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Qualitative and quantitative variation in pathogenicity of races of coffee leaf rust (Hemileia vastatrix) detected in the State of São Paulo, Brazil

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Abstract

Between 1977 and 1981, seven qualitatively distinct new races of coffee leaf rust *(Hemileia vastatrix)* were detected in breeding plots in the State of São Paulo, Brazil. Four races carry complex virulence against the resistance genes S_H1, S_H2, S_H4 and S_H5 of *Coffea arabica.* Three races match unidentified resistance genes of *C. canephora.* Two of these were isolated from cv. Kouillou and one from the hybrid population Icatu. Pending further identification, these races were indicated by the number of their type cultures (Is. 2, 10 and 11). Is. 2 and Is. 10 showed extra virulence to some coffee genotypes and decreased virulence to other coffee genotypes, suggesting stabilizing selection.

Three rust isolates were detected which differed quantitatively from the common rust race II. Is. 1 was moderately virulent to the coffee differential for S_H3 in the laboratory but avirulent in the greenhouse, indicating a host × pathogen × environtment interaction. Is. 3 and 12 showed levels of virulence intermediate between race II and Is. 2 and 10, respectively.

The results show that incomplete resistance of coffee to *H. vastatrix,* at various levels, can be race-specific. The nature of race formation of coffee leaf rust in Brazil and breeding strategies for obtaining durable resistance are discussed.

Additional keywords: incomplete resistance, durable resistance, stabilizing selection.

Introduction

More than 30 races of *Hemileia vastatrix* Berk. et Br. (orange coffee rust, coffee leaf rust) have been identified worldwide. They are maintained at the Coffee Rusts Research Center (CIFC) in Oeiras, Portugal (Rodigues et al., 1975). Race II has the widest distribution. It was the first to be detected on the American continent, in Brazil, 1970, as identified at CIFC. The rust has now invaded most American coffee producing countries (Wellman and Echandi, 1981). Nearly all cultivars of *Coffea arabica* L. grown in these countries are susceptible to race II. In Brazil, yield losses due to coffee leaf rust have been estimated to be 30%, if no control measures are taken (Monaco, 1977). Breeding efforts based on the resistance genes S_H1 to S_H4 of *C. arabica* (Bettencourt and Carvalho, 1968; Carvalho et al., 1974) were, to a great deal, frustated by the detection of the races III ($v_{1,5}$), I ($v_{2,5}$), and XV ($v_{4,5}$) in the State of São Paulo, within four years (Ribeiro et al., 1975). Presently the interest is focussed on resistance genes derived from *Coffea canephora* Pierre, available in advanced breeding lines of the hybrid populations Catimor and Icatu (Monaco, 1977). These populations contain resistance to some or all races kept at CIFC (Rodrigues et al., 1975; Marques and Bettencourt, 1979). Susceptible plants of these populations vary greatly in their level of disease in the field (Costa and Ribeiro, 1975). Incomplete minor gene resistance may confer a more durable protection to plant pathogens than complete resistance related to major genes (Vanderplank, 1968; Parlevliet and Zadoks, 1977). Durable resistance is of great importance, especially for a perennial crop such as coffee.

To study the nature of incomplete resistance the variability in the pathogen has to be taken into account. In the present research quantitative and qualitative virulence of coffee leaf rust races was studied.

Materials and methods

Definitions. 'Incomplete' resistance is here defined as a form of resistance which does not fully inhibit reproduction of the pathogen. 'Race-specific' or 'specific' resistance can be partly or totally overcome by changes in the genotype of the pathogen. 'Major gene' resistance is related to one or a few genes, each with a great effect on resistance. Major gene resistance may be complete or incomplete (Parlevliet, 1979). For the pathogen, the term 'virulence' is used to indicate the degree of specific pathogenicity. 'Incomplete virulence' is used in analogy to incomplete specific resistance. The term 'qualitative' is used here to indicate that different genotypes show easily distinguishable phenotypes and 'quantitative' when differences between genotypes are not easily distinguishable. A 'physiologic race' or 'race' contains isolates with identical virulence spectrum. In the tests one isolate (Is.) per race has been used as 'a type culture'.

Designation of virulence and resistance. The commonly adopted race designation for *H. vastatrix* has been used (Rodigues et al., 1975). Resistance genes of coffee (S_H) and virulence factors (v) of *H. vastatrix* have been postulated following the gene-for-gene theory (Noronha-Wagner and Bettencourt, 1967). Races maintained at CIFC, Portugal, are identified by the roman numerals I to XXXII. In the present paper, races with virulence to unidentified resistance genes have not been given a race number, to avoid possible confusion with future race designation at CIFC.

