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Field Selection for Endosulfan Resistance in Coffee Berry Borer (Coleoptera: Scolytidae) in New Caledonia

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ABSTRACT A direct spray technique was used to monitor the frequency of endosulfan-resistant *Hypothenemus hampei* (Ferrari) in transects across individual coffee fields that had been sprayed from the road. A rapid decrease in resistance frequency (phenotypic cline) away from the road was evident in one traditional low-density (shady) and two modern intensive (sunny) fields surveyed in 1988. Subsequent treatment of one sunny field with two applications of endosulfan in 1989 resulted in an increase in the frequency of the endosulfan-resistant phenotype by an average of 61.4% across the field. In contrast, treatment of a second sunny field with fenitrothion led to decreased frequency of the endosulfan-resistant phenotype by an average of 12% across the field. Concentration–mortality responses for beetles from particular locations in the fields (e.g., the roadside or most distant side) confirmed results obtained with the diagnostic concentration ($LC_{99.95}$ of susceptible individuals). After 1 year, frequency of the phenotype resistant to endosulfan also declined an average of 18% in a shady field treated with fenitrothion. Responses of beetles collected from the roadsides of other coffee fields confirmed that the frequency of the resistant phenotype declined an average of 47.3% at sunny fields and 34.5% at shady fields in the second year, after fenitrothion began to be used. Further applications of endosulfan raised the resistance frequency in four of five fields in the second year. Changes in frequency of the resistance phenotype in the absence of the insecticide suggest that the frequency of endosulfan resistance may not decline rapidly enough to justify reintroduction of endosulfan within several years.

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

COFFEE BERRY BORER, *Hypothenemus hampei* (Ferrari), the most important cosmopolitan pest of coffee, is highly resistant (500–1,000 fold) to endosulfan in New Caledonia (Brun et al. 1989, 1990a). In New Caledonia, coffee is grown in small plantations (usually 0.25 ha). Coffee in older plantations (30–40 yr old) is grown at a density of 400–500 trees/ha under shade provided by a mixed canopy of native trees; in more recent plantations (<8 yr old), coffee trees are cultivated in full sunlight and at high planting density (1,500 trees/ha) (shady and sunny, respectively; Brun et al. [1990b]). Production in the sunny plantations is up to three times higher (1,500 kg/ha) than in the older plantations (500 kg/ha) because of close planting and more intensive management practices (Brun et al. 1989).[1]

Compared with most other coffee-producing countries, a unique feature of *H. hampei* control in New Caledonia is the practice of spraying from the road. Accessible fields are generally treated with insecticide twice a year by a government agency, the Coffee Board (Agence de Dévéloppement Rural et d'Aménagement Foncier). Spray is applied from roadsides with vehicle-mounted air-blast sprayers, regardless of crop management practices in individual fields (e.g., removal of infested berries from the crop). Infestations begin in March–April with one to two females per berry; populations increase to 50–100 per berry before harvest in November–December. Because fertilizer is required to maintain berry production, production rapidly decreases in abandoned fields.

Endosulfan (Thiodan 35 emulsifiable concentrate [EC], Hoechst AG, Federal Republic of Germany) was routinely applied after 1974–1975 at a rate of 700 g (AI)/100 liters and a volume of 100–150 liters/ha (Brun et al. 1989). Endosulfan resistance was confirmed following significant borer infestations in 1986 and 1987 (Brun et al. 1989). Resistance was significantly more frequent in the newer, sunny plantations that had been treated with endosulfan during the preceding 12 mo compared with fields that had not been treated recently (Brun et al. 1990b). An extensive survey of >200 fields in 1988–1989 showed that the resistance problem was confined to five regions on the East Coast: Poindimié (100% of fields sampled had resistant insects present), Ponérihouen (97%), Touho (63%), Houailou

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(10%), and Heinghène (6%) (Brun et al. 1990b). In Poindimié, a significant difference in resistance level was detected between samples of insects taken from the roadside and samples taken 50 m into a coffee field (Brun et al. 1989). This difference suggested that the practice of spraying fields from only one side could have influenced the distribution and level of insecticide resistance within individual fields.

The effects of direct selection pressure from insecticides on the frequency of insecticide resistance in pests have often been used on laboratory populations because of the difficulties associated with estimating fitness in the field. In a few studies, selection acting on insecticide-resistant phenotypes over time has been investigated to estimate fitness values in the field (Krimbas & Tsakas 1971, Curtis et al. 1978, McKenzie & Whitten 1982) or under near-field conditions (e.g., Daly et al. 1988). One problem in estimating field selection parameters on natural populations is the possibility that gene flow can have a major effect on resistance frequencies and can possibly obscure the effects of selection (May et al. 1975).

