ECOPHYSIOLOGICAL VARIABILITY OF *MICROCYCLUS ULEI*, CAUSAL AGENT OF RUBBER TREE LEAF BLIGHT

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ABSTRACT

The minimum period of leaf wetness required to cause infection and the effect of temperature on the infection, incubation period, generation period, size of lesion and sporulation of six *Microcyclus ulei* isolates, from different regions of Brazil, were evaluated. At 24°C, the *M. ulei* isolate from Manicoré-AM required only 3 hr of leaf wetness for infection while both the isolates from Manaus-AM and Viana-ES required 4 hr of wetness. On the other hand, the isolates from Ituberá-BA and Registro-SP required 8 hr for infection. Concerning temperature effect on the infection, all isolates were similar, except the Viana isolate which infected and produced conidia at 16°C. These findings indicate the existence of ecotypes or ecological races of *M.ulei* able to cause disease under climatic conditions considered at present unfavorable for development of rubber tree leaf blight.

Key-words: Rubber tree, *Hevea* spp., Rubber tree leaf blight, *Microcyclus ulei*.

RESUMO

Variabilidade ecofisiológica de *Microcyclus ulei*, agente causal do mal-das-folhas da seringueira.

Determinaram-se o período mínimo de molhamento foliar (MF) necessário para o estabelecimento da infecção e os efeitos da temperatura na infecção,nos períodos de incubação e de geração, na esporulação e no tamanho das lesões incitadas por seis isolados de *Microcyclus ulei*, procedentes de diferentes locais. À 24°C, o isolado de Manicoré-AM infectou com apenas 3 horas de MF; os isolados de Manaus-AM e o de Viana-ES, com 4 horas; e o de Ituberá-BA e o de Registro-SP, com 8 horas de MF. As respostas dos isolados foram semelhantes em todas as temperaturas estudadas, exceto o isolado de Viana-ES que, a 16°C, foi mais agressivo que os demais, chegando a esporular. A variação no MF apresentada pelos isolados, a esporulação do isolado de Viana-ES a 16°C e a baixa exigência do isolado de Manicoré-AM em MF, evidenciam a existência de ecótipos ou raças ecológicas de *M.ulei* adaptadas à regiões com clima considerado adverso a esse patógeno.

INTRODUCTION

The ascomycetes *Microcyclus ulei* (P. Henn.) v. Arx, the causal agent of rubber tree leaf blight, is present in all the regions in Latin America where rubber tree is established. The presence of this pathogen in rubber plantation, located in regions where the climatic conditions are unfavorable to the epidemic development of the disease, will be able to contribute for the arising of *M.ulei*

ecologic races or ecotypes adapted in these regions. The organism populations which are more adapted in distincts ecologic environment were defined by Hill & Nelson (1976) as ecologic races.

The existence of ecologic races among plant pathogenic organism has been suggested by Schnathorst *et alii* (1958), Line & Bugbee (1964), Warren (1975), Marchetti *et alii* (1976), Hill & Nelson (1976), Cowling et *alii* (1981) and Bonde *et alii* (1985).

The high physiological variability of *M. ulei* has been confirmed by Langdon (1965), Miller (1966), Chee *et alii* (1986) and Junqueira et alii (1986). Gasparotto *et alii* (1989b, 1991) suggested a possible existence of an ecotype or ecological race of *M. ulei*, better adapted in adverses climatic condition. These authors have observed that, during the cold period in the region of Ponte Nova-MG, both the severity of rubber tree leaf blight and the sporulation of *M. ulei* were very low while in Viana-ES, both the disease severity and the sporulation of the pathogen were high.

Most of the publications on environmental effect on plant pathogens variability, has considered only the effect of the temperature. However, several authors (Rands, 1924; Langford, 1945; Kajornchaiyakul *et alii*, 1984; Gasparotto *et alii*, 1989a) have related differences among M. ulei isolates to the requirement in wetness of leaves for spores germination and infection.

