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HOST PLANT TERPENES AFFECTING AGE-RELATED DIFFERENCES IN OLFACTORY ORIENTATION OF THE CERAMBYCID BEETLE, *MONOCHAMUS ALTERNATUS* HOPE

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マツノマダラカミキリの嗅覚的定位行動における日令による差に影響を及ぼす寄主植物テルペン

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Age-related differences in laboratory flight- and walking responses by female and male adults of the cerambycid beetle, *Monochamus alternatus* HOPE, to three fractions of essential oil from paraquat-induced lightwood and α-pinene from the oil were examined in comparison with those to the oil. α-Pinene elicited responses by both immature sexes. Instead, a minor constituent fraction induced responses by both mature sexes.

パラコート誘導ライトウッドから得た精油を低温液体クロマトグラフィー及びガスクロマトグラフィーによって種々の画分に分画し，さらにα-pineneを単離した．これらの試料に対する雌雄のマツノマダラカミキリ成虫の室内飛翔反応及び歩行反応を日令との関連で調べた．これらの結果，精油中のα-pineneは雌雄の未成熟成虫を，微量成分画分は雌雄の成熟成虫を誘引することが明らかとなった．

Introduction

The cerambycid beetle, *Monochamus alternatus* HOPE, is the vector for the transmission of the pine wood nematode, *Bursaphelenchus xylophilus* (STEINER et BUHRER 1934) NICKLE 1970, which causes pine wood disease[1,2].

Recently, the sesquiterpene alcohol (+)-juniperol and the diterpene aldehyde (+)-pimaral were isolated from paraquat-induced lightwood and identified as attractants for both sexes, especially for females[3]. Besides, the monoterpene alcohol (+)-*cis*-3-pinen-2-ol from lightwood was identified as a male-specific attractant[4]. The attraction of the beetle also is caused by a synergistic effect of ethanol and monoterpene hydrocarbons[5].

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No studies, however, have been conducted on any age-related differences in the cerambycid beetle's response to the attractants. The phytophagous beetle, *M. alternatus*, requiring a long maturation feeding period after emergence[6] is interesting as an experimental insect.

The present paper deals with age-related differences in *M. alternatus* response to essential oil from lightwood and its host terpenes responsible for the differences.

Materials and Methods

Materials. Eighteen-year-old *Pinus densiflora* Sieb. et Zucc. were frill-treated with paraquat early in May 1988. Lightwood areas formed in the xylems were harvested in mid-July 1988.

Essential oil was obtained by steam distillation from freshly crushed lightwood. The oil was fractionated into a major terpene hydrocarbon fraction (*n*-pentane eluate) and a minor constituent fraction (ether eluate) by low-temperature liquid chromatography[7,8]. The terpene hydrocarbon fraction was gas chromatographed on a 10% polyethylene glycol glass column (50 to 120℃) at a program rate of 2℃/min on a semi-preparative scale[9]. α-Pinene which passed through a delivery Teflon tube kept at 90℃ was collected in cold traps. Impurities originating from the column were removed by thin layer chromatography (TLC) using *n*-pentane as a mobile phase. Gas chromatograms on a J & W DB-5 and a J & W DB-1301 fused silica capillary columns[9] of an ethereal solution of the compound, showed a single peak, except that of the solvent. Its infrared absorption spectrum and retention time coincided with those of the commercial (1*S*,5*S*)-(−)-α-pinene (assay : 98.8%), respectively. Other constituents in the terpene hydrocarbon fraction were collected as a fraction without α-pinene. Impurities were removed by TLC.

The Insect. Newly emerged adults from logs (being kept cross in outdoor cages) of pines infested in 1990 were collected every morning from June 9 to July 19, 1991. Emergence of seven and more females a day occurred June 14, 21, 23, 25, July 1, 3 (maximum mumber : 11), 5, 8, and 12. Males' : June 13, 18, 20, 25, 29 (maximum : 11), July 1, 3, 5, 9, and 15. These only adults were marked with white or yellow spot(s) and separated by sex and emergence date. Members (7 to 11 each) of the 10 male groups and 9 female ones were paired with other adults. The members of each group were kept close in age to their respective spouses (maximal difference, 4 days; average, 1.4 days). The 19 groups thus treated were held in 50 × 50 × 50-cm indoor cages containing fresh pine twigs or branches without leaves during the test period.

