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Induced Resistance of Hevea *to South American Leaf Blight by Incompatible Races of* Microcyclus ulei

ISMAIL HASHIM*

Inoculation of leaf discs of Hevea *by an isolate of* Microcyclus ulei *incompatible to a* Hevea *clone induced resistance to subsequent infection by a compatible race. Simultaneous inoculation of compatible and incompatible races reduced the size of lesions but not their numbers. Inoculation of an incompatible race 24 h prior to inoculation of a compatible race reduced both the number and size of lesions. The reduction was proportional to the concentration of the incompatible race inoculum. On intact leaves, inoculation of a compatible race prior to an incompatible race reduced the number of lesions and the conidia produced.*

Treatment of plant with pathogens or non-pathogens is known to increase resistance to compatible pathogens[1,2,3]. Prior infection by a less virulent strain of pathogen had also been shown to confer resistance to subsequent infection by the same pathogen[4,5,6]. This phenomenon of induced resistance occurs between and among viral, bacterial and fungal pathogens. Cross protection between races of a pathogen also occurs[7,8].

Microcyclus ulei (P. Henn.) v. Arx causes the economically detrimental South American leaf blight (SALB) of *Hevea*. Miller[9] identified four races of *M. ulei* among isolates from Guatemala, Costa Rica and Brazil (Belem). Chee *et al.*[10] found eight races at Una, Bahia, Brazil. *Hevea* clones resistant or susceptible to races of *M. ulei* were reported earlier[9,10]. This paper describes the effects of inoculation with incompatible races of *M. ulei* followed by compatible races on disease development.

MATERIALS AND METHODS

Leaf Disc

About seven-day-old leaves of clone FX 985 were harvested from nursery plants growing in EDJAB Experimental Station, Una, Bahia, Brazil. Twelve leaf discs (15 mm diameter), one from each leaflet, were cut and suspended with their abaxial surface up on distilled water in petri dishes.

Infected leaves of clones FX 985, FX 2804, FX 2261 and IAN 873 bearing the conidia were harvested from the same station. The inoculum was prepared by brushing conidia with an artist paint brush into distilled water. Concentrations of conidia at 1×10^4, 5×10^4 and 1×10^5 per millilitre were determined with the aid of an improved Neubauer counting chamber. The discs were inoculated by spraying (five puffs per plate), with the conidial suspensions using an Atomist atomiser and incubated in an illuminated (1200 lux) incubator maintained at 24°C.

Lesion number and size were determined on the sixth day after inoculation. Each experiment consisted of two plates and the experiment was replicated. The number of lesions resulting from inoculation was determined from ten discs per plate. Ten lesions from each disc chosen at random were determined for their sizes using a dot scale[11].

Intact Leaf of Plants in Polyethylene Bags

The middle leaflets of young copper brown leaves of clone FX 2261 grown in polyethylene bags in a glasshouse were covered with small clear polyethylene bags. The outer two unbagged leaflets were inoculated by spraying their under-surface with a conidial suspension (1×10^6 conidia per millilitre) from clone FX 2804. Clone FX 2261 is immune to infection by conidia from FX 2804[10]. The whole shoot was then covered by a polyethylene bag. The bags

*EMBRAPA/SUDHEVEA/CEPLAC/RRIM Rubber Technical Co-operation Programme, Itabuna, Bahia, Brazil

were removed after 36 h. The three leaflets were flattened on the palm and the abaxial leaf surfaces were sprayed with a conidial suspension (1 × 10^4 conidia per millilitre) from clone FX 2261. The shoots were again bagged for a further 36 h.

The number of lesions was determined by counting only sporulating lesions eight days after the second inoculation. Conidia were harvested by brushing with an artist brush into 5 ml or 10 ml of distilled water and were counted using a haemocytometer.

