Insecticidal and Fumigant Activities of *Cinnamomum cassia* Bark-Derived Materials against *Mechoris ursulus* (Coleoptera: Attelabidae)

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The insecticidal and fumigant activities of *Cinnamomum cassia* (Blume) bark-derived materials against the oak nut weevil (*Mechoris ursulus* Roelofs) were examined using filter paper diffusion and fumigation methods and compared to those of the commercially available *Cinnamomum* bark-derived compounds (eugenol, salicylaldehyde, *trans*-cinnamic acid, and cinnamyl alcohol). The biologically active constituent of the *Cinnamomum* bark was characterized as *trans*-cinnamaldehyde by spectroscopic analysis. In a test with the filter paper diffusion method, *trans*-cinnamaldehyde showed 100 and 83.3% mortality at rates of 2.5 and 1.0 mg/filter paper, respectively. At 2.5 mg/paper, strong insecticidal activity was produced from eugenol (90.0% mortality) and salicylaldehyde (88.9%), whereas *trans*-cinnamic acid revealed moderate activity (73.3%). At 5 mg/paper, weak insecticidal activity (50.0%) was produced from cinnamyl alcohol. In a fumigation test, the *Cinnamomum* bark-derived compounds were much more effective against *M. ursulus* larvae in closed cups than in open ones. These results indicate that the insecticidal activity of test compounds was attributable to fumigant action, although there is also significant contact toxicity. As a naturally occurring insect-control agent, the *Cinnamomum* bark-derived materials described could be useful as a new preventive agent against damage caused by *M. ursulus*.

Keywords: *Natural insecticide; Cinnamomum cassia; Mechoris ursulus; trans-cinnamaldehyde; salicylaldehyde; eugenol; contact toxicity; fumigation*

INTRODUCTION

Among approximately 2287 species of forest arthopod pests in Korea (Chung et al., 1995), the oak nut weevil (*Mechoris ursulus* Roelofs) is one of the most important insect pests of *Quercus* spp. (Lee and Chung, 1997; Park et al., 1998). Adults of this species emerge from mid June to late September. The female makes a hole on an acorn and oviposits one or two eggs in each acorn. If not managed properly from the growth stage of the tree, this insect species causes serious yield losses when adults and larvae excessively feed on the *Quercus* acorns which have long been considered in Korea to have natural medicinal properties and as a food source (Kim, 1996). The ecology and economic significance of this insect species have been well described by Lee and Chung (1997) and Park et al. (1998).

Control of *M. ursulus* populations is primarily dependent upon continued foliar applications of conventional insecticides such as phenthoate, fenitrothion, and cythrin (Lee and Chung, 1997). Although effective, their continued or repeated use for several decades has disrupted biological control by natural enemies and has led to outbreaks of insect pests, undesirable effects on nontarget organisms, and environmental and human health concerns. This increasing concern over adverse effects of the earlier types of insecticides have highlighted the need for the development of new types of selective control alternatives or biorational management methods without, or with, reduced use of conventional insecticides.

Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals, are often active against a limited number of species including specific target insects, are biodegradable to nontoxic products, and are potentially suitable for use in integrated pest management (IPM) programs. Therefore, many efforts have focused on plant-derived materials for potentially useful products as commercial insect-control agents or as lead compounds (Jacobson and Crosby, 1971; Elliott, 1977; Arnason et al., 1989a; Isman, 1995; Hedin et al., 1997). In a preliminary test, cinnamon oil exhibited potent insecticidal activity against *M. ursulus* when treated with 5 mg/filter paper. We have, therefore, examined the extract of the *Cinnamomum* bark for insecticidal constituents against *M. ursulus* as well as insecticidal mode of action.

MATERIALS AND METHODS

Chemicals. *trans*-Cinnamaldehyde, *trans*-cinnamic acid, salicylaldehyde, and eugenol were purchased from Sigma (St. Louis, MO). Cinnamyl alcohol was supplied by Tokyo Kasei (Tokyo, Japan). All other chemicals were of reagent grade.

Insect. The oak nut weevil (*Mechoris ursulus* Roelofs) was used in this study. Damaged acorns of *Quercus acutissima* (Carruthers) were collected at the Forestry Research Institute (Seoul) in August 1998. They had been held at room temperature for 2−3 weeks until the fourth instar larvae of *M. ursulus* were escaping from the damaged acorns.