We considered an understanding of the spatial distribution of resistance in *H. hampei* and the responses of field populations to different selection regimes to be a desirable step toward the goal of designing a valid resistance management program for this species. First, we sought to determine if the phenotypic frequency of resistance (defined as the frequency of survivors of the diagnostic concentration; i.e., the $LC_{99.95}$ of susceptible individuals) would be higher near roadsides than farther away. The presence of a gradient, or cline (Roughgarden 1979) in resistance frequency seemed probable given the directional application of endosulfan in New Caledonia and earlier results that indicated a difference in frequency at two sites within a field (Brun et al. 1989). Second, we determined whether the pattern of resistance frequency would change with continued endosulfan use or the use of the organophosphate insecticide fenitrothion (Folithion 1000 emulsifiable concentrate [EC], Bayer Australia Ltd., Sydney). The coffee berry borer has no cross-resistance to fenitrothion (unpublished data), a chemical which was introduced for use against the beetles resistant to endosulfan in 1989 (Brun et al. 1990b). A rapid reduction in resistance frequency (following the use of an alternative insecticide for 3–5 yr, for example) might permit eventual reintroduction of endosulfan; a slower rate of reversion would prevent this possibility (Roush 1989).

Materials and Methods

Insect Sampling. Fields at Poindimié (PN), Ponérihouen (PO), and Touho (TO) (East Coast) were studied over 2 yr (1988 and 1989) before and after insecticide treatments. Berries containing *H. hampei* were collected in transects across three sunny fields (PN103, 30 by 112 m, tree spacing 3 × 2.5 m; PO104, 50 by 125 m, tree spacing 3 × 2 m; PO01, 50 by 50 m, tree spacing 3 × 2 m) and one shady field (PN1401, 40 by 100 m, tree spacing 5 × 5 m). The first two sunny fields were chosen because they had similar management histories before 1989 and plants were the same age (5 yr old); trees were 2.5 m tall. The third sunny field (PO01) had been abandoned for some time after planting and was never treated with endosulfan. This field was sampled by transect in 1989 to determine the distribution of resistance in the absence of directional applications of endosulfan. This field was unusual because it had road access from both ends of the transect from a perimeter road surrounding the field. The nearest field with resistance present was 5 km away. PO01 was about 15 km from the factory at Ponérihouen; it was surrounded on three sides by native forest (10–15 m canopy).

The shady field was ≈30 yr old; trees were 2 m tall (they had been cut every 8 yr to maintain productivity). Samples of several hundred berries were taken from trees at the roadside and at intervals along transects at right angles to the roadside toward the back of the fields (50–125 m away). Between 4 and 12 sample sites were used across the fields, depending on the availability of berries at the time of sampling. Although a difficult sociopolitical situation on the East Coast during 1988–1989 limited our access in certain cases, repeated sampling within a 4-mo period (during which insecticide was not used) indicated little difference in percentage mortality from the same site (unpublished data).

The first transect at PN103 was taken in November and December 1988. Two endosulfan applications (700 g [AI]/100 liters) were made from the roadside with a B.S.E. Super Bangui (Bernhard Schulze-Eckel, Ahlen, Federal Republic of Germany) at 150 liters/ha (in January and February 1989). The field was sampled again in August 1989. At the second sunny field (PO104), a comparable transect was sampled in September 1988 before fenitrothion (1.2 kg [AI]/100 liters) was applied in January and February 1989. The transect was sampled again in July 1989. The third transect (PO01) was sampled in August 1989 to determine whether resistance was present at this site. The site had never been treated with endosulfan but was in the vicinity of other fields, 97% of which were shown to have resistant beetles present (Brun et al. 1990b). We hypothesized that resistant beetles might have arrived at this field by road transport of harvested berries from nearby affected fields but did not reach high levels because of lack of selection. This field was treated separately in all analyses and served as a control for our hypothesis on directional selection with endosulfan at other fields. Beetles were tested only at the diagnostic concentration of 400 ppm endosulfan in 1 yr to determine whether resistance was present, and if so, whether a cline in frequency was detectable.

The transect across the shady field (PN1401) was sampled in November 1988, treated twice with fenitrothion (1.2 kg [AI]/100 liters was applied in January and February 1989), and was sampled again in December 1989. All applications of insecticides were made from the roadside. Coffee berries were brought from collection sites and stored at 25 ± 1°C and 80% RH for ≈1 mo before being tested.

In addition to these transects, 16 other fields were sampled only from roadsides in 1988 and 1989. These fields were similar to the other fields; i.e., they faced the unsealed road and were otherwise surrounded by uncultivated scrub consisting of grass and bushes or native forest. Concentration–mortality responses were estimated for beetles from seven fields; beetles from the other nine fields were tested only at the diagnostic concentration. Fields in Poindimié and Ponérihouen were treated with fenitrothion from January 1989, in contrast with use of endosulfan in areas affected by resistance (except for PN103).

Direct Spray Technique. Concentration–mortality responses for a range of samples have been reported previously with the direct spray technique (Brun et al. 1989; 1990a,b). Berries were opened with a sharp scalpel to remove adult females before each test. The sex ratio strongly favored females (>10:1). Because males are smaller and flightless, they were not used. A glass ring (5 cm diameter, 2 cm high) was used to confine 30 healthy females on a piece of filter paper during application. Each experiment was replicated two to eight times, resulting in a mean of 90 and a range of 60–240 beetles per test. A Potter spray tower (Potter 1952), calibrated to deliver 1.6 mg/cm^2 to the spray table, was used to apply 2 ml of liquid through the nozzle. After treatment, the glass ring was covered by a nylon screen to prevent escape of beetles. The adults were held at 25 ± 1°C and 80–85% RH under natural light for 6 h before mortality was assessed. Assessment of mortality after 6 h provides adequate separation of resistant and susceptible phenotypes (Brun et al. 1992).

