Incomplete resistance to coffee leaf rust (*Hemileia vastatrix*)

Aan mijn ouders
Para Heloisa, Chantra e Nanda

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INCOMPLETE RESISTANCE TO COFFEE LEAF RUST
(Hemileia vastatrix)

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee,
hoogleraar in de veeteeltwetenschap,
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des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen

STELLINGEN

1. Selectie op polygeen vererervende incomplete resistentie tegen ziekteverwekkers, met als doel het verkrijgen van duurzame resistentie, is veelal te ingewikkeld voor de praktische veredeling.

2. Bij het streven naar verhoging van de landbouwproduktiviteit in ontwikkelingslanden wordt het belang van teeltkundige aspecten onderschat en het belang van de veredeling overschat.

3. De op zich interessante hypothese van Nelson et al. (1970) dat genen voor 'horizontale' en 'vertikale' resistentie identiek zouden zijn, is in feite gebaseerd op een onjuiste uitleg van hun eigen onderzoeksresultaten en van het begrip 'horizontale' resistentie.

   Nelson, R.R., D.R. MacKenzie & G.L. Scheifele, 1970. Interaction of genes for pathogenicity and virulence in *Trichometasphaeria turcica* with different number of genes for vertical resistance in *Zea mays*. Phytopathology 60: 1250-1254.

4. De term 'horizontale' resistentie blijft aanleiding geven tot verwarring. De term is onnodig en zou daarom niet verder gebruikt dienen te worden.

   Dit proefschrift.

5. De door Ou (1980) vermeende instabiliteit van fysio's van *Pyricularia oryzae* berust grotendeels op een verkeerde interpretatie van het heterogene reactietype.

   Ou, S.H., 1980. Pathogen variability and host resistance in rice blast disease. Ann. Rev. Phytopath. 18: 167-187.

6. Het 'interactiemodel' beschreven door Parlevliet en Zadoks (1977) verklaart niet de variatie in pathogeniteit waargenomen op waardplanten zonder resistentiegenen.

   Parlevliet, J.E. & J.C. Zadoks, 1977. The integrated concept of disease resistance; a new view including horizontal and vertical resistance in plants. Euphytica 26: 5-21.

7. Resistentie verkregen als gevolg van transgressie in nakomelingschappen van vatbare ouders kan berusten op additief werkende 'minor' genen, zoals gesuggereerd is o.a. door Krupinsky en Sharp (1979), of op 'major' genen waarvan de expressie beïnvloed wordt door de genetische achtergrond. In het kader van het verkrijgen van duurzame resistentie is onderzoek naar beide mogelijkheden gewenst.

   Krupinsky, J.M. & E.L. Sharp, 1979. Reselection for improved resistance of wheat to stripe rust. Phytopathology 69: 400-404.
   Dit proefschrift.

8. De schattingen door Skovmand et al. (1978) van de 'heritability en van het aantal genen betrokken bij de 'slow-rusting' eigenschap voor zwarte roest bij tarwe zijn onjuist en misleidend.
   Skovmand, B., R.D. Wilcoxson, B.L. Shearer & R.E. Stucker, 1978. Inheritance of slow rusting to stem rust in wheat. Euphytica 27: 95-107.

9. Vegetatieve vermeerdering 'in vitro', antherencultuur en somatische hybridisatie van protoplasten bieden perspectief bij de koffieveredeling.

10. Het oprichten van een internationaal onderzoekcentrum voor overblijvende tropische gewassen verdient ernstige overweging.

11. Het 'tonisch' effect van bespuitingen met fungiciden, zoals aangetoond bij de koffieteelt in Kenya onder andere door Van der Vossen (1982), dient nader onderzocht te worden in de koffieproducerende landen van Amerika.
    Van der Vossen, H.A.M., 1982. Consequences of phytotonic effects of fungicide to breeding for disease resistance, yield and quality in Coffea arabica L., J. Hort. Sci. 57: 321-329.

12. Het effect van ziekte op opbrengst is redelijk bekend, terwijl er omtrent het effect van opbrengst op ziekte veel te weinig bekend is.

13. Het weglaten van de dertiende verdieping in Amerikaanse flatgebouwen vergroot de instabiliteit van de veertiende verdieping.

14. Het poneren van een stelling is belangrijker dan de juistheid ervan.

15. Er is één uitzondering op de regel 'geen regel zonder uitzondering', namelijk deze regel zelf, hetgeen de algemene toepasbaarheid van deze regel bevestigt.

Stellingen bij het proefschrift van A.B. Eskes, getiteld 'Incomplete resistance to coffee leaf rust (*Hemileia vastatrix*)' te verdedigen op 25 maart 1983 in de Aula van de Landbouwhogeschool, Wageningen.

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INTRODUCTION

The two coffee species of economic importance are *Coffea arabica* L. and *C. canephora* Pierre. The first species is responsible for about 80 % of the world coffee production (Carvalho et al., 1969). It is an allotetraploid autogamous species, growing best in tropical highlands. *C. canephora* is a diploid, allogamous species and grows best in tropical lowlands.

Coffee leaf rust or orange coffee rust (*Hemileia vastatrix* Berk. et Br.) is a major disease of coffee. The rust arrived in Brazil in 1970 and has recently spread to nearly all coffee producing countries in South and Central America. All cultivars (*C. arabica*) grown in these countries are susceptible to race II, the common race of *H. vastatrix*. Yield losses in Brazil have been estimated to be about 30%, if no control measures are taken (Monaco, 1977). For Brazil, this would represent about 500 million U.S. dollars annually in export revenues.

The rust can be controlled by chemicals. In the State of São Paulo, Brazil, 4 to 5 applications per year are recommended (Mariotto et al., 1979). The costs of the chemicals needed for full protection of the coffee crop in Brazil is yearly about 200 million U.S. dollars (Monaco, 1977), which represents 10 to 20% of the total production costs. Chemical control is not profitable for plantations with low yields. Only a small percentage of the Brazilian farmers follows the recommended spraying schedule. The above observations indicate that chemical control is relatively expensive and that its value in the control of coffee leaf rust is restricted. This emphasizes the importance of genetical control of coffee leaf rust.

Breeding for disease resistance faces the challenge of obtaining durable resistance. Most pathogens show great variability for pathogenicity, especially biotrophic pathogens such as rust fungi. Coffee leaf rust too has shown considerable variation in pathogenicity. More than 30 physiologic races have been identified (Rodrigues, 1975). Five major resistance genes present in *C. arabica* have not given an efficient control of coffee leaf rust (Eskes, 1981^a). Many other major genes are present in *C. canephora*, which have only been partly identified. Presently, in breeding for resistance to coffee leaf rust much attention is given to the hybrid populations 'Icatu' and 'Catimor', derived from crosses between *C. arabica* and *C. canephora*. These populations contain genotypes with complete resistance and others with varying levels of incomplete resistance.

Durable resistance is of special importance for perennial crops like coffee. The breeding process is slow and cultivars which have lost resistance cannot be rapidly replaced. The basis of durable resistance has been a subject of active scientific debate during the past 15 years (Vanderplank, 1968 and 1982, Simons, 1972, Robinson, 1976, Johnson and Taylor, 1976, Parlevliet and Zadoks, 1977, Nelson, 1978, Ellingboe, 1981). The history of resistance breeding suggests that polygenically inherited incomplete resistance is generally more durable than high levels of resistance based on major genes (Caldwell, 1968, Simons, 1972). Vanderplank (1968) suggested that durable and temporary resistance are based on two distinct types of resistance named by him 'horizontal' and 'vertical' resistance. Horizontal resistance was said to be race-non-specific. Subsequent debate in the literature has involved basic questions such as:

a) does race-non-specific resistance occur in nature,

b) can polygenically inherited race-specific resistance be durable,

c) can durable resistance be identified in a breeding programme, and

d) can major resistance genes be of value in obtaining durable resistance?

The objective of the present research was to characterize incomplete resistance of coffee to *H. vastatrix* and to assess its possible value in obtaining durable resistance. The present research includes all types of resistance which do not fully inhibit the reproduction of a certain strain of the pathogen (incomplete resistance). The term incomplete was preferred to 'partial' because the latter has been used more specifically to indicate incomplete resistance with a high (susceptible) reaction type (Parlevliet, 1978^{b}).

The research has resulted from a joint project of the 'Instituto Agronômico de Campinas' (I.A.C.), S.P., Brazil and the Food and Agriculture Organization of the United Nations (F.A.O.), at the initiative of drs. A. Carvalho and L.C. Monaco (I.A.C.) and dr. R.A. Robinson (F.A.O.). The research was carried out from 1976 to 1981 at the Experimental Station of the I.A.C. at Campinas, and received partial support from the 'Instituto Brasiliero de Cafe (IBC)'. In 1981/82 the Agricultural University of Wageningen has supported the research, facilitating the elaboration of the results and the preparation of the manuscripts.

The author has received co-operation from several researchers mentioned as co-authors in five of the eight publications. Dr.

Alcides Carvalho, head of the coffee breeding programme at the I.A.C., gave scientific stimulus and provided data on productivity of the coffee genotypes. Eng AgrªWaldir M. da Costa has assisted in the research on the Icatu population. Mrs. Masako Toma-Braghini served as an assisting biologist to the project from 1979 through 1981.

Acknowledgements for the co-operation received by many others are made at the end of each chapter. In addition, I like to thank Dr. ir. I. Bos and Mr. G. Heemstra for help with the statistical elaboration of the results and Mr. J.S. de Block for corrections made in the manuscript. Also thanks go to Riet Hilhorst and Walter de Milliano, whose more general support is greatly appreciated.

(For references see page 132)

1 ASSESSMENT METHODS FOR RESISTANCE TO COFFEE LEAF RUST (*HEMILEIA VASTATRIX* BERK. & BR.)

Summary

Assessment scales are proposed for quantitative recording of reaction type and infection frequency of coffee leaf rust. All scales run from *0* to *9*. The scale for reaction type includes heterogeneous reactions that can be frequently found in the derivatives of *Coffea canephora*. The scales designed for infection frequency may help the coffee breeder in making a quick assessment of partial resistance to coffee leaf rust. The advantages of a laboratory method, using leaf disc inoculations for resistance assessment, are discussed.

The use of genetic resistance to coffee leaf rust constitutes the ideal control method of this very important coffee disease. One of the basic problems for resistance breeding is that new races of the fungus, capable of overcoming resistance, may appear. This type of resistance is called vertical resistance, according to Van der Plank (1968), and its durability is often related to the extent of commercial use of the resistant cultivar.

Problems with vertical resistance also exist in relation to coffee leaf rust. The five traditionally used resistance genes of *Coffea arabica* have not provided efficient control when used in cultivars. *Hemileia vastatrix* possesses considerable variability for virulence (Rodrigues, Jr, Bettencourt and Rijo, 1975). This was demonstrated in Brazil, where, within 10 years of rust presence, 10 different races could be detected in breeding plots of the Instituto Agronomico of Campinas (Eskes, 1981). Therefore, the search for a more durable type of resistance for the perennial coffee crop should be given high priority.

More durable resistance may be obtained by applying adequate breeding strategies for vertical resistance, such as gene deployment, pyramiding of resistance genes in one cultivar or using multiline cultivars (Nelson, 1973). However, most of these strategies are difficult to implement, especially when applied to perennial crops.

An alternative may be the search for horizontal resistance, which by definition should be equally efficient against all races of the pathogen (Robinson, 1976; Van der Plank, 1968). The concept of this type of resistance is still controversial and it continues to be widely discussed in world literature (Nelson, 1973; Parlevliet and Zadoks, 1977; Van der Plank, 1978). However, a general agreement between researchers seems to exist that polygenically inherited partial resistance confers more durable protection to crop pathogens than the monogenic hypersensitive type of resistance (Parlevliet and Zadoks, 1977; Parlevliet, 1979; Simons, 1972).

Since 1976, FAO has been collaborating with the Instituto Agronomico of Campinas, Brazil, to develop a programme on durable resistance to coffee leaf rust. One important aspect of this programme was to develop an adequate methodology for quantitative measurement of the level of resistance, including the establishment of quick assessment methods for routine resistance screening.

In this paper, a series of illustrated assessment scales, from *0* to *9*, are presented. The *0* to *9* scale was proposed by McNeal *et al.*, (1971) for cereal rust research, and has been

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widely adopted for epidemiological and resistance observations (Zadoks and Schein, 1979). This scale has the advantage that the data are computable. A uniform recording system is necessary for comparing research data from different locations or experiments.

REACTION TYPE

THE TRADITIONALLY USED SCALE FOR REACTION TYPE

The Centre for Coffee Leaf Rust Research, CIFC, in Oeiras, Portugal, has adopted a recording system for disease reaction type that is similar to the traditionally used system for cereal rusts (d'Oliveira, 1954-57). This system includes the following reaction types: *i* (immune), *fl* (flecks), *t* (tumefactions), *;* (necrosis), *o* (chlorosis), *1* (rare sporulation), *2* (small pustules), *3* (medium to large pustules), *4* (large pustules), and *X* for the heterogeneous reaction. It has served to differentiate the more than 30 rust races known and to choose 24 differential coffee genotypes (Rodrigues, Jr, Bettencourt and Rijo, 1975). Often the classification is simplified into four types: *R* (resistant), *MR* (moderately resistant), *MS* (moderately susceptible) and *S* (susceptible).

The following comments on reaction types of coffee leaf rust should be borne in mind:

a) Reaction type 2, with small-size pustules, is only rarely found in coffee (d'Oliveira, 1954-57; personal observations). It should preferably be considered as an intermediate reaction type between *1* and *3*. The *1* to *4* reaction types then present a continuous scale with an increasing rate of sporulation in relation to the diseased or chlorotic area.

b) Reaction type *t* (tumefaction), which is frequently observed in Rubiaceae, consists of swollen spongy tissue cells (Rijo, 1972). In coffee, it is often associated with incompatible combinations and has been called a hypersensitive reaction type. The authors have observed, however, that tumefactions can be found also in compatible combinations. Recent studies in Portugal confirm that the *t* reaction is not a specific resistance reaction. It can be induced by heat treatment of compatible reactions (C.J. Rodrigues, Jr, personal communication) and by wash-outs of uredospores of a compatible race (Rodrigues, Jr, Rijo and Medeiros, 1981).

c) Whatever the reaction type, necrosis is rarely observed in young lesions, but it may occur when the lesions are ageing. Environmental as well as genetic factors may induce early necrosis. Therefore, necrosis does not seem to be an entirely suitable criterion for determining reaction types of coffee leaf rust.

d) As necrosis and tumefaction are less appropriate to indicate resistance, the basic criteria for the reaction type of individual lesions should be the size of the discoloured area (flecks, o, o^+ reaction types), and the intensity of sporulation in proportion to the extension of the chlorotic area (*1* to *4* reaction types).

e) In derivatives of *C. canephora*, a mixture of susceptible and resistant reaction types is often found on the same leaf. This occurs generally on plants with an intermediate resistance level. Results indicate that this reaction may not be a result of variation in the fungus, but is rather a response of incomplete resistance that can be affected by light intensity and leaf age (Eskes, Rivera and Da Costa, 1978; Eskes, Kroon and Van de Weg, 1980; Eskes, 1981). Consequently, for the description of the resistance of a certain coffee genotype toward a certain rust genotype, it will be necessary to include heterogeneous reaction types in the assessment scale.

f) At CIFC, inoculations are generally made only on young tender leaves, as it is much more difficult to produce disease on older leaves (d'Oliveira, 1954-57). Work in Campinas showed that some types of resistance are better expressed in the young or adult leaves, as old leaves are more susceptible (Eskes, Rivera and Da Costa, 1978; Eskes, Kroon and Van de Weg, 1980). For a complete appreciation of the resistance of a plant it would be necessary to observe the reaction type of leaves of varying ages.

A *0* TO *9* SCALE FOR REACTION TYPES OF COFFEE LEAF RUST

The adoption of a *0* to *9* scale for reaction type is proposed, following the international efforts on uniform reporting of research data (McNeal *et al.*, 1971). The proposed scale reflects the aforementioned observations on reaction types. The scale is made to be used for resistance determinations of individual leaves or of entire plants. It includes a wide range for heterogeneous types of resistance, in view of the frequent occurrence of these types, especially in interspecific hybrids. A description of the proposed *0* to *9* scale is given in the table below.

Index value	**CIFC classification for individual lesions**	**Description of reaction types for leaves or entire plants**
0	*i*	Immunity, no visible reaction.
1	fl^-, t^-	Minute "chlorotic spots" often associated with small tumefactions. Sometimes only visible with a hand lens or when holding the leaf against the light.
2	*fl, t, o*	Larger chlorotic spots, often associated with tumefactions. No uredospore production.
3	*fl, t, o,* o^+	A mixture of various sizes of chlorotic spots, including very large chlorotic areas. Fewer tumefactions. No uredospore production.
4	*fl, t, o, 1*	A mixture of chlorotic spots of various sizes, with some uredospore formation on large and chlorotic lesions. Sporulation of less than 25 percent of all lesions. Few tumefactions may occur. Early necrosis of lesions is sometimes observed.
5	*fl, t, o-2*	As in *4*, but with more uredospore formation. Sporulation of less than 50 percent of all lesions.
6	*fl, t, o-3*	As in *5*, but with increased uredospore production. Sporulation of less than 75 percent of all lesions.
7	*fl, t, o-4*	As in *6*, but with abundant uredospore production. Sporulation of up to 95 percent of all lesions.
8	*t, 2-4*	A mixture of lesions with a varying degree of sporulation, sometimes associated with a few tumefactions.
9	*4*	Only lesions with abundant sporulation, without marked chlorosis at the lesion border.

Reaction types *4* to *7* indicate intermediate types in which increasing sporulation intensity per lesion is generally associated with an increasing proportion of sporulating lesions.

When no computing of the data is needed, the notation can be simplified by using the symbols *R*, *MR*, *MS* and *S* to indicate the following reaction types:

R = *1*, *2* and *3*
MR = *4* and *5*
MS = *6* and *7*
S = *8* and *9*

ASSESSMENT OF RESISTANCE

DETERMINATION OF THE COMPONENTS OF PARTIAL RESISTANCE

All components of partial resistance behave cumulatively to decrease the reproductive capacity of the pathogen. The individual components, however, may have different effects on the epidemiological development of the disease in the field (Parlevliet, 1979; Parlevliet *et al.*, 1980; Zadoks and Schein, 1979). Component analysis is therefore helpful in determining efficient selection criteria for practical breeding work (Parlevliet, 1976; Zadoks and Schein, 1979). It is also important in achieving a better understanding of the underlying resistance mechanisms. In inoculation experiments with coffee leaf rust, significant variation has been found for all components of partial resistance, i.e., infection frequency, latency period, sporulation intensity, and duration of sporulation or sporulation period.

Infection frequency is the number of lesions obtained per unit of leaf area. A uniform inoculation method should be used to determine genetic differences for infection frequency. The inoculation method of CIFC, by which dry uredospores are applied first and the leaf is wetted afterwards (d'Oliveira, 1954-57), is not suitable to measure differences in infection frequency, as the quantity of spores applied is not controlled. A quantitative inoculation method, using uniform spore suspensions in water, is to be preferred.

