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Volatile components of Japanese cedar cultivars as repellents related to resistance to *Cryptomeria* bark borer

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Abstract The response of the essential oils and their components to *Cryptomeria* bark borer has been studied. The oils of inner bark and sapwood of resistant cultivars acted as a repellent to *Cryptomeria* bark borer rather than as an attractant, whereas those of susceptible cultivars acted as an attractant. α-Terpineol, nerolidol, δ-cadinene, β-eudesmol, terpinolene, and cedrol showed high repellent activity. The relative contents of the former four compounds were larger in resistant cultivars than in sensitive cultivars. It suggests that these four compounds might be one of the causes of resistance to *Cryptomeria* bark borer. The contents of terpinolene and cedrol were small, and these two compounds may have no or a small influence on resistance. Although some of compounds acted as attractants for *Cryptomeria* bark borer, it was not attributable to the difference in the bark borer response of Japanese cedar because of the relatively low ratio of these compounds in each essential oil and the small differences in the ratios between resistant and susceptible cultivars.

Key words *Cryptomeria japonica* · *Semanstus japonicus* · Essential oils · Japanese cedar cultivars · Terpenes

Introduction

Japanese cedar (*Cryptomeria japonica* D. Don) is one of the most widely grown trees in Japan and is of chemotaxonomic interest because the genus *Cryptomeria* is monospecific.[1] Japanese cedar covers a large mountainous area in Japan[2] and is important for lumber.[3] Recently a great deal of damage[4,5] has been caused by the *Cryptomeria* bark borer[6,7] (*Semanstus japonicus* Lacordaire, Coleoptera, Cerambycidae), one of the most serious pest insects of Japanese cedar. Eggs laid underneath the outer bark during the spring season hatch within a short time, and larvae feed on the soft inner bark. They then get into the sapwood to feed on it during the summer. In late summer they make their pupal chambers there and pupate. After emerging by autumn, adults live in the sapwood and feed on it until they emerge from the trunk the following spring. *Cryptomeria* bark borer causes damage throughout Japan, except Hokkaido. Injured wood is discolored or decayed by fungi during growth, greatly reducing its commercial value.

Some cultivars are more resistant than others to this insect pest.[8–10] Several potential causes of resistance of Japanese cedar to *Cryptomeria* bark borer have been presumed. The most likely is the resin productivity of the traumatic resin canal, as many dead larvae have been found in the resin.[11] Japanese cedar does not have resin canals unless it has been injured. After injury by larvae, resin is exuded from the traumatized resin canal. The existence of traumatic resin canals was not examined in this experiment; rather, we were interested in whether essential oils act as repellents. To investigate the role of essential oils of cultivars chemically resistant to bark borers, we studied differences in the volatile compounds of three resistant (sambusugi, bokasugi, yabukuguri) and two susceptible (kumotoshi, urasebaru) cultivars and examined the effects of essential oils on the bark borer.

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Materials and methods

Japanese cedar cultivars

The sample cultivars in which external injuries were not found were cut down at the Kanto Forest Tree Breeding

Station (Mito, Japan). Their age was 15 years, and their height and the breast height diameter ranged from 8 to 16 m and 9 to 23 cm, respectively. Two trees of each cultivar were sampled in August, and samples 1–2 m above the ground were used for this experiment.

Essential oils

About 150 g of the inner bark and sapwood, respectively, ground by a Wiley mill to pass through a 5 mm sieve, were subjected to hydro-distillation for 8 h using methods standardized by the Association of Official Agricultural Chemists.[12] Leaf oils were obtained in the same way from about 150 g of leaves cut into a small pieces (1–2 cm in length) as mentioned in an earlier study.[13]

Identification of oil components

Components were identified by gas chromatography-mass spectrometry (GC-MS) analysis. The GC was equipped with flame ionization detector (FID) and was on a 25 m × 0.2 mm polyethylene glycol (PEG) 20M glass capillary column. The temperature of the GC was kept at 60°C for 30 min and then raised from 60°C to 175°C at 5°C/min. The flow rate was 0.6 ml/min. Mass spectra were obtained at 20 eV, and the peak identity was confirmed by comparison with a standard.