The objective of this study is to demonstrate the existence of ecophysiological variability among *M.ulei* isolates from differents regions of Brazil, in relation to leaf wetness and temperature required for infection and sporulation.

MATERIAL AND METHODS

The *M. ulei* isolates and its respective origins, groups of physiological specialization (Junqueira *et alii*, 1986), and the rubber tree clones utilized in this study are presented in Table 1. The *M. ulei* isolates inoculated were specific to each rubber clone, according to its pathogenic specialization.

Rubber clones, were cultivated in black polyethylene bags, containing 10 kg of substrate, and were grown under local environmental conditions at the Centro de Pesquisa Agroflorestal da Amazônia Ocidental (CPAA), Manaus, Amazonas State, Brazil. The inoculations were done on the abaxial leaf surface of developmental stage B1/B2 (Hallé *et alii*, 1978), using a Paashe air brush model H3, Chicago USA, according to Junqueira *et alii* (1988). The experiments were done using 5 leaflets per clone/pathogen combination. The inoculation were carried out with 2 x 10^5 conidia/ml.

In spite of *M. ulei* produces conidia in culture media (Junqueira *et alii*, 1984), the isolates present different sporulation rates. This fact makes difficult for obtaining larger amount of inoculum of all *M.ulei* isolates at the same time. Thus, the conidia of the different *M. ulei* isolates were produced and maintained on living leaves of specific rubber clones growing in the greenhouse.

1. Effect of temperature and leaf wetness on the initial process of infection by different isolates of *Microcyclus ulei*.

The rubber clones were inoculated with specific *M. ulei* isolate and kept in moist chambers at 16, 20 and 24°C for 3, 4, 6, 8,10 and 12 hr periods of leaf wetness. In each combination of temperature x wetness period, 5 leaflets per leaf flush, were inoculated. After each wetness period, the plants were transferred to an air-conditioned room to evaporate the water on the leaf sufarce. After the complete evaporation of the leaf surface the plants were kept in a growth chamber until completing 12 hr. Subsequently, the plants were maintained at 24°C inside a room with 85 to 90% RH and 12 hr of day light. The number of lesion per 9 cm^2 of leaf surface was evaluated after 15 days of pathogen inoculation.

2. Effect of temperature on the initial infection process by different *Microcyclus ulei* isolates and its consequence on the post-infection phase of the disease development.

After the inoculation of each rubber clone with a specific *M.ulei* isolate, the plants were maintained under fluorescent light in a incubation chamber for 12 hr at 16, 20, 24 and 28°C and 90% RH for 24 hr. Afterward, they

TABLE 1 - Origins of *Microcyclus ulei* isolates utilized.

Isolates[1]	Origin		Group	Inoculated clones
	Municipality	State		
VI	Viana	Espiríto Santo	I	IAN 717
IT	Ituberá	Bahia	I	IAN 717
MI	Manicoré	Amazonas	IV	CNS AM 7665
MA-1	Manaus	Amazonas	II	Fx 4098
MA-2	Manaus	Amazonas	I	IAN 717
RG	Registro	São Paulo	II	IAN 873

[1] Isolates: VI= Viana-ES, IT= Ituberá-BA, MI= Manicoré-AM, MA= Manaus-AM and RG= Registro-SP

were transferred to another chamber where they were maintained for 12 hr light periods daily at 24°C and 90% RH for 15 days.

