Evaluation of field resistance to *Microcyclus ulei* of a collection of Amazonian rubber tree (*Hevea brasiliensis*) germplasm

Vincent Le Guen¹; Dominique Garcia²; Carlos Raimundo Reis Mattos³ and André Clément-Demange⁴ ¹CIRAD, BP 701, 97387 Kourou Cedex, France; ² Plantações E. Michelin, Caixa Postal 80, CEP 78700-090, Rondonópolis, MT, Brazil; ³ Plantações Michelin da Bahia, Caixa Postal 2, CEP 45477-000, Igrapiúna, BA, Brazil; ⁴ Cirad-cp, Boulevard de la Lironde, 34398 Montpellier cedex 5, France. (* Corresponding Author. E-mail: leguen.v@kourou.cirad.fr)

ABSTRACT

South American Leaf Blight (SALB) field resistance to the fungus *Microcyclus ulei* (P. Henn.) v. Arx was observed on an *ex-situ* germplasm collection of rubber trees [*Hevea brasiliensis* (Willd. ex Adr. De Juss.) Muell.-Arg.], planted both in French Guyana and in Brazil, wich included 298 accessions from a survey in the Brazilian states of Acre, Mato Grosso and Rondônia. Two sets of observations were made in French Guyana, in early 1999 and late 2000, but only one set was made in Brazil in early 1999. Results showed a high ratio of SALB-susceptible clones among Mato Grosso origins (up to 81%), whereas clones from Acre or Rondônia were the most resistant. The resistance of the Mato Grosso clones seems to be more unstable than that found in of the Acre or Rondônia clones. This result is consistent with the data reported on genetic diversity of *H. brasiliensis*, and can explain the lack of resistance to *M. ulei* found in the Asiatic clones.

KEY WORDS: Hevea brasiliensis, Microcyclus ulei, resistance, germplasm, French Guyana, Mato Grosso.

INTRODUCTION

Though originating in the Amazon basin, rubber tree [Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell.-Arg.] cultivation in all of Latin America represents no more than 2% of worldwide natural rubber production. The occurrence in South and Central America of an endemic disease called South American Leaf Blight (SALB), which attacks the rubber tree, is the principal reason for this low production percentile. This disease, caused by an ascomycete fungus called Microcyclus ulei (P. Henn.) v. Arx, originates in the Amazon basin. Presently restricted to the American continent, it can induce severe leaf loss in susceptible clones. In case of repeated SALB attacks, the trees may suffer drastic dieback, which can lead to death.

The ascomycete fungus, first described in the beginning of twentieth century (Ule, 1905), is considered the principal cause of numerous failures in trying to establish rubber tree estates in South America, and represents for all other rubber growing countries an important potential threat, as the majority of cultivated clones in Asia and Africa are considered very susceptible to this disease.

The first genetic improvement work which aimed at producing clones with good SALB resistance and high rubber yielding began in the thirties. They consisted of identifying in the Amazon forest apparently SALB-free native trees, and trying to introduce this resistance into high-yielding cultivated clones through backcross schemes (Peralta et al., 1990). The results did not match with the substantial resources invested in this research, certainly due to lack of knowledge of modern experimental genetics and phytopathology theories.

Our purpose in the present study was to evaluate field resistance of various non-selected *Hevea* clones, submitted to a natural *Microcyclus ulei* inoculum pressure in two different locations.

MATERIAL AND METHODS

Vegetal material

The vegetal material was collected during an international survey which took place in 1981 under the International Rubber Research and Development Board (IRRDB) authority in the Brazilian states of Acre (Lins et al., 1981), Rondônia (Gonçalves, 1981) and Mato Grosso (Paiva, 1981). The aim of this survey was to collect a diversificated rubber tree germplasm, in order to provide genetic variability for rubber tree breeding programs all around the world. The vegetal material collected during this survey was divided among three countries: Brazil, Ivory Coast and Malaysia, each one being provided with a germplasm

sample representative of the studied area.

In Ivory Coast, 2,847 accessions which originated from this survey were submitted to agronomic evaluation (Clément-Demange et al., 1998), as well as genetic diversity studies, using molecular markers (Besse et al., 1994). These studies allow the setting up of a working collection list, restricted to 298 accessions which were introduced in French Guyana in 1995 and planted in a living collection, with 10 trees per clone. From this collection, 49 clones were introduced to Brazil and planted in a clonal collection (12 trees per clone), in an rubber tree industrial estate located south of Mato Grosso State.

The number of observed clones on these two sites, as well as their distribution according to geographical origin is represented in Table 1.

