Monitoring of endosulfan and lindane resistance in the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae) in New Caledonia

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Abstract

Endosulfan resistance in the coffee berry borer *Hypothenemus hampei* (Ferrari) was discovered in New Caledonia after six years of lindane and 12 years of endosulfan biannual applications. The direct spray technique was used to study cross resistance with lindane and to monitor resistance to both pesticides in 15 regions of New Caledonia. An easier monitoring technique was developed for early detection by field laboratories. Results from this simple method, using twice the LC_{99} of endosulfan to susceptibles (400 ppm) showed a comparable distribution of resistance to the direct spray method in five regions where resistance was detected. The percentage of surveyed fields containing resistant insects in these areas was: Poindimié, 100%, Ponérihouen, 97%; Touho, 63%; Houailou, 10%; Hienghène, 6%. Resistant insects were significantly more frequent in the new sunny fields than in older shady plantations ($P<0.05$), and fields with endosulfan used in the preceding year ($P<0.001$). The possible reasons for the observed distribution of resistance are discussed.

Introduction

A major problem in controlling *Hypothenemus hampei* (Ferrari) has arisen since the 1985 harvest in New Caledonia, where it has been present since 1948. This appeared in large outbreak populations in the north-eastern regions with up to 90% of coffee berries being attacked (Brun *et al.*, 1989) and resulted in downgrading of a significant proportion of the coffee production from affected areas.

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Management of *hampei* is organized by the Coffee Board (Department of Agriculture). Systematic biannual sprays have been applied from roadsides since the late 1960s, to all plantations which are easily accessible by vehicles with motorized air-blast sprayers. Lindane (Lindane 90 wettable powder (WP)) was used until 1974–1975 at the application rate of 900 g (AI) per 100 litres. Lindane has since then been replaced by endosulfan (Thiodan 35 emulsifiable concentrate (EC)) applied at a rate of 700 g (AI) per 100 litres.

Both chemicals were applied at rates of 100 to 150 l/ha, directly to trees. Spraying operations organized by the Coffee Board occurred during January and February, the wettest months. Spray operations began from the north-east part of the island, with one month between the two treatments.

High levels of resistance of endosulfan (> 1000 fold) have been detected in New Caledonia (Brun *et al.*, 1989), resulting in high levels of infestation despite endosulfan use.

There is a need for a better understanding of the conditions surrounding this case of resistance and for a rapid early detection method to provide the foundation for an insecticide resistance management programme. Desirable characteristics of a standardized early detection method include low cost, reliability, ease of use with various chemicals, and precision under a range of conditions.

In this study we aimed to determine the geographic distribution of endosulfan resistance in New Caledonia, using two methods. Lindane resistance was also surveyed in order to confirm the probable cross resistance between these insecticides. We also studied the influence of management history on the frequency and level of resistance by characterizing the fields according to plantation type and recent history of insecticide use.

Materials and methods

Insect strains

Coffee berries were collected during 1987 and 1988 from the vicinity of roadsides between one and 44 coffee fields from each of 15 regions (fig. 1). Berries were placed in plastic bags with gauze windows (to reduce the humidity) and stored at 25°C for approximately one month before use. Fungal attack was reduced by exposing the berries to air circulation in gauze canisters after collection. Adult female *hampei* were obtained by breaking open the berries with a sharp scalpel just before the tests. Newly emerged females (light brown in colour) were not used. Males are smaller and flightless, so were not used. Samples were taken from the roadside edge because Brun *et al.* (1989) had shown how the level of resistance can drop from the sprayed roadside to the far end of a coffee field.

Strains from 203 different coffee fields were monitored for endosulfan resistance while lindane resistance was assessed from 177 fields. 87% of strains were from the east coast where around 90% of coffee is grown. A reference susceptible strain from La Foa had been used for method development (Brun *et al.*, in press). Fields were classified as:

1) sunny (modern production plantations) or shady (native forest canopy) and
2) with or without endosulfan treatment during the previous year.

The new sunny fields have generally been established next to older shady fields and are relatively evenly spread throughout the regions. Coffee production in the sunny fields is approximately three times higher than in the older plantations (Brun *et al.*, 1989). The native forest canopy over shady plantations is around 10 m in height.

