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Lesion Size, Latent Period and Sporulation on Leaf Discs as Indicators of Resistance of Hevea *to* Microcyclus ulei

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There were clonal differences in the rate of development of mycelium and appearance of lesions, sizes of lesions, latent period and the quantity of conidia produced on discs of Hevea *leaves. There was a positive correlation between conidial production and lesion size, and negative correlations existed between conidial production and latent period and lesion size and latent period. Latent period and sporulation are also important components for assessment of resistance.*

Clones GT 711, RRIM 501, CNS AM 7701, SIAL 842 and SIAL 263 possessed relatively smaller lesions, longer latent periods and reduced sporulation. On these clones, the differences in lesion size were significant between clones but not between races of Microcyclus ulei. *However, differences in conidial production between clones, races and the interaction between clones and races were significant.*

In earlier programmes to breed *Hevea* clones resistant to South American leaf blight (SALB) caused by *Microcyclus ulei* (P. Henn.) v. Arx, crosses were made mostly between high-yielding oriental clones and South American clones, or their hybrids which are highly resistant if not immune to some races of *M. ulei*[1-4]. Most of the resistant off-springs of these crosses are resistant to some races of *M. ulei,* hence their resistance is race-specific. Since many races of *M. ulei* had been identified[5-7], and vertical resistance had failed[8], breeding for horizontal resistance to SALB is encouraged[6,8].

In many other diseases, the components of resistance often associated with horizontal resistance are low infection frequency, long incubation and/or latent period and reduced sporulation[9-18]. In the case of *Hevea,* resistant clones have been reported to produce less spores when infected with *M. ulei*[19]. It was also observed that lesions took a longer time to develop on resistant clones than on susceptible clones[20]. Clonal variations in number and size of lesions as well as conidial production have been recorded[1,20-22].

Hevea rubber is a perennial tree crop which can benefit from a laboratory method of screening resistance. One of the advantages of a laboratory method is that it offers the possibility of controlling climatic conditions which are known to affect the level of horizontal resistance[15]. A drawback is that laboratory results may not correspond with resistance of mature plants in the field. However, laboratory and glass-house results can be useful if carefully related to field results[8]. For example, laboratory methods were used to evaluate the resistance of lettuce to mildew[15] and coffee to leaf rust and berry disease[23]. Similarly, Chee[1] had correlated sizes of lesions on leaf discs with resistance of nursery plants to *M. ulei.* This study investigated lesion sizes, latent periods and sporulation of leaf discs as indicators of resistance of *Hevea* to *M. ulei.*

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MATERIALS AND METHODS

Inoculum and Plant Materials

Conidia obtained from infected leaves harvested from EDJAB Station, Una, Bahia were used in trials where field conidia were the inoculum. When a definite race of *M. ulei* was required, conidia were obtained from pure cultures. For this purpose, the cultures were grown on potato sucrose agar amended with Panvit^{R} (a mixture of vitamins, minerals and amino acids) and Bonzo^{R} dogfood[24], incubated and light-conditioned to induce sporulation as described previously[7].

Leaves were obtained from the same station. *Hevea* clones used in this study were FX 4163, FX 985, FX 3846, FX 3864, FX 2261, FX 2804, FX 3844, FX 25, IAN 717, IAN 710, IAN 713, RRIM 600, RRIM 501, MDF 180, CNS AM 7808, CNS AM 7701, SIAL 263, SIAL 842, CA 255, GT 711 and PA 31.

Inoculation of Leaf Discs

Leaf discs cut from young *Hevea* leaves (about seven days old) were floated on distilled water in petri dishes and sprayed with a suspension of conidia (1×10^5 conidia/ml) as mentioned previously[1]. Subsequently, the dishes were incubated at 24°C under continuous light in an incubator.

Assessment of Resistance

Mycelial development. Leaf discs, 48 h after being inoculated, were submerged for 24 h in a solution containing 100 ml ethyl alcohol (95%), 25 ml lactophenol, 75 ml chloral hydrate (2 g/ml) and 0.4 g cotton blue. The discs were washed with distilled water and cleared in a solution of chloral hydrate (2 g/ml). Cleared discs were mounted in glycerine on glass slides.

