Endosulfan Resistance in *Hypothenemus hampei* (Coleoptera: Scolytidae) in New Caledonia

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ABSTRACT The Potter tower direct spray technique was used to determine the susceptibility to endosulfan of 16 strains of *Hypothenemus hampei* (Ferrari) from the East and West Coasts of New Caledonia. Strains from the drier West Coast were susceptible, whereas susceptible and resistant strains were recorded from the wetter East Coast. This is the first record of resistance in *H. hampei* and Scolytidae. High levels of up to 1,000-fold endosulfan resistance were detected from five locations. These were associated with poor field control since 1986 and highly significant increases in berry infestation from 1985 to 1987. Detection of resistance follows 6 yr of lindane use and 10 yr of biannual endosulfan application.

KEY WORDS Insecta, *Hypothenemus hampei*, endosulfan resistance, coffee

Hypothenemus hampei (Ferrari), known as coffee berry borer, was recorded for the first time in 1948 from the northern part of New Caledonia (Bugnicourt 1950). Within a few years, *H. hampei* spread throughout the island and became the major pest of coffee, which is predominantly *Coffea caniphora* var. *robusta*. The number of generations of *H. hampei* per year in New Caledonia is not known, although seven to eight generations have been recorded in other tropical locations (Le Pelley 1968). The sex ratio favors females by approximately 10: 1. Males do not disperse.

Since 1978, the Coffee Board of New Caledonia (l'Operation Café) has encouraged farmers to replace the older type of plantation grown under shade provided by a canopy of native trees with new trees cultivated in full sunlight. These new production farms were first established in Ponérihouen and Poindimié and are now being developed in all areas that grow coffee in New Caledonia. The total area covered by old coffee plantations cultivated under shade is between 1,500 and 2,000 ha, but the new intensive type of coffee fields do not cover more than 350 ha. However, berry production can be up to three times higher in the newer plantations because plants are closer together and the crop is managed more carefully. Insecticide coverage also is generally superior compared with that in the older plantations.

For 15 yr, attempts to achieve good control of the borer populations were made by careful picking of coffee "cherries" (red unripe berries) from trees after harvest and by destroying fallen berries (Cohic 1958). Although this intensive practice can reduce *H. hampei* infestation, it is not always used by farmers.

After several years of heavy infestation, the Territorial Government recommended reliance on insecticides to reduce field populations of this pest in 1966. DDT was used locally before the Department of Agriculture organized spray campaigns. Since the late 1960s, systematic biannual sprays have been applied from roadsides to all easily accessible plantations by vehicles with motorized air-blast sprayers. Lindane (Lindane 90 wettable powder [WP], Bayer AG, Federal Republic of Germany) was used until 1974–1975 at the application rate of 900 g (AI)/100 liters. Lindane was replaced by endosulfan (Thiodan 35 emulsifiable concentrate [EC], Hoescht AG, Federal Republic of Germany) applied at a rate of 700 g (AI)/100 liters. Both chemicals were applied directly to trees at rates of 100 to 150 liters per hectare. Spray operations were organized gratis by the Coffee Board and occurred during January and February, the wettest months. Spray operations began from the northeast part of the island; there was about a month interval between treatments.

Heavy outbreaks of *H. hampei* occurred in 1986 and 1987. Very high levels of berry infestation (90–100 %) were observed in several areas of the East Coast, leading to major crop losses and decreased coffee quality. No data on the responses of New Caledonian strains of *H. hampei* to insecticides are available. Active compounds to control this insect are scarce (Rhodes & Mansingh 1981). Our research was done to compare concentration–mortality data of insect populations from the northern part of the East Coast where control failure was observed with two other locations on the same coast and two areas of the West Coast without reported

