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OCCURRENCE OF EIGHT RACES OF *MICROCYCLUS ULEI* ON *HEVEA* RUBBER IN BAHIA, BRAZIL

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Conidia of *Microcyclus ulei* from infected leaves of twelve clones of *Hevea*, including *H. brasiliensis* and its hybrids with *H. benthamiana*, were inoculated to leaf disks of 19 clones of diverse genetic background. From the results, eight physiologic races of *M. ulei* were distinguished. Races 1 and 2 have previously been recorded only from Central America whereas races 4, 5 and 6 (previously 4a, 4b and 4c) have been reported from Amazonas, Belem, and Bahia respectively. The new races 7, 8 and 9 are described, and a set of six clones (IAN 710, IAN 717, FX 2261, FX 985, FX 2804 and FX 25) is proposed for differentiating the eight races.

Microcyclus ulei (P. Henn.) v. Arx (syn. *Dothidella ulei* P. Henn.) is the cause of South American leaf blight (SALB), the most serious disease of *Hevea brasiliensis* Muell. Arg. in South and Central America. Langford (1945, 1946) first reported races of *M. ulei* on hitherto resistant clones such as F 409 and F 1619 at Santarem, in the State of Para, Brazil. Langford (1945) also found races attacking progeny of F 4542 (*H. benthamiana*) in Central America. Langdon (1963) examined isolates (but did not name the races) from Guatemala and Costa Rica and found that those from Costa Rica attacked and sporulated heavily on all clones derived from F 4542, whereas those from Guatemala did not infect clones originating from F 4542, except IAN 717. Subsequently Miller (1966) identified four races, of which race 2 was Langdon's Costa Rica isolate, while 1 occurred in Guatemala, 3 in Costa Rica and Guatemala and 4 in Belem, Para. Race 4 was separated into 4a–c in Brazil (SUDHEVEA, 1971). There are two pathogenic strains in Trinidad, a virulent strain isolated from South American clones and a non-virulent strain from Oriental clones. The former produces more spores and causes more severe infection than the latter (Chee, 1978; Liyanage & Chee, 1982). Holliday (1970) described the occurrence of race 4 in Trinidad, i.e. 4c in the Brazilian classification, now race 6; and since clones IAN 717 and FX 3925 were infected, race 2 also occurred there. Information on the race structure of *M. ulei* is incomplete, and a better understanding would help in disease control and prevention. This paper describes experiments which led to the discovery of eight races of *M. ulei* in Bahia, Brazil.

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MATERIALS AND METHODS

Preparation of inoculum

M. ulei is a biotrophic parasite, and although it can be cultured (Langford, 1945), field conidia from specific clones in the EMBRAPA Station (Agricultural Organization of Brazil) and in Fazenda Piruna in the municipal of Una were used as inoculum. Conidia were also obtained from Fazenda Tres Pancadas (previously Firestone), Fazenda Cultrosa and Fazenda Rodolfinho in the municipal of Camamu, Fazenda Inferno Verde in the municipal of Itubera, and Fazenda Dos Penteados in the municipal of Ilheus (Table 1, Fig. 1). These municipals are situated in the state of Bahia, where most of the established commercial plantations in Brazil are located.

Conidia were removed from leaf lesions with an artist's brush and suspended in distilled water for immediate use. Alternatively, they were preserved by sucking them from leaf lesions into tapered

Table 1. *Source of spore inoculum (isolates) of* M. ulei

	Municipality and locality						
	Una		Camamu			Itubera	Ilheus
Clone	EMBRAPA	Piruna	Tres Pancadas	Cultrosa	Rodolfinho	Inferno Verde	Dos Penteados
FX 2261[a]	*	*	*	*	—	*	*
FX 3899[b]	*	*	—	—	—	—	—
IAN 873[a]	*	—	—	—	—	—	—
FX 2804[b]	*	—	—	—	—	—	—
FX 3846[a]	*	—	—	—	—	—	*
FX 4163[a]	*	—	*	—	*	—	—
FX 3864[a]	*	—	*	—	—	—	*
FX 985[a]	*	—	—	—	*	*	*
FX 3925[b]	*	—	—	—	—	—	—
IAN 717[b]	*	—	—	—	—	—	—
IAN 710[a]	*	—	—	—	—	—	—
FX 4098[a]	*	—	—	—	—	—	—