Mycelial development was rated into three categories: *A,* conidia germinated with short germtube, no penetration; *B,* conidia germinated and the mycelium had penetrated into the leaf tissues, however mycelial branching was absent or minimal; *C,* the conidia were associated with long and branching mycelia.

Rate of appearance of lesions. The rate of appearance of lesions was determined for eight clones: IAN 717, FX 2261, FX 2804, FX 985, FX 3864, FX 4163, FX 3844 and FX 25. For clones IAN 717, FX 2261 and FX 2804, they were inoculated with field conidia obtained from these same clones respectively. Other clones were inoculated with conidia from clone FX 985. The number of lesions appeared was counted five, six and seven days after inoculation.

Size of lesions. Unless otherwise stated, the size of lesions was determined six days after inoculation by using a dot scale[25].

Latent period. Latent period is the interval (days) between inoculation and the day sporulation is first detected.

Sporulation. The conidia were harvested at definite intervals after inoculation as mentioned in the *Results* by agitating the leaf discs in 3 ml, 5 ml or 10 ml of distilled water containing a drop of diluted Triton × 114. A haemocytometer was used to determine the concentration of conidia.

RESULTS

Mycelial Development

Microscopic observations of cleared leaf discs indicated that conidial germination and mycelial penetration into the leaf tissues occurred for both the compatible (lesion formed) and incompatible (no lesion formed) host-pathogen combinations *(Table 1).* When the rate of mycelial development was estimated at 48 h after inoculation, the growth of mycelium in the leaf discs was more advanced in the compatible host-pathogen combinations compared to the incompatible combinations as the percentage of conidia which had reached *Category C* rating of fungal development was higher for the compatible than in the incompatible combinations (*Tables 1* and *2*). In the incompatible combinations, fungal development ceased to progress following penetrations as indicated by the greater percentage of conidia still in *Category B (Table 2).* Among the compatible host-parasite combinations, mycelium development was slower in clone FX 3864 *(Table 1).*

TABLE 1. DEVELOPMENT OF *MICROCYCLUS ULEI* ON LEAF DISCS OF *HEVEA* 48 H AFTER INOCULATION

Test clone	Source of conidia	Percentage number of conidia with fungal development rating			Lesion formation
		A	B	C	
FX 985	FX 2804	30.80	69.20	0.00	–
	FX 2261	30.80	57.55	11.65	–
	FX 985	4.15	14.85	80.80	+
FX 2261	FX 2804	45.00	45.00	10.00	–
	FX 2261	14.20	23.30	62.50	+
	FX 985	30.80	64.20	5.00	–
FX 3864	FX 2804	23.35	46.65	30.00	+
	FX 2261	22.50	15.83	45.85	+
	FX 985	13.35	36.65	50.00	+
IAN 717	FX 2804	3.30	6.35	90.35	+
	FX 2261	40.00	45.85	14.15	–
	FX 985	24.20	27.00	48.30	–
PA 31	FX 2804	17.50	79.15	3.35	–
	FX 2261	35.85	64.15	0.00	–
	FX 985	30.85	67.45	1.70	–

Data are averages of two experiments and sixty germinated conidia were observed per experiment.
For fungal development rating, *see Methods.*
Lesion formation: –, no lesion formed: +, lesion formed
The conidia from FX 2804, FX 2261 and FX 985 were *Races 2, 4* and *6* respectively.

Rate of Appearance of Lesions

The rate of appearance of lesions on leaf discs of eight *Hevea* clones, which is an indication of the incubation period, is shown in *Table 3.* On these clones, lesions were obviously visible on the fifth day after inoculation. The increase in the number of lesions was greatest between the fifth and the sixth day while the difference occurring between the sixth and seventh day was small *(Table 3).* When the number of lesions developed by *Day 5* expressed as a percentage to that of *Day 7* was compared between clones, there was no significant differences between most clones except for clone FX 2261 which showed a significantly lower percentage compared to the other clones. This indicated that the incubation period was longer on clone FX 2261. Similar results were obtained when the number of lesions developed by *Day 6* was expressed as a percentage of the number of *Day 7 (Table 3).*