RESULTS

The development of a compatible race of *M. ulei* on *Hevea* leaves was affected by inoculation with an incompatible race. Leaf discs inoculated with the incompatible race (inducer) 24 h before inoculation with the compatible race (challenger) produced fewer *(Table 1)* and smaller *(Table 2)* lesions as compared to discs not induced. All the three incompatible races used reduced the number of lesions on leaf discs of FX 985, significantly (P = 0.05) with the incompatible races from

TABLE 1. NUMBER OF LESIONS ON LEAF DISCS OF FX 985 INOCULATED WITH AN INCOMPATIBLE RACE OF *M. ULEI* 24 H BEFORE BEING INOCULATED BY THE COMPATIBLE RACE

Source of incompatible race	Number of lesions								Mean
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Expt. 6	Expt. 7	Expt. 8	
Control (inoculum from FX 985 only)	36.3	44.2	71.2	33.3	70.7	35.8	50.0	35.3	47.1
FX 2804	29.5	18.4	45.3	15.4	32.1	20.7	42.0	22.5	28.2*
FX 2261	28.5	ND	52.2	32.5	30.0	23.1	49.2	28.5	34.9^{NS}
IAN 873	16.2	ND	ND	10.6	47.6	26.6	39.5	22.5	27.2*

ND = No data

Control discs were inoculated only once. Analysis of variance between control and individual incompatible race treatment indicated significance at P = 0.05 (*) or non-significance (NS) at P = 0.05.

TABLE 2. SIZE OF LESIONS ON LEAF DISCS OF FX 985 INOCULATED AT 0-H AND 24-H INTERVALS BETWEEN INOCULATION OF AN INCOMPATIBLE AND A COMPATIBLE RACE OF *M. ULEI*

Source of incompatible race	Size of lesions (μm)							
	Expt. 1		Expt. 2		Expt. 3		Mean	
	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
Control (inoculum from FX 985 only)	718	600	601	695	696	677	671.7	657.3
FX 2804	503	444	437	476	561	521	500.3*	480.3**
FX 2261	508	522	458	472	549	552	505.0*	515.3*
IAN 873	544	588	495	529	554	552	531.0*	556.3*
					L.S.D. (P = 0.05)	=	97.79	76.25
					L.S.D. (P = 0.01)	=	142.27	110.94

Analysis of variance indicated significance at P = 0.05 between treatments for both 0-h and 24-h intervals.

The difference between control and individual incompatible race treatment was significant at P = 0.05 (*) or P = 0.01 (**).

FX 2804 and IAN 873. However when the incompatible race was simultaneously inoculated with the compatible race, there was no significant difference in the number of lesions between treated and control discs *(Table 3)*. Simultaneous inoculations, nevertheless produced smaller lesions on treated discs as compared to the control discs inoculated only with the compatible race *(Table 2)*. The percentage reduction in number and size of lesions formed by the challenger was proportional to the inoculum concentration of the inducer *(Figure 1)*. The concentration of the inducer inoculum should be above 1 × 10^4 conidia per millilitre in order to be effective.

When one-half of the disc or leaf was treated with the incompatible race to be followed 24 h later by inoculation of the whole leaf or disc with a compatible race, the number and size of lesions were significantly smaller on the induced side as compared to the non-induced side *(Table 4)*. However, the size of lesions on the non-induced side (598.9 μm) was not significantly different from the lesions (604.0 μm) resulting from only the compatible race *(Table 4)*. Lesion development occurred earlier on the non-induced part of the leaf or disc compared to the induced part.

Discs inoculated by placing a drop of inoculum of an incompatible race on each half of the disc and 24 h later sprayed with a compatible race produced few small inconspicuous lesions at the immediate site inoculated with the incompatible race. Numerous distinct lesions developed around these sites. Even though a reasonably large area of the disc was not infected, the total number of lesions was not significantly different compared to control discs inoculated only with the compatible race *(Table 4)*. Moreover, the size of lesions surrounding the inoculated site was also not significantly different from that developed on control discs *(Table 4)*.

The number of lesions on intact leaflets inoculated with the incompatible race prior to inoculation with a compatible race was less compared with control leaflets inoculated only with the compatible race *(Table 5)*. Similarly, the number of conidia produced was also fewer on induced leaflets compared to uninduced leaflets *(Table 5)*. In addition, pycnidial formation was delayed on induced leaflets. The general symptom was striking in that infection was more severe on control leaflets compared to induced leaflets *(Figure 2)*. In most instances, the non-induced leaflets though diseased remained attached or abscissed at a later date.