[a] *H. brasiliensis*; [b] *H. brasiliensis* × *H. benthamiana*.

centrifuge tubes attached to a cyclone spore collector, stored in a desiccator (Chee, 1976*a*) and used within 4 weeks. For inoculum, the number of conidia in the suspension was approximately 2×10^5 ml (Chee, 1976*b*).

Preparation of leaf disks

Seven-day-old leaves were removed from plants in the clonal garden and placed in dry polyethylene bags for transport. If not wetted they remained in good condition for 24 h in an air-conditioned laboratory (25–28 °C).

Disks were cut from the leaves with a 15 mm diam cork borer. Ten disks of each clone were floated with their abaxial surfaces uppermost on distilled water in Petri dishes, and the conidia applied with an Atomist Atomiser (Chee, 1976*b*).

Assessment of infection

Leaf disks were incubated at 24° under continuous light (2600 lx) for 6 d to allow lesions to develop, and after another 5 d they were examined for sporulation. Lesion size was estimated with the help of a dot scale (Darmono & Chee, 1985) and sporulation by visual examination for the presence of conidia. Each clone was tested on 3 to 6 separate occasions. Degree of infection was recognized as one of five classes (Darmono & Chee, 1985), but for the present study class I was recorded as negative (R), with no reaction or indistinct lesions up to 250 μm diam and no sporulation; classes 2–5 were recorded as positive (S) with distinct necrotic lesions but different degrees of sporulation.

RESULTS

Conidia of 26 isolates originating from 12 clones of *H. brasiliensis* and its crosses with *H. benthamiana* from south Bahia were inoculated on to leaf disks of 19 clones of diverse genetic background. The results (Table 2) show that eight physiologic races of *M. ulei* can be distinguished. Race 1 was from clone IAN 873, race 2 from IAN 717, FX 3899 or FX 3925, race 4 from FX 2261, race 5 from FX 4098, race 6 from IAN 710 or FX 3864, race 7 from FX 985 or FX 4163, race 8 from FX 2804 and race 9 from FX 3864 (Table 2). For the purpose of simplification, the previously described races 4a–c (SUDHEVEA, 1971) are renumbered 4–6 respectively. All the six isolates from FX 2261 behave similarly, so clones were tested with 2 to 4 isolates (Tables 1, 2).

Using the differential clones IAN 873, IAN 710, IAN 713 and IAN 717 three (races 1, 2 and 6 (= 4 or 4c) of the present eight races agree with descriptions by Miller (1966)). Races 7, 8 and 9 are previously unknown. The six clones considered suitable for differentiating the eight races are IAN 710 (or IAN 713), IAN 717, FX 2261, FX 985 (or FX 4163), FX 2804 and FX 25 (Table 3). The progenies of *H. benthamiana* IAN 717 and FX 3925 are required to differentiate races 2 and 3, while to differentiate race 8, FX 2804 is necessary in addition to IAN 717. To differentiate races 4, 5, 6 and others, IAN 710 (or IAN 713) is another key clone. FX 25 enables races 7 and 9 to be differentiated. A complete set of differentials to distinguish the eight races must include also clones FX 2261, FX 985 and FX 4163. MDX 96 is