TABLE 2. DEVELOPMENT OF COMPATIBLE AND INCOMPATIBLE RACES OF *MICROCYCLUS ULEI* ON *HEVEA* LEAF DISCS

Compatibility[a]	Percentage number of conidia with fungal development rating[b]		
	A	B	C
Compatible	13.48	26.66	61.25
Incompatible	31.76	57.73	10.46
	$p<0.005$	$p<0.005$	$p<0.005$

[a] *See Table 1,* compatible combinations were when lesions formed.
[b] The rating of fungal development is given in *Methods.*

TABLE 3. APPEARANCE OF LESIONS ON LEAF DISCS OF EIGHT *HEVEA* CLONES INOCULATED WITH *MICROCYCLUS ULEI*

Clone	No. (mean) of lesions			Lesion number of Day 5 or Day 6 as % (mean) of Day 7	
	Day 5	Day 6	Day 7	Day 5	Day 6
FX 2261	9.75	22.28	40.80	22.97 (2.9)	52.53 (6.4)
IAN 717	29.72	46.48	57.58	51.25 (2.3)	83.77 (3.1)
FX 985	37.92	61.12	74.18	50.42 (7.5)	82.93 (1.9)
FX 2804	31.13	61.57	67.57	40.90 (7.6)	89.24 (4.3)
FX 3864	31.45	56.18	62.23	49.35 (5.9)	89.98 (2.4)
FX 4163	32.25	62.48	67.68	52.15 (5.3)	92.96 (2.5)
FX 3844	33.15	63.62	72.33	42.21 (5.1)	87.01 (4.2)
FX 25	40.50	81.37	100.12	39.38 (4.1)	81.92 (4.3)
		F		3.98**	0.76^{NS}
		$L.S.D._{0.05}$		13.95	17.87
		C.V. (%)		27.43	18.48

Analysis of variance indicated significance at 1% (**) or non-significance (NS).
Numbers within brackets are standard errors.

Lesion Size

There is a large clonal variation in the size of lesions developed on *Hevea* leaf discs inoculated with conidia of *M. ulei (Figure 1).* Clones MDF 180, FX 4163 and FX 3864 produced larger lesions while the lesions on clones GT 711, RRIM 501, CNS AM 7701 and SIAL 842 were smaller.

The sizes of lesions on six selected *Hevea* clones inoculated with three races of *M. ulei* are shown in *Table 4.* No lesion was observed on FX 985 and SIAL 842 inoculated with *Race 2.* The sizes of lesions developed on these clones indicated significant differences ($p = 0.01$) between clones, however the differences between races were not significant. The clone × race interaction was also not significant.

Latent Period

The length of the latent period of various clones is shown in *Figure 1.* On leaf discs, conidia were detected six days after inoculation of some clones while the latent period was longer on clone GT 711 (eleven days), RRIM 501 (thirteen days), and fourteen days for clones SIAL 842 and CNS AM 7701.

There was a negative correlation ($r = -0.86$) between lesion size and latent period *(Figure 1).* Generally, clones with smaller lesions had longer latent periods. However, some clones (FX 985, FX3846, FX 2261, IAN 717, FX 2804, CNS AM 7808, IAN 710, FX 25 and RRIM 513) possessing about similar sizes of lesions had a wider variation of latent periods varying from six to eleven days *(Figure 1).*

Sporulation

The amount of conidia produced on leaf discs varied with the clones *(Figure 2).* Sporulation was high on clones FX 4163, FX 985 and FX 3846 and very low on clones GT 711, RRIM 501, CNS AM 7701 and SIAL 842. Sporulation was influenced by sizes of lesions and latent period. There was a negative correlation ($r = -0.717$) between the quantity of conidia produced and latent period *(Figure 2),* and a positive correlation ($r = 0.643$) between lesion size and the amount of conidia produced *(Figure 3).* Clones such as FX 25, FX 3864,

