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Note

Female sex pheromone of a Japanese population of allium leafminer, *Acrolepiopsis sapporensis* (Lepidoptera: Acrolepiidae)

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Three monoenyl compounds were detected in GC/MS analysis of a hexane extract of pheromone glands from virgin female *Acrolepiopsis sapporensis*. They were identified as (Z)-11-hexadecenyl acetate (Z11-16:Ac), (*Z*)-11-hexadecenal (Z11-16:Ald) and (*Z*)-11-hexadecenol (Z11-16:OH), respectively, by chemical derivatization and synthesis of both geometric isomers. The ratio of three components in the sex pheromone gland of female moths was 100 : 10 : 23 with GC/MS analyses. In field tests, blends of the three components were evidenced to attract male moths in a Welsh onion field. Synthetic (*Z*)-11-hexadecenal was the principal component to catch more males.

Keywords: *Acrolepiopsis sapporensis*; female sex pheromone; field attraction; (*Z*)-11-hexadecenyl compound; Lepidoptera.

Introduction

Allium leafminer, *Acrolepiopsis sapporensis* Matsumura (=*Acrolepia alliella*),[1] is an economically important pest of garlic, welsh onion and green chive, belonging to the Allium species.[2] In order to clarify the population dynamics of *A. sapporensis*, an authentic female sex pheromone blend, (*Z*)-11-hexadecenyl acetate (Z11-16:Ac) and (*Z*)-11-hexadecenal (Z11-16:Ald), of *Plutella xylostella* (L.) was tentatively employed in Okabe-shi, Fukushima in 1977 and 1978,[1] and the binary blend was active against *A. sapporensis*. By field screening, more *A. sapporensis* males were captured with traps baited with a 45 : 45 : 10 blend of Z11-16:Ac, Z11-16:Ald and (*Z*)-11-hexadecenol (Z11-16:OH). Additionally, the activity of the lure was confirmed by two other groups in Tsu-shi, Mie in 1978[3] and at Hachioji-shi, Tokyo in 1978.[4] A 5 : 5 : 1[3] or 1 : 3 : 1[4] blend was found to be optimal to attract male moths, although the components of female gland extracts of a Japanese population of *A. sapporensis* have not been identified. We report here the identification of the components of female pheromone glands from *A. sapporensis* and their attractant activity against male moths in Kameoka-shi, Kyoto.

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Materials and Methods

1. Insects

A. sapporensis pupae were collected from *Allium grayi* grown wild along roadsides of rice fields in Sogabe-cho, Kameoka-shi, Kyoto Prefecture in May 2007. Immatures were sexed at the pupal stage and maintained in separate Petri dishes. Emerging adults were collected individually every 24 h and kept in containers without food.

2. Gland extraction

Extract was obtained from 3-day-old virgin females. The abdominal tip with the pheromone gland excised from the female abdomen with a fine knife was collected into a microvial (Vial Insert, Part Number 5181-1270; Agilent Technologies) and extracted with hexane (5 abdominal tips/3 μl for GC/MS, and 12 tips/10 μl for DMDS-GC/MS) for 3 min.

3. DMDS derivatization

To determine the double bond position, the crude extract (12 FE, in 10 μl of hexane) led to DMDS derivatives as follows; the extract was placed in a handmade ampoule, and the solvent was removed by N_2 stream. Freshly distilled DMDS (5 μl), in which a very small crystal of iodine had previously been dissolved to give a pale yellow color, was added and the ampoule was sealed. The ampoule was kept overnight at 60°C. The reaction mixture was subjected to GC/MS analysis with 5 min solvent cut.

4. Chemical analysis

The extract was subjected to GC-MS (Agilent Technologies 6890N Network GC System, electron impact ionization at 70 eV), using a capillary column (HP-5MS, 0.25 mm i.d.×30 m; Agilent Technologies Santa Clara, CA). The pheromone extract was analyzed with a column temperature program of 60°C for 2 min and then 10°C/min to 290°C (5 min hold). To determine geometric isomerism, synthetic compounds were analyzed by GC (Agilent Technologies 6890N Network GC System) equipped with a flame ionization detector (FID), using the polar InertCap® Wax column (0.25 mm i.d.×30 m, GL Sciences). The oven was programmed from 50°C (3 min hold) to 230°C at 10°C/min and held at this temperature for 5 min.

