Importance of resistance monitoring in Sigatoka management programs.

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IMPORTANCE DU CONTROLE PERMANENT DES RESISTANCES DANS LES PROGRAMMES DE LUTTE CONTRE LA CERCOSPORIOSE

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RESUME - En bananeraie, comme pour d'autres cultures, la résistance aux fongicides était inconnue jusqu'à il y a environ 15 ans. Aujourd'hui entre les phénomènes de résistance et l'apparition de la cercosporiose noire, le contrôle des *Mycosphaerella* spp sur bananiers et plantains est devenu plus complexe et plus coûteux.
Il y a actuellement deux classes de fongicides actifs : les benzimidazoles et les inhibiteurs de stérols, utilisables pour maîtriser ces pathogènes. Malheureusement leur grande action à faible dose est associée à un seul site d'inhibition sur le champignon, ce qui peut conduire à la résistance du pathogène.
La première résistance aux benzimidazoles fut enregistrée au Honduras en 1976. Les études sur les *Mycosphaerella* des bananiers ont démontré par la suite que :

1) il y a une résistance nucléaire pour les ascospores et les conidies et une résistance extranucléaire pour les hyphes isolés. La première est pathogène, l'autre (qui concerne le Cercospora «non virulent») ne l'est pas.
2) sans pression de sélection, les spores des races résistantes (R) diminuent dans la population. Elles ne sont pas capables d'entrer en compétition avec les variétés sensibles (sauvages).
A partir de ces données (et d'autres), des programmes de contrôle ont été élaborés et testés pour faire face à cette résistance. Les principes de bases de ces stratégies sont les suivants :
- éviter les applications répétées de produits induisant des résistances.
- faire des mélanges et/ou des rotations de produits à mode d'action différent.
- surveiller le phénomène de résistance.
- supprimer les produits à «un seul site d'inhibition» si la population résistante atteint un seuil critique.

Ces principes de base considérés dans leur ensemble constituent le système de lutte intégrée contre la cercosporiose du bananier. Beaucoup de questions restent sans réponse et indiquent la nécessité de recherches de bases plus poussées.

INTRODUCTION

The resistance problem is a recent phenomenon and is almost exclusively associated with the newer fungicides, which depend for their high fungi-toxicity on specific-site inhibition. In bananas, the benzimidazoles represent the beginning of these resistance problems.

Resistance of *Mycosphaerella fijiensis* to benomyl was first reported in Honduras in April 1976 (STOVER, 1977a), then in Belize in late 1978 and Guatemala in 1979. *Mycosphaerella musicola* resistance has been less problematic, but occurred in Suriname in 1977 to methyl thiophanate, and more recently in 1981 in the French Antilles (BUREAU et al., 1982) where benomyl and methyl thiophanate had been used for about 10 years.

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The sterol inhibitors, very active systemics, have not yet been used intensively on bananas and their fate is uncertain. But under laboratory conditions, mutants with resistance have been found and these mutants are frequently cross-resistant to other sterol inhibiting fungicides - a pattern of development similar to the benzimidazoles (JONES, 1981).

FOURCADE and LAVILLE as early as 1973 obtained benomyl-resistant strains of *Cercospora musae* in culture by inducing the appearance of resistant mutants with the use of the mutagenic agent, nitrosoguanidine. They cautioned that it was necessary to be watchful.

STOVER (1977 b) distinguished between heritable resistance through sexually produced pathogenic ascospores and extranuclear resistance found only in isolates from young lesions. The latter seems unable to transmit resistance to ascospores or conidia and spore suspensions from cultures failed to cause spotting. These isolates, fast gro-

wing in culture, were called *Cercospora* «non-virulentum».

RESISTANCE MECHANISMS

Benzimidazoles inhibit sensitive fungi by binding to protein subunits of microtubulin, thereby disrupting microtubule assembly, which is necessary for spindle formation during cell division.

This action results in alterations in hyphal tips of growing fungi in the form of (i) displacement of mitochondria from hyphal apices ; (ii) reduction of linear growth ; and (iii) metaphase arrest of all mitoses through absence of spindle formation (HOWARD and AIST, 1977 ; 1980). Resistant or (R) mutants in the natural population with less sensitive sites may be selected out by exclusive use of benzimidazole fungicides.

