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Relationship between Resistance to Microcyclus ulei *and Clonal Foliar Phenolics of Rubber Trees*

C. SANIER*, P. BERGER*, M. COUPÉ*, J.-J. MACHEIX*, J.M. PETAT**, F. RIVANO**, A. de SAINT BLANQUAT** AND J. d'AUZAC*

The extent to which resistance of Hevea brasiliensis *to* Microcyclus ulei *may be related to the presence or absence of certain foliar phenolics analysed by High Performance Liquid Chromotography (HPLC) after selective extraction was studied. Young leaves at* M. ulei-*sensitive* (B2) *stage from fourteen clones with a broad range of resistance were used. The resistance to* M. ulei *of these clones was evaluated in the field by determining the percentage of leaf abscission during a one-year period.*

No qualitative differences were observed between the foliar phenolics of various clones of Hevea brasiliensis, *and hybrid rubber trees. Except for clones PB 235 and PB 260, the total quantity of phenols and the proportion of flavans appear to be related to resistance to* M. ulei *expressed as percentage of abscission. Multiple regression analysis showed that some compounds seemed to be closely related to the percentage of abscission whereas others appeared to be correlated with resistance (kaempferol-3-rutinoside, 3'p-coumaroylquinic acid). Classification of the fourteen clones as 'sensitive' and 'resistant' shows that some phenols are present in resistant clones and others are found in susceptible clones [(+)-catechin and rutin]. Likewise, the hydroxycinnamic derivatives (HDC)/flavonols ratio, the percentage of ratin in relation to total flavonols and the ratios of certain phenols (kaempferol-3-rutinoside: kaempferol-galactoside, rutin: kaempferol-galactoside) appeared in particular to be related to clonal resistance.*

South American leaf blight (SALB) caused by *Microcyclus ulei* (P. Henn) V. Arx, prevents the creation of large, economically profitable plantations throughout the Amazon basin, where *Hevea brasiliensis* (the only cultivated species) originated. The disease is also a threat to the other rubber-growing areas in the world as most of the high-yielding clones bred in Asia are extremely susceptible.

The earlier results of Langford[1] on the biology of the pathogen, relationships with its host and characterisation of the sensitivity and resistance of the various *Hevea brasiliensis* clones and of other *Hevea* species have been the subject of numerous references[2,3,4]

The most promising method of control appears to be based on the genetic selection for disease-resistant clones which also possess high production potential. All the resistance of the clones bred since the beginning of the century seem to have been rapidly overcome by *M. ulei*. This can be explained in part by the great physiological variability of *M. ulei*[5,6,7] and also by the types of resistance of the clones selected by breeders. The reaction of these clones[8,9] is similar to a hypersensitivity phenomenon often related to vertical resistance[10].

The current trend is to seek horizontal resistance to *M. ulei* combining as many resistant characters as possible[11]. The continuation of research on possible defence

*Laboratoire de Physiologie Vegetale Appliquée, Université Montpellier 2, F-34095, Montpellier Cedex 5, France
**IRCA-CIRAD, Guyane française, BP 701, Kourou. Cedex 97387, France

mechanisms may contribute to this and provide the selection criteria.

It seems that *M. ulei* penetrates the cuticle and epidermis of all clones[8,9], which seems to indicate that resistance is not related to an anatomical barrier but to biochemical features. Phenolics may contribute to resistance to phytopathogenic fungi in several ways:

- Phenols present before the infection may have an inhibitory effect on conidial germination[12].
- Many phytoalexins are phenolic[13].
- Oxidised phenolics are the cause of the brown compounds which appear during the hyper-sensitive reaction. They are also lignin precursors and thus contribute to the strengthening of cell walls.

In addition, even if phenolics have no active role in resistance, they may possibly be used as resistance or susceptibility markers.

Phenolics in *Hevea* leaves have been studied. Aqueous extracts[14] or diffusates[6] of young resistant healthy leaves have a greater inhibitory effect on conidium germination than extracts of susceptible leaves. This inhibition was attributed[14] to a compound existing before infection, identified as a flavonol, which is probably more abundant in resistant leaves. It is probably a kaempferol derivative[15] or a quercetin derivative[16]. It has also been shown[17] that diffusates of young *Hevea* leaves contain phenols which hinder the germination of *Colletotrichum gloeosporioides* conidia.

No clear relationship has been established between total phenols or each constituent phenol analysed by HPLC and the degree of resistance of *Hevea* to *C. gloeosporioides*[18] or *M. ulei*[16]. The only difference between susceptible and resistant clones concerns the flavonol contents. These compounds may, for example, be involved in the regulation of foliar auxin-oxidase[19]. Two phytoalexins have been characterised in infected *Hevea* leaves[20]; one is a coumarin and the other scopoletin[21].

All the work carried out to date[14,16,18,21] suggests that one or more particular phenols are involved — at least in part — in the resistance of *Hevea* to pathogenic fungi. A detailed HPLC study was therefore undertaken on the phenolics of fourteen clones which, under the conditions of French Guiana, displayed a broad range of susceptibility to *M. ulei*. The report concerns only results of phenolics present in young healthy leaves at the susceptible stage *(B2)* to *M. ulei*.

MATERIAL AND METHODS

Plant Material

Young leaves of the fourteen clones: FX 3864, IAN 873, RRIC 101, PB 235, IRCA 19, PB 260 and GT 1 *(Hevea brasiliensis)*; IAN 717, CD 1078, RO 38, IAN 2878, IAN 710 and IAN 713 (interspecific hybrids) and *Hevea pauciflora* PA 31 were analysed *(Table 1)*. These clones were cultivated in a plastic greenhouse and were free of any foliar contamination.

The leaves were sampled at *Stage* B_2, as defined by Dijkman[22] or at *Stage III* as defined by Blazquez and Owen[8], from plants cultivated under plastic at the IRCA Station at Kourou (French Guiana) and frequently cut back to ensure continuous growth of young leaves. The plants were not subjected to any chemical treatment and were totally free of *M. ulei*.

Samples of 1 – 2 g fresh weight (FW) were conserved in 95% ethanol and sent to Montpellier (France) for analysis. Sampling was repeated five to seven times depending on the clone to reduce the variability of sampling.