Concentration–mortality responses were estimated where sufficient beetles were available, including samples from the transects (10 fields). When this was not possible, a diagnostic concentration of endosulfan was used (400 ppm, the $LC_{99.95}$ of susceptible beetles [Brun et al. 1990a]). A treatment with water was included as a control; control mortality was consistently <5%. The criterion for death was the absence of coordinated movement when a beetle was touched with a fine brush. Samples with any beetles surviving the diagnostic concentration were considered to include resistant individuals.

Statistical Analysis. Results were segregated according to the type of field (sunny or shady) and insecticide use. Lack of knowledge of the genetic basis of resistance was a constraint in estimating the insecticide selection pressure because we have no knowledge about dominance and fitness of genotypes. Therefore, we analyzed the data as percentages of two phenotypes (resistant and susceptible). Angular transformation was used throughout (except in the figures or unless otherwise stated) to create a linear scale and stabilize the variance of percentage mortality (the rate of change of untransformed mortality is otherwise a function of its position on the scale). Linear regression (Beaux et al. 1988) of mortality on distance from the roadside was used to describe the linear portion of transect data. χ^2 contingency tests (at $P < 0.05$) were applied on raw mortality counts at the $LC_{99.95}$ for 19 fields (including three transects) to test for differences between years. Student's t tests (Beaux et al. 1988) were performed on the difference in transformed mortality between years at the $LC_{99.95}$, to investigate whether a change in the frequency of the resistant phenotype occurred overall in roadside populations between 1988 and 1989 at different types of fields.

For the two transects where responses of samples at a range of concentrations were available (fields PN103 and PO104), we included responses at concentrations $>LC_{99}$ (200–10,000 ppm) as replicates in the analysis of differences between the 2 yr because the concentration–responses in this range are normally completely flat for samples containing resistance (e.g., Fig. 2 and 4) (Brun et al. 1989). Five concentrations were generally used for each field each year. We included mortality values up to and including 100% (not shown in Fig. 2 and 4) because the concentration range was equivalent between years. Means were back-transformed from the angular transformation for tabular presentation.

Results

A rapid change in mortality of *H. hampei* at the diagnostic concentration of endosulfan was observed in the transect samples taken across the first coffee field (PN103) in November and December 1988. Values reached an asymptote only 50 m from the roadside (Fig. 1). Regression of mortality on distance (because of the plateau in the data, we used the first six points from December 1988 only) indicated a linear response over this range (transformed mortality = −0.0411 + 0.237 [distance, m]; r^2 = 95%; $P < 0.01$). This result, which demonstrates the presence of a rapid change in resistance frequency from the roadside across the field, or phenotype cline (Roughgarden 1979), indicates that the endosul-

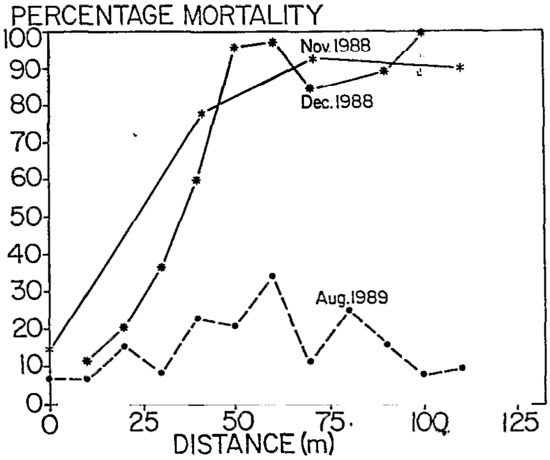

Fig. 1. Phenotypic frequency of endosulfan resistance of *H. hampei* across a modern sunny coffee field (PN103) in New Caledonia, indicated by mortality at the $LC_{99.95}$ of endosulfan (400 ppm) on three sampling occasions (November and December 1988 and August 1989). Two applications of endosulfan were applied from the roadside (0 m) in the time between transects in 1988 and 1989.

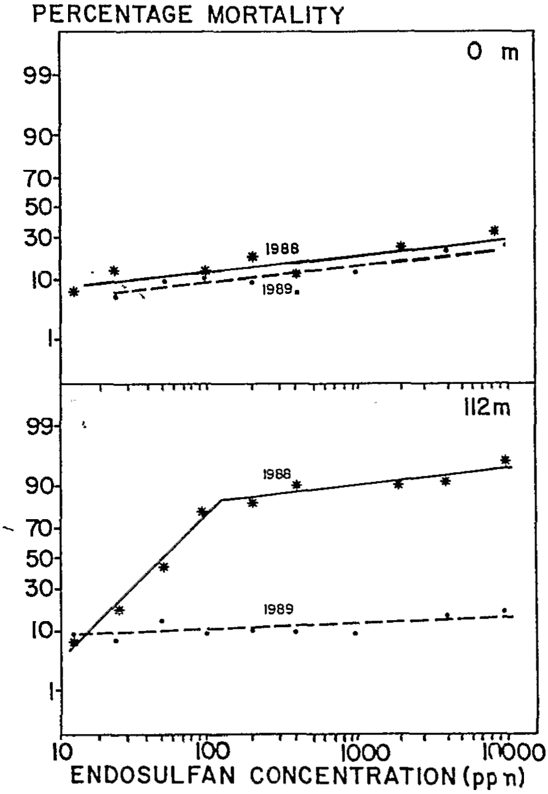

Fig. 2. Concentration–mortality responses to endosulfan of *H. hampei* with time at two positions in a modern sunny coffee field (PN103) in New Caledonia (at the roadside [0 m] and far end of the field [112 m]). Two applications of endosulfan were applied from the roadside (0 m) in the time between transects in 1988 and 1989.

