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REACTION OF ORIENTAL *HAVEA* CLONES TO ISOLATES OF *MICROCYCLUS ULEI* AND THE RESPONSE OF ISOLATES TO FUNGICIDES

By

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SUMMARY

Hevea clones from Sri Lanka, Malaysia and India were assessed in vitro for their resistance to four isolates of Microcyclus ulei. *Clones SL 26, R-14, 5B/17/4, and 1/2/56/77 were infected by all isolates of* M. ulei *tested. However, they were found to have some resistant to* M. ulei.

Propiconazole was more effective than Triforine and Triadimefon in inhibiting germination of conidia of M. ulei. *These fungicides were also effective in inhibiting lesion formation. Triadimefon (Bayleton 50 w.p.) had a better curative effect than propiconazole (Tilt 25 EC) and Triforine (Saprol). The effect of Triadimefon on germination of conidia varied between isolates while the effect varied little between isolates for propiconazole and Triforine. The effect of the fungicides on the number of lessios on leaf discs did not show major differences between isolates.*

INTRODUCTION

Breeding for resistant clones is a useful method to control South American leaf blight (SALB) caused by *Microcyclus ulei* (P. Henn.) V. Arx. SALB is absent outside the American tropics. Clones bred in Malaysia and Sri Lanka for resistance aganist SALB were previously screened in Trinidad and Tobago. The results of this screening had been reported earlier (Chee, 1976 ; Liyanage & Chee, 1981 ; Fernando and Liyanage, 1983). There exist many physiologic races of *M. ulei* (Miller, 1967 ; Chee *et al.*, 1986). It is therefore important that clones of *Hevea* be screened against as many races as possible. There are only one or may be two races of *M. ulei* in Trinidad and Tobago, while many more are found in Brazil (Chee *et al.*, 1986).

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Use of fungicides is another approach to combat SALB. Zhang and Chee (1986) indicated that the effect of Benomil and Thiophanate methyl varied with races of *M. ulei*. These fungicides had been used for many years in Brazil to control SALB. Triforine has recently been found to be effective against SALB (Santos & Pereira, 1985).

MATERIALS AND METHODS

Preparation of leaf discs

Discs (15 mm diam) were obtained from young, apparently healthy leaves (7-day old) harvested from EDJAB Experimental Station, Una, Bahia, Brazil. Twelve discs were suspended, lower surface up on water in petri plates. After specific treatments the plates were incubated under continuous light (2500 lux) in an incubator maintained at 25°C

Assessment of resistance

Leaf discs were inoculated by spraying a suspension (2×10^5 spores/ml) of field conidia obtained from clones FX 985, FX 2804, FX 3846 and FX 2261 using an atomist atomiser. These conidia constituted different isolates, which probably were race 2 (FX 2804), 4(FX 2261), 7 (FX 3846) and 9 (FX 985) (Chee *et al.*, 1986). Resistance was assessed by estimating the size of lesions on the sixth day after inoculation. With the aid of a dot-scale (Darmono & Chee, 1985), 100 lesions were measured per clone and each clone was tested at least twice. Clones prefixed SL or C were from Sri Lanka, RRIM or unnamed were from Malaysia and RRII were from India.

Effect of fungicide on germination of conidia

An aqueous suspension of conidia from either clone FX 985; FX 2261, FX 2804 FX 3846, FX3864 or IAN 873 were mixed with an equal part of a solution of either triadimefon, triforine and propiconazole giving a final fungicide concentration of either 10, 20 or 40 mg/l and conidia concentration of 1×10^5 spores/ml. The conidial fungicide suspension was pipeted onto glass slides and incubated at room temperature for about 14h.

Effect of fungicides on lesion number on leaf discs

Leaf discs from clone FX 3864 floated on solutions of either triforine (10 mg/l), propiconazole (10 mg/l) and triadimefon (20 mg/l) were inoculated with conidia from either clone FX 3864, FX 985, FX 2261 or FX 2804. On the sixth day after inoculation the number of lesions were counted from 10 discs. The experiment was replicated thrice, and was repeated once. In the control, the leaf discs were suspended on distilled water.

To test the effect of time of fungicide treatment on lesion development, leaf discs of clone FX 3864, were inoculated with conidia from clone FX 985. At various intervals *viz.* (Oh, 24 h, 48h) after inoculation, the discs were subsequently sprayed with solution of either propiconazole (10 mg/l), triadimefon (20 mg/l), or triforine (10 mg/l). The number of lesions were counted on the sixth day after inoculation.