Evaluation of Susceptibility to *M. ulei*

Susceptibility was estimated using trees grown in a comparative clone trial

TABLE 1. THE DIFFERENT CLONES STUDIED

Clone	Country of origin	Species	% Abscission
PA 31	Brazil	Aspecific *H paucıflora*	0.001
IAN 717	Brazil	Interspecific *H. benthamiana* × *H. brasiliensis*	0.001
CD 1078	Guatemala	Interspecific *H paucıflora* × *H. brasiliensis*	0 8
RO 38	South America	Interspecific *H. benthamiana* × *H. brasiliensis*	1.5
IAN 2878	Brazil	Interspecific *H benthamiana* × *H. brasiliensis* × *H. brasiliensis*	1.6
IAN 710	Brazil	Interspecific *H. benthamiana* × *H. brasiliensis*	4 5
FX 3864	Brazil	Intraspecific *H. brasiliensis*	4.5
IAN 873	Brazil	Intraspecific *H. brasiliensis*	4.5
RRIC 101	Sri Lanka	Intraspecific *H brasiliensis*	11.6
PB 235	Malaysia	Intraspecific *H. brasiliensis*	20 4
IRCA 19	Ivory Coast	Intraspecific *H. brasiliensis*	30 8
PB 260	Malaysia	Intraspecific *H. brasiliensis*	40
IAN 713	Brazil	Interspecific *H. benthamiana* × *H. brasiliensis*	45
GT 1	Java	Intraspecific *H. brasiliensis*	45

The percentage of leaf abscission measured in one year was used as the criterion of sensitivity to *M. ulei*. The dividing line between resistant and sensitive clones was fixed arbitrarily at 10%. This classification is valid in the eco-climatic conditions of the French Guiana and for the *M ulei* races present in this country

established from 1982 to 1987 at the IRCA-CIRAD station in French Guiana.

The susceptibility score awarded to each clone is the average abscission of young leaves in 1989; this was recorded for all the trees every two months *(Table 1)*. According to Rivano (personal communication), this score gives the best description of the degree of tolerance to the disease. In *Table 1* are listed clones which appear to be resistant or susceptible to all the races of *M. ulei* collected in Guiana. It may be possible that in another eco-climatic condition or for other races of *M. ulei*, the classification will be different.

Extraction of Phenolics

The leaves conserved in 95% ethanol were drained, frozen in liquid nitrogen, crushed in a ball grinder and agitated cold for 20 min in the conservation ethanol diluted to 80% and to which 0.5% sodium disulfite had been

added. After filtration through No. 4 fritted glass, the solid residue was extracted once more for 20 min in 80% ethanol containing 0.5% $Na_2S_2O_5$. The filtrates were combined and the ethanol evaporated under partial vacuum using a rotary evaporator at 35°C.

The residual aqueous phase was bleached four times with half a volume of petrol ether; $(NH_4)_2SO_4$ 40% (v/v) and metaphosphoric acid 20% (v/10) were added before four ethyl acetate (v/v) extractions. Ethanol (95%) was added during the final extraction to complete the transfer of the phenolic compounds to the ethyl acetate. The organic phase was dehydrated by filtration on 1PS Whatman paper and evaporated to dryness. The residue was re-suspended in 5 ml of HPLC methanol and filtered on a Millipore HVLP membrane (porosity 0.45 μm). The purified phenolic extract was stored at −18°C. The variability coefficient for the extraction method was 10% – 12%.

HPLC

HPLC analyses were performed with a Waters 600 E apparatus equipped with a C_{18} column (Macherey–Nagel Nucleosil 5 μm, 250 × 4 mm). The injection volume was 20 μlitre (10 μlitre of extract + 10 μlitre of phloretic acid used as an external standard; peak *E* on the chromatograms). Elution was performed with a pH 2.6 acetonitrile–water gradient. The separated components were detected by Waters 990 diode array detector.

The peak area was integrated at 280 nm for flavans (*Peaks A3* to *A22*), 314 nm for hydroxycinnamic derivatives (*Peaks B3* to *B10*) and 350 nm for flavonol (*Peaks C11* to *C21*). These wavelengths are the maximum absorption zones for the three groups of compounds. The peak areas were then converted into micromoles equivalent levels of (+)-catechin (flavans), p-coumaric acid (hydroxycinnamic derivatives, HCD) and quercitrin (flavonols). When the flavan peaks were partially superimposed on the HCD peaks, the 280 nm flavan values were corrected by subtracting the HCD absorption values at this wavelength.

In addition, an overall phenol content (in micromoles phenolic equivalent per gramme FW) was obtained by adding the quantities determined for each of the three large separation groups above.

The HPLC variability was less than 10% in all the cases.

Statistical Analyses

The analyses below take into account all the results for each of the fourteen clones using five to seven different samplings, each of which was subjected to three HPLC analyses.

Principal component analysis (PCA) was performed using the amount of each phenol in order to examine the relationship between the molecules. Supplementary variables such as combinations of the different groups of phenols and the abscission percentage were introduced in the PCA.

A second set of analyses was carried out using multiple linear regression. A relation was sought between the explained variable (the abscission percentage in this case) and the explanatory variables (abundance of phenols). Use of the method was justified as the number of observations (84) was more than double the number of explanatory variables (2 × 32).

The model is therefore written as follows:

Percentage abscission =

$$a + bA1 + cA2 + dA3 + \ldots$$

in which $A1$, $A2$ ... represent the levels of the different phenols expressed in micromoles equivalent phenol per gramme FW. A stepwise procedure was used to maximise the determination coefficient R^2.

Analysis of variance was also performed on the phenols (quantitative character) according to the nature of the clone (qualitative character). When this analysis showed differences between clones for a particular phenol, the average contents of these phenols were determined using the Duncan test. The advantage of such analysis of variance is that no hypothesis concerning

the sensitivity of the clones to *M ulei* is required

The clones were also classified as being sensitive or resistant according to their percentage of abscission (more or less than 10%) Analysis of variance was then performed to compare the phenol contents of the two classes of clones

The results of these different analyses of variance do not necessarily correspond fully since different qualitative criteria are taken into account (clones in one case and sensitivity class in the other) Analyses of the correlation between the percentage of abscission and abundance of each of the phenols and the different ratios between phenolics were also carried out

Computations were made with 'Statistical analysis system Inc' (USA) and SYSPAD programs from COREF (Paris)

RESULTS

Direct Analysis of HPLC Results

Analysis using thin-layer chromatography (not reported here) and HPLC co-injection with commercial standards [(+)-catechin, chlorogenic acid, rutin] or standards extracted from other plant material[23] show that *Hevea* phenolics belong to three main groups flavans [(+)-catechin, (−)-epicatechin and derived substances] which are only detected by HPLC at 280 nm (*A* peaks), HCD whose maximum absorption is close to 314 nm (*B* peaks) and finally flavonol heterosides whose detection is maximum at 362 nm (*C* peaks) Several compounds were identified in each of these three groups when possible *(Table 2)*

Individual study of the different peaks of phenolics on HPLC chromatograms did not reveal any qualitative differences (*Figures 1* and *2*) Only clone CD 1078 displayed an extra peak *(C12)*

Several observations can be made concerning the quantitative results

- The flavan group forms the largest proportion of the phenols with an average of nearly 66% resistant clones also appeared to display a high rate *(Table 3)*
- In contrast, HCD were not abundant but the percentage in relation to the overall phenol content was greater in sensitive clones, except in clones PB 235 and PB 260 *(Table 3)*
- The HCD/flavan ratio was also higher in sensitive clones, except PB 235 and PB 260 as above

