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Distinguishing Hevea *Clones Resistant to Races of* Microcyclus ulei *by Means of Leaf Diffusate*

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Leaves of Hevea *clones were induced to produce diffusates by inoculating them with spore suspension of* Colletotrichum gloeosporioides *and* Phytophthora *sp. The diffusates subsequently exerted different degrees of inhibition of conidial germination of four physiologic races of* Microcyclus ulei, *enabling the resistance or susceptibility to South American leaf blight of the test clones to be determined.*

As part of a continuing programme of developing effective preventive methods against South American leaf blight [*Microcyclus ulei* (P. Henn.) Arx.] of *Hevea* rubber, clones bred in the Far East (where the disease is absent) are sent to South America for evaluation against SALB. Malaysia and Sri Lanka formerly sent budwood to the SALB Unit of the Rubber Research Institute of Malaysia in Trinidad for this purpose. However, the transfer of the Unit to Brazil has necessitated a reduction in disease screening. This paper describes a simple laboratory method to distinguish susceptible clones from resistant clones, so that only the latter need to be sent to Brazil for field trials.

MATERIALS AND METHODS

Ten- to twelve-day-old leaves of clones with a known reaction to races 4,6,7 and 8 of *M. ulei*[1] *(Table 1)* were collected from the EMBRAPA Station in Una, Bahia. Ten leaflets of each clone were inoculated with a spore suspension of *Colletotrichum gloeosporioides* or *Phytophthora* sp. immediately after collection. Spores of *C. gloeosporioides* were scraped from a one-week-old culture on PDA and suspended in water. A zoospore suspension of *Phytophthora* sp. was obtained by flooding a one-week-old culture on PDA with water and chilling it for 15 min. The number of spores in the suspensions was not determined. A dropper was used to place ten 2-mm^3 drops of spore suspension on the abaxial surfaces of leaves of each test clone. The drops or diffusates containing some original spore inoculum were collected after 18 h to 24 h

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incubation in a humid chamber. 'Diffusates' were also obtained by placing drops of water on leaves and pricking the leaves under water, followed by incubation for 18 h to 24 h. The diffusates thus obtained were either used immediately or left to dry in the laboratory and resuspended after six days.

Conidia of *M. ulei* were obtained from leaf lesions of clones FX 2261, FX 3864, FX 985 and FX 2804, representing physiologic races 4, 6, 7 and 8 respectively[1], and suspended in water. The conidial suspension (2×10^5 spores per millilitre) was mixed with the diffusate in equal proportions. Drops of the mixture were placed on microscope slides and incubated in a humid chamber for 6 h before spore germination was counted. Portions of the spore suspension of *C. gloeosporioides* and *Phytophthora* sp. were left to stand for 18 h to 24 h and germination of conidia of *M. ulei* subsequently added served as control. All tests were conducted three to four times.

RESULTS

The results in *Table 1* show that when the races of *M. ulei* were tested, clones which are known to be resistant to that race exhibited greater inhibition to spore germination by both the diffusates induced by *C. gloeosporioides* or *Phytophthora* sp. Conversely, diffusates from suspectible clones were less inhibitory to the germination of conidia of *M. ulei.* In general, the inhibition of resistant clones was > 30% (mean 40.85%), whereas that of susceptible clones was < 30% (mean 14.55%). Diffusates left to dry and resuspended after six days gave the same results as diffusates tested immediately *(Table 2).* 'Diffusates' from pin-prick wounds exhibited no inhibition to spore germination, irrespective of whether the clone was resistant or susceptible. The spore inoculum and bacteria contaminants in the diffusates did not affect conidial germination. The unique morphological characteristic of the conidia of *M. ulei* allowed them to be differentiated from other fungus spores.