Resistance to ergosterol biosynthesis inhibiting (EBI) fungicides is thought to be based on a decreased affinity of the enzymes involved in ergosterol synthesis for this type of fungicide. With ergosterol inhibitors, initial spore germination may be normal, but germ tubes become heavily distorted and hyphae are swollen and/or excessively branched (De WAARD and FUCHS, 1980). Other effects include highly vacuolated germ tube cells, and balloon-like vesicles may form at the growing tip which rupture to release cell contents.

MONITORING METHODS

Before we examine why resistance monitoring is important, we should ensure that the methods for monitoring are reliable and lend themselves to some measure of realistic interpretation.

There are three methods as far as I am aware :

1. Ascospore germination is the most widely used method and was developed by STOVER, United Brands (JACOME, 1981 ; Du pont Bulletin, 1983). The procedure is standardised on the basis of the following :

   - 4 or 5 different leaf pieces per area or farm in each plate at each fungicide concentration.

   - Water agar concentration 2%. A better nutritive base may increase % normal germination and germ tube length causing % resistance to be unusually high.

   - Agar is amended with a freshly prepared benomyl (Benlate ®) suspension to concentrations of 0.1 to 200 ppm.

   - Agar pH should be near to the optimum of 6.7 for ascospore germination. Use sterile distilled water. pH 7 or pH 6.5 causes reduction in germ tube length.

   - Incubation temperature 26°C in controlled-temperature incubator.

   - Resistance is recorded as % normal germination of ascospores vs. the check.

2. The hyphal growth technique is used by IRFA and is an extension of the ascospore discharge and germination method (BUREAU et al., 1982). Twenty-four hours after discharge unto water agar, germinated ascospores are placed on V8 juice agar and incubated for 4 to 5 days. Each colony is divided into two and only one-half is transferred to V8 juice agar with 5 or 10 ppm benomyl. A few days later, growth on amended or initial unamended agar is compared and the percentage of resistant colonies is calculated. This technique gives somewhat higher results.

3. Conidial germination, the third technique, is utilized in the Windward Islands (K. CRONSHAW, personal communication). It requires incubating leaf tissue with young lesions in a damp chamber, streaking conidia on benomyl-amended water agar and counting normally germinated conidia as for the ascospore method.

COMPARISON OF TECHNIQUES

Ascospore germination	Hyphal growth	Conidial germination
Quick results	Takes longer time	Similar to ascospore germination
Ascospore may fail to discharge	Similar to ascospore germination	Bacterial contamination possible
Presence of non-pathogenic ascospores	Probably much less contamination	Other saprophytic fungi possible
Measures % of spore population	Measures % of growing colonies	Measures % of spore population

INTERPRETATION OF DATA

Realistic interpretation of laboratory resistance data in terms of loss of disease control has been difficult. Members of the Fungicide Resistance Action Committee (FRAC) at a Brussels meeting expressed concern about reports of resistance based only on laboratory studies. They proposed the use of the term field resistance in cases where a reduction in disease control is obtained, under practical conditions. BRUIN and EDGINGTON (1982) emphasize that fungi growing in a living plant may react differently than when growing on artificial media.

Interpretation of laboratory resistance information requires :

- Exclusion of other contributing factors to unsatisfactory

control such as poor coverage, favourable environment for epidemics.

- Knowledge of a base-line figure for the fungus on agar, for example, 0.1 ppm benomyl is the minimum inhibitory concentration for ascospore germination.

- Knowledge of the critical resistance levels in the field measured by a standard method that corresponds to failure to control or correlation of laboratory resistance with disease control effectiveness.

- Utilisation of a critical resistance ratio where the resistance ratio

$$= \frac{\text{LD resistant strain}}{\text{LD sensitive strain}}$$

IMPORTANCE OF MONITORING

Assuming that we now have reliable technique for measurement and interpretation of resistance monitoring, why is resistance monitoring an important component of disease control programs ?