5. Chemicals

We synthesized (*E*)- and (*Z*)-isomers of 11-hexadecenyl acetate (E11-16:Ac and Z11-16:Ac) and 11-hexadecenol according to the process described.[5] The isomers (*E*)- and (*Z*)-11-hexadecenal (E11-16:Ald and Z11-16:Ald) were prepared by Collins oxi-

dation of the corresponding alcohol (*E*)- and (*Z*)-11-hexadecenol (E11-16:OH and Z11-16:OH), respectively.

6. Field tests

The field test was carried out in Kyoto (Kameoka-shi) between 5 and 12 July 2007. The chemicals were mixed in several different ratios to impregnate polyethylene septa (7 mm OD, white; Scienceware®) by applying 20 μl of hexane solution and were used as dispensers on the upper side of fly-catch sticky-trap ribbon (27 cm×5 cm; Kamoi Kakoshi Co.). Traps were set 40 cm above the ground at about 5-m intervals beside a Welsh onion field. Tests were conducted with one trap for each lure, and the numbers of catches were recorded daily for 3 nights. Trap data were analyzed by ANOVA, and treatment means were compared using Tukey's test.

Results and Discussion

As shown in Fig. 1, compounds A, B and C were eluted at t_R=17.06, t_R=17.71 and t_R=18.97, respectively. Hydrocarbons (a, b, d, f, g and h) and fatty acids (c and e) were detected as contamination derived from the surface and inside the body of female moths. In the mass spectrum for compound A, a base ion peak was observed at *m*/*z* 55 (100%), although the molecular ion at *m*/*z* 238 was undetectable. The fragmentation pattern of compound A was virtually identical to that of hexadecenal derived from Agilent NIST05 Mass spectral library search. For compound B, characteristic ions were *m*/*z* 222 (M^+-18, 10%) and 55 (100%). The ion of M^+-H_2O at *m*/*z* 222 and the fragmentation pattern of compound B from the mass spectral library suggested that compound B was hexadecenol. For compound C, characteristic fragment ions were observed at *m*/*z* 222 (M^+-60, 19%), 82 (100%) and 61 ($CH_3CO_2H+H^+$, 14%). Although the molecular ion at *m*/*z* 282 was not observed, $M^+-CH_3CO_2H$ ion at *m*/*z* 222

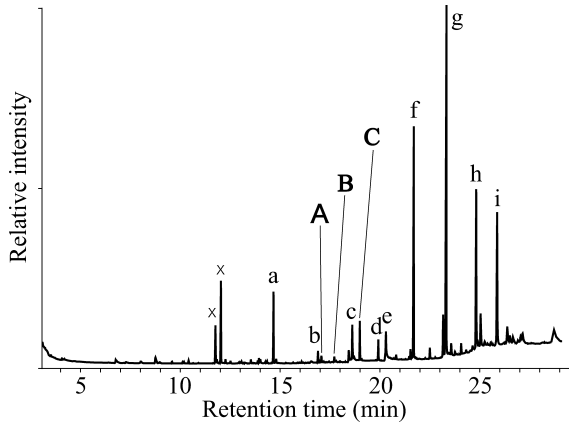

Fig. 1. GC-MS analysis of the pheromone gland extracts of *Acrolepiopsis sapporensis* females (5 FE). GC column: HP-5MS. Identification of peaks: A, (*Z*)-11-hexadecenal (Z11-16:Ald); B, (*Z*)-11-hexadecenol (Z11-16:OH); and C, (*Z*)-11-hexadeceny acetate (Z11-16:Ac). a, hexadecene; b, octadecene; c, hexadecanoic acid; d, heneicosane; e, octadecenoic acid; f, tricosane; g, pentacosane; h, heptacosane; i, squalene.

and $CH_3CO_2H_2^+$ at *m*/*z* 61 indicated that compound C was hexadecenyl acetate. The DMDS adduct derived from compound C at t_R=24.50 min showed molecular ions at *m*/*z* 376, and diagnostic fragment ions at *m*/*z* 259 ($[CH_3SCH(CH_2)_{10}OCOCH_3]^+$) and *m*/*z* 117 ($[CH_3(CH_2)_3CHSCH_3]^+$), which indicated the double bond at the 11-position of the C_{16} chain. Unfortunately, the DMDS adducts of compound A and B were not detected due to the small quantity and many impurities. Based on the biosynthetic aspect, all three compounds were presumed to have a double bond at the 11-position.