Latency period is defined here as the number of days from inoculation until 50 percent of the lesions sporulate. If all the lesions do not sporulate then the latency period should be calculated in relation to the final number of sporulating lesions, and not in relation to the total number of all visible infection sites.

Sporulation intensity is the number of spores produced per infection unit or per leaf area unit.

Sporulation period is the time interval from completion of the latency period until cessation of sporulation (necrosis of lesions or leaf shedding).

THE USE OF ASSESSMENT SCALES FOR PARTIAL RESISTANCE

Observations on the individual components of partial resistance are time consuming and would not be practical for routine resistance screening. Therefore, assessment scales are proposed that serve the breeder in carrying out observations more quickly. All scales are made for determinations on disease incidence (number of lesions per leaf area unit) or disease severity (proportion of diseased leaf tissue).

These scales will measure the combined action of all resistance components when applied to polycyclic experiments with successive natural disease cycles in the field or nursery. When applied to monocyclic inoculation experiments, the scales will only assess the infection frequency. If infection frequency is well correlated to the other components of resistance, the proposed assessment scales may also be applied to measure the overall resistance level in monocyclic experiments.

In specific inoculation experiments, it may be of interest to determine the individual components of resistance separately. No assessment scale can be designed for measuring latency period. Instead of actually counting all sporulating lesions one by one, the observations may be simplified by estimating the day on which 50 percent of the apparently "susceptible type" lesions that will probably sporulate actually do so. A leaf or whole plant is used as a reading unit.

Sporulation intensity may be assessed by the scale for reaction type, if large differences in sporulation are present. If smaller differences in sporulation intensity need to be observed, spore collecting and counting should be carried out.

The duration of sporulation is determined by leaf shedding or lesion necrosis. Necrosis can be induced by the environment (extreme temperatures, low relative humidity), by the coffee genotype or simply by ageing of the lesions. In certain *C. arabica* coffee types, leaf retention after inoculation may be a very important factor in determining field resistance (Eskes *et al.*, 1979). This parameter can be assessed in artificial inoculation experiments by applying an arbitrary scale running from *0* to *9* (see section on greenhouse and nursery observations).

FIELD OBSERVATIONS

Epidemiological parameters. Incidence of coffee leaf rust has been generally estimated by the following parameters:

1 = number of lesions per leaf
2 = percentage of diseased leaves
3 = number of lesions per diseased leaf.

These parameters correlate well. Observations on 100 *C. arabica* cv. Catuai plants showed the following correlation coefficients (*r* values), all significant at $P = 0.01$.

	parameter *2*	parameter *3*
$\log_{10}$ (parameter *1*)	.88	.83
parameter *2*		.56

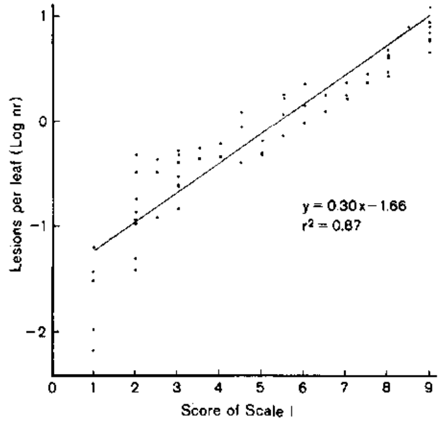

***Figure 1.** *Correlation of the logarithm of real disease incidence (no. of lesions per leaf) with the disease assessment values of Scale I*

*The log number of lesions per leaf (independant variable) should have been plotted on the adsissa and the scores of scale I on the ordinate. The formula for linear regression becomes then $y = 3.33\ x + 5.53$.

These data indicate that the number of lesions per leaf is the most reliable parameter for measuring disease incidence in the field (see Fig. 1).

Assessment Scales I and II. For measuring resistance of large field populations, two types of disease-assessment scales have been designed. Scale I (see Fig. 2) is based on the whole tree as a reading unit, whereas for Scale II a branch has been taken as a reading unit (see Fig 3). Both scales have been designed to give comparable results. Scale I is especially suited for Brazilian growing conditions, in which the epidemic builds up from the lower part of the tree.

Each point on the *0* to *9* scale should indicate equal differences in resistance. The relationship between the scale points and actual disease incidence should follow the logit [$\ln (x/1 - x)$] curve (Van der Plank, 1963, 1968; Zadoks and

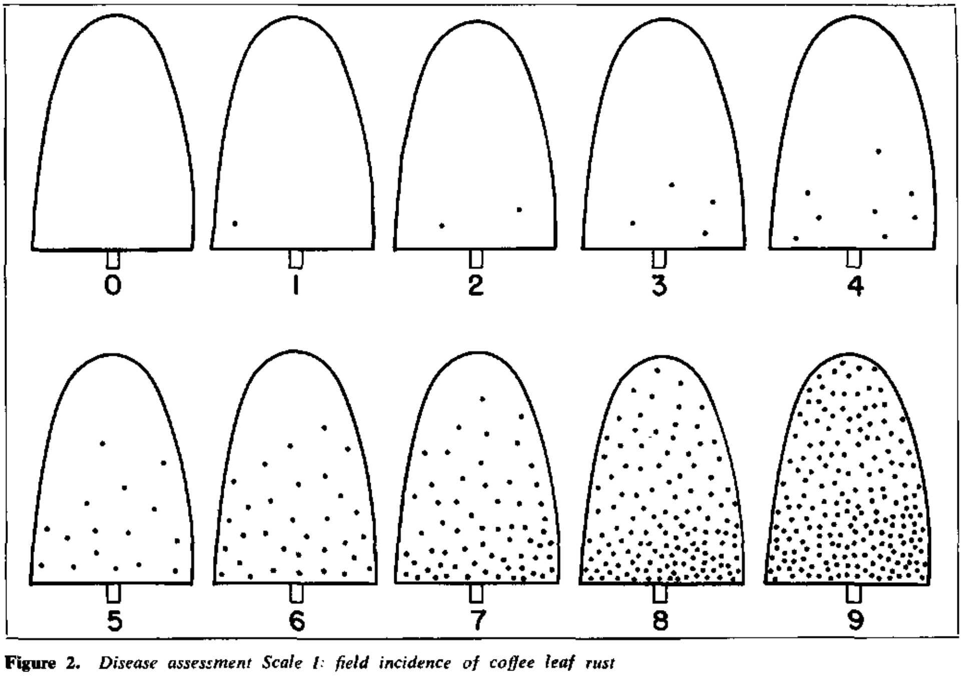

Figure 2. *Disease assessment Scale I: field incidence of coffee leaf rust*

Schein, 1979). This curve corresponds to the normal exponential curve for low values of x. As the breeder is especially interested in the lower part of the epidemiological curve, the number of dots chosen for each scale value follow a 2^n exponential curve. For scale value *9*, only 192 dots were placed instead of 256, as a massive leaf fall is normally induced, which limits the exponential increase of the lesion number per leaf, at this level of disease incidence.

When using the scales, only the quantity of sporulating lesions should be considered. The observation can be made either on one side of the coffee shrub or by two readers simultaneously on both sides. In Brazil, the southeastern side of the coffee shrub normally shows a higher degree of attack than the northwestern side, probably due to differences in microclimate.

Description of Scale I. The observation unit of this field scale is the entire coffee shrub. Each dot on the drawing represents the number of diseased branches seen on the shrub at first look (see Fig. 2). More detailed observations are only made to confirm the *0* and *1* readings.

Scale value	Description
0	Absence of sporulating lesions; confirmation necessary by pushing aside branches of the lower canopy and more detailed observation of the branches inside the shrub.
1	Presence of one diseased branch at first look on any side of the shrub. Pushing aside of lower canopy branches may be necessary for confirmation. On detailed observation, more than one diseased branch may be found.
2 to *8*	These values represent a gradual increase in the number of diseased branches per tree, seen at first look. Value *7* corresponds with an average attack on susceptible coffee cultivars in Brazil at harvest time.
9	This scale value indicates the maximum of disease incidence, corresponding to the level of disease of the very susceptible Harar coffee type in Brazil at harvest time. Normally, susceptible cultivars may drop leaves before reaching this level. Therefore, an *8*-value associated with intensive leaf shedding should be scored as a *9*.

Description of Scale II. The observation unit here is the average disease incidence per diseased branch (see Fig. 3). Readings should not involve very detailed observations. Each small dot represents a sporulating lesion. Bigger dots represent a coalescence of two lesions. As, in the field, older leaves are generally more severely infected than younger ones, more dots have been placed on the former.

The number of lesions for each scale value and the percentage of leaf area they occupy are given below.

Scale value	No. of lesions	Percentage of leaf area diseased
0	0	0
1	1	0.06
2	2	0.12
3	4	0.24
4	8	0.47
5	16	0.94
6	32	1.89
7	64	3.78
8	128	7.56
9	192	11.51

Scale value	Description
0	Absence of sporulating lesions. More detailed observation, by removal of lower branches, needed for confirmation.
1	Average number of lesions per diseased branch is about *1*.
2-8	Increasing number of lesions per diseased branch. Value *7* is commonly found for normally susceptible cultivars in Brazil at harvest time.
9	This value indicates maximum disease incidence, occurring frequently on the Harar coffee type in Brazil at harvest time. An *8*-value that is associated with intensive leaf shedding should be classified as a *9*.

Experimental results. Scales I and II were tested during June 1981 on 75 coffee plants with a varying degree of disease intensity.

Readings were made by two trained observers. The relationships between the average scores, using Scale I, of the two observers were plotted against the logarithm of the disease intensity as determined by detailed assessment (see Fig. 1).

For the individual observers the r^2 values were 0.86 and 0.85. The degree of agreement between two observers was high, with equal scores obtained in 67 percent of the observations, one scale value difference in 30 percent and two scale values differences in 3 percent.

The readings using Scale II were very similar to those using Scale I, with the following parameters of regression obtained: $y = 0.31x - 1.69$ and $r^2 = 0.88$, with x being the score of Scale II and y the logarithm of the number of lesions per leaf. Here also the degree of agreement between the observers was high. In 64 percent of the observations, scoring was equal. In 34 percent of the observations, one scale value, and in 2 percent of the observations two scale values difference were obtained.

Scale I is recommended for Brazilian conditions. It is less time-consuming than Scale II. The rate of observations using the former is about 200 readings per hour for an experienced observer.

AN ASSESSMENT SCALE FOR GREENHOUSE AND NURSERY OBSERVATIONS

Greenhouse and nursery observations are generally made on one-year-old coffee seedlings. The degree of natural infection can be assessed by Scale II, if the drawing is held upside down.

Another scale has been designed to measure

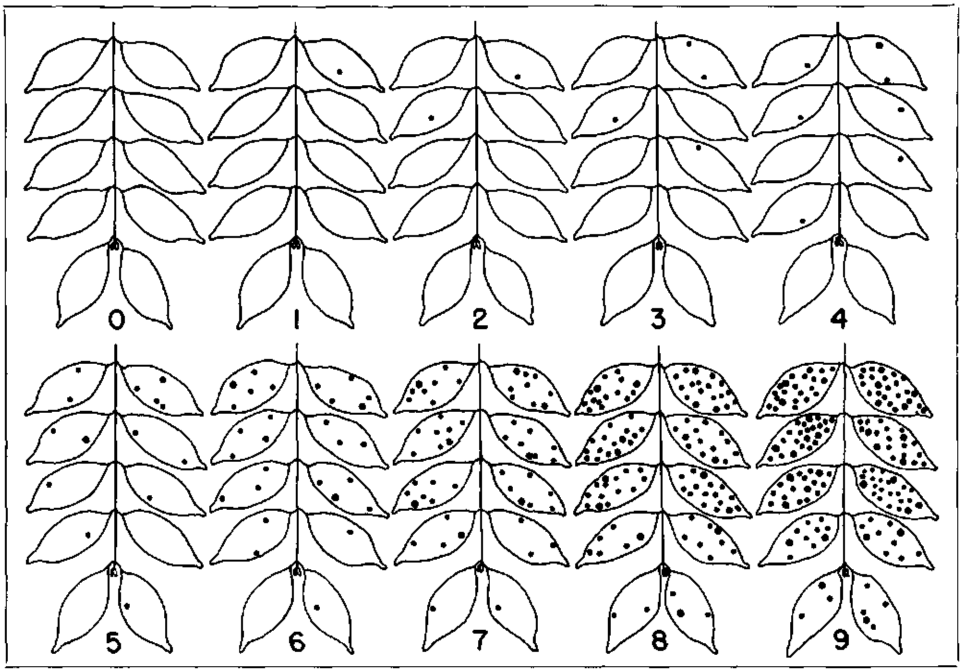

Figure 3. *Disease assessment Scale II: field incidence of coffee leaf rust*

infection frequency obtained in artificial inoculation experiments (see Fig. 4). For this scale, a deviation from the 2^n exponential relationship had to be included, to correct for the overlapping of lesions. The correction was made by applying the following formula (Garwood, 1947; Justesen and Tammes, 1960):

$$r = (1 - s/m)^n,$$

where r = disease-free area, s = surface of one lesion, m = surface of the leaf, and n = number of lesions per leaf.

Furthermore, the leaf area vulnerable to infection is considered to be only 90 percent of the total area. No lesions develop on the veins and few lesions at the tip and the border of the leaf. Taking these assumptions into account, the following number of lesions and percentage of diseased area have been calculated for each scale value:

Scale value	No. of lesions	Percentage of diseased area
0	0	0
1	1	0.6
2	2	1.3
3	4	2.5
4	8	5.2
5	15	9.7
6	29	18.5
7	52	33.1
8	84	54.1
9	118	75.6

Scale III will be useful when screening a large number of plants. When few observations are to be made, it will be more accurate to count

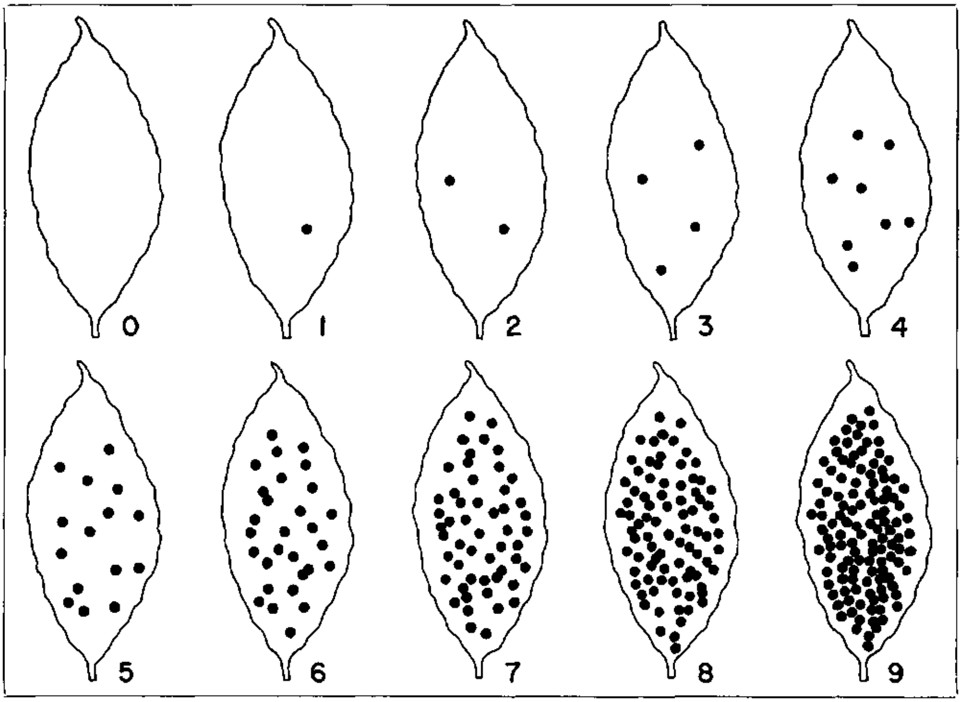

Figure 4. *Disease assessment Scale III: infection frequency of coffee leaf rust on individual leaves*

the actual number of lesions per unit leaf area.

In addition to assessments of infection frequency, it may be of interest to observe leaf retention after inoculation. This parameter can be important in determining field susceptibility in some *C. arabica* coffee types (Eskes *et al.*, 1979). An arbitrary *0* to *9* scale can be used, giving a *5* value for the commercial coffee cultivar. Higher values would indicate a longer leaf retention, lower values a shorter leaf retention. Both very high and low values may not be desirable. High values will give an inoculum build-up and, thus, a high apparent susceptibility, whereas low values may be correlated with low production capacity.

LABORATORY INOCULATION TESTS

Leaf disc inoculations. In the past, leaf disc inoculations have been sporadically used to measure vertical resistance (Narasimhaswamy, Narayanan Nambiar and Sreenivasan, 1961; CIFC, personal communication). Recently, this method has been extensively tested at the Instituto Agronomico of Campinas (Eskes, 1977, 1978; Eskes, Rivera and Da Costa, 1978; Eskes and Toma-Braghini, 1979).

The inoculation method used consists of applying a 0.025-ml droplet of a uniform uredopore suspension to the centre of coffee leaf discs 1.7 cm in diameter. The discs are incubated in the dark for 24 h, after which the droplets are allowed to dry. The discs are then maintained at 100 percent humidity, a temperature of 22 ± 2°C and moderate light intensity.

The method has given reliable results for different types of experiments, i.e., determination of vertical resistance and race differentiation, determination of partial resistance of field plants, determination of partial resistance of coffee lines grown in the nursery, determination of environmental effects on the expression of resistance, and determination of the effect of leaf age on the expression of resistance.

The advantages of the leaf disc inoculation method, compared with inoculations of intact plants, are as follows: very little inoculum and space are needed; the inoculation conditions are controlled, allowing for comparisons between locations and different inoculation data; plants selected in the breeding programme that come from distant locations can be tested by taking leaves to a central testing laboratory; resistance of field plants may be better determined in leaf discs than by field inoculations, as a random sample of leaves can be used for the inoculations; host/pathogen interactions can be studied very precisely by using discs of the same leaves for each combination; and in one experiment a large number of host/pathogen combinations can be studied under uniform conditions.

Assessment scale for disease severity on leaf discs. The observation unit for this scale is a group of 10 leaf discs. Scale IV (see Fig. 5) has been designed for measuring disease severity, using the same mathematical principles as those applied for Scale III. The size of one leaf disc is taken to be 2.54 cm^2, with the area of the infection droplet being 0.78 cm^2 (1 cm in diameter). Each dot on the scale represents a diseased area of about 3.5 mm^2.

Observations can be made once or repeatedly during the development of the lesions. Readings may include total area of diseased tissue or total sporulating area.

The assessment scale has given good correlations with other parameters for disease severity. For the number of lesions per disc, the correlation coefficient was $r = 0.86$ ($n = 84$) and, for the percentage of diseased discs, $r = 0.84$ ($n = 96$).

The degree of agreement between two trained observers was good. No difference in readings was obtained for 68 percent of the observations, one point difference for 30 percent and two points difference for 2 percent.

FACTORS AFFECTING THE RESISTANCE LEVEL

Partial resistance to coffee leaf rust may be affected by environmental as well as physiological factors. Experiments have shown a marked effect on resistance of productivity, leaf age, and light intensity (Eskes, 1977, 1978; Eskes, Rivera and Da Costa, 1978; Eskes and

Toma-Braghini, 1979; Eskes *et al.*, 1979; Eskes, Kroon and Van de Weg, 1980). These factors combined may produce substantial seasonal differences in resistance, and will affect the efficiency of selection for partial resistance to coffee leaf rust.