Evaluations were done by determining the incubation period (time from inoculation until the appearance of visible lesions) and the generation period of pathogen (time from inoculation until the production of conidia). The number of lesions per 9 cm^2 leaf area, size of lesions and spore production were evaluated after 15 days of the inoculation using the following diagramatical scale proposed by Junqueira *et alii* (1988):

type 0 - Clorotic lesions (fleck) less than 1 mm diameter with no sporulation

type 1 - Necrotic lesions less than 1 mm diameter with no sporulation

type 2 - Necrotic lesions with 1 to 2 mm diameter with no sporulation

type 3A - Necrotic lesions more than 2 mm diameter with no sporulation

type 3B - Non necrotic lesions with no sporulation

type 4A - Lesions less than 2 mm diameter with some sporulation occurring only at the lesion border region.

type 4B - Lesions more than 2 mm diameter with some sporulation occurring only at the lesion border region.

type 5A - Lesions less than 2 mm diameter with partial sporulation (low number of spores) occurring over the entire lesion area.

type 5B - Lesions more than 2 mm diameter with partial sporulation (low number of spores) occurring over the entire lesion area.

type 6 - Lesions less than 1,5 mm diameter with high spores production on the lesions, but only on the leaf surface.

type 7 - Lesions with 1,5 to 2,5 mm diameter with high spore production on the lesions, but only on lower leaf surface.

type 8 - Lesions with 1,5 to 2,5 mm diameter with very high spore production on lower leaf surface and partial sporulation occurring on the upper leaf surface.

type 9 - Lesions more than 2,5 mm diameter with very high spore production only on lower leaf surface.

type 10 - Lesions more than 2,5 mm diameter with very high spore production on both leaf surface.

3. Effect of temperature on post-infection phase of different isolates of *Microcyclus ulei*.

After the inoculation of each rubber clone with a specific *M.ulei* isolate, the plants were kept in growth chamber under 12 hr artificial light, at 24°C, and 90% RH during 24 hr. Then, the clones were transferred to other growth chambers, where they were maintained under 12 hr light period and 90% RH at 16, 20, 24 and 28°C for 18 days.

The evaluation was done by determining the incubation period, generation period, lesion size and sporulation according to Junqueira *et alii* (1988).

4. Effect of temperature variation on the post-infection phase of different isolates of *Microcyclus ulei*.

The objective of this study, was to evaluate the effect of the variation of temperatures on the post-infection phase of *M. ulei* isolates, in young leaves of different rubber clones. The rubber clones inoculated with each specific *M. ulei* isolates were kept in moist chambers under 12 hr light period, at 24°C, 90% RH for 24 hr. Subsequently these plants were submitted to the following temperature variation systems:

a) Plants were kept daily at alternated temperature periods of 16°C for 20 hr followed by 24°C for 4 hr;

b) Plants were kept daily at alternated temperature periods of 16°C for 16 hr followed by 24°C for 8 hr;

c) Plants were kept daily at alternated temperature of 20°C for 20 hr followed by 24°C for 4 hr;

d) Plants were kept daily at alternated temperature of 20°C for 16 hr followed by 24°C for 8 hr.

The evaluation was done by determining the incubation period and generation period of *M. ulei*. After 15 days of inoculation, the lesion size and sporulation were determined according to a diagramatical scale proposed by Junqueira *et alii* (1988).

RESULTS

1. Effect of temperature and leaf wetness period on the initial process of infection by different isolates of *Microcyclus ulei*.

The number of lesions per 9 cm^2 of leaf area varied considerably with the leaf wetness period and among the *M. ulei* isolates (Fig. 1 and Table 2). The number of lesions increased with the increase of the leaf wetness period. At 16°C, the isolate of *M. ulei* Manaus-1 required 6 hr of wetness to cause infection whereas the other isolates required at least 8 hr except the isolate of Ituberá that needed 10 hr of leaf wetness. At 20°C, only the Manaus isolates required 6 hr of wetness to cause infection whereas the others required at least 8 hr. At 24°C, the Manicoré and Viana isolates of M. ulei required respectively, only 3 and 4 hr of leaf wetness to cause infection whereas the Manaus isolates required 6 hr and the other isolates required 8 hr.

2. Effect of temperature on the initial infection process by different *Microcyclus ulei* isolates and its consequence on the post-infection phase of the disease development.