To determine the position and geometry of the double bond of the compounds and to provide preparations for field bioassays, we synthesized (*E*)- and (*Z*)-isomers of 11-hexadecenyl acetate (E11-16:Ac and Z11-16:Ac), 11-hexadecenal (E11-16:Ald and Z11-16:Ald) and 11-hexadecenol (E11-16:OH and Z11-16:OH). Each pair of geometric isomers of the synthetic 11-hexadecenyl acetate, 11-hexadecenal and 11-hexadecenol was indistinguishable on the HP-5MS column under GC-MS conditions; however, the retention time and mass spectra of the three compounds were identical to those of the natural components. To determine geometric isomerism, the synthetic compounds were analyzed by GC using the polar InertCap® Wax column. Isomers E11-16:Ac and Z11-16:Ac were easily resolved (15.00 min and 15.57 min, respectively). Similarly, separation of E11-16:Ald and Z11-16:Ald (24.39 min and 24.52 min), and E11-16:OH and Z11-16:OH (18.89 min and 19.21 min) was achieved by using the same capillary column. The corresponding compounds A, B and C in the gland extract (10 abdominal tips/3 μl) had the same retention times as those of (*Z*)-isomers. Based on these results, the three compounds were identified as Z11-16:Ac (*ca.* 2.1 ng/female), Z11-16:Ald (*ca.* 0.29 ng/female) and Z11-16:OH (*ca.* 0.69 ng/female), at a ratio of 100 : 10 : 23.

Previous studies have described that the major component was a binary blend of Z11-16:Ac and Z11-16:Ald to attract *A. sapporensis* males in field tests.[1,3,4] In our field test, very few *A. sapporensis* males were captured with single compounds or two-component lures of Z11-16:Ac and Z11-16:Ald (Fig. 2). When 20% of Z11-16:OH was added to the two-component lures, more males were captured with the 20 : 140 blend than with other blends. The change of the Z11-16:OH dose to the corresponding binary blends did not increase trap catches (Fig. 3). According to previous field tests by Ando *et al.* in Hachioji-shi (Tokyo), *A. sapporensis* were attracted more strongly to the 1 : 3 : 1 blend of Z11-16:Ac, Z11-16:Ald and Z11-16:OH.[4] In our field tests in Kameoka-shi (Kyoto), the ratio of 1 : 7 : 2 (=20 : 140 : 40) was the most successful in attracting males among the lures tested, although strong attraction was also observed in a ratio of 1 : 3 : 1 (=40 : 120 : 40). The amount of Z11-16:Ald was significant in the blend ratio of three components. The pheromone gland of *A. sapporensis* includes a trace quantity of Z11-16:OH, although the compound was as essential as Z11-16:Ac to capture more males. The ratio of the ternary blend derived from virgin female moths did not parallel the attraction in our field tests. We presented identification and field experiments of the sex pheromone gland components of *A. sapporensis* at an international academic meet-

