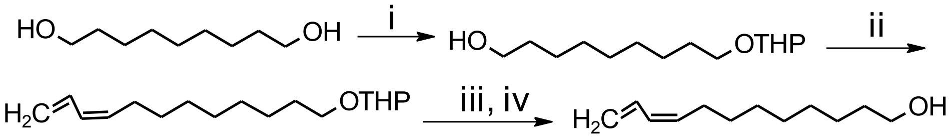

Figure 3. Synthetic pathway to (Z)-9,11-dodecadien-1-ol (Z9,11-12:OH). i, 3,4-dihydro-2H-pyran; ii, $CH_2=CHCH=PPh_3$/THF; iii, p-TsOH/EtOH; iv, $(CN)_2C=C(CN)_2$/benzene.

(15) $[M^+-H_2O]$, 121 (13), 108 (8) $[M^+-C_2H_5OH]$, 107 (13), 95 (20) $[C_7H_{11}^+]$, 93 (20), 82 (26), 81 (42), 80 (71), 79 (85) $[C_6H_7^+]$, 77 (13), 69 (22), 68 (47), 67 (100), 65 (11), 57 (14), 55 (25), 54 (58) , 53 (15), 41 (74) $[CH_2=CHCH_2^+]$, 31 (28) $[CH_2OH^+]$; Z9,11-12:OH: m/z 182 (2) $[M^+]$, 164 (3) $[M^+-H_2O]$, 135 (5), 121 (8), 107 (9), 95 (19) $[C_7H_{11}^+]$, 93 (18), 82 (27), 81 (50), 80 (34), 79 (51) $[C_6H_7^+]$, 77 (11), 69 (14), 68 (63), 67 (100), 65 (15), 57 (8), 55 (63), 54 (75), 53 (19), 41 (72) $[CH_2=CHCH_2^+]$, 31 (25) $[CH_2OH^+]$. All compounds were >98% pure based on gas chromatography. All reagents and solvents were from Fisher Chemicals (Fair Lawn, NJ, USA).

Field tests

Field trials were conducted in peach trees in Luanxian County (39°66'N, 118°72'E) during 2014 and 2015. Pheromone traps were placed during the moth flight seasons. The traps were baited with green rubber septa (190 × 80 mm; Baoji Guangren Biotechnology Co., Shaanxi, China) loaded with test compounds dissolved in n-hexane. We used a randomized block design with six replicates in each trial. The distance between traps within a replicate was ≥50 m. The controls were a trap with hexane only and a net cage containing two-day-old virgin females. The trap catches were counted (i.e., moths stuck to the septa); the sticky septa were replaced daily and the traps were moved at three-day intervals. In experiments 1-4, the traps were placed around trees, approximately 1.5 m above the ground. In experiments 1-3, various pheromone blends were tested in sticky delta traps to determine the optimum ratio and dose. In experiment 4, we compared wing, triangle, and water-basin traps. In experiment 5, we placed traps 0.5, 1.5, and 2.5 m above the ground, corresponding to below the canopy, the middle canopy, and above the canopy of orchard trees, respectively.

Data analysis

A statistical analysis was performed using SPSS 19.0 software (IBM Corp., Armonk, NY, USA). We compared capture data from the field experiments. The Friedman test that is the corresponding test in the case of non-parametric statistical was used. Traps with zero moth catches complicated the initial analysis. Thus, the data only of the treatments that presented caught used a generalized linear model with Poisson and then made the Bonferroni corrections (dividing the desired probability by the number of tests performed). The fact that some compounds were effective allowed us to reject the null hypothesis. In this manner, we effectively considered compounds that did not attract moths. The level of significance for all tests was set at P = 0.05.

Results

Experiment 1: The number of components

Field traps baited with all three components attracted significantly more males than those baited with one or any two components, or virgin females (figure 4). The Japanese pheromones did not attract Chinese moths. A mixture of E8-10:OH, Z9,11-12:OH, and Z7,9-10:OH at a ratio of 10:2:2 (w/w/w) was optimally effective.