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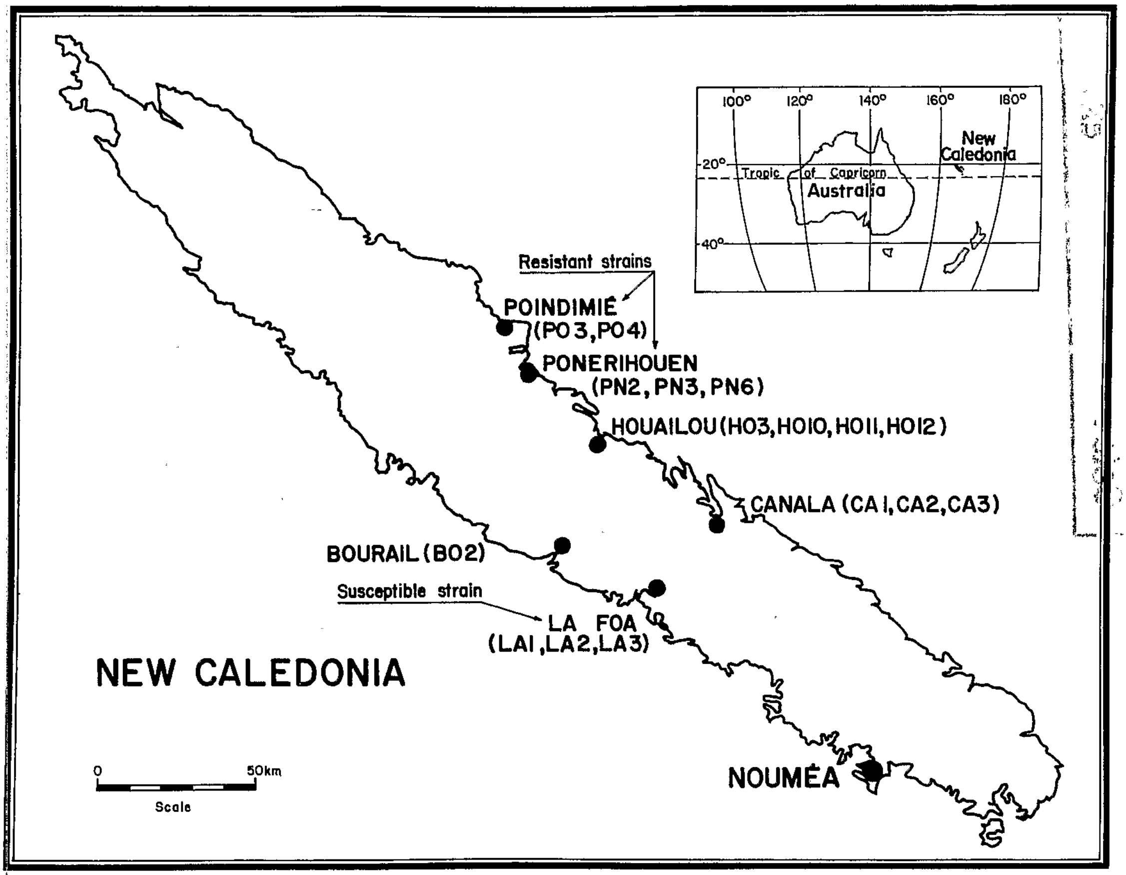

Fig. 1. Location of collection sites in New Caledonia of *H. hampei* resistant and susceptible to endosulfan.

control problems. We sought to document resistance development and crop loss and to provide background data as the basis for developing a resistance management strategy to regain control of the pest.

Materials and Methods

Insect Strains. Six well-separated geographical sites on the West and East Coasts (Fig. 1) were selected for insect collection. The responses of 16 populations to endosulfan were compared. Bourail (BO2) and La Foa (LA1, LA2, and LA3) were on the West Coast, and the remaining collections were on the wetter East Coast of the island where 90% of coffee is grown. Samples were taken from sunny locations. All sites were treated with endosulfan (Poindimié [colonies PO3 and PO4], Ponérihouen [colonies PN2 and PN6], Houailou [colonies HO10, HO11, and HO12], Canala [colony CA1], and La Foa [colony LA3]). Samples were also taken from shady plantations of the traditional type, which were either treated with endosulfan (colonies LA1, CA3, and PN3) or untreated (colonies LA2, BO2, CA2, and HO3). *H. hampei* from colony LA2 were collected from an abandoned field located about 5 km from other treated areas of La Foa. Other untreated sites were approximately 100 m from treated plantations. Most sites were located near (<1 km) the sea, except for LA1 and LA3 (which were near Saramea), and PN3 and PN6 (which were in Nimbaye and Tchamba Valleys), all of which were about 15 km from the sea. Berries from colony PN6 were divided into two groups. The first group of berries (PN6A) came from the roadside edge of the plantation where insecticide was repeatedly applied, and the second group of berries (PN6B) came from the bottom end of a long plantation, >50 m from the roadside and out of range of the area where most insecticide application occurred.