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Table 1. Insecticidal Activity of *C. cassia* Bark-Derived Compounds against *M. ursulus* Larvae, Filter Paper Diffusion Method, 48 h

rate, mg/paper	compound[a]	*n*	mortality, % (mean ± SE)[b]
5.0	CA	35	100.0a
	CM	35	50.0 ± 5.8c
	CN	35	86.7 ± 3.3b
	EN	35	100.0a
	SA	35	100.0a
2.5	CA	35	100.0a
	CN	35	73.3 ± 3.3b
	EN	35	90.0 ± 5.8ab
	SA	35	88.9 ± 1.1ab
1.0	CA	35	83.3 ± 3.3a
	CN	35	53.3 ± 3.3b
	EN	35	66.7 ± 6.7ab
	SA	35	74.4 ± 2.9ab

[a] CA, *trans*-cinnamaldehyde; CM, cinnamyl alcohol; CN, *trans*-cinnamic acid; EN, eugenol; SA, salicylaldehyde. [b] Means within a column followed by the same letter are not significantly different ($P = 0.05$, Scheffe's test). Mortalities were transformed to arcsine square-root before ANOVA. Means (±SE) of untransformed data are reported.

Isolation and Identification. The *Cinnamomum cassia* bark (3.6 kg) purchased as a commercially available product was dried in an oven at 60 °C for 2 days, finely powdered, extracted twice with methanol (10 L) at room temperature, and filtered (Toyo filter paper No. 2, Japan). The combined filtrate was concentrated in vacuo at 35 °C to give a yield of ~10% (based on the dry weight of the bark). The extract (20 g) was sequentially partitioned into hexane (3.9 g), chloroform (4.5 g), ethyl acetate (1.9 g), and water-soluble (9.7 g) portions for subsequent bioassay. The organic solvent portions were concentrated to dryness by rotary evaporator at 35 °C, and the water portion was freeze-dried. For isolation, 5 mg of each *Cinnamomum* bark-derived portion in acetone was applied to filter paper, as described below.

Because of its strong insecticidal activity against *M. ursulus* larvae, the hexane portion (10 g) was chromatographed on a silica gel column (Merck 70−230 mesh, 500 g, 5.5 i.d. × 70 cm) and successively eluted with a stepwise gradient of hexane/ethyl acetate (100/0, 90/10, 70/30, 50/50, 20/80, and 0/100). The insecticidal fraction (50/50) was chromatographed on a silica gel column and eluted with hexane/ethyl acetate (2:1). Column fractions were collected and analyzed by TLC (hexane/ethyl acetate, 9:1). Fractions with similar TLC pattern were pooled. The insecticidal fraction was chromatographed on a silica gel column and eluted with hexane/ethyl acetate (8:2). For further separation of the insecticidal constituent(s), a prep HPLC (Waters Delta Prep 4000) was used. The column was 29 i.d. × 300 mm Bondapak C_{18} (Waters) using methanol/water (3:7) at a flow rate 10 mL/min and detection at 260 nm. Finally, an insecticidal principle (100 mg) was isolated.

Structural determination of insecticidal principle isolated was made by spectroscopic analysis. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a Bruker AM-500 spectrometer (Rheinspettem, Germany). Ultraviolet spectra were obtained on a Waters 490 spectrometer (Milford, MA), IR spectra on a Biorad FT-80 spectrophotometer (CA), and mass spectra on a JEOL JMS-DX 30 spectrometer (Tokyo, Japan).

Bioassay. A filter paper diffusion method was used to test the insecticidal properties of compounds used. Three rates (1.0, 2.5, and 5.0 mg) of test compounds in 1 mL of acetone were applied to filter papers (Whatman No. 2, 50-mm diameter). Controls received 1 mL of acetone. After drying in a hood for 2 min, each paper was placed in the bottom of a polyethylene cup (50 × 35 mm), and then *M. ursulus* larvae (fourth instar) were placed in each cup and covered with a lid.