Test procedures. Hourly individual tests for flight responses of the entire member of a female or a male group of an age to each of test samples were conducted a night using a 6.8 × 2.6 × 3.8-m chamber type olfactometer according to a previous method[3]. Aliquots (50 μl × 4) of a *n*-pentane solution of a sample were delivered at 15-min intervals. Additional tests of the samples with other three groups of the same sex which reached the same age were followed in the same manner. Thus, quartet tests were conducted by sex and age. In fact, either 21 to 28 females or 21 to 27 males of three different ages were placed in the olfactometer.

The walking response of one individual randomized from a famale or a male group of an age was observed for 10 min a night using a 200 × 9 × 9-cm walkway type olfactometer, as previously described[10]. An aliquot (50 μl) of a *n*-pentane solution of a sample was presented. In the aggregate, walking responses of seven to eight individuals of the group to the sample were observed in the night using the two walkway type olfactometers. Additional tests of the same sample were run some night using seven to eight individuals of another group of the same sex which reached the same age. Thus, duplicate tests were done by sex and age.

When the number of members of a group decreased to six, the group was not used for any test. The tests for flight- and walking responses were closed September 8 and 10, respectively. Responsive activity, a percentage of the beetles responding to a test sample, was represented by the mean of the four or two replications.

Results and Discussion

Response to essential oil. *n*-Pentane was not active to both sexes of any ages tested (Fig. 1-F). Significant flight responses (about 70%) of both sexes at least 10 hours to 2 days old to the essential oil from paraquat-induced lightwood occurred in the chamber type olfactometer (Fig. 1-A). The responses diminished with age, dropped to a level of about 26%, 10 days after emergence. Females 14 to 56 days old and males 12 to 49 days old exhibited responses of 23% and more. Particularly, both sexes about 30 to 43 days old kept levels of 37% and more. Females over 56 days old and males over 49 days old showed limited responses only.

Response to minor constituent fraction. No flight responses of both sexes of less than about 13 days to the minor constituent fraction were observed by using the chamber type olfactometer (Fig. 1-B). No walking responses of such young adults to the fraction also were observed by using the walkway type olfactometer, except that limited those by both sexes one day old occurred (Fig. 2-A). Flight responses of both sexes to the fraction occurred about 14 days after emergence, increased gradually, and reached a long stretch (about 3 to 8 weeks after emergence) of levels of 24% and more (Fig. 1-B). Especially the adults of about 30 to 43 days kept conspicuous levels (average : about 40%). No walking responses of both sexes less than 14 days old also were observed, except that limited responses by adults one day old occurred (Fig. 2-A). Conspicuous walking responses of both sexes occurred 14 days after emergence, increased gradually, reached a plateau (about 25 to 42 days after emergence) of levels of 65 to 70%, and diminished (Fig. 2-A). Copulation and oviposition begin 10 days and about 3 weeks after emergence, respectively[11]. Oviposition reaches its peak 45 days after emergence[6]. The adults which exhibited the laboratory flight- or walking responses to the minor consutituent fraction, thereby, appear to be reproducible ones. It has been confirmed that, of many constituents present in the fraction, only (+)-juniperol, (+)-pimaral, and (+)-*cis*-3-pinen-2-ol are attractants for the beetle of uncertain age[3,4,9]. The present results, therefore, indicate that the three compounds are attractive to mature adults, but not to immature ones.

Attraction of α-pinene without other terpene hydrocarbons. In contrast to the results obtained with the minor constituent fraction, newly emerged adults were significantly attracted to the terpene hydrocarbon fraction containing α-pinene (Fig. 1-C). The responses diminished with age and dropped to a level of about 7%, 16 days after emergence. Being over such an age-grade, the adults were indifferent to the fraction, although limited responses were rarely observed. Similar results were obtained with the walkway type olfactometer (Fig. 2-B).