Bioassay for *Cryptomeria* bark borer

Newly emerged *Cryptomeria* bark borers were collected at the experimental forest of Forestry and Forest Products Research Institute in Tsukuba, Ibaraki, Japan during early April. Figure 1 shows the olfactometer used for bioassays. Two plastic boxes (30, 30, and 20 cm in length, width, and height, respectively) were connected to a small cylindrical container (10 cm diameter, 5 cm height) by two plastic pipes (3 cm diameter, 40 cm length). The two boxes and the cylindrical container (a pit container for bark borers) were placed in a straight line. Filter paper with 1 μl of a tested sample was put in one of the boxes, and a piece of untreated filter paper was put in the other box as a control. Four or five *Cryptomeria* bark borers were then placed in the cylindrical container, and the apparatus was kept in a dark room maintained at 20°C. After 24 h the borers in the two boxes and pit container were counted. Each determination was made with six replicates. The *t*-test was used to analyze the differences in the activities between the control and test samples.

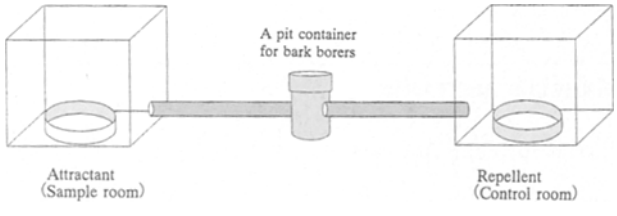

Fig. 1. Olfactometer used for bioassays

Results and discussion

Components of the essential oils of inner bark

Altogether, 57 compounds were identified by GC-MS, as shown in Table 1. There were four main components in the five cultivars: α-pinene, Δ-3-carene, limonene, and δ-cadinene. The total contents of these four compounds in the oils of bokasugi, sambusugi, yabukuguri, kumotoshi, and urasebaru were 63.31%, 42.26%, 62.61%, 77.06%, and 71.75%, respectively. Components other than these four compounds were present in small amounts.

α-Pinene was contained in the highest concentration in the inner bark oils of the five cultivars. The susceptible cultivars contained larger percentages of α-pinene than did the resistant cultivars. The α-pinene contents of the susceptible cultivars (kumotoshi and urasebaru) were 52% and 45%, respectively, accounting for nearly half of their essential oil contents. Although the main components of the oils were similar among the cultivars, there were differences in the minor components. Some compounds were present in larger amounts in the resistant cultivars than in the susceptible ones. They were α-terpineol, α-muurolene, humulene, δ-cadinene, nerolidol, T-cadinol, and β-eudesmol.

Bioassay of the essential oils for *Cryptomeria* bark borer

The inner bark oils of all resistant cultivars acted as repellents to keep away *Cryptomeria* bark borer, whereas those of susceptible cultivars acted as attractants (Fig. 2). In the case of kumotoshi, male bark borers stayed in the pit container during the test, and their bar graph was not included in Fig. 2. In each test, the sum of repellent and attractant was less than 100% because certain bark borers stayed in the pit container.

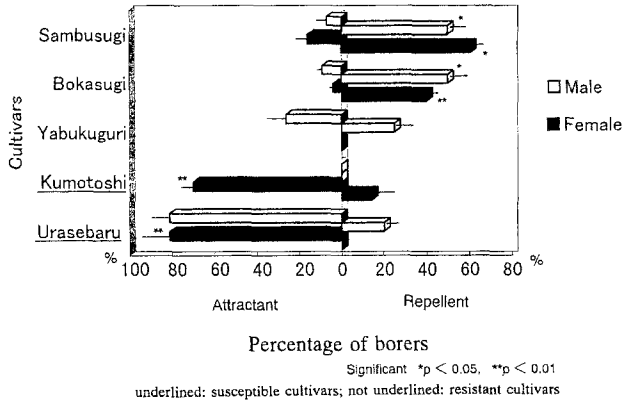

Fig. 2. Response of *Cryptomeria* bark borer to Japanese cedar inner bark essential oils