The numbers of lesions per 9 cm^2 of leaf area were not altered when the inoculated plants were kept in moist chambers at 16, 20, 24 and 28°C, during 24 hr, and immediately transferred to other moist chamber at 24°C

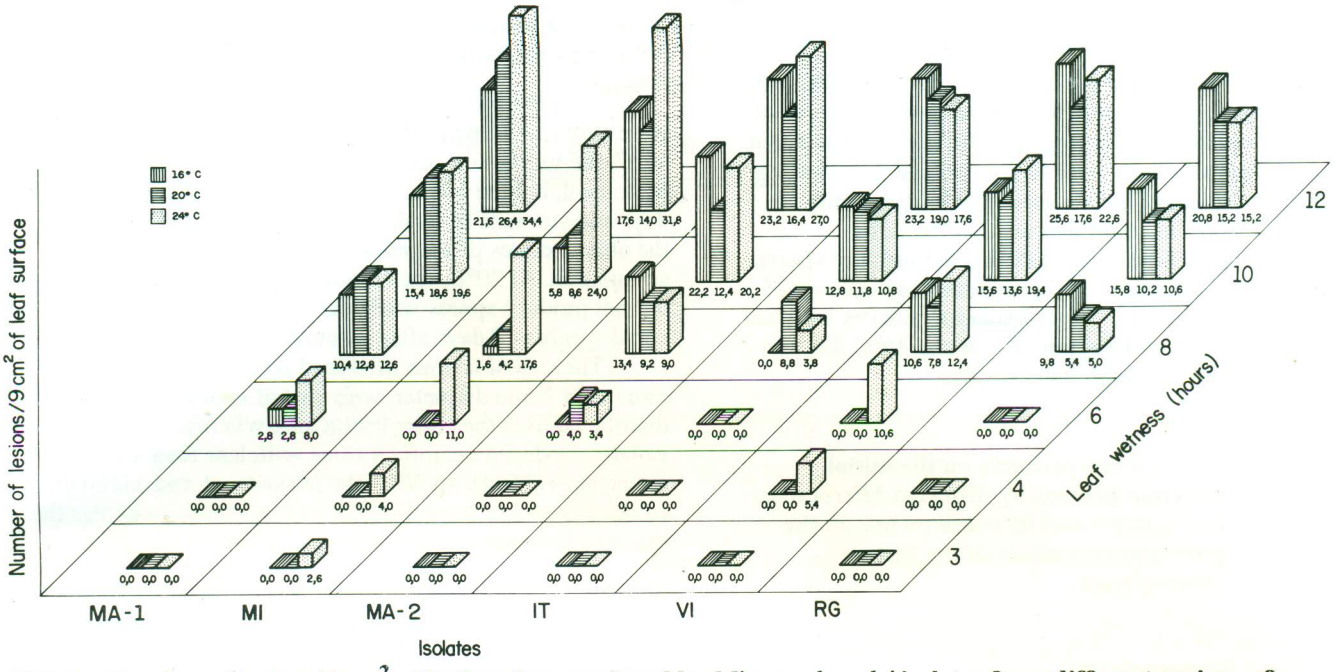

FIG. 1 - Number of lesions/9 cm^2 of leaf surface produced by *Microcyclus ulei* isolates from different regions of Brazil under different temperatures and leaf wetness. Isolates: MA = Manaus-AM, Mi = Manicoré-AM, IT = Ituberá-BA, Vi = Viana-ES and RG = Registro-SP.

TABLE 2 - Effect of temperature and leaf wetness period on the initial process of infection by different isolates of *Microcyclus ulei*.