Coffee genotypes. The differential genotypes of *C. arabica* (Tables 1 and 2) belong to the collection of the Department of Phytopathology at the I.A.C. They are cuttings from differentials maintained at CIFC in Portugal. *C. arabica* cv. Mundo Novo was used as a susceptible check in most experiments. Cv. Ibaarê (LC 1133-2) is derived from a *C. arabica* accession from the Harrar province in Ethiopia. Cv. Ibaarê is more susceptible in the field than cv. Mundo Novo. Both culivars carry the S_H5 resistance gene . The Icatu and Catimor populations consist of advanced tetraploid breeding lines derived from an artificial (Icatu) and natural (Catimor) hybrid between *C. canephora* ($2x = 22$) and *C. arabica* ($4x = 44$). *C. canephora* cv. Kouillou is commercially grown in the State of Espirito Santo, Brazil. Although most plants of cv. Kouillou are susceptible to race II of *H. vastatrix,* great variation in disease level in the field can be observed between plants. Field plants used were about 10 years old

(Icatu, Catimor, *C. arabica* cvs) or about 45 years old (cv. Kouillou). Cuttings from field plants were grown under moderate light conditions in the greenhouse and used intests when about one year old.

Rust genotypes. Single-lesions isolates from race II (v_5), III ($v_{1,5}$), I ($_{2,5}$) and XV ($v_{4,5}$), obtained from the Department of Phytopathology of the I.A.C., were used. Field isolates were taken either from many lesions or single lesions. Single-lesion isolates can, with high probability, be considered to be pure as indicated by observations of J.G.J. Hoogstraten (personal communication). Isolates 1,2, 3, 10, 11 and 12 were obtained from coffee genotypes which, under the conditions of isolation, were completely resistant to race II. Therefore, they can be considered to represent races different from race II.

Isolates and races were maintained on susceptible plants in the greenouse, and urediospores were stored in a refrigerator at 52% relative humidity. Purity of races and isolates was checked regularly by inoculation on differential coffee plants.

Inoculation methods. Inoculations were carried out in the field, greenhouse or laboratory by applying suspensions of urediospores to the abaxial side of coffee leaves (Eskes, 1982a). The laboratory method consists of inoculations of leaf disks (1.8 cm diameter), each with a 25 μl droplet of a suspension of urediospores in destilled water with densities of 0.5 to 1.5 ml^{-1}.The reliability of this method in assessing resistance to coffee leaf rust is shown by Eskes (1982a). The temperature in the laboratory was kept at 22 ± 2 °C.

The number of disks per treatment used for the determination of qualitative differences in resistance was 15 to 25 (Tables 1 and 4) and for quantitative differences 60 (Table 2), 120 (Table 3) and 45 (Table 5). Disks taken from the same leaf were distributed over different treatments in order to decrease residual variance. For field tests rust free leaves of two branches were used. These tests were carried out when the level of disease in the field was low to avoid contamination by natural infection. For greenhouse tests (Tables 2 and 6) between 6 and 12 leaves were used per treatment.

Inoculation dates. Results shown were obtained during 1978 to 1981 (Table 1), 1979-1980 (Table 2), November 1980 and March 1981 (Table 3), March 1981 (Table 4), and May 1981 (Table 5).

Disease assessment. Reaction types were scored by a 0-9 scale (Eskes and Toma-Braghini, 1981). Scale value 0 indicates absence of visible symptoms, values 1 to 3 variation within resistant reaction types (small flecks and tumefactions to large chlorotic areas without sporulation), values 4 to 7 heterogeneous reaction types with increasing sporulation intensity and percentage of sporulating lesions, and values 8 and 9 indicate susceptible reaction types with moderate (8) to high (9) sporulation intensity. Reaction types indicated by R (resistant) represent scale values 0 to 3, by MR (moderately resistant) 4 and 5, by MS (moderately susceptible) 6 and 7, and by S (susceptible) 8 and 9 (Table 1). Scoring was done when no further development of reaction types occurred. Detailed information on how resistance components were observed is given by Eskes (1982a).

Statistics. Use was made of SPSS (Statistical package for the Social Sciences). Scores for reaction type using the 0 to 9 scale were normally distributed if no extremes were involved. Therefore, linear regression models were applied in the computations for reaction type shown in Table 5.

Results

Races in relation to C. arabica. The sequence of detection of 11 distinct races in the State of São Paulo, between 1971 and 1980, and their qualitative reaction in leaf disk tests are shown in Table 1. All local *C. arabica* cultivars and most breeding lines of *C. arabica* are homozygous for the S_H5 resistance allele. They are susceptible to race II (v_5), which arrived in the state of São Paulo in 1971. Till 1974, races I ($v_{2,5}$), III ($v_{1,5}$) and XV ($v_{4,5}$) were isolated from *C. arabica* cv. Mundo Novo (Ribeiro et al., 1975). From 1976 to 1981, the author assessed annually rust incidence in breeding plots of the Experimental Station of the I.A.C. at Campinas. During this period, coffee plants carrying the resistance genes S_H1, S_H2 or S_H4 showed low to moderate (S_H4) or moderate to high (S_H1 and S_H2) disease levels in the field. Therefore, races I, III and XV were widely present in the breeding plots.

