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Differential Sensitivities of Physiologic Races of Microcyclus ulei *to Fungicides*

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Benomyl, thiophanate methyl, chlorothalonil, and mancozeb were tested against Races 4, 6, 7, *and* 8 *of* Microcyclus ulei *for inhibition of conidial germination, leaf disc infection, and field disease control.* Races 6 *and* 8 *were less sensitive to benomyl and thiophanate methyl than* Races 4 *and* 7. *Spraying benomyl (25 mg and 50 mg per litre) in the nursery gave satisfactory control of South American leaf blight on clones FX 2261 and FX 985 infected by* Races 4 *and* 7 *respectively, but not FX 3864 and FX 2804 infected by* Races 6 *and* 8 *respectively.*

South American leaf blight (SALB) of *Hevea* rubber caused by *Microcyclus ulei* (P. Henn.) Arx is indigenous to South America where it is a serious impediment to the development of a natural rubber industry. In Bahia, the annual expense of controlling SALB is equivalent to some 20% of the value of the crop. In one estate in Belem, Para chemical control of SALB represents 40% of the operational cost. Work on methods of chemical control represents some three-quarters of total research and development effort on SALB in Brazil. From 1970 onwards large-scale applications of fungicides have been carried out annually over several thousand hectares of rubber in Bahia, under the sponsorship of a Government Agency — PROMASE/SUDHEVEA. As a result, many areas of debilitated rubber have been rehabilitated. Chemicals used are the systemic compounds benomyl and thiophanate methyl, and the contact fungicides chlorothalonil and mancozeb. They are applied either from a helicopter or from the ground using a thermal fogger when the trees are refoliating. Applications are usually made on six to eight occasions at intervals of four to seven days but twelve or even more applications may be needed if climatic conditions and the phenology of the trees warrant it. Even then, control is not always completely effective and research effort is still being devoted to improving it.

This paper describes the effect of fungicides on the germination of conidia of different races of *M. ulei*, as well as the pathogenicity of these races on leaves in the laboratory and their control in the field.

MATERIALS AND METHODS

Fungicide and Inoculum

The fungicides tested were benomyl (Benlate), thiophanate methyl (Cercobin), chlorothalonil (Daconil), and mancozeb (Dithane M45). They were diluted with tap water to give concentrations of 200 mg, 50 mg and 4 mg per litre, and mixed in equal volumes with a suspension (obtained by brushing conidia off lesions) containing 2×10^5 spores per millilitre, so that

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final concentrations of both spores and fungicide were half those stated, *i.e.* 100 mg, 25 mg and 2 mg per litre. Conidia of *Race 4* were obtained from clone FX 2261, *Race 6* from FX 3864, *Race 7* from FX 985 and *Race 3* from FX 2804[1].

Conidial Germination

Drops of the spore/fungicide mixtures, and of spore suspension diluted with distilled water, were placed on microscope slides and incubated in a humid chamber at 24°C for 6 h before germination of 200 spores was counted. Conidia were considered to have germinated when the germtubes were more than half the length of the spores. Each experiment was carried out three to six times.

Prophylactic Treatment of Leaf Discs

Leaf discs 15 mm diameter were cut with a cork borer from healthy seven-day-old leaves of clones FX 2261, FX 3864, FX 985 and FX 2804 and floated with the abaxial surface uppermost[2] on distilled water in petri dishes. The fungicide/spore suspension mixtures were sprayed on the discs using an atomiser, and the dishes were incubated at 24°C under light (2600 lux) for seven days. The inoculation was carried out twice, each time using ten leaf discs. Assessment of infection was based on the percentage area of each disc which was covered with lesions, using a scale of 0%, 1% – 5%, 6% – 15%, 16% – 30%, and > 30%. The effectiveness of each fungicide was measured by the reduction of infection compared with the fungicide-free treatment. The experiments were carried out three to six times. For the method of calculating percentage control, see *Formula 2* of the later section.

Effect of Fungicide Concentration and Inoculum Density

Benomyl and thiophanate methyl were mixed with conidia of *Races 6, 7* and *8* to give mixtures containing 12.5 mg or 50 mg per litre of fungicide and conidial concentrations of 2×10^5 (high) and 4×10^4 (low) spores per millilitre. The mixtures were used as described above.