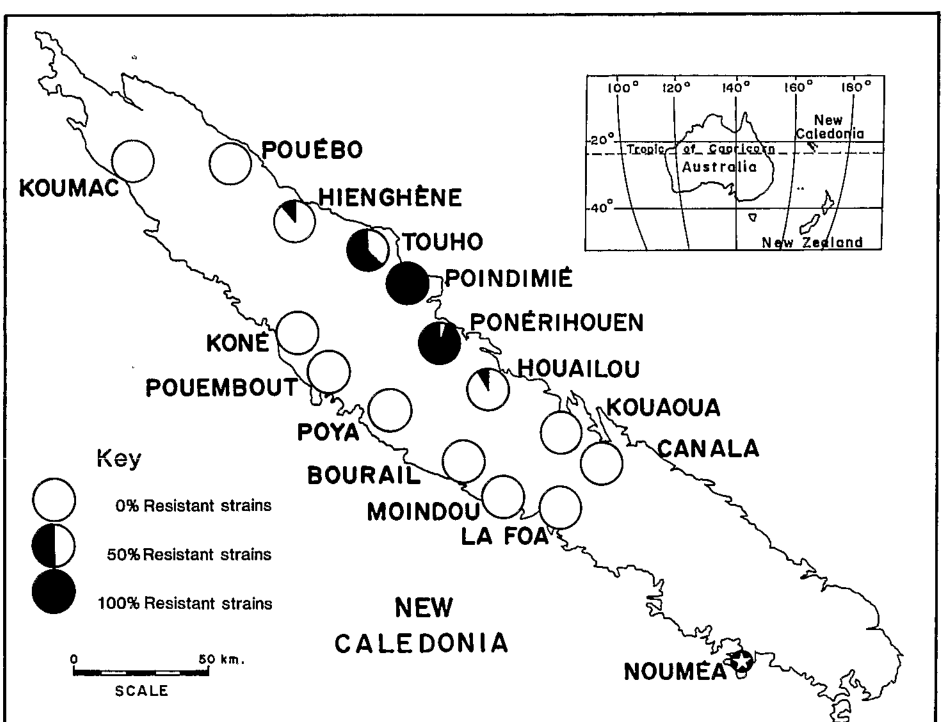

Fig. 1. Map of New Caledonia showing the 15 regions from which *H. hampei* were sampled and tested for endosulfan resistance.

Direct spray technique

A glass ring (5 cm diameter, 2 cm high) was used to confine 20 healthy females on filter paper during insecticide spraying. The glass ring was covered by a nylon screen to prevent escape after spraying. An aqueous suspension of Lindane 6 EC or Thiodan 35 EC (Hoechst AG) was used for each test and 2 ml of liquid 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 FAO (FAO, 1980) for stored products coleopterous pests, since this time period proved adequate for separation of phenotypes, due to the rapid expression of mortality of susceptible insects at the diagnositic dosages. The criterion for death was the absence of co-ordinated movement when the beetle was touched with a fine paintbrush, i.e. the inability to move more than its own body length. Control mortality was consistently <10%. The diagnostic dosages used to detect the resistant phenotype were 400 ppm of endosulfan, or 300 ppm of lindane, i.e. slightly over twice the LC_{99} for the reference susceptible strain (Brun *et al.*, in press). For direct sprays of endosulfan, the fields were considered to contain resistant individuals when any *hampei* survived a diagnostic dosage of $LC_{99.95}$ of susceptibles, i.e., twice the LC_{99}. The resistant phenotype was defined as capable of surviving the 400 ppm diagnostic dosage.

The direct spray technique was used on 380 samples for assessment of *hampei* resistance to endosulfan and lindane. A total of between 45 and 250 adult females from each strain were tested.

Indirect exposure technique

A specially made cage was devised for this test, consisting of a series of five layers ensuring that *hampei* was caged above treated filter paper but exposed only to the insecticide vapour. The bottom layer was a piece of solid rectangular (80 × 140 × 3 mm) perspex. A rectangular piece of filter paper (80 × 140 mm) that had been treated with endosulfan solution (400 ppm) and left for an hour to dry, was placed on this base. Next, a piece of fine white nylon gauze (80 × 140 mm) was placed on the filter paper, and a second piece of perspex of the same dimensions, but with two rows of five holes (20 mm diamater), was placed on the gauze layer, and 15 female *hampei* added per hole. The use of nylon gauze ensured that the beetles did not eat the filter paper (Brun *et al.*, in press). The nylon gauze also prevented direct contact with the insecticide deposits. Finally, a thin sheet of transparent plastic (80 × 140 mm) with 10 holes of 15 mm diameter (centred on the large holes) was used to partly cover the testing arenas to prevent escape. Each test was repeated at least twice and a water only treatment was included for each batch of tests. Mortality was assessed after 6 h exposure at 25°C and 80–85% RH. Any test with more than 10% mortality was discarded, but control mortality was generally lower than 5%. The indirect exposure technique was used on 150 samples. The diagnostic dosage (400 ppm) was slightly greater than the $LC_{99.99}$ of susceptibles when exposed by this method (Brun *et al.*, in press). The criteria for the presence of resistance in a sample, was the occurrence of any survivors of this dosage, defined as of resistant phenotype.