In 1977, a small rust focus was found on a plant with genotype S_H1s_H1 S_H2s_H2 S_H5S_H5. This plant stood between plants of genotype S_H1s_H1 s_H2s_H2 S_H5S_H5, which were heavily attacked by rust. Isolates from this rust focus belonged to race XVII ($v_{1,2,5}$). In 1978, when rust incidence in the field was low, the size of the focus did not increase much. In 1979, the rust incidence in the field was high and nearly all plants of genotype S_H1s_H1 S_H2s_H2 S_H5S_H5 in different breeding plots, scattered over the Experimental Station, became moderately or highly diseased.

In 1979, race X ($v_{1,4,5}$) was isolated from S_H1s_H1 S_H4s_H4 S_H5S_H5 plants, which showed low to moderate disease levels for the first time.

In the same year moderate rust attacks were found on plants with genotype S_H2s_H2 S_H4s_H4 S_H5S_H5, but only race I ($v_{2,5}$) could be isolated from these plants. The result suggests that genotypes heterozygous for S_H4 are not completely resistant in the field to supposedly avirulent rust races. A similar result was obtained in the nursery (Eskes, 1982b). In 1980, the rust incidence on S_H2s_H2 S_H4s_H4 S_H5s_H5 plants had increased considerably and race XXIV ($v_{2,4,5}$) could be isolated. In the same year this race was isolated by the Instituto Biológico in São Paulo (Fanucchi et al., 1980).

Race XXIII ($_{1,2,4,5}$) was isolated in 1980 from a *C. arabica* field plant with unidentified resistance genotype.

Races in relation to C. canephora. In 1979 and 1980, races with virulence to resistancefactors of *C. canephora* were found for the first time in Brazil. In 1979, some formerly resistant Icatu plants showed low to moderate rust incidence. Is. 2 (Table 1) was obtained from plant H3851-4-40, which in 1980 and 1981 became as much rusted as *C. arabica* cv. Mundo Novo. In 1980, Is. 2 caused moderate to high levels of disease on about 40 formerly resistant or moderately resistant plant, in a field with about 1500 resistant Icatu plants. A few of these Icatu plants had shown disease before, in 1975, but not in 1976 and 1977. They became diseased again between 1977 and 1980. Therefore, Is. 2 is supposed to have been present in Icatu in 1975 already.

Table 1. Reaction types of rust races, detected in the State of São Paulo, Brazil, between 1970 and 1981, on leaf disks of seven coffee genotypes.

Race or isolate	Coffee genotypes							Virulence factors	Year of detection	Reference of detection
	C. arabica differentials				Icatu	*C. canephora* cv. Kouillou				
	resistance genes									
	S_H1	S_H2	S_H4	S_H5	H3851-4-40	C66-13	C67-1			
II	R[1]	R	R	S	R	R	MR	v_5	1971	–
III	S	R	R	S	R	R	–[2]	$v_{1,5}$	1974	Ribeiro et al., 1975
I	R	S	R	S	R	R	–	$v_{2,5}$	1974	Ribeiro et al., 1975
XV	R	R	S	S	R	R	–	$v_{4,5}$	1974	Ribeiro et al., 1975
XVII	S	S	R	S	R	R	–	$v_{1,2,5}$	1977	Eskes et al., 1979
X	S	R	S	S	R	R	–	$v_{1,4,5}$	1979	Eskes et al., 1980
XXIV	R	S	S	S	R	R	–	$v_{2,4,5}$	1980	Fanucchi et al., 1980
XXIII	S	S	S	S	R	R	–	$v_{1,2,4,5}$	1980	Personal observation
Is. 2	R	R	R	MS	MS	R	MR	$v_{5+?}$	1979	Eskes et al., 1981
Is. 10	R	R	R	S	R	S	R	$v_{5+?}$	1980	Eskes et al., 1981
Is. 11	R	R	R	S	R	R	S	$v_{5+?}$	1980	Eskes et al., 1981

[1] Reaction types are indicated by S (susceptible), MS (moderately susceptible), MR (moderately resistant) and R (resistant).
[2] A dash (–) indicates no observation or no reference.

Tabel 1. Reactietypen van roestfysio's, ontdekt in de staat São Paulo, Brazilië, tussen 1970 en 1981, op bladschijven van zeven koffiegenotypen.

It may have disappeared temporarily due to frost in the winter of 1975. Is. 2 also overcomes the resistance of a few Catimor genotypes (Table 3). Out of 60 genotypes tested, two were more susceptible to Is. 2 than to race II.

