Spray deposition in relation to endosulfan resistance in coffee berry borer (*Hypothenemus hampei*) (Coleoptera: Scolytidae) in New Caledonia

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Abstract *Hypothenemus hampei* (Ferrari), in *Coffea canephora* var. *robusta* grown in New Caledonia, is controlled by applying endosulfan using vehicle-mounted mistblowers operating from roadsides. Spray deposit profiles across fields of shaded and unshaded coffee were measured by a surface fluorescence technique. In unshaded mature coffee plantations a small mistblower deposited 90% of the spray within the first 10 m from the roadside, whereas a large mistblower deposited 50% to 15 m and 90% to 30 m. The range of this large sprayer was reduced in shaded traditional plantations and was severely affected by windy conditions. Susceptible and resistant beetles, arranged in transects perpendicular to the roadside, were exposed to a standard application of endosulfan. Complete mortality of susceptible beetles occurred over the first 20 m for beetles contained in filter paper packets, compared with 10 m for beetles in green berries and 5 m for beetles in dry berries. Resistant beetles had significantly greater survival at all distances. Differential selection pressure, estimated by the differential mortality of resistant and susceptible beetles, was at maximum 6 m from the roadside. The correlation between the heaviest spray deposit and greatest selection for resistance near the point of application, is discussed.

Keywords *Hypothenemus hampei*; coffee; endosulfan; resistance; pesticide application; air-assisted spraying; mistblowers

Introduction

Endosulfan resistance is present in *Hypothenemus hampei* (Ferrari), known as coffee berry borer (CBB), in New Caledonia (Brun *et al.*, 1989). As this species is the only insect pest requiring control in coffee in New Caledonia, and coffee is the major cash crop of Melanesian farmers, CBB control is conducted nationally during the months of January and February as a service of the Agence de Développment Rural et d'Aménagement Foncier (ADRAF).

The resistance is currently confined to five regions on the East Coast of the island, although it is already very widely distributed in the two largest coffee-growing areas around

The aim of this study was to measure spray deposit patterns in newer unshaded plantations and traditional shaded plantations, and to relate these patterns to the efficacy of endosulfan. From this, it was hoped to gain insight into the selection pressure resulting from the roadside spraying of the coffee. An earlier paper (Parkin *et al.*, 1991) reported on some measurements of spray distribution, and examined the variability of deposit; this paper examines more closely the spray distribution patterns, presents comparative efficacy data and examines the link between deposit and endosulfan resistance.

Materials and methods

Mascinenfabrik, Ahlen, Germany), mounted on a light truck, is used. This consists of a petrol engine-driven centrifugal fan which generates an air-jet of 1.5 $m^3 s^{-1}$ at 113 $m s^{-1}$ velocity (nominal). Four 2.5 mm orifice diameter hollow-cone nozzles mounted on a ring around the fan outlet project spray into the air-jet. The height of the outlet,

used to assess the response of susceptible and resistant populations present in dry berries.

Field 3 was 100 m to the north-west of field 2, and was another unshaded plantation. It was also chosen to study spray deposition with the large mistblower. The trees were 2 m high, following pruning to ground level in 1987. The of tracer per unit area. The calibration was carried out using a Potter Tower technique (Potter, 1952). Calibration details were given by Parkin *et al.* (1991).

Sampling transects were established perpendicular to the roadsides. In all but one of the spray deposition experiments, at each sample location 10 leaves were sampled from positions around the tree. For the experiment in field 4 it was considered that there might be greater variability in deposits in a traditional shaded plantation, so 20 leaves per location were taken. For the experiments at La Foa (field 1), and the first experiment at Ponérihouen (field 2), samples were taken at three levels in the crop, but in the other fields samples were taken at two levels. Two replicate transects were sampled in the first spray deposit experiments at both La Foa (field 1) and Ponérihouen (field 2), but at all other sites, as there was little variation between transects, it was decided to restrict the samples to a single transect. Spray deposition was measured at three locations on the adaxial and abaxial surfaces of each leaf. The results for each surface, crop level and sampling point were then grouped. Thus, at least 30 measurements were made for each data point. The location of the sampling points along a transect varied between treatments and was based on visual assessment of the spray deposit and related to field size.

