

ISOLATION, IDENTIFICATION AND FIELD TESTS OF THE SEX PHEROMONE OF THE CARAMBOLA FRUIT BORER, *Eucosma notanthes*

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Abstract—Two components, (Z)-8-dodecenyl acetate (Z8-12:Ac) and (Z)-8-dodecenol (Z8-12:OH), were isolated from sex pheromone glands of the carambola fruit borer, *Eucosma notanthes*, and were identified by GC, and GC-MS, chemical derivatization, and comparison of retention times. The ratio of the alcohol to acetate in the sex pheromone extracts was 2.7. However, synthetic mixtures (1 mg) in ratios ranging from 0.5 to 1.5 were more effective than other blends in trapping male moths in field tests.

Key Words—Carambola fruit borer, *Eucosma notanthes*, sex pheromone, (Z)-8-dodecenyl acetate, (Z)-8-dodecenol.

INTRODUCTION

The carambola fruit borer, *Eucosma notanthes* Meyrick (Lepidoptera: Tortricidae), is a key pest of carambola (also known as star fruit), *Averrhoa carambola* L., in Taiwan (Ho, 1985). This insect has 8 generations per year and may attack carambola fruits all year. Its population density remains high from July to

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November. At fruiting time, the moths lay eggs on the surface, and the larvae penetrate into the fruits, causing severe damage. Damaged fruits drop or become unedible and unmarketable. Although farmers usually apply insecticides or bag the fruits to protect them from attack by fruit flies and borers, the fruit damage rate varies from 29 to 77% (Ho, 1988a,b).

Recently, (Z)-8-dodecenyl acetate (Z8-12:Ac) was found to be an effective sex attractant for this borer in field tests and laboratory bioassays (Hwang et al., 1987, 1996; Hwang and Hung, 1994). High concentrations of this component used as a mating disruptant resulted in significant control of the borer (Hwang and Hung, 1997). Therefore, Z8-12:Ac was suspected to be one of the sex pheromone components of the carambola fruit borer. However, the natural sex pheromone components and their blend for this borer were still not yet known. In this study, two components of the sex pheromone were extracted from calling virgin females and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Attraction of the moth to different blend ratios of the sex pheromone components was determined in field tests.

METHODS AND MATERIALS

Insects. Experimental colonies of *E. notanthes* were initially collected from carambola orchards at Changhua, Taiwan. Larvae were mass-reared on a diet at $25 \pm 1^\circ\text{C}$, $70 \pm 5\%$ relative humidity, and 12 hr photoperiod (Hung and Hwang, 1991). Sexes were identified at the pupal stage and maintained separately in 200-ml plastic jars until eclosion. Adult moths were supplied with 5% honey in water and maintained in plastic bags (30 \times 40 cm).

Preparation of Sex Pheromone Gland Extracts. At the maximum calling and mating time between 1–3 hr after lights on, ovipositors of 1–4-day-old virgin females were excised (Hung et al., 1997). Pheromone glands were immersed in *n*-hexane for 5 sec and the extract was stored at -19°C .

Sources and Purity of Authentic Samples. Authentic samples used for comparison and bioassay were as follows: decanol (Lancaster, 98%), dodecyl acetate (Aldrich, 97%), (Z)-7-dodecenyl acetate (Z7-12:Ac) (Instituut voor Planteziektenkundig Onderzoek, the Netherlands, >99%), (Z)-8-dodecenyl acetate (Z8-12:Ac) (Sigma, 96.8%), (Z)-8-dodecenyl acetate (Instituut voor Planteziektenkundig Onderzoek, >99%), (Z)-9-dodecenyl acetate (Z9-12:Ac) (Instituut voor Planteziektenkundig Onderzoek, >99%), (E)-8-dodecenyl acetate (E8-12:Ac) (Sigma), dodecanol (12:OH) (Lancaster, 97%), (E)-8-dodecenol (E8-12:OH) (Chemtech B. V., the Netherlands), (Z)-8-dodecenol (Z8-12:OH) (Instituut voor Planteziektenkundig Onderzoek, >99%), 1-tetradecanol (Fluka, 97%), 1-hexadecanol (Aldrich, 99%), 1-octadecyl acetate (TCI, 99%) and 1-octadecanol (Lancaster,

97%). Three additional authentic samples, decyl acetate, 1-tetradecyl acetate and 1-hexadecyl acetate, were synthesized from decanol, 1-tetradecanol, and 1-hexadecanol, respectively.