Two races in relation to genotypes of *C. canephora* cv. Kouillou were isolated in 1980. Is. 11 was obtained from genotype C67-1. This plant was heavily rusted during the whole observation period, so Is. 11 has been present at least from 1976 onward. Is. 10 was obtained from genotype C66-13, which in 1980 showed rust for the first time, be it on one leaf only. In 1982 the rust incidence on this plant was still relatively low. In the observed population of 65 'Kouillou' genotypes, nine were highly resistant to race II and four out of these nine were suceptible to either Is. 10 or Is. 11.

Low virulence to S_H3*.* About 200 field plants, homozygous or heterozygous for the S_H3 resistance allele, were observed annually. On homozygous plants no rust was found during the observation period. In July 1977, a few heterozygous plants, showed some rust lesions in the lower leaf canopy, with moderate to intense sporulation. Small rust foci reappeared in the following years, generally on other plants also heterozygous for S_H3, at the end of the growing season (May,June) when the rust was more severe. Between years, some variation in number and size of these foci occurred, depending on the general disease level in the field, but no consistent increase was observed over the years. Is. 1, used as the type culture isolated from these foci, was tested twice in 1979 and 1980 in the greenhouse and nursery and gave similar results (Table 2). In the greenhouse tests, Is. 1 was avirulent on clone CIFC 33/1 (S_H3S_H3 S_H5S_H5), although its reaction type was higher than that of race II. In the laboratory tests, moderate to high virulence of Is. 1 on clone CIFC 33/1 was observed. On intact leaves, some well sporulating lesions developed among lesions of resistant types. Spores from these lesions were reused for inoculations in the greenhouse, but again avirulence was observed on clone CIFC 33/1. On cv. Mundo Novo no difference between Is. 1 and race II was observed.

Tabel 2. Range of reaction types in laboratory and greenhouse tests of the differential CIFC 33/1 and of cv. Mundo Novo with *H. vastatrix*, race II (v_5) and isolate 1 (Is. 1).

Coffee genotype	Resistance factors	Race or isolate	Reaction type (range)		
			laboratory		greenhouse
			leaf disks	attached leaves	attached leaves
CIFC 33/1	$S_H3S_H3S_H5S_H5$	Race II	0-1	0-2	0-1
		Is. 1	6-8	4-5	2-3
Cv. Mundo Novo	$s_H3s_H3S_H5S_H5$	Race II	8-9	–	8-9
		Is. 1	8-9	–	8-9

Tabel 2. Variatie in reactietype in laboratorium- en kastoetsen van het differentiërende genotype CIFC 33/1 en van cv. Mundo Novo met H. vastatrix, fysio II (v_5) en isolaat 1 (Is. 1).

In addition, isolates with virulence similar to Is. 1 were obtained twice in leaf disk tests in low frequencies from rust cultures belonging to race II.

It is concluded that Is. 1 is a stable rust genotype, distinct from race II, with incomplete virulence to S_H3. Is. 1 occurs apparently rather frequently in the rust population of Campinas. Its presence can only be detected under specific test conditions, indicating a host × pathogen × environment interaction.

Isolate 2. Is. 2 was isolated in the field from the Icatu genotype H3851-4-40, which was completely resistant to race II. Table 3 summarizes the differences in virulence between race II and Is. 2 observed in two field tests (1980, 1981) and in one laboratory test (1981). Differences in virulence between the rust isolates was somewhat greater in the laboratory than in the field but the same tendency was observed in both tests.

Is. 2 was more virulent than race II on several Icatu genotypes and on a few Catimor genotypes (e.g. C2012-19, Table 3). Besides, Is. 2 was less virulent than race II on *C. arabica* cv. Mundo Novo and avirulent to some Catimor genotypes (e.g. C2477-2, Table 3) to which race II was virulent. Similar results have been obtained in several greenhouse tests.

Is. 2 showed a lower virulence than race II also on other *C. arabica* cultivars, like cvs Catuai, Ibaarê and Matari, as observed in the greenhouse. Even on the highly susceptible cv. Ibaarê sometimes a lower reaction type was obtained with Is. 2, especially on young leaves.

Table 3. Average reaction type (RT, 0 to 9 scale) and latency period (LP, in days) of coffee genotypes inoculated in the field and in the laboratory with rust race II and isolate 2 (Is. 2). Is. 2 was obtained from Icatu genotype H3851-4-40 in the field.

Coffee genotype	Disease score in the field (1-5 scale)		Field test[1]		Laboratory test[1]			
			RT		RT		LP	
	1975	1979	race II	Is. 2	race II	Is. 2	race II	Is. 2
C. arabica								
cv. Mundo Novo	5	5	9.0	7.5	9.0	8.5	24	27
Icatu:								
H8351-2-689	3	5	7.5	9.0	7.5	9.0	35	25
H4782-10-203	2	5	5.3	7.8	4.0	8.0	39	25
H3851-4-40	1	4	3.3	5.5	1.0	8.0	–	32
Catimor:								
C2477-2	–	3	–	–	8.0	2.5	30	–
C2012-19	–	5	3.5	7.0	1.0	8.0	–	30

[1] Each entry is based on leaves from 3 branches (field test) or on 15 leaf disks (laboratory test).