Biological assessment

Insect strains. The reference susceptible strain was the same as that reported by Brun *et al.* (1989), from La Foa (LA2). Both green and dry berries containing insects from this strain were used in the experiment at La Foa, whereas only dry berries were used at Ponérihouen. Two resistant strains were collected: the first, for the packet bioassay at La Foa, was from Ponérihouen (PN402), whereas the second resistant strain, tested in the berry bioassay at Ponérihouen, was collected near Poindimié (PO01). The phenotypic frequency of resistance for the strains was determined from 120 beetles from each strain using the diagnostic concentration of 400 p.p.m. endosulfan (the $LC_{99.95}$ of susceptibles) by the direct spray method of Brun *et al.* (1989).

Packet bioassay. A pilot study was conducted in Decem-

tained under similar conditions nearby. A spray application of endosulfan was made by the BSE Super IV mistblower mounted on a light truck. Packets were removed from the field after 6 h, and CBB mortality, corrected for control mortality by Abbott's formula (Abbott, 1925), was assessed at 10 and 25 h post-treatment, with interim storage at 25°C.

Berry bioassay. Infestation levels of berries by CBB in the field show a wide variation. This has led to the development of a technique where dry berries, which are known to have high infestation levels, are collected and exposed to the spray. As some escape of beetles after spraying could not be prevented, in order to avoid introducing the resistance to the West Coast only berries from the susceptible strain were used in the test at La Foa (field 1). However, both resistant and susceptible strains were used on the East Coast. Green berries, which characteristically have a much lower infestation rate than dry berries, were also used at La Foa to investigate whether there are differences in mortality caused by berry condition. In this experiment at each position along a transect, two enclosures, each containing 20 dry berries infested with a mean of 28 CBB per berry (s.e.m. = 4), giving totals of 920–1509 susceptible CBB per transect position, were exposed to a field treatment of endosulfan. In addition, at each position, two enclosures each containing 30 green berries, with a mean of 0.9 CBB per berry (s.e.m. = 0.1), giving totals of 38–87 susceptible CBB per position, were exposed.

The resistant (PO01) and susceptible (LA2) strains were used in the transect at Ponérihouen (field 2). At each position of this transect, for each strain, there were two enclosures containing 15 dry berries. There was a mean of 34 CBB per berry (s.e.m. = 2) in berries from the susceptible strain area and 30 CBB per berry (s.e.m. = 2) in berries from the resistant strain area, giving total numbers per transect position of 433–747 for the susceptible strain and 322–564 for the resistant strain.

In each case, berries were contained in fully ventilated flat plastic mesh enclosures (100 mm × 160 mm), consisting of large-gauge mesh (5 mm apertures). The CBB enclosures were hung on stakes at each of two heights (1 m and 2 m). The stakes were set within the row, close to the normal location of the berries. After spray application, berries

Table 1. Summary of field experiments

Description	Location	Date	Temp. (°C)	Humidity (%)	Wind[a] (m s^{-1})
Pilot study. Packet bioassay. Susceptible and resistant strains. Mature unshaded plantation. Small sprayer.	La Foa (field 1)	14 December 1989	29	96	+0.7
Spray deposition. Mature unshaded plantation. Small sprayer.	La Foa (field 1)	9 January 1990	31	55	−1.5[b] +1.25
Berry bioassay. Susceptible strain. Mature unshaded plantation. Small sprayer.	La Foa (field 1)	11 January 1990	32	62	+1.5
Spray deposition. Mature unshaded plantation. Large sprayer.	Ponérihouen (field 2)	17 January 1990	29	78	+1.86
Spray deposition. Young unshaded plantation. Large sprayer.	Ponérihouen (field 3)	17 January 1990	29	78	+2.98
Spray deposition. Mature shaded plantation. Large sprayer.	Ponérihouen (field 4)	17 January 1990	29	78	Calm
Berry bioassay. Susceptible and resistant strains. Mature unshaded plantation. Large sprayer.	Ponérihouen (field 2)	18 January 1990	29	86	+0.89

[a]Wind component in direction of spraying; [b]plot sprayed both with and against wind (i.e. from each end of field)

Spray experiments

A summary of all the experiments is given in *Table 1*. To allow comparison, spray deposition and comparable bioassay experiments were conducted under similar meteorological conditions.

