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MÉTHODE PROVISOIRE POUR DÉPISTER LA RÉSISTANCE À  
L'ENDOSULFAN CHEZ LE SCOLYTE DE LA CERISE DU CAFÉ,  
*HYPOTHENEMUS HAMPEI* (COLEOPTERA: SCOLYTIDAE)

Résumé

Cet article décrit et compare trois méthodes de mesure de la résistance à l'endosulfan du scolyte du café, *Hypothenemus hampei*: la pulvérisation directe des femelles avec la tour de Potter et l'exposition directe ou indirecte à un papier filtre préalablement traité. La similarité des résultats obtenus suggère que la tour de Potter sera utilisée pour toutes les études toxicologiques et que la méthode d'exposition indirecte aux insecticides conviendra aux programmes visant à détecter précocement de nouveaux cas de résistance ou à gérer une résistance déjà établie.

METODO PROVISIONAL PARA DETECTAR LA RESISTENCIA AL  
ENDOSULFAN EN LA BROCA DEL CAFE, *HYPOTHENEMUS HAMPEI*  
(COLEOPTERA: SCOLYTIDAE)

Resumen

En el presente artículo se comparan tres métodos para determinar la resistencia de *Hypothenemus hampei* al endosulfán: la técnica de pulverización directa, ya descrita en otra publicación, y los métodos de exposición directa e indirecta utilizando papel de filtro tratado. Los resultados indican que la eficacia es parecida con los tres métodos. El método indirecto, que utiliza la acción del vapor mediante la exposición de los insectos en un espacio cerrado por encima del papel de filtro tratado, fue el más fácil de aplicar. Se recomienda este método como sistema normal provisional para la detección de resistencia al endosulfán en esta especie. En un estudio comparativo entre la pulverización directa y la exposición indirecta sólo se observó una diferencia en el diagnóstico de la resistencia en 162 cepas estudiadas.

Provisional method for  
detecting endosulfan resistance  
in coffee berry borer,  
*Hypothenemus hampei*  
(Coleoptera: Scolytidae)

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**Summary.** This paper compares three methods for assessment of endosulfan resistance in *Hypothenemus hampei*: the direct spray technique reported previously, and direct and indirect exposure methods using treated filter paper. The results indicate comparable accuracy with all three methods. The indirect method, using vapour action by exposing insects in a confined space above the treated filter, was the easiest to use. This method is recommended as the provisional standard method for detection of endosulfan resistance in this species. A comparative survey of the direct spray and indirect exposure methods showed only one difference in diagnosis of resistance out of 162 strains tested.

Coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari), was reported for the first time in 1948 from the northern part of New Caledonia (Bugnicourt, 1950). *H. hampei* spread rapidly and became the major pest of coffee, which is predominantly composed of *Coffea caniphora* var. *robusta*. CBB is controlled throughout the world predominantly with the use of a single cyclodiene insecticide, endosulfan (Huttenbach, 1982). Alternative insecticides are generally less effective and not widely used (Rhodes and Mansingh, 1981).

The discovery of high levels of resistance to endosulfan in New Caledonia may indicate future problems elsewhere, given similar pat-

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terns of usage (Brun *et al.*, 1989). Field control of CBB without insecticides is currently difficult, and high levels of infestation despite endosulfan use, similar to untreated situations, have occurred in New Caledonia where the resistance is present. An insecticide resistance management programme is being formulated by ORSTOM and DSIR (Department of Scientific and Industrial Research, New Zealand) in cooperation with the Coffee Board in New Caledonia, which requires information on the current distribution of resistance as the basis for action. In the long term, integration of biological control agents and other control tactics may lead to successful alternatives to endosulfan.

Although other bioassay methods have been used for comparing insecticide efficacy against CBB, techniques such as berry dipping (Rhodes and Mansingh, 1981) have disadvantages for resistance surveys. In particular, problems include lower precision and difficulty in mortality assessment of beetles inside berries.