Tabel 3. Gemiddeld reactietype (RT, 0 tot 9 schaal) en latentieperiode (LP, in dagen) van koffiegenotypen die in het veld en in het laboratorium zijn geïnoculeerd met roestfysio II en isolaat 2 (Is. 2). Is. 2 werd in het veld gevonden op Icatu genotype H3851-4-40.

The differences between race II and Is. 2 were qualitative as well as quantitative: differences in reaction type were associated with differences in latency period, as shown in the laboratory test (Table 3). The Icatu genotypes shown in Table 3 had different levels of resistance to race II, but all were more susceptible to Is. 2. Apparently, the resistance factor matched by Is. 2 can be more or less effective depending on the coffee genotype. Also seasonal variation in reaction was observed. Virulence was generally higher in summer than in winter. Furthermore, differences in virulence or resistance were more evident in young leaves than in older leaves (Eskes and Toma-Braghini, 1982).

It is concluded that Is. 2 differs from race II in three aspects: 1) it has an extra virulence factor to certain Icatu and Catimor genotypes, 2) it lacks a virulence factor, present in race II, to other Catimor genotypes, and 3) it is slightly less virulent than race II on *C. arabica* cultivars.

Isolate 3. A race with virulence intermediate between that of race II and Is. 2 was found in the Icatu population. Is. 3, obtained from H4782-10-203, showed such intermediate virulence in many tests. In a laboratory test (Table 4) intermediate virulence of Is. 3 was observed in relation to two Icatu genotypes. In this test 6 single-lesions isolates from Is. 2 and 10 single-lesions isolates from Is. 3 were used. No marked differences between single-lesion isolates within Is. 2 and Is. 3 were observed. Intermediate virulence was also observed for some single-lesion field isolates from H3851-4-41, a genotype with moderate resistance to race II. In a greenhouse test with cuttings from this genotype, the field isolates showed three levels of virulence, similar to that of race II (low virulence), Is. 3 (intermediate virulence) and Is. 2 (moderate virulence). Apparently, the three races may occur together in the field on the same genotype.

The results suggest that Is. 3 represents a race with virulence intermediate between race II and Is. 2. This race may persist in future, besides Is. 2, in the Icatu population, because it is more virulent than Is. 2 on cv. Mundo Novo (Table 4).

Table 4. Average reaction type in a leaf disk test of three Icatu genotypes and of cv. Mundo Novo, inoculated with *H. vastatrix*, race II, and single-lesion isolates from H4782-10-203 (Is. 3) and H3851-4-40 (Is. 2).

Coffee genotype	Race or isolate		
	race II	Is. 3	Is. 2
Icatu:			
H3851-4-40	1.0	2.3	7.0
H4782-7-923	2.0	4.3	7.8
H4782-10-203	4.0	7.7	7.8
Cv. Mundo Novo	9.0	8.5	7.5

Tabel 4. Gemiddeld reactietype in de bladschijftoets van drie Icatu genotypen en cv. Mundo Novo, geïnoculeerd met fysio II van H. vastatrix, en éénlesie-isolaten van H4782-10-203 (Is. 3) en H3851-4-40 (Is. 2).

Table 5. Reaction type (RT) and percentage of leaf disks with sporulation (PDS) of 14 genotypes of *C. canephora* cv. Kouillou and of *C. arabica* cv. Mundo Novo inoculated in the laboratory with race II and three isolates (Is.) of *H. vastatrix*.

Coffee genotype	RT				PDS[1]			
	race or isolate				race or isolate			
	race II	Is. 11	Is. 12	Is. 10	race II	Is. 11	Is. 12	Is. 10
Cv. Kouillou:								
C70-12	0.0	0.0	0.0	0.0	0	0	0	0
C69-5	0.3^{a}[2]	0.3^{a}	3.3^{b}	5.7^{c}	0^{a}	0^{a}	9^{b}	65^{c}
C66-13	0.0^{a}	0.0^{a}	5.3^{b}	7.7^{c}	0^{a}	0^{a}	11^{b}	58^{c}
C68-11	0.0^{a}	0.0^{a}	7.3^{b}	7.7^{b}	0^{a}	0^{a}	45^{b}	80^{c}
C67-12	6.3^{a}	4.0^{b}	3.3^{a}	11^{a}	11^{a}	42^{b}	11^{a}	2^{a}
C67-1	5.0^{b}	7.0^{c}	3.7^{b}	2.0^{a}	24^{b}	62^{c}	11^{ab}	0^{a}
C69-7	5.3^{a}	6.7^{b}	4.7^{a}	4.0^{a}	51^{b}	78^{c}	31^{ab}	22^{a}
C69-15	6.0^{b}	6.7^{b}	4.0^{a}	4.0^{a}	60^{b}	91^{c}	18^{a}	13^{a}
C68-7	5.5^{b}	5.0^{ab}	3.7^{a}	3.7^{a}	38^{b}	22^{ab}	7^{a}	7^{a}
C69-10	6.0^{ab}	6.3^{b}	5.0^{ab}	4.0^{a}	64^{b}	73^{b}	31^{a}	27^{a}
C68-10	7.0^{a}	7.0^{a}	6.0^{a}	5.7^{a}	60^{b}	60^{b}	49^{ab}	29^{a}
C66-3	6.3^{a}	6.3^{a}	5.3^{a}	6.3^{a}	51^{a}	65^{a}	51^{a}	49^{a}
C66-12	8.0^{a}	8.7^{a}	7.7^{a}	8.3^{a}	44^{a}	51^{a}	40^{a}	42^{a}
C70-14	6.3^{ab}	6.3^{ab}	6.7^{b}	5.3^{a}	24^{a}	29^{a}	42^{a}	22^{a}
Cv. Mundo Novo	8.9^{a}	8.8^{a}	8.6^{a}	8.8^{a}	62^{a}	65^{a}	66^{a}	59^{a}