The level of natural disease in the field is the final test for partial resistance. However, observations should be repeated over several years, taking into account the biannual bearing habit of the coffee tree. Plants will show a higher degree of attack in a production year than in a non-production year. Selected plants should have a good yield and a low disease intensity.

In the *C. canephora* cv. Kouillou and in derivatives of the *C. canephora* × *C. arabica* cross, a marked effect of leaf age on resistance was found for several plants (Eskes, Rivera and Da Costa, 1978; Eskes, 1981). Generally, old leaves become more susceptible to coffee leaf rust. Inoculations using young leaves only may not be sufficient to indicate the real level of resistance of the plants.

Natural disease occurring in the nursery may be very valuable in determining the resistance of coffee populations. However, plants should be placed under moderate shade levels. Full sunlight may increase susceptibility to coffee leaf rust, but it may also kill the young lesions by overheating of the leaf (Eskes, Kroon and Van de Weg, 1980).

The environmental effect on disease severity is considerable in greenhouse inoculation experiments. The coefficient of variation of a

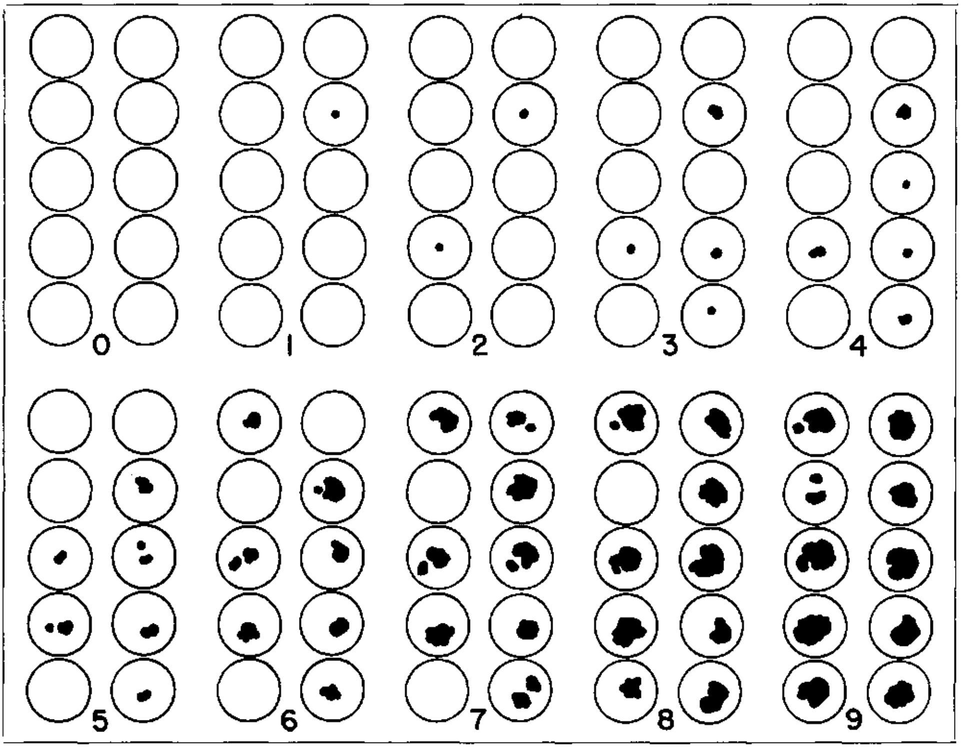

Figure 5. *Disease assessment Scale IV: disease severity of coffee leaf rust on leaf discs*

genetically homogeneous population of *C. arabica* plants may be over 100 percent. This variation can be reduced by predisposing the coffee seedlings to similar light conditions and by choosing seedlings of similar height. However, even then, the efficiency of individual plant selection for disease severity is considered low in greenhouse or nursery inoculation tests.

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2 THE USE OF LEAF DISK INOCULATIONS IN ASSESSING RESISTANCE TO COFFEE LEAF RUST *(HEMILEIA VASTATRIX)*

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ABSTRACT

The suitability of inoculations of leaf disks of 1.8 cm diameter in determining resistance of coffee to *Hemileia vastatrix*, the causal agent of coffee leaf rust, was studied. Results obtained by this method were similar to those obtained by greenhouse tests with respect to reaction types of coffee plants with complete and/or major gene resistance. The efficacy of the method in assessing incomplete resistance was tested on 19 plants of *Coffea canephora* cv Kouillou, which varied in level of disease in the field. Four series of inoculation were carried out in four different months of the year, and six components of resistance were assessed. The analysis of multiple correlation, applied to the average data of the four series, indicated that 79% of the variation in disease in the field could be explained by the observations in the leaf disk test. For the individual series this percentage varied from 58 to 70. The coefficients of correlation between the six components were significant and high. The percentage of leaf disks with sporulating lesions was found to be the most suitable component for assessing incomplete resistance.

The number of lesions per leaf disk was affected substantially by the hour of the day of leaf collection and by light intensity in the field. No effect was observed of the size of the disks (1 to 2 cm in diameter) and of the leaf wetness period after inoculation (24 and 48 h). Results were more consistent when the inoculum was applied in droplets of 0.025 ml than when the inoculum was sprayed onto the disks. No genotype × treatment interactions were observed for the hour of leaf collection, for the size of the leaf disk, for the inoculation method or for the leaf wetness period.

It is concluded that the leaf disk method, if adequately standardized, can be a very useful tool in breeding for coffee leaf rust resistance and also in basic research on the coffee - *H. vastatrix* relationship.

Additional keywords: laboratory tests, greenhouse tests, components of resistance, light intensity, leaf wetness period, urediospore density.

INTRODUCTION

Coffee leaf rust or orange coffee rust, *Hemileia vastatrix* Berk. et Br., has recently invaded many South and Central American countries. All *Coffea arabica* cultivars grown in America are susceptible to the common race II of *H. vastatrix*. Yield losses in Brazil owing to the disease have been estimated at 30%, if no control measures are taken (Monaco, 1977). Breeding for resistance has been hampered by the appearance of new rust races. Within three years of rust presence in Brazil the resistance genes S_H1, S_H2 and S_H4 lost their effectiveness (Monaco, 1977).

By the joint effort of the Instituto Agronômico of Campinas, Brazil, and of the Food and Agricultural Organization of the United Nations, a program was started in 1976 to investigate incomplete resistance to coffee rust, with the aim to obtain durable resistance. The present study resulting from this effort was carried out at the Genetics Department of the Instituto Agronomico.

Methods to determine incomplete resistance are time and space consuming and results may vary according to the environmental conditions. Inoculations of leaf disks, performed under controlled conditions, may reduce these inconveniences.

Detached plant parts have long been used to determine resistance to plant pathogens (Yarwood, 1946). Incomplete resistance to biotrophic pathogens has been determined with this method, reportedly with success (Hodgson, 1961; Umaerus and Lihnell, 1976; Verma and Petrie, 1978; Shain and Cornelius, 1979), although susceptibility ratings may be higher for detached plant parts than for intact plants (Atif and Wilcoxson, 1975; Zadoks, 1963). Retardants of senescence such as benzimidazoles have been applied to keep the tissues in a good state.

Inoculations of detached leaves or leaf parts of coffee have occassionally been used to study the biology of *H. vastatrix* (Ward, 1882; Nutman and Roberts, 1963; Saccas and Charpentier, 1971) and to assess major gene resistance (Mayne, 1932; B.d'Oliveira, personal communication; Narasimhswamy et al., 1961). Coffee leaf disks were incubated by placing them on moist filter paper or by floating them on water. Costa et al. (1978) found that leaf disks could be easier kept in a good state than entire detached leaves. Conservation of the disks, when placed on moist filter paper, was satisfactory for more than 100 days and no extra advantage of the use of benzimidazole was observed.

The present study was undertaken to evaluate the leaf disk method for its efficacy in determining complete as well as incomplete resistance to *H. vastatrix*. Also studies are reported on factors which may influence the results of the leaf disk test.

MATERIALS AND METHOD

Definitions. 'Complete resistance' is considered here to be a form of resistance that fully inhibits the reproduction of the pathogen. 'Major gene resistance' results from the action of one or a few, generally dominant genes, which individually have a great effect on resistance, but is not necessarily complete resistance. 'Incomplete resistance' is defined here as a form of resistance that allows for at least some reproduction of the pathogen.

Coffee genotypes. Plants of *C. arabica* cv Mundo Novo, *C. canephora* cv Kouillou and of the Icatu hybrid population were used. The 'Kouillou' field plants were about 45 years old and the Icatu plants were planted in the field in 1970. Icatu consists of backcrosses of an artificial hybrid between *C. arabica* (4 x = 44) and *C. canephora* (2 x = 22) with cultivars of *C. arabica* (Monaco, 1977). Most of the Icatu and Kouillou plants used showed medium or low levels of attack of coffee rust in the field, in comparison to cv Mundo Novo.

For the evaluation of major gene resistance the coffee differentials carrying the S_H1, S_H2, S_H3 and S_H4 genes of the Coffee Rusts Research Center (CIFC) in Oeiras, Portugal, were used, and also two plants of Icatu and Kouillou with complete race-specific resistance (Eskes et al., 1981).

Rust material. Unless stated otherwise, greenhouse or field isolates of race II (v5) of *H. vastatrix* were used. These isolates were maintained on living plants in the greenhouse. Spores were stored in the refrigerator at 52% relative humidity. Germination percentages of the urediospores were checked before each experiment by placing small quantities of urediospores on droplets of distilled water. Incubation was done in darkness at 22 ± 2 °C and germination counts were made after 14 hours. Only spore batches with more than 10% germination were used in experiments.

Standard method of leaf disk inoculation. Random samples of full-grown leaves were obtained from field, nursery or greenhouse plants, the use of old and damaged leaves being avoided. Leaves collected between 08.00 and 10.00 AM were temporarily stored in moist plastic bags. Disks were cut with a cork borer of 1.8 cm diameter early in the afternoon. These were placed in plastic boxes of size 48 × 64 × 10 cm, with the upper leaf side down, on a plastic foam layer of 2 cm thickness saturated with tapwater. The disks were kept moist by spraying distilled water until inoculation time, at the end of the afternoon.

Uniform inoculum was obtained by suspending urediospores in distilled water and shaking or stirring during five to ten minutes. Thereafter the suspension was kept in motion by a magnetic stirrer, to avoid deposition of the spores. For inoculation, samples containing 10 ml were taken from the suspension with a polyethylene micropipette (Beckton and Dickinson nr 5688 disposable pipette). One droplet of about 0.025 ml was placed onto each disk. Due to the moist surface of the disk the droplets spread to cover an area of about 0.5 - 0.7 cm^2. The coefficient of variation for the number of urediospores per droplet, as determined in 48 droplets, was 10.5 percent. Preparation of the inoculum and inoculation was always carried out under low light intensities.

Urediospore densities applied in the experiments on complete and/or major gene resistance varied from 0.8 to 1.2 mg ml^{-1}, and in the experiments on incomplete resistance from 0.1 to 0.5 mg ml^{-1}, according to the germination percentage of the spore batch. The number of spores present in one mg is about 1.5×10^5.

After inoculation the boxes were closed with glass lids and incubated in the dark, at 22 ± 2 °C, during 20 hours. Then the lids were removed in order to allow the inoculum droplets to evaporate. In an air-conditioned room this was completed within 3 to 5 hours. Care was taken to avoid drying out of the disks. After evaporation of the droplets, the disks were slightly wetted again and the closed boxes were placed under moderate light conditions (fluorescent or indirect day light of 500 - 1000 lux) with daily a 12 hour dark period. During incubation the water level in the plastic boxes was checked weekly and the disks moistened slightly. It was avoided that the edge would come into contact with liquid water. Temperature was not controlled in the experiments of which the results are shown in Table 2, 3, 5 and 7. In the other experiments temperature was maintained at 22 ± 2 °C by the use of an air-conditioner. This improved longevity of the disks and was adopted as a standard, although adequate results have also been obtained at less controlled temperatures (Costa et al., 1978).

Greenhouse and field tests. Greenhouse tests were carried out by spraying a urediospore suspension with a Steula I paint sprayer, pressurized with a small pump, onto the abaxial surface of healthy leaves, usually belonging to the youngest two leaf pairs. The spore densities used varied from 0.5 to 1.5 mg urediospores per ml. Afterwards, the plants were incubated in the dark at 100% relative humidity and 22 ± 2 °C during 24 to 48 hours. For field experiments a manual De Vilbiss atomiser was used to apply the urediospore suspension. The reservoir of the atomiser was protected against the light by aluminium foil. After inoculation the leaves were covered immediately by an inner plastic bag, containing some liquid water, and an outer paper bag. Field inoculations were carried out in the late afternoon and the bags were removed early in the next morning.

Inoculation dates. The dates on which the experiments shown in Table 2 to 7 were started are respectively: 2 May and 29 September 1977, 12 April, 1 January and 10 January 1978 and 2 May 1977.

Observations. The following components of resistance have been recorded in the leaf disk tests:

1. the first day of sporulation on a disk (FDS),
2. the latency period (LP), which is the time in days from inoculation until 50 percent of all finally sporulating disks have come to sporulation,
3. the number of visible lesions per disk (NLD),
4. the percentage of disks with lesions (PDL),
5. the percentage of disks that come to sporulation (PDS),
6. the percentage of disks with sporulating lesions as a percentage of the total number of disks with lesions (PDSDL = 100·PDS/PDL),
7. the spore production per lesion or disk (SP) determined 10 days after LP is completed,
8. the percentage of disks with necrotic lesions (PDN), and
9. the reaction type (RT).

When the first lesions appeared, records were taken every second day on the number of disks with sporulating lesions, using a hand lens. NLD was determined at the onset of sporulation, when the individual lesions were best recognizable. If lesions overlapped, an estimation of the number of lesions was made based on the size of separate lesions. Symptoms of infection counted as lesions included tumefactions (groups of swollen spongy tissue cells, Rijo, 1972), flecks (tiny chlorotic spots) and larger chlorotic areas, with or without sporulation. To determine SP, urediospores were collected by means of a small vacuum pump into a known volume of water. Spores were counted using a haemocytometer for high densities or droplets of 0.025 ml placed on microscope slides for low densities. RT was assessed by means of a 0 to 9 scale (Eskes and Toma-Braghini, 1981). Scale value 0 indicates absence of visible symptoms, values 1 to 3 indicate variation within resistant reaction types (no sporulating lesions), values 4 to 7 indicate heterogeneous reaction types with increasing sporulation intensity and increasing percentage of sporulating lesions, and values 8 and 9 indicate susceptible reaction types with large lesions with moderate (8) to intense (9) sporulation. Greenhouse and field observations included determination of LP (number of days from inoculation till 50% of the final number of sporulating lesions sporulated) and lesion density (LD = number of lesions per leaf or leaf area unit). Assessment of natural infection in the field was made using a 1 to 5 scale. Value 1 indicates absence of sporulating lesions and values 2 to 5 indicate increasing rust incidence and increasing reaction type. Field scores were made annually, for Icatu plants, and twice a year (February and August), for Kouillou plants, from 1976 through 1981.

Longevity of the disks. Using the standard inoculation method the leaf disks stayed green and apparently healthy for more than three months. The percentage of deteriorated leaf disks was less than 1 percent for the reported experiments. Best results were obtained during the months October through April, when the coffee plants were actively growing. Disks taken in a period of natural leaf fall may become yellow and senescent within a few weeks.

Occasionally infections other than those of coffee leaf rust occurred, mostly due to coffee leaf miner (*Perileucoptera coffeella*). When dirty leaves were used, a grayish fungal growth developed sometimes at the side of the infection droplet, interfering with the results. In one experiment a darkbrown wet rot developed which destroyed the disks. This was no longer observed when autoclaving of the plastic foam was adopted routinely. In one case, an infestation of mites was observed, which consumed freshly produced urediospores. Control was obtained by applying an acaricide. The occurrence of *Verticillium hemileiae* was frequently observed, but this hyperparasite of coffee rust appeared so late that it did not interfere with the results.

Statistics. For the leaf disk test the plastic boxes have been considered as replications. Generally 20 disks have been used per replication. To elaborate the results of the leaf disks tests applied to the Kouillou cultivar (Tables 8 and 9) SPSS (Statistical Package for the Social Sciences), has been used.

RESULTS

Evaluation of complete and/or major gene resistance. Great similarity in RT was observed when comparing the results of leaf disk tests with the results of greenhouse inoculations (Table 1). The resistance of gene S_H4 was not always complete in the leaf disk test. This finding is in accordance with the relative instability of the S_H4 gene under nursery conditions (Eskes, 1979).

The precision of the leaf disk test in determining RT was estimated in an experiment including seven plants of cv Kouillou and three rust races. Seven of the 21 combinations were compatible ones. The experiment was done in three replications, each containing 15 leaf disks. The $LSD_{0.05}$ value for RT was 1.10 for the comparison between combinations. If either 2 replications or no replications would have been used, the $LSD_{0.05}$ values would have been respectively 1.34 and 1.90. This result suggests that, as far as qualitative differences in resistance are concerned, observations based on 15 leaf disks will provide a fair estimate.

Table 1. Variation in reaction type (RT), as observed on a 0 to 9 scale, in leaf disk and greenhouse tests of six coffee genotypes with compatible and incompatible races of H. vastatrix. For the leaf disk test each figure is based on 15 to 30 inoculated disks.

Coffee genotype	Resistance gene involved	Leaf disk test		Greenhouse test	
		Compatible	Incompatible	Compatible	Incompatible
CIFC 87/1	S_H1	8-9	0-2	7-9	0-2
CIFC 32/1	S_H2	8-9	1	8-9	0-2
CIFC 33/1	S_H3	-	0-1	-	1-2
CIFC 110/5	S_H4	8-9	2-5	8-9	2-3
Icatu H3851-4-40	?	5-8	1-2	5-8	1-2
Kouillou C66-13	?	8	0-1	9	0

Tabel 1. Variatie in reactietype (RT), waargenomen op een schaal van 0 tot 9, bij bladschijf- en kastoetsen van zes koffiegenotypen met compatibele en incompatibele fysio's van H. vastatrix. Bij de bladschijftoets is ieder getal gebaseerd op 15 à 30 geïnoculeerde bladschijven.

Factors that may influence the results of the leaf disk test. The effect of urediospore density and that of the addition of Tween 20 to the urediospore suspension were studied for cv Mundo Novo. The LP was slightly shorter at high inoculum densities, whereas NLD, PDS and PDN were linearly related to density (Table 2). Only the increase in NLD was not completely linear, because at 400 mg l^{-1} overlapping of lesions occurred. Necrosis of sporulating lesions started about ten days after the onset of sporulation. The addition of Tween 20 reduced NLD and PDS (Table 2). This was unexpected, because germination of the urediospores can be enhanced by addition of Tween (Stahmann et al., 1976).

Table 2. Latency period (LP, in days), number of lesions per disk (NLD), percentage of disks with sporulating lesions (PDS), and percentage of disks with necrotic lesions (PDN, observed 50 days after inoculation) of leaf disks of cv Mundo Novo inoculated with four urediospore densities. For the 200 mg l^{-1} density the addition of Tween 20 was also tested. The germination percentage of the spores was 21. Each entry is based on 360 inoculated disks.

