

EFFECT OF LABORATORY SELECTION FOR ENDOSULFAN RESISTANCE IN THE COFFEE BERRY BORERL.O. BRUN¹ and D. M. SUCKLING²¹*Institut de Recherche Scientifique pour le Développement en Coopération (ORSTOM), B.P. A 5, Nouméa, New Caledonia and*²*The Horticulture and Food Research Institute of New Zealand Ltd., P.O. Box 51, Lincoln, Canterbury, New Zealand***ABSTRACT**

The level of endosulfan resistance of male *Hypothenemus hampei* was compared with reciprocal crosses between resistant and susceptible parent strains. Resistant males had c. 1000-fold higher LC₅₀ values than susceptibles. Male progeny of susceptible female x resistant male crosses responded like susceptible males (ie. recessive). In contrast, progeny of resistant female x susceptible male crosses responded like resistant males (ie. dominant). Selection and backcrossing did not result in segregation of different levels of resistance in either sex. These results support the proposition that a single major sex-linked gene is primarily responsible for the high level of endosulfan resistance in this species.

Keywords: insecticide resistance, coffee berry borer, endosulfan, genetics, selection

INTRODUCTION

The coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) is the major pest of coffee worldwide (Le Pelley 1968). Endosulfan is the primary means of control because of its superior efficacy and lower environmental hazard than the current alternatives. High levels of endosulfan resistance have been reported from New Caledonia (Brun *et al.* 1989; Brun and Suckling 1992). Cross resistance to lindane, which was used until the late 1970s, may have contributed to the high endosulfan resistance frequencies observed in the field (Brun *et al.* 1990).

A sound understanding of the mode of inheritance is desirable for monitoring resistance. The genetic system in this insect is unusual, with a 10:1 sex ratio in favour of females (Le Pelley 1968). Males are dwarf and flightless. Recent work suggests that endosulfan resistance is inherited with functional haplodiploidy (Brun *et al.* 1995a). The preliminary evidence from a single dose of endosulfan shows that, unlike females, males cannot be functionally heterozygotic. Hence they are functionally R (resistant) or S (susceptible), not RS or SR, because they express n chromosomes, rather than 2n like females. In this genetic system, males can only exhibit half of the genotype of their mother (ie. males are S as long as their mother was SS or SR, or R if their mother was RR or RS). Cytological evidence (Brun *et al.* 1995b) indicated that this is caused by the compaction of the paternal male chromosome. Establishing the mode of inheritance and genetic basis of the resistance should help to understand the population genetics of resistance in the field. Here we present data on the responses to endosulfan of males and females from reciprocal and repeated backcrosses.

In addition to classical backcrosses to indicate the mode of inheritance (Brun *et al.* 1995a), it has been possible to develop a molecular probe for a single major gene that causes cyclodiene resistance, based on homology with *Drosophila* (French-Constant *et al.* 1994). While the results for inheritance of the resistance in female beetles implicated a single major gene, the possibility of a second gene could not be overlooked. Hence we undertook the selection experiments reported here.

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MATERIALS AND METHODS

Berries were collected in 1990 from fields in the Poindimié (resistant strain PN106), and La Foa regions (susceptible strain). Berries were stored at $25 \pm 1^\circ\text{C}$ (80% relative humidity) for 1-2 months before initial testing and insecticide selection of females. We selected females in preference to the relatively rare males.

Direct spray technique

We removed adult females from berries for each test. A glass ring confined 30 healthy females on a piece of filter paper during the application. We used two to nine replicates (60-270 beetles) per test. A Potter spray tower (Potter 1952) was used to apply 2 ml of aqueous endosulfan (400 ppm ai., the $\text{LC}_{99.95}$ of susceptibles) onto each sample. Beetles were held at $25 \pm 1^\circ\text{C}$ and 80-85% RH under constant darkness for 6 h before mortality assessment. Assessment after 6 h provided separation of genotypes (Brun *et al.* 1995b), but bioassays were also assessed after 7 days, since this assessment was more likely to provide a better estimate of the full effect of the insecticide. For selections, survivors of each concentration were reared on artificial diet (Brun *et al.* 1993).