In a separate experiment, susceptibilities of *M. ursulus* larvae to test compounds were investigated according to the method of Ahn et al. (1998) for fumigant action in further detail. Each filter paper (Whatman No. 2, 50-mm diameter) treated with 5.0 mg of each test compound in 1 mL of acetone was placed in the bottom of the polyethylene cup (50 × 60 mm). Each cup was packed with 40 g of sterile sea sand B (Nakalai, Japan) moistened with water, and then *M. ursulus* larvae were placed in each cup either with (method A) or without a lid (method B) to prevent direct contact of the larvae with each test compound. In case of method C, each paper treated with 5.0 mg of each test compound in 1 mL of acetone was placed in the bottom of the polyethylene cup, and then the larvae were placed in each cup and covered with a lid. Controls received 1 mL of acetone.

All treated materials were held at 25 ± 1 °C, 50−60% relative humidity, and a photoregime of 16L:8D. Mortalities were determined 48 h after treatment. Test insects were considered dead if appendages did not move when prodded with a camel's hair brush. Each assay was conducted in triplicate. Data from all bioassays were corrected for control mortality using Abbott's (1925) formula.

Statistical Analysis. The percentage mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Means were compared and separated by Scheffe's test at the $P = 0.05$ (SAS Institute, 1996). Means (±SE) of untransformed data are reported.

RESULTS

Identification. When the methanol extract from the *Cinnamomum* bark was subjected to bioassay, we observed the insecticidal activity against *M. ursulus* larvae. For further separation, the extract was sequentially partitioned into four portions. At a rate of 5 mg/filter paper, the hexane portion showed potent insecticidal activity (100% mortality).

Purification of the biologically active constituent(s) from the hexane fraction was done by using silica gel column chromatography and HPLC. Finally, an active principle was obtained. Structural determination of the isolate was made by spectroscopic analysis and by direct comparison with authentic reference compound, and it was characterized as *trans*-cinnamaldehyde.

Insecticidal Activity. The insecticidal activity of the *Cinnamomum* bark-derived *trans*-cinnamaldehyde against *M. ursulus* larvae was determined and compared to that of other commercially available constitu-

Table 2. Insecticidal Activity of *C. cassia* Bark-Derived Compounds against *M. ursulus* Larvae by Different Application Methods, 5 mg/Filter Paper, 48 h

	mortality, % (mean ± SE)[b]							
method[a]	*n*	CA[c]	*n*	CN	*n*	EN	*n*	SA
A	35	73.3 ±3.3b	35	66.7 ± 3.3a	35	83.3 ± 3.3b	35	100.0a
B	35	48.9 ± 5.9c	35	36.7 ± 3.3b	35	36.7 ± 3.3c	35	76.7 ± 3.3b
C	35	100.0a	35	77.8 ± 2.2a	35	100.0a	35	100.0a

[a] A, polyethylene cup contains filter paper treated with test compounds, sea sand, and lid; B, polyethylene cup contains filter paper treated with test compounds, sea sand, and no lid; C, polyethylene cup contains filter paper treated with test compounds and lid. [b] Means within a column followed by the same letter are not significantly different ($P = 0.05$, Scheffe's test). Mortalities were transformed to arcsine square-root before ANOVA. Means (± SE) of untransformed data are reported. [c] For explanation, see Table 1.

ents (eugenol, salicylaldehyde, *trans*-cinnamic acid, and cinnamyl alcohol) of the *Cinnamomum* bark (Table 1). At a rate of 5 mg/filter paper, very strong insecticidal activity (100% mortality) was produced from *trans*-cinnamaldehyde, eugenol, and salicylaldehyde. *trans*-Cinnamic acid exhibited strong insecticidal activity (86.7%), whereas weak activity was observed in cinnamyl alcohol (50.0%). At 2.5 mg/filter paper, insecticidal activity was more pronounced in *trans*-cinnamaldehyde (100%), compared to eugenol (90.0%), salicylaldehyde (88.9%), and *trans*-cinnamic acid (73.3%). Some difference in insecticidal activity among test compounds was also observed at $P = 0.05$ when treated with 1 mg/filter paper: *trans*-cinnamaldehyde showed more insecticidal activity than the other test compounds.