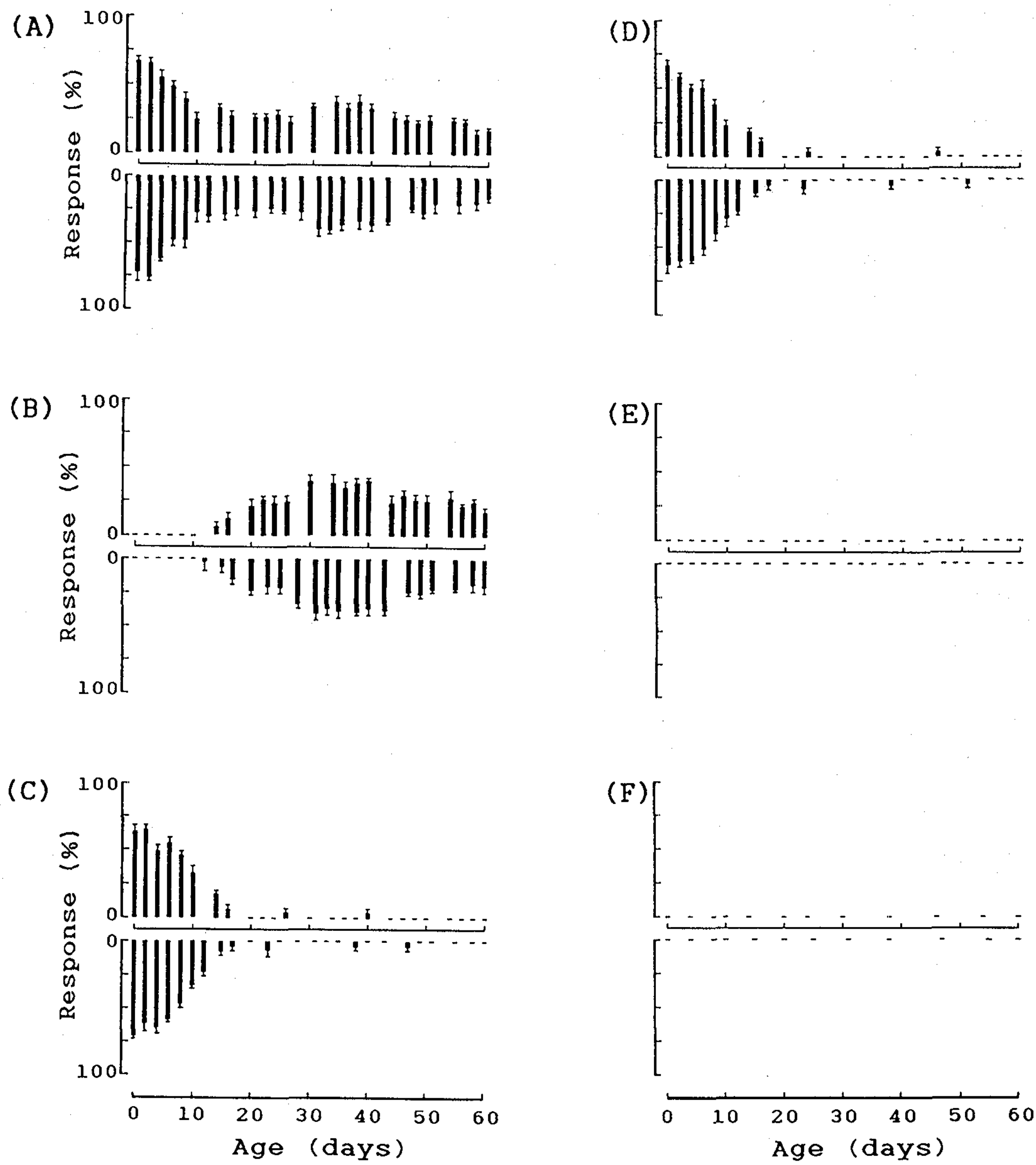

Fig. 1 Age-related flight responses of *M. alternatus* to essential oil (A), minor constituent fraction (B), terpene hydrocarbon fraction containing α-pinene (C), α-pinene (D), terpene hydrocarbon fraction without α-pinene (E), and *n*-pentane (F).
Upward bar, female; downward, male. Dose (50 μl/15 min) : (A), solutes (34.8 mg) in *n*-pentane(10 μl); (B), 272 μl in *n*-pentane (50 μl); (C), 34.5 mg in *n*-pentane (10 μl); (D), 23.0 mg in *n*-pentane (24 μl); (E), 6.27 mg in *n*-pentane (43 μl). The doses are based on the quantitative constituent-composition of the essential oil. Blank; air only. Only three females (0-, 6-, 34-d-old) and males (0-,4-, 33-d-old) responded to blank on a series of the tests of A; one female (40-d-old) and two males (35-, 43-d-old), on B; one female (2-d-old) and male (4-d-old) each and two males (0-d-old), on C; two females (2-, 4-d-old) and males (0-, 4-d-old), on D. These numbers, however, were not taken into account.