fan-resistant phenotype was most frequent near the roadside where spray treatments had been applied. However, by August 1989, after two endosulfan spray applications were applied from the roadside in January–February 1989, a major increase in resistance frequency had occurred, resulting in a high frequency right across the field. χ^2 contingency tests indicated no significant difference between years at the first two transect points with paired data at the diagnostic concentration (13 and 23 m), but all other paired transect points (Fig. 1) had highly significant differences in response between years (χ^2 = 27.99, 33.29, 89.54, 104.20, 126.29, and 126.72; df = 1; $P < 0.001$. This pattern suggests that a very rapid spread of high-resistance frequency occurred away from the roadside across the entire field during the year after only two endosulfan applications.

Samples from two positions in this field (PN103) (the roadside [0 m] and far end of the field from the roadside [112 m]) were tested before and after endosulfan field applications were made in 1989. The results (Fig. 2) showed flat responses at high concentrations of endosulfan similar to those reported by Brun et al. (1989). The same pattern at the diagnostic concentration was observed in another sample where the roadside sample exhibited significantly less mortality (i.e., had more resistance) than the sample collected at the far end of the field away from the spray application. A significant decrease in mortality occurred in samples of beetles from the roadside treated with approximately the LC_{99} (200 ppm) (t test on transformed mortality; t = −3.61, df = 3, $P < 0.05$). At 90%, the frequency of the resistant phenotype at the roadside of this field was close to the maximum level previously observed (Fig. 2 and Brun et al. 1990b). The difference in untransformed mean percentage mortality between years was only 1%, suggesting little effect from continued selection. In samples from the far side of the field, the decrease in mortality across a range of concentrations was much more significant (t = −33.41, df = 5, $P <$ 0.001), with a mean decrease in mortality (at concentrations from 200 to 10,000 ppm) of 61.4%.

In 1988, a similar pattern of mortality at the diagnostic concentration was evident in samples from the second sunny field (PO104). Very low mortality (16%) occurred at the roadside and increased to >90% at the far side of the field, 125 m from the roadside (Fig. 3). Linear regression of mortality on distance was significant (transformed mortality = 0.4551 + 0.0076 [distance, m]; r^2 = 98%, $P < 0.001$).

PO104 was treated with fenitrothion in early 1989, and further samples were taken 10 mo after the 1988 samples. A loss in resistance was evi-

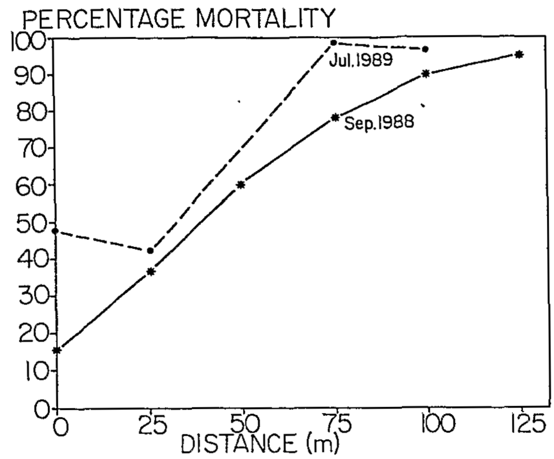

Fig. 3. Phenotypic frequency of endosulfan resistance of *H. hampei* across a modern sunny coffee field (PO104) in New Caledonia, indicated by mortality at the $LC_{99.95}$ of endosulfan (400 ppm). Two applications of fenitrothion were applied from the roadside (0 m) in the time between transects in 1988 and 1989.

dent between 1988 and 1989. The increase in percentage mortality at the diagnostic concentration was significant at the roadside ($\chi^2 = 33.02$, df $= 1$, $P < 0.001$), 75 m ($\chi^2 = 23.38$, df $= 1$, $P < 0.001$), and 100 m away at the far end of the field ($\chi^2 = 4.36$, df $= 1$, $P < 0.05$), but not at 25 m at the single diagnostic concentration (Fig. 3). However, sufficient beetles were available from four positions at PO104 to test a wider range of insecticide concentrations in both years (Fig. 4). Student's t tests on mortality at the LC_{99} of susceptible individuals (200 ppm) and higher concentrations indicated significantly more mortality in the second year at all four positions in the field (0 meters, $t = 11.78$, df $= 2$, $P < 0.01$; 25 m, $t = 3.48$, df $= 3$, $P < 0.05$; 75 m, $t = 13.42$, df $= 4$; $P < 0.001$; 100 m, $t = 4.07$, df $= 3$, $P < 0.05$). Several samples from 1989 showed 100% mortality at higher concentrations of endosulfan (>10,000 ppm, not shown in Fig. 4), but in 1988 the maximum mortality had reached only 87% at concentrations up to 10,000 ppm in this field. The mean decrease in frequency of resistant phenotype was 12% across the field.

