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Cross resistance between insecticides in coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae) from New Caledonia

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Abstract

The responses of adult females of *Hypothenemus hampei* (Ferrari) from New Caledonia, which were either resistant or susceptible to endosulfan, were compared to a range of insecticides. High levels of cross resistance were present to organochlorines (aldrin, dieldrin and lindane). No cross resistance was evident to carbaryl or organophosphates (descending order of toxicity: fenitrothion > pirimiphos-methyl > chlorpyrifos > diazinon=malathion). Carbaryl, a carbamate, was the least toxic, and avermectin, a macrocyclic lactone, was the most toxic insecticide tested on *H. hampei*. No evidence of synergy from esterase (DEF) or microsomal oxidase (piperonyl butoxide) inhibitors was present.

Introduction

Coffee berry borer, *Hypothenemus hampei* (Ferrari) (*Coleoptera : Scolytidae*) is the most important cosmopolitan pest of cultivated coffee (*Coffea arabica canephora*, var. *robusta*) (Waterhouse & Norris, 1989), with the potential for high levels of infestation, and significant losses in yield and quality. Endosulfan is the main insecticide used for its control worldwide. This insecticide has several key advantages, including a relatively low level of environmental hazard, low cost, and generally good efficacy against *H. hampei*.

High levels of resistance to endosulfan (500 to 1000-fold) have been detected in New Caledonia, (Brun *et al.*, 1989a, 1989b; Brun & Suckling, 1992). Similar problems could conceivably develop elsewhere, resulting in comparably high levels of infestation despite the use of endosulfan. Several factors have been shown to be associated with endosulfan resistance in the field, including cross resistance to lindane, which was used for control prior to 1975 (Brun *et al.*, 1990).

The responses of *H. hampei* to a range of insecticides have been reported, where bioassays were designed to compare relative efficacy for field control (e.g. Mansingh & Rhodes, 1983). This paper reports a comparison of the responses of endosulfan resistant and susceptible strains of *H. hampei* exposed to organochlorines, organophosphates, a carbamate, and avermectin, a macrocyclic lactone (Campbell *et al.*, 1983), in order to further characterize cross resistance. Tests with synergists were used to investigate possible mechanisms of resistance.

Materials and methods

Insect strains

Coffee berries infested with *H. hampei* were harvested from a reference susceptible site (La Foa, called LA2 in Brun *et al.*, 1989a) on the west coast of New Caledonia and from

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a site on the east coast having insects with a high level of resistance to endosulfan (PN103, Néavin Valley, Poindimié). Samples of *H. hampei* from LA2 were maintained at 25°C for approximately one month before testing, and this procedure was also followed for the tests with synergists on PN103. The PN103 strain was used for the tests of cross resistance, after selection in the laboratory with endosulfan at 10,000 ppm for eight generations. Survivors of Potter tower spraying were placed on artificial diet for oviposition and larval rearing (Brun *et al.*, in press). This was done in order to determine the level of cross resistance in a more homozygous resistant strain than was available directly from the field. Voucher specimens were deposited in the ORSTOM Entomology Section Museum, Nouméa.

Insecticide tests

The following insecticides were tested: endosulfan (Thiodan 35% EC, Hoechst AG, Germany); chlorpyrifos (45% WP Kumiai Chemical Industry Co. Ltd, Japan); lindane (Elgecide Concentrate 6% EC, G. Truffault, Vineuil, France); malathion (50% EC Kumiai Chemical Industry Co. Ltd Japan); fenitrothion (Folithion 100% EC, Bayer Australia Ltd); pirimiphos-methyl (Actellic 45%, ICI, UK); avermectin B1 (0.03% MSD Agvet Ltd, New Zealand); diazinon (40%, Ciba-Geigy, Switzerland). Samples of dieldrin (15% EC) and aldrin (60% EC) were obtained from the Biological and Chemical Research Institute, Sydney.

The Potter tower direct spray technique and concentration-mortality responses for a range of strains of *H. hampei* have been reported previously (Brun *et al.*, 1989). In this method, a glass ring (5 cm diameter, 2 cm high) was used to confine 30 healthy females on the filter paper during insecticide spraying, with at least two replicates per test. A Potter spray tower (Potter, 1952) calibrated to deliver 1.6 mg/cm^2 was used to apply 2 ml of liquid onto each sample of insects. After treatment, the glass ring was covered by a nylon screen to prevent escape. The adults were held at 25 ± 1°C and 80-85% RH under constant illumination. An exposure time of 6 h was used, with further readings after 24 h and 7 days. The criterion for death was the absence of movement when the beetle was touched with a fine paint-brush. Control mortality was consistently under 5%. Two synergists were also tested in combination with endosulfan, at a high but constant dose of synergist which did not cause mortality in either strain (Scott, 1990). Six ml of 1% solution of DEF (0.1 ml S,S,S-tributylphosphorotrithoate) or piperonyl butoxide was sprayed onto beetles as above, which were kept for 2 h before being re-sprayed with endosulfan. Probit analysis was done to determine the responses of susceptible and resistant insects using POLO (Leora, 1987). Pairs of lines were compared using the likelihood ratio test (Savin *et al.*, 1977).