RESULTS AND DISCUSSION

The clones prefixed RRISL and C had been tested in Trinidad and Tobago and were found to be immune or moderately resistant to SALB (Chee, 1976 ; Liyanage & Chee, 1981 ; Liyanage & Chee, unpublished). The races of *M. ulei* present in Trinidad and Tobago were probably race 2 and 6 (Chee *et al*, 1986). When these clones were tested against more races of *M. ulei* present in Bahia, Brazil some of the clones still maintained the ratings of moderate to resistant while others were susceptible (Table 1). Five clones (C-8, RRIM 725, 1/3/56/77, 1/67 and 4/7 reacted race specifically, as they were not infected by isolates of *M. ulei* from FX 2804 (race 2) which usually attack progenies of *H. benthamiana* clone F 4542. Clone 6/185 was not infected by any of the races tested. Except for clones 6/185 and RRIM 725 all the *Hevea* clones tested was infected by isolates from FX 985, FX 2261 and FX 3846. Even though more inoculations with other races are needed to prove that the reaction of clones to *M. ulei* are not race specific, the results so far obtained indicated that clones SL 26, R-14, 5B/17/4 and 1/2/56/77, were more resistant to the four races of *M. ulei*. These clones should be further studied to verify their reactions to all the available races of *M. ulei*.

Propiconazole was more effective than triadimefon and triforine in inhibiting conidial germination (Table 2). Propiconazole at 10 mg/l gave 90.42% inhibition while triadimefon (40 mg/l) and triforine (40 mg/l) produced 43.80% and 63.22% inhibition, respectively.

Table 1. *Susceptibility of oriental clones planted in UNA, Bahia, Brazil to 4 races of* Microcyclus ulei.

Clone	Size of lesion (/μm) on clones inoculated with conidia From FX 985	FX 2261	FX 2804	FX 3846	Mean lesion size (/μm)	Susceptibility rating *
SL 30	434	341	322	N.A.	366	3
SL 26	300	328	328	N.A.	319	2
6/185	N.R.	N.R.	N.R.	N.R.	0	Immune
C - 8	335	288	N.R.	N.A.	312	2
3/2	327	358	404	371	365	3
64B 203	372	319	412	329	358	3
SL 29	432	321	394	N.A.	382	3
RRIM 725	340	N.R.	N.R.	N.A.	340	2
6/267	421	483	339	411	414	3
64B 808	591	414	506	555	517	4
RRII 33	577	501	438	N.A.	505	4
RRII 203	347	324	416	518	401	3
SL 28	488	499	431	442	465	4
62C 225	467	339	314	296	354	3
1/3/56/77	384	257	N.R.	351	331	2
1/67	508	440	N.R.	420	456	4
7/15	389	332	345	420	372	3
1/13/56/77	494	512	522	516	511	4
12/10/56/77	459	286	262	392	350	3
56/64/72	506	462	470	457	473	4
R - 14	302	319	316	412	336	2
1/14/56/77	463	502	425	N.A.	463	4
1/10/56/77	540	437	380	433	448	3
5B/17/4	338	381	276	375	343	2
1/2/56/77	296	306	276	375	313	2
1/33	357	357	331	447	373	3
4/7	515	375	N.R.	N.A.	445	3
3/1	371	392	N.A.	N.A.	382	3

Standard error or lesion size = 7.1821 *1-5 scale of decreasing resistance.

NR = No reaction

NA = Not available

1 – Highly resistant (0–250/ μm)

2 – resistant (251–350/ μm)

3 – Moderately resistant (351–450)

4 – Susceptible (451–550) and

5 – Highly susceptible (>551/ μm)

N.R. – Indicates no lesions detected.

N.A. – Not Available

Table 2. *Effect of fungicide on germination of* Microcyclus ulei *conidia*

Treatment	Concentration mg/l	Percentage inhibition * of germination of conidia obtained from clones						
		FX 985	FX 2804	FX 2261	FX 3864	IAN 873	FX 3846	MEAN
	10	94.19 (76.08)	91.27 (72.29)	88.76 (70.49)	100 (90)	77.83 (62.10)	90.51 (72.08)	90.42
TILT (PROPICONAZOLE)	20	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100
	40	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100
	10	18.07 (25.15)	3.86 (11.29)	8.56 (15.83)	4.63 (12.42)	9.19 (17.59)	9.78 (18.16)	9.01
TRIADIMEFON	20	21.41 (27.52)	4.08 (14.38)	16.39 (23.80)	5.81 (13.58)	10.28 (18.61)	13.09 (20.03)	11,84
	40	50.07 (45.04)	34.99 (36.26)	63.01 (52.60)	45.29 (42.30)	44.97 (42.12)	24.48 (34.55)	43.80
	10	15.46 (22.85)	17.66 (24.42)	8.64 (17.06)	14.52 (22.39)	10.45 (18.86)	13.28 (21.36)	13.33
TRIFORINE	20	35.72 (36.66)	25.76 (30.48)	25.99 (30.58)	23.93 (29.56)	18.07 (25.14)	31.92 (34.15)	26.89
	40	71.86 (57.99)	76.73 (61.31)	52.49 (46.43)	71.95 (58.15)	48.30 (44.00)	58.02 (49.62)	63.22