On the East Coast, the annual rainfall range is greatest at Poindimié and Ponérihouen (2,500–2,600 mm) and at Houailou and Canala (1,700–1,800 mm), whereas on the West Coast the mean annual rainfall at sites La Foa and Bourail is around 1,150 mm.

Direct Spray Technique. Coffee berries were brought from collection sites and stored at 25°C for approximately one month before use. Adult females were removed just before the test by breaking open the berries with a sharp scalpel. The age of the females used is not known. For each con-

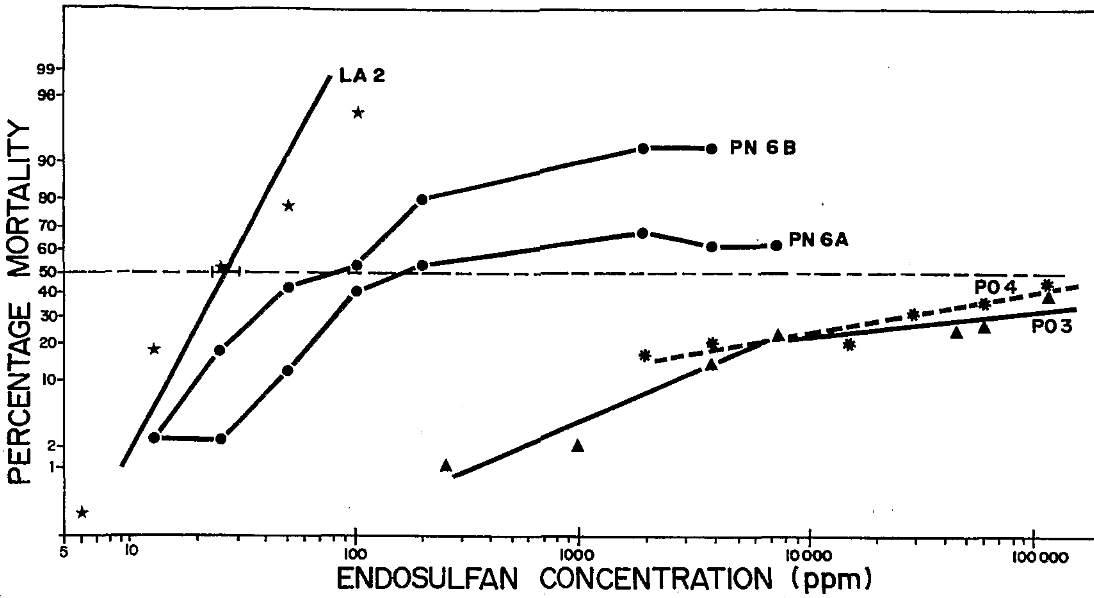

Fig. 2. Concentration–mortality lines for resistant and susceptible female *H. hampei* from New Caledonia to endosulfan. The PN6A site was located at the roadside from which endosulfan had been routinely applied, and PN6B was 50 m across the field from this point.

centration, 20 healthy females were transferred onto a filter paper disk. A glass ring (5 cm diameter, 2 cm high) was used to confine them on the filter paper during insecticide spraying. After spraying, the glass ring was covered by a nylon screen to prevent escape. Five or six serial dilutions of an aqueous suspension of Thiodan 35 EC were used for each test and 2 ml of liquid was sprayed with a Potter spray tower (Potter 1952) calibrated to deliver 1.6 mg/cm^2. Each test was replicated two to four times, and a treatment with water was included in each replicate as a control.