Fungus inoculum

In both cases, only natural infestation occurred on the observed trees. In French Guyana, the germplasm collection was planted among other clones of a larger collection, in the Combi Experimental Station, district of Sinnamary. This station is located close to the native Amazonian forest, in which some *Hevea guianensis* trees are naturally infected by *Microcyclus ulei*. The first rubber trees were planted in the experimental station in 1982, and infected a few years later by this primary native inoculum. Afterwards, this inoculum was self-maintained, and probably diversificated on the numerous cultivated or primary clones existing in the experimental station. All the observed material is therefore submitted to a high level of parasitory pressure.

The Mato Grosso plantation, though established outside of natural rubber tree distribution, is also submitted to parasitory pressure from *M. ulei*. During the rainy season (December to April), the conidiospores produced by more than 8,500 ha of cultivated susceptible clones surrounding the observed trees provide a huge parasitory environment.

Variability of *M. ulei* strains in both locations has been studied (Mattos et al., in press) and appeared to be very large. Thus, in both locations, observed trees are naturally infected by a mixed pathogen population.

Table 1. Characteristics of test sites, description of study material and ages of measurements.

Site			Combi 05°17' N 52°56'W		Itiquira 17°21'S 54°44'W	
		Lat				
		Long				
		Annual rain fall (mm)	2,879		1,708	
		Inoculum source	Native H. guianensis		Various cultivated	
			trees a	and other clones	clones	planted on a
			in collection		8,500 ha estate	
Plantation	date		1995		1997	
Observation date			Feb. 1999 to Oct. 2000		Dec. 1998 to Feb. 1999	
Material						
State	District	Abbreviation	Numb	er of accessions	Number of accessions	
Acre	Brasileia	AC/B	17		2	
	Feijo	AC/F	7		1	
	Feijo	AC/FA	5		1	
	Feijo	AC/FB	3		1	
		AC/I	2		1	
	Sena Madureira	AC/S	31		4	
	Tarauaca	AC/T	7		0	
	Xapuri	AC/X	6		1	
Total		•	78		11	
Mato Grosso	Aracatuba	MT/A	3		0	
	Rio Juruena	MT/C	70		9	
		MT/I	2		0	
	Itauba	MT/IT	45		4	
	Vila Bela	MT/VB	1		0	
Total			121		13	
Rondônia	Ariquemes	RO/A	11		4	
	Calama	RO/C	22		6	
	Costa Marques	RO/CM	18		2	
		RO/I	5		0	
	Jaru	RO/J	4		4	
	Jiparana	RO/JP	21		6	
	Ouro Preto	RO/OP	6		1	
	Pimenta Bueno	RO/PB	12		2	
Total		·	99		25	

Observation method

The symptoms which can be observed following a *M. ulei* infection are dependent upon the clonal resistance level and the foliar stage.

Colonization of the foliar tissues by the fungus can only occur during the young foliar stage, named B stage (Hallé and Martin, 1968). If the infection is rapidly stopped by the host's tissues, only chlorotic or necrotic lesions are observed, generally of small size, and without spore production. In this case, it is assumed that the infection process was unsuccessful, and the clone is considered as a resistant one, in the agronomic sense of the word.

If the infection process cannot be stopped at a early stage, the fungus develops within host's tissues and gives rise to sporulating lesions with conidial production. This stage can only be detected during a short period of foliar development. Beyond this stage, the maturation of lesion and foliar tissues does not allow further detection with any certainty as to whether conidial production did or did not occur.

Finally, if the fungus meets favourable conditions for growth and continues its biological cycle, one may observe on the upper part of leaflets black stromatas made by the accumulation of peritheces, in which ascospores are produced. These stromatas are easy to detect and remain on the leaves until their natural fall. The presence of stromatas on an old leaf thus indicates a successful infection which has evolved into sporulating lesions of asexual type (conidial production), and then continued its biological cycle until sexual reproduction (ascospores).

Two criteria of observation were defined:

- a) Infection on young, not yet mature stages, denominated as C stage (Hallé and Martin, 1968), was annotated by the reaction type (RT) on a 0 to 6 scale adapted from Junqueira et al. (1986):
- 0: No visible symptoms; 1: Necrotical lesion (without conidial production); 2: Chlorotical lesion (without conidial production); 3: Weakly sporulating lesion; 4: Lesion with heterogeneous sporulation; 5: Lesion with abundant and homogeneous sporulation on all its area; 6 = Lesion with abundant and homogeneous sporulation on the upper part and on the lower part of the leaf.

