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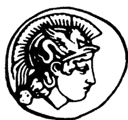

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Cross-resistance of a chlorpyrifos-methyl resistant strain of *Oryzaephilus surinamensis* (Coleoptera: Cucujidae) to fumigant toxicity of essential oil extracted from *Eucalyptus globulus* and its major monoterpene, 1,8-cineole

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Abstract

The fumigant toxicities of eucalyptus essential oil and 1,8-cineole, the major component of eucalyptus oil, were tested against a chlorpyrifos-methyl resistant strain and a reference strain of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.). The resistant strain showed 1.9- and 2.2-fold higher tolerance against essential oil and 1,8-cineole fumigation toxicity, respectively, relative to the susceptible strain. The increased tolerance for the essential oil may be the result of cross-resistance. The resistance mechanisms in the resistant strain are discussed in relation to elevated detoxifying enzymes such as cytochrome P450 and esterases. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Cross-resistance; *Eucalyptus globulus*; Essential oil; 1; 8-Cineole; Fumigant toxicity; *Oryzaephilus surinamensis*

1. Introduction

Chemical management of stored grain insect pests is a widely used method of pest control. Effective procedures include the use of fumigants and protectants. The ideal fumigants used

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should leave no hazardous residues and should not adversely affect the nutritional quality, flavor, or processing characteristics of stored grains (Plimmer, 1982). They should be biologically active, sufficiently volatile to be removed by aeration, not absorbed by the grain, not flammable and inert with metals. Currently, few chemicals are available for use as fumigants. Methyl bromide and phosphine have been used as fumigants in integrated pest management of stored grain insect pests. However, the use of methyl bromide will be restricted soon due to its ozone depleting properties (WMO, 1991) and it must be used after careful consideration because of its very high toxicity to warm-blooded animals (Dansi et al., 1984). Phosphine fumigation may be also limited in use because evidence is increasing that stored product insects are becoming resistant to the compound in more than 45 countries (Chaudhry, 1995; Bell and Wilson, 1995). Therefore, an effort is needed to develop a new compound to replace the conventional fumigants.

Essential oils are potential alternatives to current fumigants because of their low toxicity to warm-blooded mammals, high volatility, and fumigation toxicity to stored grain insect pests (Shaaya et al., 1991, 1997; Regnault-Roger et al., 1993). In a fumigation toxicity test of essential oils and monoterpenes, the alcohol and phenolic monoterpenes had the greatest activity against *Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle (Shaaya et al., 1991).

In this paper we report the fumigation toxicity of essential oil from *Eucalyptus globulus* Labill and its main component 1,8-cineole against two different strains of *O. surinamensis*, and demonstrate possible cross-resistance to the fumigants in a chlorpyrifos-methyl resistant strain of the insect.

2. Materials and methods

2.1. Insects and materials

The two strains of *O. surinamensis* used in this study were supplied by Dr P.J. Collins, Department of Primary Industries, Queensland, Australia. VOS48 is an insecticide-susceptible reference strain that has been in laboratory culture since 1973. QVOS102 is a composite field strain resistant to chlorpyrifos-methyl, CM, at the time of collection and maintained in the laboratory in Queensland at 28°C under CM selection. This work was done in the Department of Crop Science, The University of Sydney, Australia. The essential oil of *E. globulus* was purchased from Essence Co. (Sydney, Australia) and 1,8-cineole was purchased from Aldrich Chemical Co. (Milwaukee, WI).

2.2. Chemical analysis

Compounds of eucalyptus essential oil were identified by comparison of gas chromatography (GC) retention times to those of the standards and by gas chromatography–mass spectrometry (GC–MS) data.

2.2.1. GC

A Hewlett–Packard 5890 Series II gas chromatograph equipped with a flame ionization detector was used. The GC conditions were as follows: column, DB-5 (J&W) 30 m $\times$ 0.53 mm i.d. 0.15 mm; N_2 carrier gas flow rate, 1.0 ml/min; temperature program, isotherm 3 min at 100°C, 15°C/min gradient to 250°C, isotherm 5 min; injection temperature, 250°C; detector temperature, 250°C.

2.2.2. GC–MS

A Shimadzu QP-5000 GC/MS system was used. The mass data were analyzed by a Shimadzu CLASS 5000 system. The GC conditions were as follows: column, CBP5 25 m $\times$ 0.25 mm; He carrier gas flow rate, 0.25 ml/min; split ratio, 50:1; temperature program, isotherm 3 min at 100°C, 15°C/min gradient to 250°C, 10°C/min gradient to 280°C, isotherm 20 min; injection temperature, 250°C; detector temperature, 250°C.