Chemical Analysis. Gas chromatography analyses were performed on a Hewlett-Packard 5890A GC (Avondale, Pennsylvania) with a capillary DB-Wax column (30 m \times 0.25 mm; 0.25- μ m; J&W Scientific, Folsom, CA). The oven temperature was set at 150°C for 20 min, then increased at 8°C/min to final temperature, 240°C. GC-MS spectra were recorded on a Finnigan MAT GCQ system equipped with an ion trap mass analyzer and a DB-Wax capillary column. The temperature program was the same as above. Electron impact (EI) and chemical ionization (CI) mass spectral data of natural and authentic compounds were recorded at 70 eV with the transfer line at 230°C and the ion source at 200°C. Methane was used as a reagent gas for CI analysis. The extract solution of 52,820 ovipositors prepared as described above was subsequently concentrated to 1 ml, and analyzed with the external standard method.

Derivatization. Retention times and retention indices were determined with (Z)- and (E)-8-12:Ac, and (Z)- and (E)-8-12:OH as described above in GC analysis. Double bond position and configuration were determined by the mass spectral fragmentation patterns of their dimethyl disulfide (DMDS) derivatives, which were synthesized following the procedure of Buser et al. (1983) and Leonhardt and DeVilbiss (1985). GC-MS was equipped with a CP-Sil 8 CB fused silica capillary column (Chrompack, the Netherlands) (30 m \times 0.25 mm; 0.25 μ m). Chromatography was carried out with a temperature program initially at 80°C for 2 min, then increasing at 20°C/min to 140°C, followed by increasing at 4°C/min to 240°C.

Field Tests. To verify the attractiveness of the pheromone components to males of *E. notanthes* and to develop an optimized trap lure, seven field experiments were conducted. The numbers of males captured in traps baited with different components or blend ratios of two authentic compounds were calculated and evaluated. In an attempt to realize the relationship between the attractiveness and the double bond position in the components, three analogs, Z7-12:Ac, Z8-12:Ac and Z9-12:Ac, were used for attraction tests. In addition, both Z8-12:Ac and Z8-12:OH were suspected to be the main pheromone components; therefore, various blends of these two components were also tested. Since the synthetic Z8-12:Ac always contains an impurity of E8-12:Ac, which could affect the attractiveness of Z8-12:Ac, 7 blends of Z8-12:Ac and E8-12:Ac were examined.

Field tests were conducted in carambola orchards at Changhua, Taiwan, between August 1996 and October 1997. Each lure comprising 1 mg of content was absorbed on a rubber septa (Aldrich, Z-12435-4) and glued to a wing sticky trap (Taiwan Jia-Fu Co.) (Hung et al. 1999). Each trap was hung at 180–200 cm above the ground, and spaced 1–2 trees (10 ~ 15 m) between traps. A randomized

complete block design was used for the field tests. Four orchards spaced 100 ~ 200 m apart were used as blocks. The area of each orchard was between 0.2 and 0.5 ha. Each orchard had all treatments including pheromone formulations and blank. Treatments within a block were randomized. Traps were checked weekly for 4 ~ 8 weeks and treatments within a block were rotated one position each time (Hwang et al., 1987; Hwang and Hung, 1994).

Statistical analysis was conducted with the SAS system. Trap catch data were subjected to an analysis of variance, and treatment means were separated by Duncan's multiple range test.

In the first experiment, each of the three isomers, i.e., Z7-12:Ac, Z8-12:Ac and Z9-12:Ac, was tested. Each isomer was mixed individually with Z8-12:OH in a ratio of 1 : 1 and tested for the second experiment. In the third experiment, Z8-12:Ac and Z8-12:OH were assayed individually in three orchards. In the fourth experiment, five different ratios of Z8-12:Ac to Z8-12:OH were tested, namely, 100 : 0, 100 : 1, 100 : 100, 100 : 200, and 0 : 100. In the fifth and sixth experiments, ratios of Z8-12:Ac and Z8-12:OH were reevaluated in order to obtain the optimum ratio of these two components for maximum capture of males in pheromone-baited traps. Additionally, five and three ratios of Z8-12:Ac and Z8-12:OH in 100 : 5, 100 : 10, 100 : 50, 100 : 100, 100 : 150, and 100 : 100, 100 : 150, 100 : 270, respectively, were assayed. In the last experiment, seven ratios of Z8-12:Ac and E8-12:Ac in 100 : 0, 99.5 : 0.5, 98 : 2, 96 : 4, 94 : 6, 92 : 8, or 0 : 100 were tested.