Transect samples were collected from a single shady field with native forest canopy (PN1401) in 1988 and 1989, and beetles were tested at the diagnostic concentration (Fig. 5). In contrast with the first two sunny fields, the relationship between mortality and distance from the roadside was not as evident in 1988 ($P = 0.08$), although a significant correlation was apparent in 1989 (transformed mortality $= 1.267 + 0.0026$ [distance, m]; $r^2 = 83\%$, $P < 0.05$). The field was treated with fenitrothion in the period between sampling. Only a moderate level of resistance was evident in 1988, and mortality differed

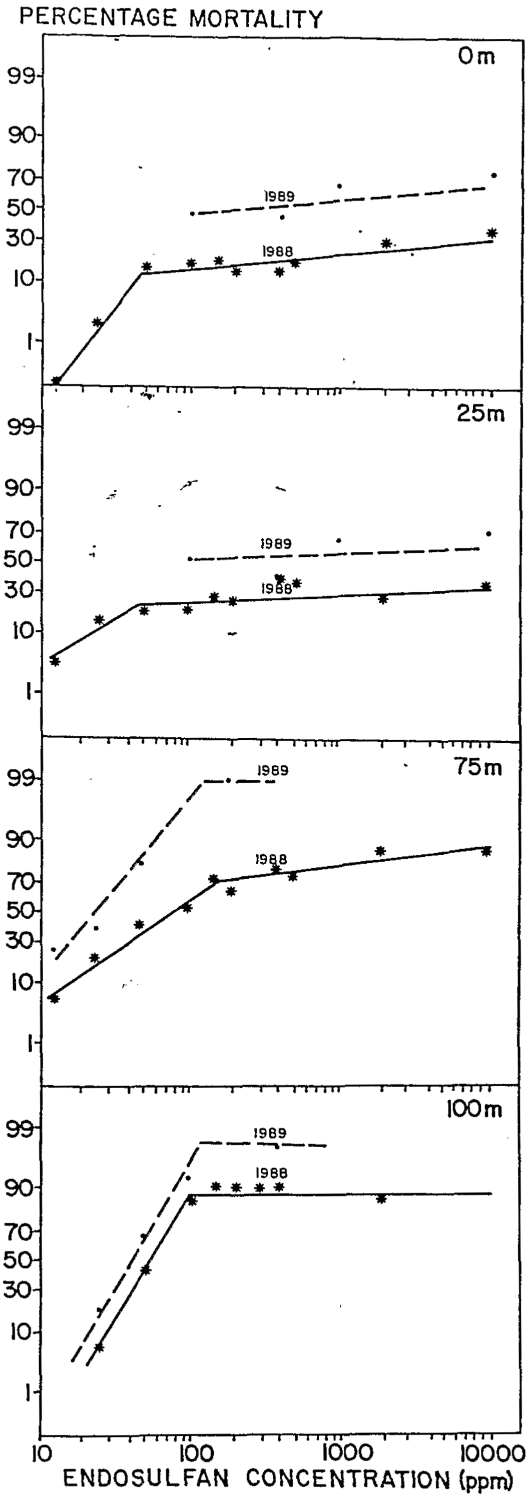

Fig. 4. Concentration–mortality response to endosulfan of *H. hampei* with time at four positions in a modern sunny coffee field (PO104) in New Caledonia (at the roadside [0 m] and across the field [25, 75, and 100 m away]. Two applications of fenitrothion were applied from the roadside (0 m) in the time between transects in 1988 and 1989.

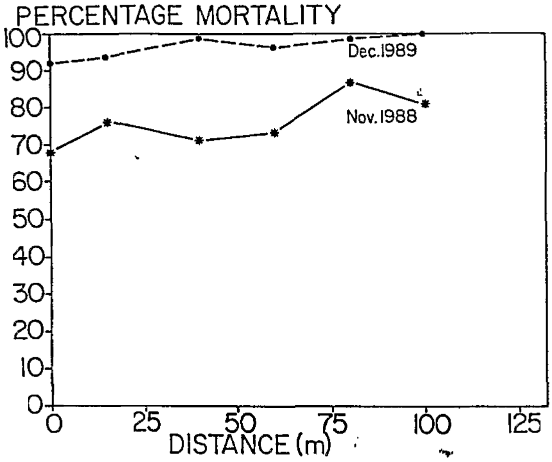

Fig. 5. Phenotypic frequency of endosulfan resistance of *H. hampei* across a shady coffee field (PN1401) in New Caledonia, indicated by mortality at the $LC_{99.95}$ of endosulfan (400 ppm). Two applications of fenitrothion were applied from the roadside (0 m) in the time between transects in 1988 and 1989.