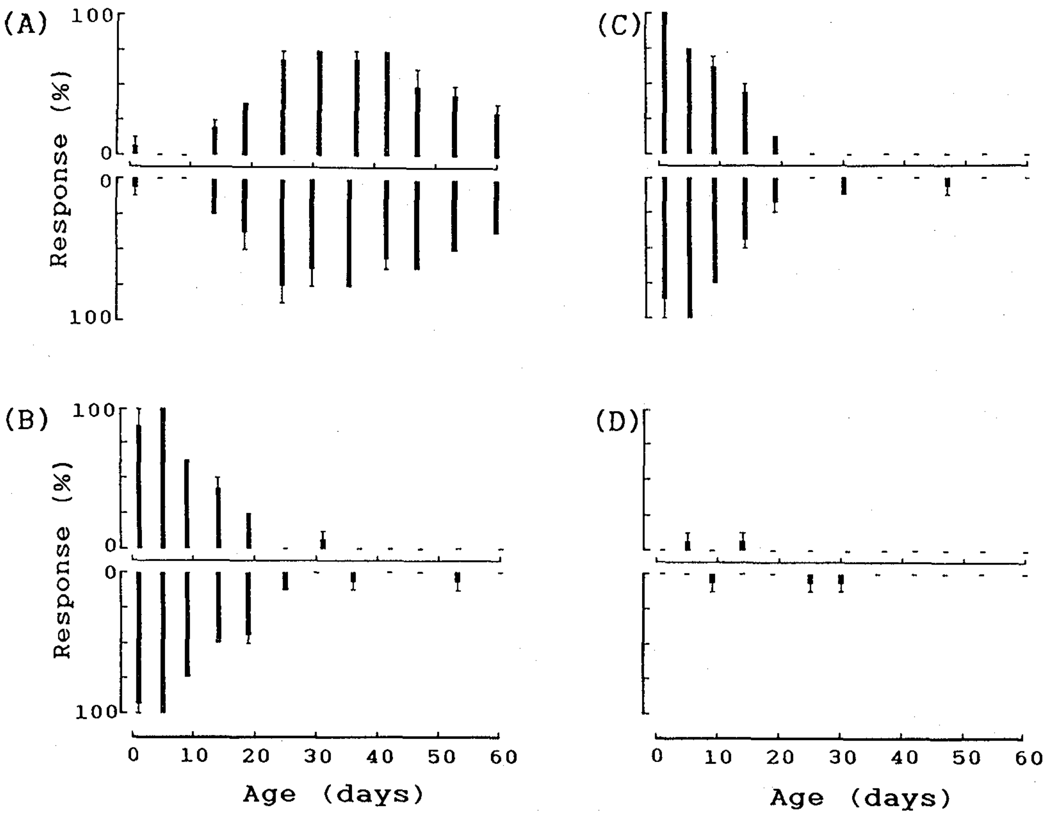

Fig. 2 Age-related walking responses of *M. alternatus* to minor constituent fraction (A), terpene hydrocarbon fraction containing α-pinene (B), α-pinene (C), and terpene hydrocarbon fraction without α-pinene (D)
Upward bar, female; downward, male. Dose (50 μl/10 min) : (A), solutes (272 μg) in *n*-pentane (50 μl); (B), 34.5 mg in *n*-pentane (10 μl); (C), 23.0 mg in *n*-pentane (24 μl); (D), 6.27 mg in *n*-pentane (43 μl). The doses are based on the quantitative constituent-composition of the essential oil.

α-Pinene predominated in the terpene hydrocarbon fraction from lightwood : the fraction consisted of α-pinene (64.6%), camphene (1.2%), sabinene (<0.1%), β-pinene (13.3%), myrcene (1.8%), α-phellandrene (0.2%), α-terpinene (<0.1%), *p*-cymene (<0.1%), limonene (9.9%),γ-terpinene (<0.1%), terpinolene (0.4%), longifolene (5.8%), and others (2.4%). α-Pinene isoltated from the terpene hydrocarbon fraction induced significant flight responses by immature adults, more especially newly emerged ones (Fig. 1-D). The age-ralated differences in the flight responses to α-pinene (Fig. 1-D) were strikingly similar to those to the terpene hydrocarbon fraction containing α-pinene (Fig. 1-C). Furthermore, the profiles of the walking responses to α-pinene (Fig. 2-C) reflected those of the flight responses (Fig. 1-D). Instead, the terpene hydrocarbon fraction without α-pinene stimulated no flight responses by the adults of any ages tested (Fig. 1-E). Limited walking responses to this fraction occurred only.