Results and discussion

Spray distribution

At La Foa (field 1), for the small mistblower on mature unshaded coffee, spray deposit patterns were measured along two transects. As there was little difference between results from the two transects, results from only one transect are shown (*Figure 1*). It should be noted that the field was sprayed from both the upwind and downwind field edges. The spray deposit patterns from the large mistblower in mature unshaded coffee at Ponérihouen (field 2), are shown in *Figure 2*. Again, only one transect is shown because there was a similar pattern in each transect. The results of the other deposit experiments with the large mistblower are given in *Figures 3* and *4*. Only the adaxial surface results are shown since there was a similar relationship between surfaces to the results in Figure 2.

The spray distribution from the small mistblower was very limited, even with a following wind (*Figure 1*), as >90% of the spray deposited in the crop was deposited in the first 10 m. This was undoubtedly because the emission height of the mistblower was at, or slightly below, the tops of the trees and the air output volume is relatively low.

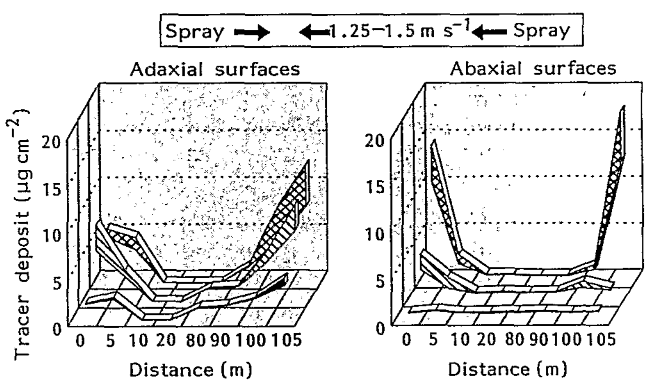


Figure 1. Deposit of tracer (95% confidence interval of the mean) on adaxial and abaxial surfaces in an unshaded plantation with distance from point of application for the small mistblower at La Foa (field 1). Level in crop: ■, lower; ▧, middle; ▩, upper

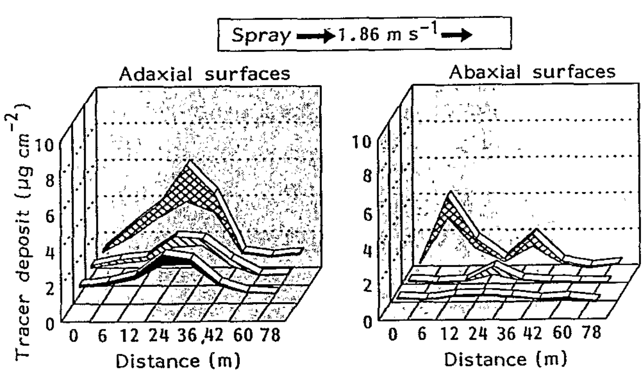


Figure 2. Deposit of tracer (95% confidence interval of the mean) on adaxial and abaxial surfaces in an unshaded plantation, with distance from point of application for the large mistblower at Ponérihouen (field 2). Level in crop as in *Figure 1*

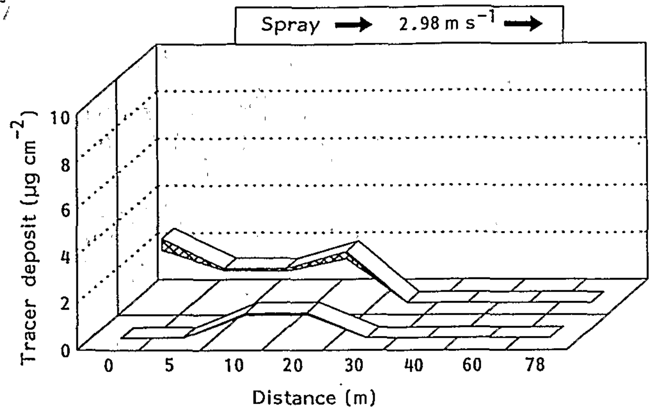


Figure 3. Deposit of tracer (95% confidence interval of the mean) on adaxial surfaces in an unshaded plantation, with distance from point of application for the large mistblower at Ponérihouen (field 3). Level in crop: ■, lower; ⊠, upper

