

スギノアカネトラカミキリおよびトゲヒゲトラカミキリ(甲虫目:カミキリムシ科)の花の香り成分に対する嗅覚反応

誌名	日本林學會誌 = Journal of the Japanese Forestry Society
ISSN	0021485X
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発行元	日本林學會
巻/号	75巻2号
巻号補足	
掲載ページ	p. 108-112
発行年月	1993年3月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター

Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council
Secretariat



 論 文

Olfactory Responses of *Anaglyptus subfasciatus* PIC and *Demonax transilis* BATES (Coleoptera: Cerambycidae) to Flower Scents

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IKEDA, Toshiya, OHYA, Eiji, MAKIHARA, Hiroshi, NAKASHIMA, Tadakazu, SAITOH, Akira, TATE, Kazuo and KOJIMA, Kohichiro: **Olfactory responses of *Anaglyptus subfasciatus* PIC and *Demonax transilis* BATES (Coleoptera: Cerambycidae) to flower scents.** J. Jpn. For. Soc. 75: 108~112, 1993 Fourteen chemicals, being known as flower scents, were screened initially for possible attraction for two species of flower visiting Coleoptera, *Anaglyptus subfasciatus* PIC and *Demonax transilis* BATES. The response was examined on an electro-antenogram (EAG), on an olfactometer, and then with field bioassay using traps. *A. subfasciatus*, one of the most harmful insect pests against Japanese cedar (*Cryptomeria japonica* D. DON) and Japanese cypress [*Chamaecyparis obtusa* (SIEB. et ZUCC) ENDL.], responded most to benzyl acetate in the field test, followed by phenylethyl propionate, and linalool. *D. transilis*, that gives much less damage to cedar and cypress, also responded most to benzyl acetate, followed by linalool and phenylethyl propionate.

池田俊弥・大谷英児・榎原 寛・中島忠一・斉藤 諦・館 和夫・小島耕一郎：スギノアカネトラカミキリおよびトゲヒゲトラカミキリ（甲虫目：カミキリムシ科）の花の香り成分に対する嗅覚反応 日林誌 75：108~112, 1993 訪花昆虫であるスギノアカネトラカミキリとトゲヒゲトラカミキリに対する誘引性について、花の香り成分として知られる14種の化合物が検索された。反応は触角電図、嗅覚計さらにトラップを用いた野外試験で調べられた。日本のスギおよびヒノキの最も有害な害虫の一つであるスギノアカネトラカミキリは、野外試験において、ベンジルアセテートに最も強く誘引され、続いてフェニルエチルプロピオネート、リナロールに反応した。スギ、ヒノキの枯枝で生育することが知られているトゲヒゲトラカミキリもまたベンジルアセテートに最も強く反応し、続いてリナロール、フェニルエチルプロピオネートに誘引された。

I. Introduction

Anaglyptus subfasciatus PIC (Coleoptera: Cerambycidae) is one of the most harmful insect pests against Japanese cedar (*Cryptomeria japonica* D. DON) and Japanese cypress [*Chamaecyparis obtusa* (SIEB. et ZUCC.) ENDL.]. The female lays eggs on dead twigs, and the hatched larva bore into the twigs and then migrates toward the trunk. Vertical feeding by the larva on sapwood followed by a fungus infection causes discoloration and decay of the wood, resulting in a deterioration of the quality and the value of the wood on the market. Adults of both sexes are flower visitors feeding on nectars and pollens. Flowers identified, on which *A. subfasciatus* has been collected, range over 13 families of woody plants including 48 species and four families of herbaceous plants including five species (SAITOH *et al.*, 1987). Most of them are characterized by having many white to whitish yellow colored flowers with small petals, such as *Stephanandra incisa* ZEBEL

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and *Viburnum dilatatum* THUNB.

Generally, scents in diurnal flowers are not strong and act as close-in attractants to entice the landings of insects (KEVAN and BAKER, 1983). However, the cooperative scents of large stands of flowers may act over considerable distances (BOGDANY and TABER, 1979; KEVAN and BAKER, 1983). This might be the present case of *A. subfasciatus*. Many chemicals, included in wide-ranging species of flowers as host locations, are supposed to act as attractants to *A. subfasciatus*. Thus, the chemicals known as components of flower scents in the literature were screened in bioassays.