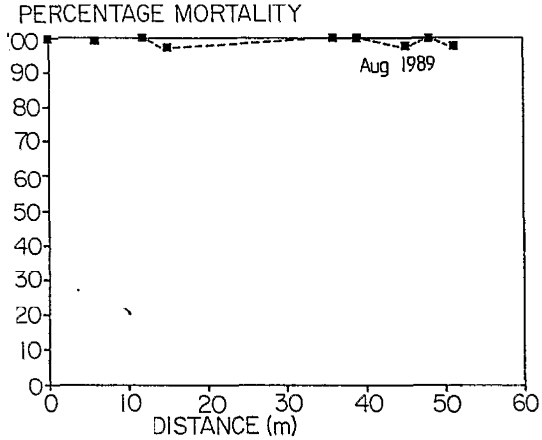

Fig. 6. Phenotypic frequency of resistance to endosulfan of *H. hampei* across an abandoned, unsprayed, modern sunny coffee field (PO01) in New Caledonia in August 1989.

among samples from six locations in the field (χ^2 = 14.81, df = 5; $P < 0.05$). This result provided some evidence for differential selection pressure with distance from the roadside, as we had observed in the sunny fields. A significant loss in resistance occurred between years at all positions across the field (0 m, χ^2 = 17.07, df = 1, $P < 0.001$; 20 m, χ^2 = 10.70, df = 1, $P < 0.01$; 40 m, χ^2 = 12.39, df = 1, $P < 0.001$; 60 m, χ^2 = 18.29, df = 1, $P < 0.001$); 80 m, χ^2 = 7.88, df = 1, $P < 0.01$; 100 m, χ^2 = 19.77, df = 1, $P < 0.001$). The mean decrease in frequency of resistant phenotype was 18% across the field.

Resistance was detected at the abandoned sunny field, but a trend with distance from the perimeter road at either end of the field was not apparent (Fig. 6). Only a few beetles were present in the center of this field, suggesting limited colonization from the perimeter road. Berry production was also very low because of the lack of fertilizer. The overall frequency of resistance in the field was 1% (n = 5,906 beetles tested). This result corroborates the hypothesis of Brun et al. (1989, 1990b) of movement of resistant beetles within the affected areas (probably by road transport) and limited establishment of resistance independent of insecticide use.

Concentration–mortality responses were estimated for beetles collected from roadside berry samples from seven more fields in 1988 and 1989. In all cases, the results followed the pattern described above (Fig. 2 and 4); i.e., flat concentration mortality responses at >200 ppm of endosulfan, with an increase or decrease in mortality between years depending on insecticide use in the field. The mortality at $LC_{99.95}$ was a reliable indicator of the results at higher concentrations because of the flat concentration–mortality response resulting from high resistance levels. For brevity, we present the results only for tests with this concentration along with those for nine fields for which samples were tested only at this concentration.

The direction and amount of change in level of resistance in samples of beetles tested at the diagnostic concentration depended on insecticide treatment (Fig. 7). Although Student's t tests indicated that together, the two untreated sunny fields did not show a significant increase in mean resistance frequency (Table 1), an increase in mortality was evident in one of the fields according to χ^2 tests (Fig. 7, Field 2). We expected loss of resistance in both the untreated fields equivalent to that seen in the fields treated with fenitrothion. Field-to-field variation could result from differences in beetle phenology, dispersal characteristics related to differences in beetle density or management of coffee production, or microclimate. A general similarity in resistance levels was evident at many fields in 1988 (e.g., fields 9–19, Fig. 7).

Further treatment with endosulfan led to significantly decreased mortality ($P < 0.05$) for three of five individual sunny and shady fields (Fig. 7, fields 3–7). The difference between years was not significant when all fields were examined together (Table 1) due in part to the small change in resistance frequency in field 5. Although the resistance level at the roadside was already near the maximum observed anywhere and may have had little prospect of increasing further (Fig. 2), mortality in field 5 (PN103, reported earlier) was significantly different between years at most sites, or when ranges of concentrations were compared. The largest change in mortality seen was at field 3, where the frequency of resistance after further endosulfan use increased from 2 to