Urediospore density mg l^{-1}	Parameters of infection			
	LP	NLD	PDS	PDN
50	44	0.23	21	0
100	43	0.45	31	4
200	43	0.84	44	8
400	40	1.32	57	12
200 + 0.02% Tween 20	43	0.21	17	1

Tabel 2. Latentieperiode (LP, in dagen), aantal lesies per bladschijf (NLD), percentage bladschijven met sporulatie (PDS) en percentage bladschijven met necrotische lesies (PDN, waargenomen 50 dagen na inoculatie) van bladschijven van cv Mundo Novo geïnoculeerd met vier urediosporedichtheden. Bij 200 mg/l werd ook de toevoeging van Tween 20 getoetst. Het percentage gekiemde sporen bedroeg 21. Ieder getal is gebaseerd op 360 geïnoculeerde bladschijven.

The effect of the size of the leaf disk was studied for two coffee genotypes (Table 3). The analysis of variance detected a significant effect of genotype on LP and PDS, but not of disk size and of interaction. The 1 cm diameter size is considered to be too small for routine experiments, because the 1 cm disks deteriorated rapidly after the onset of sporulation.

Table 3. Latency period (LP, in days) and percentage of disks with sporulating lesions (PDS) of leaf disks of different sizes of two coffee genotypes. Each entry is based on 60 inoculated disks.

Coffee genotype	Disease score in the field (1-5 scale)	Diameter of disk (cm)	Component of resistance	
			LP	PDS
Cv Mundo Novo	4.5	1.0	24	78
		1.5	27	72
		2.0	27	59
Cv Kouillou C68-4	3.1	1.0	33	16
		1.5	33	16
		2.0	36	26

Tabel 3. Latentieperiode (LP, in dagen) en percentage bladschijven met lesies (PDS) van bladschijven van verschillende grootte van twee koffiegenotypen. Ieder getal berust op 60 geïnoculeerde bladschijven.

The effect of leaf wetness period on germination of the urediospores, appressorium formation and infection was studied for three coffee genotypes (Table 4). The percentage of urediospore germination (PGU) was similar for the 9 and 24 h treatment. Appressorium formation (PAF) was near to zero after 9 h and had a mean value of 62 percent after 24 hours. No significant effect of genotype and of the genotype × treatment interaction was obtained for PGU and PAF.

Table 4. Percentage of germinated urediospores (PGU), percentage of appressoria in relation to PGU (PAF), and percentage of disks with sporulation (PDS) observed for three leaf wetness periods with three coffee genotypes. Entries are based on 10 (PGU, PAF) or 90 (PDS) inoculated disks.

Coffee genotype	Disease score in the field (0-5 scale)	Wetness period						
		9 hours			24 hours			48 hours
		PGU	PAF	PDS	PGU	PAF	PDS	PDS
Cv Mundo Novo	4.5	17.2	0.4	1	17.4	60	71	65
Icatu H3851-2-291	4.8	17.5	0.4	11	18.1	64	86	86
Cv Kouillou C69-14	2.0	15.9	0.4	0	18.1	63	21	16
Mean		16.9	0.4	4	17.9	62	59	56

Tabel 4. Percentage gekiemde urediosporen (PGU), percentage appressoria betrokken op PGU (PAF) en percentage bladschijven met sporulatie (PDS) waargenomen voor drie bladnatperiodes bij drie koffiegenotypen. De getallen berusten op 10 (PGU, PAF) of 90 (PDS) geïnoculeerde bladschijven.

Germination and appressorium formation could not be determined after 48 hours, due to abundant growth of other fungi in the inoculation droplet. The PDS values were very low for the 9 hours treatment, in accordance with the PAF values observed for this treatment. For the

24 and 48 hours treatment, no significant differences were observed for PDS either between treatments or between genotypes.

The standard inoculation method was compared to inoculation by spraying for two coffee genotypes at two urediospore densities (Table 5). About equal amounts of inoculum were applied per disk with both methods. The inoculation method did not significantly affect LP. The results for NLD were less consistent with the spraying method than with the droplet method. For the Kouillou genotype lesion formation occurred mainly at the edge of the disk with the spraying method. These edge lesions showed an abnormal development. Therefore, the droplet method was preferred as the standard inoculation method.

Table 5. Latency period (LP, in days), number of lesions per disk (NLD), and number of urediospores produced per lesion (SP, × 10^3) of two coffee genotypes inoculated in two different ways with two urediospore densities. The germination percentage of the urediospores was 24. Each entry is based on 166 inoculated disks.

Coffee genotype	Disease score in the field (0-5 scale)	Urediospore density (mg l^{-1})	Inoculation method					
			Spraying			Droplets		
			LP	NLD	SP	LP	NLD	SP
Cv Mundo Novo	4.5	33	30	1.13	3.5	29	0.73	4.8
		100	30	1.10	3.8	30	1.42	5.4
Cv Kouillou C68-15	4.3	33	45	0.14	0.4	41	0.33	0.6
		100	45	0.44	0.5	44	0.79	1.1

Tabel 5. Latentieperiode (LP, in dagen), aantal lesies per bladschijf (NLD) en aantal urediosporen geproduceerd per lesie (SP, × 10^3) van twee koffiegenotypen geïnoculeerd op twee manieren met twee urediosporedichtheden. Het kiempercentage van de urediosporen was 24. Ieder getal berust op 166 geïnoculeerde bladschijven.

The effect of the hour of the day at which leaves were collected and of the time span between collection of the leaves and cutting of the disks was studied for two genotypes (Table 6). The experiment was carried out on a day with bright sunshine. The analysis of variance detected significant genotype and treatment effects on PDS, but the interaction was not significant. A significant increase in PDS occurred when leaves were collected later on the day (treatments 1 to 3). When some hours elapsed between the collection of the leaves and the cutting of the disks, a decrease in PDS occurred (treatments 4 to 6 in comparison to 1 and 2). The difference between treatments 4 and 5, which represent the variation observed when the standard inoculation method is applied, was not significant. For latency period no significant effects were observed in this experiment.

Table 6. The percentage of disks with sporulation (PDS) of leaves of two coffee genotypes collected at three different hours of the day and of which disks were cut at different time spans from collection. In all treatments disks were inoculated at 17.30. The disease score in the field of cv Mundo Novo and cv Kouillou C70-11, was 4.3 and 1.8 respectively. Each entry is based on 80 inoculated disks. Different letters indicate significance of differences according to the $\mathrm{LSD}_{0.05}$ value.

	Treatment		PDS		
			Coffee genotype		
Number	Collection of leaves (hour)	Cutting of disks (hour)	Cv Mundo Novo	Cv Kouillou C70-11	Mean
1	8.30	9.00	35	16	26b
2	13.30	14.00	48	29	38c
3	16.30	17.00	76	55	66d
4	8.30	14.00	23	8	15ab
5	8.30	17.00	18	9	14ab
6	13.30	17.00	14	13	13a
Mean			35	22	29

Analysis of variance:

	DF	MS	F	P
Genotype (G)	1	552.0	45.3	<0.01
Treatment (T)	5	698.3	57.3	<0.01
G × T	5	21.3	1.8	n.s.
Error	12	12.2		
Total	24			

Tabel 6. Het percentage bladschijven met sporulatie (PDS) van bladeren van twee koffiegenotypen geplukt in het veld op verschillende uren van de dag en waarvan schijven werden geponst op verschillende tijdstippen na het plukken. De ziektescore in het veld van cv Mundo Novo en cv Kouillou C70-11 was 4.3, respectievelijk 1.8. Ieder getal berust op 80 geïnoculeerde schijven. Verschillende letters duiden op significantie van verschillen volgens de $\mathrm{LSD}_{0.05}$ waarde.

Table 7 shows the effect of light intensities to which leaves of cv Mundo Novo were exposed in the field, before collection, and to which disks were exposed in the laboratory. LP was only slightly affected by light intensity in the field, but far more infection developed on disks of sun exposed leaves than of shaded leaves. This result shows the importance of using leaves grown under similar light conditions for the leaf disk test. The light intensity treatments in the laboratory did not affect LP, NLD, and PDS, but it did affect the longevity of the disks. At low light intensity in the laboratory, sporulation intensity was low and the infected disks soon became necrotic, sometimes even before sporulation began. Therefore, light intensities in the laboratory above a certain minimum seem to be a requisite for success with the leaf disk method.

Table 7. Latency period (LP, in days), percentage of disks with sporulation (PDS), and percentage of disks with necrotic lesions (PDN, 50 days after inoculation) of disks of cv Mundo Novo from shaded and sun-exposed leaves in the field placed at different light intensities (LI) in the laboratory (indirect daylight). Each entry is based on 400 inoculated disks.

LI of leaves in the field	LI in the laboratory					
	100 lux			500 lux		
	LP	PDS	PDN	LP	PDS	PDN
Exposure to shade	44	11	2	43	12	0
Exposure to sunlight	42	59	45	41	53	7

Tabel 7. Latentieperiode (LP, in dagen), percentage schijven met sporulatie (PDS) en percentage schijven met necrotische lesies (PDN, 50 dagen na inoculatie) van bladschijven van cv Mundo Novo verkregen van schaduw- en zonnebladeren in het veld geplaatst bij verschillende lichtintensiteiten (LI) in het laboratorium (indirect daglicht). Ieder getal is gebaseerd op 400 geïnoculeerde schijven.

The efficacy of the leaf disk method in assessing incomplete resistance. In three experiments the leaf disk test was compared to greenhouse or field inoculations. In the first experiment six leaves of each of eight plants of cv Kouillou were inoculated in the field. The opposite leaves were used for the leaf disk test (10 disks per leaf). The coefficient of correlation between the two inoculation methods was significant at $P \leq 0.05$ for the number of lesions ($r = 0.66$) but not for the latency period ($r = 0.06$). In the second experiment, leaf disk and greenhouse tests were carried out on 25 seedlings of cv Mundo Novo. Two leaves per seedling were used for the leaf disk test (14 disks per plant) and the opposite leaves were inoculated in the greenhouse. The coefficients of correlation between the two methods were low but significant at $P \leq 0.05$, both for latency period and for the number of lesions per leaf (0.43 and 0.56, respectively). In the third experiment the leaf disk test was applied to seedlings of 37 F_3 progenies of the cross H7317 (Agaro C 1164-19 × cv Catuai), grown in the nursery. For these inoculations race XV (v_4v_5) has been used instead of race II (v_5) because of the occurrence of S_H4 in the F_3 progenies. Fifty six leaf disks, obtained from 20 randomly choosen leaves per progeny, were inoculated in February 1978. Two months later four leaves of each of 15 plants per progeny were used for the greenhouse inoculations. The coefficients of correlation between the results of the two methods were significant at $P \leq 0.01$ for latency period and for the number of lesions (0.80 and 0.66, respectively).

In a fourth experiment the efficacy of the leaf disk method in assessing the level of disease in the field of 19 plants (genotypes) of cv Kouillou, was tested. The standard inoculation method was applied four times in different months of 1978 and 1979 (four series). For each series, 40 randomly chosen leaves were used per plant, in 4 or 5 replications with 20 disks per replication. Inoculation and incubation of the disks was done in an air-conditioned room at a temperature of 22 ± 2 °C.

The mean PDS values of cv Mundo Novo were 35, 81, 19 and 86 respectively for series 1 to 4. For the 19 Kouillou genotypes PDS varied from 1 to 69, 9 to 68, 1 to 55 and 16 to 100 in the four series. The mean level of disease in the field was 4.4 for cv Mundo Novo and varied from 1.6 to 5.2 for the Kouillou genotypes. All simple and multiple coefficients of linear correlation between the components of resistance observed in the leaf disk test and the level of disease in the field were significant at $P \leq 0.05$ (Table 8). The proportion of the variance for disease level in the field explained by the observed components, as assessed by the R^2 values, varied

Table 8. Coefficients of simple and multiple linear correlation between components of resistance, observed in four series of leaf disk tests, and the average disease score in the field of 19 plants of cv Kouillou. For each series 80 to 100 leaf disks per genotype were inoculated. The components of resistance observed were the number of days from inoculation till first sporulation (FDS), latency period in days (LP), number of lesions per disk (NLD), percentage of disks with lesions (PDL), the percentage of disks with sporulation (PDS), and the percentage of disks with sporulation relative to PDL (PDSDL = 100 · PDS/PDL). Simple r is significant at $P \leq 0.05$ when $r \geq 0.46$.

Coefficient of correlation	Component of resistance	Series number and inoculation date				Coefficients for the means of series 1 to 4
		1 December 1978	2 March 1979	3 May 1979	4 October 1979	
Simple r	FDS	-.66	-.67	-.64	-.58	-.76
	LP	-.69	-.72	-.54	-.67	-.77
	NLD	.48	.55	.54	.71	.78
	PDL	.51	.61	.57	.60	.79
	PDS	.66	.70	.63	.69	.82
	PDSDL	.63	.55	.52	.53	.61
Multiple R		.84	.78	.76	.81	.89
R^2		.70	.61	.58	.66	.79

Tabel 8. Coëfficiënten van enkelvoudige en multipele correlatie tussen resistentiecomponenten, waargenomen in vier series van bladschijftoetsen, en de gemiddelde ziektescore in het veld bij 19 planten van cv Kouillou. Voor ieder genotype werden per serie 80 à 100 bladschijven geïnoculeerd. De waargenomen resistentiecomponenten zijn: het aantal dagen vanaf inoculatie tot eerste sporulatie (FDS), latentieperiode in dagen (LP), het aantal lesies per bladschijf (NLD), het percentage bladschijven met lesies (PDL), het percentage bladschijven met sporulatie (PDS) en het percentage bladschijven met sporulatie betrokken op PDL (PDSDL = 100 · PDS/PDL). Enkelvoudige r waarden zijn significant bij $P \leq 0.05$ als $r \geq 0.46$.

from 0.58 to 0.70 for the individual series. This proportion was 0.79 when the means of the four series were used for the calculations. All coefficients of correlation were higher for the means of the 4 series than for the individual series. The coefficients of correlations between the observed components were generally high, especially between FDS and LP and between NLD and PDL (Table 9).

For the pooled analysis of variance over the four series a transformation of the percentage data into the arcsine of their square roots was applied. The effects of series, genotypes, and interactions were significant for all components. The coefficients of variation observed for FDS, LP, NLD, PDL, PDS and PDSDL were 17, 14, 41, 20, 24 and 23%, respectively.

Table 9. Coefficients of linear correlation between the means of six components of resistance observed in four series of leaf disk inoculations applied to 19 plants of cv Kouillou. For explanation of abbreviations see Table 8. Coefficients are significant at $P \leq 0.01$ when greater than 0.57.

Components of resistance	Components of resistance				
	LP	NLD	PDL	PDS	PDSDL
FDS	.95	-.77	-.73	-.92	-.86
LP		-.69	-.63	-.85	-.83
NLD			.95	.87	.54
PDL				.86	.53
PDS					.87

Tabel 9. Coëfficiënten van lineaire correlatie tussen de gemiddelden van zes resistentiecomponenten waargenomen in vier series van bladschijftoetsen bij 19 planten van cv Kouillou. Voor verklaring van de gebruikte afkortingen zie tabel 8. De coëfficiënten zijn significant bij $P \leq 0.01$ als ze groter zijn dan 0.57.

DISCUSSION

Results. The leaf disk inoculation method appeared to be adequate in assessing complete and/or major gene resistance (Table 1). Observation of as few as 15 disks can give reliable results, if sufficiently high infection percentages are attained in the susceptible control. At urediospore densities of 0.8 to 1.2 mg ml^{-1} infection of the control cv Mundo Novo was generally sufficiently high (PDS values of 70 to 100%). The use of leaves, harvested from twigs exposed to high light intensities, helped to assure high infection percentages (Table 7) and is, therefore, recommended.

For assessment of incomplete resistance the use of 2 to 4 replications, with 20 disks each, is recommended. Urediospore densities should be calibrated according to the percentage of germinated urediospores, in order to obtain that level of infection at which the discriminative capacity of the method is highest (50 to 70% sporulating disks of the susceptible control). In our experiments the calibration was done according to the results shown in Table 2.

The average number of germinated urediospores needed to produce, in average, one lesion on disks of cv Mundo Novo was about 100, but variation between experiments was considerable. Extreme average values were 52 (Table 5, droplet method) and 193 (Table 2). This compares well to inoculations of intact leaves of cv Mundo Novo, where this figure ordinarily varies between 100 and 300 (personal observations).

Latency periods of leaf disks are generally about five to ten days shorter than those of intact leaves on greenhouse or field plants. When temperatures in the laboratory were not controlled the latency period of cv Mundo Novo in leaf disks varied from 24 (Table 3) to 44 (Table 1), attaining highest values in the winter. When temperatures in the laboratory were controlled, at 22 ± 2°C, the observed variation in LP between summer and winter was smaller (about 23 to 30 days). For seedlings of this cultivar, grown in the open, a variation from 31 days (summer) to 54 days (winter) was reported (Moraes et al., 1976).

Certain factors were shown to greatly affect the results of the leaf disk test (Tables 2 to 7), but no important interactions between coffee genotypes and these factors were observed (Table 3 to 6). The absence of interaction indicates that the leaf disk method can be used to assess incomplete resistance but the variability of the results dictates that the method be rigourously standardized. The experience gained so far suggests that this also holds for other, more laborious, inoculation methods.

Table 8 shows the efficacy of the standardized leaf disk method in assessing incomplete resistance of 19 cv Kouillou plants. Between 58 and 70% of the variance for disease level in the field could be explained by the results of individual tests, which is considered satisfactory. Among the components of resistance, PDS gave the highest coefficients of correlation (r). According to the r^2 values, this component alone could explain 40 to 49% of the variance in disease in field. Lowest but still significant r values were obtained for PDSDL, which is an indicator of reaction type. Apparently, part of the variation for incomplete resistance among the Kouillou plants is related to reaction type.

Accurate observations on components of resistance are laborious. For routine applications of the leaf disk test, Eskes and Toma-

Braghini (1981) proposed to use assessment scales running from 0 to 9 for scoring lesion incidence and reaction type.

Applications. The leaf disk method seems suitable for various types of research on the coffee - *H. vastatrix* relationship, including race identification, biology of the rust, assessment of complete and incomplete resistance, and the study of environmental and physiological factors. The breeder could use the method as an early screening method of progenies grown in the nursery or of individual field grown plants.

Limitations. The limitations of the leaf disk method mentioned hereafter possibly apply also to inoculations of intact leaves. Environment may influence incomplete resistance. In Table 8, the highest coefficient of variation was observed for NLD, indicating that resistance expressed by a low lesion density is particularly affected by environment and, therefore, is difficult to assess.

The leaf disk method gives more reliable results the greater the differences in resistance are. If small differences in resistance must be measured, repetition of the experiment in time will be necessary.

The test cannot be recommended to select for incomplete resistance of individual nursery plants. More plants per genotype are needed to obtain satisfactory high coefficients of correlation.

Certain forms of incomplete resistance may not be observable in leaf disks, as is the case for resistance related to leaf retention, reported for some coffee populations (Eskes et al., 1979).

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SAMENVATTING

Het gebruik van bladschijfinoculaties voor het bepalen van resistentie tegen koffieroest (Hemileia vastatrix).