Hence there is a need for a simplified standard method for resistance surveys to assist early detection of incipient problems. The authors report here the comparison of three methods at 25°C, 80-85 percent R.H., in order to choose the best standard method for future recommendation. Fuller details of the change in concentration-mortality responses of susceptible and resistant strains of CBB with temperature, relative humidity and time will be reported elsewhere (Brun *et al.*, in preparation).

## MATERIALS AND METHODS

**Insect strains.** Coffee berries were collected from the roadside edge of coffee fields in plastic bags with gauze windows (to reduce the humidity) and stored at 25°C for approximately one month before use. The importance of testing samples from identical collection sites during a comparison of methods as reported here, was shown by the difference in resistance level within the same coffee plant-

ation (Brun *et al.*, 1989a, b). Fungal attack was reduced by exposing the berries to air circulation in gauze cannisters after collection. Adult females 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. The susceptible reference strain was obtained from La Foa, and the resistant strain came from Poindime (see Table). For comparison of success at detection of endosulfan resistant *Hypothenemus hampei*, 162 samples were collected throughout New Caledonia.

**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 Thiodan 35 EC (HOECHST AG, Frankfurt) was used for each test and 2 ml of liquid (400 ppm) were sprayed with a Potter spray tower (Potter, 1952) calibrated to deliver 1.6 mg/cm<sup>2</sup>. 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 percent R.H. under constant illumination. An exposure time of 6 h was used, as recommended by FAO (FAO, 1980) for stored product coleopterous pests. A rapid increase in control mortality was often observed after 6-7 h. The criterion for death was the absence of coordinated 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 less than 10 percent. The diagnostic dosage used was 400 ppm of endosulfan (twice the LC<sub>99</sub> for the reference susceptible strain).

**Residue exposure.** A piece of rectangular perspex (80 × 140 × 3 mm) with two rows of five holes (20 mm diameter) was used to contain 15 females per hole. This plate was placed on filter paper (80 × 140 mm) treated with 0.6 ml of Thiodan solution and left on a laboratory bench for one hour to dry; the liquid was

Comparison of dosage-mortality responses of endosulfan-susceptible and resistant strains of *Hypothenemus hampei*

Strain	N <sup>3</sup>	Slope (SE)	LC <sub>50</sub> (95% CL)	LC <sub>90</sub> (95% CL)	LC <sub>99</sub>
Direct spray <sup>1</sup>					
S	1 380	2.87(0.14)	28(24-32)	78(66-97)	180
R	240	0.46(0.75)	*** <sup>2</sup>	*** <sup>2</sup>	*** <sup>2</sup>
Indirect exposure					
S	300	4.67(0.79)	64(38-82)	119(91-266)	200
R	480	0.59(0.14)	26 900 (5.592 - 1 × 10 <sup>6</sup> )	1 × 10 <sup>6</sup> (1.9 × 10 <sup>5</sup> - *** <sup>2</sup> )	*** <sup>2</sup> *** <sup>2</sup>
Residue					
S	90	4.29(0.88)	50(32-66)	99(74-148)	173
R	150	0.51(0.35)	*** <sup>2</sup>	*** <sup>2</sup>	*** <sup>2</sup>

<sup>1</sup> Reported in Brun *et al.*, 1989.

<sup>2</sup> Resistance level too high to calculate parameter by probit analysis.

<sup>3</sup> Number of females tested.

pipetted on filter paper with a rotational motion.

A thin sheet of transparent plastic (75 × 135 mm) with ten holes (15 mm diameter) was used to cover partly the testing arenas to prevent escape. Any test with more than 10 percent mortality was discarded, but control mortality was generally lower than 5 percent. Mortality was assessed after 6 h exposure at 25°C and 80-85 percent R.H.