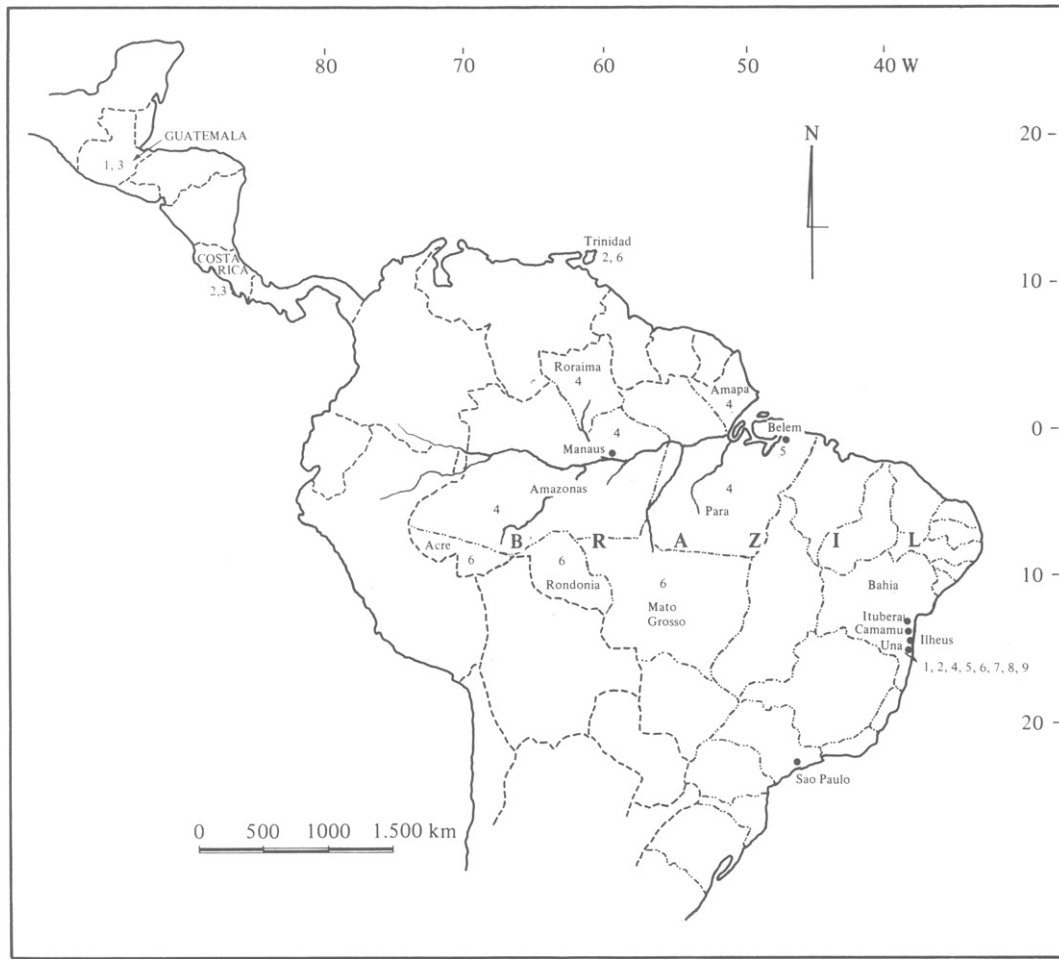

Fig. 1. Present knowledge of occurrence of races of *M. ulei* in Brazil and Central America.

resistant to all the eight races tested and is worth including for detecting new races.

Conidia were also collected from clones available in four estates (Fazenda) in Camamu and Itubera (*ca* 300 km from Una) (Table 1) and one estate in Ilheus (*ca* 60 km from Una) (Table 1) and inoculated on leaf disks of different clones from Una. The results show that races 4, 6 and 7 are present on clones FX 2261, FX 3864 and FX 4163 respectively in Fazenda Tres Pancadas. In Fazenda Cultrosa, race 4 was found among 6 rows of FX 2261 in two separate nurseries. In Fazenda Rodolfinho, FX 985 and FX 4163 were both infected by race 7. In Fazenda Inferno Verde, race 4 was found on FX 2261 and race 7 on FX 985. In Fazenda Dos Penteados, races 4, 6, 7 and 9 were found on FX 2261, FX 3864, FX 985 and FX 3846 respectively. In the EMBRAPA Station in Una, where many clones are grown, 8 races from 1, 2 and 4 to 9 were found. Table 4 and Fig. 1 summarize the occurrence of races of *M. ulei* as they are known to date in south Bahia as well as other States in Brazil and Central America.