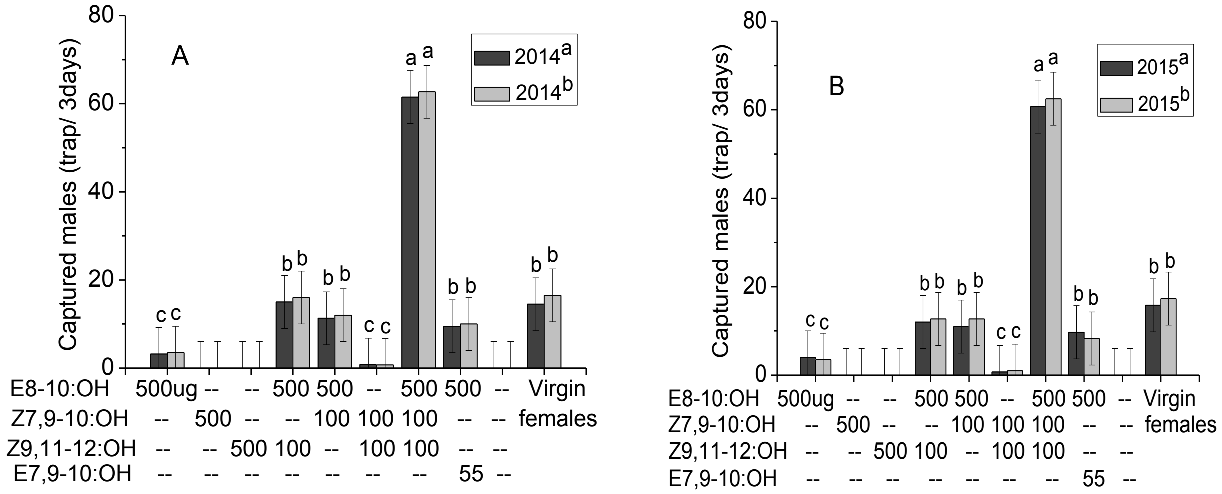

Figure 4. Attraction of *M. flavescens* males to lures baited with synthetic pheromones. [a]Test data from June 1st to June 30th (the first *M. flavescens* flight season). [b]Test data from August 1st to August 30th (the second *M. flavescens* flight season). All field data were analyzed using the non-parametric Friedman test (2014[a]; H = 50.0, d.f. = 9, P = 0.000; 2014[b]; H =49.3, d.f. = 9, P = 0.000; 2015[a]; H =49.3, d.f. = 9, P = 0.000; 2015[b]; H =50.7, d.f. = 9, P = 0.000). Data that presented caught only were analyzed using a generalized linear model with Poisson model (2014[a]; G = 720.7, d.f. = 6, P = 0.000; 2014[b]; G = 729.1, d.f. = 6, P = 0.000; 2015[a]; G = 682.0, d.f. = 6, P = 0.000; 2015[b]; G = 727.3, d.f. = 6, P = 0.000 after application of the Bonferroni correction [P < 0.05/6]). Different letters on the error bars indicate that the data differed significantly.