Statistical analysis

Probit analysis was carried out with POLO (Robertson *et al.*, 1980) to determine the LC_{99} of susceptible insects with each technique. The influence of management history on resistance level was examined by analysis of variance of angular transformed mortality (SAS, 1985) after a plot of residuals on fitted valves indicated no deviation from the expected model of uniform variability. Means and standard errors were then back-transformed for presentation.

Results and discussion

Comparative survey of endosulfan and lindane resistance

Very little difference in the estimated proportion of fields with resistance was found when the sample populations were tested by the Potter tower direct spray or by indirect exposure methods. Both methods showed very good agreement in the geographical distribution of resistance in five regions of the East Coast (table 1). No resistance was detected in samples from the West Coast or from Canala, Kouaoua, and Pouebo on the East Coast (fig. 1). The proportion of strains with resistance was low (6–12%) at Hienghène and Houailou, according to both methods. In contrast, both Ponérihouen and Poindimié had resistance present at most sites surveyed (97% and 100%, respectively). Touho appeared to have an intermediate frequency of resistance, with about half of the samples tested showing some level of survival at the diagnostic dosages. There was a minor discrepancy in the number of samples classified as resistant to endosulfan with direct spray and indirect exposure methods from Hienghène (table 1). This discrepancy was only due to one survivor, which caused one field (of 17) to be classified as resistant by one method, and susceptible by the other. The diagnostic dosages used could be expected on theoretical grounds to permit susceptible individuals to survive on rare occasions. For the direct spray of enosulfan applied at the $LC_{99.95}$ of susceptibles, five insects in 10,000 would be expected to survive from susceptible strains. For the diagnostic dosage using the indirect exposure ($LC_{99.99}$) only one survivor, in 10,000 treated insects would be expected. A check of direct spray results from the West Coast (which is presumed not to have any resistance present) indicated no survivors, in over 1700 insects tested (table 1), indicating no evidence of a false positive diagnosis. Given the large samples screened (over 10,000 with each method), a discrepancy of this order due to one insect is not cause for concern. We have also demonstrated considerable variation in the phenotypic frequency of resistance across coffee fields (Brun *et al.*, 1989; Suckling & Brun, 1989), and it is also conceivable that the discrepancy could be due to sampling error of a rare resistant allele within the strain.

It is possible that the diagnostic dosages we used killed a small proportion of the resistant insects (i.e.

Table 1· Geographic distribution of lindane and endosulfan resistance in *H. hampei* in New Caledonia.

	Lindane			Endosulfan					
	Direct spray technique			Direct spray technique			Indirect exposure		
Location	No. strains tested	No. *hampei* tested	% R strains	No. strains tested	No. *hampei* tested	% R strains	No. strains tested	No. *hampei* tested	% R strains
West Coast									
La Foa	13	600	0	16	1020	0	1	60	0
Moindou	1	45	0	3	180	0	–	–	–
Bourail	3	135	0	5	300	0	–	–	–
Poya	1	45	0	1	60	0	1	60	0
Pouembout	1	45	0	1	60	0	1	60	0
Kone	1	45	0	1	60	0	1	60	0
Koumac	1	45	0	1	60	0	1	27	0
East Coast									
Canala	13	585	0	13	780	0	11	660	0
Kouaoua	6	270	0	7	480	0	5	300	0
Houailou	34	1620	12	36	2319	8	32	1980	10
Ponérihouen	30	1620	97	32	2503	97	30	2060	97
Poindimié	23	1230	100	23	1835	100	12	1040	100
Touho	30	2265	53	44	2915	63	38	2923	63
Hienghène	17	780	6	17	1005	12	17	1170	6
Pouebo	3	180	0	3	180	0	–	–	–
Total	177	8850		203	12326		150	10122	