[1] For statistical analysis of PDS transformations into the arcsine of the square root of PDS have been applied.

[2] Different letters indicate significant differences, within horizontal rows, at LSD 0.05.

Tabel 5. Reactietype (RT) en percentage bladschijven met sporulatie (PDS) van 14 genotypen van C. canephora cv. Kouillou en van C. arabica cv. Mundo Novo getoetst in het laboratorium met fysio II en drie isolaten (Is.) van H. vastatrix.

Isolates from C. canephora cv. Kouillou. In 1980, rust isolates 10, 11 and 12 were obtained in the field from the 'Kouillou' genotypes C66-13, C67-1 and C68-11, respectively. Leaf disks from 29 Kouillou genotypes were inoculated with Is. 10, Is. 11 and Is. 12, using three replications with 15 disks each. Table 5 shows the reaction type (RT) and percentage of disks with sporulating lesions (PDS) of 14 representative genotypes and of cv. Mundo Novo. PDS is a resistance component correlating with the disease level in the field (Eskes, 1982a).

In Table 5 the 14 genotypes have been divided into 5 groups. One Kouillou genotype (C70-12) was completely resistant to all isolates. The second group of genotypes (C69-5, C66-13 and C68-11) showed susceptibility to Is. 10 and Is. 12, and complete resistance to Is. 11 and race II. Genotypes of the third group (C67-12 to C69-15) were more susceptible to Is. 11 than to race II, Is. 10 or Is. 12. In this group, the differences in reaction were more quantitative than qualitative. Is. 10 was often significantly less virulent than race II to genotypes of the third and fourth group. In the fifth group (C66-3, C66-12 and C70-14) differences between rust isolates were small and rarely significant. Cv. Mundo Novo reacted equally to all isolates. Is. 12 was often intermediate between race II and Is. 10 with respect to genotypes of the second, third and fourth group.

It is concluded that complete and incomplete race-specific resistance to coffee leaf rust occurs in the 'Kouillou' population which is matched by Is. 10 and Is. 11, respectively. The level of incomplete race-specific resistance matched by Is. 11 (third group of genotypes, Table 5) varied between Kouillou genotypes. Is. 10 was more virulent than race II to some 'Kouillou' genotypes and less virulent to other genotypes, indicating that the dissemination of Is. 10 in the 'Kouillou' population may be hampered to some extent. This is in accordance with the slow increase of Is. 10 observed in the field between 1979 and 1982.

Discussion

Race formation in Brazil. Between 1976 and 1981, seven qualitatively distinct races of coffee leaf rust were found in the breeding plots of the Instituto Agronômico at Campinas. Most of the new races appeared in 1979 and 1980, years in which disease level in the field was high. The total number of races detected in Brazil in now eleven. All races contain virulence to the S_H5 resistance gene that is present in the Brazilian *C. arabica* cultivars. Eight races contain, in addition, single or combined virulence to the genes S_H1, S_H2 and S_H4. Three races match yet unidentified resistance genes of *C. canephora*. The type cultures of these races (Is. 2, 10 and 11) have been sent to CIFC, Portugal, for further identification.

The rust strain which arrived in Brazil in 1970 was identified at CIFC as race II (v_5). This race is still by far the most common race in Brazil. Apparently, the other 10 races detected in the State of São Paulo have originated from race II. Mutation is generally considered as the driving force in creating variability in asexually propagated fungi (Person et al., 1976). For yellow rust of wheat, it has been shown that stepwise mutations can explain the formation of races in the field with increasing numbers of virulence factors (Stubbs, 1968; Stubbs, 1972). Similarly, the stepwise increase in virulence of coffee leaf rust in Brazil (Table 1) could well be explained by subsequent mutations.