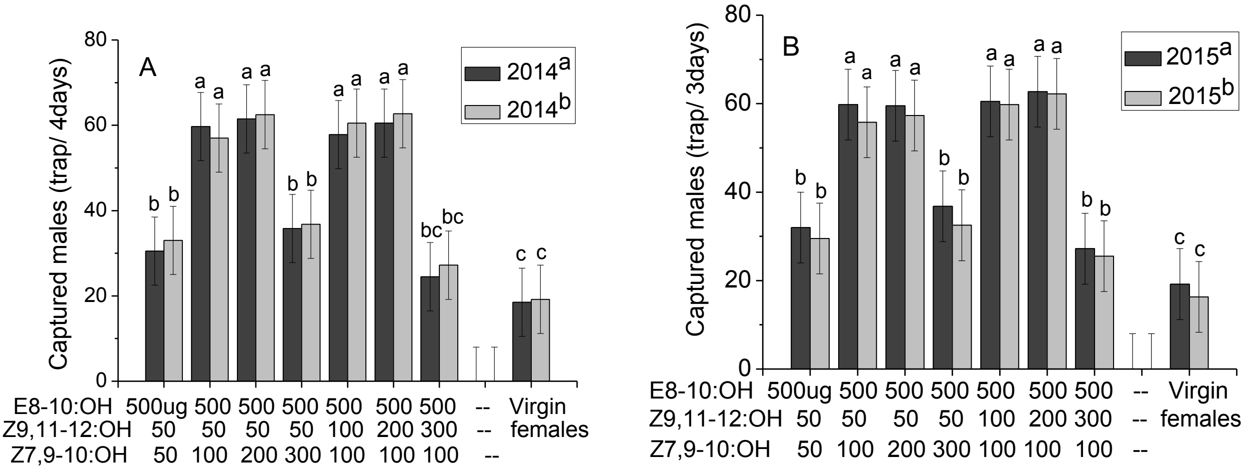

Figure 5. Attraction of *M. flavescens* males to lures baited with synthetic pheromones at different weight ratios. [a]Test data from June 1st to June 30th (the first *M. flavescens* flight season). [b]Test data from August 1st to August 30th (the second *M. flavescens* flight season). All field data were analyzed using the non-parametric Friedman test (2014[a]; H = 39.0, d.f. = 8, P = 0.000; 2014[b]; H =35.1, d.f. = 8, P = 0.000; 2015[a]; H =42.1, d.f. = 8, P = 0.000; 2015[b]; H = 40.8, d.f. = 8, P = 0.000). Data that presented caught only were analyzed using a generalized linear model with Poisson model (2014[a]; G = 337.1, d.f. = 7, P = 0.000; 2014[b]; G = 313.8, d.f. = 7, P = 0.000; 2015[a]; G = 334.7, d.f. = 7, P = 0.000; 2015[b]; G = 352.5, d.f. = 7, P = 0.000 after application of the Bonferroni correction [P < 0.05/7]). Different letters on the error bars indicate that the data differed significantly.

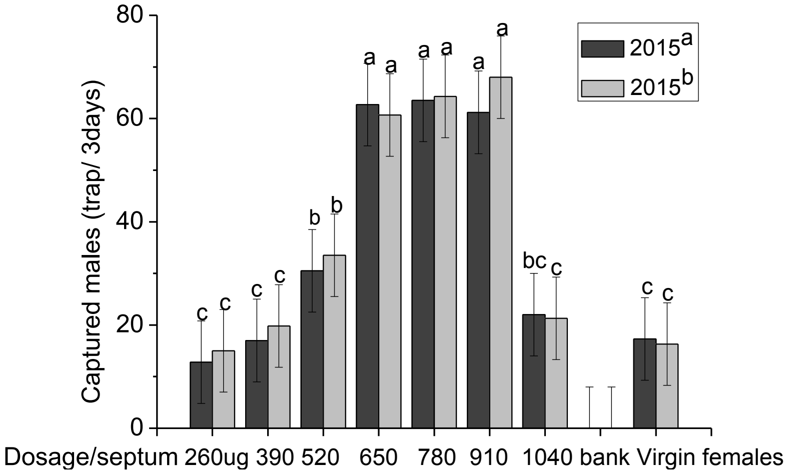

Figure 6. Attraction of *M. flavescens* males to lures baited with different amounts of a 10:2:1 (w/w/w) ratio of E8-10:OH, Z7,9-10:OH, and Z9,11-12:OH. [a]Test data from June 1st to June 30th (the first *M. flavescens* flight season). [b]Test data from August 1st to August 30th (the second *M. flavescens* flight season). All field data were analyzed using the non-parametric Friedman test (2015[a]; H = 43.6, d.f. = 8, P = 0.000; 2015[b]; H = 41.3, d.f. = 8, P = 0.000). Data that presented caught only were analyzed using a generalized linear model with Poisson model (2015[a]; G = 595.7, d.f. = 7, P = 0.000; 2015[b]; G = 595.4, d.f. = 7, P = 0.000 after application of the Bonferroni correction [P < 0.05/7]). Different letters on the error bars indicate that the data differed significantly.

Experiment 2: Optimum ratios of synthetic pheromones

Ratios (w/w/w) of 10:2:4, 10:4:1 and 10:2:1 of E8-10:OH, Z7,9-10:OH, and Z9,11-12:OH were optimal, and attracted males better than did virgin females (figure 5). The numbers of males attracted did not differ significantly between the three ratios; we recommend the use of a 10:2:1 (w/w/w) ratio.

Experiment 3: Optimum pheromone dose

Totals of 650, 780 or 910 µg of the three components (at a 10:2:1 w/w/w ratio) afforded equivalent optimal capture rates (figure 6); we used 650 µg in our subsequent experiments.

Experiment 4: Trap type

We tested three different types of traps using the optimal blend and dose. The number of males caught by wing traps was higher than that caught by delta or water- basin traps, but the latter two trap types were equally effective (figure 7).

Experiment 5: Trap height

No significant difference in catch was evident among traps placed at heights of 0.5, 1.0, and 2.5 m (figure 8).