TABLE 2 IDENTIFICATION OF SEVERAL PEAKS OF PHENOLICS SEPARATED BY HPLC

Classes	Peak	Nature of the compounds
Flavans	A7	(+)-Catechin
	A10	(−)-Epicatechin (partly)
HCD	B5	*cis* 3' *p*-coumaroylquinic acid
	B6	*trans* 3' *p*-coumaroylquinic acid
	B9	*cis* 4' *p*-coumaroylquinic acid (+ Feruloylglucose ?)
	B10	*trans* 4' *p*-coumaroylquinic acid
Flavonols	C14	Rutin (Quercetin-3-rhamnoglucoside)
	C16	Isoquercitrin (Quercetin-3-glucoside)
	C19	Kaempferol-3-Rutinoside (= Kampferol-3-rhamnoglucoside)
	C21	Kaempferol-galactoside

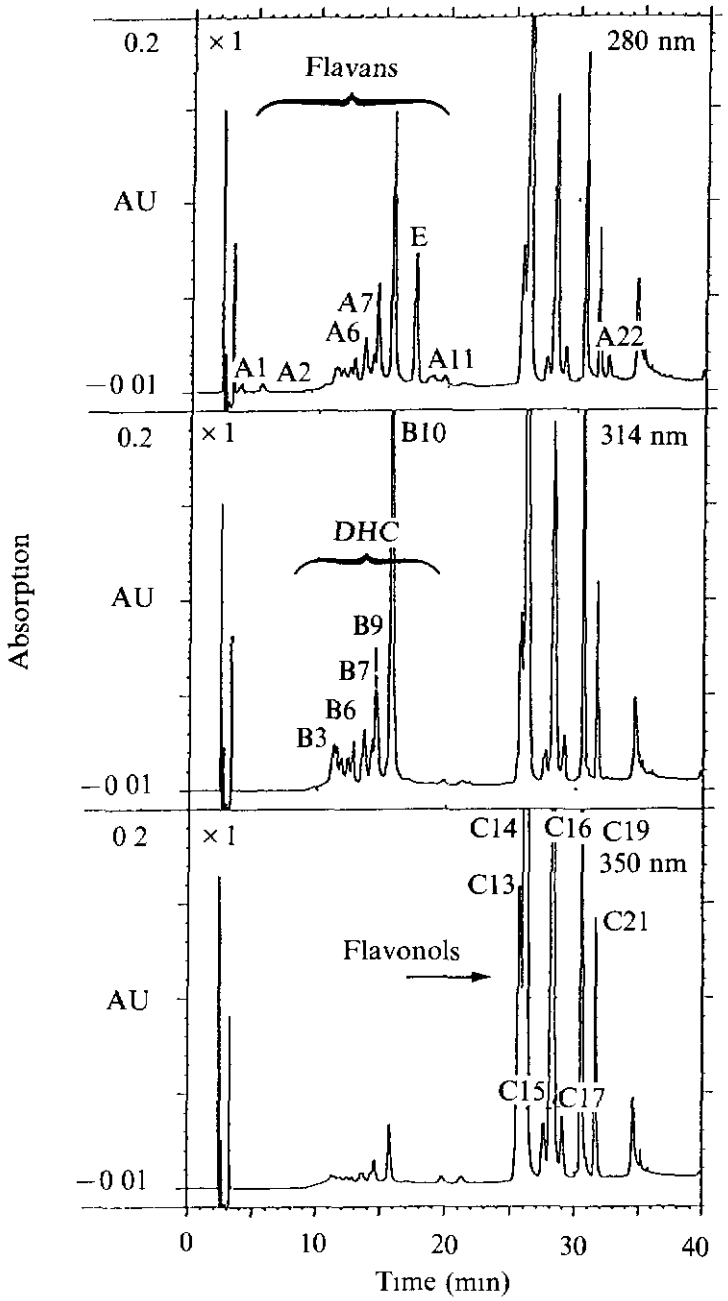

Figure 1 HPLC chromatogram of the phenols in a foliar sample of GT 1(S), (3.24 μg FW)

Individual analyses of each of the compounds separated by HPLC show that certain flavans *(A5, A7, A8, A22)* and the flavonol *(C14)* were more abundant in the resistant clones (*Tables 4* and *5*). In contrast, the HCD *B3, B4, B5, B6, B9* and *B10* and

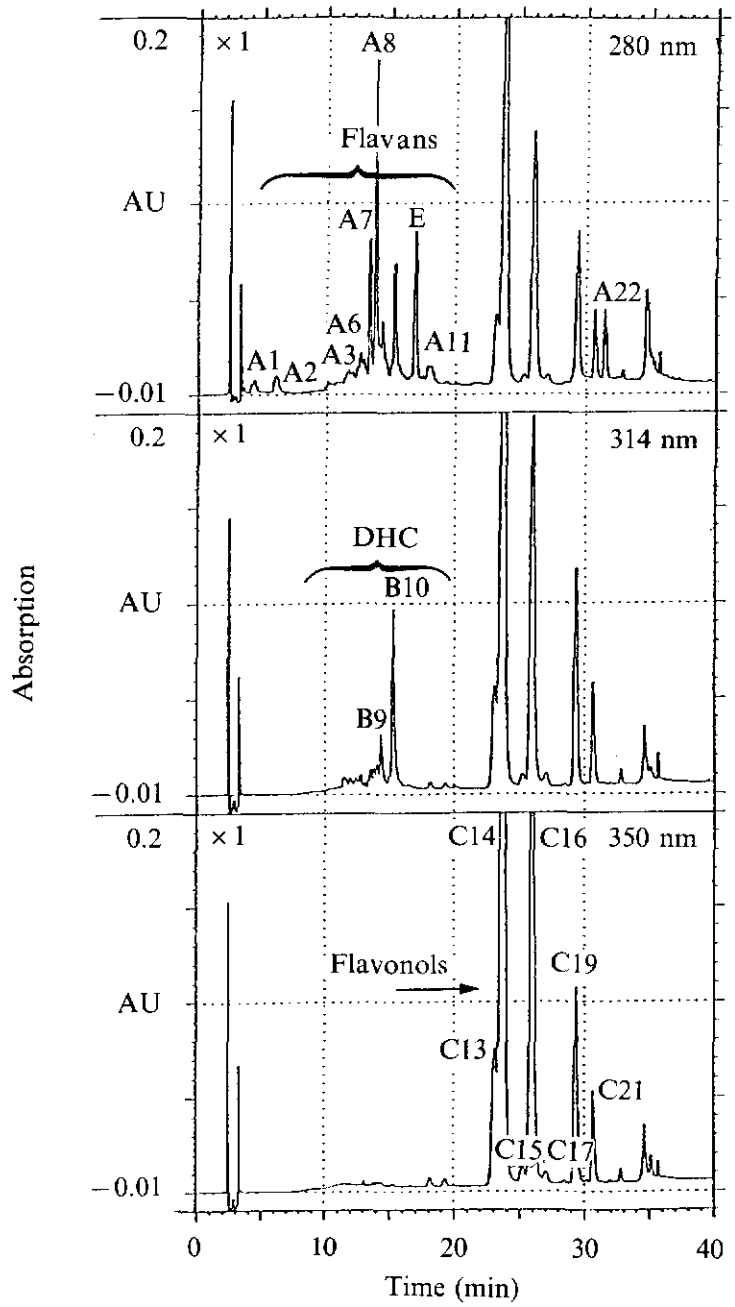

Figure 2. HPLC chromatogram of the phenols in a foliar sample of IAN 717(R), (4 μg FW).

the flavonols *C13, C15, C17* and *C18* were more abundant in the sensitive clones (*Tables 5* and *6*).