De geschiktheid van inoculaties van bladschijven van 1.8 cm diameter voor de bepaling van resistentie van koffie tegen *Hemileia vastatrix*, de veroorzaker van koffieroest, werd nagegaan. Voor het bepalen van het reactietype van koffieplanten, met complete en/of monogene resistentie, bleek de bladschijfmethode resultaten op te leveren die vergelijkbaar waren met die van kasproeven. De geschiktheid van de methode voor het bepalen van onvolledige resistentie werd beproefd bij 19 planten, behorende tot het ras Kouillou van *Coffea canephora*, die varieerden in veldaantasting. Vier inoculatie series werden uitgevoerd in vier verschillende maanden van het jaar, waarbij zes resistentie-componenten werden bepaald. Multipele correlatie voor de gemiddelden van de vier series toonde aan dat 79% van de variatie in veldaantasting te verklaren was door de waargenomen componenten in de bladschijftoets. Voor de individuele series varieerde dit percentage tussen de 58 en 70. De zes componenten vertoonden onderling een sterke mate van correlatie. Het percentage bladschijven met sporulatie bleek de meest geschikte component te zijn voor het schatten van onvolledige resistentie.

Het aantal lesies per bladschijf werd duidelijk beïnvloed door het uur van de dag waarop de bladeren werden geplukt en de hoeveelheid licht waaraan de bladeren waren blootgesteld in het veld. De grootte van de bladschijven (1 à 2 cm in diameter) en de bladnatperiode na inoculatie (24 en 48 uur) bleken hierop geen effect te hebben. De inoculatiemethode, waarbij druppels van 0,025 ml. werden gebruikt, bleek meer consistente resultaten te geven dan de methode waarbij het inoculum op de bladschijven werd gespoten. Er werd geen genotype × behandeling interactie waargenomen voor het uur van de dag waarop de bladeren werden geplukt, de grootte van de bladschijven, de inoculatiemethode en de duur van de bladnatperiode.

Geconcludeerd wordt dat de bladschijfmethode, mits toegepast in gestandaardiseerde vorm, een zeer bruikbaar hulpmiddel kan zijn bij de veredeling van koffie op roestresistentie en bij het onderzoek naar de relatie tussen koffie en *H. vastatrix*.

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3 THE EFFECT OF LIGHT INTENSITY ON INCOMPLETE RESISTANCE OF COFFEE TO *HEMILEIA VASTATRIX*

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ABSTRACT

Resistance of coffee to race II of *Hemileia vastatrix* was tested in different environments at light intensities (LI) from 17 to 100% of total outdoor radiation. Nine treatments, in which three levels of LI before inoculation were combined with three levels of LI after inoculation, were applied to seedlings of the susceptible cv Mundo Novo. Higher LI before inoculation induced a significant increase in lesion density, whereas the opposite was observed for treatments after inoculation. Maximum differences in lesion density were three-fold. The interaction between pre- and post-inoculation treatments was also significant. Necrosis of lesions occurred under extremely high LI after inoculation.

Genotypes of the Icatu population and of *Coffea canephora* cv Kouillou, which varied in disease level in the field, were tested in different environments, constant LI being applied before and after inoculation. The resistance of most genotypes was higher at low LI than at high LI, paralleling the results obtained for the control cv Mundo Novo. With cv Kouillou, sporulating lesion density, latency period and reaction type were significantly affected by LI and genotype. The interaction effect was significant for sporulating lesion density and reaction type, mainly because the most resistent genotype was not affected, or affected in opposite direction, by LI.

Environment affected the expression of the resistance gene S_H4. Observations on a segregating F2 population indicated dominant gene action in the greenhouse (low LI) and incomplete dominant to nearly recessive gene action in the nursery (high LI). This incomplete dominance was expressed by heterogeneous to susceptible reaction types of heterozygote plants (S_H4s_H4), under high LI.

Some ecological and breeding aspects of the observed effect of LI on resistance to coffee leaf rust are discussed.

Additional keywords: complete resistance, major gene resistance, temperature, heterogeneous reaction type, components of resistance.

INTRODUCTION

The influence of environment is a common aspect of plant disease (Colhoun, 1973). Knowledge of how environment affects disease is of great importance to the plant breeder, who must choose a suitable environment for resistance screening. It is also essential for a basic understanding of the physiology and ecology of host-pathogen relationships.

Incomplete resistance is generally more affected by environment than complete, major gene resistance. Polygenically inherited incomplete resistance, even when partially race-specific, could provide a more durable protection to diseases than complete monogenic resistance, but this idea is still being discussed in the literature (e.g. Simons, 1972; Vanderplank, 1975; Parlevliet and Zadoks, 1977). Durable resistance to orange coffee rust or coffee leaf rust (*Hemileia vastatrix* Berk. et Br.) is considered to be of great value for the perennial coffee crop.

Effects of light intensity (LI) on resistance were reported for a number of plant diseases, e.g. yellow rust of wheat and barley (Bever, 1934; Manners, 1950; Stubbs, 1967), late blight of potato (Umaerus, 1970; Schumann and Thurston, 1977) and powdery mildew in oats (Jones, 1975). These reports refer to major gene resistance as well as to 'field resistance' or 'adult plant resistance'.

Little is known about the influence of environment on resistance to coffee leaf rust. According to d'Oliveira (1957), who selected for coffee rust resistance, LI and temperature may affect symptom expression. Monaco et al. (1973) observed inhibition of lesion growth when plants were temporarily exposed to a temperature of 40 °C. Rodrigues (1975) found treatments of 45 °C during 1.5 h to cause predisposition to coffee rust. He mentioned that these treatments resulted in a higher susceptibility of compatible and of some normally incompatible combinations.

The studies reported in the present paper were undertaken following observations in the greenhouse and the field, which indicated an effect of shade on resistance to coffee leaf rust.

MATERIALS AND METHODS

Definitions. Complete resistance is considered here as any form of resistance in which reproduction of the pathogen is completely inhibited. Incomplete resistance allows for at least some reproduction of the pathogen. Major gene resistance is related to one or a few genes each of which has a great effect on resistance. Major gene resistance is not always complete resistance (Parlevliet, 1979).

Location. All experiments were carried out at the experimental station of the Instituto Agronômico at Campinas, S.P., Brazil.

Coffee genotypes. One year old seedlings of *Coffea arabica* cv Mundo Novo, cv Catuai and of the F2 population of cross H 7317 (Agaro C 1164-19 × cv. Catuai) were used in experiments 1 to 4. Cv Mundo Novo and cv Catuai are genetically related cultivars both susceptible to race II of *H. vastatrix*. The Agaro C1164-19 plant is homozygous for the S_H4 resistance gene. In experiments 5 and 6 cuttings from field plants of *C. canephora* cv Kouillou and of Icatu were used. Icatu consists of tetraploid plants of advanced breeding generations of an artificial hybrid between *C. canephora* (2x = 22) and *C. arabica* (4x = 44). Cv Kouillou is commercially grown under the name of 'Conilon' in the state of Espirito Santo, Brazil. All the Kouillou and Icatu genotypes showed at least some infection in the field.

Rust material. In all experiments isolates of race II of *H. vastatrix*, which has the v5 virulence factor, were used. The isolates were maintained in the greenhouse on susceptible coffee plants and spores were stored in the refrigerator at 52% relative humidity. Racial purity was regularly controlled by inoculation on the coffee differentials for S_H1, S_H2, S_H3 and S_H4. Before each experiment, the germination percentage of the urediospores in distilled water was determined. Spore batches with less than 10% germination were not used. According to the germination percentage of the spore batch, the urediospore densities applied in the experiments varied from 0.5 to 1.5 mg ml^{-1} (75 x 10^3 to 225 x 10^3 spores per ml).

Standard inoculation method. Uniform dilution of the urediospores in distilled water was obtained by shaking and stirring the suspension during 10 min. Inoculations were carried out by spraying the suspension on the abaxial surface of the coffee leaves with a Steula I paint sprayer pressurized by a small pump. The whole leaf surface was uniformly covered with small droplets, at a rate of about 0.3 ml per 10 cm^2 leaf area. The coefficient of variation of the spore deposite as determined on glass slides was 23%. The number of inoculated leaves per plant varied from 3 to 5, for seedlings, and from 8 to 36 for cuttings. After inoculation the plants were placed in a humid dark room at 22 ± 2 °C for 24 to 48 h.

Inoculation dates. Inoculations of experiments 1 and 2 were done in September, and of experiment 3 in November, 1979. Inoculations of experiments 5 and 6 were made in January and March, 1981.

Light intensity treatments. Different LI were applied by placing the plants in the greenhouse, in the nursery, or in two different screenhouses. In these environments radiation was respectively 17, 66, 24 and 43% of total radiation, as determined with an Epplin radiometer on a bright day, when the total radiation was 2200 $J.cm^{-2}$. Average radiation in Campinas during the summer months is about 1900 to 2500, and during the winter months 1000 to 1500 $J.cm^{-2}$. Shade was provided by chalk on the window panes of the greenhouse, by wooden lathing in the nursery, and by black nylon 'Sombrite' net of different mesh sizes in the screenhouses. Differences in air temperature between the four environments did not exceed 2 °C.

LI treatments started 1.5 month before inoculation (exps 3,5 and 6) or after inoculation (exps. 2 and 3) and were prolonged till the end of the observation period (45 to 60 days after inoculation).

Observations. To determine the components of resistance, observations were made every two or three days after appearance of the first symptoms. Lesion density (LD = number of lesions per leaf area unit) was determined at the onset of sporulation and sporulating lesion density (SLD = number of sporulating lesions per leaf area unit) at the moment that no further increase in the number of sporulating lesions occurred. Latency period (LP) is the time in days from inoculation till 50% of all finally sporulating lesions have come to sporulation. The reaction type (RT) was scored, between 50 and 60 days after inoculation, using a 0 to 9 scale (Eskes and Toma-Braghini, 1981). Scale value 0 indicates absence of visible symptoms, values 1 to 3 variation within resistant reaction types, values 4 to 7 heterogeneous reaction types with increasig sporulation intensity and percentage of sporulating lesions, and values 8 and 9 indicate highly susceptible reaction types, with some variation in sporulating intensity. RT scores were applied to individual leaves or to a whole plant or genotype. Natural infection in the field was assessed by means of a 1 to 5 scale adapted from Costa and Ribeiro (1975). Value 1 indicates absence of sporulating lesions, values 2 to 5 increasing incidence of sporulating lesions, associated with an increase in reaction type. These observations were made annually for Icatu plants and every 6 months for Kouillou plants.

Data on field infection are means of these observations over the period 1976 to 1981.

RESULTS

In the first three experiments the effect of LI on the susceptible coffee cultivars Mundo Novo and Catuai was investigated. Experiment 1 showed that different LI, applied during some hours immediately before inoculation, did not significantly affect the lesion density and latency period of seedlings of cv Mundo Novo in the greenhouse (Table 1).

Table 1. Lesion density (LD = number of lesion per leaf) and latency period (LP, in days) of C. arabica cv Mundo Novo seedlings, grown in the greenhouse, treated at different light intensities immediately before inoculation.

Treatment before inoculation	LD	LP
Darkness for 6 h	17.2	38
Shade of the greenhouse	10.4	37
Full sunlight for 1 h	8.9	37
Full sunlight for 6 h	9.2	38

Tabel 1. Lesiedichtheid (LD = aantal lesies per blad) en latentieperiode (LP, in dagen) van zaailingen van het C. arabica ras Mundo Novo, opgegroeid in de kas, geplaatst bij verschillende lichtintensiteiten vlak voor inoculatie.

In experiment 2, different levels of LI after inoculation were applied to cv Catuai. The number of lesions per leaf and the percentage of necrotic lesions were affected significantly, but not so the latency period (Table 2).

Table 2. Infection of seedlings of C. arabica cv Catuai placed, after inoculation, at different light intensities, expressed as % of total radiation. Different letters indicate significant differences according to the Scheffé test at P = 0.05.

Parameters of infection	Light intensity (%)		
	24	43	100
Number of lesions per leaf	36 a	22 a	13 b
Percentage of necrotic lesions	30 a	28 a	94 b
Latency period	43 a	48 a	41 a

Tabel 2. Infectie van zaailingen van het C. arabica ras Catuai geplaatst, na inoculatie, bij verschillende lichtintensiteiten, uitgedrukt als % van de totale instraling. Verschillende letters duiden significantie van verschillen aan voor iedere parameter, getoetst volgens de Scheffé test bij P = 0.05.

At 100% LI most lesions became necrotic before sporulation began. Only those lesions that were not directly exposed to sunlight came to normal sporulation. Therefore, the 100% LI treatment after inoculation was replaced by the 66% LI treatment in following experiments.

The effects of LI before as well as after inoculation were studied together in experiment 3 for cv Mundo Novo. LI treatments were initiated 1.5 month before inoculation. The results (Table 3) showed significant but opposite effects of LI before and after inoculation on lesion density and latency period. Also, the interaction between treatments was significant. In general latency period was not greatly affected and the differences could partly be explained by the observed negative correlation with lesion density (see Table 3). In this experiment, three leaves of each seedling were inoculated.

Table 3. Lesion density (LD = number of lesions per leaf) and latency period (LP, in days) of C. arabica cv Mundo Novo seedlings placed at three different light intensities (LI = percentage of total radiation) before and after inoculation.

	LD				LP			
LI before inoculation	LI after inoculation				LI after inoculation			
	24	43	66	Mean	24	43	66	Mean
24	16	19	15	17	36	36	39	37
43	32	21	11	21	35	36	39	37
100	49	37	20	35	31	34	38	34
Mean	33	26	15	25	34	35	39	36

Analysis of variance:	LD				LP			
Source	DF	MS	F	P ≤	DF	MS	F	P ≤
LI before inoculation	2	12033	38.6	0.001	2	296.2	17.6	0.001
LI after inoculation	2	8741	28.0	0.001	2	595.5	35.5	0.001
Interaction	4	2011	6.5	0.001	4	42.9	2.6	0.05
Residual	357	312			341	16.8		
Total	365				349			

Tabel 3. Lesiedichtheid (LD = aantal lesies per blad) en latentieperiode (LP, in dagen) van zaailingen van het C. arabica ras Mundo Novo geplaatst bij drie verschillende lichtintensiteiten (LI) vóór en ná inoculatie.

The youngest leaf was formed during the pre-inoculation treatments. For this leaf, the greatest difference in lesion density between the treatments was about a tenfold, which was much more than for the other two leaves, where these differences were about a fivefold (second leaf) and a twofold (third leaf). The necrosis of lesions seen in experiment 2 was hardly noticed in experiment 3.

In experiment 4, seedlings of an F2 population, segregating for resistance gene S_H4, were inoculated in the nursery and in the greenhouse. In the greenhouse the expected 1S : 3R ratio was obtained (Table 4). However, in the nursery no clear distinction of the plants into S and R could be made. Many plants had an intermediate reaction type (MR), characterized by a mixture of sporulating and non-sporulating lesions. At 80 days after inoculation more plants were classified as S than at 50 days after inoculation. At both observation dates all plants of the susceptible parent cv Catuai (s_H4s_H4) were of the S type and all plants of the resistant parent Agaro (S_H4S_H4) were of the R type. For this reason, and because of the observed segregation ratio, it was concluded that the plants heterozygous for S_H4 were incompletely resistant in the nursery and completely resistant in the greenhouse. The difference between the two environments was probably due to differences in LI because air temperatures were very similar in both environments. This conclusion was further confirmed by results of the inoculation of two cuttings of the F1 plant of the Agaro × cv Catuai cross, which was heterozygous for S_H4, at high and low LI (Table 5). A heterogeneous reaction type was observed under high LI and a completely resistant reaction type under low LI.

Table 4. Segregation for resistance to race II (v5) of H. vastatrix among F2 seedlings derived from C. arabica cross H 7317 (S_H4S_H4 × s_H4s_H4) in nursery and greenhouse environments. Resistance groups are indicated by: S (susceptible, sporulating lesions only), MR (moderately resistant, a mixture of resistant type lesions and sporulating lesions), and R (resistant, no sporulating lesions).

Environment	Light intensity (% of total radiation	Inoculation date	Observation period (nr of days after inoculation)	Number of seedlings observed	Frequency of seedlings (%)		
					S	MR	R
Nursery	66	8-2-79	50	197	28.4	33.5	38.1
		8-2-79	80	192	57.8	14.6	27.6
		2-5-79	64	409	26.2	36.2	37.7
Greenhouse	17	25-4-79	60	250	26.6	0	73.4

Tabel 4. Uitsplitsing voor resistentie tegen fysio II (v5) van H. vaststrix bij F2 zaailingen van C. arabica kruising H 7317 (S_H4S_H4 × s_H4s_H4) in de kwekerij en in de kas. Resistentiegroepen zijn aangeduid met: S (vatbare planten met alleen sporulerende lesies), MR (matig resistente planten met zowel lesies van het resistente type als sporulerende lesies) en R (resistente planten zonder sporulerende lesies).

Table 5. Lesion density (LD = number of lesions per leaf) and reaction type (RT) of cuttings of eight coffee genotypes with varying disease scores in the field placed at three different light intensities (LI, in percentage of total radiation). Each figure is based on one or two cuttings, with 10 to 36 inoculated leaves each.

Genotype	Disease score in the field (1-5 scale)	LD			RT		
		LI before/after inoculation 24/24	43/43	100/66	LI before/after inoculation 24/24	43/43	100/66
C. canephora cv Kouillou:							
C69-14	2.1	4	8	26	5.0	6.5	8.0
C67- 5	3.5	11	9	53	6.5	7.5	7.5
Icatu:							
H 3851- 2-437	2.0	10	28	33	3.0	4.0	5.0
H 3849- 9- 27	3.4	4	31	52	4.0	6.0	7.0
H 4782-13- 72	4.2	56	44	108	8.5	8.0	8.0
H 3851- 2-513	5.0	112	152	135	8.0	9.0	9.0
C. arabica cv Mundo Novo	4.4	38	68	77	8.0	8.0	9.0
H 7317 (S_H4s_H4)	-	10	-	73	1.0	-	6.0

Tabel 5. Lesiedichtheid (LD, in aantallen lesies per blad) en reactietype (RT) van stekken van acht koffiegenotypen, met een variërende ziektescore in het veld, geplaatst bij drie verschillende lichtintensiteiten (LI, in percentage van de totale instraling). Ieder getal is gebaseerd op 1 of 2 stekken. Per stek werden 10 tot 36 bladeren geïnoculeerd.

Table 6. Sporulating lesion density (SLD = number of sporulating lesions per 15 cm^2 leaf surface), latency period (LP, in days), mean reaction type (RT), and ranges for RT of seven coffee genotypes observed in the greenhouse (Gre) and nursery (Nur).