Table 1. Components of essential oils of Japanese cedar inner barks

Compound	R_t	Resistant cultivars			Susceptible cultivars	
		Bokasugi	Sambusugi	Yabukuguri	Kumotoshi	Urasebaru
Tricyclene	0.96	0.06	–	0.06	0.09	0.07
α-Pinene	1.00	35.60	16.18	39.95	52.14	44.77
M^+ 136 (93)	1.07	0.26	0.10	0.22	0.30	0.64
Camphene	1.09	0.31	0.20	0.30	0.48	0.62
β-Pinene	1.22	1.46	0.57	1.83	1.16	1.32
Sabinene	1.27	–	–	0.09	1.32	1.08
Δ-3-Carene	1.38	7.27	10.01	6.57	7.84	13.16
β-Phellandrene	1.46	3.73	1.73	2.68	3.61	2.63
γ-Terpinene	1.47	t	t	t	t	t
Limonene	1.61	12.57	7.42	8.38	13.35	9.89
M^+ 136	1.64	0.36	–	0.53	–	–
Myrcene	1.73	t	–	–	t	–
M^+ 134 (93)	1.85	0.05	–	–	t	t
p-Cymene	1.98	0.04	–	0.05	0.09	0.17
Methyl enanthate	2.06	0.21	0.39	0.09	0.08	0.13
Terpinolene	2.11	0.07	–	0.06	0.12	–
α-Pinene oxide	2.70	0.02	–	t	–	0.02
cis-Thujone	2.89	–	–	–	t	0.01
4-Isopropenyl toluene	3.06	0.05	–	t	0.01	t
Isomenthone	3.15	–	–	–	t	–
Limonen-1,2-oxide	3.20	–	–	–	–	0.01
α-Cubebene	3.35	0.85	0.30	0.65	0.29	0.29
Campholenic aldehyde	3.41	–	–	–	t	–
α-Ylangene	3.49	0.02	–	0.02	–	–
Copaene	3.56	0.28	0.10	0.35	0.23	0.31
Camphor	3.70	0.01	0.51	–	0.02	–
Linalool	3.91	0.26	0.10	0.31	0.09	–
M^+ 204	3.97	0.11	–	0.20	0.07	t
Linalyl isobutylate	4.02	0.08	–	0.10	0.06	t
Linalool-3,6-oxide acetate	4.05	0.61	–	–	0.13	–
Longifolene	4.07	0.29	–	–	–	–
Bornyl acetate	4.08	–	1.03	–	1.24	1.23
Terpine-4-ol	4.27	0.15	0.82	–	0.24	0.75
β-Cubebene	4.28	0.28	–	2.00	0.36	0.62
β-Caryophyllene	4.30	0.62	0.30	0.20	0.38	0.30
p-Menth-2,8-dienol-1	4.39	0.07	–	t	–	–
Thujyl alcohol-2	4.42	–	–	–	0.01	0.01
Linalool-3,7-oxide acetate	4.49	0.05	–	–	0.02	–
Pinocarveol	4.51	–	–	–	–	0.15
M^+ 204	4.65	0.50	–	0.15	0.01	–
Humulene	4.81	2.03	0.37	1.94	0.55	0.08
α-Terpineol	4.95	3.27	5.02	2.00	0.78	0.89
α-Terpinyl acetate	5.01	1.08	–	1.00	0.95	1.50
p-Menth-6-en-3,8-diol	5.10	–	–	–	–	0.20
Germacrene-D	5.15	1.05	0.82	0.99	0.78	0.64
α-Muurolene	5.28	2.21	2.60	1.65	1.12	1.44
Geranyl acetate	5.35	0.15	–	0.11	0.14	–
M^+ 204	5.40	0.12	–	–	–	0.10
δ-Cadinene	5.54	7.87	8.65	7.71	3.73	3.93
M^+ 204	5.68	0.65	0.20	0.58	0.24	0.17
M^+ 204	5.70	0.03	–	0.08	t	–
Carveol	5.77	t	–	–	t	0.20
M^+ 202	5.91	–	–	0.09	–	–
p-Cymen-8-ol	5.98	t	0.10	–	0.01	0.01
Calamenene	6.02	0.46	3.73	0.76	0.99	2.27
Cubebol	6.77	0.80	0.84	0.92	0.45	0.52
M^+ 200	6.97	0.02	–	0.13	t	0.06
Caryophellenoxide	6.97	–	0.35	0.20	0.21	0.41
Nerolidol	7.57	2.13	3.69	0.90	0.94	0.60
Spachulenol	7.71	0.13	–	0.13	0.06	–
M^+ 205	7.80	–	–	–	–	0.17
Cedrol	7.85	–	–	0.97	–	–
T-Cadinol	8.36	0.55	1.13	0.75	0.25	0.42
Torreyol	8.44	0.53	1.18	0.62	0.29	0.58
Kusunol	8.53	–	1.59	–	0.30	–
α-Eudesmol	8.54	0.80	1.19	0.83	1.29	0.96
β-Eudesmol	8.59	0.80	2.89	1.62	0.34	0.71
Farnesol	8.76	0.14	–	–	0.10	0.06
M^+ 205	9.08	–	t	0.11	–	0.01

Figures show percentages in essential oils based on the peak area determined by gas chromatography R_t, retention times are relative to those of α-pinene