In addition, the chromatograms of the various clones appeared to show interesting variations between compounds *C19*

TABLE 3 CLONAL ANALYSIS OF THE THREE MAIN PHENOLIC GROUPS FLAVANS, HYDROXYCINNAMIC ACIDS (HCD) AND FLAVONOLS, AND THE OVERALL PHENOL CONTENT

Clone	% abscission	Total (μmol phenol eq/ g FW)	Flavans (μmol (+) catechin eq/ g FW)	Flavans (% sum of phenols)	HCD (μmol p coumaric acid eq/g FW)	HCD (% sum)	Flavonols (mmol quercitrin eq /g FW)	Flavonols (% sum of phenols)	HCD/ Flavans $\times 10^{2}$
PA 31	0 001	37 2 abcd	24 3 abcdef	66 9 ab	1 8 a	4 9 cd	11 1 ab	28 2 bcd	7 3 cd
IAN 717	0 001	36 2 abcd	26 1 abcdef	67 9 ab	0 9 e	2 9 cd	9 3 bcd	29 2 bcd	3 3 ab
CD 1078	0 8	49 7 abc	39 6 ab	77 8 a	1 5 abcd	3 2 cd	8 6 cde	19 0 cd	3 9 abc
RO 38	1 5	32 3 bcde	20 7 bcdef	62 6 ab	1 0 e	3 3 cd	10 6 abc	34 1 b	4 4 abc
IAN 2878	1 6	33 7 bcde	26 5 abcde	73 6 ab	1 6 ab	6 3 bc	5 6 de	20 1 bcd	6 3 cd
IAN 710	4 5	37 6 abcde	31 7 abcde	79 3 a	1 2 cde	5 0 cd	4 8 e	15 7 d	3 8 abc
FX 3864	4 5	51 6 ab	37 3 abc	72 2 ab	1 1 de	2 1 d	13 2 a	25 7 bcd	2 9 a
IAN 873	4 5	26 1 de	18 4 cdef	64 1 ab	1 3 bcde	6 4 bc	6 4 de	29 5 bcd	6 8 cd
Mean		37 7	27 7	70 2 A	1 3 B	4 2 B	8 8	25 6 B	4 3 A

TABLE 3 CLONAL ANALYSIS OF THE THREE MAIN PHENOLIC GROUPS FLAVANS, HYDROXYCINNAMIC ACIDS (HCD) AND FLAVONOLS, AND THE OVERALL PHENOL CONTENT (CONTD)

Clone	% abscission	Total (μmol phenol eq/ g FW)	Flavans (μmol (+) catechin eq/ g FW)	Flavans (% sum of phenols)	HCD (μmol p coumaric acid eq/g FW)	HCD (% sum)	Flavonols (mmol quercitrin eq /g FW)	Flavonols (% sum of phenols)	HCD/ Flavans $\times 10^2$
RRIC 101	11 6	20 9 e	9 7 f	41 2 c	1 8 a	10 3 a	9 3 bcd	48 4 a	17 7 d
PB 235	20 4	47 8 abcd	34 0 abcd	64 0 ab	1 7 ab	4 6 cd	12 1 ab	31 4 bc	5 3 bcd
IRCA 19	30 8	25 2 e	16 5 def	58 2 b	2 0 a	9 1 ab	6 8 de	32 7 bc	12 3 d
PB 260	40	57 2 a	43 9 a	73 4 ab	1 9 a	3 8 cd	11 5 ab	22 8 bcd	4 3 abc
IAN 713	45	27 8 cde	19 3 bcdef	67 6 ab	1 6 abcd	6 2 bc	7 0 cde	26 2 bcd	7 3
GT 1	45	20 5 e	13 2 ef	59 9 b	1 6 abc	9 4 ab	5 8 de	30 6 bc	12 7 d
Mean		32 4	22 0	60 3 B	1 8 A	7 4 A	8 6	32 3 A	7 9 B
Global mean		35 6	24 8	65 6	1 5	5 7	8 7	28 6	6 1
p clone		0 0012	0 0026	0 0002	0 0001	0 0001	0 0001	0 0002	0 0001
p sens		0 1943	0 1286	0 0023	0 0001	0 0001	0 8254	0 0134	0 0001

The overall phenol content was calculated by totalling the values in micromole equivalent for the three groups p clones, p sensitivity probability of null hypothesis between clones and between sensitivity classes a, b, c, *etc* , the figure followed by the same letters refer to clones which are not statistically different (Duncan test)

TABLE 4. MEAN LEVELS OF THE MAIN FLAVANS (μMOL EQUIVALENT (+)-CATECHIN/g FW)

Clone	% abscission	A3	A4	A5	A6	A7	A8	A9	A10	A11	A22
PA 31	0.001	0.56 ab	2.41 ab	0.97 ab	1 04 cd	3.54 bc	3.98 b	2 27 bc	2 66 bcd	4.09 cdef	2.78 ab
IAN 717	0.001	0.71 ab	1.00 c	0 74 ab	2.80 abc	4.79 bc	3.83 b	2.51 bc	2.40 bcd	4.60 cdef	2.76 ab
CD 1078	0.8	0 81 ab	1 59 bc	2.22 a	3.50 a	9.57 a	9.19 a	3.24 b	3.10 bcd	1.96 ef	4.40 a
RO 38	1.5	0 31 ab	0.97 c	0.99 ab	1 93 abcd	3.68 bc	3 40 b	1 40 bc	2 15 bcd	2.88 def	3.00 ab
IAN 2878	1 6	0 14 b	0.71 c	1.87 ab	2.13 abcd	4.34 bc	3.95 b	2.71 bc	2.24 bcd	5.24 bcde	3 16 ab
IAN 710	4.5	0.13 b	1.17 bc	1.13 ab	2.01 abcd	5.60 abc	4.64 b	1.89 bc	6.25 ab	5 82 bcd	2.62 ab
FX 3864	4.5	0.56 ab	1.44 bc	1.94 ab	2.74 abc	6 91 ab	4.80 b	2.36 bc	4.75 abc	7.83 ab	3.93 ab
IAN 873	4.5	0 b	0 96 c	0.64 ab	1 40 bcd	4.31 bc	3.54 b	0 52 bc	2.45 bcd	3 43 def	1.19 ab
Mean		0.40	1.28	1.27 A	2.16	5.29 A	4.56 A	2.06	3.25	4.48	2.95 A