Dose responses of males

Male beetles from susceptible or resistant strains were removed from berries, and exposed to direct spray of a range of concentrations, before mortality assessment after 6 h or 7 days. At least three replicates were used per test, with generally 15-30 males tested per concentration. Reciprocal crosses between susceptible and resistant strains were made, the progeny reared, and adult males tested as above.

Selection and backcrossing of males

Females from the resistant strain were mated with males from the susceptible strain to produce F_1 generation females (RS), which were then backcrossed to susceptible males (RS x S). The male offspring from backcross generations F_2 and F_3 were then exposed to a range of endosulfan concentrations to determine their dose-responses (insufficient males were available for testing in subsequent generations). Selection of female parents at the $\text{LC}_{99.95}$ of the SS genotype ensured that each generation cross was similar in composition.

Selection and backcrossing of females

As above, resistant females were mated with males from the susceptible strain, to produce the F_1 generation females (RS), which were then backcrossed to susceptible males (RS x S), to give a 50% mixture of female offspring of RS and SS genotypes. The dose response of these F_2 offspring was then determined. Survivors of the $\text{LC}_{99.95}$ of the SS genotype were presumed to be entirely of the RS genotype, and were mated with susceptible males, and tested up to F_5 . In practice, the mating preceded selection, although the result was the same, with survival limited to RS females.

Selection of strains at different concentrations

Females from another field strain (collected from Poindimié, strain PN103) were selected and inbred over 10 generations at seven concentrations of endosulfan (those in Table 1 plus 100,000 ppm) and the survivors from each selection used to maintain the colony. Dose-responses were generated at the 3rd, 5th, 7th, 9th and 10th generations. Probit analyses were performed using POLO (LeOra 1987).

RESULTS AND DISCUSSION

Dose response of males

Dose responses of resistant and susceptible males indicated ca. 1000 fold differences in LC_{50} s (Fig. 1), a similar resistance factor to females (Brun *et al.* 1989; 1995b). Control mortalities were often over 20% in males after 7 days, so results from 6 h are presented (Fig. 1). The responses of male progeny from the reciprocal crosses (F_1) were very different. Males from heterozygote resistant female (RS) x susceptible male crosses (S) responded in a similar fashion to males of the resistant parent strain (R, fully dominant). In contrast, male progeny of susceptible female (SS) x resistant male (R) crosses responded in a similar fashion to the susceptible parent strain (S, fully recessive).

Selection and backcrossing

Male progeny of female heterozygote (RS) x susceptible (S) male crosses showed similar dosage responses in the F₂ and F₃ generations, and were very close in response to males of the F₁ (RR x S) and parent resistant strain (Fig. 1). These results indicate that males expressed only a single gene for resistance or susceptibility, coming from the maternal side (ie. R). In contrast, female progeny of backcrosses showed a plateau slightly above 50% mortality, close to the heterozygote responses (intermediate dominance).

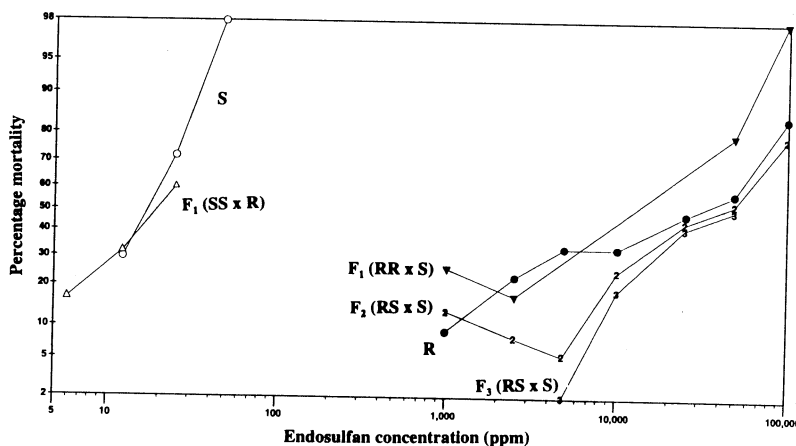


FIGURE 1: Dose responses of male *H. hampei* 6 h after being sprayed with endosulfan, showing susceptible males (S), resistant males (R), F₁ (progeny of SS x R and the very different RR x S), and backcrosses (RS x S, at F₂ and F₃).