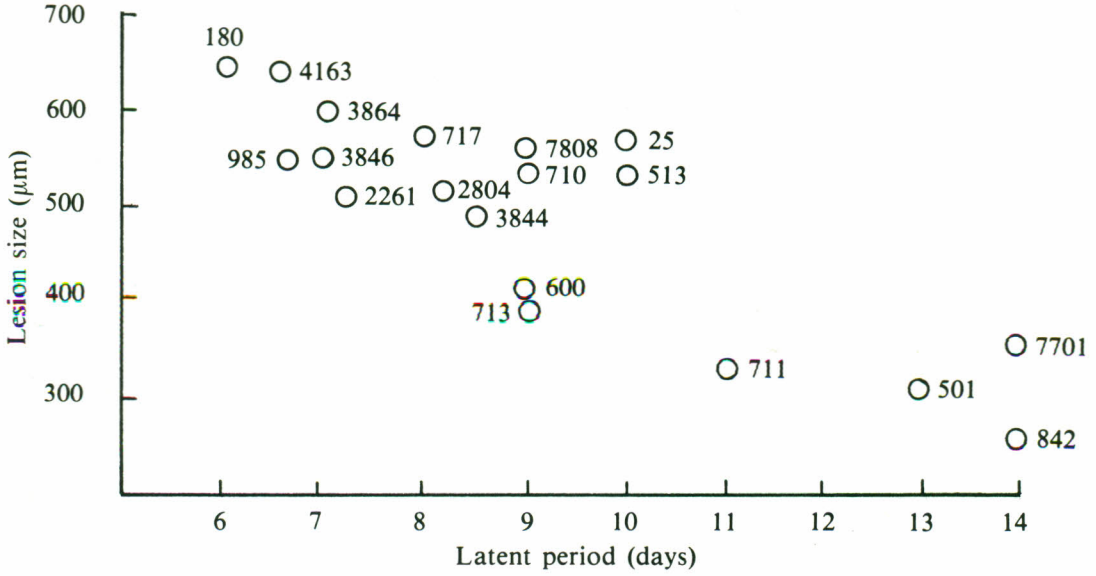

Figure 1. Relationship between lesion size and latent period on leaf discs of different Hevea *clones inoculated with* M. ulei.

TABLE 4. LESION SIZE AND SPORULATION ON LEAF DISCS OF SIX *HEVEA* CLONES INOCULATED WITH THREE RACES OF *MICROCYCLUS ULEI*

Clone	Mean size of lesion (μm)				Conidia produced/disc ($\times 10^4$)[a]			
	Race 2	Race 5	Race 6	Mean	Race 2	Race 5	Race 6	Mean
SIAL 263	363.0	263.3	341.7	322.8	5.32	2.12	1.89	3.11
GT 711	288.7	303.3	317.0	303.0	4.26	4.19	3.06	3.84
RRIM 501	200.0	272.0	249.0	240.3	0.21	2.71	0.58	1.17
SIAL 842	NL	227.7	231.0	229.4	0.00	0.64	0.24	0.29
FX 985	NL	449.0	427.7	438.5	0.00	23.47	4.33	9.27
CNS AM 7701	200.0	254.3	212.7	222.3	0.14	0.39	0.15	0.23
Mean	175.3	294.9	296.5		1.66	5.59	1.71	
Analysis of variance								
Source	df	MS		p	df	MS		p
Replicate	2	118.74		NS	2	2.3615		NS
Clone	5	35 955.35		**	5	104.8580		**
Error (a)	10	458.56			10	0.9371		
Race	2	87 052.07		NS	2	91.9335		*
Race × clone	10	34 674.69		NS	10	78.8521		**
Error (b)	24	30 137.88			24	1.1175		

NS = Not significant; *Significant at $P<0.05$; **Significant at $P<0.01$

NL = No lesion formed

[a]Conidia were harvested nine days after inoculation for clone FX 985 and fourteen days after inoculation for the other clones.