**Indirect exposure.** The technique used was essentially the same as the residue exposure method, but a fine white nylon gauze was placed above the filter paper prior to adding the beetles to each hole. In this way the CBB were only exposed to the vapour of endosulfan. The test was repeated twice with each sample and a water-only treatment was included for each batch of tests. This procedure ensured that the beetles did not eat the filter paper, which occasionally occurred with the residue exposure method. Exposure time and mortality assessment were the same as above. The diagnostic dosage used was 400 ppm of endosulfan.

**Statistical analysis.** Probit analysis was done with POLO (Robertson, Russell and Savin, 1980) to determine the LC<sub>99</sub> of susceptible insects with each technique. The diagnostic dosages were set at approximately twice the LC<sub>99</sub> of susceptibles. Sites with any beetles surviving the diagnostic dosage were considered to include resistant individuals.

## RESULTS AND DISCUSSION

**Comparison of methods.** A very close similarity of endosulfan toxicity to susceptible insects was evident with the three methods which overlap in confidence limits between "residue" and "indirect exposure" methods (see Table). This similarity in responses of susceptible CBB between techniques (for slopes and LC<sub>50</sub> S, see Table) led us to use the same endosulfan concentration (400 ppm) as a diagnostic dosage for the survey using "direct" spray and indirect exposure methods.

The high level of resistance 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 estimated at over one thousand fold by graphical analysis for direct spray and residue tests, while the resistance factor was 572 fold (see Table).

The authors have assessed mortality of resistant strains at 400 ppm for longer periods of time (up to 96 h). No substantial increase in mortality of resistant females was observed between 6 and 48 h, so a period of 6 h was chosen as recommended by FAO (FAO, 1980). The increase in mortality of treated insects after 48 h was at the same rate as in untreated control insects. The advantage of this short time period is that results are rapid.

The main limitation of the Potter tower direct spray technique is the need for specialized spray application equipment. The residue exposure technique was developed with the aim of reproducibility, low cost, and ease of use. However, an occasional problem occurring with the residue exposure technique was beetles eating or shredding the filter paper. To eliminate this possibility, the beetles were caged above the treated surface using fine gauze. We found that it was easier to get clear results with this indirect exposure method. Mortality assessment was also made easier, since the beetles could not become entangled in the paper because of the behaviour of the scolytid, which is inclined to bore holes in berries. Beetle mortality in this type of bioassay relies entirely on the high vapour action of endosulfan (Knauf, 1982).

The close similarity of endosulfan toxicity evident with the three methods, suggests that toxicity from exposure to the "gas phase" may be of primary importance in all three testing methods.

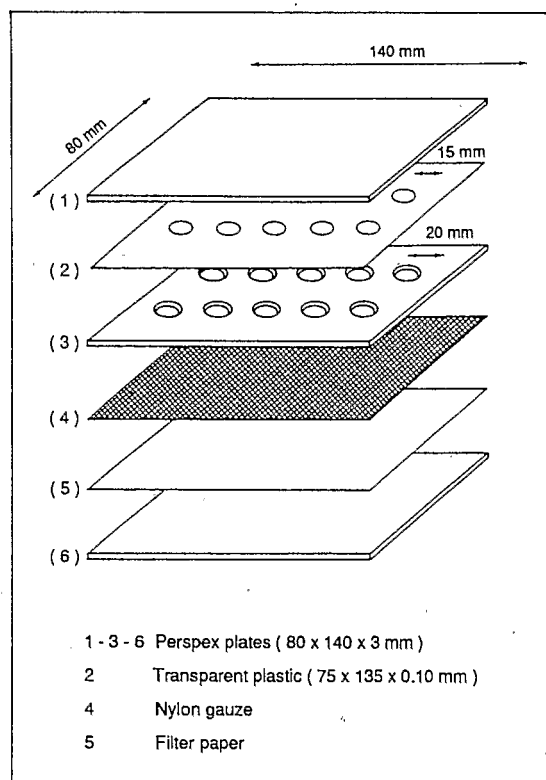
**Survey results.** The survey included 162 collection sites throughout New Caledonia covering the West Coast, East Coast, Northern Region, Middle Region and Southern Region, but resistance was detected only on the East Coast. The two detection methods showed excellent agreement, with a difference in detection of resistance at only one site. Resistance was present at 43.2 percent of the sites according to

the direct spray method, and at 42.6 percent according to the indirect exposure method.