R = resistant

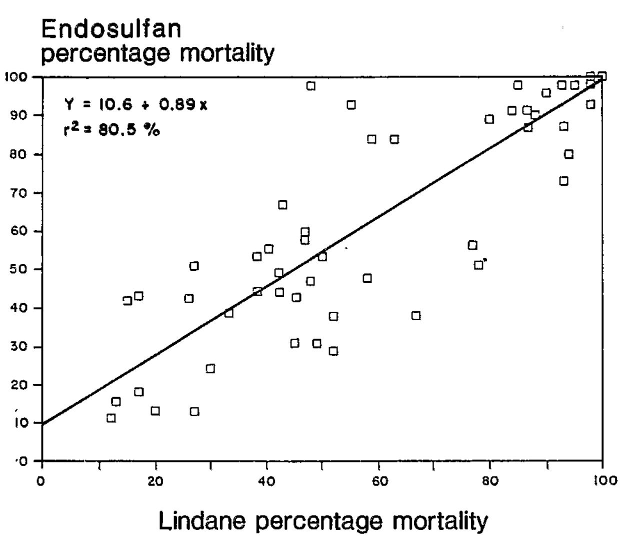

Fig. 2. Relationship between percentage mortalities of *H. hampei* after dosing with diagnositic dosages of endosulfan and lindane.

heterozygotes in the event of monogenic inheritance), thereby resulting in an underestimation of the frequency of resistance. It has not yet been possible to determine the degree of dominance of the resistance, so this possibility cannot yet be investigated.

Tests with diagnostic dosages of the two insecticides showed full cross resistance between lindane and endosulfan, with a regression coefficient of r^2=80.5% (fig. 2) between mortalities for each strain. These data suggest that the previous use of lindane in New Caledonia (from the late 1960s up to 1974/75) may have enhanced the risk of development of endosulfan resistance in *hampei*, since selection would have acted over the combined period of use of these insecticides.

Phenotype frequency

Samples taken from sites within the five regions where resistance was detected can also be compared to determine whether the frequency of the resistance phenotype within samples was the same in all five regions. The average percentage survival of females from sites with resistance present varied significantly ($P<0.05$, χ^2 goodness of fit) between the regions (table 2). This indicates that the phenotypic frequency of resistance varied significantly within regions where resistance was present. The average survival of *hampei* taken from Poindimié was highest (in all three data sets from endosulfan and lindane tests) showing that these strains contained the highest frequency of resistant individuals. Strains from both Ponérihouen and Touho had comparatively less resistance present, while strains from Houailou had the lowest survival on average and showed the lowest frequency of fields with resistance of any region.

The frequency distributions of survival of samples from Poindimié and Ponérihouen were similar (fig. 3), indicating an even spread of samples showing a low to high level of survival, and therefore a wide range of

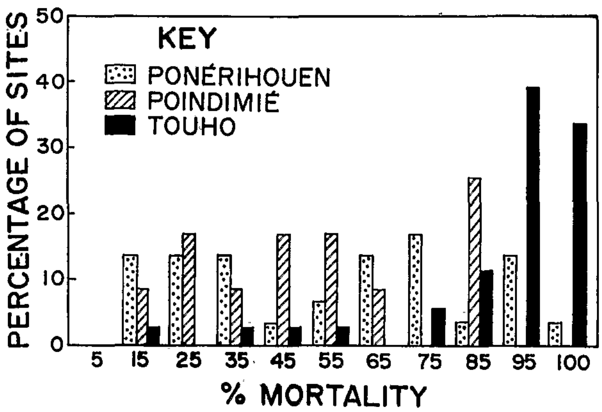

Fig. 3. Difference in proportion of resistant *H. hampei* females between three regions worst affected by endosulfan resistance in New Caledonia, according to a survey using the indirect exposure technique with a diagnostic dosage of 400 ppm endosulfan. Fields at Touho had less resistance present in the samples, as well as fewer samples with any resistance.

Table 2. Percentage survival of adult female *H. hampei* from sites with endosulfan resistance, after exposure to the diagnostic dosage with endosulfan (400 ppm) or lindane (300 ppm) with direct spray or indirect exposure methods.