A reaction type of 1 or 2, i.e. without sporulating lesion, corresponds to clones called resistant or highly resistant by Junqueira et al.(1988). Actually, if such a clone does not suffer a reduction of photosynthetic capacity of the leaves, nor participate in infestation propagation, then no epidemic situation is likely to naturally occur on it.

b) Infection on mature foliar stages (D stage), was registered by the abundance of stromatas (STR), in a 0 (no stromatas) to 3 (stromatas very abundant on majority of leaves) scale.

All living trees of each clone were observed, but for each criteria only one value was given to each clone and per observation date. This value corresponds to the maximal one registered for all observed trees.

In Combi, the observations were gathered in two sets: the first one from September 1998 to February 1999 (four observation dates) and the second one in October 2000. No fungicide treatment was done on the studied clones from June 1999 to October 2000. In Itiquira, only one set of observations was performed (from December 1998 to February 1999).

RESULTS

In Combi, for both observation sets, the results are shown according to geographical origin of clones (Table 2). The number of susceptible clones for each origin was registered according to three criteria:

- a) For reaction type (RT), number of clones with value superior to 2, i.e. clones with sporulating lesions;
- b) For stromatas (STR), a value superior to 0, i.e. stromatas detected even in small quantities;
- c) Globally diseased clones, that is RT>2 or STR>0.

A great difference can be observed in Table 2 between clones of the three origins. Whatever criteria are considered (reaction type, or stromatas), the Mato Grosso origin shows the greater number of diseased clones, up to 59% in February 1999, and 81% in October 2000.

The infestation evolution between February 1999 and October 2000 demonstrates an increase of diseased clones: from 107 to 138 clones on a total of 298, which represents a 10% increase. However, this rise is unequally distributed between origins, with more than 22% for Mato Grosso clones (from 59 to 81%), a slight evolution from 19% to 26% for Rondônia origin, and a stagnation at 20% level for Acre origin.

Considering only heavily affected clones during the second set of observations, i.e. clones with high conidial sporulation (RT 5 and 6), or high stromatas production (STR 2 and 3), the distinction between origins becomes even more clear (Table 3): one part of Mato Grosso origin 62% of heavily affected clones, and the other part of Acre and Rondônia origins with 13% strongly affected clones. A more detailed

Table 2. Number of diseased clones according to origin (Combi site).

Criteria			RT>2		STR>0	Total disea	sed clones*	Total clone
Obs. period		1st set	2nd set	1st set	2nd set	1st set	2nd set	number
Acre	Nb	13	11	14	13	17	14	78
	%	16.7	14.1	17.9	16.7	21.8	17.9	
Mato Grosso	Nb	47	67	65	98	71	98	121
	%	38.8	55.4	53.7	81.0	58.7	81.0	
Rondônia	Nb	11	16	14	24	19	26	99
	%	11.1	16.2	14.1	24.2	19.2	26.3	

^{*} total diseased clones = number of clones with RT>2 or STR>0.

Table 3. Number of severely attacked clones according to origin Combi site, 2nd set of observations (2000).

		Crite	eria	Total attacked	%attacked	Total clone
State	District	RT>4	STR>1	clones	clones	number
Acre	AC/B	1	0	1	5.9	17
	AC/F	1	1	1	14.3	7
	AC/FA	1	1	1	20.0	5
	AC/FB	0	0	0	0.0	3
	AC/I	1	2	2	100.0	2
	AC/S	3	4	4	12.9	31
	AC/T	0	0	0	0.0	7
	AC/X	1	1	1	16.7	6
Total		8	9	10	12.8	78
Mato Grosso	MT/A	2	2	2	66.7	3
	MT/C	17	38	38	54.3	70
	MI/I	1	2	2	100.0	2
	MI/IT	27	31	33	73.3	45
	MT/VB	0	0	0	0.0	1
Total		47	73	75	62.0	121
Rondônia	RO/A	0	0	0	0.0	11
	RO/C	4	4	4	18.2	22
	RO/CM	1	1	1	5.6	18
	RO/I	0	0	0	0.0	5
	RO/J	2	2	2	50.0	4
	RO/JP	0	0	0	0.0	21
	RO/OP	0	1	1	16.7	6
	RO/PB	3	5	5	41.7	12
Total		10	13	13	13.1	99

analysis on districts represented with more than 10 clones shows:

- a) Global high susceptibility for Mato Grosso districts (54% for MT/A and 73% for MT/IT);
- b) Heterogeneity for Rondônia districts: from 0% of severely affected clones (RO/A, RO/JP) to 42% (RO/
- PB) with intermediate situations (RO/CM, RO/C);
- c) Good homogeneity of resistant clones ratio in Acre (6% for AC/B, 13% for AC/S).