The multiple differences between race II and Is. 2 and 10 (Tables 3 and 5) could be better explained by somatic recombination (Tinline and Mac Neill, 1969) than by mutation. However, then one might also expect changes in virulence in relation to the resistance genes S_H1, S_H2 and S_H4, which did not occur with the isolates (Table 1). Another possible explanation might be the occurrence of interallelic interactions for vilulence in *H. vastatrix.*

Three rust isolates (1, 3 and 12) have incomplete virulence to the corresponding coffee genotypes (Tables 2, 4 and 5). These isolates are considered as genotypes distinct from race II, but not as distinct races, because differences in virulence were quantitative rather than qualitative. The occurrence of incomplete virulence suggests that, in order to achieve complete virulence, more than one change in the genotypes of *H. vastatrix* may be necessary. Intermediate virulence in dikarytotic rust fungi has been observed earlier (Watson and Luig, 1968; Statler and Jones, 1981) and can be most simply explained by assuming incomplete dominance of the avirulence allele.

Is. 1 is moderately virulent to the differential 33/1 (S_H3S_H3 S_H5S_H5) in the laboratory, while race II is avirulent. When tested at CIFC, Portugal, Is. 1 showed an R reaction type (2-3) on clone 33/1 (C.J. Rodrigues Jr., personal communication). The type culture of race VII ($v_{3,5}$), maintained at CIFC, is not completely virulent to clone 33/1, giving an MS reaction type, while other races with the v_3 virulence factor give reaction types S on this differential (Rodrigues et al., 1975). These observations suggest the existence of four level of virulence (R, MR, MS and S) in relation to the S_H3 resistance gene. The existence of more than three levels of virulence to major resistance genes has been reported for other rust fungi (Watson and Luig, 1968; Statler and Jones, 1981). Schwartzbach (1979) reported four virulence levels of the monokaryotic mildew fungus to the ml-o resistance gene in barley. The authors ascribed this wide variation in virulence to allelism for virulence or to non-allelic interactions in the pathogen.

The complex nature of virulence of *H. vastatrix* to the S_H3 resistance gene might explain why this resistance gene is till effective in Brazil. In India, where the S_H3 gene has been applied in commercial cultivars from 1946 onwards, it has taken a relatively long period before the rust developed high levels of virulence on these cultivars (Narasimhaswamy, 1961; Visveswara, 1974).

The future race formation of coffee leaf rust in Brazil might largely depend on the selection pressure for virulence as determined by the extension and mode of the use of resistant genes in cultivars. Besides, several factors in the fungus whose importance cannot be foreseen may play a role. The frequency of mutant alleles for virulence is determined by the relative fitness of the allele and by mutation rate, which both can vary between loci (Person et al., 1976). Virulence may also have a complex genetic base (see above) and require multiple changes in the rust genotype. The more modifications are needed, the longer it will take for virulence to appear, and the greater the chance is that te fitness of the strain is adversely affected.

Stabilizing selection. Two of the new rust races (Is. 2 and 10, Tables 3 and 5) show, in comparison to race II, increased virulence to some coffee genotypes and decreased virulence to other genotypes. This result indicates stabilizing selection, in the sense that multiplication of Is. 2 and Is. 10 will be hampered to some extent in the Icatu and Kouillou population, respectively. This has been confirmed so far in the field, where the dissemination of Is. 2 and Is. 10 was relatively slow in comparison to the other new rust races. The basis for this stabilizing effect cannot be known. It could be related to a direct effect of the virulence (or avirulence) allele (Vanderplank, 1968), to interallelic interactions for virulence or to an unbalance in the rust genotype created by somatic recombination or mutation.

Resistance breeding. New races of coffee leaf rust appeared when only very few genotypes with the corresponding resistance genes were present in the breeding plots (e.g. Is. 10 and Is. 11, Table 1). Resistance, applied in cultivars planted at a large scale will create a much higher selection pressure for virulence than resistance present in small breeding plots only. This emphasizes the need for obtaining durable resistance to coffee leaf rust.

The durability of resistance may be influenced by the type of resistance or by the management of the resistance genes. The results indicate that the resistance genes S_H1, S_H2 and S_H4, used singly or in combination, will not provide the coffee crop with a lasting protection against coffee leaf rust. The experience with the S_H3 gene gained in Brazil and India indicates that this gene can be more useful, especially in combinations with other resistance genes.

The quick appearence of complex races from simple ones in Brazil (Table 1) makes the efficiency of multilines of coffee questionable. A multiline could facilitate, like the breeding plots in Campinas have appararently done, the build up of complex races by stepwise increases in virulence. Besides, the perennial coffee crop lacks the flexibility of an annual crop needed for management of resistance genes in a multiline. A multiline could have some efficiency if cross-protection between races with different virulence spectra is of importance under field conditions. No pertinent information is available for coffee leaf rust. Research in this direction is desirable.