	Lindane	Endosulfan	
Region	Direct spray technique	Direct spray technique	Indirect exposure
Houailou:			
mean survival	6.9%	6.5%	2.8%
n[1]	4	3	3
SEM	4.7	3.6	1.1
Ponérihouen:			
mean survival	17.1%	18.8%	17.5%
n	29	31	29
SEM	4.3	4.1	4.2
Poindimié:			
mean survival	37.1%	44.5%	38.7%
n	23	23	12
SEM	4.8	5.7	7.7
Touho:			
mean survival	18.1%	19.3%	16.7%
n	16	26	23
SEM	4.6	4.4	4.7
Hienghène:			
mean survival	6.7%	8.6%	20%
n	1	2	1
SEM	–	7.0	–

n[1] = number of samples, each with between 45 and 250 adult females.

different frequencies of the resistant phenotype within strains. In contrast, samples from Touho predominantly showed low levels of survival, from 0 to 10%. However, a few samples from this region showed high levels of survival (up to 85% resistant phenotype), indicating the potential for the problem to get worse in this area (fig. 3).

Samples were collected near roadside edges, which tend to have the highest phenotypic frequency of resistance, according to transects across fields (Brun *et al.*, 1989; Suckling & Brun, 1989). Hence, our estimates of phenotypic frequencies are likely to overestimate actual frequencies present throughout individual fields. However, it appears that continued treatment with endosulfan can rapidly increase the frequency of the resistant phenotype (Brun & Suckling, unpublished data).

Comparison of methods in monitoring resistance

The high level of resistance (>1000 fold) to endosulfan, determined from the Potter tower direct spray technique, has been reported previously (Brun *et al.*, 1989). The resistance factor ($LC_{50}R/LC_{50}S$) was more than 500 fold with the indirect exposure and is of the same order as that determined by the direct spray method. The main limitation of the Potter tower direct spray technique is the need for specialized spray application equipment. The indirect exposure technique was developed with the aim of providing an easy field laboratory technique.

A very close similarity of endosulfan toxicity to susceptible insects was evident with the direct spray technique and indirect exposure methods (Brun *et al.*, in press). This led us to use the same endosulfan concentration (400 ppm) as a diagnostic dosage for the survey using direct spray and indirect exposure methods, even though the slight differences in slope resulted in slightly different probabilities of survival ($LC_{99.95}$ and $LC_{99.99}$ respectively). The precision of the bioassays was similar, according to the magnitudes of the standard errors (table 2).

The indirect exposure technique offers reproducibility at a low cost, and ease of use. Monitoring for resistance in *hampei* in other coffee growing countries is now possible with the development of this simplified method. The efficiency of the technique permits extensive screening of strains and should allow early detection of resistance problems and reduces the cost to developing countries. The method is proposed as a provisional method for early detection of resistance (Brun *et al.*, in press).

Effect of management history

Brun *et al.* (1989) have speculated that transport of coffee berries infested with resistant *hampei* might partially account for the rapid spread and patchy distribution of resistance in remote valleys, in view of the reportedly limited natural dispersal ability of this insect (Le Pelley, 1968). The high frequency of resistance which occurred in two regions (table 1, fig. 1) may be related to locally important factors. A centralized coffee processing factory and a coffee research station (Institut de Recherche du Café et du Cacao, IRCC) are located at Ponérihouen. The research station was the first location to introduce both insecticide applications and the modern sunny plantations, suggesting that the longest history of selection pressure may have been in this region. The depot nearby at Poindimié would also be at risk due to the arrival of trucks containing coffee berries from a wide catchment area.

The proportion of fields with endosulfan resistance

Table 3. Percentage of fields with endosulfan resistant *H. hampei* present, in relation to management history. The number of fields is included in parenthesis.

	Untreated fields	Treated fields
Shady fields	30%	79%
	(10)	(34)
Sunny fields	80%	94%
	(5)	(47)

Table 4. Percentage survival of adult female *H. hampei* from fields with endosulfan resistance, after direct spray exposure to the diagnostic dosages of endosulfan (400 ppm) or lindane (300 ppm) using the direct exposure technique, divided according to sunny or shady fields, with or without applications of endosulfan within the preceding 12 months.

	Untreated fields	Treated fields
Direct spray of endosulfan		
Sunny fields	8.8%	41.3%
(SEM)	(1.4)	(0.2)
Shady fields	3.3%	25.4%
(SEM)	(0.7)	(0.2)
Direct spray of lindane		
Sunny fields	15.8%	33.8%
(SEM)	(1.8)	(0.2)
Shady fields	3.2%	24.8%
(SEM)	(0.7)	(0.2)
Indirect exposure to endosulfan		
Sunny fields	19.8%	34.4%
(SEM)	(8.2)	(0.3)
Shady fields	1.7%	24.4%
(SEM)	(1.0)	(0.4)

present (based on both direct and indirect exposure methods) were compared for Poindimié, Ponérihouen, and Touho, using criteria of sunny or shady plantations, with or without endosulfan treatment in the preceding 12 months (table 3). Regions with little or no resistance were not included in this analysis, as it was necessary to assume an equal likelihood of resistance throughout fields of all types in order to investigate the influence of management factors. There is no reason to discount this assumption for these three regions, since the geographic distribution of different types of fields was more or less random within regions.