Some preliminary conclusions can now be drawn concerning the distribution of races of *M. ulei* in south Bahia and elsewhere in Brazil.

Race 1. This is the wild type to which nearly all material bred in South America is resistant. Possible exceptions are clones with resistance from *H. brasiliensis* Ford selection FA 1717, from the Acre territory of Brazil. An example is the first-generation clone IAN 873. The distribution of

Table 2. *Interaction between* Hevea *clones and races of* M. ulei

		Race							
		1	2	4	5	6	7	8	9
		Source							
Test clone	Parentage	IAN 873	IAN 717 FX 3899 FX 3925	FX 2261	FX 4098	FX 3864 IAN 710	FX 985 FX 4163	FX 2804	FX 3846
IAN 717	PB 86 × F 4542	R	S	R	R	R	R	R	R
FX 3925	F 4542 × Avros 363	R	S	R	—	—	—	R	—
FX 3899	F 4542 × Avros 363								
FX 2804	F 4542 × Tjir 1	R	S	R	R	R	R	S	R
IAN 710	PB 86 × F 409	R	R	S	S	S	R	R	R
IAN 713	PB 86 × F 409								
IAN 873	PB 86 × FX 1717	S	R	S	R	R	R	R	R
FX 2261	F 1619 × Avros 183	R	R	S	S	R	R	R	R
FX 985	F 315 × Avros 183	R	R	R	—	R	S	R	S
FX 4163	Tjir 1 × F 170								
FX 3844	Avros 183 × B 45	R	R	R	—	R	S	R	S
FX 3846	Avros 183 × B 45								
FX 4098	PB 86 × B 74	R	R	R	S	R	S	R	—
FX 25	F 351 × Avros 49	R	R	R	—	S	R	R	S
MDF 180	Madre de Dios, *H. brasiliensis*	R	R	R	—	R	R	S	—
MDX 96	Avros 308 × M. de Dios	R	R	R	R	R	R	R	R
FX 3864	PB 86 × B 38	—	—	S	—	S	S	S	S
RRIM 614	Tjir 1 × RRIM 509	S	—	S	—	S	S	S	—
RRIM 628	Tjir 1 × RRIM 527								

R = resistant; no reaction or indistinct lesion < 250 μm. S = susceptible; distinct necrotic lesions. —, no data.

Table 3. Hevea *clones recommended for differentiating races of* M. ulei *in Bahia*

	Differential clone						
Races	IAN 717	FX 2804	IAN 710	FX 2261	FX 985	FX 25	MDX 96
1	R	R	R	R	R	R	R
2	S	S	R	R	R	R	R
4	R	R	S	S	R	R	R
5	R	R	S	S	S	—	R
6	R	R	S	R	R	S	R
7	R	R	R	R	S	R	R
8	R	S	R	R	R	R	R
9	R	R	R	R	S	S	R

—, no data.
Race 3 has not yet been found in Bahia.

the wild type is presumed to correspond with that of the disease.

Race 2. This attacks progeny with resistance from the *H. benthamiana* clone F 4542 including IAN 717, FX 3925 and FX 3810. Clones with F 409 parentage such as IAN 710 and IAN 713, and clones IAN 873, FX 25 and Madre de Dios material are resistant. The race was first reported from Costa Rica.

Race 3. This is also reported to attack clones with resistance from F 4542, notably IAN 717, but it appears to differ from race 2 in that it does not generally and seriously infect selections with resistance from this source, such as FX 3925 and FX 3810. IAN 710, FX 25 and Madre de Dios material are resistant. It has been reported from Costa Rica and Guatemala.