The best approach for the use of specific resistance genes in coffee might be to combine several genes into one cultivar. All genes should give resistance to the entire rust population. Genotypes with only one or two genes should be avoided in such a cultivar in order to make it more difficult for the rust to build up virulence gradually. It is realized that by current breeding methods such an approach is difficult to achieve. Modern methods of massive vegetative propagation of coffee (Söndahl and Sharp, 1977; Custers, 1980), may be a help in the future to produce hybrid cultivars which contain many resistance genes.

Another breeding approach, which has been initiated at the I.A.C., Campinas, Brazil, is the search for durable resistance by selecting for incomplete resistance with polygenic inheritance. So far, promising genotypes have been identified within the *C. canephora* cv. Kouillou and within some *C. arabica* breeding lines (Eskes, 1981b). The present results show the occurrence of gene-specific incomplete virulence in coffee leaf rust and race-specific incomplete resistance in coffee (Tables 2 to 5). Hence, selection for incomplete resistance based on field observations alone will not be sufficient to indicate the durbability of the resistance. In addition, information on the polygenic nature of the resistance is required (Parlevliet, 1979; Eskes, 1981a). Although this approach is not very simple, it is recommended that it should be continued.

The results suggest still another breeding approach to durable resistance. Tables 3 and 5 show that the extra virulence of Is. 2 and Is. 10, in comparison to race II, is associated with a loss in virulence to certain coffee genotypes. In the coffee literature a similar case was found. The type cultures, kept at CIFC, Portugal, of seven distinct rust races of different origins but all with virulence towards the *C. congensis* 263/1 differential, are only moderately virulent to *C. arabica* differentials and lack the v_5 virulence factor present in most other rust races (Rodrigues et al., 1975). The above observations suggest a difficulty of *H. vastatrix* in combining certain virulence factors. Possibly, the breeder could take advantage of this by combining the corresponding resistance genes into one genotype. For instance, the resistance of the 263/1 differential could be introduced into *C. arabica* cultivars with the S_H5 gene, and resistance of Icatu H3851-2-40 could be combined with that of Catimor 2477-2 (Table 3). In case the rust overcomes both resistance, resulting virulence can be expected to be low. Technically, this approach consists of selection for the combination of the two resistance factors in the cross progeny, which should be relatively easy because probably major genes are involved.

It is realized that this latter approach is new and based on assumptions. However, some indications in literature are in support of such an approach. Durable or broadly based resistance has been related to a combination of specific resistance genes (Johnson and Taylor, 1976; Wolfe and Schwartzbach, 1978; Ahn and Ou, 1982). Inter-allelic interactions for virulence in the pathogen could hamper complete virulence to combinations of certain resistance genes. Recently the occurrence of dissociation of virulence genes in rust fungi has been suggested (Vanderplank, 1982). This suggestion also leads to the assumption that certain combinations of race-specific resistance can be useful in obtaining durable resistance.

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Samenvatting

Kwalitatieve en kwantitatieve variatie in pathogeniteit van fysio's van koffieroest (Hemileia vastatrix) gevonden in de staat Sã Paulo, Brazilië

Tussen 1977 en 1981 werden zeven kwalitatief verschillende nieuwe fysio's van koffieroest *(Hemileia vastatrix)* gevonden in veredelingsproefvelden in de staat São Paulo, Brazilië. Vier fysio's bevatten complexe virulentie tegen de resistentiegenen S_H1, S_H2, S_H4 en S_H5 van *Coffea arabica.* Drie fysio's neutralizeren nog ongeïdentificeerde resistentiegenen van *C. canephora.* Twee van deze fysio's werden van cv. Kouillou geïsoleerd en één van de hybride populatie Icatu. In afwachting van verdere

identificatie zijn deze drie fysio's aangeduid met hun isolaatnummer (Is. 2, 10 en 11). Is. 2 en 10 vertoonden naast extra virulentie t.o.v. bepaalde koffiegenotypen verminderde virulentie t.o.v. andere genotypen, hetgeen kan wijzen op stabiliserende selectie.

Tevens werden drie roestisolaten ontdekt die kwantitatief verschilden van het gangbare fysio II. Is 1 was matig virulent op de differentiërende kloon voor het S_H3 resistentiegen in het laboratorium maar avirulent in de kas, wat duidt op een waardplant × fysio × milieu-interactie. De virulenties van Is. 3 en 12 lagen in tussen die van fysio II en Is. 2, respectievelijk fysio II en Is. 10. De resultaten tonen aan dat incomplete resistentie van koffie tegen koffieroest, van variërend niveau, fysiospecifiek kan zijn.

De aard van het ontstaan van de nieuwe roestfysio's in Brazilië en veredelingsstrategieën voor het verkrijgen van duurzame resistentie tegen koffieroest worden besproken.

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