Demonax transilis BATES (Coleoptera : Cerambycidae) has a much wider host range, not only in coniferous but in broad-leaved trees. The larva feeds on dead twigs and seldom bores into the trunks of Japanese cedar and cypress. A much larger numbers of the beetles, however, have been collected on the same flowers that *A. subfasciatus* occupy. The flower scents relating to *D. transilis* also are being investigated and discussed in contrast to those for *A. subfasciatus*.

II. Methods and Materials

1. Study sites

Field tests were conducted in Japanese cedar stands (0.5~2 ha) in Kanagawa Prefecture, Trials 1 and 2, Yamagata Prefecture, Trials 3 and 4, Hokkaido, Trial 5, and Nagano Prefecture, Trial 6.

2. Chemicals

Fourteen chemicals having been identified as flower scents (Table 1) were supplied by Takasago Perfumery Co., Ltd. All chemicals, except citral, were confirmed to maintain sufficient purity (more than 96%) until the time for the screening tests.

3. Electroantennogram (EAG) response

Adult beetles collected on flowers of *S. incisa* on the forest margins of Japanese cedar in Odawara were reared individually in plastic containers ($\phi 3 \times 4$ cm) with cotton wicks impregnated with water for less than a few days until the time for use. The technique used for the EAGs was a modification of that described by ROELOFS and COMEAU (1971). An antenna was cut off at the second segment from the head, and the distal tips (one segment or less) were removed. Each antenna was placed between the tips of two Pasteur pipets containing saline solution. Ag-AgCl wires were inserted into the saline solution from the other end of the pipet and connected to an amplifier.

Linalool and linalyl acetate were used as standard compounds. Each test chemical ($1\mu\text{l}$), dissolved in $10\mu\text{l}$ of *n*-hexane, was applied to 1 cm^2 piece of filter paper. The filter paper impregnated with a test chemical was placed at the end of a glass syringe (5 ml) whose needle was introduced into a glass tube through a small hole (3 mm diameter). The syringe was used to blow a 2 ml-puff and to mix the compound into a continuous air flow (about 5 ml/sec). The tip of the glass tube was positioned 1 cm from the antenna. A minimum of 30 sec was allowed between each stimulus. At least three replicates were recorded for each chemical. Fourteen chemicals having relatively greater response out of fifty-two chemicals initially tested were submitted to following olfactory and field assays.

4. Behavioral assay on an olfactometer

Adult beetles of *A. subfasciatus*, emerging from Japanese cedar logs in a field cage, were collected every few hours or less, to eliminate the chance of copulation, and were kept individually in a plastic case (10 cm diameter $\times$ 7 cm height) at 10°C for less than three days until the time for use. Adult beetles of *D. transilis* were collected from flowers and maintained in the same manner as in the EAG experiment. The olfactometer device was a glass tube ($\phi 15 \times 80$ cm long) covered with gauze at both ends. It was placed in a cabinet (1.1 $\times$ 2.7 $\times$ 1.8 m) where a charcoal-filtered air flow was maintained. The flow rate in the olfactometer was approximately 10 cm/sec. A dark green paper was positioned about 10 cm above the olfactometer (to make it dark) and 5 cm from the down wind end (to keep it lighter than the other part of the glass tube), so that an adult insect remained in position until getting a stimulus. Test insects kept at 10°C were transferred into the cabinet, and the temperature was raised gradually to 25°C at the time of the experiment. A single insect and a paper disk, impregnated with $10\mu\text{l}$ of each test compound, was put on the down and upwind ends. The

glass tube was divided into eight parts of equal lengths, and the maximum part that an insect reached during 30 min was recorded. The insect reaching the upwind end was, therefore, scored eight. Two to four replicates were recorded for each chemical.