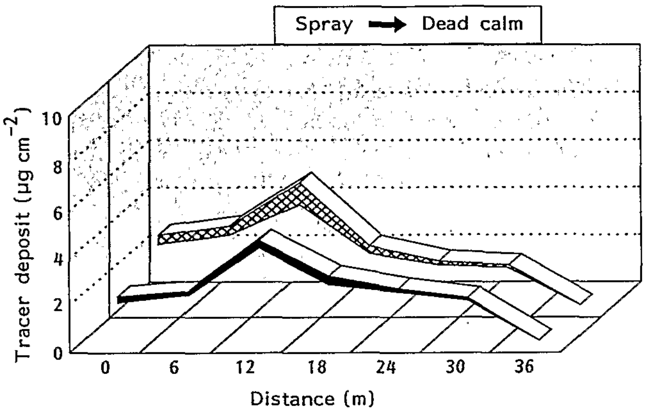


Figure 4. Deposit of tracer (95% confidence interval of the mean) on adaxial surfaces in a shaded plantation, with distance from point of application for the large mistblower at Ponérihouen (field 4). Level in crop as in *Figure 3*

The large mistblower, having both a greater volume of air output and a higher emission point, projected spray across the top of the canopy and provided better spray distribution (*Figure 2*). It was estimated, from the adaxial upper level measurements, that 50% of the spray was deposited within 15 m of the road and 90% within 30 m of the road. Its performance could be adversely affected by wind, as seen in the experiment in field 3 at Ponérihouen (*Figure 3*). Here, a relatively strong following wind appears to have dissipated the air-jet and diluted the spray cloud sufficiently to produce an unacceptable result. These conditions are not uncommon during routine spraying. Presumably most of the spray in this experiment drifted out of the field.

Where an air-jet projects spray across the top of a crop, as with the large mistblower, the deposition mechanism is almost entirely sedimentation. The air-jet carries spray across the top of the crop and provides the basic spray dispersal but, at some point determined by the strength and size of the air-jet, drop size and atmospheric conditions, the spray sediments into the crop. This can be seen by comparing data from the large and small mistblowers in mature unshaded plantations. With the small mistblower (*Figure 1*), spray deposition on the abaxial and adaxial surfaces close to the emission point was similar. However, with the large mistblower (*Figure 2*), the adaxial surfaces received significantly more spray than the abaxial, indicating a more vertical, and thus sedimentary, trajectory for the spray. The advantages of having an air outlet discharging above a crop, and emitting a larger air volume, can be clearly seen.

Most coffee in New Caledonia is still grown in shaded plantations (Brun *et al.*, 1989), so the result shown in *Figure 4* is significant in that it represents the most common form of application in the most common type of plantation. It appears that spray coverage in this type of plantation is still limited to areas close to the road. It was estimated that 50% of the spray was deposited within 10 m of the road and 90% within 27 m of the road.

Thus, as predicted by Decazy (1988), most of the spray from all the treatments is deposited close to the point of emission, giving a very heavy overdose of active ingredient in the first 10 or 20 m of the fields. It appears that far sides of the coffee fields receive only a small fraction of the expected dosage of endosulfan and expressions of field dosage rates per hectare are effectively meaningless in this case.

Biological assessment

Insect strains. As described earlier, the LA2 strain of CBB has been found to be endosulfan susceptible. The frequency of the resistant phenotype surviving the diagnostic dose in the PN402 strain was 64.4%, and 49% in the case of the PO01 strain.