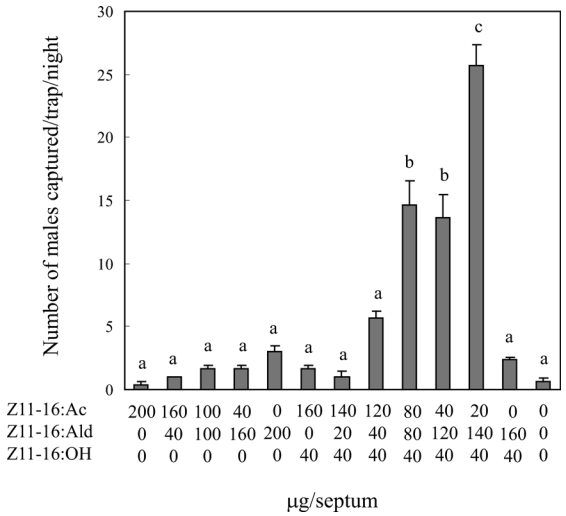

Fig. 2. Captures of *Acrolepiopsis sapporensis* males with synthetic sex pheromone in a Welsh onion field (5–12 July 2007, Kameoka, Kyoto). Values are the means of 1 trap for 3 nights. Means capped with the same letter are not significantly different by Tukey's HSD test ($p<0.05$). Bars represent the mean±S.E. (n=3). (*Z*)-11-hexadeceny acetate (Z11-16:Ac), (*Z*)-11-hexadecenal (Z11-16:Ald), (*Z*)-11-hexadecenol (Z11-16:OH).

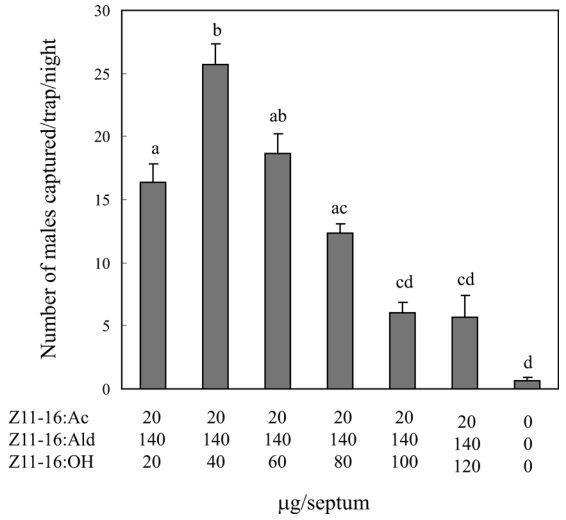

Fig. 3. Captures of *Acrolepiopsis sapporensis* males with various ratios of Z11-16:OH in binary blends of Z11-16:Ac (20 μg) +Z11-16:Ald (140 μg). Values are the means of 1 trap for 3 nights. Means capped with the same letter are not significantly different by Tukey's HSD test ($p<0.05$). Bars represent the mean±S.E. (n=3).

ing in 2007.[6]

In previous trials in Japan, a 45 : 45 : 10 (Okabe-shi, Fukushima)[1] or 5:5:1 (Tsu-shi, Mie)[3] blend of Z11-16:Ac, Z11-16:Ald and Z11-16:OH was found to be optimal for attracting a Japanese population of *A. sapporensis* males. Our most successful blend ratio for attracting males was 1 : 7 : 2, containing mainly Z11-16:Ald. In contrast, males of a Korean population were strongly attracted to a 65:25:10 or 75:25:10 blend of Z11-16:Ac, Z11-16:Ald and Z11-16:OH, containing relatively large amounts of acetate.[7] These results were nearly identical to the natural ratio (100 : 33 : 14) of the ternary blend from the Korean population of *A. sapporensis* females. Consequently, the results of the field tests showed different trends not only in East Asia but also in Japanese regions, suggesting significant geographic variation.

The monoenyl compounds, which might be biosynthesized *via* the Δ11-desaturation of palmitic acid,[8] are common pheromone components of many lepidopteran species, particularly the noctuid species. In the genus *Acrolepiopsis*, the female sex pheromone of *A. assectella* (Zeller) has been identified as Z11-16:Ald,[9] and an improved formulation was determined to be a 1 : 10 blend of Z11-16:Ac and Z11-16:Ald.[10] Three components, Z11-16:Ac, Z11-16:Ald and Z11-16:OH, were identified in female extracts of *A. nagaimo* and the blends of the former two compounds attracted more males in field tests.[11] The addition of Z11-16:OH to the binary sex pheromone blends of Z11-16:Ac and Z11-16:Ald, however, did not affect captures of *A. nagaimo* males. The composition of pheromone gland components of *A. sapporensis* females is similar to that of *A. nagaimo* females. The example of *A. sapporensis*, for which three components, Z11-16:Ac, Z11-16:Ald and Z11-16:OH, are indispensable for male attraction, has never been seen in the genus *Acrolepiopsis*. The optimal ratio of ternary blends for attracting *A. sapporensis* males should be determined by region in Japan and other areas of the world.

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