RESULTS

Chemical Analysis. A small amount of sex pheromone was isolated from a total of 4,043 females. Only four peaks were found in the gas chromatogram; two major peaks, 1 and 4, and two minor peaks, 2 and 3 (Figure 1B). The retention times of these four peaks matched those of 4 authentic samples; peaks 1 and 4 gave the same retention times as Z8-12:Ac and Z8-12:OH, respectively, while peaks 2 and 3 were also coincided with 12:OH and E8-12:OH, respectively (Figure 1A, B). The gland extracts and 14 authentic samples were analyzed by GC-MS (Figure 1C, D). The retention times of 14 authentic samples are shown as peak 1 to 14 in Figure 1C. Each peak was identified as follows: 1 (10:Ac, 5.02), 2 (10:OH, 6.38), 3 (12:Ac, 10.47), 4 (E8-12:Ac, 11.82), 5 (Z8-12:Ac, 12.35), 6 (12:OH, 13.98), 7 (E8-12:OH, 16.02), 8 (Z8-12:OH, 16.72), 9(14:Ac, 22.37), 10 (14:OH, 24.87), 11 (16:Ac, 27.72), 12 (16:OH, 28.95), 13 (18:Ac, 30.85) and 14 (18:OH, 31.75 min). The sex pheromone extracts show 12 peaks in the gas chromatogram (Figure 1D). Their retention times were as follows: 1(9.52), 2(10.13), 3(11.30), 4(12.33), 5(16.83), 6(25.88), 7(29.77), 8(30.15), 9(31.50), 10(31.73), 11(31.83), and 12(33.08 min). Comparison of the retention times and EI and CI mass spectra of the fourth and fifth peaks of the extracts with those of synthetic Z8-12:Ac and