Packet bioassay. CBB mortality in the packets increased slightly, between 10 and 25 h; hence only the results from the second reading are presented here (*Figure 5*) because these represent a better estimation of the full insecticide efficacy. Greater mortality resulted at 2 m, compared with the 1 m height, but the results have been grouped ($n = 60$) for presentation. All susceptible beetles were killed at distances up to and including 20 m from the roadside, whereas resistant insects survived at all locations.

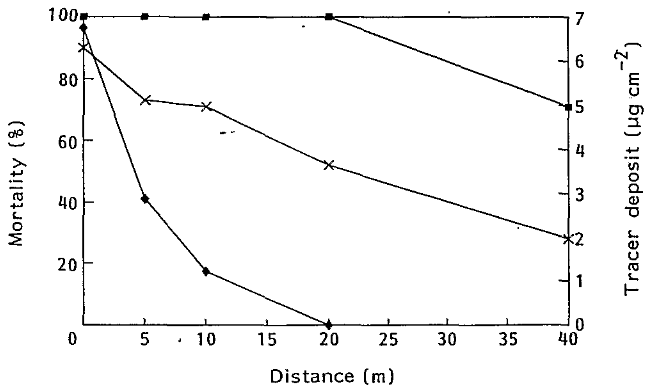


Figure 5. Percentage mortality of adult female susceptible (■) and resistant (X) *H. hampei* in filter paper packets and mean tracer deposit (all surfaces and levels, ◆), with distance from the point of endosulfan application at La Foa (field 1)

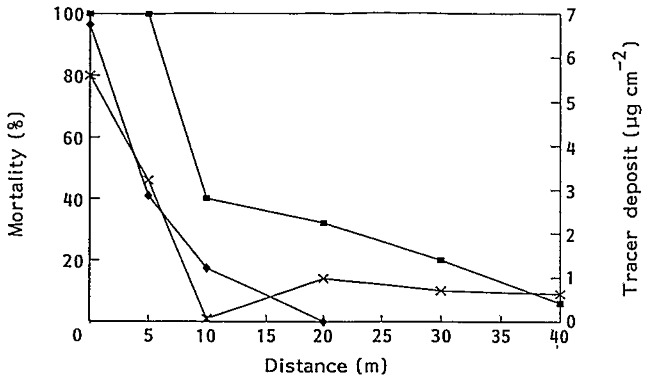
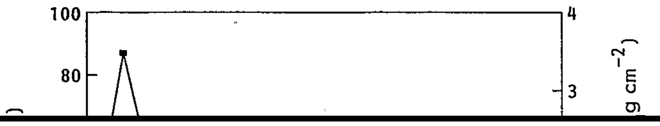


Figure 6. Percentage mortality of adult female susceptible *H. hampei* in green (■) and dry (X) coffee berries and mean tracer deposit (all surfaces and levels, ◆), with distance from the point of endosulfan application at La Foa (field 1)



(liquid and vapour) and the packets were positioned between rows, allowing for better spray penetration and vapour movement.

The differential selection pressure on susceptible and resistant populations of CBB from the experiment at Ponérihouen is shown in *Figure 7*. The difference in mortality between strains was significant at allocations in the field according to the χ^2 goodness of fit test (the minimum difference occurred at 78 m, $\chi^2 = 18.7$, 1 d.f., $p < 0.001$). There appears to be a low level of mortality of the susceptible population even at the downwind edge of the field. This could be explained in terms of a fumigant action. Brun *et al.* (1991) found that indirect exposure bioassays gave rapid mortality of susceptible but not resistant strains after 2–3 h at 400–1000 p.p.m. It is, therefore, likely that this mortality, with little apparent direct spray deposition, was the result of gaseous phase activity. This probably also explains why overspraying the crop at a height of 3.3 m from the roadside has little effect on the distribution of mortality in the immediate vicinity of the sprayer.