These results are consistent with observations made in Brazil (Table 4). The same clones observed in Combi and in Itiquira get similar classification, with a shift toward increased susceptibility: clones completely resistant in Combi are generally registered as partially resistant in Itiquira, and those partially resistant in Combi are considered as highly susceptible in Itiquira. Only four clones (two from Acre and two from Rondônia) are registered as completely resistant in Itiquira. Mato Grosso is the most susceptible origin, with a 100% of highly susceptible clones.

DISCUSSION

Few works are available in the literature which study rubber tree resistance to M. ulei under natural infestation conditions and with such a large number of clones (either from spontaneous or cultivated origin), and length of observation. In Trinidad, Chee (1976) studied 188 clones (Brazilian and Asiatic cultivated clones) under natural infestation conditions, as well as under controlled inoculation conditions. Results on resistance under natural infestation conditions for 106 Brazilian cultivated clones in the state of Bahia are presented by Darmono and Chee (1985). Bos and MacIndoe (1965) mentioned the breeding work conducted by the Firestone company in Guatemala, on 7,542 genotypes coming from crosses between Brazilian and Asiatic clones. Gonçalves (1968) has tested eight well-known and resistant Brazilian clones in five different locations, and stated that it is generally in the Amazonas and Pará states where the more severe attacks occurred. The most similar work to ours was done by Paiva et al. (1985) in which the resistance of 36 clones from the 1981 IRRDB¹ survey (from six districts in Acre, two in Mato Grosso and six in Rondônia) was evaluated under natural conditions in Manaus. These last authors reached the conclusion that all clones without exception are highly susceptible, whatever their origin. In the Combi site, a previous experimentation carried out on 31 different clones during three successive years (Rivano, 1992) showed that seven clones (all Brazilian cultivated ones) were totally resistant; some sensitive clones evolved from moderately to highly sensitive between the first and the third year of observation.

In contrast to our predecessor's works, the interest of the present study was to follow the disease's evolution over a two year period and on a more significant number of primary unselected clones, thus enabling the formulation of a hypothesis regarding resistance determinism and genetic structure.

Resistance determinism

The first attempts to characterize the various SALB resistance types in rubber trees (Chee and Wastie, 1980) made distinctions between major gene hypersensitivity reaction, which was assumed to be unstable, and horizontal resistance, which may have expressed itself in restricted lesions. By testing 33 Hevea clones against 16 geographical strains of M. ulei in controlled conditions, Junqueira et al. (1988) differentiated the clones according to presence of complete resistance against some races and the level of incomplete resistance to other races of M. ulei, assuming that complete resistance is a vertical one, and incomplete resistance is a horizontal one, by reference to Vanderplank's works (1982). It was thus implicit that complete or vertical resistance would be a mono (or oligogenic) and race-specific, whereas horizontal or incomplete resistance would be polygenic and non race-specific. However, Young (1996) states that simply because a resistant trait is polygenic does not indicate whether or not the underlying genes are race-specific. More recent works

Table 4. Comparison between	Itiquira and Combi	observations according	to clonal origin ¹⁷ .
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	Site of		Number of clones			
Material origin	observation	CR	PR	HS	Total	
Acre	Itiquira	2	4	5	11	
	Combi	6	0	5	11	
Mato Grosso	Itiquira	0	0	13	13	
	Combi	0	3	9	12	
Rondônia	Itiquira	2	15	8	25	
	Combi	16	4	5	25	

 $^{^{1/}}$ CR: Completely resistant = TR<3 and STR = 0; PR: Partially resistant = TR<5 and STR<2 and HS: Highly susceptible = TR>4 or STR>1.

(Lespinasse et al., 2000) on molecular mapping of Quantitative Trait Loci of resistance to *M. ulei* have shown that complete resistance could rely on a four-to-five genes. In other words, it does not seem more obvious in the *Hevea/Microcyclus ulei* pathosystem that a complete resistance is automatically monogenic, unstable and race-specific, and that partial or incomplete resistance is polygenic, stable and non race-specific.

In our study, it was rather difficult to try to infer the nature of resistance control because of a lack of knowledge of the natural inoculum population which has effectively infected the observed clones.

Nevertheless, global results were very consistent in Combi between both observation sets, and also between the Combi and Itiquira data. They clearly show that the percentage of susceptible Mato Grosso clones is much higher than for Acre or Rondônia clones. This indicates a higher frequency of resistance, either complete or incomplete, among Acre or Rondônia origins than among Mato Grosso origin.