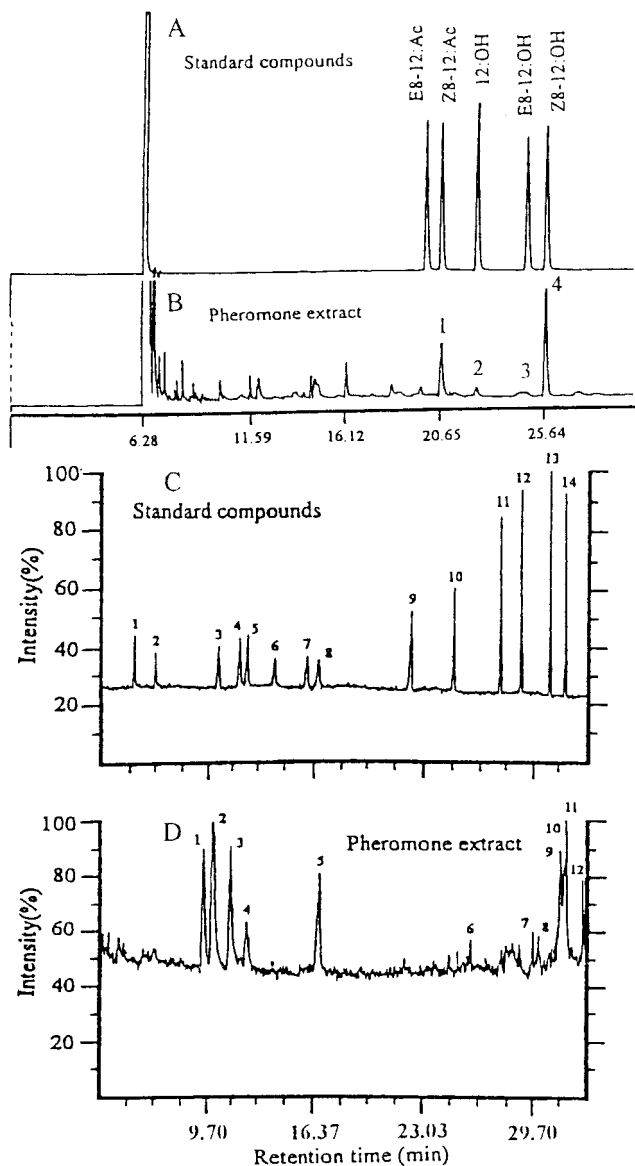


FIG. 1. Comparative GC (A, B) and GC-MS (C, D) chromatograms of pheromone extracts produced by females of *Eucosma notanthes* and standard compounds. Fourteen standard compounds are shown as peaks 1 to 14 in C: 1 (10:Ac), 2 (10:OH), 3 (12:Ac), 4 (E8-12:Ac), 5 (Z8-12:Ac), 6 (12:OH), 7 (E8-12:OH), 8 (Z8-12:OH), 9 (14:Ac), 10 (14:OH), 11 (16:Ac), 12 (16:OH), 13 (18:Ac) and 14 (18:OH).

TABLE 1. CI MASS SPECTRAL DATA FOR THE FOURTH AND FIFTH PEAKS OF *Eucosma notanthes* PHEROMONE EXTRACTS AND STANDARD COMPOUNDS OF Z8-12:AC AND Z8-12:OH

Compound	Source	Mass spectral data, m/z (Intensity, %)
Z8-12:Ac	Synthetic	227($M+1^+$, 12.31), 165(9.23), 137(9.23), 123(21.54), 111(70.77), 97(78.46), 81(81.54), 69(100), 55(73.85), 43(23.08)
	Natural (peak 4)	227($M+1^+$, 5.88), 165(2.94), 151(2.94), 137(5.88), 123(14.71), 109(38.24), 95(61.76), 81(94.12), 67(100), 55(44.12), 43(8.82)
Z8-12:OH	Synthetic	185($M+1^+$, 2.60), 173(1.30), 165(1.30), 137(5.19), 123(18.18), 109(48.05), 95(67.53), 81(76.62), 69(100), 55(89.61), 41(74.03)
	Natural (peak 5)	185($M+1^+$, 1.11), 173(1.11), 165(1.11), 149(1.11), 141(1.11), 137(5.56), 123(14.44), 109(38.89), 95(64.44), 91(4.44), 85(2.22), 81(92.22), 71(4.44), 67(100), 55(68.89), 43(8.89)

Z8-12:OH suggested that peak 4 and 5 were Z8-12:Ac and Z8-12:OH, respectively (Table 1). The EI mass spectra of Z8-12:Ac have two characteristic peaks, one with $m/z = 61$, which is a typical fragmentation peak of acetates, (AcOH_2^+), and the other with $m/z = 166$, which corresponds to ($M\text{-AcOH}$) $^+$. In contrast, the EI mass spectra of Z8-12:OH do not show any peak with $m/z = 61$ (AcOH_2^+). Therefore, it can be differentiated from acetate to alcohol. The results of our EI mass spectra of peak 4 and 5 are consistent with Z8-12:Ac and Z8-12:OH. The CI mass spectra of Z8-12:Ac and peak 4 both have a mother molecular ion peak with $m/z = 227$ ($M+1^+$) and all identical fragmentation patterns (Table 1). Similarly, the CI mass spectra of Z8-12:OH and peak 5 also have a mother molecular peak with $m/z = 185$ ($M+1^+$) and are consistent with the spectral data (Table 1). These data indicate that peak 4 corresponds to Z8-12:Ac and peak 5 to Z8-12:OH. The total amounts of Z8-12:Ac and Z8-12:OH obtained from the extraction of 52,820 females were 63.37 and 169.32 μg , respectively. This is equivalent to a ratio of Z8-12:OH:Z8-12:Ac of 2.7.

Mass spectral analysis of DMDS derivatives resolved the isomer identification of the dodecenyl acetate and dodecenol. Comparison with the data from Buser et al. (1983) and Leonhardt and DeVilbiss (1985), our derivatized standards showed the diagnostic peaks to be [M^+320 (29.2%), A^+103 (2.1%), B^+217 (64.4%)] for Z8-12:Ac at a retention time of 27.77 min; [M^+320 (25.2%), A^+103 (2.0%), B^+217 (61.9%)] for *E*8-12:Ac at 28.03 min; [M^+278 (44.7%), A^+103 (2.5%), B^+175 (29.6%)] for Z8-12:OH at 25.23 min; and [M^+278 (39.3%), A^+103 (2.6%), B^+175 (28.1%)] for *E*8-12:OH at 25.53 min. The derivatives of two components from the pheromone gland extracts also gave diagnostic peaks of [M^+320 (31.7%), A^+103

(2.2%), B⁺217 (69.0%)], at a retention time of 27.78 min; and [M⁺278 (39.7%), A⁺103 (2.3%), B⁺175 (27.5%)], at 25.30 min, confirming their identities as Z8-12:Ac and Z8-12:OH, respectively.