5. Field trapping tests

The field tests were conducted from May to July in 1987. Two types of trap were used. A two-vaned (30 × 30 cm) L-shaped, pale brown sticky trap was used in Trial 1, and a white- or yellow-colored plastic trap was used in Trials 2~6 (Fig. 1). Plastic containers ($\phi 6 \times 2.5$ cm) were used wherein cotton balls impregnated separately with 10 g of each candidate chemical were placed per trap. As for the plastic traps, water with salt also was changed at the same times. Traps were hung on a tightened string between trees 1.5 m above the ground just before the emergence periods of beetles. The numbers of beetle trapped were counted, and the chemicals were renewed every two weeks for 1~2 month.

III. Results

1. Electroantennograms (EAGs) generated by flower scents

There were large sexual differences in EAGs of both insect species, although they both responded to many test compounds. In Table 1, it appears that the EAG responses of the female *A. subfasciatus* and the male *D. transilis* to all compounds tested were much stronger than those of the opposite sexes. Of these, linalool, α -terpineol, and benzyl acetate induced greater amplitudes of EAGs for the female *A. subfasciatus* than other compounds tested, whereas benzyl acetate, borneol, linalool, phenylethyl butyrate, and citronellal did the same for the male *D. transilis*.

2. Olfactory responses of walking insects to flower scents

There were no such significant differences between sexes for all test compounds as was shown by EAGs. Benzyl acetate and camphor had greater attractivenesses for walking female adults of *A. subfasciatus* than did other compounds tested, although their attractivenesses for the males were not significantly different from others. As for *D. transilis*, linalool and phenylethyl butyrate had greater attractivenesses for both sexes, whereas benzyl acetate was attractive for only males and citronellal and α -pinene was attractive for only females (Table 2).

3. Field trapping tests

Trap catches of beetles were examined with various combinations of test chemicals in various



Fig. 1. The white plastic trap for field tests

Table 1. EAG responses of *A. subfasciatus* and *D. transilis* to odorous compounds

Test compounds	EAG responses (mv) ^{a)}			
	<i>A. subfasciatus</i>		<i>D. transilis</i>	
	Males	Females	Males	Females
Alcohol				
Borneol	0.3	0.6	1.2	0.3
Linalool	0.5	1.4	1.2	0.3
Nerol	0.5	0.5	0.5	0.2
α -Terpineol	0.5	1.0	0.4	0.2
Ester				
Benzyl acetate	0.3	1.0	1.6	0.3
Linalyl acetate	0.4	0.6	0.3	0.0
Neryl acetate	0.4	0.5	0.4	0.0
Phenylethyl propionate	0.3	0.4	0.3	0.0
Phenylethyl butyrate	0.4	0.6	1.2	0.2
Aldehyde and ketone				
Camphor	0.5	0.8	0.8	0.3
Citral	0.5	0.8	0.8	0.4
Citronellal	0.6	0.8	1.0	0.5
Hydrocarbon				
α -Pinene		0.4	0.7	0.2
Others				
Japanese cedar oil	0.6	0.8	0.6	0.4

^{a)} Figures are the average of more than three replicates of EAG responses.

places (Table 3). Relative comparisons of trap catches among the test compounds showed that benzyl acetate, followed by linalool or phenylethyl propionate, was most attractive to both species, although there were exceptions only for *D. transilis* in Trials 1 and 3 (Table 3).

IV. Discussion

In nature, both *A. subfasciatus* and *D. transilis* focus on wide ranges of flower species by responding to various flower scents. These behaviors also were suggested on EAGs by the responses of beetle's antennae to various candidate chemicals. As it is not always the case that the larger values of EAGs correspond to stronger attractivenesses, the screening tests on an olfactometer were made.

Among the chemicals tested, only camphor and benzyl acetate showed the great attractiveness to *A. subfasciatus* on the olfactometer bioassay. On the other hand, linalool, benzyl acetate, phenylethyl butyrate, and citronellal showed greater attractivenesses to *D. transilis*, whereas borneol, camphor, and citral showed little attractivenesses and some of them even showed repellencies on different bioassays using flying beetles (unpublished).