TABLE 4 MEAN LEVELS OF THE MAIN FLAVANS (μMOL EQUIVALENT (+)-CATECHIN/g FW) (CONTD)

Clone	% abscission	A3	A4	A5	A6	A7	A8	A9	A10	A11	A22
RRIC 101	11 6	0.22 b	0.49 c	0.24 b	0.68 d	2.63 c	3.30 b	0 35 c	0 d	1 04 f	0 79 b
PB 235	20.4	0 53 ab	1 61 bc	1.34 ab	3.04 ab	6.77 ab	4.07 b	1.28 bc	5.56 ab	7 16 abc	2 58 ab
IRCA 19	30 8	0.32 ab	0 48 c	0 40 b	1.71 abcd	3.08 bc	2.08 b	0.97 bc	0.96 cd	3.01 def	3 45 ab
PB 260	40	1.45 a	2.90 a	0 83 ab	3 26 a	3.99 bc	3.20 b	8 13 a	7.42 a	10.3 a	2.35 ab
IAN 713	45	0 38 ab	1.05 c	0.87 ab	2.18 abcd	2.64 c	1 33 b	0.97 bc	2.92 bcd	6.34 bcd	0.61 b
GT 1	45	0 07 b	0 79 c	0.47 ab	0.94 d	3.21 bc	2.37 b	0.37 c	0 d	3 25 def	1.71 ab
Mean		0.48	1 18	0.67 B	1.91	3 58 B	2.67 B	1 96	2.66	5 05	1.83 B
Global mean		0.44	1 23	0 97	2.04	4.43	3 61	2 10	2.96	4 77	2.39
p clones p.sens		0.3087 0 6522	0 0026 0 7060	0 2914 0.0359	0.0041 0.4473	0 0063 0 0167	0 0043 0.0026	0 0001 0 8635	0.0013 0.4573	0 0001 0 4590	0 2865 0.0463

The overall phenol content was calculated by totalling the values in micromole equivalent for the three groups. p clones, p sensitivity: probability of null hypothesis between clones and between sensitivity classes a, b, c, *etc.*; the figure followed by the same letters refer to clones which are not statistically different (Duncan test).

TABLE 5 MEAN LEVELS OF THE MAIN FLAVONOLS (μMOL EQUIVALENT QUERCITRIN/g FW)

Clone	% abscission	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C19/ C21	C14/ C21
PA 31	0.001	0.15 b	0 b	1.21 bc	3.36 cdc	0 33 bc	3.13 abc	0.31 a	0.016 bc	1 32 ab	0 05 ab	1 22 a	1.085	2 762
IAN 717	0.01	0.05 cdc	0 b	0 68 d	4.61 bc	0.10 de	2.50 abcde	0.08 defg	0.005 cd	0.83 cde	0 03 ecd	0 38 cdc	2.197	12.247
CD 1078	0 8	0.44 a	0.16 a	0 72 d	1 93 f	0.41 ab	2.67 abcde	0.23 b	0 d	1.03 bc	0.03 bcd	0 97 ab	1.061	1.990
RO 38	1.5	0.02 de	0 b	0 63 d	5 32 b	0.10 de	2.89 abcd	0.10 cdef	0.013 bc	1.00 bc	0.03 bc	0.49 cde	2.032	10.813
IAN 2878	1.6	0 e	0 b	0 50 dc	2.70 def	0.08 de	1 39 ef	0.11 cde	0.017 bc	0.57 de	0.02 cd	0.20 e	2 841	13.283
IAN 710	4.5	0 e	0 b	0.30 ef	2.63 ef	0.03 e	1 08 f	0 05 efg	0 013 bc	0.54 c	0.02 cd	0.18 e	2.994	14.665
FX 3864	4.5	0.07 cd	0 b	0 f	7.71 a	0.04 e	3 38 ab	0 03 fg	0.005 cd	1.41 a	0.01 cd	0.55 bcde	2.547	13.924
IAN 873	4 5	0 e	0 f	0 f	3.18 def	0.01 e	1.39 ef	0.02 g	0 d	1.29 ab	0.01 cd	0.45 cde	3 180	7 040
Mean		0 10		0 50 B	4.03 A	0.13 B	2.33	0.11 B	0.009 B	1.01	0.03	0.55	2.242	9.603

TABLE 5 MEAN LEVELS OF THE MAIN FLAVONOLS (μMOL EQUIVALENT QUERCITRIN/g FW) (CONTD)

Clone	% abscission	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C19/ C21	C14/ C21
RRIC 101	11 6	0 01 de	0 b	1 07 c	3 41 cde	0 27 bc	2 10 bcdef	0 25 ab	0 02 b	1 41 a	0 04 bc	0 76 bcd	1 858	4 513
PB 235	20 4	0 09 c	0 b	1 54 a	4 08 bcd	0 51 a	3 51 a	0 30 ab	0 01 bed	1 28 ab	0 02 cd	0 78 bc	1 643	5 221
IRCA 19	30 8	0 03 de	0 b	0 71 d	2 49	0 22 cd	1 95 cdef	0 16 c	0 01 ecd	0 76 cde	0 02 cd	0 41	1 837	6 048
PB 260	40	0 09 c	0 b	1 47 ab	3 54 cde	0 52 a	3 82 a	0 23 b	0 04 a	0 92 cd	0 07 a	0 76 bcd	1 208	4 655
IAN 713	45	0 06 cde	0 b	0 44 de	3 31 cdef	0 12 de	1 75 def	0 07 defg	0 01 cd	0 81 cde	0 01 d	0 44 cde	1 851	7 555
GT 1	45	0 e	0 b	0 59 de	2 46 ef	0 11 de	1 37 ef	0 13 cd	0 01 bcd	0 76 cde	0 03 bcd	0 31 de	2 463	7 951
Mean		0 05		0 94 A	3 21 B	0 28 A	2 36	0 19 A	0 02 A	1 00	0 03	0 57	1 810	5 990
Global mean		0 08		0 70	3 61	0 21	2 35	0 15	0 01	1 00	0 03	0 56		
p clones		0 0001	0 0001	0 0001	0 0001	0 0001	0 0001	0 0001	0 0001	0 0001	0 0001	0 0001		
p sens		0 1060	0 0465	0 0001	0 0282	0 0005	0 9027	0 0017	0 0152	0 8253	0 4052	0 8374		

The overall phenol content was calculated by totalling the values in micromole equivalent for the three groups p clones, p sensitivity probability of null hypothesis between clones and between sensitivity classes a, b, c, *etc* , the figure followed by the same letters refer to clones which are not statistically different (Duncan test)