Race 4. The first variant to be reported occurs in

Table 4. *Geographical distribution of races of* M. ulei

Race (previous numbering in parentheses)	Present in	Clones	
		Susceptible	Resistant
1	Guatemala Brazil (Bahia)*	IAN 873	IAN 710, IAN 713, progeny of F 4542
2	Costa Rica Trinidad* Brazil (Bahia)*	Progeny of F 4542	IAN 710, IAN 713, IAN 873, FX 2261
3	Guatemala Costa Rica	IAN 717	IAN 710, IAN 713, FX 3925, FX 25
4(4a)	Brazil (Amazonas, Lower Amazonas, Bahia*)	IAN 710, IAN 713, FX 2261	Progeny of F 4542, FX 4098
5(4b)	Brazil (Belém–Bragança, Bahia*)	IAN 710, IAN 713, FX 2261, FX 4098	Progeny of F 4542
6(4c)	Brazil (Bahia, Acre, Rondonia, Mato Grosso) Trinidad*	IAN 710, IAN 713	Progeny of F 4542, FX 2261, FX 4098
7	Brazil (Bahia)*	FX 4098, FX 985	Progeny of F 4542, IAN 710, IAN 713, FX 2261, FX 25
8	Brazil (Bahia)*	FX 2804	FX 710, IAN 713, IAN 717
9	Brazil (Bahia)*	FX 3846, FX 3844, FX 25	IAN 710, IAN 713

* New information.

Brazil (Belterra) and now in Bahia, but may be absent from Central America. It breaks down resistance from *H. brasiliensis* upper Amazon selections, notably that from F 409. Clone FX 2261 is susceptible, but it does not attack FX 4098 and clones with resistance from F 4542. Race 4 was originally 4a (SUDHEVEA, 1971).

Race 5. Originally race 4b, it occurs in Belém and Bahia and attacks selections from F 4542 and clone FX 2261 and FX 4098. Selections derived from F 4542 are resistant. The difference between races 4 and 5 is that race 4 does not attack F 4098.

Race 6. This race was reported from Acre, Rondonia, Mato Grosso, possibly Trinidad and now Bahia. Selections from F 4542 are resistant; so are clones FX 2261 and FX 4098. It breaks down the resistance from F 409.

Race 7. It occurs in Bahia, and is a new race in Brazil. Selections from F 4542 and F 409, and clones FX 2261, IAN 873 and FX 25 are resistant. Clones FX 985, FX 4163 and FX 4098 are susceptible.

Race 8. Like race 7, it occurs in Bahia and is also a new race. Selections from F 4542 (excepting FX 2804) and F 409, and clones IAN 873, FX 985 and F 4163 are not attacked.

Race 9. This is a new race occurring in Bahia, also a new race from Brazil. It does not break the resistance from F 4542, including FX 2804 and F 409. Clones IAN 873 and FX 2261 are resistant. It differs from race 7 in that it attacks FX 25.

DISCUSSION

Miller (1966), in his study of race differentiation of *M. ulei*, used four categories – R, HR, MR and S – to score disease susceptibility. In the present study, although five disease classes (Darmono & Chee, 1985) were recognized, it was found practical to consider class 1 as resistant (R) and classes 2–5 as susceptible (S). Although little progress has previously been made in the study of the race structure of *M. ulei*, largely due to the difficulty of working with a biotrophic parasite, the leaf disk method originally developed for testing *Hevea* clones for susceptibility (Chee, 1976*b*) was found to be a useful technique.

Race 4 was previously recorded in Brazil (SUDHEVEA, 1971) as races 4a, 4b and 4c, which now become 4, 5 and 6. Race 4 was found in Amazonas and Lower Amazonas, race 5 in Belém-Bragança, and race 6 was more widespread, occurring in Bahia, Acre, Rondonia and Mato Grosso (Fig. 1). The present study has confirmed the presence of race 6 in Bahia, and has revealed three new races (7, 8 and 9) as well as races 1 and

2, which were originally reported from Guatemala and Costa Rica (Miller, 1966).