It is essential because :

- Constant surveillance is necessary to detect presence of resistance and to avoid surprise by sudden and unexplained control failures.

- It helps in determining critical levels of resistance in the laboratory and field.

- It documents the occurrence of resistance and allows for statistical analyses to develop correlations between resistance levels and disease control.

- It is an indispensable tool to study the dynamics and epidemiology of resistant populations.

- It provides information to determine which chemicals to use.

- It aids in preventing introduction of resistant strains from other countries or other zones.

Three years ago, it was customary to avoid benomyl use once resistance was suspected, even though not proven. Today we realise that there are increased costs associated with precipitate moves to other fungicides and that there is a decline in the number of potential new products that can economically be used. Also the new fungicides, all in the sterol-inhibiting group, are also specific-site inhibitors and pose an imminent threat of a new and different form of resistance.

RESULTS OF RESISTANCE MONITORING STUDIES

Distribution of resistance within plantations or zones appears random with regard to occurrence and levels of resistance (Table 1)

Information to date indicates that once it occurs, resistance persists at low levels and may increase again when benzimidazole sprays are re-introduced (WOODS et al., 1982). When the applications are stopped, the populations shift back from resistance towards sensitivity (Fig. 1).

From 1978 to 1980, no benomyl was used commercially in the Sula Valley by United Brands and resistance declined to less than 1.0 ppm in most areas. Fig. 2 shows % of farms with resistance in 1980. This percentage increased during the rainy season in the absence of benomyl selection pressure and declined in the dry season. The fungus is multiplying much more rapidly during the rainy season and therefore this time of year requires a greater use of chemical tactics.

From June, 1981, commercial use of benomyl/mancozeb «cocktails» with intercycles of mancozeb was recommended. By January, 1982, after 7 months, resistance at unsafe levels in some cases up to 200 ppm was found on farms where resistance had first developed (Fig. 1). During 1982, resistance levels again dropped from a high of 200 ppm. By April/May of that year about 80 % of farms had levels of less than 10 ppm. Resistance tended to develop relati-

TABLE 1 - Resistance, occurrence and disease control.

Farm	June 81 to Jan. 82 «cocktails»	Youngest Leaf Spotted, 1981											
		J	F	M	A	M	J	J	A	S	O	N	D
COPEN	9	R 9.8	R 9.6	R 10.2	R 11.2	12.3	R 12.8	14.5	R 14.0	11.8	R 12.5	12.5	R 13.3
TACAMICHE	9	11.5	11.0	8.1	12.5	13.4	13.7	13.4	11.0	11.3	R 11.3	11.6	R 10.3
GUARUMA	8	9.1	R 9.0	R 9.9	R 11.1	12.5	14.7	15.0	R 10.8	R 11.9	R 13.5	R 14.0	12.3

«R» indicates resistance was detected at minimum 0.1 ppm benomyl.

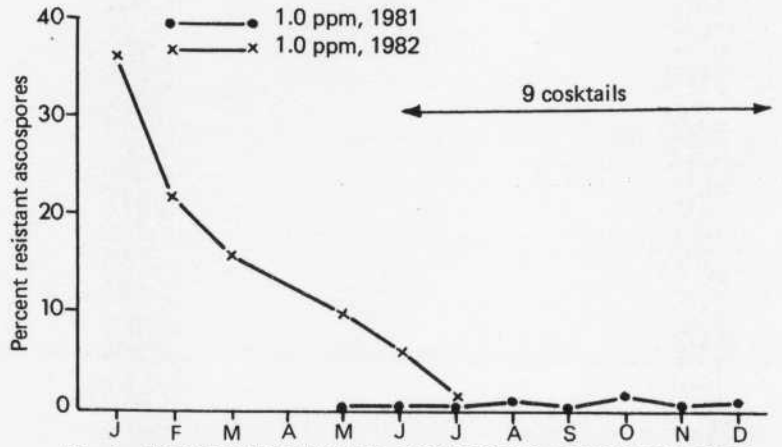

Fig. 1 - PERCENT RESISTANT ASCOSPORES FOR COPEN FARM.