Results and discussion

After laboratory selection, the level of endosulfan resistance expressed after 6 h post-treatment was very high (>2600-fold, table 1), similar to values reported by Brun *et al.* (1989a, 1989b). The toxicity of lindane, dieldrin and aldrin was also very low against the endosulfan-resistant strain of *H. hampei*, indicating a very high degree of cross resistance to members of the organochlorine group. Such high levels of resistance prevented estimation of the resistance factors in these cases. With dieldrin, no resistant *H. hampei* were dead 6 h after treatment with 150,000 ppm, while 600,000 ppm of aldrin caused only 2% mortality after 6 h (which rose to only 38% after 7 d) (table 1). Mortality of the susceptible strain was within the range expected for these insecticides. Carbaryl was slightly (2.5-fold) less toxic

Table 1. The responses of endosulfan susceptible and resistant females of *Hypothenemus hampei* to direct spray of insecticides (ppm) in a Potter tower (6 h post-treatment)

Insecticide	Strain	n	Slope ± SE	LD_{50} (ppm)	95%CL	RF[1]
Endosulfan	S	300	2.70 ± 0.52	42.3	16.4–61.2	
	R	705	2.29 ± 0.26	109 800	90 600–142 400	2614
Lindane	S	440	4.03 ± 0.35	92.0	59–149	
	R	570	1.26 ± 0.09	6574	3872–11 062	71.5
Dieldrin	S	180	2.14 ± 0.46	510	276–749	
	R	600	NE[2]	NE	NE–NE	High[3]
Aldrin	S	330	2.92 ± 0.33	284	176–402	
	R	225	NE	NE	NE–NE	High[4]
Malathion	S	270	2.30 ± 0.24	104	55–169	
	R	345	2.99 ± 0.34	135	63–201	1.3
Chlorpyrifos	S	255	3.88 ± 0.43	94	47–143	
	R	225	2.93 ± 0.34	83	47–129	0.9
Pirimiphos-methyl	S	240	5.67 ± 0.92	22	18–25	
	R	225	2.72 ± 0.31	18	9–34	0.8
Fenitrothion	S	180	3.42 ± 0.42	17.7	15.1–20.8	
	R	180	3.30 ± 0.52	22.8	18.9–27.8	1.3
Diazinon	S	225	3.88 ± 0.49	131	85–188	
	R	225	3.17 ± 0.54	137	69–196	1.0
Avermectin	S	270	2.18 ± 0.24	4.0	2.7–5.6	
	R	225	2.24 ± 0.28	5.5	2.6–8.7	1.4
Carbaryl	S	375	2.76 ± 0.32	722	427–1027	
	R	345	2.72 ± 0.25	1832	1545–2175	2.5

[1]Resistance factor (LD_{50}R/LD_{50}S); [2]NE Not estimated due to very high levels of resistance; [3]3% killed at 150,000 ppm after 7 days; [4]2% killed at 300,000 ppm after 6 h, 38% killed after 7 days.

Table 2. The responses of endosulfan susceptible and resistant females of *Hypothenemus hampei* to direct spray of endosulfan with and without synergists (ppm) in a Potter tower (6 h post-treatment)

Strain & synergist	n	Slope ± SE	LD_{50} (ppm)	95% CL	TR[1]
S	300	5.46 ± 0.68	73.2	*	
S with PB	240	3.29 ± 0.34	69.0	*	1.0
R	478	0.69 ± 0.09	1050	590–1930	
R with PB	480	0.86 ± 0.08	1220	670–2700	0.9
S	300	2.70 ± 0.52	42.3	16.4–61.2	
S with DEF	300	3.96 ± 0.45	46.7	29.7–66.1	0.9
R	460	0.50 ± 0.10	2710	445–27 000	
R with DEF	476	0.48 ± 0.08	2460	889–13 800	1.3

[1]Toxicity ratio (LD_{50} insecticide/LD_{50} insecticide & synergist).

to the endosulfan resistant strain ($P < 0.05$), although the responses were parallel. It is unclear whether this difference is linked with the endosulfan resistance.

Brun *et al.* (1991) noted that the rapid increases in mortality of susceptible *H. hampei* from endosulfan over several hours, and similarly rapid toxicity was observed with the organochlorines in these experiments against the susceptible strain. For example, 100% of susceptible beetles were killed by 100 ppm of aldrin and dieldrin after 7 d. For resistant strains, readings after 7 d were generally not substantially different from those reported here. The presence of cross resistance between endosulfan and lindane was previously demonstrated by Brun *et al.* (1990), with a close correlation (r^2=80%) in mortality at diagnostic dosages ($LD_{99.95}$) of the two insecticides in samples from 177 fields.

Similarity in responses between strains after 6 h indicated that no cross resistance was present to organophosphates (descending order of toxicity: fenitrothion > pirimiphos-methyl > chlorpyrifos > diazinon = malathion, table 1). Carbaryl was the least toxic, and avermectin, was the most toxic insecticide tested, with no cross resistance to endosulfan in either case. Prospects of using avermectin or other contact insecticides against *H. hampei* would not seem very promising, despite the high toxity, since most mortality from field applications of current insecticides results from fumigation of larvae and adults inside berries (Parkin *et al.*, 1992).

The use of esterase DEF (S,S,S,-tributylphosphorotrithioate) or microsomal oxidase (piperonyl butoxide) inhibitors combined with endosulfan did not show synergism with either susceptible or resistant strains of *H. hampei* at the LD_{50}. The toxicity ratio (LD_{50} insecticide/LD_{50} insecticide & synergist) varied only from 0.9 to 1.3 (table 2). Combined with the lack of cross resistance to the above mentioned insecticides, this suggests that metabolic resistance may not be important as a resistance mechanism. It is therefore more probable that penetration, binding or target site insensitivity may be present (Matsumura, 1983). In particular, it is possible that the cyclodiene resistance is due to insensitivity of the cyclodiene/picrotoxinin binding site on the γ-aminobutyric acid (GABA) gated chloride channel as in *Drosophila* (ffrench-Constant *et al.*, 1991, 1993). Recent experiments reporting this possibility are reported elsewhere (ffrench-Constant *et al.*, in press).

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