Insufficient data exist to relate the race structure to any pattern of host genotype. The eight races are therefore simply differentiated with reference to the six clones listed in Table 3.

The races appear to belong to two groups: Group 1 constitutes races 1, 2, 3, 7, 8 and 9, i.e. all the new races in Brazil, which do not attack progeny of F 409 (IAN 710, IAN 713). Group 2 consists of races 4–6 which attack progeny of F 409; while progeny of F 4542 (*H. benthamiana*) are immune (Table 4). In Trinidad, the fungus attacked IAN 710 and also clones with resistance from F 4542 (Liyanage & Chee, 1982), suggesting that races 4–6 as well as 2 are present.

The races in Belém, Amazonas, Rondonia, Acre and Mato Grosso are likely to be more numerous than presently recorded, should a detailed study be conducted, and they may differ from those of Bahia and the Amazonas States. This may be the reason why FX 2261 was very susceptible in Belém and much less so in Bahia (Chee & Wastie, 1980). Rogers & Peterson (1976) reported that in Granja Marathon (Goodyear estate) in Belém, FX 4098 was attacked in 1970, followed in 1972 by FX 3925 and in 1973 by FX 2261, IAN 710, IAN 713 and FX 25. Thus, more than one race must be present. Indeed, Pinheiro & Libonati (1976) noted that IAN 873 was very susceptible in Belém, suggesting, in addition to race 5, the presence of races 1 and 4. Similarly, Lins & Brito (1980) reported that FX 2261 was most susceptible in Acre, suggesting, in addition to race 6, the presence of race 4 or 5 as well. Although race 3 has not been detected in the EMBRAPA Station in Una, the possibility exists of finding it among clone IAN 717 in the nearby Fazenda Piruna. Clone IAN 717 in that plantation was attacked severely in 1982/1983 and required routine fungicide protection (Chee, 1984). The EMBRAPA Station in Una has the largest collection of clones in Bahia, so the eight races found in the station possibly represent most of those occurring in Bahia. In the present study, the conidial inoculum was from 12 clones representing 80% of the plants in the station. Other places in Brazil where there are large collections of clones include the National Research Centre of Rubber and Oil Palm (CNPSD) in Manaus, Amazonas, and the EMBRAPA Station in Belém (former IAN = Instituto Agronomico do Norte, later IPEAN = Instituto de Pesquisas e Experimentação Agropecaurias do Norte) in the State of Para. A similar study in these places should yield interesting results. Parallel with our study, a major investigation on the physiological variability of isolates of *M. ulei* was conducted by Junqueira *et al.* (1984).

In 1984 in the EMBRAPA Station in Una, race 8 (from clone FX 2804) did not make its appearance until June, when races 6 and 7 had already been prevalent for over a month, and by November, towards the end of the disease season, race 2 was consistently present on clone FX 2804, suggesting that more than one race may be present simultaneously on the same clone, or that the race changes with the season.

Table 2 shows that the same race can occur on clones of different genotypes, e.g. race 7 on FX 985 and FX 4163. Similarly, clones of similar parentage (P 10 × PB 86) can be either resistant (IAN 6475) or susceptible (IAN 6540) to different races (Darmono & Chee, 1985). Some clones are susceptible to most of the races, and some races infect more clones than others. This pattern of interaction suggest that many resistance genes of the host and many virulence genes of the pathogen are involved.

When clones such as FX 3844 and FX 4163 were inoculated with the four races to which they are susceptible, diseases of different severity resulted (Darmono & Chee, 1985), indicating that races exhibit different degrees of aggressiveness. The same authors show that the source of a race may influence its degree of infection on a given test clone.

New races of *M. ulei* are responsible for hitherto resistant clones succumbing to SALB (Simmonds, 1982). Miller's work (1966) on races of *M. ulei* suggests the presence of vertical resistance and indicates that host resistance is likely to fail due to this reason. In view of this, it is better to look for horizontal or field resistance. The possibility of building up horizontal resistance by breeding has been predicted by Simmonds (1982).

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