TABLE 6 MEAN LEVELS OF THE MAIN HYDROXYCINNAMIC DERIVATIVES (μMOL EQUIVALENT P-COUMARIC ACID/g FW)

Clone	% abscission	B3	B4	B5	B6	B7	B8	B9	B10
PA 31	0 001	0 08 dc	0 08 a	0 06 ab	0 07 abcd	0 23 a	0 22 a	0 17 abcd	0 89 abc
IAN 717	0 001	0 05 e	0 04 de	0 04 d	0 05 d	0 06 ef	0 10 def	0 11 d	0 43
CD 1078	0 8	0 05 e	0 02 e	0 03 d	0 06 bcd	0 05 ef	0 07 cd	0 17 abcd	1 05 ab
RO 38	1 5	0 08 de	0 04 de	0 04 bcd	0 05 cd	0 08 def	0 09 fe	0 11 d	0 46 e
IAN 2878	1 6	0 13 ab	0 05 bcd	0 16 a	0 07 abc	0 08 cdef	0 10 def	0 17 abcd	0 97 ab
IAN 710	4 5	0 10 cd	0 07 ab	0 07 a	0 05 cd	0 09 bcde	0 12 cde	0 12 d	0 54 de
FX 3864	4 5	0 06 de	0 04 cde	0 04 cd	0 05 cd	0 04 f	0 12 cde	0 13 cd	0 60 cde
IAN 873	4 5	0 16 a	0 08 a	0 07 a	0 07 abc	0 10 bcde	0 13 cde	0 15 bcd	0 55 de
Mean		0 09 B	0 05 B	0 05 B	0 06 B	0 09	0 12	0 14 B	0 67 B

TABLE 6 MEAN LEVELS OF THE MAIN HYDROXYCINNAMIC DERIVATIVES (μMOL EQUIVALENT P-COUMARIC ACID/g FW) (CONTD)

Clone	% abscission	B3	B4	B5	B6	B7	B8	B9	B10
RRIC 101	11 6	0 12 bc	0 06 abc	0 07 a	0 08 a	0 13 bc	0 09 fe	0 20 abcd	1 09 ab
PB 235	20 4	0 16 a	0 07 ab	0 07 a	0 08 a	0 09 cde	0 19 ab	0 25 a	0 82 abcd
IRCA 19	30 8	*0 15* ab	*0 05* bcd	*0 06* abc	*0 09* a	*0 12* bcd	*0 16* bc	*0 22* abc	*1 13* a
PB 260	40	0 14 ab	0 07 ab	0 06 ab	0 08 ab	0 13 b	0 16 bc	0 23 ab	1 05 ab
IAN 713	45	0 12 bc	0 07 ab	0 06 a	0 07 abc	0 09 cde	0 14 cd	0 19 abcd	0 81 bcd
GT 1	45	0 16 a	0 08 a	0 06 ab	0 07 abc	0 09 bcde	0 13 cde	0 21 abc	0 81 bcd
Mean		0 14 A	0 07 A	0 06 A	0 08 A	0 11	0 14	0 21 A	0 95 A
Global mean		0 11	0 06	0 06	0 07	0 10	0 13	0 18	0 81
p clone p sens		0 0001 0 0001	0 0001 0 0047	0 0001 0 0010	0 0002 0 0001	0 0001 0 2413	0 0001 0 0509	0 0039 0 0001	0 0001 0 0001

The overall phenol content was calculated by totalling the values in micromole equivalent for the three groups p clones, p sensitivity probability of null hypothesis between clones and between sensitivity classes a, b c *etc* the figure followed by the same letters refer to clones which are not statistically different (Duncan test)

(kaempferol-3-rutinoside) and *C21* (kaempferol-galactoside). The ratio of these compounds was 25% higher on the average in the resistant clones except for PA 31 and CD 1078; these were *H. pauciflora* and an interspecific *H. pauciflora* × *H. brasiliensis* hybrid *(Table 5)*. GT 1 was the only exception among the sensitive clones. Similar results were found for the rutin/kaempferol-galactoside ratio *(C14/C12)*, which was higher in the resistant clones *(Table 5)*, and for the rutin/total flavonol ratio (45.3% instead of 38.1%), again with the exception of clones CD 1078 and PA 31.

Statistical Interpretation of the Results

The phenol contents of the fourteen clones studied, with five to seven replications per clone, were processed using different statistical methods.

Principal component analysis (PCA). PCA was applied to the phenols. Supplementary variables were introduced: total flavans *(A)*, HCD *(B)*, flavonols *(C)*, the sum of *A1+A2 (Y)*, the sum of total phenols *Y+A+B+C(T)*, HCD/flavonols ratio *(BC)*, flavans/flavonols ratio and the abscission percentage *(X)*.

As a whole, the first three axes accounted for 58.2% of total variability: 28.3% by *Axis 1*, 18.8% by *Axis 2* and 11% by *Axis 3*. In simple terms, *Axis 1* was characterised by total phenol content and *Axis 2* was more related to the flavans *(Figure 3)*.

Unfortunately, variable *X* (percentage abscission) was only very weakly determined by *Axis 2* ($\cos^2 = 0.1720$, *i.e.* 65.5°C). This PCA does not give an overall picture of the relation between percentage abscission and the abundance of phenols.

Comparison of clones. The abundance of each phenol was investigated by analysis of variance (GLM procedure) according to the nature of the clone and hence without pre-conceptions concerning susceptibility to *M. ulei*. *Tables 4, 5* and *6* show significant clonal differences in the various phenolics analysed. For twenty compounds, the differences were significant at $P = 0.0001 - 0.0002$.

In addition, comparison of the classification of the clones for each of the phenols given by the Duncan test with their classification of susceptibility to *M. ulei* shows that differences between susceptible and resistant clones are due, for a given phenol, to a few clones. For example, amounts of *C14* are significantly higher in CD 1078 and FX 3864 than in the other twelve clones. Likewise, significantly low levels of *B4* are noticed only in IAN 717, RO 38, CD 1078 and FX 3864. Conversely, the resistant clone like IAN 2878 displays no differences in phenolic composition with the susceptible clones *(Table 7)*.

Comparison of susceptible and resistant clones. The data above reveal interclonal differences and suggest certain links between phenolics and sensitivity or resistance to *M. ulei*. A closer approach to the preliminary information was carried out by complementary statistical analysis of the sensitive and resistant clones according to whether the abscission percentage was lower (resistant) or higher (susceptible) than 10%.

Variance analysis *(Table 8)* highlights compounds *B3, B6, B9, B10* and *C13* and to a lesser extent *B4, B5, C15* and *C17* in the most sensitive clones. In contrast, phenolics *A5, A7, A8, A22* and *C14* were more abundant in the more resistant clones.