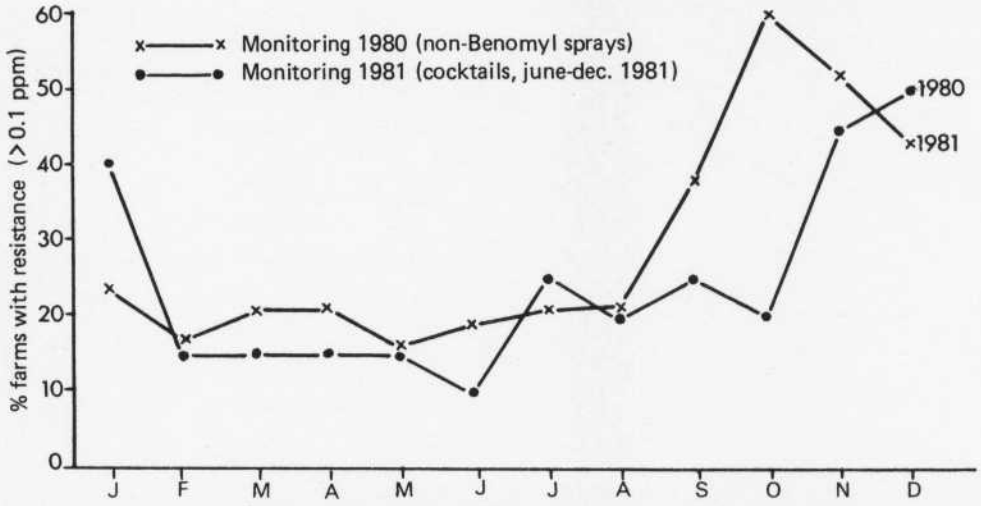

Fig. 2 - OCCURRENCE OF RESISTANCE UNITED BRANDS FARMS.

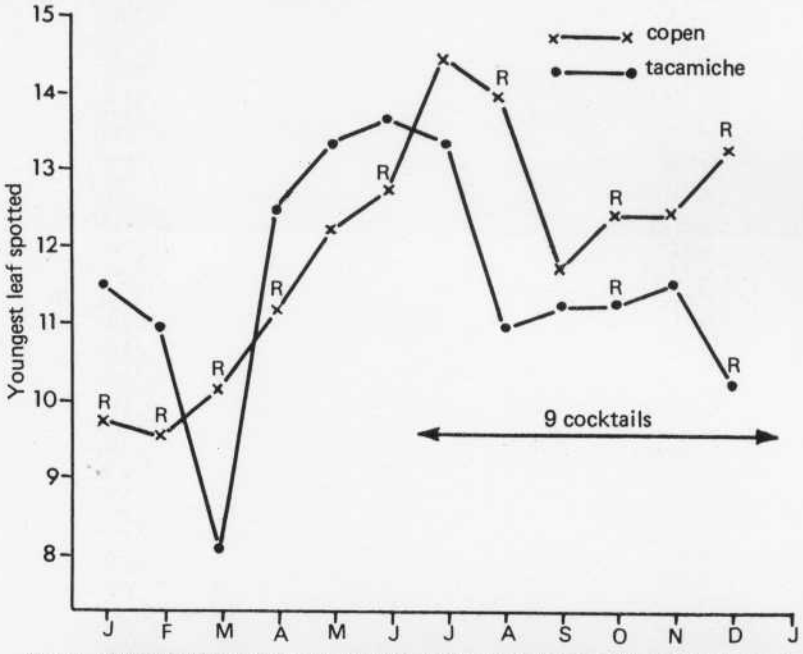

Fig. 3 - RESISTANCE OCCURRENCE AND DISEASE CONTROL IN 1981.