The present results indicate that the attraction of the terpene hydrocarbon fraction for immature adults is caused by α-pinene. Figure 1 shows that the profiles of the age-related responses to essential oil from praquat-induced lightwood are almost equivalent to a composite of the profiles of the responses to the minor constituent fraction and those to α-pinene or the terpene hydrocarbon fraction containing α-pinene. It is evident from Figures 1 and 2 that the correspondence of the walking response to the flight response is not limited to the tests with α-pinene. No sex-related differences in the response profiles were detected here, except small differences.

Although considerable information on plant attractants has been acquired[12], no studies have dealt with the attractants affecting age-related differences in phytophagous insects' olfactory orientations. The present paper, however, showed host plant terpenes affecting the age-related differences. It is not sure whether the age-related differences in *M. alternatus* response are dependent on the function of sensory systems or odor quality coding.

References

(1) MORIMOTO, K. and IWASAKI, A. : Role of *Monochamus alternatus* (Coleoptera : Cerambycidae) as a vector of *Bursaphelenchus lignicolus* (Nematoda : Aphelenchoididae), *J. Jpn. For. Soc.*, **54**, 177-183 (1972).

(2) MAMIYA, Y. and ENDA, N. : Transmission of *Bursaphelenchus lignicolus* (Nematoda : Aphelenchoididae) by *Monochamus alternatus* (Coleoptera : Cerambycidae), *Nematologica*, **18**, 159-162 (1972).

(3) SAKAI, M. and YAMASAKI, T. : (+)-Juniperol and (+)-pimaral : Attractants for the cerambycid beetle, *Monochamus alternatus* HOPE, *J. Chem. Ecol.*, **16**, 3383-3392 (1990).

(4) SAKAI, M. and YAMASAKI, T. : (+)-*cis*-3-Pinen-2-ol : Attractant for male cerambycid beetle, *Monochamus alternatus* HOPE, *J. Chem. Ecol.*, **17**, 757-765 (1991).

(5) IKEDA, T., ENDA, N., YAMANE, A., ODA, K., and TOYODA, T. : Attractants for the Japanese pine sawer, *Monochamus alternatus* HOPE (Coleoptera : Cerambycidae), *Appl. Entomol. Zool.*, **15**, 358-361 (1980).

(6) ENDA, N. and NOBUCHI, A. : Studies on the pine bark and wood boring beetles. Maturation of the ovary and its parasitic nenatodes, *Trans. 81st Ann. Meet. Jpn. For. Soc.*,1970, p. 274-276 (in Japanese).

(7) YAMASAKI, T., SAKAI, M., KANAMORI, T., and SOGO, M. : Low temperature liquid chromatographic separation of oxygenated terpenes from terpene hydrocarbons, *Chromatographia*, **21**, 478-479 (1986).

(8) SAKAI, M. and YAMASAKI, T. : The attraction of minor constituents, present in the volatile oil from paraquat-induced lightwood, for *Monochamus alternatus* HOPE (Coleoptera : Cerambycidae), *Mokuzai Gakkaishi*, **34**, 246-250 (1988).

(9) SAKAI, M. and YAMASAKI, T. : Screening of attractants for the beetle, *Monochamus alternatus* HOPE, from the volatile oil present in paraquat-induced lightwood, *Mokuzai Gakkaishi*, **35**, 537-542 (1989).

(10) YAMASAKI, T. and SUZUKI, N. : Luring of Japanese pine sawyer *Monochamus alternatus* HOPE by paraquat-treated pine trees (II). The attractiveness of volatile oil from lightwood, *J. Jpn. For. Soc.*, **64**, 340-345 (1982).

(11) OCHI, K. : Ecological studies on cerambycid injurious to pine trees (II) Biology of two *Monochamus* (Coleoptera, Cerambycidae), *J. Jpn. For. Soc.*, **51**, 188-192 (1969).

(12) WAAGE, S. K. and HEDIN, P. A. : Plant stimulants and attractants (kairomones), in "Handbook of natural pesticides," **VI**, ed. by MORGAN, E. D. and MANDAVA, N. B., CRC Press, Florida, 1990, pp.161-180.

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