Field Experiments

Young flushes of leaves of clones FX 2261, FX 3864, FX 985 and FX 2804 planted in the EMBRAPA Station in Una, Bahia exposed to natural infection were sprayed to run-off using a 5-1 pressurised hand sprayer, with benomyl, at concentration of 25 mg or 50 mg per litre. The flushes were sprayed four times at weekly intervals until the leaves were mature. Unsprayed flushes served as controls. Flushes were cut one week after the last application and brought back to the laboratory where 300 leaves were assessed for severity of infection based on a 0 to 4 scale:

0 : No infection

1 : Lesions small and sparsely scattered, affected leaf area ≤ 5%

2 : Lesions mostly small and scattered, affected leaf area 6% – 15%

3 : Lesions large and dense, sporulation and leaf distortion, affected leaf area 16% – 30%

4 : Lesions rampant, confluent, leaf torn, affected leaf area > 30%.

The severity of infection (%) was calculated by *Formula 1,* and the percentage control by *Formula 2:*

$$\frac{\text{No. of leaves in different disease classes} \times \text{Score of the class}}{\text{No. of leaves in different disease classes} \times 4} \times 100 \qquad \ldots 1$$

$$\frac{\text{Severity of infection with no treatment} - \text{Severity of infection with treatment}}{\text{Severity of infection with no treatment}} \times 100 \qquad \ldots 2$$

RESULTS

Germination of Conidia

Table 1 shows that chlorothalonil (1 mg per litre) was strongly inhibitory to germination of conidia of all races of *M. ulei,* and mancozeb (12.5 mg per litre) slightly less inhibitory. However, the inhibitory effect of mancozeb suspension diminished somewhat after 24 h storage. Conidia of the four races were affected to different degrees by benomyl and thiophanate methyl. *Races 6* and *8* were much less susceptible to thiophanate methyl than *Races 4* and *7. Race 6,* and to a lesser extent *Races 4* and *8* were also less affected by benomyl than *Race 7.*

Prophylactic Treatment of Leaf Discs

Table 2 shows that the effect of fungicides on leaf disc infection differed among four races, especially with benomyl. Benomyl was much less effective in suppressing infection by *Races 6* and *8* than *4* and *7* when tested at 12.5 mg per litre. Even at 50 mg per litre, benomyl was about half as effective on infec-

TABLE 1. PERCENTAGE GERMINATION OF CONIDIA OF FOUR RACES OF *M. ULEI* IN FOUR FUNGICIDES

Fungicide	Concentration (mg/litre)	Percentage germination[a]			
		Race 4	Race 6	Race 7	Race 8
1. Chlorothalonil	1.0	0	0	0	0
2. Mancozeb	12.5	0	4.1	0	28.4
3. Benomyl	12.5	42.6	81.7	24.6	48.7
4. Thiophanate methyl	12.5	28.4	53.5	17.2	46.6
L.S.D. (Between 3 and 4)		9.23	7.68	4.26	6.21
L.S.D. (Between 2 and 3)					6.81

[a]As compared to control converted to 100%

TABLE 2. PERCENTAGE CONTROL OF LEAF DISC INFECTION BY CONIDIA OF FOUR RACES OF *M. ULEI* IN THE PRESENCE OF THREE FUNGICIDES AT TWO CONCENTRATIONS

Fungicide	Concentration (mg/litre)	Percentage control			
		Race 4	Race 6	Race 7	Race 8
Mancozeb	12.5	64.4	67.0	67.7	39.6
	50.0	84.3	83.3	94.1	51.7
Benomyl	12.5	54.2	26.8	61.4	30.7
	50.0	71.0	41.7	79.2	52.1
Thiophanate methyl	12.5	49.6	47.8	60.7	45.9
	50.0	83.9	62.2	76.9	57.5
L.S.D. (Chemical)		12.87	4.81	5.22	6.49
L.S.D. (Concentration)		10.51	3.92	4.26	5.30

tion caused by *Race 6* than on infection caused by *Races 4* and *7*. Mancozeb was less effective in controlling infection caused by *Race 8* than by *Races 4, 6* and *7*, whereas thiophanate methyl was less effective against *Races 6, 8* and *4* at low fungicide concentration.