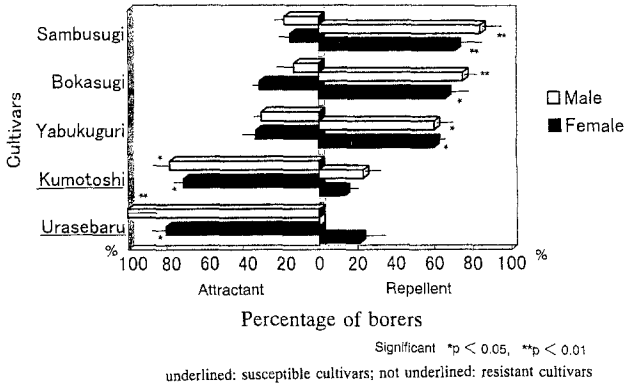

Fig. 3. Response of *Cryptomeria* bark borer to Japanese cedar sapwood essential oils

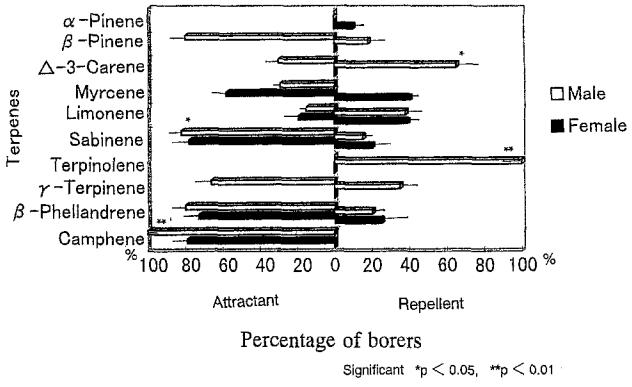

Fig. 4. Response of *Cryptomeria* bark borer to terpenes

The sapwood oils showed results similar to those of the inner bark oils. That is, in susceptible cultivars they acted mostly as attractants, whereas in resistant cultivars they were repellents (Fig. 3). No further investigation of sapwood oils was done because they were present in too small amounts.

The above results indicate that the behavior of the bark borer is affected by certain components of essential oils of Japanese cedar. Therefore, the activities of the components as attractants or repellents vis-à-vis the bark borer were studied.

Figures 4 and 5 show the response of *Cryptomeria* bark borers to the main terpenes of Japanese cedar oils. In the case of inner bark oils, α-terpineol, nerolidol, δ-cadinene, and β-eudesmol, which were present in resistant cultivars in larger amounts than in sensitive ones, showed high repellent activity. Therefore, it is possible that the resistance of the three cultivars resistant to Japanese bark borer may be attributable to these compounds. Although terpinolene and cedrol also showed high repellent action, they made up only a small percentage of the inner bark oils. Hence they might have had little or no effect on the resistance to *Cryptomeria* bark borer. A series of bioassays showed differences in activity between males and females, but the reason is not clear.

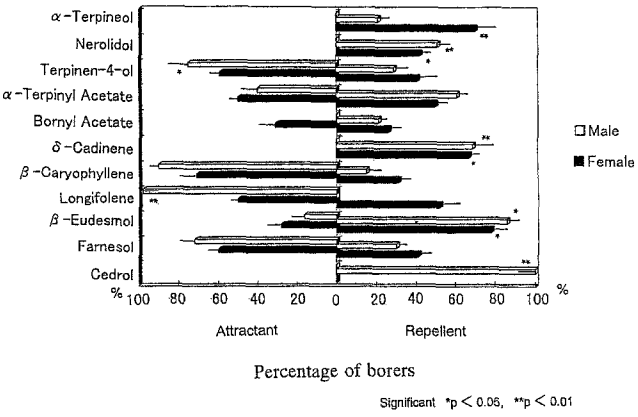

Fig. 5. Response of *Cryptomeria* bark borer to terpenes

Some of the compounds acted as attractants for *Cryptomeria* bark borer rather than as repellents (e.g., β-pinene, sabinene, β-phellandrene, camphene, terpine-4-ol, β-caryophyllene, and longifolene). However, these compounds might not have been involved in the bark borers' response to Japanese cedar because the ratio of these compounds in each essential oil was relatively low and the difference in the ratio of those compounds in resistant and susceptible cultivars was not large.

We studied the resistance of Japanese cedar to the feeding behavior of the bark borer from the viewpoint of essential oil composition. Japanese cedar does not naturally have resin canals, but it can form them in its inner bark in response to wounding or a fungus attack.[14,15] It may thus be assumed that resin productivity of traumatic resin canals is related to repellent activity against bark borers. The relation between the amount of essential oils in inner bark and repellent activity as well as the activity of nonvolatile compounds other than essential oils against bark borers, will be the next topics of study.

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