2.3. Resistance to chlorpyrifos-methyl

Insecticide resistance was assessed by the standard FAO impregnated filter paper assay method (Champ and Dyte, 1976) to determine dose response for the resistant and susceptible strains of *O. surinamensis* to CM. The insecticide was dissolved in a mixture of ondina oil:acetone:petroleum ether (1:1:3, v/v). Insecticide solutions (0.5 ml) of different concentrations were applied to Whatman No 1 filter papers and the acetone and petroleum were allowed to evaporate. Aluminum rings (4.3 cm i.d. and 2.9 cm high) coated inside with Fluon (polytetrafluoroethylene) to contain the adult beetles were placed on each filter paper. Two replicates of 40 adult beetles each were exposed to a series of six concentrations of CM for 24 h at 28°C. The LD_{50} and LD_{95} values were calculated by probit analysis (Finney, 1971). Control mortality was accounted for by Abbott's formula (Abbott, 1925). Resistance factors (RF) were calculated as the ratio of LD_{50} and LD_{95} values for a resistant strain to LD_{50} and LD_{95} values, respectively, for the susceptible strain, VOS48.

2.4. Fumigant toxicity

The insecticidal test was based on 20 adult insects confined in a 3.4-liter glass flask with round bottom, closed with a glass stopper fitted with a hook. The test materials were applied together with the insect cages, in the fumigation chamber. To obtain even distribution of the test materials during the treatment, a magnetic stir bar was placed on the bottom of each flask which was placed on a magnetic stirrer. Twenty insects, aged 10–15 days, were placed in each of three cages (4 cm in length and 2 cm diameter), which were perforated with small holes enabling penetration of the gas. Small amounts of polished oats were placed in each cage. Each treatment was duplicated. Mortality was examined after 24 h of treatment. The LD_{50} and LD_{95} values were calculated by probit analysis (Finney, 1971). Control mortality was accounted for by Abbott's formula (Abbott, 1925). Resistance factors (RF) were calculated as the ratio of LD_{50} and LD_{95} values for a resistant strain divided by the LD_{50} and LD_{95} values for the susceptible strain, VOS48, respectively.

3. Results

3.1. Chemical analysis

Three main components of eucalyptus essential oil were identified by comparison of GC retention times to those of standards and by GC–MS data. The content of 1,8-cineole was ca. 79.4%, the major component. Content of α-pinene and α-terpineol was ca. 3.65% and ca. 3.04%, respectively. Other components were less than 13.9% in total.

3.2. Resistance level of *O. surinamensis* to chlorpyrifos-methyl

The LD_{50} and LD_{95} values of CM against the two strains of *O. surinamensis* are shown in Table 1. The CM-resistant strain, QVOS102, had a 4.9-fold higher LD_{50} value than VOS48, the reference strain.

3.3. Fumigant toxicity

The LD_{50} values of eucalyptus oil and 1,8-cineole against the insects and RF values are shown in Table 1. QVOS102 showed 1.9-fold and 2.2-fold higher tolerance than VOS48 to the essential oil and to 1,8-cineole fumigation toxicity, respectively. It demonstrated that there might be possible cross-resistance in a CM-resistant strain of *O. surinamensis* to the eucalyptus oil and to 1,8-cineole fumigation toxicity.

4. Discussion

The major components of essential oil of *E. globulus* are 1,8-cineole, α-pinene and α-

Table 1
The LD_{50} and LD_{95} values for two strains of *O. surinamensis* in response to chlorpyrifos-methyl, 1,8-cineole and eucalyptus essential oil. Resistance factor (RF) for the resistant strain has been calculated relative to the susceptible strain, VOS48. LD_{50} and LD_{95} values for chlorpyrifos-methyl are in g l^{-1} of ondina oil. LD_{50} and LD_{95} values for 1,8-cineole and eucalyptus essential oil are in g l^{-1} of air

Strain	*n*	Slope	LD_{50} (95% FL) (g l^{-1})	r.f.	LD_{95} (95% FL) (g l^{-1})	r.f.	χ^2
Chlorpyrifos-methyl							
VOS48	454	1.1 (±0.11)	0.0045 (0.0033–0.0063)	1	0.099 (0.054–0.18)	1	9.23
QVOS102	543	2.0 (±0.13)	0.023 (0.019–0.028)	5	0.13 (0.071–0.22)	1.3	2.15
Eucalyptus essential oil							
VOS48	483	3.9 (±0.38)	0.13 (0.10–0.17)	1	0.32 (0.21–0.50)	1	1.52
QVOS102	474	3.5 (±0.40)	0.25 (0.19–0.32)	1.9	0.67 (0.40–1.10)	2.1	2.15
1,8-cineole							
VOS48	476	4.0 (±0.51)	0.12 (0.09–0.15)	1	0.28 (0.18–0.42)	1	2.51
QVOS102	480	3.3 (±0.50)	0.25 (0.20–0.33)	2.1	0.74 (0.43–1.29)	2.6	3.02

terpineol. The eucalyptus essential oil has been used as a medicinal agent, industrial oil, perfumery and flavoring oil (Boland et al., 1991). Several reports have also shown that the oil has insect repellent activity and inhibits insect growth (Morrow and Fox, 1980; Stampoulos, 1991).