Effect of Fungicide Concentrations and Inoculum Density

As expected, the results in *Table 3* show that high fungicide concentration (50 mg per litre) combined with low inoculum density (4×10^4 spores per mililitre) reduced infection more than low fungicide concentration (12.5 mg per litre) combined with high inoculum density (2×10^5 spores per millilitre). Percentage control of leaf disc infection by benomyl in the former combination with *Races 6, 7* and *8* were 65.0, 86.6 and 68.6 respectively, whereas in the latter they were 27.2, 53.4, and 35.5 respectively. However, the results differed little when the comparisons were between high fungicide concentration with high inoculum density, and low fungicide concentration with low inoculum density *(Table 3)*. Irrespective of fungicide concentrations and inoculum densities, *Races 6* and *8* were consistently less sensitive to benomyl and thiophanate methyl than *Race 7*.

Field Control

The results in *Table 4* show that under field conditions, benomyl at 25 mg and 50 mg per litre were most effective against SALB on FX 2261 (infection by *Race 4*), slightly less effective on FX 985 (infection by *Race 7*), and least effective on FX 3864 (infection by *Race 6*) and FX 2804 (infection by *Race 8*). Field results were consistent with laboratory finding in that benomyl was effective against certain races of *M. ulei* and not others at the concentrations tested.

DISCUSSION

Fungicides had hitherto been tested against *M. ulei* without regard to possible differences in the sensitivity of races to the chemicals. The present study demonstrated that benomyl,

TABLE 3. PERCENTAGE CONTROL OF LEAF DISC INFECTION BY *M. ULEI* IN DIFFERENT COMBINATIONS OF CONIDIA DENSITIES AND FUNGICIDE CONCENTRATIONS

Fungicide	Concentration (mg/litre)	Conidia density	Percentage control Race 6	Race 7	Race 8
Benomyl	12.5	High	27.2	53.4	35.5
		Low	40.0	67.3	47.1
	50.0	High	42.3	76.4	53.5
		Low	65.0	86.6	68.6
Thiophanate methyl	12.5	High	36.4	60.2	36.1
		Low	40.0	67.3	45.3
	50.0	High	45.5	80.3	50.0
		Low	65.0	86.6	65.9
Transformed data					
L.S.D. (Chemical)			5.50	5.91	2.67
L.S.D. (Concentration)			3.89	4.18	1.89
L.S.D. (Chemical × Concentration)			7.78		

TABLE 4. EFFECT OF BENOMYL ON THE CONTROL OF SALB CAUSED BY FOUR RACES OF *M. ULEI*

Clone	Race	Severity of infection				Percentage control as compared to no treatment	
		Treatment		No treatment			
		Expt. 1 25 mg/litre	Expt. 2 50 mg/litre	Expt. 1 25 mg/litre	Expt. 2 50 mg/litre	Expt. 1 25 mg/litre	Expt. 2 50 mg/litre
FX 2261	4	19.1	12.3	51.5	57.9	62.9	78.8
FX 3864	6	39.9	45.5	38.0	51.4	0	11.9
FX 985	7	44.0	25.9	77.3	61.8	43.4	58.1
FX 2804	8	57.5	39.2	62.9	50.0	8.6	21.6

which is commonly used against SALB, was more effective against some races of *M. ulei* than others. Thus *Races 6* and *8* were tolerant to benomyl while *Races 4* and *7* were sensitive. Hence benomyl gave satisfactory control of SALB on FX 2261 (infection by *Race 4*) and FX 985 (infection by *Race 7*), and poor or no control on FX 3864 (infection by *Race 6*) and FX 2804 (infection by *Race 8*). This finding helped to explain some of the inconsistent results obtained in the treatment of SALB in Bahia with benomyl.

It is important therefore, that along with a study of the race structure of *M. ulei* in Brazil, research should also be conducted on the effect of fungicides, especially the systemic compounds, on controlling infection caused by separate races of *M. ulei*. There are many different clones of *Hevea* in commercial plantings and it is likely that they are differently susceptible to each of the races present[1] and therefore the degree of control given by benomyl can vary from clone to clone. Benomyl has been used for SALB control in Bahia for over ten years, and it is not known if certain races of *M. ulei* have developed resistance to it. Rao *et al.*[3] in Bahia tested benomyl at 10 mg per litre and found that conidial germination was merely 4%. In the present study however, germination averaged 49.9% when tested against benomyl at 12.5 mg per litre. Chee[4] in Trinidad found no germination with conidia of *Race 6* (25 mg per litre), whereas in the present study, germination of the same race was 28.6% (50 mg per litre). If indeed some races do become tolerant to benomyl through repeated usage there is a need to consider alternating or combined use of conventional fungicides, such as chlorothalonil, for the control of SALB.

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