After spray application, the adults were held at 25 ± 1°C and 80–85% RH under constant illumination. An exposure time of 6 h was used, as recommended by Food and Agriculture Organization (1980) for stored-products coleopterous pests. After six hours, rapid increase in control mortality was observed. The criterion for death was the absence of movement when the beetle was touched with a fine paintbrush. Control mortalities were <10%. Observed mortality data from colonies PO and PN were corrected for control mortality by Abbott's (1925) formula for data of PO and PN and plotted (Fig. 2). Probit analysis was done with POLO (Robertson et al. 1980). Sites with large numbers of beetles surviving the LC_{99} of susceptible beetles were considered to include resistant individuals.

Coffee Infestation. Counts of *H. hampei* infestation were made by specialists of the Coffee Board. Each 300-g sample comprised numerous subsamples taken from bags of dried beans from each district. The coffee had already been through an air flotation process to remove most of the infested beans. Samples contained approximately 1,800 beans/300 g, and the counts were made visually without magnification. Analysis of variance was done on the number of infested beans in samples from 1985–1987 from collection sites Poindimié and Ponérihouen.

Results and Discussion

Direct Spray Technique. Among the 16 *H. hampei* populations tested, five strains located at Poindimié and Ponérihouen in the northeastern part of the island were resistant, and 11 strains exhibited similar susceptibility to endosulfan as our reference susceptible strain at LA2 (Table 1).

LA2 (collected from an untreated field on the West Coast) had an LC_{50} (95% CL) of 25 ppm (22–29). From treated areas, a group of samples from the drier West Coast (BO2, LA1, and LA3) showed responses very similar to those of colony LA2. On the East Coast, samples from three locations from Canala (CA1, CA2, and CA3), the southernmost coffee-producing location, and from four sites at Houailou (HO3, HO10, HO11, and HO12), also were very similar in their response to endosulfan. Among the susceptible strains, responses were predominantly parallel; however, HO10 had a slope that was steeper than normal. Although LC_{50}s among these sites varied, no trend towards tolerance associated with a history of recent insecticide use was detected (Table 1).

In Jamaica, Rhodes & Mansingh (1981) and Mansingh & Rhodes (1983) used a different bioassay technique (berry dipping) to assess the susceptibility of larvae and adults of *H. hampei* to various insecticide formulations. LC_{50}s for endosulfan to

Table 1. Concentration–mortality responses of female *H. hampei*

Collection site and colony	No. ♀♀ Tested	No. ♀♀ Control	Slope (SE)	LC_{50}	(95% CL), ppm	LC_{90}	(95% CL), ppm	χ^2	df
				West Coast					
La Foa									
LA3	360	60	4.24 (0.45)	24	(17–33)	49	(35–97)	10.0	4
LA2	420	80	3.17 (0.28)	25	(22–29)	64	(54–80)	1.8	4
LA1	600	100	2.48 (0.16)	33	(26–44)	110	(77–192)	9.3	4
Bourail									
BO2	300	60	3.68 (0.48)	47	(39–54)	104	(86–136)	5.6	8
				East Coast					
Canala									
CA1	540	100	2.26 (0.17)	30	(22–41)	111	(74–220)	13.9	5
CA2	580	100	2.86 (0.28)	33	(22–43)	92	(67–163)	12.9	5
CA3	600	100	3.02 (0.37)	46	(26–62)	122	(88–274)	9.6	4
Houailou									
HO11	220	40	2.99 (0.35)	17	(14–21)	47	(37–65)	1.0	4
HO3	240	40	3.24 (0.35)	22	(19–26)	55	(44–74)	3.9	4
HO12	360	60	2.79 (0.25)	22	(16–29)	62	(43–122)	8.1	4
HO10	200	40	6.64 (1.60)	29	(24–33)	45	(39–63)	2.4	4
Ponérihouen									
PN6B	280	60	2.24 (0.25)	72	(48–123)	268	(147–1,255)	5.4	3
PN6A	220	60	2.22 (0.31)	145	(116–194)	549	(360–1,117)	2.0	3
PN3	220	40	0.38 (0.10)	2,092	NE	NE	NE	2.8	6
PN2	180	40	0.81 (0.23)	238,969	NE	NE	NE	2.1	5
Poindimié									
PO3	300	40	2.21 (0.75)	NE	NE	NE	NE	23.6	8
PO4	240	40	0.46 (0.75)	NE	NE	NE	NE	15.2	6