Temperature (°C)	Time of leaf wetness (h)	Mean number of lesions per 9 cm^2 of leaf surface					
		MA-1[1]	MI	MA-2	IT	VI	RG
16	3	0,0 a[2]	0,0 a	0,0 a	0,0 a	0,0 a	0,0 a
	4	0,0 a	0,0 a	0,0 a	0,0 a	0,0 a	0,0 a
	6	2,8 b	0,0 a	0,0 a	0,0 a	0,0 a	0,0 a
	8	10,4 c	1,6 a	13,4 b	0,0 a	10,6 b	9,8 b
	10	15,4 d	5,8 b	22,2 c	12,8 b	15,6 c	15,8 c
	12	21,6 e	17,6 c	23,2 c	23,2 c	25,6 d	20,8 d
20	3	0,0 a	0,0 a	0,0 a	0,0 a	0,0 a	0,0 a
	4	0,0 a	0,0 a	0,0 a	0,0 a	0,0 a	0,0 a
	6	2,8 b	0,0 a	4,0 b	0,0 a	0,0 a	0,0 a
	8	12,8 c	4,2 b	9,2 c	8,8 b	7,8 b	5,4 b
	10	18,6 d	8,6 c	12,4 d	12,8 c	13,6 c	10,2 c
	12	26,4 e	14,0 d	16,4 e	19,0 d	17,6 d	15,2 d
24	3	0,0 a	2,6 a	0,0 a	0,0 a	0,0 a	0,0 a
	4	0,0 a	4,0 a	0,0 a	0,0 a	5,4 b	0,0 a
	6	8,0 b	11,0 b	3,4 b	0,0 a	10,6 c	0,0 a
	8	12,6 c	17,6 c	9,0 c	3,8 b	12,4 c	5,0 b
	10	19,6 d	24,0 d	20,2 d	10,8 c	19,4 d	10,6 c
	12	34,4 e	31,8 e	27,2 e	17,6 d	22,6 e	15,2 d

Date are mean of 5 replications
[1] Isolates:VI= Viana-ES, MA= Manaus-AM, Mi= Manicoré-AM, IT= Ituberá-BA and RG= Registro-SP
[2] Means in column followed by the same letter are not significantly different (P= 0,01), according to Duncan test.

and 90% RH and alternated light of 12 hr daily. The generation period of *M. ulei* isolates in the rubber leaves also did not vary among the temperatures tested (Table 3). On other hand, at 16 and 20°C, the diameter of lesions of all *M.ulei* isolates, except the Viana isolate, were reduced. When inoculated plants were kept at 16 and 20°C during 24 hr, only the Viana isolate produced high quantity of spores and larger lesions (Table 3). At the same temperatures, the Registro isolate did not produce spores whereas the isolate Manaus - 1 produced low amount of spores and the Manaus - 2 and Manicoré isolates were not influenced by low temperature. At 24 and 28°C the sporulations of all isolates were similar.

TABLE 3 - Effect of temperature on the initial infection process by different *Microcyclus ulei* isolates and its consequence on the post-infection phase of the disease development.

Isolates[1]	Temperature (°C)	Generation period (days)	Lesion diameter (mm)	Lesion types[2]
MA-1	16	06	1,0	5A
	20	06	2,0	5B
	24	06	3,0	09
	28	06	3,0	09
MI	16	08	1,0	06
	20	07	2,0	07
	24	07	3,5	09
	28	07	3,5	09
MA-2	16	04	2,0	07
	20	04	2,0	07
	24	04	2,5	08
	28	04	2,5	08
IT	16	05	1,0	07
	20	05	1,0	07
	24	05	2,5	08
	28	05	2,5	08
VI	16	04	3,0	10
	20	04	3,5	10
	24	04	3,5	10
	28	04	3,0	10
RG	16	08	2,0	02
	20	08	2,0	02
	24	08	2,5	07
	28	08	2,5	07

Date are mean of 5 replications
[1] Isolates:VI= Viana-ES, MA= Manaus-AM, Mi= Manicoré-AM, IT= Ituberá-BA and RG= Registro-SP.
[2] Lesion types according to Junqueira *et alii* (1988).

3. Effect of temperature on post-infection phase of different isolates of *Microcyclus ulei*.

The post-infection phases of different *M.ulei* isolates were strongly influenced by low temperatures (Table 4). At 16°C, the incubation periods of all *M. ulei* isolates were prolonged, but the Viana isolate produced conidia at 12 days and stromata at 18 days after the inoculation whereas the other isolates produced necrotic lesions without sporulation. At 20°C, the Manaus - 1 and Manicoré isolates did not produce spores while the other isolates produced conidia only at 8 days after inoculation.