Data in parentheses are transformed values using Sin-1, L.S D $_{0.05}$ = 7.29

*Germination in water - germination in fungicide X 100
Germination in water

Propiconazole effectively inhibited germination of all isolates of *M. ulei*. Analysis using Sin^{-1} transformation of data showed that for isolates from FX 2804, FX 3864 FX 3846 and IAN 873 inhibition increased uniformly with concentrations of both triadimefon and triforine with propiconazole having a higher level of inhibition at all concentrations. In the case of triadimefon, the percentage inhibition of germination of conidia from FX 3846 and FX 2804 was lower as compared to the other isolates (Table 2). Benomyl and thiophanate methyl had also been shown to have variable effects on germination of conidia of different isolates of *M. ulei* (Zhang and Chee, 1986). They concluded that these variations were due to the difference in resistance of the fungus to these fungicides as they had been used for many years to control SALB. Similarly, triadimefon could have the same effect as it also have been used for sometime to control the disease. In the case of triforine, its use in the field is only recent, while propiconazole is yet to be used in the field.

When leaf discs were suspended on solutions of fungicides, the three fungicides effectively inhibited lesion development on leaf discs with triforine showing significantly higher level of inhibition than propiconazole (Table 3). On the other hand, when these fungicides were sprayed onto the leaf surface, tridimefon and propiconazole were significantly more effective than triforine in inhibiting lesion formation (Table 4). This discrepancy could be due to the difference in the method used to apply the fungicides as well as the difference in the protective and eradicant action of these fungicides. Conidia would come in direct contact with the fungicides sprayed on the leaves, whilst they would not come in direct contact with the fungicides, when leaf discs were suspended on fungicide solutions. Propiconazole had a stronger protective action than triforine ; as propiconazole had a greater inhibition of germination than triforine (Table 2). There were no significant differences in the effect of the fungicides on germination of different isolates except for propiconazole which had a lower inhibition of the isolate from FX 2804 (Table 3).

Table 3. *Effect of fungicide on the number of lesions on* Hevea *leaf disc inoculated with* M. ulei

Fungicide	Percentage inhibition of lesion number on leaf disc inoculated with conidia from					Transformed mean *
	FX 985	FX 2261	FX2804	FX3864	Mean	
PROPICONAZOLE (10 /^{u}g/ml)	88.92	93.83	74.49	91.52	87.19	70.70
TRIADIMEFON (20/^{u}g/ml)	97.41	93.91	96.82	90.09	94.55	77.05
TRIFORINE (10 /^{u}g/ml)	97.60	97.41	95.10	98.42	97.13	80.88

*Using Sin^{-1} transformation; L S D $_{0.05}$ = 7.18

Table 4. *Percentage inhibition of lesion number by fungicide applied at* different hours after inoculation

Fungicide	Percentage inhibition of lesion number by fungicide applied at various intervals after inoculation		
	0 h	24 h	48 h
PROPICONAZOLE	97.58	89.31	62.93
(10/ᵘg/ml)	(81.05)	(70.97)	(52.59)
TRIADIMEFON	100 *	100 *	100 *
(20/ ᵘg/ml)	(90.00)	(90.00)	(90.00)
(TRIFORINE	53.71	45.50	13.29
(10/ᵘg/ml)	(47.13)	(42.35)	(21.33)

data in parentheses are transformed values using $\sin^{-1}$, $LSD_{0.05} = 6.55$

*Assessment was taken 9th day after inoculation.

Propiconazole was effective in inhibiting conidial germination, and on lesion development. It was effective when sprayed at 0 and 24 h after inoculation and less effective when sprayed 48 h after inoculation. Triadimefon which was less effective in inhibiting conidial germination was more effective in inhibiting lesion development even when applied 48 h after inoculation (Table 4). Nursery trials indicated that triadimefon and triforine were effective in controlling SALB (Santos and Pereira, 1985).

SALB could be managed through the use of resistant clones and fungicides. The results indicated that there are clones which were bred in the East which are less susceptible to several isolates of *M. ulei*. Inhibition of lesion development on leaf disc could be a better method to screen for effective systemic fungicides for controlling *M. ulei*.

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