DISCUSSION

Induced resistance has been studied on several plants using various inducers and challengers. The potential of this field of research includes the possibility of understanding plant immunity as well as the likely use of elicitors of induced resistance for selecting resistant clones[3]. There

TABLE 3. NUMBER OF LESIONS ON LEAF DISCS OF FX 985 SIMULTANEOUSLY INOCULATED WITH COMPATIBLE AND INCOMPATIBLE RACES OF *M. ULEI*

Source of incompatible race	Number of lesions					Mean
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	
Control (inoculum from FX 985 only)	54.4	64.6	48.0	54.3	35.1	49.3
FX 2804	45.7	47.3	42.5	46.1	17.9	39.9
FX 2261	54.4	58.8	47.0	52.7	32.2	49.0
IAN 873	41.6	ND	ND	61.6	22.7	42.0

ND = No data

Analysis of variance indicated no significant difference between treatments.

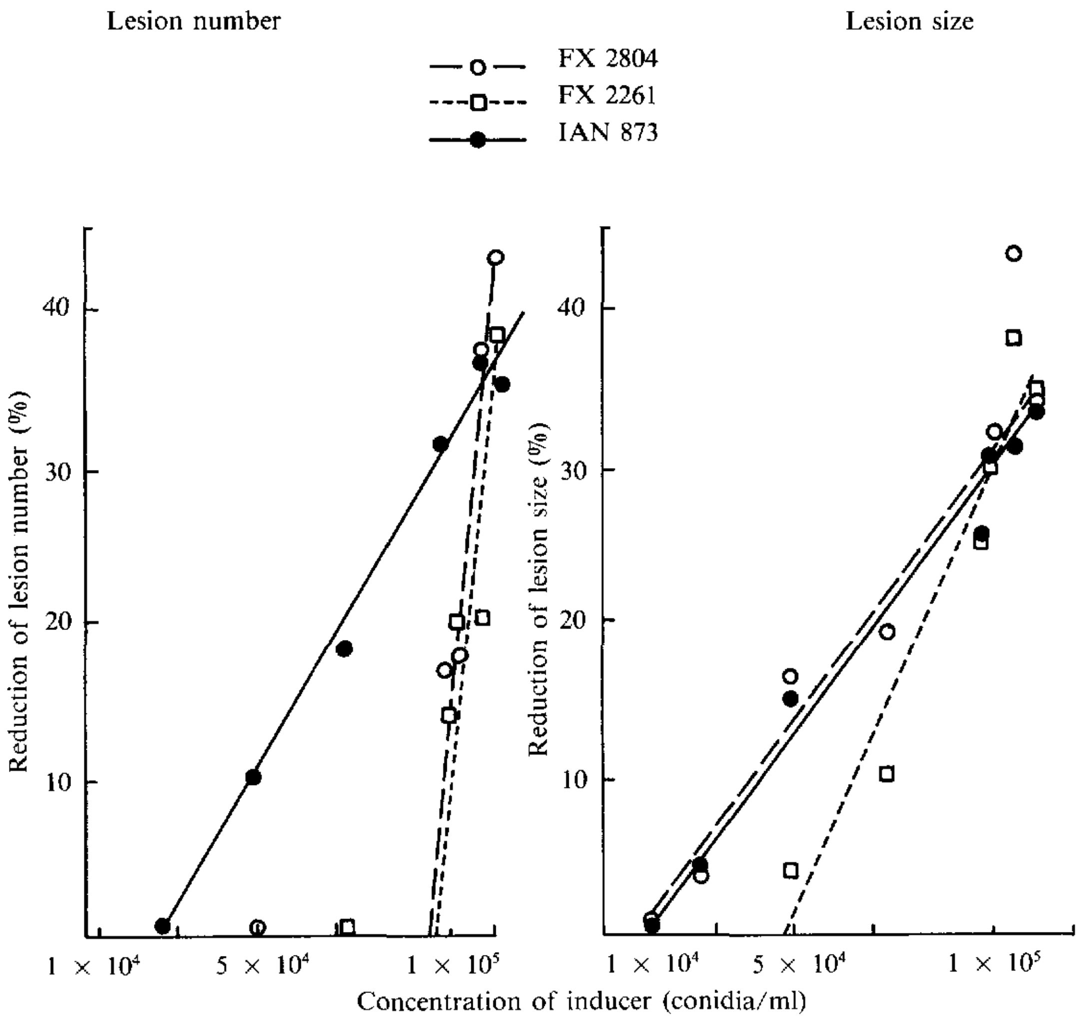

Figure 1. Effect of inoculum concentration of an incompatible race of M. ulei *from clones FX 2804, FX 2261 and IAN 873 on the number and size of lesions developed by a compatible race.*