Genotype	Disease score in the field (1-5 scale)	SLD		LP		RT (means)		RT (ranges)	
		Gre	Nur	Gre	Nur	Gre	Nur	Gre	Nur
C. canephora									
cv Kouillou:									
C67- 7	1.3	16	3	56	52	3.5	3.1	2.1-4.5	2.0-3.4
C66- 1	1.6	2	19	60	54	2.0	5.2	1.0-4.0	4.0-6.4
C70-11	1.8	18	34	52	38	5.6	8.4	3.6-7.0	8.3-8.5
C66- 3	2.2	9	13	52	50	4.7	5.9	3.8-5.4	4.9-7.3
C68- 4	3.1	23	45	45	39	6.6	9.0	5.3-8.1	8.9-9.0
C68-15	4.3	35	43	41	37	8.1	9.0	7.9-8.8	8.6-8.9
C. arabica									
cv Mundo Novo:	4.4	1	7	44	38	8.3	9.0	8.0-9.0	8.6-9.0
Means		15	23	50	44	5.5	7.1		

Analysis of variance; significance of F (P values $\leq$):

Source	SLD	LP	RT
Environment	0.001	0.001	0.001
Genotype	0.001	0.001	0.001
Interaction	0.035	0.199	0.001
Coefficient of variation (%)	59	8	11

Tabel 6. Dichtheid van sporulerende lesies (SLD = aantal sporulerende lesies per 15 cm^2 bladoppervlakte), latentieperiode (LP, in dagen), gemiddeld reactietype (RT) en variatiebreedte voor RT van zeven koffiegenotypen waargenomen in de kas (Gre) en in de kwekerij (Nur).

In experiment 5, two-year-old cuttings of field plants of cv Kouillou and of Icatu were inoculated under three light regimes. The cuttings were adapted to the environments 1.5 month prior to inoculation. The results showed an increase in susceptibility with increased LI (Table 5). The relative increase was greater for the more resistant plants than for the more susceptible ones. No statistical analysis was performed because only one or two cuttings were used per treatment. The differences in disease score of the plants in the field appeared to be well related to the number of lesions per leaf in all three environments, but this relation was less clear for reaction type.

Experiment 6 consisted of inoculations of cuttings from six genotypes of cv Kouillou and of seedlings of cv Mundo Novo in the greenhouse and nursery. The cuttings were placed in these environments 6 weeks prior to inoculation. The age of the inoculated leaves varied from a few weeks to a few months. The results (Table 6) indicated a significant environment and genotype effect on SLD, LP and RT. Most genotypes were considerably more susceptible in the nursery environment. Interaction between environment and genotype, signicifant for SLD and RT, was mainly due to the different response of the C67-7 genotype of cv Kouillou. The reaction of this genotype was characterized by flecks and large chlorotic areas, which mostly became necrotic before sporulation started. The residual variation was large for SLD and relatively low for LP and RT, as indicated by the coefficients of variation (CV) of these components (Table 6). However, the CV values for LP and RT may have been underestimated, because more cuttings were used of the genotypes with relatively stable LP and RT (C68-15 and cv Mundo Novo) than of the genotypes with a more variable reaction. In this experiment the number of lesions of cv Mundo Novo was abnormally low in relation to the 'Kouillou' genotypes. A possible explanation for this unexpected result is that the seedlings of cv Mundo Novo were transplanted 1.5 month prior to inoculation and the cuttings of cv Kouillou were not.

DISCUSSION

C. arabica cultivars. The susceptibility to coffee leaf rust of the Brazilian cultivars Mundo Novo and Catuai appeared to be significantly affected by LI. Treatments of high LI during 6 weeks before inoculation increased lesion density of cv Mundo Novo threefold (Table 3) in comparison to low LI treatments. This effect was not found when high LI was applied only a few hours immediately before inoculation (Table 1). Therefore physiological differences which are induced by a more long term adaptation of the plants to the environments may have been involved.

High LI after inoculation had effects opposite to those of high LI before inoculation (Tables 2 and 3). Different treatments with an equal LI before and after inoculation were applied to cv Mundo Novo in experiments 3, 5 and 6. In experiment 3 no significant differences were observed between these treatments (Table 3), but in experiments 5 and 6 more lesions developed in environments with a higher LI than with a lower LI. This indicates that the pre-inoculation effect was more important than the post-inoculation effect in experiments 5 and 6. The balance resulting from the two opposing effects is expected to vary according to the intensity of the treatments applied.

The S_H4 resistance gene. This resistance gene has been reported to be a dominant gene (Rodrigues et al., 1975). The present results indicate that the action of this gene may depend on environment. In environments with low LI it was dominant indeed, but at high LI incomplete dominance was observed (Tables 4 and 5). The incomplete dominance was expressed by a heterogeneous reaction type which, as time evolved, became rather a susceptible reaction type.

C. canephora cv Kouillou and Icatu. The resistance of most genotypes was better expressed in environments with low LI. Genotypes with intermediate levels of resistance appeared to be most affected by environment and also displayed the greatest residual variation (Tables 5 and 6). Only one genotype of cv Kouillou, with the highest level of incomplete resistance, was not affected by environment, and caused a significant environment × genotype interaction for SLD and RT.

Relation between LI and temperature. The observed effect of LI may be confounded with an effect of temperature. Although the difference in average air temperature between the environments was not greater than 2 °C, the leaf temperatures in environments with a high LI must have been higher than in environments with low LI. Gomez and Jaramillo (1974) observed that the average temperature of sun exposed leaves of coffee was 3 to 5 °C higher than that of shaded leaves. In one case, a difference of 10 °C was observed. As reported by Monaco et al. (1973), lesion growth of *H. vastatrix* was completely inhibited

when coffee plants were treated at 40 °C during 4 h on five successive days. Leaf temperatures nearly as high as 40 °C may occur in Campinas, Brazil, during the summer months, when the air temperature frequently reaches 30 to 35 °C. Therefore, the depressive effect of high post-inoculation LI on lesion development
(Table 2 and 3) might be explained by the effect of temperature.

High temperature prior to inoculation may induce a higher susceptibility of plants to several rust fungi (Heath, 1979; Vanderplank, 1978). Rodrigues (1975) reported an increased susceptibility of compatible and incompatible coffee/*H. vastatrix* combinations when leaves were exposed to 45 °C during 1.5 h prior to inoculation. Therefore, the increased susceptibility induced by high LI before inoculation (Table 3) may, at least in part, be due to a temperature effect.

Types of resistance involved. The coffee genotypes used in this study had either incomplete resistance, the genetic base of which is unknown (Tables 1, 2, 3, 5, and 6), or major gene resistance provided by S_H4, which is race-specific (Table 4). The incomplete resistance of two plants of Icatu shown in Table 5 (H 3851-2-437 and H 3851-2-513) is known to be race-specific. This resistance was lost by the appearance of a new and more compatible race in the Icatu population in 1978. The other Icatu and Kouillou genotypes were not affected by this race and they did not change their level of disease in the field during the years 1976 to 1981 (personal observations). Therefore, it appears that LI may affect various forms of resistance, which probably depend on different genetic mechanisms.

Ecological considerations. Field resistance of potatoes to late blight (Umaerus, 1970; Schumann and Thurston, 1977) and partial adult plant resistance of oats to powdery mildew (Jones, 1975) are reduced when lower LI are applied. Resistance of wheat to yellow rust also may decrease at lower LI, although Stubbs (1967) classified some differentials as photolabile and others as photostable. With coffee leaf rust the tendency appears to be opposite: an increase in resistance was observed at lower LI. It could be argued that this discrepancy is caused by the different conditions under which these species normally grow in the wild. Both *C. arabica* and *C. canephora* are forest species, whereas potatoes and, certainly, cereals normally grow in the open. As observed by Sylvain (1955) 'coffee rust is rare under natural forest conditions in Ethiopia, but in cases where the upper story of the forest has been removed, leaf rust and *Cercospora coffeicola* may spread quite rapidly'. This is in agreement with the present results showing that coffee plants at high LI tend to become more susceptible. It seems plausible that resistance of plants to diseases will be optimal under conditions representing the natural environment of the species.

However, it should not be concluded that shading of susceptible *C. arabica* cultivars would help to control coffee rust. The results of Tables 2 and 3 show that pre- and post-inoculation LI treatments had opposite effects, thus suggesting that intermediate levels of LI may be optimal for rust development. These intermediate levels may predominate in the leaf canopy of coffee trees, when medium levels of shade are applied. Shading of coffee plantations will also change other environmental factors, among which relative humidity, and these may favour disease development in stead of controlling it.

Practical implications for the coffee breeder. The observed incomplete dominance of the SH4 resistance gene under nursery conditions may have practical implications for resistance screening and race identification. Coffee differentials heterozygous for S_H4 are available for detection of rust races with complex virulence (Rodrigues, et al., 1975). Care should be taken in using these differentials, because their resistance will not be complete in all environments.

The coffee breeder interested in screening for incomplete resistance may prefer to work under relatively low LI, which make this type of resistance more easily detectable. Similar LI should be applied to all plants in order to decrease the residual error of experiments.

Other environmental factors. In experiments not reported here a seasonal influence on resistance to coffee leaf rust was observed. For example, inoculations of certain Icatu plants were much more successful in summer than in winter. In addition, some races of coffee leaf rust were more difficult to grow during winter than in summer. Seasonal effects may be explained by separate or combined effects of LI, temperature, relative humidity, and leaf age. These factors deserve further investigation.

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SAMENVATTING

Effect van lichtintensiteit op incomplete resistentie van koffie tegen Hemileia vastatrix.

Resistentie van koffie tegen fysio II van *Hemileia vastatrix* werd getoetst in milieus bij lichtintensiteiten (LI), die varieerden van 17 tot 100% van de totale instraling. Negen behandelingen, bestaande uit de combinaties van drie niveaus van LI vóór inoculatie en drie ná inoculatie, werden toegepast op zaailingen van het vatbare *Coffea arabica* ras Mundo Novo. Toenemende LI vóór inoculatie veroorzaakte een significante toename in lesiedichtheid, terwijl het tegenovergestelde werd waargenomen bij de behandelingen na inoculatie. Maximale verschillen in lesiedichtheid waren drievoudig. De interactie tussen behandelingen vóór en ná inoculatie was ook significant. Bij extreem hoge LI ná inoculatie trad necrose van de lesies op.

Genotypen van de Icatu populatie en van het *C. canephora* ras Kouillou, met verschillende ziektescores in het veld, werden beproefd in verschillende milieus, waarbij een constante LI voor en na inoculatie werd toegepast. De resistentie van de meeste genotypen kwam beter tot uiting bij lage LI dan bij hoge LI, wat ook waargenomen werd voor het controle ras Mundo Novo. Bij het ras Kouillou werden de dichtheid van sporulerende lesies, de latentieperiode en het reactietype significant beïnvloed door LI en genotype. De interactie tussen LI en genotype was ook significant voor dichtheid van sporulerende lesies en voor reactietype, voornamelijk doordat het meest resistente genotype niet, of in de omgekeerde richting, beïnvloed werd door LI.

De expressie van het resistentiegen S_H4 bleek ook afhankelijk van het milieu. Waarnemingen aan een uitsplitsende F2-populatie duidden op een dominante genwerking in de kas (lage LI) en een incompleet dominante, of bijna recessieve, genwerking in de kwekerij (hoge LI).

Deze incomplete dominantie uitte zich d.m.v. heterogene tot vatbare reactietypes van heterozygote planten (S_H4s_H4) onder hoge LI.

Enkele ecologische en veredelingstechnische aspecten van de waargenomen invloed van LI worden besproken.

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4 THE EFFECT OF LEAF AGE ON INCOMPLETE RESISTANCE OF COFFEE TO *HEMILEIA VASTATRIX*

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ABSTRACT

The influence of leaf age on incomplete resistance to race II of coffee leaf rust (*Hemileia vastatrix*), which is the most common race in Brazil, was tested in the greenhouse and in the field. Three stages of leaf age were distinguished: 'young' leaves which had just fully expanded and still had a shiny appearance on the day of inoculation, 'adult' leaves of 1 to 4 months old and 'old' leaves of 6 to 12 months old which were formed in the foregoing growing season.

With the susceptible *Coffea arabica* cvs Mundo Novo and Catuai no important effect of leaf age on latency period and reaction type was observed. Lesion density, depending on inoculation method, was affected to some extent, older leaves showing lower values. With genotypes of the *C. canephora* cv. Kouillou, which varied in level of incomplete resistance, adult leaves appeared to be more resistant than young and old leaves. This adult leaf resistance was expressed mainly by a lower lesion density, but also by a longer latency period and a lower reaction type.

Within the hybrid populations Icatu and Catimor, genotypes with race-specific incomplete resistance were tested and others of unknown specificity. Resistance was expressed mainly by a low reaction type. Generally, resistance decreased with increasing leaf age, which was occasionally associated with a complete change in reaction type. Contrary to cv. Kouillou, in Icatu and Catimor no higher susceptibility of young leaves than of adult leaves was observed.

The incomplete resistance to race I of the differential CIFC H152/3, heterozygous for S_H4, was also better expressed in young leaves, older ones becoming gradually more susceptible.

It is concluded that for screening incomplete resistance to coffee leaf rust leaves of different ages should be tested.

Additional keywords: Coffee leaf rust, coffee differentials, durable resistance, heterogeneous reaction type, components of resistance.

INTRODUCTION

Resistance to pathogens is often affected by the developmental stage of the plant or of the plant organs. Generally, plants become more resistant when they mature (Dickinson and Crute, 1974; Hooker, 1967; Hyde, 1977, Zadoks, 1961), but conflicting results have been reported (Hooker, 1967). The increase in resistance during maturation may be followed by a decrease in resistance with further ageing (Jones and Hayes, 1971; Luttrell et al., 1974; Mence and Pegg, 1971; Parlevliet, 1975, Umaerus, 1970). Padmanabhan and Ganguly (1954) observed that resistance of rice to *Helminthosporium* decreased with age but that resistance to rice blast increased with age.

For perennial crops the age of plant organs may be more important than the age of the whole plant. Reports exist on the effect of leaf age on resistance to pathogens (Sharma et al., 1980, Weinhold and English, 1964; Williams and Kuć, 1969). The developmental stage of coffee berries appeared to influence resistance to coffee berry disease (Mulinge, 1970, van der Graaff, 1981). Little is known about the influence of leaf age on resistance to orange coffee rust or coffee leaf rust (*Hemileia vastatrix* Berk. et Br.). D'Oliveira (1957), screening for resistance to coffee rust, mentioned that mature leaves are more resistant than succulent young leaves.

Results are reported here on the effect of leaf age on incomplete resistance to coffee leaf rust at various levels.

MATERIALS AND METHODS

Details on resistance terminology, coffee and rust genotypes used, standard inoculation methods and observations on natural and artificial infection are described by Eskes (1982).

Location. All experiments were carried out at the Experimental Station of the Instituto Agronômico of Campinas (I.A.C.), S.P., Brazil.

Coffee genotypes. The coffee plants used were one-year-old seedlings or cuttings from field plants. The age of the field plants was 10 years with Icatu and with the *Coffea arabica* cvs Mundo Novo and Catuai, 6 years with Catimor, and about 45 years with the *C. canephora* cv. Kouillou. Icatu and Catimor consist of advanced breeding generations derived from crosses between *C. arabica* (4x = 44) and *C. canephora* (2x = 22) (Monaco, 1977; Rodrigues et al., 1975). Cvs

Mundo Novo and Catuai are equally susceptible to the common race II of *H. vastatrix*. The genotypes used from cv. Kouillou and from the Icatu and Catimor populations varied in level of resistance. The cuttings of the coffee differentials used in experiment 6 belonged to the collection of differentials of the Phytopathology Department of the I.A.C. The seedlings of the Agaro C1164-4 introduction, used in the same experiment, were two years old.

Rust materials. Unless stated otherwise, race II of *H. vastatrix* has been employed. Isolate 2, used in experiment 5, was obtained in 1979 from an Icatu field plant with complete resistance to race II.

Inoculation dates and method. Experiments 1 to 5 were inoculated on 15 December 1976; 25 February, 27 March and 24 March 1981, and 10 November 1980, respectively. Standard inoculation was done by spraying suspensions of urediospores on the abaxial leaf surface, using densities of 0.5 to 1.5 mg urediospores per ml. The number of spores in one mg is about 1.5×10^5. In experiment 1B dry urediospores were applied with a scalpel on the abaxial leaf surface and spread with a small brush. Subsequently the spores were wetted by spraying distilled water on the leaf. After inoculation the plants were always incubated in a humid dark room at 22 ± 2 °C during 24 to 48 hours.

Leaf age treatments. Coffee leaves can remain on the plant for more than one year. In Campinas, rapid vegetative growth occurs during the wet summer (October to April), when 4 to 8 new leaf pairs may develop on each branch. Growth ceases during the dry winter. Natural and also rust-induced leaf fall is highest at the onset of the dry season (May, June), which coincides with harvest time.

Three stages of leaf age were considered. 'Young' leaves were just fully developed leaves which were shiny and succulent. 'Adult' leaves varied in age from a few weeks to a few months at the day of inoculation and had lost the shiny appearance. 'Old' leaves, formed in the foregoing growing season, varied in age from 6 to 12 months.

A second way of identifying leaf age was by position on the branch. Leaf position 1 generally indicated a young leaf, higher positions adult or old leaves.

Observations. Observations on the components of resistance were made every two or three days after appearance of first symptoms. Reaction type (RT) was scored between 50 and 60 days after inoculation when no further increase in sporulation occurred, using a 0 to 9 scale (Eskes and Toma-Braghini, 1981). Value 0 indicates absence of visible symptoms, values 1 to 3 variation within resistant reaction types, 4 to 7 heterogeneous reaction types with increasing sporulation intensity and percentage of sporulating lesions, and 8 and 9 susceptible reaction types with moderate (8) to high (9) sporulation intensity.

Assessment of natural infection in the field was by scoring on a 1 to 5 scale. Value 1 indicates absence of symptoms and values 2 to 5 increasing numbers of sporulating lesions, associated with an increase in reaction type. These observations were made annually with Icatu plants and every 6 months with cv. Kouillou plants. Data on field infection represent means over the years 1976 to 1981.

Statistics. For elaboration of the results, SPSS (Statistical Package for the Social Sciences) has been applied. The Friedman test (Siegel, 1956) has been used when the distribution of the data deviated from the normal distribution.

RESULTS

In experiment 1A and 1B, the effect of leaf position on the branch on susceptibility of cv. Mundo Novo was studied with two inoculation methods. Four or five leaf pairs of six branches were inoculated. With the standard suspension inoculation method only leaves of position 5 showed a significantly lower lesion density (LD) than the other leaves, whereas latency period (LP) was only slightly affected (Table 1). Results were somewhat different when the inoculation was done with dry urediospores and subsequent wetting by spraying. Then, the youngest leaf had the highest LD value and LD declined more rapidly with increasing leaf position than with the suspension method. The variation in LP was small but significant, due to the low coefficient of variation (CV) for this component. The average CV values for LD and LP in the experiments were 88 and 6 %, respectively.

In experiment 2 the effect of leaf age was studied with cv. Kouillou genotypes and cv. Mundo Novo under field conditions. Of each genotype all leaves of four branches were inoculated. All five genotypes of cv. Kouillou showed a significantly higher level of resistance in adult than in old leaves (Table 2). Differences with genotype C67-12 were smallest and least significant. With cv. Mundo Novo no significant differences were observed for the resistance components. With both cultivars no difference was observed between leaves of similar age group but with different positions on the branches. The percentage of infected leaves that dropped from the branch before 70 days after inoculation varied from 0 to 26 and from 57 to 100 with adult and old leaves, respectively (Table 2).