NE, no value could be estimated.

larvae were 20 to 30 ppm, depending on the stage of berries used. For adult females, an LC_{50} of 30 ppm was estimated when beetles were in green endosperm; however, large variations in LC_{50}s (20-fold) were estimated. These LC_{50}s approximate responses that we obtained with susceptible strains when adults were sprayed directly.

A diagnostic concentration of twice the LC_{99} of susceptible strains should indicate resistance in field populations better than any comparison based on the LC_{50} (Roush & Miller 1986). Our data for susceptible strains indicated that the appropriate concentration for early detection of resistance by the direct spray technique would be approximately 400 ppm.

Samples from five collection sites on the East Coast (PO3, PO4, PN2, PN3, and PN6) showed that the resistance to endosulfan was very high in the wettest area of the island (the north). Because departure from linearity was evident in the data of some of the insect populations, and because the level of resistance was high, the probit model was not appropriate in most cases (Table 1). Significant χ^2 values were obtained only for PN6A and PN6B when probit analysis included all data points (not shown in Table 1). We plotted responses of colonies PO2, PO3, PN6A, and PN6B (Fig. 2) for comparison with our reference susceptible strain (LA2). Responses of strains PN2 and PN3 also indicated high resistance (Table 1) but are not shown. With the exception of PN6A and PN6B, the level of resistance to endosulfan was so high in the insect strains that mortality was <50%, even if higher concentrations or longer exposure times were used (L.O.B., unpublished data). Resistance factors could not be calculated from these data, but appear to be >1,000-fold.

Responses from colony PN6 indicated that this strain, although resistant to endosulfan, does not contain the same high frequency of resistant individuals present in strains from the nearest valleys (PN2 and PN3). Probit analysis of data from the five lowest concentrations (which gave a linear response) showed that responses of *H. hampei* populations from the two locations 50 m apart (Table 1) were significantly different ($P < 0.05$), with the more tolerant insects found at the treated site (PN6A) as expected. Mortality at higher concentrations showed different plateau for the two sites, with 25 to 35% greater survival at PN6A (Fig. 2). These results probably indicated effects of selection pressure on the endosulfan resistance near the roadside. Although sites PN6A and PN6B received the same number of sprays, a difference in selection pressure could be caused by heavier coverage of the closest trees during spraying operations.

Coffee Infestation. Quality monitoring by the Coffee Board graders during the 1985, 1986, and 1987 harvests show a highly significant increase in *H. hampei* infestation in samples from Poindimié ($F = 26.51$; df = 2, 31; $P < 0.01$) and Ponérihouen ($F = 11.99$; df = 2, 23; $P < 0.01$) (Table 2), sites

Table 2. Mean level of coffee berry infestation ($\bar{x}$ (SEM) beans attacked per 300 g) by *H. hampei*

Year	n	Pondimié[a]	n	Ponérihouen[a]	n	La Foa[b]	
1985	16	8.2 (1.8)	13	17.2 (1.6)	4	0.8	(0.5)
1986	12	29.5 (6.3)	11	29.6 (3.4)	2	3.0	(1.0)
1987	7	73.1 (10.5)	3	60.6 (21.7)	1	14	—

[a] Resistant.
[b] Susceptible.

where our results showed that highly resistant beetles were present. Samples from La Foa (the reference susceptible site) were not numerous enough for analysis of variance, but showed 5–10 times less infestation than the above sites (Table 2). The few data from La Foa resulted from the fact that the Coffee Board sometimes does not take records when the coffee quality is consistent. A t test between infestation levels in 1985 and 1986 showed no significant difference in infestation between those years ($P = 0.29$) at La Foa. The high level of infestation at sites Poindimié and Ponérihouen is more surprising because the quality assessment occurs after mechanical removal of most infested berries through air flotation. Coffee samples are downgraded if >50 infested beans are detected after processing.