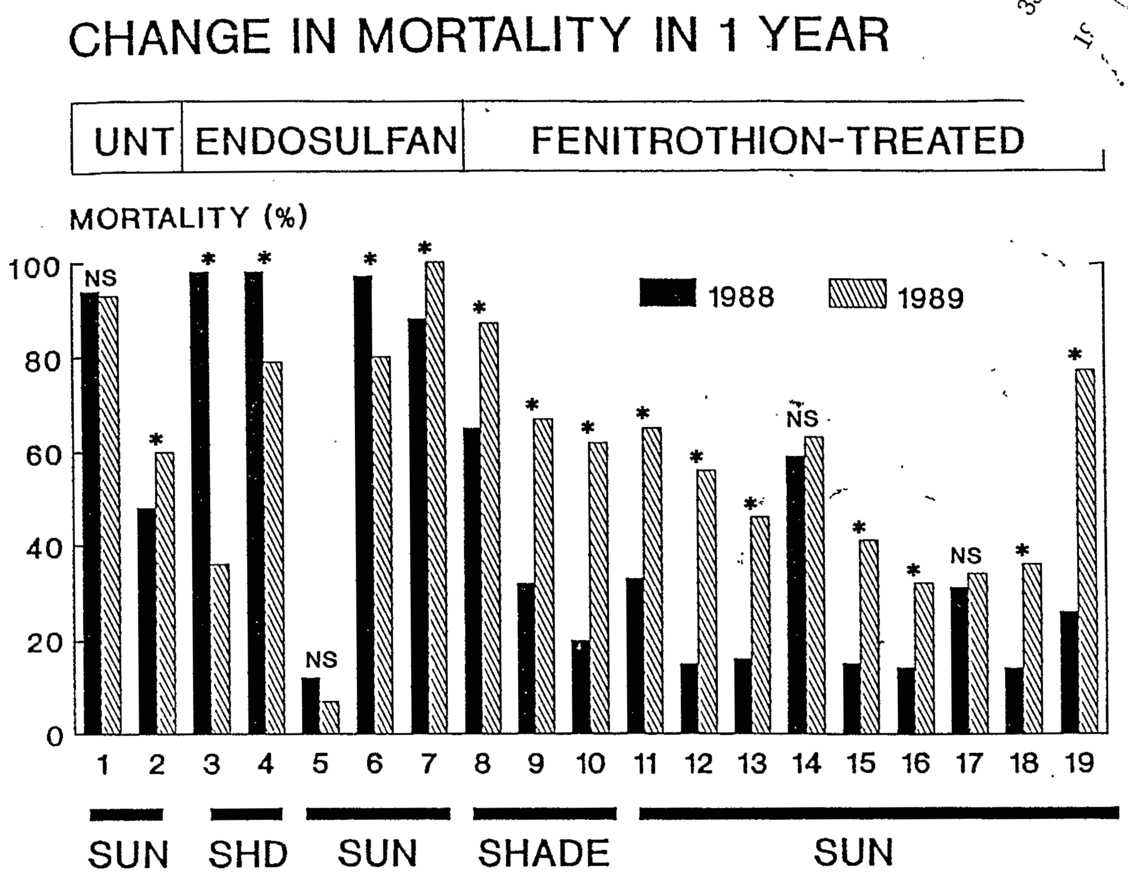

Fig. 7. Phenotypic frequency of endosulfan resistance of *H. hampei* from sunny and shady coffee fields in New Caledonia, indicated by mortality at the $LC_{99.95}$ of endosulfan (400 ppm); treatments between sampling occasions are indicated as untreated (UNT), endosulfan-treated, or fenitrothion-treated. Stars show fields where a significant change in resistance frequency was detected ($P < 0.05$); NS, not significant.

64%. Unfortunately, the samples from the one field which showed an unexpected result (a significant increase in mortality from 88 to 100% despite endosulfan use [field 7, Fig. 7]) were not adequate for further testing. The lack of fields treated with endosulfan within the regions containing resistant beetles was due to our recommendation to the Coffee Board to avoid the use of endosulfan in affected areas; only a special arrangement permitted us to study the effect of further endosulfan use on the resistance level at these fields.

Table 1. Mean percentage mortality of *H. hampei* tested at the $LC_{99.95}$ of endosulfan from collections in 1988 and 1989 from roadsides of coffee fields in New Caledonia under different management regimes[a]

Management regime	Field treatment					
	Endosulfan		Fenitrothion		Untreated	
	1988	1989	1988	1989	1988	1989
Sunny	70.0	69.2NS	23	50.1**	74.8	79.0NS
t	−0.05		4.96		1.57	
n	3		9		2	
Shady	98.0	58.3NS	38.3	72.8**	—	—
t	−2.43		6.78		—	
n	2		3		—	

[a] *n*, numbers of fields. Significance levels (*, $P < 0.05$; **, $P < 0.01$; NS, not significant) relate to *t* tests of angular transformed mortality for $\mu = 0$.

A switch to the use of fenitrothion led to a significant loss of endosulfan resistance frequency after 1 yr in both sunny ($P < 0.01$) and shady fields ($P < 0.05$) (Table 1), with two exceptions where the drop in resistance frequency was not significant (Fig. 7). For sunny fields (fields 11–19, Fig. 7), the mean drop was 47.3%; for shady fields (fields 8–10), the mean drop was 34.5%.

Discussion

Directional selection from roadside spraying may account for the phenotypic clines in resistance frequency observed in the sunny fields in 1988. Apparently our samples in 1988 were taken at a fortuitous time (relatively soon after the arrival of the resistant genotypes, possibly by road transport during harvest) (Brun et al. 1989, 1990b), so that we were able to detect the clines as a smooth increase in mortality across the fields. The presence of endosulfan resistance at unsprayed fields located en route to other fields (Brun et al. 1990b) (Fig. 6) provides evidence for inoculation from berries being transported along the road. Our results clearly show that the distribution of endosulfan resistance in New Caledonia is not stable. Evidence for this conclusion comes from the rapid changes in frequency that we observed (Fig. 7), the relatively recent detection of resistance following control failure in 1985–1987 (Brun et al. 1989), and its occurrence at 97–100% of surveyed fields in Ponérihouen and Poindimié in 1988–1989 but lower frequencies elsewhere (Brun et al. 1990b).

Extensive dispersal and gene flow within a coffee field would be expected to lead to a uniform level of mortality with distance from the roadside, once directional selection ceases. This pattern was observed at the abandoned field PO01 (Fig. 6), where transport of beetles from nearby fields during harvesting probably led to the accidental inoculation of the field with barely detectable levels of resistance. Our best available estimate, given the cycle of coffee production and climate in New Caledonia, is between four to six (overlapping) generations per year. Possibly, dispersal behavior may be limited to certain times of year so that not all generations are dispersive. This would slow the process of mixing of phenotypes.