DISCUSSION

The experiments demonstrated that leaf diffusates induced by the ubiquitous *Hevea* leaf pathogens, *C. gloeosporioides* or *Phytophthora* sp., gave different degrees of inhibition of germination of conidia of races of *M. ulei,* depending on the clones from which the diffusates were obtained. Other leaf pathogens of *Hevea* could possibly be used as diffusate inducers as well. Indeed, the present investigation[2, 3] shows that leaf diffusate (induced by *M. ulei*) from clones resistant to this fungus inhibited spore germination to a greater extent than diffusates from susceptible clones. It is possible that the same leaf diffusates could also be used to screen clones resistant or susceptible to other leaf pathogens. The diffusate method provides a practical and fast means for pre-screening clones for SALB resistance. The diffusates prepared in South-East Asia could be air-dried in the laboratory in which they are prepared and sent to Brazil for germination tests with conidia of *M. ulei.* The advantage of this method is that it can be made in large batches, stored until needed and easily sent across international boundaries without the risk of introducing new diseases associated with living tissues. The dis-

TABLE 1. RATE OF INHIBITION OF CONIDIAL GERMINATION OF *M. ULEI* IN *HEVEA* LEAF DIFFUSATES INDUCED BY *C. GLOEOSPORIOIDES* AND *PHYTOPHTHORA* SP.

Clone	Reaction to races of *M. ulei*				Rate of inhibition to diffusate, induced by[a] *C. gloeosporioides*				*Phytopthora* sp.
	4	6	7	8	4	6	7	8	6
FX 2261	S	R	R	R	7.1	46.5	38.9	36.5	37.9
FX 3864	S	S	S	–	17.3	19.0	12.5	–	5.2
FX 3844	S	S	S	S	20.7	20.8	15.5	5.0	15.4
MDX 96	R	R	R	R	41.7	46.1	37.8	33.9	39.1
FX 2804	R	R	R	S	49.7	37.7	36.7	13.4	41.7
FX 985	R	S	S	R	50.8	22.6	11.6	55.9	20.4
IAN 710	–	S	–	–	–	15.6	–	–	9.9
FX 25	–	S	–	–	–	16.7	–	–	13.3
IAN 717	–	R	–	–	–	13.3	–	–	38.0
FX 3899	–	R	R	–	–	36.9	37.7	–	40.2

Mean of resistant clones = 40.85%
Mean of susceptible clones = 14.55%
S = Susceptible; R = Resistant
[a]Rate of inhibition of conidial germination of

$$M.\ ulei = \frac{\text{Germination of control} - \text{Germination of treatment}}{\text{Germination of control}} \times 100$$

advantage is that it cannot be used to screen a segregating population, since the bioassay does not clearly discriminate between moderately and highly resistant individuals.

For consistent results, the leaves used for diffusate preparation should be uniform in age (ten to twelve days old) and should be used immedately after collection. Poor results have been obtained when older leaves were used. Hashim *et al.*[4] observed that *M. ulei* can initiate infection on any *Hevea* clones, but in resistant clones the mycelium is stopped from spreading further within the host tissue. It seems that the first reaction of the host in response to infection of any rubber pathogens is to produce a non-specific phyto-toxin. The resistance and susceptibility of the host to a specific pathogen depends subsequently on whether the phyto-toxin is toxic or not to the pathogen.

TABLE 2. COMPARISON OF RATE OF INHIBITION OF CONIDIAL GERMINATION OF *M. ULEI* BETWEEN FRESHLY PREPARED AND AIR-DRIED DIFFUSATES

Clone	Reaction to races of *M. ulei*		Rate of inhibition by diffusates[a]			
	6	7	Fresh	6 days	Fresh	6 days
FX 25	S	–	6.7	12.7	–	–
FX 985	S	S	7.7	8.8	19.7	12.5
FX 3864	S	–	10.9	8.4	–	–
FX 3844	S	S	16.5	9.2	16.0	18.6
MDX 96	R	R	32.6	41.7	47.3	35.3
FX 2804	R	R	36.5	37.9	56.1	38.0
FX 3899	R	R	36.7	39.1	45.7	37.2
FX 2261	R	R	37.8	38.5	46.1	38.5

Mean of resistant clones = 40.31%
Mean of susceptible clones = 12.31%
S = Susceptible; R = Resistant
[a]Rate of inhibition of conidial germination of

$$M.\ ulei = \frac{\text{Germination of control} - \text{Germination of treatment}}{\text{Germination of control}} \times 100$$

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