Table 1. Lesion density (LD = number of lesions per leaf) and latency period (LP, in days) of leaves of cv. Mundo Novo at different positions on the branch inoculated in the greenhouse by two methods.

Experiment	Method of inoculation	Component of resistance	Leaf position [1]				
			1	2	3	4	5
1A	Spraying of a suspension (standard method)	LD	27^{a}[2]	33^{a}	37^{a}	29^{a}	11^{b}
		LP	35^{a}[2]	35^{a}	35^{a}	35^{a}	36^{b}
1B	Applying dry urediospores with subsequent wetting	LD	38^{a}	19^{ab}	20^{ab}	10^{b}	-
		LP	32^{a}	34^{b}	33^{ab}	34^{b}	-

[1] The age of leaves of position 1 to 4 increased from a few weeks to 3 months, respectively, while leaves of position 5 were about 7 months old on the inoculation date.

[2] Different letters indicate significant differences within rows according to the LSD 0.05 values.

Tabel 1. Lesiedichtheid (LD = aantal lesies per blad) en latentieperiode (LP, in dagen) van bladeren van cv. Mundo Novo met verschillende positie aan de tak geïnoculeerd in de kas op twee verschillende manieren.

Leaf fall of symptomless leaves, from the same experiment, was about 0 and 20 percent for adult and old leaves, respectively. Therefore, the pronounced leaf fall of old leaves was apparently induced by the rust infections.

Six leaves used in this experiment were considered as young leaves, because they still had a shiny appearance on the day of inoculation. Average lesion density of these leaves was about 2 to 4 times higher than of adult leaves, which is in agreement with the higher susceptibility of young leaves observed in the following experiment.

In experiment 3, one-year-old cuttings of the same plants of cv. Kouillou as used in experiment 2 were inoculated in the greenhouse. The cuttings had grown vigorously during the two months prior to inoculation. Four to ten cuttings were inoculated per genotype, using one branch per cutting with three leaf pairs each. The leaves of the first or top pair were called young and of the second and third pairs were called adult. Generally, differences between leaf

Table 2. Latency period (LP, in days), lesion density (LD = number of lesion per 10 cm^2 leaf surface), sporulating lesion density (SLD), reaction type (RT) and leaf fall (LF, in percentage observed 70 days after inoculation) of adult and old leaves of cv. Kouillou genotypes and of cv. Mundo Novo, inoculated in the field.

Genotype	Disease score in the field (1-5 scale)	LP		LD		SLD		RT		LF	
		Leaf age[1]		Leaf age		Leaf age		Leaf age		Leaf age	
		Adult	Old	Adult	Old	Adult	Old	Adult	Old	Adult	Old
Cv. Kouillou:											
C66- 1	1.6	-	59	0.32^{a}[2]	3.70^{b}	0.00^{a}	0.95^{b}	2.3^{a}	5.3^{b}	0^{a}	56^{b}
C70-11	1.8	-	50	0.09^{a}	1.15^{b}	0.00^{a}	0.60^{b}	3.0^{a}	4.3^{b}	0^{a}	100^{b}
C60- 3	2.2	61^{a}	48^{a}	0.04^{a}	1.50^{b}	0.04^{a}	1.50^{b}	4.3^{a}	6.5^{b}	0^{a}	57^{a}
C67-12	3.0	66^{a}	43^{a}	1.50^{a}	1.75^{a}	0.48^{a}	1.19^{a}	4.0^{a}	5.8^{b}	0^{a}	47^{a}
C68-15	4.3	53^{a}	41^{b}	3.73^{a}	7.48^{b}	2.70^{a}	7.48^{b}	5.8^{a}	8.0^{b}	17^{a}	100^{b}
Mean	-	-	-	1.14^{a}	3.12^{b}	0.64^{a}	2.35^{b}	3.9^{a}	6.0^{b}	3^{a}	72^{b}
Cv. Mundo Novo	4.5	34^{a}	34^{a}	10.10^{a}	12.80^{a}	10.10^{a}	12.80^{a}	8.5^{a}	8.5^{a}	26^{a}	94^{b}

[1] Adult leaves varied in age from 1 to 3 months, old leaves were over 8 months old.

[2] Different letters indicate significant differences between leaf ages, within genotypes, according to the analysis of Friedman at P = 0.05.

Tabel 2. Latentieperiode (LP, in dagen), lesiedichtheid (LD = aantal lesies per 10 cm^2 bladoppervlak), dichtheid van sporulerende lesies (SLD), reactietype (RT) en bladval (LF, in percentage waargenomen 70 dagen na inoculatie) van volwassen en oude bladeren bij cv. Kouillou genotypen en bij cv. Mundo Novo, geïnoculeerd in het veld.

Table 3. Latency period (LP, in days), lesion density (LD = number of lesions per 15 cm^2 leaf surface), sporulating lesion density (SLD), and reaction type (RT) of young and adult leaves with five genotypes of cv. Kouillou observed in the greenhouse.

Genotype	Disease score in the field (1-5 scale)	LP		LD		SLD		RT	
		Leaf age[1]		Leaf age		Leaf age		Leaf age	
		Young	Adult	Young	Adult	Young	Adult	Young	Adult
C66- 1	1.6	61^{a}[2]	64^{a}	52^{a}	16^{b}	2^{a}	2^{a}	2.8^{a}	1.7^{a}
C70-11	1.8	51^{a}	56^{a}	72^{a}	22^{b}	40^{a}	12^{b}	6.0^{a}	5.3^{a}
C66- 3	2.2	53^{a}	60^{b}	46^{a}	14^{a}	13^{a}	7^{a}	4.5^{a}	3.8^{a}
C67-12	3.0	47^{a}	51^{a}	41^{a}	23^{a}	31^{a}	17^{a}	5.7^{a}	4.8^{a}
C68-15	4.3	35^{a}	43^{b}	52^{a}	32^{a}	50^{a}	30^{a}	8.3^{a}	8.2^{a}
Means		50^{a}	55^{b}	53^{a}	21^{b}	27^{a}	13^{b}	5.5^{a}	4.8^{a}

[1] 'Young' leaves were generally leaves which had just expanded and 'adult' leaves varied in age from about one to three months on the day of inoculation.

[2] Different letters indicate significant differences, between leaf ages, within genotypes, according to the Friedman test at P = 0.05.

Tabel 3. Latentieperiode (LP, in dagen), lesiedichtheid (LD = aantal lesies per 15 cm^2 bladoppervlak), dichtheid van sporulerende lesies (SLD) en reactietype (RT) van jonge en volwassen bladeren bij vijf genotypen van het ras Kouillou waargenomen in de kas.

ages within genotypes were not statistically significant (Table 3), but the differences between the means were significant, except for RT, which indicates a tendency. The difference in susceptibility was most expressed by lesion density, which in average was 53 for young leaves and 21 for adult ones.

In experiment 4, leaves of different positions on one year old seedlings of Catimor families were used. The families segregated for complete as well as incomplete resistance. Only seedlings with incomplete resistance were considered, 6 to 12 per family. Table 4 shows that the effect of leaf position on reaction type varied according to the family. The most remarkable effect was observed for the C2502 family, which showed nearly complete resistance in the younger leaves whereas older leaves were normally susceptible. The difference in response between families is probably not due to a difference in age of the leaves of similar positions, because growth rate of the seedlings was about equal with all families. The control cv. Catuai did not show a significant effect of leaf position on reaction type.

Table 4. Reaction type of leaves of different age of seedlings with incomplete resistance to coffee leaf rust for five families derived from the cv. Caturra × Hybrid of Timor cross (Catimor) and of cv. Catuai observed in the greenhouse.

Origin of family	Leaf position [1]					Mean
	1	2	3	4	5	
Catimor:						
UFV386-19	8.5^{a}[2]	8.3^{a}	8.4^{a}	8.7^{a}	8.8^{a}	8.5
C2502	4.0^{a}	6.5^{b}	7.9^{c}	8.3^{c}	8.4^{c}	7.0
UFV386-C798-A	5.2^{a}	5.9^{ab}	6.1^{ab}	6.6^{ab}	6.7^{b}	6.1
UFV386-C798-B	3.7^{a}	4.3^{ab}	4.3^{ab}	4.7^{b}	5.4^{b}	4.5
C2501	2.9^{a}	3.2^{a}	3.6^{a}	3.3^{a}	3.0^{a}	3.2
Cv. Catuai	8.5^{a}	8.4^{a}	8.5^{a}	8.5^{a}	8.9^{a}	8.6

[1] The age of the leaves of position 1 to 5 ranged from a few weeks to about 5 months on the day of inoculation, respectively.

[2] Different letters indicate significant differences between leaf positions within families, according to the LSD 0.05 value.

Tabel 4. Reactietype van bladeren van verschillende leeftijd bij zaailingen van vijf families die afstammen van de kruising tussen cv. Caturra × Hybrid of Timor (Catimor) en van cv. Catuai, waargenomen in de kas.

Experiment 5 was carried out in the field and included four coffee plants with differential reactions to two races of *H. vastatrix* (Table 5). For each genotype/race combination all leaves of two branches, growing in the shade, were inoculated. The resistance to race II of adult leaves of the genotypes H 4782-10-203 and C2012-19 was nearly complete, whereas old leaves were quite susceptible. Similar but smaller effects of leaf age were observed with other combinations. The differences in pathogenicity between race II and isolate 2 were more expressed in adult than in old leaves.

Table 5. Latency period (LP, in days), percentage of sporulating lesions (PSL) and reaction type (RT) of adult and old leaves for four coffee genotypes inoculated in the field with H. vastatrix, race II and isolate 2.

Genotype	Race or isolate	LP		PSL		RT	
		Leaf age[1]		Leaf age		Leaf age	
		Adult	Old	Adult	Old	Adult	Old
Icatu:	Race II	-	50	1	80	3	7
H 4782-10-203	Isolate 2	45	39	75	98	7	8
Icatu:	Race II	39	39	75	100	8	9
H 3851-2-689	Isolate 2	36	35	94	100	9	9
Catimor:	Race II	-	54	0	63	2	5
C 2012-19	Isolate 2	45	46	44	73	6	7
Cv. Mundo Novo	Race II	38	35	97	98	9	9
	Isolate 2	48	38	59	92	6	8

[1] The age of the adult leaves was 1 to 2 months and that of old leaves over 6 months on the day of inoculation.

Tabel 5. Latentieperiode (LP, in dagen), percentage sporulerende lesies (PSL) en reactietype (RT) van volwassen en oude bladeren bij vier koffiegenotypen geïnoculeerd met twee isolaten van H. vastatrix in het veld (race II en isolate 2).

Table 6 shows the results of inoculations, carried out in different months and different years, of three differential clones with the resistance genes S_H2 and S_H4 and of seedling progeny of the Agaro C1164-4 introduction. Adult leaves of the differential CIFC 110/5 were not always susceptible when inoculated with the 'compatible' race XV; with one inoculation predominantly flecks and chlorotic lesions developed and sometimes early necrosis of apparently susceptible lesions occurred. These reactions were not observed with the progeny of the Agaro C1164-4 introduction, which is also homozygous for the dominant allele S_H4. Therefore, the unstable adult leaf resistance of the 110/5 differential must be due to a resistance factor other than S_H4. Table 6 also shows the relative susceptibility to race I of old leaves in relation to young leaves of differential H152/3, which is heterozygous for S_H4. Resistance due to gene S_H2 was not affected by leaf age or heterozygozity.

Table 6. Variation in reaction type observed on adult and old leaves of three coffee differentials and of the Agaro C1164-4 introduction, inoculated with two races of H. vastatrix in the greenhouse. Each entry is based on 6 to 12 leaves.

Differential or introduction	Genotype	Race I (v2V4)		Race XV (V2v4)	
		Leaf age		Leaf age	
		Adult	Old	Adult	Old
CIFC 32/1	$S_H2S_H2\ s_H4s_H4$	8-9	8-9	0-1	0-1
CIFC H152/3	$S_H2s_H2\ S_H4s_H4$	2-4	4-7	0-1	0-1
CIFC 110/5	$s_H2s_H2\ S_H4S_H4$	2-3	2-3	4-8	7-9
Agaro C1164-4	$s_H2s_H2\ S_H4S_H4$	2-4	2-3	8-9	8-9

Tabel 6. Variatie in reactietype waargenomen aan volwassen en oude bladeren van drie differentiërende koffieplanten en van de Agaro C1164-4 introductie, geïnoculeerd met twee fysio's van H. vastatrix in de kas. Ieder gegeven is gebaseerd op 6 à 12 bladeren.

DISCUSSION

C. arabica cultivars. Leaf age or position did not greatly influence the susceptibility of cvs Mundo Novo and Catuai (Tables 1, 2, and 4). Only lesion density was affected to some extent by leaf position (Table 1). The decrease in lesion density of leaves at position 4 and 5 may be partly due to the effect of light intensity (see below). The two inoculation methods applied in experiment 1 appeared to interact with the effect of leaf position. This interaction might be related to differences in leaf surface characteristics. Dry urediospores may adhere better to the waxy surface of young leaves than to the harder and drier surface of older leaves. Besides, water droplets coalesce and runn off more easily from young than from older leaves.

Other coffee genotypes. Tables 2 and 3 suggest the existence of three phases of susceptibility during the development of leaves of *C. canephora* cv. Kouillou. Adult leaves were relatively more resistant than young or old leaves. Results with Icatu and Catimor genotypes (Tables 4 and 5) showed higher resistance of young or adult leaves than of old leaves. With some genotypes resistance was only expressed in young leaves, older ones being normally susceptible, a combination which was never observed with the cv. Kouillou genotypes.

Types of incomplete resistance involved. Complete monogenic resistance is often race-specific resistance, as is also the case with coffee leaf rust (Rodrigues et al., 1975). Tables 5 and 6 show how certain types of incomplete resistance to coffee leaf rust may also be race-specific. Nothing is known about the specificity of the resistance of the Catimor families shown in Table 4.

Within Catimor families quite distinct phenotypes were observed with either complete resistance, intermediate resistance or high susceptibility. Therefore, these families could have mono- or oligogenic resistance with incomplete dominance, as observed with the S_H4 resistance gene (Table 6).

The genetic base of the resistance shown by most genotypes of cv. Kouillou seems complex. Some specificity of resistance has been observed with genotype 67-12, which was more susceptible to a rust race detected in the Kouillou population in 1980 than to race II. The other genotypes have maintained their resistance in the field from 1976 to 1981. Any inference about the durability of this resistance is speculative. However, the pecularities of the resistance of these genotypes of cv. Kouillou resemble those of other reportedly stable pathosystems (Jones and Hayes, 1971; Parlevliet, 1975, Umaerus, 1970). Therefore, the resistance of certain Kouillou plants could be of great value in obtaining durable resistance to coffee rust.

Effect of leaf age on complete resistance. Most results shown in this paper relate to incomplete resistance, though also genotypes with complete resistance were included in the experiments. Complete resistance is common in the Icatu and Catimor populations, but is less frequent in cv. Kouillou (personal observations). As far as observed, leaf age does not much affect the expression of complete resistance. However, certain genotypes with complete resistance showed symptoms on young leaves (flecks and tumefactions), while adult leaves were symptomless.

Other factors influencing incomplete resistance. As reported earlier high light intensities may predispose coffee plants to coffee leaf rust (Eskes, 1982). Leaves of different positions on the branch will receive different light intensities. Therefore the results of Tables 1, 3, and 4 may be confounded with an effect of light intensity, though shading of leaves was avoided as much as possible by suitable spacing of the plants.

Seasonal effects on resistance, which could not be explained by differences in leaf age, have been observed with the genotypes of Icatu mentioned in Table 5. Resistance of these genotypes to race II was higher in winter and spring than in summer and autumn. The same was observed for the relative resistance of cv. Mundo Novo to isolate 2. The resistance of the genotypes of cv. Kouillou in experiment 2 was higher than in other field inoculations of these genotypes. It is supposed that the relatively hot and dry weather, which prevailed during experiment 2, may have increased resistance of the plant in the field.

Epidemiological aspects. Rust epidemics in cv. Kouillou usually start early in the season but subsequent development is slower than in cv. Mundo Novo and Catuai (Cadena-Gomez and Buritiĉa-Céspedes, 1980; personal observations). This can be explained, at least in part, by the difference in effect of leaf age between these cultivars (Tables 1, 2, 3, and 4).

The high pathogen-induced leaf fall of old leaves also has epidemiological connotations. Leaf rust in Brazil has to overwinter on old leaves which drop easily upon infection (Table 2), thereby decreasing the carry-over of inoculum to the next growing season.

Breeding aspects. Screening for resistance to coffee leaf rust is often done by inoculating young, recently expanded leaves (e.g. D'Oliveira, 1957). The results of the present paper suggest that, with certain coffee genotypes, this inoculation method may under- or overestimate the real level of resistance of the entire plant. The breeder who is interested in measuring incomplete resistance with precision will have to inoculate leaves of different ages.

The coffee differential heterozygous for S_H4 (Table 6) was incompletely resistant when inoculated with the 'incompatible' race I. Therefore, this differential should be used with care in race identification studies.

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SAMENVATTING

Het effect van bladleeftijd op incomplete resistentie van koffie tegen Hemileia vastatrix.

Het effect van bladleeftijd op incomplete resistentie van koffie tegen fysio II van koffieroest (*Hemileia vastatrix*), het algemeen voorkomende fysio in Brazilië, werd getoetst in de kas en in het veld. Drie stadia in de bladleeftijd werden onderscheiden: 'jong' blad dat net volgroeid was en nog glansde op de dag van inoculatie, 'volwassen' blad van 1 tot 4 maanden oud en 'oud' blad van 6 tot 12 maanden oud dat gevormd was in het voorafgaande groeiseizoen.

Bij de vatbare *Coffea arabica* rassen Mundo Novo en Catuai werd geen belangrijke invloed waargenomen van bladleeftijd op latentieperiode en reactietype. Lesiedichtheid werd, afhankelijk van de inoculatiemethode, wel enigszins beïnvloed. Bij vijf genotypen van het *C. canephora* ras Kouillou, die varieerden in niveau van incomplete resistentie, bleek volwassen blad veelal resistenter dan jong en oud blad. Deze resistentie uitte zich voornamelijk door een lagere lesiedichtheid maar ook door een langere latente periode en een lager reactietype.

Van de hybride populaties Icatu en Catimor werden enkele genotypen met fysiospecifieke incomplete resistentie getoetst alsmede genotypen waarvan de specificiteit van de resistentie onbekend was. Verschillen in resistentie uitten zich voornamelijk door verschillen in reactietype. Over het algemeen nam de resistentie af bij toenemende bladouderdom. Anders dan bij de cv. Kouillou werd bij Icatu en Catimor geen grotere vatbaarheid van jong ten opzichte van volwassen blad waargenomen. Bij sommige genotypen kwam resistentie zelfs alleen maar tot uiting in het jonge blad, terwijl het volwassen blad normaal vatbaar was. Een soortgelijk effect van bladleeftijd werd waargenomen bij inoculatie van de koffiedifferentieel CIFC H152/3, die heterozygoot is voor het resistentiegen S_H4, met het incompatibele fysio I.

Er wordt geconcludeerd dat voor het bepalen van incomplete resistentie tegen koffieroest bladeren van verschillende ouderdom getoetst dienen te worden.