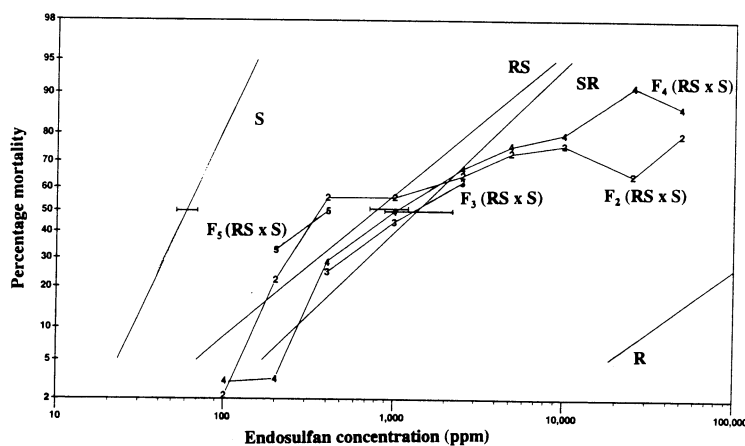


FIGURE 2: Dose responses of female *H. hampei* 6 h after being sprayed with endosulfan, showing susceptible (SS), resistant (RR), F₁ (SR and RS), and backcrosses (RS x S, at F₂, F₃, F₄ and F₅). Dose responses of homozygotes and heterozygotes (Brun *et al.* 1995a) are represented for comparison.

The repeated backcrossing and selection for heterozygote females using the LC_{99.95} to remove homozygous susceptible females did not result in major changes in the responses of the F₂, F₃, F₄, or F₅ generations (Fig. 2, which for reference includes the responses of female parent strains, Brun *et al.* 1995a). At the 400 ppm dose, no significant difference from the expected values was present across these four generations ($\chi^2= 4.0$, $df=3$, $P=0.26$). The lack of segregation of additional phenotypes under backcrossing, which might be expected under multiple locus models (Roush and Daly 1990) agrees with the prediction of a single major resistance gene.

Selection at different concentrations

An increase in LC₅₀ was evident from the parental F₀ to F₁₀ generations, at all concentrations including 400 ppm of endosulfan (the LC_{99.95} of the SS genotype) (Brun *et al.* 1995a), indicating a lack of homozygosity in the field-collected insects. However, no trend was evident in LC₅₀ with increasing selection concentration (Table 1). Overlap of 95% confidence limits did not occur for all pairs of treatments. The line of insects selected with 100,000 ppm (or 10% pure endosulfan) showed a similar response to the other selected lines, but died out after nine generations. This could have been due to inbreeding. Results from previous generations were similar, but have not been presented for brevity.

TABLE 1: Dose responses of female *H. hampei* to endosulfan assessed after 7 days, following 10 generations of selection with endosulfan at the indicated concentrations.

Selection concentration (ppm)	Number of insects ¹	Slope	SE	LC ₅₀	95% Confidence limits		H ²
					lower	upper	
F ₀	1095	1.27	0.07	2,950	201	4,040	1.86
400	270	3.22	0.32	23,200	19,700	26,700	0.71
1,000	330	3.09	0.27	17,700	17,600	32,500	4.70
5,000	330	2.83	0.25	16,100	13,700	18,700	0.23
10,000	285	3.15	0.33	18,600	9,200 ³	28,800	3.68
25,000	270	4.59	0.49	24,300	13,000	37,300	2.08
50,000	165	2.14	0.49	7,200	2,800	11,000	0.24

¹Tested at all concentrations

² heterogeneity (χ^2/df)

³ 90% Confidence limits

An increase in slope was also evident in all selected lines, compared to the F₀ generation. This increase in slope corresponds to a decrease in genetic variability after selection, and reflects the reduction in the level of susceptibility. The lack of a trend in slope or LC₅₀ with selection concentration suggests that all concentrations produced similar changes in the genetic composition of the strains. This similarity indicates no evidence of a second gene for endosulfan resistance, as a function of selection concentration.

These results, particularly those with males from reciprocal crosses, support the findings of Brun *et al.* (1995a,b) that functional haplodiploidy is present in the coffee berry borer, and that a single major gene appears to be responsible for the endosulfan resistance. Insecticide resistance genes can be useful markers for elucidating the mode of inheritance in insects.

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