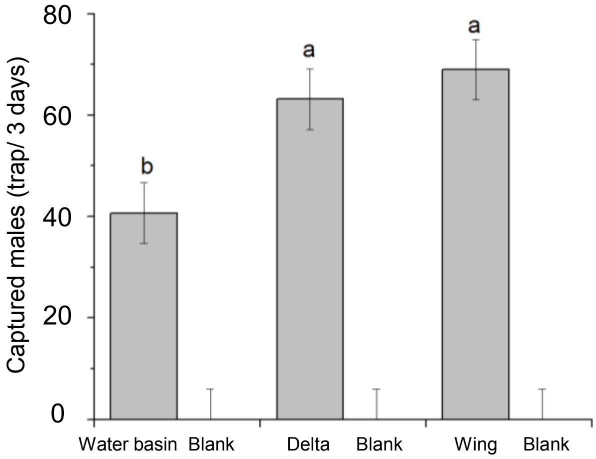

Figure 7. Field data derived using three types of traps baited with a ternary blend of 500 µg of E8-10:OH, 100 µg of Z7,9-10:OH, and 50 µg of Z9,11-12: OH, or empty traps, from June 2nd to August 30th of 2015. All field data were analyzed using the non-parametric Friedman test (H = 29.2, d.f. = 5, P = 0.000). Data that presented caught only were analyzed using a generalized linear model with Poisson model (G = 49.3, d.f. = 2, P = 0.000 after application of the Bonferroni correction [P < 0.05/2]). Different letters on the error bars indicate that the data differed significantly.

Discussion

Synthetic lures trapped male moths efficiently. A ternary blend of E8-10:OH, Z7,9-10:OH, and Z9,11-12:OH (w/w/w ratio 10:2:1) was more effective than were the individual components or binary blends. The composition and optimal weight ratio of our lures differed significantly from the binary mixture of E8-10:OH and E-7,9-10:OH (9:1 w/w) employed by Shibasaki *et al*. (2013). This suggests that pheromone dimorphism is in play in *M. flavescens* males. Similar results were found in studies of *Sparganothis sulfureana* Clemens (Zhu *et al*., 2009) and *Ascotis selenaria* Denis et Schiffermuller (Choi *et al*., 2012). We found that Z9,11-12:OH was an essential lure component. The optimal field lure contained 500 µg of E8-10:OH, 100 µg of Z7,9-10:OH, and 50 µg of Z9,11-12:OH. Wing and delta traps were more efficient than water-basin traps.

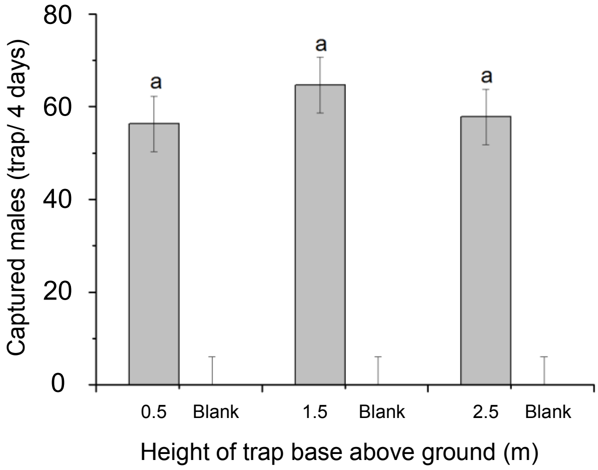

Figure 8. Catches of *M. flavescens* males in delta traps baited with a ternary blend of 500 µg of E8-10:OH, 100 µg of Z7,9-10:OH, and 50 µg of Z9,11-12:OH, or empty traps placed at different heights above the ground from June 2nd to August 30th of 2015. All field data were analyzed using the non-parametric Friedman test (H = 26.9, d.f. = 5, P = 0.000). Data that presented caught only were analyzed using a generalized linear model with Poisson model (G = 3.9, d.f. = 2, P = 0.140 after application of the Bonferroni correction [0.05/2 < P]). Different letters on the error bars indicate that the data differed significantly.

Sometimes, scales were evident on the traps, indicating that some moths had escaped. The adhesive may thus have been inadequately strong. Delta traps deployed at heights of 0.5, 1.5, and 2.5 m were equally effective. For practical field work, we recommend the use of sticky delta traps baited with a total of 650 µg of a ternary blend of E8-10:OH, Z7,9-10:OH, and Z9,11-12:OH at a ratio 10:2:1 (w/w/w), conveniently hung about 1.5 m above the ground. Blends of sex pheromones and attraction antagonists can be very effective tree protectants (Sanders, 1997; Fadamiro and Baker, 2002; Ryne *et al*., 2006) when employed in environmentally friendly integrated pest management programs.

However, some problems remain. Component synthesis must become more convenient and scalable. Also, septum life, trap volume, and component volatility require optimization.

Acknowledgements

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