It is also worthwhile to notice that the ratio of Mato Grosso clones newly attacked between early 1999 and late 2000 is higher than those of either Acre or Rondônia. Differences of resistance from one year to another are quite frequent in natural infestation conditions, and generally reflect variations of climate or other environmental factors. In that way, one can infer that resistance of Acre or Rondônia clones is more stable than resistance of Mato Grosso clones.

Genetic structure

Works on genetic diversity among wild and cultivated populations of Hevea brasiliensis made by Besse et al (1994) have clearly shown that genetic structure of populations is very consistent with their geographical localization. The different marker types used (isozymes, RFLP, ribosomic DNA) allow the differentiation of accessions from Acre, Rondônia or Mato Grosso, highlighting a variability which matches with hydrographic basins. In particular, Asiatic clones of the Wickham group are genetically very close to Mato Grosso clones, and furthermore have a geographical origin just downstream in the same river basin, the Tapajós river, which is an Amazon tributary. An other interesting fact is that according to this study, the Vila Bela district (Mato Grosso) is genetically closer to other Rondônia origins, whereas the Pimenta Bueno district (Rondônia) is related to Mato Grosso clones (Seguin et al., 1999). This result is consistent with the fact that in our study only a single clone coming from Vila Bela is resistant, while a majority of Rondônia clones are resistant. It is also consistent with the high ratio of very susceptible Pimenta Bueno clones, as it is the case for the Mato Grosso clones (Table 3).

This genetic proximity between Wickham and Mato Grosso origins, and the fact that more than 80% of Mato Grosso clones were considered susceptible or highly susceptible in October 2000, provide a simple explanation to the lack of resistance sources – even weak ones – among Asiatic cultivated clones. It seems very likely that the majority, if not totality of Wickham prospected material in 1876 in Santarém, near the Tapajós river, and which became the origin of all Asiatic cultivated rubber tree clones (Serier, 1993), was made of seeds proceeding from trees without any kind of durable resistance to SALB. There is thus no need of invoking any genetic dilution of eventual resistance genes under a SALB-free selection pressure to explain the extreme susceptibility of Asiatic clones to M. ulei.

On the other hand, the fairly good SALB resistance of clones originated from tributaries of the upper part of Amazon basin has been a well established fact for years (Chee and Holliday, 1986). Other populations may certainly be more systematically investigated in order to identify new durable resistance sources. For instance, some individuals which originated from a survey made by scientists of Firestone company in Madre de Dios region (Peruvian Amazon forest) appear to be genetically close to Rondônia clones (Seguin et al., 1999) and have exhibited in experimental designs a worthwhile and remarkably stable resistance, which could be transmitted to their progenies by crossing (Mattos, personal communication).

CONCLUSIONS

This study clearly shows an important difference of resistance to *M. ulei* between Mato Grosso clones and Acre and Rondônia clones. The ratio of susceptible clones is much higher among Mato Grosso origins than among Rondônia and Acre origins. Furthermore, the resistance in Mato Grosso clones seems to be unstable, contrary to the Acre and Rondônia clones, which remained stable from one year to the next. This fact suggests important differences in resistance mechanisms between both cases, but does not permit any hypothesis due to the lack of information on the composition of the

parasitic population. However, when one considers that only a few number of Mato Grosso clones have a real and stable resistance, and the genetic proximity between Mato Grosso and Wickham clones, it becomes easy to explain the total absence of resistance to *Microcyclus* in all Asiatic high-yielding clones.

RESUMO

Avaliação de campo da resistência de clones de seringueira da Amazonia ao fungo *Microcyclus ulei*

Uma coleção de 298 clones de seringueira [Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell. Arg.] oriundos dos estados de Acre, Mato Grosso e Rondônia, plantada na Guiana Francesa e no Brasil, foi avaliada pela sua resistência ao fungo Microcyclus ulei (P. Henn.) v. Arx, em condições naturais de infestação. Duas séries de observações foram realizadas na Guiana Francesa, a primeira no início de 1999 e a segunda no final de 2000, e apenas uma série de observações no Brasil, início de 1999. Todos os resultados obtidos demostram uma quantidade elevada de clones sensíveis dentro dos clones do Mato Grosso (até 81%), enquanto clones de Acre ou de Rondônia são em maioria resistentes. A resistência dos clones do Mato Grosso parece ser menos durável do que a dos clones do Acre ou de Rondônia. Esses resultados estão de acordo com aqueles já conhecidos sobre a variabilidade genêtica de H. brasiliensis, e podem fornecer uma explicação à ausência completa de resistência ao M. ulei nos clones asiáticos cultivados.

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