Field Tests. In the initial comparison of three isomers, Z7-12:Ac, Z8-12:Ac and Z9-12:Ac, as trap lures, the greatest numbers of *E. notanthes* males were captured in traps baited with Z8-12:Ac. Other isomers either failed to capture any male or the number of males captured was less than that in unbaited traps (ANOVA $F = 3.96$, $df = 79$, $0.01 < P < 0.001$) (Figure 2A). The total number of *E. notanthes* males captured was 134.

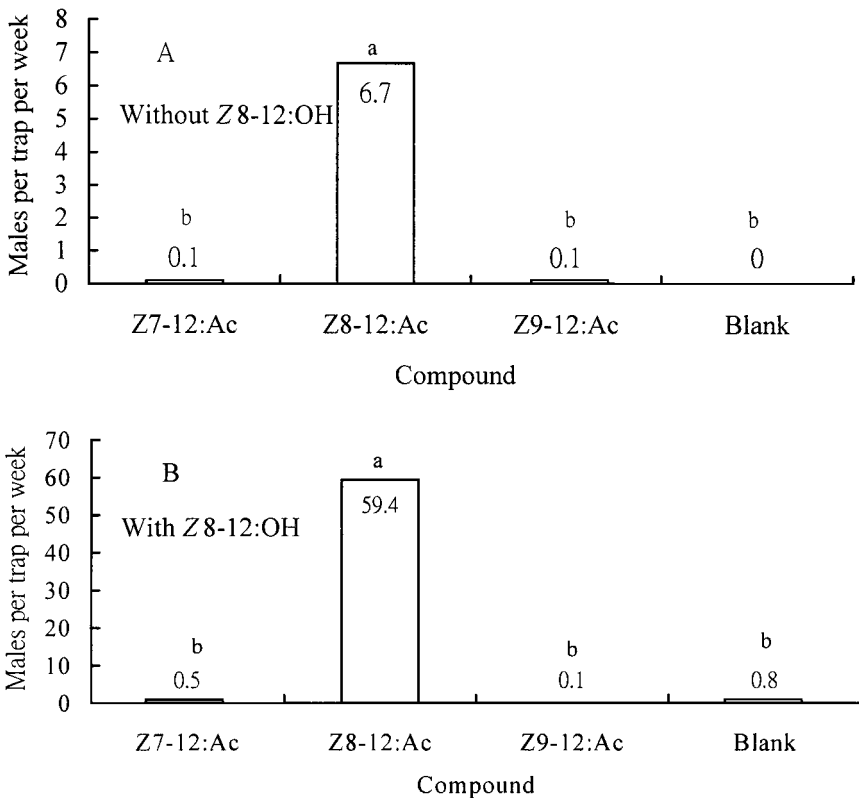


FIG. 2. Mean numbers of male *Eucosma notanthes* moths captured in carambola orchards in traps baited with three isomers, Z7-12:Ac, Z8-12:Ac, and Z9-12:Ac, alone (A) or with equal amount Z8-12:OH (B). Experiments were conducted at Changhua, Taiwan, on the following dates: experiment A, July 2–August 6, 1997; experiment B, August 6–September 10, 1997. Bars with the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).

In the second experiment, where three isomers, Z7-12:Ac, Z8-12:Ac and Z9-12:Ac, were mixed individually with Z8-12:OH in ratio of 1 : 1. The greatest numbers of *E. notanthes* males were captured in traps baited with Z8-12:Ac and Z8-12:OH. In the other two mixtures, the numbers of males captured were nearly 0 and not different from those in unbaited traps (ANOVA $F = 31.41$, $df = 79$, $P < 0.001$) (Figure 2B). The total number of males captured in this experiment was 1,214.

In the third experiment, in which Z8-12:Ac and Z8-12:OH were compared, the greatest numbers of males were captured in traps baited with Z8-12:Ac, ranging from 13 to 208 males/trap/week (mean 60.1), while those captured with Z8-12:OH and in the blank were only 1.1 and 0 males/trap/week, respectively (ANOVA $F = 15.4$, $df = 35$, $P < 0.001$) (data not shown). The total number of males captured was 734.