Fumigant Activity. We were interested to determine whether the insecticidal activity of the *Cinnamomum* bark-derived materials against *M. ursulus* larvae was attributable to contact toxicity or fumigant action. Therefore, three different treatment methods (A, B, and C) were tested (Table 2). The responses varied with both treatment method and compound used. There was significant difference ($P = 0.05$) in insecticidal activity of *trans*-cinnamaldehyde between with lids (A, 73.3% mortality) and without lids (B, 48.9%) when packed with sand. When covered with lids, significant difference in insecticidal activity of *trans*-cinnamaldehyde between with sand (A, 73.3%) and without sand (C, 100%) was also observed. Similar results were also obtained in treatment with eugenol, *trans*-cinnamic acid, and salicylaldehyde. However, the fumigant activity was more pronounced in salicylaldehyde than the other compounds.

DISCUSSION

In the laboratory study with *M. ursulus* larvae, the extract of the *Cinnamomum* bark revealed potent insecticidal activity when tested by the filter paper diffusion method. This plant species belongs to the family Lauraceae. Jacobson (1989) already pointed out that the most promising botanical insect-control agents are in the families Annonaceae, Asteraceae, Canellaceae, Labiatae, Meliaceae, and Rutaceae. Some investigations have demonstrated that *C. cassia*-derived materials have insecticidal and antifeeding effects against insect pests (Ben-Yakir et al., 1995; Huang and Ho, 1998) as well as a rodent-repellent effect (Lee et al., 1999), although this plant is not only important as a spice but has also long been considered in East Asia to have natural medicinal properties such as a stomachic, an astringent, and a carminative (Namba, 1993; Kim, 1996).

Various compounds, including phenolics, terpenoids, and alkaloids, exist in plants. Jointly or independently, these may contribute to the protection of plants against herbivores, although some herbivores have counteradpted to them. It has been well recognized that plant-derived insect-control agents could be developed into products suitable for IPM, because they are selective to pests, have no or little harmful effects on nontarget organisms and the environment, act in many ways on various types of pest complex, and may be applied to the plant in the same way as other agricultural chemicals (Chapman, 1974; Arnason et al., 1989a; Hedin et al., 1997). For example, derivatives of neem (*Azadirachta indica* A. Juss) belonging to the family Meliaceae are found to have a variety of biological activities including insecticidal activity against nearly 200 species of insects without any adverse effects on most nontarget organisms (Saxena, 1989; Lowery and Isman, 1995). Additionally, plant-derived materials are found to be highly effective against insecticide-resistant insect pests (Arnason et al., 1989b; Kwon et al., 1996; Ahn et al., 1997). Derivatives of *Ginkgo biloba* L. (Ginkgoaceae) leaves had potent insecticidal activity toward three strains of *Nilaparvata lugens* resistant to carbofuran, fenobucarb, and diazinon (Ahn et al., 1997). Much concern has, therefore, been focused on the distribution, nature, and practical use of chemical substances having the insecticidal activity for insects in plants. In our study, the insecticidal constituent of the *Cinnamomum* bark was identified as *trans*-cinnamaldehyde. Strong insecticidal activity was also obtained in the commercial eugenol and salicylaldehyde which are constituents of the *Cinnamomum* bark. Additionally, the *Cinnamomum* bark-derived compounds were much more effective against *M. ursulus* larvae in closed cups than in open ones. These compounds have a characteristic odor and are highly volatile (Budavari et al., 1989). These results indicate that the insecticidal mode of action of the *Cinnamomum* bark-derived compounds may be largely attributable to fumigant action, although there is also significant contact toxicity based on our results. The fumigant activity was compound-dependent. The fumigant activity was more pronounced in salicylaldehyde than the other compounds, suggesting that difference in physicochemical properties might be involved. Fumigant action of the *Cinnamomum* bark-derived compounds might be of great importance for controlling soil insect and stored-product insect pests because most of the residue is deposited on their shell or surface while protectant insecticides such as malathion are applied to stored products and organic fumigants such as methyl bromide and chlorpicrin are very toxic to humans. However, the *Cinnamomum* bark-derived materials have low toxicity (Budavari et al., 1989). Especially, considering that hatched larvae in late July feed in *Quercus* acorns for ~20 days, escape from the acorn and overwinter as fourth instar larvae in 6−15 cm of soil depth (Park et al., 1998), good potential for the control of field populations of *M. ursulus* would be expected to be obtained when the *Cinnamomum* bark-derived compounds apply to soil in early to mid August.

In conclusion, the *Cinnamomum* bark-derived materials described could be of practical use as an insect-control fumigant for *M. ursulus* for a relatively long time, provided that a carrier producing a slow-release effect can be selected or developed.

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