were reports that induced resistance was successful as a method of disease control[1,3]. Specifically, water-melon seedlings induced by *Colletotrichum lagenarium* in the laboratory were resistant to further infection by the fungus in the field[12].

Results presented in this paper indicated that incompatible races of *M. ulei* induced resistance to *Hevea* against compatible races. This was indicated by reduced size and number of lesions as well as less number of conidia produced. The induced leaflets suffered less severe disease. Studies performed on other host-parasite interactions explain the mechanism of induced resistance[8,12]. In the case of *Hevea,* induction of resistance by incompatible races of *M. ulei* against compatible races seems to be localised in action. This was supported by observations that inoculation of a portion of leaf disc did not reduce the number or size of lesions on other portions. Similarly, induction of resistance to the outer two leaflets did not confer resistance to the middle uninduced leaflets. Resistance was

TABLE 4. EFFECT OF SITE OF INOCULATION OF AN INCOMPATIBLE RACE OF *M. ULEI* ON THE NUMBER AND SIZE OF LESIONS DEVELOPED BY A COMPATIBLE RACE

Treatment	Lesion No.	S.E.	Lesion size (μm)	S.E.
When incompatible was inoculated on one-half of leaf discs[a]				
Inoculated half	18.32	1.61	420.36	41.19
Uninoculated half	36.41	2.88	598.91	18.76
	$P < 0.001$		$P < 0.001$	
When one drop of incompatible race inoculum was placed on each half of disc[a]				
Inoculated	60.75	4.40	549.90	22.35
Uninoculated	65.78	4.38	604.40	21.37
	N.S. at $P = 0.05$		N.S. at $P = 0.05$	

[a]The data are means of 10-13 replicates.

TABLE 5. NUMBER OF LESIONS AND CONIDIA PRODUCED ON INTACT *HEVEA* LEAVES INOCULATED WITH AN INCOMPATIBLE RACE OF *M. ULEI* PRIOR TO A COMPATIBLE RACE

Treatment	Lesion No./cm^2	Sporulation	
		No./cm^2 ($\times 10^4$)	No./lesion ($\times 10^3$)
Induced	11.48	1.81	1.64
Non-induced	19.53	4.08	2.63
	$P < 0.001$	$P < 0.001$	$P < 0.02$

conferred to the area where the inducer was applied. Localised resistance was attributed to host cell collapse or production of phytoalexins by the affected cells[3]. This might be the case with induced resistance of *Hevea* by incompatible races of *M. ulei* against compatible races. It was earlier observed that hypersensitive cell collapse occurred at sites of penetration of highly resistant clones[13]. Leaf diffusate obtained at sites of penetration of resistant leaves was also inhibitory to conidial germination suggesting the presence of toxic substances[14].

It may not be practical to control SALB with incompatible races of *M. ulei*. However, it might be able to reduce the incidence or effect of the disease. This could be achieved by planting a mixture of suitable clones with race-specific resistance to *M. ulei*. In the event of disease, the conidia produced by one particular clone, when landed on the young leaves of another clone could protect it against races in which it is susceptible. Subsequent reduction in lesions and sporulation presumably would diminish the inoculum and hence disease. Of course, the concentration of the inducer needs to be large as the intensity of induced resistance was dependent on the concentration of the inducer. The above postulate was based on the success on cereal crops in which disease was

Figure 2. Disease symptoms on Hevea *leaflets induced (outer two leaflets) or uninduced (middle leaflets) with an incompatible race of* M. ulei.

lower where a mixture of varieties was planted as compared to the pure stand[8,15,16,17]. Among other reasons, induced resistance was attributed to the reduction in disease severity[18].