In the fourth experiment, in which comparison of various ratios of Z8-12:Ac and Z8-12:OH were made, the greatest numbers of males were captured in traps baited with Z8-12:Ac and Z8-12:OH at a load ratio of 100 : 100. Significant numbers of males were also captured in traps baited with ratios of 100 : 1 and 100 : 200. Capture of males with the remaining ratios were rather small and did not differ from those in unbaited traps (ANOVA $F = 47.87$, $df = 119$, $P < 0.001$) (Figure 3A). The total number of males captured was 2,420.

In the fifth experiment, where the ratios of Z8-12:Ac and Z8-12:OH were 100 : 5, 100 : 10, 100 : 50, 100 : 100 and 100 : 150, the numbers of *E. notanthes* males captured in traps baited with a load ratio of 100 : 100 did not differ from those with 100 : 50 and 100 : 150, while they were greater than those captured in traps baited with two other load ratios of 100 : 5 and 100 : 10. (ANOVA $F = 12.69$, $df = 143$, $P < 0.001$) (Figure 3B). The total number of males captured was 2,115.

In the sixth experiment, where three ratios of Z8-12:Ac and Z18-12:OH were 100 : 100, 100 : 150, and 100:270, the numbers of *E. notanthes* males captured in traps baited with a load ratio of 100 : 100 did not differ from those with 100 : 150, while they were greater than those baited with 100 : 270. (ANOVA $F = 16.96$, $df = 99$, $P < 0.001$) (Figure 3C). The total number of males captured was 4,539.

In the last experiment, where Z8-12:Ac and E8-12:Ac were used, in ratios ranging from 100 : 0 to 0 : 100, the greatest numbers of males were captured in traps baited with a load ratio of 100 : 0. This was not greater than numbers of males captured in traps baited with these compounds at a load ratio of 99.5 : 0.5. Almost no moths was captured in traps baited with these compounds in other load ratios (ANOVA $F = 3.93$, $df = 253$, $P < 0.001$) (Figure 4). A total of 1,285 males were captured.

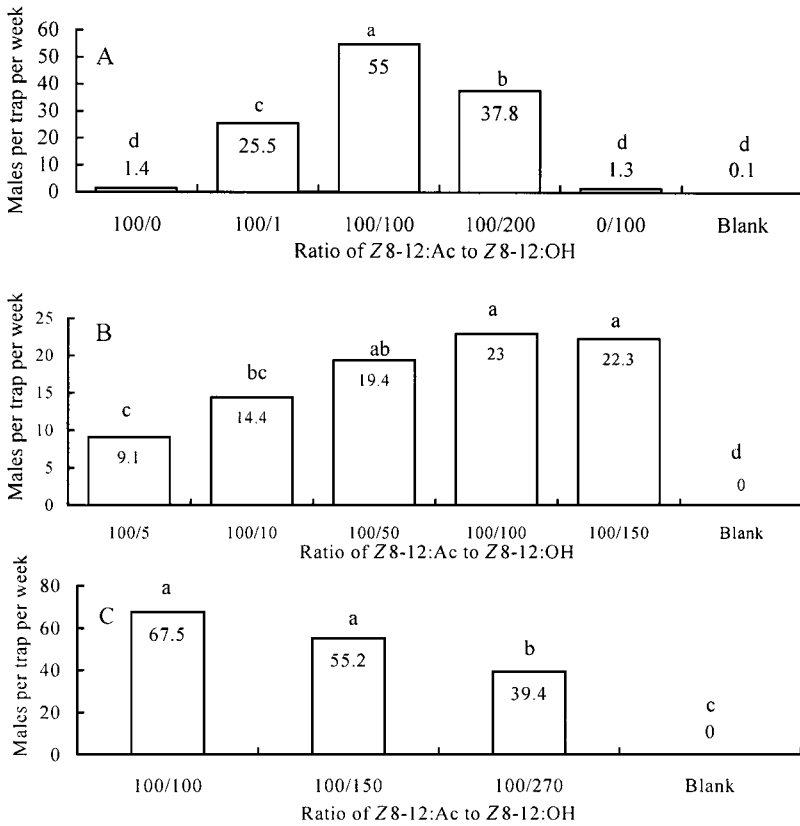


FIG. 3. Mean numbers of male *Eucosma notanthes* moths captured in traps baited with different blend ratios of Z8-12:Ac mixed with Z8-12:OH in carambola orchards. The first comparison (A) consisted of a broader range of ratios, while comparison of (B) and (C) consisted of a range of ratios closer to that found in the best efficiency lure for trapping carambola fruit borer. Experiments were conducted at Changhua, Taiwan, on the following dates: experiment A, March 19–April 23, 1997; experiment B, April 30–June 11, 1997; experiment C, December 22, 1999–February 10, 2000. Bars with the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).