The frequency of resistance was significantly higher among treated sunny, compared to treated shady fields ($P<0.05$, χ^2 goodness of fit). While a similar trend appeared to hold for the untreated fields (table 3), sample sizes were too small for a statistical comparison. It would appear that sunny or treated fields were more likely to have successful establishment of resistant *hampei* than untreated or shady fields. However, the occurrence of resistance at several untreated shady fields provides support for the suggested role of road transport. At least two shady fields known to have never been treated with endosulfan (due to their isolation) had resistant *hampei*. The very high proportion of treated sunny fields with resistance present (93%) adds further weight to this interpretation.

Analysis of variance on data from direct spray of endosulfan from samples collected at fields with resistance present at Poindimié, Ponérihouen, and Touho indicated that sample site ($P<0.001$), recent endosulfan treatment ($P<0.001$), and plantation type (sunny or shady) ($P<0.05$) were all significant factors affecting the phenotypic frequency of resistance, shown by percentage survival of the diagnostic dose (table 4). No interaction between treatment and plantation type was present. Analysis of results from direct spray of lindane and indirect exposure to endosulfan confirmed these findings.

The influence of recent endosulfan treatment significantly increased both the probability of the presence of resistance at a particular field (table 3), and the phenotypic frequency of resistance (table 4), compared to fields which had not been treated recently, due to factors such as abandonment by farmers as well a proximity to residences, inaccessibility to the truck-mounted sprayers, etc. If, as has been assumed, all fields were equally at risk of infestation by resistant *hampei*, then our data suggest that endosulfan selection pressure may be necessary to reach and maintain the higher resistance frequencies seen in treated fields, indicating that fitness levels are lower in resistant insects. Alternatively, dilution of the resistance frequency from mixing with susceptible insects present in the same fields could be important.

The influence of sunny compared to shady fields could act in several ways to increase the frequency of resistant insects. Endosulfan has a positive temperature coefficient (Knauf, 1982), and it seems likely that the warmer temperatures present in sunny fields at the time of spray applications would increase the selection pressure for resistance compared to the cooler temperatures and consequently lower toxicity in the shady plantations. The physical interruption of spray penetration by canopy trees could also be important in reducing the effectiveness of insecticide applications in shady fields.

Coffee production in the two worst affected regions (Poindimié and Ponérihouen) amounts to around one-third of the total in New Caledonia and in 1987 was 150 tonnes (B. Chambon, pers. comm.). The loss in coffee quality due to the resistance in these two regions in 1987 has been estimated by the Coffee Board as between 9×10^6–16.5×10^6 Pacific Francs (approximately £53–98000), a loss of up to 30% of the value of the crop. Losses in other regions with resistance present (Touho, Hienghéne and Houailou) have not been estimated but would appear to have been minor based on our data. However, our work suggests that in the absence of any changes to control measures, the potential exists for losses in coffee quality and value for Touho, Hienghéne and Houailou. For this reason, the use of organophosphates has been recommended for the five affected regions.

Conclusions

1) Both the Potter tower direct spray and indirect exposure methods showed extremely good precision at estimating the frequency of resistant strains in different regions of New Caledonia using a diagnostic dosage of 400 ppm of endosulfan.
2) Lindane and endosulfan resistance were closely

linked. Resistance to endosulfan would be more likely to be present at sites with a history of lindane usage.

3) Resistance was present at five of fifteen coffee growing regions in New Caledonia. Very high frequencies of resistance were found at Ponérihouen and Poindimié. Over half the fields tested at Touho had resistance present. Low frequencies of resistance were present at Hienghène and Houailou.
4) Samples from fields with resistance present varied considerably in the proportion of resistant individuals in the samples, suggesting the potential for the problem to worsen in these regions.
5) Sunny fields, and those with a recent history of endosulfan treatment were more likely to have resistance present and had higher frequencies of resistant *hampei* when resistance was present.

Acknowledgements

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