Hevea clones with race specific resistance to *M. ulei* are numerous. Some of these are presently utilised commercially usually planted in mono-clone blocks. These clones are healthy in the absence of a compatible race and often are heavily diseased when the compatible race is present with suitable climatic conditions. It is therefore worthwhile to pursue planting of a mixture of clones, if clones with race-specific resistance are continuing to be used. This of course would demand proper selection of clones for suitability to agronomic practices as well as the right host-fungal race specificity.

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REFERENCES

1. KUC, J. AND PREISIG, C. (1984) Fungal Regulation of Disease Resistance Mechanisms in Plants. *Mycologia,* **76,** 767–784.
2. OUCHI, S. (1983) Induction of Resistance or Susceptibility. *A. Rev. Phytopathol.,* **21,** 289–315.
3. SEQUEIRA, L. (1983) Mechanism of Induced Resistance in Plants. *A. Rev. Microbiol.,* **37,** 51–80.
4. ELLISTON, J., KUC, J. AND WILLIAMS, E.B. (1976) Protection of *Phaseolus vulgaris* against Anthracnose by *Colletotrichum* Species Non-pathogenic to Bean. *Phytopath. Z.,* **86,** 117–126.
5. KUC, J., SHOCHLEY, G. AND KERNEAY, K. (1975) Protection of Cucumber against *Colletotrichum lagenarium* by *C. lagenarium. Physiol. Pl. Pathol.,* **7,** 159–199.
6. LITTLEFIELD, C.J. (1969) Flax Rust Resistance Induced by Prior Inoculation with an Avirulent Race of *Melampsora lini. Phytopathology,* **59,** 1323–1328.
7. NELSON, R.R. AND TUNG, G. (1973) Cross-protection by Race 0 against Race T of *Helminthosporium maydis. Pl. Dis. Reptr,* **51,** 971–973.

8. KOCHMAN, J.K. AND BROWN, J.F. (1975) Studies on the Mechanism of Cross-protection in Cereal Rusts. *Physiol. Pl. Pathol.*, **6**, 19-27.
9. MILLER, J. (1966) Differential Clones of *Hevea* for Identifying Races of *Dothidella ulei*. *Pl. Dis. Reptr*, **50**, 187-190.
10. CHEE, K.H., ZHANG, K.M. AND DARMONO, T.W. (1986) The Occurrence of Eight Races of *Microcyclus ulei* on *Hevea* Rubber in Bahia, Brazil. *Trans. Br. mycol. Soc.*, **87**, 15-21.
11. DARMONO, T.W. AND CHEE, K.H. (1985) Reaction of *Hevea* Clones to Races of *Microcyclus ulei* in Brazil. *J. Rubb. Res. Inst. Malaysia*, **33(1)**, 1-8.
12. KUC, J. (1977) Plant Protection by the Activation of Latent Mechanism for Resistance. *Neth. Pl. Pathol.*, **83**, 463-471.
13. HASHIM, I., CHEE, K.H. AND DUNCAN, E.J. (1978) Reactions of *Hevea* Leaves to Infection with *Microcyclus ulei*. *J. Rubb. Res. Inst. Malaysia*, **26(2)**, 67-75.
14. CHEE, K.H., DARMONO, T.W., ZHANG, K.M. AND LIEBEREI, R. (1985) Leaf Development and Spore Production and Germination after Infection of *Hevea* leaves by *Microcyclus ulei*. *J. Rubb. Res. Inst. Malaysia*, **33(3)**, 124-137.
15. JOHNSON, R. AND ALLEN, D.J. (1975) Induced Resistance to Rust Diseases and its Possible Role in the Resistance of Multiline Varieties. *Ann. appl. Biol.*, **80**, 359-364.
16. LEONARD, K.J. (1969) Factors affecting Rates of Stem Rust Increase in Mixed Plantings of Susceptible and Resistant Oat Varieties. *Phytopathology*, **95**, 1845-1850.
17. STITCH, C. AND WHITTINGTON, W.J. (1983) The Effect of Variety Mixtures on the Development of Swede Powdery Mildew. *Pl. Pathol.*, **32**, 41-46.
18. CHIN, K.M. AND WOLFE, M.S. (1984) The Spread of *Erisiphe graminis* f. sp. hordei in Mixture of Barley Varieties. *Pl. Pathol.*, **33**, 89-100.