The size of lesions produced by Viana isolate was more than 2 mm diameter, with partial sporulation distributed over the entire lesion area whereas the other isolates produced necrotic lesions with less than 1 mm diameter (Table 4). At 20°C the Manaus - 1 and Manicoré isolates produced larger lesions with no spores whereas the other isolates remained unaffected (Table 4).

4. Effect of temperature variations on the post-infection phase of different isolates of *Microcyclus ulei*.

The incubation periods of the isolates were slighty affected by thermic variations (Table 5). On other hand, the fungal generation periods of all *M. ulei* isolates were prolonged when inoculated plants were kept daily at alternated temperature periods of 16°C during 20 hr and 24°C for 4 hr (system A), or under alternated temperature periods of 16°C for 16 hr and 24°C for 8 hr (system B). Under system A, the Ituberá, Registro and Manaus - 2 isolates produced a low number of spores occurring only at the lesion border region whereas Viana isolate produced a partial sporulation (low number of spores) distributed over the entire lesion area (Table 5). Under system B the lesion types were slighly affected in comparison to systems C and D. The systems C and D did not affected the types of lesion, sporulation and lesion size of the different *M. ulei* isolates (Table 5).

DISCUSSION

The results of this study indicate the existence of ecophysiologic variability among the *M. ulei* origins as far as the minimum leaf wetness period required for infection as the effect of low temperatures on the diseases development in post- infection phases of the pathogen isolates.

The effect of leaf wetness period on the infection by *M. ulei* has been reported by several authors (Rands, 1924; Langford 1945; Kajornchaiyakul *et alii*, 1984; Gasparotto *et alii*, 1989a). However, in spite of these authors have worked with only one isolate, their results indicate differential response to wetness among isolate of this pathogen. The *M. ulei* isolate tested by Rands (1924) needed, at least, 10 hr of wetness for infection while the isolates tested by Langford (1945), Kajornchaiyakul et alii (1984) and Gasparotto (1989a) needed, respectively 8, 8 and 6 hr for infection. The data obtained in this study presented a differential response of the *M. ulei* isolates to requirements of wetness.

TABLE 4 - Effect of temperature on post-infection phase of different isolates of *Microcyclus ulei*.

Isolates[1]	Incubation period (days)				Generation period (days)				Lesion types[3]			
	16[2]	20	24	28	16	20	24	28	16	20	24	28
MA-1	9	4	3	2,5	*	*	6	6	1	3A	9	10
MI	7	5	2,5	3	*	*	5	6	1	3A	9	9
MA-2	7	4	4	3	**	8	6	5	1	10	10	10
IT	7	4	4	3	**	8	6	6	1	9	9	9
VI	5	4	4	3	***	8	5	5	5B	7	8	8
RG	7	4	4	3	**	9	6	8	1	10	10	7

Date are means of 5 replications
[1] Isolates:MA= Manaus-AM, MI= Manicoré-AM, IT= Ituberá-BA, VI= Viana-ES and RG= Registro-SP.
[2] Temperature (°C)
* No esporulation; lesions were necrosed at 14 days after inoculation
** No sporulation; lesions were necrosed at 18 days after inoculation
*** Partial sporulation at 12 days after inoculation and initial stromata formation at 18 days after inoculation.
[3] Lesion types according to Junqueira *et alii* (1988).

TABLE 5 - Effect of temperature variations on the post-infection phase of different isolates of *Microcyclus ulei*.