The major component in eucalyptus essential oil, 1,8-cineole, has fumigant activity against *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.) and *O. surinamensis* (Shaaya et al., 1991). The second most abundant compound of eucalyptus essential oil tested, α-pinene, has shown a strong fumigant toxicity against the bean bruchid beetle, *Acanthoscelides obtectus* (Say) (Regnault-Roger and Hamraoui, 1995). They also determined that this compound inhibited oviposition, female fecundity, and the development of neonate and intracotyledonal larvae of the insect. However, there is no direct information of α-pinene fumigant toxicity on *O. surinamensis*. Our results showed a fumigant toxicity of eucalyptus essential oil and 1,8-cineole against two strains of *O. surinamensis*. These data showed that QVOS102 might become resistant against the eucalyptus oil and to 1,8-cineole fumigation. As shown in Table 1, QVOS102 also had moderate resistance, about 4-fold higher than in VOS48, after selection with CM. This resistance to CM detected in *O. surinamensis* is caused by enhanced detoxifying enzymes such as cytochrome P450-dependent monooxygenase, glutathione *S*-transferase and esterase (S.E. Lee, unpublished data).

The mode of action of fumigant toxicity of essential oil or monoterpene against insects may be inhibition of acetylcholinesterase (AChE) (Ryan and Byrne,1988). They determined that five monoterpenes inhibited AChE activity in the electric eel and killed adults of the red flour beetle, *Tribolium castaneum* (Herbst). 1,8-Cineole was the most potent inhibitor of eel AChE among the monoterpenes tested. This inhibition may be a mode of action for essential oil and monoterpene fumigation toxicity against stored grain insect pests as well.

Monoterpenes can be degraded by the cytochrome P450-dependent monooxygenase system. Cytochrome P450LM2 derived from rabbits is involved in the metabolism of several monoterpenes including geraniol and nerol by hydroxylation process (Licht and Coscia, 1978). In insects, 1,8-cineole was metabolised to 2β-hydroxycineole when the pyrgo beetle, *Paropsisterna tigrina* Chapuis, was fed leaves of the Australian tea tree, *Melaleuca alternifolia* (Maiden and Betche) Cheel (Southwell et al., 1995). Essential oils or monoterpenes can induce the concentration and aldrin epoxidase activity of cytochrome P450-dependent monooxygenase in rats and insects (Hiroi et al., 1995; Allanson, 1982; Brattsten and Wilkinson, 1977). Allanson (1982) demonstrated that monoterpenes with an accessible hydroxyl functional group significantly induced microsomal P450 and aldrin epoxidase activity in the cluster caterpillar, *Spodoptera litura* (F.). Brattsten and Wilkinson (1977) also determined that α-pinene, a bicyclic monoterpene, was the strongest inducer among the tested secondary plant substances for N-demethylase activity in the midgut of the southern armyworm, *S. eridania* Cramer. These reports may demonstrate the involvement of cytochrome P450-dependent monooxygenase in detoxification of essential oil or monoterpene in the insect body even though precise information on what the monoterpenes and essential oil do to metabolic processes is not available. QVOS102 has 186.5-fold higher epoxidase activity than VOS48 (S.E. Lee, unpublished data). Collins et al. (1992) also reported the 21.9-fold higher aldrin epoxidase activity and 12.5-fold higher concentration of cytochrome P450 in a CM-resistant strain,

VOSCM in comparison to VOS48. Therefore, cytochrome P450 monooxygenase activity is presumably related to the detoxification of essential oil or monoterpenes in *O. surinamensis*.

Another possible biochemical detoxification mechanism is non-specific hydrolases. The active site of esterase is similar to AChE, thus its activity may be inhibited by essential oil or monoterpene. Esterase activity was 30.5-fold higher in QVOS102 than in VOS48 when p-nitrophenyl acetate was used as a substrate (S.E. Lee, unpublished data), resulting in a conclusion that the toxic essential oil might find it hard to reach the target site, AChE. Glutathione *S*-transferase activity is another metabolic detoxification system. This enzyme is not specific, but QVOS102 has 2.61-fold higher glutathione *S*-transferase activity when compared to VOS48 (S.E. Lee, unpublished data). Therefore, a general increase in the level of detoxifying enzymes in QVOS102 might play an important role in the resistance mechanism for fumigation.

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