It was also sought to correlate the abscission percentage — as a sensitivity criterion — with the amounts of flavans, HCD and flavonols, the respective percentages of the various groups in relation to total phenols and to the ratios of the different groups to each other. This analysis gave the following information *(Table 9)*:

- *A11, B3, B4, B5, B6, B8, B9* and *B10* are correlated to abcission.
- *A7, A8, C14* and *C19* are negatively correlated with it.

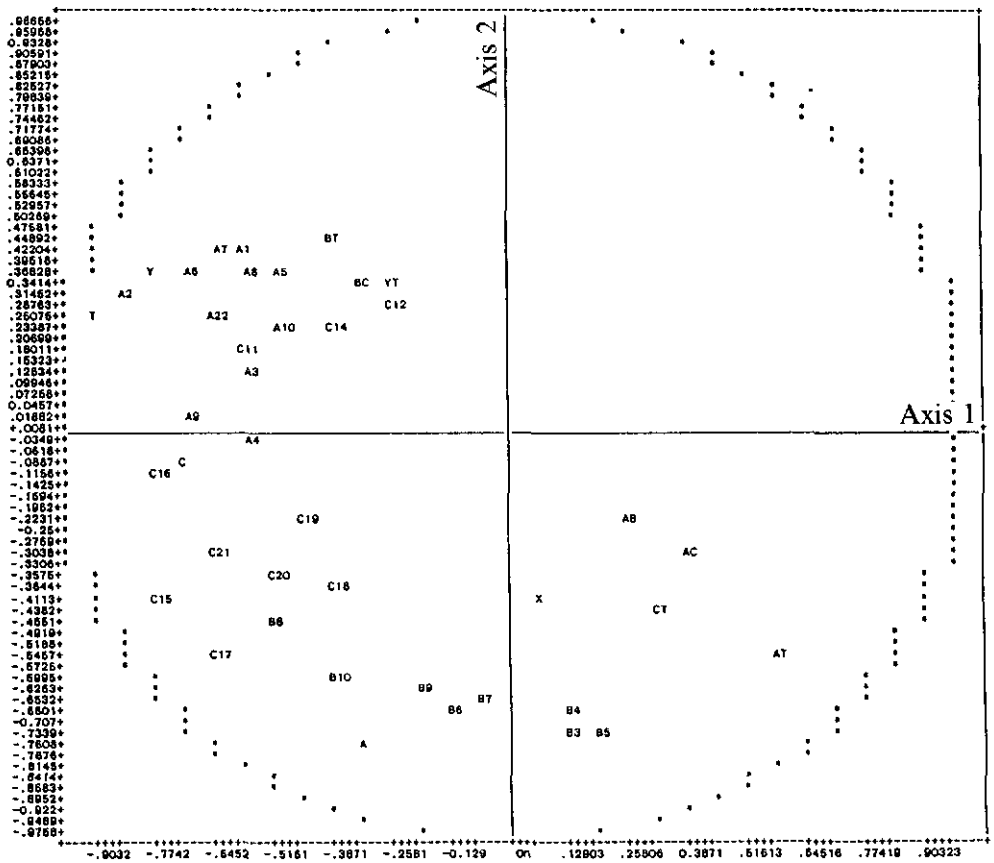

Figure 3. Correlation circle given by principal component analysis. Variable codes: A3 to A10 = flavans; B3 to B10 = hydroxycinnamic acid derivatives; C11 to C21 = flavonols; T = sum of phenols; A = sum of flavans; B = sum of HCD; C = sum of flavonols; Y = sum of A1+A2; AB = ratio of flavans/HCD; AC = ratio of flavans/flavonols; BC = ratio of HCD/flavonols; YT = ratio of A1+A2/total phenols; AT = flavans/total phenols; BT = HCD/total phenols; CT = flavonols/total phenols; X = % abscission. Superposed points: A2 (B), A3 (A11), C17 (C13).

- The HCD percentage was correlated positively to the abscission percentage with extremely high probability; a high level of HCD would indicate a susceptible clone. The HCD/total phenols ratio should also be noted; this varies like the HCD level. Only the HCD/total flavonols ratio was correlated negatively.

Successive multiple regression. This method showed that a linear correlation of several peaks significantly characterised the degree of susceptiblity of the clones *(Table 10)*. Starting with the HCD *B3*, which had already been noted for its abundance in susceptible clones, the following, in descending order of importance, were phenols *C19* and *A8*, which were negatively correlated

TABLE 7. ANALYSIS OF VARIANCE OF PHENOLIC COMPOUNDS

Clone	% abscission	A 5	A 7	A 8	A 22	B 3	B 4	B 5	B 6	B9	B 10	C 13	C 14	C 15	C 17	C 18
PA 31	0.001					+									+	
IAN 717	0.001					+	+	+	+	+						+
CD 1078	0.8	+	+	+	+	+	+	+					+			+
RO 38	1.5					+	+		+	+	+					
IAN 2878	1.6															
IAN 710	4.5					+				+	+	+		+	+	
FX 3864	4.5					+	+	+				+	+	+	+	+
IAN 873	4.5										+	+		+	+	+
RRIC 101	11.6	+	+		+			+							+	
PB 235	20.4					+		+	+			+		+	+	
IRCA 19	30.8	+						+		+				+		
PB 260	40							+	+			+				+
IAN 713	45		+		+											
GT 1	45					+										

Clones which have the higher contribution in the differences between sensitive and resistant clones.

TABLE 8. COMPARISON OF THE PHENOLIC LEVELS IN SENSITIVE AND RESISTANT CLONES

Phenolic level (m mol/g FW)	Sensitive clones	Resistant clones	Probability p
A5	0.67	1.27	0.036
A7	3.58	5.29	0.017
A8	2.67	4.56	0.0026
A22	1.83	2.95	0.046
B3	0.14	0.088	0.0001
B4	0.067	0.053	0.005
B5	0.062	0.049	0.001
B6	0.078	0.058	0.0001
B9	0.212	0.14	0.0001
B10	0.954	0.67	0.0001
C12	0	0.017	0.047
C13	0.94	0.50	0 0001
C14	3.20	4.03	0.028
C15	0.28	0.13	0.0005
C17	0.19	0.11	0.0017
C18	0 015	0.008	0.015

TABLE 9. CORRELATIONS BETWEEN THE PHENOLIC CONTENT AND PERCENTAGE FOLIAR ABSCISSION

Phenol	r	ρ
A5	−0 178	0.105
A7	−0.248	0.023
A8	−0.352	0.001
A11	0.225	0.040
A22	−0.195	0.076
B3	0.525	0.0001
B4	0.338	0.002
B5	0.252	0.021
B6	0 392	0.0002
B8	0.236	0.031
B9	0.409	0.0001
B10	0.300	0.006
C11	−0.170	0.122
C12	−0 208	0.057
C13	0 192	0.080
C14	−0.237	0.030
C15	0 191	0.080
C18	0 167	0 128
C19	−0.220	0.045
DHC	0.382	0 0003
DHC/Sum	0.292	0 007
DHC/Flavonol	−0.382	0.022

with abscission and hence positively with resistance, and then phenols *A11* and *B9*, which were positively correlated with abscission and hence with susceptibility. *C19* alone has only a slight influence on susceptibility *(Table 9)*, but in combination with other phenolics, the analysis shows its significance.