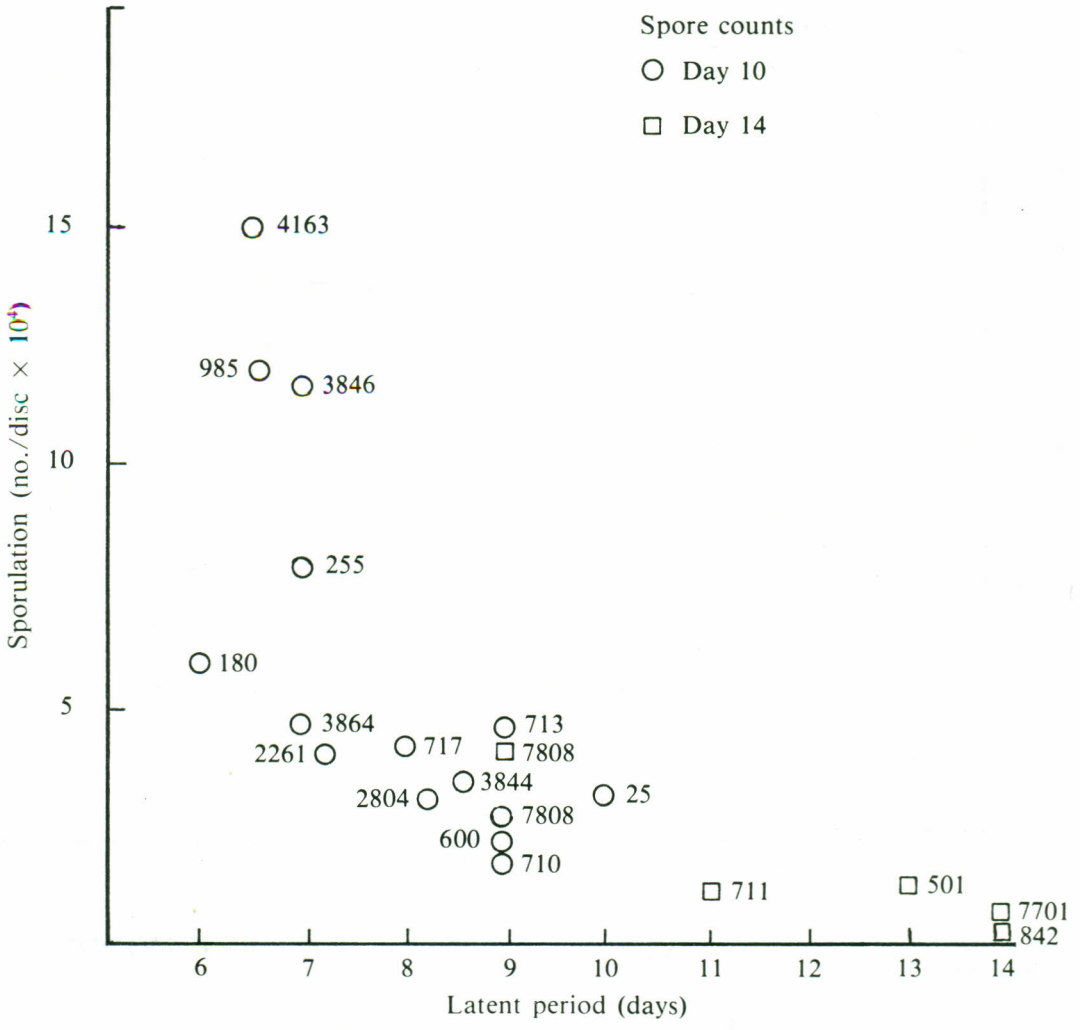

Figure 2. Relationship between latent period and sporulation of M. ulei *on leaf discs of different* Hevea *clones. Spore counts were done on* Day 10 *or* Day 14 *after inoculation.*

MDF 180 and CNS AM 7808 produced large lesions; however, the amount of conidia produced was low *(Figure 3)*. Similarly, results shown in *Table 5* indicated that clones with slight variation in the sizes of lesions, indicated significant differences when the amount of conidia produced was determined. In fact, the sizes of lesions on clones such as FX 2261, FX 2804 and FX 3844 were smaller than on clone FX 25; however, the amounts of conidia produced on these clones were greater than on FX 25 *(Table 5)*.

The amounts of conidia produced on six selected clones inoculated with three races of *M. ulei* are shown in *Table 4*. The amount of conidia produced showed significant differences both between clones and between races. The interaction between clone and race was also significant.

