The Chemical Relationship between Fungus and Beetles on Ponderosa Pine

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Pinus ponderosa, Ischnodactylus loripes Lewis, Alphitophagus plagiatus Marseul, Bolitophagus felix Lewis, Cryptoporus volvatus (Pk.) Hubb.

Volatile components of Cryptoporus volvatus attracting three pine beetles (Ischnodactylus loripes Lewis, Alphitophagus plagiatus Marseul, Bolitophagus felix Lewis, family Tenebrionidae) were isolated and found to be a mixture of two monoterpenes and an aliphatic hydrocarbon by means of GC-MS. The attractivity of the fungus for the beetles can be induced by synergistic action of (+)-isopinocamphone, (+)-trans-pinocarveol, and 1, 3E, 5Z-undecatriene.

Introduction

Cryptoporus volvatus (Pk.) Hubb. belonging to the Polyporaceae is distributed in Japan, China, and North America (Borden et al., 1970). The fungus grows on the trunk of Ponderosa pine which are weakened or killed by insect or fire. The glo- bular sporophores (2–5 cm broad, 2–3 cm thick) are formed annually and grow from insects holes in the bark. When the sporophore of the fungus is nearly matured, a ventral aperture is formed from the inner surface of the fungus and at that time the fruit body secretes odoriferous compounds. The chamber provides an ideal refuge and feeding site for many beetles. Mature specimens were found at intervals between May to July. Some beetles (Ischnodactylus loripes Lewis, Alphitophagus plagiatus Marseul, Bolitophagus felix Lewis, family Tenebrionidae) infest the fruit body as soon as they matured. Occurrence of sporophores of the fungus at holes produced by bark beetles suggest that the fungus might be disseminated by them (Castello et al., 1976).

As evidenced in a recent review, numerous reports have considered the antileukemic polysaccharides (Kim et al., 1982) and bitter sesquiterpenoids (Hashimoto et al., 1987) of the fungus. However, relatively few reports have dealt with the role of the volatile components of the fungus. In the present work, we focus on the role of volatile components between the fungus and beetles on Ponderosa pine.

Experimental

Beetles

The three beetle species (Ischnodactylus loripes Lewis, Alphitophagus plagiatus Marseul, Bolitophagus felix Lewis) used in this study were field-collected in spring from the trunks of the Ponderosa pine in Hiroshima. The beetles were reared in a plastic box (24×34×5 cm) covered with a 40-mesh wire net for aeration. The conditions of rearing were as follows: temperature 25–30 °C, atmospheric moisture 70% relative humidity, 12 h light, 12 h dark. Fifty to one hundred beetles were placed in each plastic box containing dry fruit bodies of Cryptoporus volvatus as a food substrate. Beetles used in this study, as subjects attracted by a volatile source, were 5 or more days old and were starved for 12 h prior to the experiment.

Biological procedure

All experiments were done in an olfactometer, composed of a vacuum pump connected through a flowmeter, to a Y-shaped glass tube (stem 3×11 cm, each arm 3×9 cm). Each of the two arms led to a spherical glass trap, followed by a glass bulb containing a volatile source. The assay beetles (males and females together) were released individually at the starting point and will walk against the air current toward the attractant source. The airflow along the stem tube and in both arms was 1.5 l/min. A beetle without food for one night was placed in one arm of the olfactometer vs. no volatile source in the other. Beetles which chose the
direction of the trap and fell into the trap within 5 min were recorded as a response, while those that did not choose a certain arm within 5 min or moved to the opposite direction were scored as no response. After every seven runs (using 20 specimens), the olfactometer was turned 180° in order to change the direction of the volatile sources, to avoid bias from uncontrolled directional factors.

Attraction activity index (AAI)

The preference activity of the sample on males and females together is expressed as the AAI and is calculated as follows: AAI = (S − R)/(S + R + N). S denotes the number of the beetles on the sample side, R the number of the beetles on the control side, and N the number of the beetles not attracted by both sides. All index values lie within the range +1 to −1. Positive values indicate that more attractions were observed in the sample side than in the control, and conversely more attractions in the control than in the sample side result in a negative AAI.

Extraction and fractionation of the fungus material

The isolation procedure of active substances is shown in Fig. 1. Cryptoporus volvatus obtained from a pine forest in Hiroshima was minced and extracted with n-hexane. The n-hexane extracts showed the attractive activity for the beetles. The hexane extracts were then separated into two fractions (n-hexane soluble fraction and ethyl ether soluble fraction) by silica gel column chromatography with n-hexane-ethyl ether. The individual components were isolated by silica gel column chromatography and high performance liquid chromatography (HPLC).

Instrumentation

GC-MS was performed using a Shimadzu GC-9A gas chromatograph-mass spectrometer using the OV-101 capillary column (0.25 mm x 50 m), initial temperature at 100 °C, then 2 °C/min to 200 °C with helium as a carrier gas. Ionization voltage: 70 eV. Chamber temp.: 200 °C.