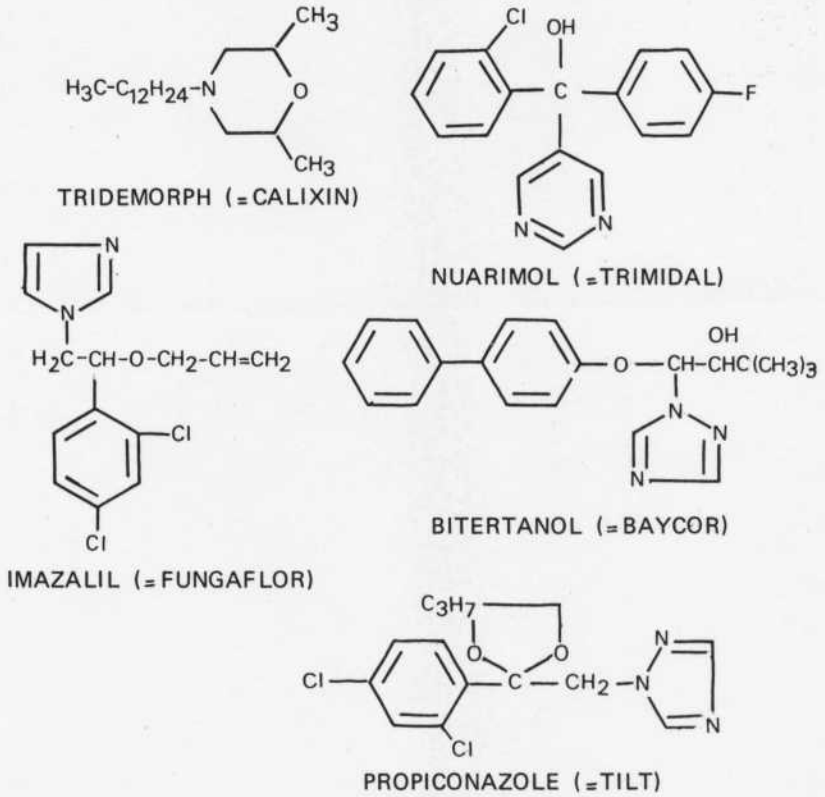

Fig. 4 - STRUCTURE OF ERGOSTEROL BIOSYNTHESIS INHIBITORS.

vely quickly on those farms with a history of resistance from 1976-77, but there was no correlation between levels of benomyl resistance and degree of disease control (Fig. 3). Through monitoring, benomyl use was postponed until resistance levels declined again. On other farms with no resistance history, up to 10 applications of «cocktail» were made with no resistance development. It now appears that in areas with a previous history of resistance 8 to 9 benomyl/mancozeb «cocktails» did not lead to resistance levels that affected Sigatoka control (STOVER, personal communication and Table 1).

CHEMISTRY AND USE OF FUNGICIDES

As mentioned before, there are two categories of fungicides available for Sigatoka control : specific-site and multi-site inhibitors. The ability of the fungus to develop resistance to multi-site inhibitors such as mancozeb is extremely low. Thus, multi-site inhibitors make excellent companions for the benzimidazoles in «cocktail» tank mixtures. Currently, it is thought that other specific-site inhibitors with different modes of action can also be used in mixtures with the benzimidazoles (DELP, 1981).

Other factors of importance in use are the ratio of the fungicides in mixtures and the necessity for rotation with multi-site inhibitors used alone. Tank-mixing benomyl with mancozeb may improve efficacy, but use of low mancozeb rates may encourage resistance development, especially as the mancozeb residues decline in the second week of the spray interval. Of course, fungi with cross resistance (to similar fungicides) or multiple resistance (to unrelated fungicides) would preclude combination of fungicides in these two categories.

Again, stability of the fungicide and frequency of use are factors favouring the build-up of resistance. Hence, there is a greater probability of resistance development where more sprays are needed, e.g., Black Sigatoka.

ERGOSTEROL INHIBITORS

Since 1967 a new family of specific-site, systemic fungicides has been developed which specifically interfere with ergosterol biosynthesis. They all have a N-containing heterocyclic ring and at least one asymetric carbon atom, resulting in different stereoisometric forms (Fig. 4).

The following groups are distinguished on the basis of the chemical nature of the N-containing heterocyclic ring. Only those compounds which have been tested for possible use in controlling Sigatoka are listed (Table 2).