DISCUSSION

Parlevliet[10] stated that often there was no resistance to infection, germination, appressorium formation and penetration by bio-

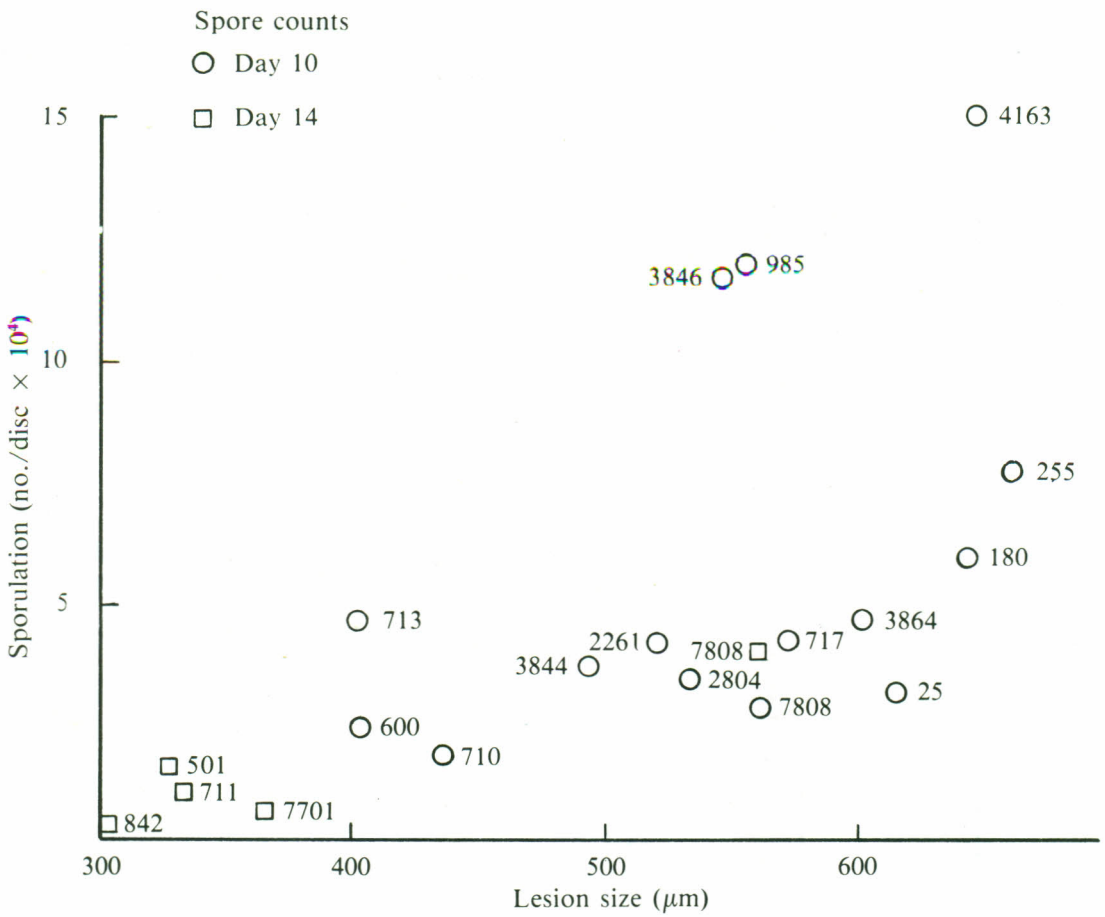

Figure 3. Relationship between lesion size and sporulation of M. ulei *on leaf discs of different* Hevea *clones.*

trophic pathogens. In conformity, this study confirms an earlier observation[26] that conidial germination and mycelial penetration occurred on all clones tested irrespective of their resistance. However, no fungal development occurred beyond penetration and slight mycelial growth in incompatible host-parasite combinations *(Table 1)*. This suggests that in order to assess varietal resistance of *Hevea* to *M. ulei,* fungal development beyond penetration of the epidermal layer needs to be compared.