Analysis of active substances

As a small amount of the three active components (1, 3E, 5Z-undecatriene, isopinocamphone, trans-pinocarveol) could be isolated in pure state by means of column chromatography and HPLC, the three components were further synthesized by the following methods and can be used to test the biological activity. The purity of tested compounds

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**Fig. 1. Isolation of active components from Cryptoporus volvatus.**
was greater than 98% chemically and geometrically pure.

**Synthesis of 1, 3E, 5Z-undecatriene**

Reaction of 1-heptyl zinc chloride with 1E-dibromoethylene gave the 1-bromo-1E-nonen-3-yn in a 36.6% yield. Coupling with ethylene zinc chloride under tetrais(triphenylphosphine) palladium catalyst gave 1, 3E-undecadien-5-yn in a 18.0% yield. Reaction of one triple bond with palladium catalyst led to 1, 3Z, 5E-undecatriene in a 40.2% yield (Andreini et al., 1987).

**Preparation of (+)-isopinocamphone and (+)-trans-pinocarveol**

Chromic acid oxidation of (-)-isopinocamphol gave (+)-isopinocamphone (Brown et al., 1961) in a 63% yield and the oxidation of β-pinene with selenium dioxide gave (+)-trans-pinocarveol (Joshel et al., 1942) in a 9.4% yield.

**Results and Discussion**

Biological activity of the components is expressed by the attractive activity index (AAI). The AAI of the crude n-hexane extracts of *Cryptoporus volvatus* for *Ischnodactylus loripes* was +0.52, showing the positive response was tested as demonstrated in Fig. 2. The n-hexane extracts, therefore, contained attractants for the beetle. The n-hexane extracts were separated into two fractions (n-hexane fraction, ethyle ether fraction) by silica gel column chromatography over n-hexane and ethyl ether. No significant attraction to n-hexane fraction or ethyl ether fraction was demonstrated. When the n-hexane fraction and ethyl ether fraction were combined the AAI indicated a significant positive response to the beetle. Individual components of the two fractions were separated by means of column chromatography and HPLC, and identified by means of GC-MS as α-pinene, δ-3-carene, limonene, longifolene, γ-cadinene, 1, 3E, 5Z-undecatriene, 1, 3E, 5E-undecatriene, 3E-undecene, 7-octen-4-ol, 2-ethyl-hexenol, acetophenone, 2-octenal, *trans*-pinocarveol, isopinocamphone, 4-terpineol, myrtenol, piperitone, 2-decenol, undeca-2,4-dienol, 2,4-decadienol, 4-heptenol, 6,10-dimethylundeca-5,9-dien-2-one. Among the constituents, 1, 3E, 5Z-undecatriene was containing up to 45% (w/w) of the volatile components. When assayed alone or in combination of two, AAI of these components gave no positive response. AAI of the mixture of the three components (trans-pinocarveol, isopinocamphone, 1, 3E, 5Z-undecatriene) in original

![Fig. 2. Attractive activity index of *Cryptoporus volvatus* for *Ischnodactylus loripes*.](image-url)
Temporal changes of the attractants and AAI of *Cryptoporus volvatus* for the response of *Ischnodactylus loripes* are shown in Fig. 3. Changes of the attractants of *Cryptoporus volvatus* for the beetles were examined during three stages (young, mature, old) of the fungus, and samples were collected during three months from May to July. The samples thus included three stages of the fungus. The components of the fruit body in three stages showed quantitative compositional differences. The mature fruit body contained a large amount of $1, 3E, 5Z$-undecatriene (46.6%, w/v) and trans-pinocarveol (22.6%) together with isopinocamphone (6.3%) comparing with those of young and old stages. AAI of the mature fruit body of *Cryptoporus volvatus* showed a significant positive response for the beetles. In contrast, the fruit body of the young and old stages contained a small amount of attractants only compared to that of the mature stage.

The AAI of the three beetles in several concentrations is shown in Fig. 4. When the attractants were tested at different concentrations, significant differences were detected in the AAIs for the three beetles. A maximum AAI for *Alphitophagus plagiatus* and *Bolitophagus felix* was evident at $10^{-3}$ mg per ml, but the AAI dropped slightly at higher concentrations ($10^{-3}$ to $10^{-1}$ mg per ml). In contrast, the AAI of *Ischnodactylus loripes* gradually increased from $10^{-4}$ to $10^{-1}$ mg per ml. *Alphitophaga*

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**Fig. 3.** Temporal changes of the active components and attractive activity index of *Cryptoporus volvatus* for *Ischnodactylus loripes*.

**Fig. 4.** The attractive activity index of *Cryptoporus volvatus* for three beetle species at several concentrations.
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