Benzyl acetate showed the greatest attractiveness to both insect species in all field tests. Trap catches in the field tests seemed to be affected greatly by the

Table 2. Olfactory responses of *A. subfasciatus* and *D. transilis* to odorous compounds

Test compounds	Olfactory responses ^{a)}			
	<i>A. subfasciatus</i>		<i>D. transilis</i>	
	Males	Females	Males	Females
Alcohol				
Borneol	3.5	2.5	3.0	1.5
Linalool	1.5	5.0	8.0	6.8
Nerol		3.0		
α -Terpineol	0.0	4.3		
Ester				
Benzyl acetate	4.0	6.8	5.5	4.5
Linalyl acetate	4.0	2.0		
Neryl acetate		1.0		
Phenylethyl propionate		3.0		
Phenylethyl butyrate		3.0	5.8	5.4
Aldehyde and ketone				
Camphor	4.0	7.5	0.0	3.8
Citral		1.5	2.8	3.0
Citronellal	2.0	4.5	4.8	7.5
Hydrocarbon				
α -Pinene		4.0	1.5	5.8
Others				
Japanese cedar oil	4.0	5.5	1.5	2.8
Control	1.8	2.5	2.0	2.4

^{a)} Responses are shown by the means of maximum scores that each insect obtained within 30 min of test period.

Table 3. Trap catches of *A. subfasciatus* and *D. transilis* by odorous compounds

Chemicals	Number of beetles caught per trap ^{a)}									
	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5	
	A ^{b)}	D	A	D	A	D	A	D	A	D
Borneol	0.2	0.2			1.0	5.5				
Linalool	0.3	4.3	1.8	344.5	6.5	611.0	1.3	28.3	0.0	21.3
Nerol	0.0	0.0								
α -Terpineol	0.0	0.0					0.0	8.3		
Benzyl acetate	1.0	1.3	3.7	388.8			5.3	98.5	11.8	313.2
Linalyl acetate	0.2	0.0								
Neryl acetate	0.5	0.0	1.5	96.3						
Phenylethyl propionate	0.3	0.2	2.7	158.2	6.8	6.5	2.8	8.3	4.5	1.0
Phenylethyl butyrate							0.3	1.8		
Camphor	0.2	0.2					0.3	1.8		
Citral	0.0	0.5								
Citronellal	0.3	0.2								
α -Pinene	0.0	0.0			0.5	20.5				
Japanese cedar oil	0.0	0.0			0.5	4.5				
None	0.0	0.0	1.7	62.5					0.0	

^{a)} The number and color of traps were; six pale brown sticky traps for Trial 1, three white and three yellow plastic traps for Trial 2, four white, four white, six white and three white plastic traps for Trials 3, 4, 5, and 6, respectively.

^{b)} Legend: A, *A. subfasciatus*, and D, *D. transilis*.

population densities of the beetles as well as the colors and shapes of traps. The poor trap catches in Trial 1 (Table 3) might not be due to the population density of the beetles, but due to the trap itself (pale brown, L-shaped sticky trap), because the area of Trial 1 was adjacent to that of Trial 2 in the same stand. Nevertheless, the data showed that benzyl acetate was most attractive to *A. subfasciatus* as it did in other trials.

After the series of screening tests in the laboratory, it was possible to nominate potential chemicals which could show attractivenesses to beetles, and it was effective to some extent to select candidate chemicals for field bioassay tests. However, the responses to the chemicals on EAG did not correlate with those on the olfactometer, so that a comparison of the attractivenesses of test chemicals was dependent on field tests using traps.

Benzyl acetate, a main component of jasmine oil and a common chemical included in various flower species, had the strongest attractiveness for both *A. subfasciatus* and *D. transversalis* among the chemicals tested. However, it was not included in the flowers of *S. incisae* and *V. dilatatum* which were frequently visited by both insect species.

Acknowledgements

We thank the researchers at the Forest Experimental Stations or Centers of Aomori, Akita, Iwate, Niigata, and Wakayama Prefectures for their cooperation on screening of the chemicals in the field tests, and the City of Odawara, Kanagawa Prefecture, for its kind presentation of experimental fields. We also thank the Takasago Perfumery Company and the Sankei Chemical Company for providing us test chemicals and traps, respectively, and Dr. K. NAKAMUTA, Forestry and Forest Products Research Institute, for his critical review of the manuscript and his helpful discussions.

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*The title is tentative translation from the original Japanese title by the author of thin paper.

(Received June 19, 1992)