As the bioassay of the PO01 strain showed a 49%

Implications for managing resistance

Relatively few attempts have been made to estimate selection resistance in the field. Selection for insecticide resistance in sheep blowfly has been demonstrated to change over time, owing to changes in viability of different genotypes with decaying residues (McKenzie and Whitten, 1982). One interesting outcome of this work was that the period of selection was considerably greater than the period of protection from fly strike. This concept could have a parallel in our case, where selection for resistance appears to extend a greater distance from the roadside than the distance over which the insecticide is effective at controlling susceptible beetles. In another example, resistance was not constant over time; Daly, Fisk and Forrester (1988) showed that the selective mortality of resistant *Helicoverpa (Heliothis) armigera* could be minimized by targeting very young larvae that were as susceptible to pyrethroids as were susceptible larvae, making resistance functionally recessive by correct insecticide timing. Unfortunately, no parallel period (or location) of minimum selection is likely to exist in this case, owing to overlapping generations and other factors.

Clearly, the distribution of spray within the fields is a contributory factor in the development of resistance. A more even application would be beneficial in delaying the onset of resistance, but achieving this may not be easy. Spray application to individual trees by, for example, motorized knapsack mistblower, could provide a solution, but this would require considerably more resources and organization than are currently employed. It would also increase the likelihood of pesticide contamination either through use, because of the dense canopy and uneven ground, or in mixing. It is, therefore, unlikely that this form of application could be adopted except, perhaps, on a limited scale in inaccessible areas. Thus, the current technique of using vehicle-mounted mistblowers operating from roadsides will remain the primary technique for some years. It is vital, therefore, that it is optimized and improved. It is also important that planting patterns and access to the fields be improved to achieve better application.

Any improvement in spray distribution would not necessarily delay the onset of resistance (depending on the fitness of the genotypes), but immediate steps could be taken to reduce the problem. Alternative pesticides, such as fenitrothion, must be investigated and a resistance management strategy that cycles pesticides of differing chemical types and groups should be implemented (Brun *et al.*, 1989).

The mean numbers of female CBB per green and dry berry collected during the experiments were 1 and 31.5 respectively. Thus, a large potential for reinfestation exists if dry berries are not removed at the end of the growing season. Selection for resistance is a function of the population size and level of kill. Increases in resistance frequency will depend on the relative fitness of different genotypes, with and without the presence of insecticide (Georghiou and Taylor, 1977). A higher rate of kill of CBB was observed in green berries, compared with dry berries, and mortality extended further across fields. The application of endosulfan normally occurs at the green berry stage in New Caledonia (January and February). The higher rate of kill of beetles in green berries suggests that the resistance of the high level found in New Caledonia could be more likely to result from the selection of beetles attacking green berries. It would take longer for the population to recover, but it would be more resistant, assuming equal fitness, than a lower level of kill of beetles at higher infestation levels in dry berries. The relatively slow rate of reversion suggests that the fitness differential may not be that great in the absence of endosulfan (Brun and Suckling, 1992). Thus, the physical removal of dry berries from trees, and from the plantation floor, could have an important role in reducing the size of the population under selection, both in dry berries and as a source of infection of green berries.

Future work

Spray deposit measurements, although indicating trends, should be improved to measure directly spray deposition on berries. In addition, deposition studies should be extended to *Coffea arabica*, which is also grown in some areas of New Caledonia and has a different growth form, leaf shape and berry development. Tracer deposit studies could be confirmed by using gas–liquid chromatography technique: this would enable the amount of tracer deposited to be directly related to the quantity of endosulfan on each berry; this in turn could be related to bioassay and field efficacy results.

It may also be possible to improve the field estimation of selection pressure further by using the berry bioassay technique, as this technique has shown the ability to provide differential mortality and is sufficiently robust for field use.

It is important that biological control alternatives continue to be investigated alongside the search for improvements in spray application techniques, and the selection of alternative environmentally acceptable insecticides.

Conclusions

The link between the endosulfan resistance profile across fields and spray deposition has been clearly demonstrated, as the current models of mistblowers appear to deposit most of the spray within 20 m of the point of application. Improvements in application technique are therefore required, although it is thought that the widespread use of spray application to individual trees by pedestrian-operated sprayers is not feasible in New Caledonia.

There is a high selection pressure exerted near the roadside as a result of the high deposits and atmospheric concentration of endosulfan there, not because of the frequency of application (twice yearly). It has also been shown that the number of CBB exposed to selection in green and dry berries may be very different, and would be expected to change as a function of distance from the point of application.

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