TABLE 2 - Ergosterol inhibitors tested for Sigatoka control.

morpholines	tridemorph (Calixin)
pyrimidines	nuarimol (Trimidad = EL228)
imidazoles	imazalil (Fungaflor)
triazoles	bitertanol (Baycor = KWG599)
triazoles	propiconazole (Tilt = CGA64250)

Commercial application of these fungicides has not so far led to any widespread resistance in the field. However, increased levels of resistance of *Erysiphe graminis* f. sp. *hordei* to tridemorph have been reported in both glasshouse and field experiments (De WAARD and FUCHS, 1980).

STRATEGIES FOR RESISTANCE MANAGEMENT

Strategies for resistance management utilise two basic approaches : moderation and multiple attack.

Several programs are now utilised to control Sigatoka disease. Some are based mostly on the use of multi-site inhibitors (Table 3).

TABLE 3 - Multi-site - Inhibitor fungicides.

Oil (fungistat)
Chlorothalonil (= Bravo) + water
Mancozeb (= Manzate ® 200, Dithane M-45, Dithane F) + water
Mancozeb + oil
Mancozeb + oil + water

Other programs developed to deal specifically with the problem of resistance include combinations of multi- and specific-site inhibitors in tank-mix cocktails (Table 4). It is best to use combination programs before resistance occurs.

TABLE 4 - Programs based on combinations of specific- and multi-site inhibitors.

Benomyl (= Benlate®) + oil or o/w
Benomyl + mancozeb + o/w
Tridemorph + mancozeb + o/w
Imazalil + oil

The objective of these programs is to delay resistance or to manage it by preventing exposure of the pathogen to continuous selection pressure of the resistant-prone fungicide.

Examples of integrated Sigatoka management programs are given in Table 5 and are categorised on the basis of type of Sigatoka and secondly on whether resistance has been detected.

PRINCIPLES OF RESISTANCE MANAGEMENT

Experience to date has led to certain guidelines for resistance management.

- Avoid consecutive applications of benzimidazole sprays but if essential to prevent loss of control, no more than 3-4 consecutive cycles.
- Separate benzimidazole sprays by one or more non-benzimidazole containing cycles.
- Use combinations of different mode of action fungicides. If the specific-site inhibitor is more fungitoxic or has longer residual (e.g. benomyl) rotation with the multi-site inhibitor (e.g., mancozeb) is necessary.
- Withdraw the specific-site inhibitor when resistance reaches levels that may result in loss of disease control.
- Monitor for resistance.

TABLE 5 - Example of recommended Sigatoka Management Programs.

Yellow Sigatoka

- Resistance at significant levels (5% at 10 ppm). Postpone benzimidazole containing sprays until the resistant population declines to insignificant levels.
- Resistance absent or at insignificant levels.
  - a. B/O → I/O → B/O → I/O → probability of resistance low to benzimidazole because of reduced selection pressure
  - b. B/O → T/O → B/O → T/O → "
  - c. B/O → M/O → B/O → M/O → "

Black Sigatoka

- Resistance at significant levels. Postpone benomyl sprays until the resistant population declines to insignificant levels.
- Resistance absent or at insignificant levels.
  - a. C → C → C → B/M/O → M → probability of resistance moderate to low to benzimidazole
  - b. B/M/O → M → M → B/M/O → M → "

B : benzimidazole ; C : chlorothalonil ; I : imazalil ; M : mancozeb ; T : tridemorph ; O . oil

FUTURE

Future prospects of coping with resistance will require cooperative efforts among the segments of society involved in using pesticides, for example, the chemical industry, governments and universities, regulatory bodies and agricultural producers. They will need to :

- Conduct investigations to find the best multi- or specific-site companions for the benzimidazole or other systemics.

- Develop adequate laboratory facilities for identification of pathogen variation and methods of resistance monitoring, e.g., germination studies on leaf discs instead of agar.

- Conduct investigations on the epidemiology of resistant strains.

I am happy to report that some of these activities are already under-way, including attemps to get support from regional institutions (IICA, UPEB) or from international organisations such as FAO.

Also, as a result of a symposium on resistance last year, the Fungicide Resistance Action Committee (FRAC) was established to coordinate the efforts of several working groups dealing specifically with resistance problems. Ciba-Geigy, Bayer and Eli Lilly are focusing attention on potential problems with their sterol inhibitors.

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