Resistance Development. The high level of resistance recorded and the extent of the phenomenon in such well-separated localities of the East Coast of New Caledonia suggest that resistance may have developed some years ago. The first indication of poor efficacy of endosulfan may have been observed as early as 1984 at the Institut de Recherches du Café et du Cacao, at Ponérihouen (Fig. 1). Conceivably, resistance was spread by flat-bed trucks that contained loose mature coffee berries and that visited farms on the way to the processing factory at Poindimié. Except for this possibility, it is not easy to see how resistance could have reached such a high level in only some locations because the distribution of resistance is somewhat patchy, despite uniform application of insecticide under the auspices of the Coffee Board.

In Uganda, an endosulfan concentration of 0.2% (AI) was recommended (Ingram 1968), whereas most countries in Central America use an application rate of 0.3 to 0.5% (AI) (M. Chambon, Operation Café, Noumea, personal communication). The higher concentration used in New Caledonia (0.7% [AI] endosulfan) may represent significantly greater selection pressure than elsewhere.

Because most of the life cycle of *H. hampei* occurs within the berry, only dispersing females contact pesticide droplets or residues directly. However, modes of action other than contact toxicity might be important. Preliminary laboratory experiments have shown that exposure of susceptible *H. hampei* to endosulfan vapor alone can cause 100% mortality of adult females within a few hours following treatment, even at very low concentrations (L.O.B., unpublished data). The possible role of this vapor effect of endosulfan has yet to be confirmed in the field. If this mechanism is involved, the high concentration used in New Caledonia would have had a greater chance to enhance resistance development through greater selection pressure on life stages present inside coffee berries. In addition, lack of crop hygeine, which results in a significant residual population of all *H. hampei* life stages in old berries, means that selection might affect large numbers of insects, particularly in the newer sunny plantations where the berry density is greater. Compared with shaded plantations, better insecticide coverage and penetration as well as warmer temperatures, are more likely in sunny plantations. Warmer temperatures might result in greater fumigant action in newer plantations.

Despite the same history of pesticide use as in other regions, resistance first appeared in Poindimié and Ponérihouen, two locations with higher rainfall than elsewhere. Higher rainfall also was associated with the development of resistance to ethion in the southern cattle tick, *Boophilus microplus* (Canestrini) (Brun et al. 1983) on the East Coast of New Caledonia. Variations in ecological factors can induce differences in population ecology, and those factors sometimes determine how rapidly resistance evolves (Tabashnik & Croft 1985).

An increasing number of abandoned plantations are present in New Caledonia, and surrounding unsprayed plantations might act as refuges of susceptibility. However, coffee yield typically drops to zero after two years without fertilizer because of the very poor soils. The Coffee Board continues to apply insecticides for one season after the first year without harvest. The possibility of the avoidance of pesticide application within coffee fields exists, although mortality of all stages inside the berries is generally thought to be high after treatment.

Although this is the first recorded case of resistance of *H. hampei* to endosulfan, it has occurred after 6 and 10 yr of application of two related organochlorine chemicals, lindane and endosulfan. Several other factors may have contributed to *H. hampei* resistance in New Caledonia, and more research is needed to determine relationships between population biology, ecology, genetics, and insecticide use.

Resistance to endosulfan in *H. hampei* in New Caledonia suggests the significant risk of resistance development in this pest elsewhere, particularly where the number of insecticide applications is higher (e.g., Rhodes & Mansingh 1981), and alternative measures are not used to reduce the endosulfan selection pressure. Wherever practical and economic considerations permit, rotation of pesticides with no cross-resistance might delay or prevent resistance to endosulfan in *H. hampei.*

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