DISCUSSION AND CONCLUSIONS

As a preliminary approximation, this investigation did not establish simple, clearly-defined relationships between the constituent phenolic compounds in young healthy leaves of clones cultivated under plastic and the resistance of these clones to *M. ulei* estimated by the foliar abscission percentage induced by the parasite in the same adult clones in a plantation. However, a number of interesting indications emerged concerning some phenolics and some ratios of levels of the different groups of phenols.

The overall phenol content and the flavan content appear to be related to resistance, except in clones PB 235 and PB 260 which, although classified as susceptible, contain high levels of these compounds. High HCD and flavonol levels — except in the same two clones — appear to be related to susceptibility. Nevertheless, some of the flavonol ratios *(C12/C21, C14/C21)* and the rutin content appear to be related to resistance.

Overall, the various statistical analyses performed confirm these preliminary observations. Analysis by successive multiple regression characterised the phenolics positively correlated with susceptibility (compounds *B3*, *A11* and *B9*, HCD/flavan ratio, HCD level). In contrast, a number of compounds appear to be more related to resistance (kaempferol-3, rutinoside, *A8*, rutin).

The different statistical analyses result in data displaying coherence for the results as a whole *(Table 11)*. Indeed, the same phenolics are often the most abundant in susceptible clones while others are more abundant in resistant clones.

The investigation shows that there are no marked differences in the nature of the constituent phenolic compounds in the various clones or species of *Hevea*. Only quantitative differences were shown between the susceptible and resistant clones. However, certain difficulties in interpretation may result from the action of factors which increase intraclonal variability:

- sampling distributed irregularly in time

TABLE 10. ANALYSIS USING STEP-WISE MULTIPLE LINEAR REGRESSION

Step	Variable entered	Variable removed	R^2 Partial	R^2 Model	C(P)	F	Prob > F
1	B3		0.2760	0.2760	21.1096	31.2595	0 0001
2	C19		0 0494	0.3253	16 2138	5.9287	0.0171
3	C15		0.0588	0.3842	10.0049	7 6357	0 0071
4	A8		0 0533	0.4374	4.5627	7.4837	0.0077
5	A11		0.0401	0.4775	0.9686	5.9798	0 0167
6	B9		0.0233	0.5008	−0.2803	3.5881	0 0620
7	B5	C15	0.0226	0.5234	−1.4429	3.6113	0.0612
8			0.0115	0.5119	−1.8380	1 8327	0.1798
9	A3		0.0136	0.5255	−1 7390	2 1805	0 1439

Regression curve equation:

% abscission = 8.0 + 0.0027 A3 − 0.0020 A8 + 0.0011 A11 + 0.17 B3 − 0.24 B5 + 0.076 B9 − 0.010 C19

C(P) is the Mallow's statistic.

TABLE 11. COMPARISON OF THE PHENOLIC COMPOSITION OF SENSITIVE AND RESISTANT CLONES USING DIFFERENT METHODS OF STATISTICAL ANALYSIS

Table	Type of analysis	Characteristics of clones	
		Sensitive	Resistant
3	Variance analysis (Classes of sensitivity	% Flavans HCD/Flavans	HCD HCD/Sum of phenols % Flavonol
4, 8	Variance analysis (Classes of sensitivity)		A5, A7, A8 A22
5, 8	Variance analysis (Classes of sensitivity)	B3, B4, B5, B6, B9, B10	
6, 8	Variance analysis (Classes of sensitivity)	C13, C15, C17, C18	C14
9	Linear regression	A11, B3, B4, B5, B6, B8, B9, B10 HCD HCD/Sum HCD/Flavonol	A7. A8, C14, C19
10	Step-wise regression	A3, A11, B3, B9, C15	A8, B5, C19

- physiological changes, especially in the phenolic metabolism of the plants under plastic; in this case, the material analysed may not fully express the potential of the trees in the plantation used to establish the clonal susceptible scores.

Should the importance of (+)-catechin *(A7)* and other flavans and rutin *(C14)* in relation to the flavans as a whole be confirmed, the results would agree overall with those reported in the literature. The fungi-toxicity of extracts of young leaves has been attributed to a yellow compound —

probably a flavonol — which appears to be more abundant in IAN 710 (relatively resistant here and in Guiana) than in GA 1126 (susceptible)[14] The greatest abundance of aglycone quercetin was reported in resistant clones[16] (with a few exceptions) This would appear to support our remark on the percentage of rutin in relation to total flavonols, assuming that the compound referred to by Figari[14] and Hashim *et al*[16] is rutin and not kaempferol-rhamnodiglucoside, as was reported by Martins *et al*[15], who worked on old leaves, unlike Figari[14] and ourselves

With regard to the possible importance of (+)-catechin *(A7)* and certain other flavans *(A8, A22)*, these results might be similar to data showing that sorghum seeds resistant to grain mould complex have a higher flavan content than sensitive seeds[24] It has been suggested that the catechin present in onion bulb coats before infection contributes to resistance to *Colletotrichum circinans* by inhibiting spore germination[25] It has also been shown that catechin has an inhibitory effect on the germination of *M ulei* conidia[14]

In spite of the positive aspects reported above, our results nevertheless seem to show that most constituent phenolics do not play a major role in the resistance of young *Hevea* leaves to *M ulei* However, they may be involved in the setting up of induced resistance mechanisms Hashim[26] showed that it is possible to induce resistance to SALB using incompatible *M ulei* races He observed that the resistance remains localised in the induced zone and may therefore be caused by host cell collapse or the production of phytoalexins The first mechanism was observed by Blazquez and Owen[8] and Hashim *et al*[9] and the second by Gieseman *et al*[21] for scopoletin Scopoletin is considered a phytoalexin in *Hevea* and, logically, was not detected in our uninfected material

As the criterion of susceptibility to *M ulei* used here was abscission percentage, there may be links between constituent phenols and abscission which depend only indirectly on the action of pathogens in general and *M ulei* in particular As has been pointed out, some phenols activating auxin-oxidase[19] may thus enhance leaf abscission in leaves where pathogen attack has favourably changed the auxin-ethylene ratio

Finally, it can be considered that even if the constituent phenolics in young *Hevea* leaves (with the possible exception of *C14, A8* and *A22*) do not participate directly in defence mechanisms against *M ulei*, some may nevertheless be retained as sensitivity markers Elsewhere, the total phenolics present in the leaves may contribute to passive resistance even if there is a lack of correlation in their content Phenolics may be important especially in hypersensitive reactions

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