DISCUSSION

Our results indicate that the sex pheromone of *E. notanthes* comprises two compounds, i.e., Z8-12:Ac and Z8-12:OH. Both compounds in combinations or Z8-12:Ac alone can capture males, indicating that these compounds are sex pheromone components of this species.

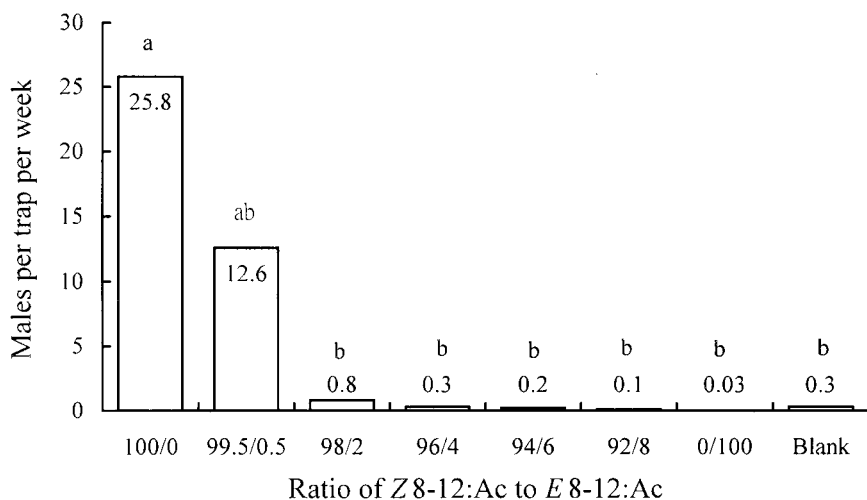


FIG. 4. Mean numbers of male *Eucosma notanthes* moths captured in traps baited with different blend ratios of Z8-12:Ac mixed with E8-12:Ac in carambola orchards at Changhua, Taiwan, from August 1 to November 11, 1996. Bars with the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).

The compound, Z8-12:Ac, is an attractant for more than 40 insect species, and has been found in females of three species, *Cryptophlebia leucotreta*, *Grapholita molesta* and *Hedya nubiferana*, in which it was found with other components including E8-12:Ac, 12:OH, 12:Ac, E5-12:Ac, and Z8-12:OH. However, only Z8-12:OH is reported to be an attractant of four insect species, and was identified as a sex pheromone component for the Oriental fruit moth, *G. molesta* (Kydonieus et al., 1982). The sex pheromone components of *G. molesta* have been reported to be Z8-12:Ac and E8-12:Ac, and Z8-12:OH and 12:OH (Roelofs et al., 1969; Cardé et al., 1979; Lacey and Sanders, 1992). In this study, two of these four components, i.e., Z8-12:Ac and Z8-12:OH, were identified as sex pheromone components of *E. notanthes*. Ratios of Z8-12:Ac and Z8-12:OH ranging from 1:0.5 to 1:1.5 were more attractive to *E. notanthes* males in orchards, whereas the ratio of these two compounds was 1:2.7 in hexane extracts of sex pheromone glands. A concentration of 0.5% or more of E8-12:Ac inhibited attraction to *E. notanthes* by the lures containing Z8-12:Ac, while 6–7% and 2.2% of E8-12:Ac was active as a pheromone component with Z8-12:Ac in attracting *G. molesta* and the lesser apple worm, *Grapholita prunivora*, respectively. However, when the amount of E8-12:Ac reached 23% in lures, the trap catch to males of both species decreased (Roelofs and Cardé, 1974). Therefore, isomeric purity of Z8-12:Ac is important for attractiveness of lures to *E. notanthes*.

In conclusion, we identified two compounds, Z8-12:Ac and Z8-12:OH, to be sex pheromone components of *E. notanthes*. Furthermore, based on field tests, Z8-12:Ac is regarded as the main component, while Z8-12:OH per se does not attract males.

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