The rapid rise in resistance level beyond 30 m from the roadside of PN103 between 1988 and 1989 (Fig. 1) could be due to dispersal of resistant individuals selected at the roadside or the effects of insecticide selection across the field. Dispersal of *H. hampei* is apparently not extensive (Waterhouse & Norris 1989), although resistant beetles were detected 20 m into an untreated field (PO01). Furthermore, application of endosulfan from roadsides on coffee fields in New Caledonia resulted in very heavy deposition of insecticide near the roadside but no detectable deposits after only 30–40 m (Parkin et al. 1992). However, field bioassays showed that mortality of susceptible beetles inside coffee berries extended to 78 m across the field. Resistant beetles showed complete survival only 16 m from the point of treatment with a decreasing difference in mortality and consequently reduced selection pressure with distance across the field (Parkin et al. 1992).

Parkin et al. (1992) demonstrated that the vapor action of endosulfan (Knauf 1982) was involved in the field efficacy of this insecticide against *H. hampei* (the mortality of beetles extended considerably farther than the detectable spray deposition). Mortality of beetles within berries caused by vapor action during two applications 1 mo apart could explain the relatively even changes in mortality with distance. Diffusion would reduce the variability in exposure experienced by the insects compared with the variability in deposits on the surfaces of coffee trees (Parkin et al. 1992). The desired rate of application (700 g/ha) apparently is considerably exceeded near the roadside following direction applications, potentially raising the selection pressure considerably above the level experienced under more uniform application conditions.

Site-specific factors (e.g., tree height, density, topography) and meteorological conditions (especially temperature, wind velocity, and wind direction) at the time of application are apparently important in determining the distance and extent of the selection pressure exerted by a particular directional application. These operational factors (Georghiou & Taylor 1977) affect resistance. Spray deposits in shady fields were more evenly distributed after roadside spraying than were deposits in sunny fields, possibly because of more laminar flow conditions (Parkin et al. 1992). More even deposition probably explains the smaller difference in resistance level from the roadside across the shady field (PN1401, Fig. 5). The difference in temperature at 1.5 m between sunny and shady fields averages 3°C during January and February on the East Coast of New Caledonia (unpublished data). Slightly cooler daytime temperatures in shady fields could be expected to reduce mortality from endosulfan (Brun et al. 1992), possibly contributing to the lower levels of resistance recorded in shady plantations (Brun et al. 1990) (Table 1). A difference of 3°C would correspond to a 25% decrease in LC_{50} of susceptible beetles in sunny over shady fields (Brun et al. 1992) and, therefore, less intensive selection for resistance at shady fields because of the lower mortality from the same field rate.

The effects of high-dose and low-dose strategies on the development of resistance are contradictory, depending on the efficacy of kill of different genotypes achieved and the ability of the population to recover (Georghiou & Taylor 1977). Application of insecticide deposits across a wide range of rates would be expected within coffee fields treated from a single direction, as in New Caledonia. This could lead to changes in the kill of heterozygotes with distance. If the observed high level of resistance expression (500–1,000 fold, Brun et al. [1989]) is due to a single major gene, then our results would suggest that a high selection coefficient (Christiansen & Feldman 1986) had operated on the original roadside population. Furthermore, overdosing near roadsides probably favors newly arrived, resistant beetles, allowing a high frequency of resistance to spread rapidly across fields as appears to have happened in PN103.

The highest resistance levels were associated with a management history of recent endosulfan use in sunny plantations (Brun et al. 1990b). In our study, we have shown how rapidly resistance levels can rise across a sunny field under the continued use of endosulfan (Fig. 1). Reversion of resistance in the absence of endosulfan use was evident, although the rate of reversion could be expected to decrease with generation following removal of insecticide selection (Keiding 1967). Our results do not suggest that reintroduction of endosulfan in fields with detectable levels of resistance would provide adequate control for an extended period. A slow rate of reversion of resistance suggests that the difference in fitness between resistant and susceptible beetles in the absence of endosulfan may not be great (Crow 1957) and therefore reduces the likelihood of insecticide rotations in a realistic time frame of 3–5 yr.

Clinical variation in invertebrates has been studied relatively extensively (e.g., see Lees [1981] for a review of industrial melanism), but not in relation to insecticide resistance. Spatial variation in insecticide resistance has been commonly detected. Despite several recent studies on the influence of insecticide use on regional patterns of resistance (e.g., Heim et al. 1990, Knight & Hull 1990), clines in resistance seem not to have been reported widely. This could be due to lack of a gradient in insecticide selection (e.g., Roush et al. 1990), gene flow considerably in excess of selection, or lack of appropriate sampling. Clines in insecticide resistance frequency over short distances are also known for organophosphate-resistant *Epiphyas postvittana* (Walker) (Suckling et al. 1987; D.M.S., unpublished data) and *Typhlodromus pyri* (Scheuten) resistant to pyrethroids (N. P. Markwick & D.M.S. unpublished data).

Study of phenotypic clines seem to offer an advantage in estimating selection over time alone. Such studies could lead to descriptions of wave phenomena associated with the rate advance of resistance genes, or their stability in the presence of gene flow (May et al. 1975). Accurate estimation of field selection for resistant genotypes (e.g., Wood & Cook 1983) should enable predictions of resistance development and spread in future. In the case of *H. hampei*, a requirement for such accurate estimation will be the demonstration of the genetic basis of endosulfan resistance and a diagnostic concentration for heterozygotes, if monogenic resistance is present.

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