Isolates[1]	Incubation period (days)				Generation period (days)				Lesion types[3]			
	A[2]	B	C	D	A	B	C	D	A	B	C	D
IT	5	5	4	4	15	12	7	7	4A	7	9	10
RG	5	4	4	3	13	11	6	6	4A	7	9	9
MA-2	5	5	3	3	15	11	6	5	4A	7	9	9
VI	4	3	3	3	11	8	5	4	5A	7	10	10

Date are means of 5 replications
[1] Isolates: IT= Ituberá-BA, RG= Registro-SP, MA= Manaus-AM and VI= Viana-ES
[2] Regime of temperature variations: A= Inoculated plants were kept daily at alternated temperature of 16°C for 20 hr followed by 24°C for 4 hr; B= Inoculated plants were kept daily at alternated temperature of 16°C for 16 hr followed by 24°C for 8 hr; C= Inoculated plants were kept daily at alternated temperature of 20°C for 20 hr followed by 24°C for 4 hr; D= Inoculated plants were kept daily at alternated temperature of 20°C for 16 hr followed by 24°C for 8 hr.
[3] Lesion types according to Junqueira *et alii* (1988).

The level of infection, colonization and sporulation of all *M. ulei* isolates except the Viana isolate, were strongly affected by low temperatures (Table 2). At 16 and 20°C, the lesion numbers were reduced and the wetness period required for infection was increased. When the inoculated plants were kept in moist chambers during 24 hr at 16, 20, 24 and 28°C and later on transferred to a room at 24°C, the lesion numbers produced by all isolates were similar. These results clearly demonstrated that at lower temperatures, the time required for conidia germination and to cause infection was increased.

Concerning the effect of temperature on post-infection phase, all isolates, except the Viana isolate, had a similar behavior although slight differences in the sporulation among the isolates, were observed. At 20°C the Manaus - 1 and Manicoré isolates did not produce spores while the other isolates only produced spores at 8 days after inoculations (Table 4). The shortest incubation and generation periods, the largest lesions size and the highest conidia production by Viana isolate at 16°C, indicated that this isolate is more adapted at low temperatures than other isolates tested till now. On other hand, it was also shown that at 24°C, the Manicoré-AM isolate needed only a leaf wetness period of 3 hr for infection.

When inoculated plants were kept daily at temperature system of 16 or 20°C for 20 or 16 hr followed by 4 or 8 hr at 24°C, all the isolates produced spores, but the Viana isolate produced the highest numbers of spores (Table 5). These findings have indicated the existence of ecologic races or ecotypes of *M. ulei* less exigent in wetness and more adapted to low temperature conditions. Similar results were also obtained by Gasparotto *et alii* (1991). These authors observed that, in Viana, Espírito Santo State, the rubber tree leaf blight severity was not affected during the cold periods. On the other hands, Gasparotto *et alii* (1989b) observed in Ponte Nova, Minas Gerais State, that the leaf blight severity was reduced at the same time when the minimum temperature was lowered or vice versa. Langford (1945) working at Turrialba-Costa Rica, reported that *M. ulei* caused severe leaf fall in susceptible rubber plants when the minimum and mediun temperatures were respectively, 11 and 21°C. These results

show that the sensitivity of *M. ulei* to lower temperatures depend on the pathogen isolate and its origin.

The data obtained in this study indicate that the leaf blight incidence or severity may be enhanced in rubber plantations established in regions were nocturnal temperatures are lower (16 - 18°C) and diurnal temperatures are high (24 ± 2°C).

The sexual phase of *M. ulei* takes place throughout the year in all regions of Brazil where rubber plantations were established. According to Pontecorvo, cited by Alexopoulos & Mims (1979) the recombination rate occurring in ascomycetes is over 500 times than the parassexual mechanism. Wolf *et alii* (1987) observed that sexual recombination in *Saccharomyces cerevisiae* was more efficient under varying environmental conditions. This fact may also occurs with *M. ulei* considering the distribution of this pathogen in regions presenting high climatic variations.

The higher severity of rubber tree leaf blight in Viana during lower temperature periods (Gasparotto *et alii*, 1991) and the results obtained in this study lead to a substancial evidence on the existence of ecological races of *M. ulei*.

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