Inoculation of leaf discs is useful in determining the occurrence of vertical resistance *(Table 1),* and it could also measure the quantitative resistance in disease resistance especially when latent period and sporulation were considered. Earlier, Chee[1] assessed resistance of *Hevea* to *M. ulei* in the laboratory by measuring only the sizes of lesions on leaf discs. The present study indicated that generally the clonal variations between lesion size, latent period and sporulation were associated. A higher correlation existed between lesion size and latent period as both components measure the rate of growth of mycelium in the host tissues. In addition, Johnson and Taylor[9] considered that resistance to growth of mycelium and resistance to production of spores could also be closely related. However, there were exceptions in these relationships. Some *Hevea* clones which developed larger lesions had longer latent

TABLE 5. PRODUCTION OF CONIDIA ON LEAF DISCS OF *HEVEA* INOCULATED WITH *MICROCYCLUS ULEI*

Clone	Lesion size (μm)	Latent period (days)	Conidia harvested on Day 9			Conidia harvested on Day 11		
			No./disc ($\times 10^4$)	No./lesion ($\times 10^2$)	No./lesion/ day ($\times 10^2$)	No./disc ($\times 10^4$)	No./lesion ($\times 10^2$)	No./lesion/ day ($\times 10^2$)
FX 2261	509	7–9	4.12 (2.2)	8.79 (3.7)	6.27 (2.1)	10.09 (1.0	23.73 (1.6)	5.93 (0.4)
FX 2804	515	7–9	3.27 (0.3)	4.43 (0.6)	3.48 (0.4)	3.72 (0.7)	5.12 (0.9)	2.56 (0.5)
IAN 717	569	8–9	4.24 (1.0)	5.62 (1.0)	5.62 (1.0)	—	—	—
FX 985	549	6–7	12.37 (1.1)	14.45 (1.7)	5.76 (1.4)	9.23 (2.3)	14.49 (2.4)	3.62 (0.6)
FX 3864	599	7–8	4.72 (0.6)	8.11 (1.5)	4.06 (0.7)	6.26 (0.5)	8.81 (0.4)	2.20 (0.1)
FX 4163	641	6–7	15.31 (2.7)	17.37 (0.9)	6.68 (0.7)	16.46 (8.3)	27.29 (4.0)	6.25 (0.5)
FX 3844	489	7–9	3.57 (0.7)	3.97 (1.0)	3.12 (0.8)	1.63 (0.3)	1.95 (0.1)	0.98 (0.1)
FX 25	573	9–11	0.42 (0.1)	0.41 (0.1)	0.41 (0.1)	0.70 (0.4)	0.66 (0.4)	0.66 (0.4)
F			13.86**	11.72**	3.86*	2.26^{NS}	24.70***	27.66***
$L.S.D._{0.05}$			4.09	4.93	3.19	10.84	6.07	1.20

Numbers within brackets are standard errors.

Analysis of variance indicated significance at 5% (*), 1% (**), 0.1% (***) or non-significance (NS)

– = No data

periods and sporulated poorly. Thus latent period and sporulation, the components of resistance which are commonly determined to assess for horizontal resistance in other diseases[9-18], should also be considered when clones of *Hevea* are assessed for resistance, especially horizontal resistance. After all, Langford[20] observed that complete or partial inhibition of sporulation of *M. ulei* on *Hevea* is an expression of resistance as consistent as resistance to leaf damage and defoliation and hence suggested that sporulation should be given even or greater merit in the selection of *Hevea* clones for resistance to *M. ulei.* In other diseases[9,17,18], sporulation is considered a more sensitive component of resistance to diseases. Sporulation is also a sensitive test for differential interaction with races of a pathogen which is often due to race specific resistance[9]. This is clearly shown in *Table 4* where the clonal interaction with races of *M. ulei* was not significant when lesion size was analysed while the interaction was significant when sporulation was considered. Moreover, clones with slight variation in lesion size possessed greater differences in conidia production *(Table 5)* and latent period *(Figure 1).*

When resistance of *Hevea* to *M. ulei* was assessed in the laboratory, determination of the latent period and amount of conidia produced enhanced the reliability of measuring only lesion size especially when horizontal resistance was selected. Inoculation of leaf discs or probably a detached leaf is a useful tool in the early selection of clones for resistance, even for horizontal resistance.

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