Further details of the distribution of resistance in New Caledonia are reported by Brun *et al.* (in preparation).

## CONCLUSIONS

The indirect exposure technique offers reproducibility, low cost, and ease of use. It is recommended for future surveys of endosulfan resistance in the coffee berry borer. The efficiency of the technique permits extensive screening of strains and should allow early detection of resistance problems. Kits for the indirect exposure method (see Figure) are available from the authors, ORSTOM, Nouméa, New Caledonia.



Components of the test method, showing the different layers of Perspex, filter paper and gauze. The layers are assembled as shown

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# OUTBREAKS AND NEW RECORDS

## ATTAQUES ET NOUVEAUX ENNEMIS SIGNALÉS

## NUEVOS FOCOS DE ENFERMEDADES Y PLAGAS

### BHUTAN

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#### Black leaf streak of banana *Mycosphaerella fijiensis* Morelet

Black leaf streak of banana, *Mycosphaerella fijiensis* Morelet, was recognized in 1963 as a distinct and more severe disease than yellow Sigatoka, *M. musicola* Leach ex Mulder.<sup>1</sup>

The authors now report the presence of black leaf streak in the Himalayan kingdom of Bhutan, with the fungal identity confirmed in 1985 by the CAB International Mycological Institute as *Paracercospora fijiensis* (Morelet) Deighton state, (*Mycosphaerella fijiensis* Morelet), IMI No. 290376.

Since bananas are only a backyard crop in Bhutan, the disease is not of economic significance there. Yellow Sigatoka is still more common. However, the route taken by black leaf streak into the country is of considerable interest.

As the disease is not known to occur in the Indian subcontinent, one possible route is from Malaysia or Thailand via Myanmar, with which country Bhutan shares an eastern border. The authors' record, however, was made in southwest Bhutan and in an area where cross-border trade and exchange of planting materials with India is normal. It is possible therefore that the disease may already be in India or another country.

Its advent in Bhutan reinforces the importance of an effective plant quarantine system, especially in countries with an agriculture-based economy. The situation should be reviewed in the light of the explosive spread of some other diseases, such as groundnut rust (*Puccinia arachidis*) throughout Asia and Africa, following its discovery in Brunei in 1970.<sup>2</sup>

<sup>1</sup> MEREDITH, D.S. & LAWRENCE, J.S. 1969. Black leaf streak disease of banana (*Mycosphaerella fijiensis*): symptoms of disease in Hawaii and notes on the conidial state of the casual fungus. *Transactions of the British Mycological Society*, 52: 459-476.

<sup>2</sup> PEREGRINE, W.T.H. 1971. Groundnut rust (*Puccinia arachidis*) in Brunei. *PANS*, 17: 3.

### CANADA

Reported to FAO by D. Coates-Milne, Director, Plant Protection Division, Canada.

#### Eradication of potato spindle tuber viroid (PSTV) in the Canadian provinces of Prince Edward Island and New Brunswick

Scientific surveys conducted from 1984 to 1987 in New Brunswick and Prince Edward Island, Canada, have confirmed the absence of potato

spindle tuber viroid in seed and ware potato fields. Leaf samples taken during the survey were tested by means of polyacrylamide gel electrophoresis (PAGE), nucleic acid hybridization (dot-blot) and return-polyacrylamide gel electrophoresis (R-PAGE). These laboratory results corroborate field inspection and post-harvest testing data collected by Canada's Plant Protection Division over the past seven years, during which no PSTV was detected.

The eradication of this infectious pathogen was accomplished through rigorous screening