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5 QUALITATIVE AND QUANTITATIVE VARIATION IN PATHOGENICITY OF RACES OF COFFEE LEAF RUST *(HEMILEIA VASTATRIX)* DETECTED IN THE STATE OF SAO 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 × environment 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 (Rodrigues 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, 1980). 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, frustrated 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 (Rodrigues 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 cultivars 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 in tests when about one year old.

Rust genotypes. Single-lesion isolates from race II (v_5), III ($v_{1,5}$), I ($v_{2,5}$) and XV ($v_{4,5}$), obtained from the Department of Phytopathology of the I.A.C., were used and in the 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 greenhouse, 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, 1982[a]). The laboratory method consisted of inoculations of leaf disks (1.8 cm diameter), each with a 25 µl droplet of a suspension of urediospores in distilled water with densities of 0.5 to 1.5 mg ml^{-1}. The reliability of this method in assessing resistance to coffee leaf rust is shown by Eskes (1982[a]). 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 (1982[a]).

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 genotypes 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, 1982[b]). 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 Biologico in São Paulo (Fanucchi et al., 1980).

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	R[1]	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.

Race XXIII ($v_{1,2,4,5}$) 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 resistance factors 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 plants, 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. 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 must have 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 susceptible 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

Table 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).

			Reaction type (range)		
Coffee genotype	Resistance factors	Race or isolate	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).

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.

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,

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 genotypes	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.

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.

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.

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).

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 has the advantage of being more virulent than Is. 2 on cv. Mundo Novo (Table 4).

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, 1982[a]).

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

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	4.0^{a}	6.3^{b}	4.0^{a}	3.3^{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*.

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 is 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 virulence 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 dikaryotic 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 levels 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 still 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 might be that the 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 appearance of complex races from simple ones in Brazil (Table 1) makes the efficiency of multilines in coffee questionable. A multiline could facilitate, like the breeding plots in Campinas have apparently 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 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, 1981[b]). The present results showed the occurence 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 durability of the resistance. In addition, information on the polygenic nature of the resistance is required (Parlevliet, 1979, Eskes, 1981[a]). 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, was 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 resistances, 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 São 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, fysio-specifiek 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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6 VARIATION FOR INCOMPLETE RESISTANCE TO *HEMILEIA VASTATRIX* IN COFFEA ARABICA

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INDEX WORDS
Coffee Leaf Rust, durable resistance, components of resistance, leaf retention period, yield.

SUMMARY

Variation for incomplete resistance to coffee leaf rust was studied in *Coffea arabica*. Disease level in the field was scored by a 0 to 9 scale. Components of resistance observed in laboratory and greenhouse tests were latency period (LP), lesion density (LD) and leaf retention period (LRP). LRP determines the duration of sporulation.

Lines of 'Mundo Novo' and 'Catuai' showed relatively small but significant differences for disease level in the field. Heritibility was low (0.31) and part of the variance (34%) was explainable by a significant correlation between disease level and yielding capacity of the lines.

The high susceptibility of 'Ibaarê', in comparison to other cultivars, was best explained by longer LRP values for 'Ibaarê'. Also its LP was generally shorter than that of other cultivars.

Among coffee accessions from Ethiopia great variation was observed for disease level in the field. A significant part of the variance (35%) was explained by differences in yielding capacity. Variation for resistance components was observed between accessions, tested in the greenhouse and laboratory. However, results of the two tests were inconsistent.

Transgressive segregation for incomplete resistance was observed in F_3 populations of the cross between Agaro C1164-19 and 'Catuai', tested in the laboratory and greenhouse. Resistance was expressed by a longer LP, a lower LD, a certain percentage of non-sporulating lesions and, in some populations, by early necrosis of lesions.

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Prospects for breeding for incomplete resistance to *H. vastatrix* in *C. arabica* are discussed. Factors which may hamper selection progress are: a) the positive correlation between yield and disease level in the field, b) the relative small genetic variation for incomplete resistance among *C. arabica* cultivars and productive breeding lines, and c) inconsistency of results between resistance tests.

INTRODUCTION

The centre of origin of *Coffea arabica* L., an autogamous tetraploid species, is Ethiopia. In this country coffee leaf rust (*Hemileia vastatrix* Berk. et Br.) is endemic but is no serious disease problem (Sylvain, 1955). Rust severity is generally greater at low altitudes than at high altitudes. The way *C. arabica* has been distributed for cultivation has led to coffee populations with relatively limited genetic variation in most coffee producing countries. Breeding efforts have been undertaken, including interspecific hybridization, to increase variation (Monaco, 1977).

In Brazil, the two most grown cultivars are 'Mundo Novo' and 'Catuai'. They derive from varietal crosses and variation is present for yield and other characteristics between lines of these cultivars (Carvalho, 1967, Carvalho and Monaco, 1972). Both cultivars are susceptible to race II of *H. vastatrix*. Race II is the most common rust race and the first one detected in Brazil in 1970. Yield losses due to coffee leaf rust are estimated to be about 30% (Monaco, 1977). In the State of São Paulo, rust epidemics develop yearly from December to July, when the maximum rust incidence is observed (Figueredo et al., 1974). Rust incidence declines during the dry season (May-September) mainly due to rust- and environment-induced leaf fall.

Breeding for resistance to *H. vastatrix*, based on major genes in *C. arabica*, has not been effective (Ribeiro et al., 1975, Eskes, 1983). Incomplete, polygenically inherited resistance might be more durable than resistance related to major genes (Vanderplank, 1968, Parlevliet and Zadoks, 1977). Little is known about the variation for incomplete resistance in *C. arabica*. Sylvain (1955) and van der Graaff (1981) reported differences in the level of resistance between selections from different localities in Ethiopia. Ribeiro et al. (1981) reported a consistently higher disease incidence in the field of 'Ibaarê' than of 'Mundo Novo' or 'Catuai' in Brazil. Eskes (1983) showed that the major resistance genes S_H3 and S_H4 may confer incomplete resistance in the field to 'incompatible' rust races.

The present paper describes variation for incomplete resistance to *H. vastatrix* in *C. arabica*, unrelated to known major genes.

MATERIALS AND METHODS

Coffee genotypes in the field were 4-15 year old trees of *C. arabica* accessions from Ethiopia, cultivars and breeding lines. The accessions are selections made by P.G. Sylvain (Sylvain, 1955) and by an F.A.O. mission in 1964 (Meyer et al., 1968). Cvs Mundo Novo and Catuai consist of advanced generations derived from the varietal crosses 'Bourbon Vermelho' × 'Sumatra' and 'Mundo Novo' × 'Caturra Amarelo', respectively. 'Caturra' is a mutant with semi-dwarf growth, probably with a 'Bourbon' genetic background. 'Amarelo' (yellow) and 'Vermelho' (red) indicate the color of the ripe berries, which is a monogenic trait (Carvalho, 1967, Carvalho & Monaco, 1972).

Symbols used to indicate the origin of a coffee genotype refer to the type of selection (L = Line, H = Hybrid) or the place of selection (C = Campinas, M = Mococa, P = Pindorama, R = Ribeirão Preto) and are followed by a number with one or more dashes (generations). 'Ibaarê', is a selection (LC1133-2) from an accession (C1133) from the province of Harrar in Ethiopia. It has a high yielding capacity (Monaco, 1977), but is very susceptible to rust (Ribeiro et al., 1981, Sylvain, 1955). The accessions C1164-4 and C1164-19 come from Agaro, Ethiopia. They are homozygous dominant for the S_H4 allele. All cultivars and breeding lines used are homozygous dominant for the S_H5 resistance allele (Bettencourt & Carvalho, 1968). Some of the accessions from Ethiopia possess the S_H1 allele (Meyer et al., 1968).

In all tests, compatible rust races were employed with the virulence factors v_5 (race II), $v_{1,5}$ (race III) or $v_{4,5}$ (race XV). Isolates from these races were maintained on plants of 'Ibaarê' and 'Mundo Novo' in the greenhouse. Purity of races was checked regularly by inoculation on differential coffee genotypes.

Inoculations shown in Table 1, 2 and 6 were made in October 1977, August 1979, and May 1979, respectively. Inoculation methods follow those specified by Eskes (1982^a). In greenhouse tests, 10 to 14 one-year-old seedlings were used per progeny, inoculating 4 leaves per seedling, except in the experiment shown in Table 1, where 6 two-year-old seedlings were used per progeny, with 8 leaves each.

The disease level in the field was assessed by counting the number of rust lesions per leaf (rust incidence) or was scored by a 0 to 9 scale (disease score, DS) (Eskes & Toma-Braghini, 1981). Value 0 idicates absence of sporulating lesions and 1 to 9 increasing incidence of sporulating lesions. Components of incomplete resistance were observed as described by Eskes (1982[a]). Latency period (LP) is the time, in days, between inoculation and sporulation of 50% of the lesions. Lesion density (LD) is the number of lesions per leaf area unit. The spore production per lesion (SP) has been measured by diluting urediospores in a known volume of water and subsequent counting with a haemocytometer. The duration of sporulation was determined by the period, in days, between sporulation of 50% of the lesions and leaf abscission (leaf retention period, LRP). LRP was positively correlated with LD. Therefore, the table values of LRP shown are corrected values obtained by analysis of covariance, with LD as the covariate. For statistical elaboration use was made of computer programmes from the Statistical Package for the Social Sciences (SPSS). For calculations of LD values, a square root transformation was applied to improve normality of the data.

RESULTS AND DISCUSSION

Lines of C. arabica cultivars. Eleven lines of old and new Brazilian cultivars were tested in the greenhouse (Table 1). These lines have similar levels of disease in the field, except 'Ibaarê' which is substantially more affected by rust than the other cultivars. Table 1 shows that the differences between lines for latency period (LP) and spore production (SP) were non-significant and for lesion density (LD) barely significant at the 5% level. Highest significance was observed for the leaf retention period (LRP). 'Ibaarê' showed a 20 to 35 days longer LRP than the other cultivars. The coeffients of variation (C.V.) were extremely high for LD and SP.

Accessions from Ethiopia. In 1975 a field trial was established in Campinas with 200 accessions selected by the F.A.O. mission in 1964 in Ethiopia (Meyer et al., 1968). Each accession consists of seed progeny from individual trees. They are planted in 6 replications with 3 trees each. Great variation in vegetative vigour, yield, growth habit, earliness and adaptability is observed. The average yield of the progenies is about 30% of the yield of 'Mundo Novo' or 'Catuai'. The coffee leaf rust disease level varies greatly; some progenies show very few disease symptoms others are more diseased than 'Mundo Novo' or 'Catuai'.

Table 1. Components of resistance of 11 lines of *C. arabica* cultivars inoculated with rust race II (v_5) in the greenhouse.

Cultivar	Line number	Components of resistance[1]			
		LP	LD	SP	LRP
Ibaarê	LC1133-2	39	1.0	3.3	97
Catuai	H2077-2-5-81	40	1.4	3.4	60
Mundo Novo	LCP474-7	41	0.6	3.0	59
Caturra Vermelho	-	42	0.4	2.8	73
Bourbon Amarelo	-	42	1.0	3.5	62
Erecta	-	43	1.3	3.9	77
Mundo Novo	LCMP376-4-22	43	0.7	2.6	63
Catuai	H2077-2-5-66	44	0.9	2.4	66
Arabica	LC12-1-18-2	45	0.3	2.3	67
Sumatra	-	45	0.5	1.7	68
Catuai	H2077-2-5-99	46	0.6	2.0	63
Mean		42	0.8	2.8	68
C.V. (%)		7	65	48	24
F value of line effects		2.0	2.2	1.1	3.7
Significance of F		$P>.05$	$.05<P<.01$	$P>.05$	$P<.001$

[1] LP = latency period, in days, LD = lesion density, number of lesions per cm^2, SP = spore production per lesion, × 10^4, during 19 days from the day LP was completed, LRP = leaf retention period, in days, from the day LP was completed till abscission of the leaves.

Productivity appeared to influence the level of disease in the field. The coefficient of correlation between yield till 1980 and disease score in the field (0 - 9 scale) of the progenies in 1980 was significant ($r = 0.59$). Therefore, about 35% of the variance for disease level in this population can be explained by yield. Some progenies showed low productivity and a high disease level, but the opposite was not observed.

Greenhouse tests were carried out with race III of *H. vastatrix* on 10 progenies with varying disease levels in the field (Table 2). Significant differences were observed for LP, LD and LRP. Only two progenies differed significantly from 'Mundo Novo' for LP and none for LD (Duncans test at $P \leq 0.05$). Five introductions had a significantly longer LRP than 'Mundo Novo' at $P \leq 0.05$ (t-test). The coefficients of correlation (r) between the disease score in the field and the components of resistance and yield were low and non-significant ($r = -0.31$, 0.15, 0.50 and 0.47, for LP, LD, LRP and yield, respectively). The coefficient of multiple correlation (R) was higher (0.72), but still not significant. The results indicate that significant variation for LP or LD observed in the greenhouse may not be correlated with the disease level in the field. Variation for LRP and yield appear to be better indicators for the disease

Table 2. Disease level, yield (kg fresh berries per tree), and components of resistance of 10 C. arabica introductions from Ethiopia and of 'Mundo Novo' inoculated with rust race III ($v_{1,5}$) in the greenhouse).

Introduction or cultivar	Field		Progeny test in the greenhouse[3]		
	Disease score[1] (0 - 9 scale)	Yield (1978-81)	LP	LD	LRP
LC2038	4.1[2] a	9.1	53	26	55
LC2181	4.1 a	6.0	54	16	57
LC2109	5.7 bc	14.5	46	27	67
LC2063	6.3[2] bc	12.9	46	29	62
LC2111	6.3 bc	14.8	49	34	52
LC2066	6.5 bc	10.3	50	38	56
LC2157	6.5 bc	6.9	50	23	59
'Mundo Novo'	7.0 cd	21.2	50	26	53
LC2026	8.0 de	18.1	52	22	66
LC2025	8.4 e	13.7	49	25	68
LC2106	8.4 e	10.3	50	26	64
Mean	6.5		50	27	61
C.V. (%)	14		9	58	20
F value	15.9		6.4	2.5	3.4
Significance of F (P)	<.001		<.001	.007	.001

[1] Average of two readings made in June 1980 and 1982. Different letters indicate significant differences according to the $LSD_{0.01}$ value.

[2] These values may have been slightly underestimated due to the presence of the S_H1 gene in some plants of these lines.

[3] LP = latency period, in days, LD = lesion density (number of lesions per leaf), LRP = leaf retention period, in days, from the day LP was completed till leaf abscission.

level in the field.

In December 1979, 65 genotypes were tested in the laboratory with race III. Fourty leaf disks, collected from field plants with relatively good yield and/or low disease incidence, were inoculated. The suitability of the laboratory test in assessing incomplete resistance is shown by Eskes (1982[a]). The components of resistance LP and LD, expressed as percentages in relation to the average values of 'Mundo Novo' and 'Catuai' (= 100%) ranged from 90 - 130% and 20 to 180%, respectively. In May 1980, a greenhouse test was carried out on seedling progenies from the same 65 field plants. Ranges observed for LP and LD were similar to those in the laboratory test. However, the coefficients of correlation between the tests were low, insignificant for LP (r = 0.12) and barely significant at the 5% level for LD (r = 0.26), indicating a genotype × test interaction. The coefficients of correlation between LP and LD were significant but not very high (-0.61 and -0.41 for the laboratory and greenhouse tests, respectively).

Based on the results of the resistance tests 10 accessions were selected and crossed with cv. Catuai. In the cross progenies transgression for resistance might occur (see hereafter). Selections with good yielding capacity and increased incomplete resistance might be obtainable in these progenies.

Cvs Catuai and Mundo Novo. The Catuai cultivar consists of selected lines derived from a cross (H2077) between 'Mundo Novo' and 'Caturra Amarelo'. In May 1978, rust incidence was observed in the field with 18 F_3, F_4 and F_5 lines, each with 6 replicates of single plants. For 17 lines, rust incidence varied from 3.3 to 6.5 lesions per leaf. Differences between these lines were not significant. One line (H2077-2-5-50) was significantly more diseased than the rest, with an average of 12.3 lesions per leaf.

In June 1979, 1980 an 1981 the disease level was scored with 99 lines, derived from twelve different mother tree selections of 'Mundo Novo' and one of 'Catuai' (Table 4). Per line six replications of two plants each were observed. For individual years, the analysis of variance detected a significant line effect for disease score but coefficients of correlation between successive years were low or even negative (Table 3). Disease scores (DS) were positively correlated with yields (Y), in the same year, r values ranging from 0.67 to 0.69, and negatively correlated with yields in the foregoing or following year. Yield in 1980 was negatively correlated with yield in 1979 and 1981 (Table 3), illustrating the effect of biennial bearing of the coffee crop. Some lines were in a different biennial bearing pattern than others. To account for the differences

Table 3. Matrix of coefficients of linear correlation (r) between average yield ($\bar{Y}$) and coffee leaf rust disease score ($\overline{DS}$) of 88 lines of 'Mundo Novo' and 11 lines of 'Catuai' for three years (year 1 = 1979, year 2 = 1980, and year 3 = 1981). Significance of r = ± 0.20 (P = 0.05) and ± 0.254 (P = 0.01).

	$\overline{DS}_2$	$\overline{DS}_3$	$\bar{Y}_1$	$\bar{Y}_2$	$\bar{Y}_3$	$\bar{Y}_1 + 2\bar{Y}_2 + \bar{Y}_3$	Total yield (1972-1981)
$\overline{DS}_1$	.19	.56	.67	-.21	.43		.47
$\overline{DS}_2$		-.28	-.04	.67	-.57		.40
$\overline{DS}_3$			.31	-.52	.69		.23
$\overline{DS}_1 + 2\overline{DS}_2 + \overline{DS}_3$						.44	.58
$\bar{Y}_1$				-.18	.41		.52
$\bar{Y}_2$					-.78		.34
$\bar{Y}_3$							.01
$\bar{Y}_1 + 2\bar{Y}_2 + \bar{Y}_3$							.68

Table 4. Average coffee leaf rust disease level in the field and total average yield (kg fresh berries per tree) of lines from 12 mother trees of 'Mundo Novo' (MN) and from 1 mother tree of 'Catuai' (C).

Mother tree selection number	Number of lines tested	Disease score (1979-1981)		
		0 - 9 scale	Estimated number of lesions per leaf	Yield (1972-1981)
MN-474	14	6.8 a[1]	2.4	38.0 b
MN-471	3	6.8 a	2.5	35.3 ab
C-H2077-2-5	11	7.0 a	2.8	35.3 a
MN-502	9	7.3 b	3.6	42.2 de
MN-467	10	7.3 bc	3.7	39.8 bc
MN-500	8	7.4 bc	3.8	39.2 bc
MN-501	3	7.4 bcd	3.9	41.0 cde
MN-480	4	7.5 bcd	4.0	38.6 bc
MN-464	6	7.6 bcd	4.3	40.5 cd
MN-515	5	7.6 bcde	4.5	41.9 cde
MN-475	6	7.6 cde	4.5	44.4 e
MN-388	12	7.7 de	4.6	43.6 e
MN-376	8	7.9 e	5.6	44.4 e
Mean		7.4	3.9	40.3

[1] Different letters indicate significant differences according to Students t-test at $P \leqq 0.01$.

in bearing pattern, average DS and Y values were estimated by the formulas $(DS_1 + 2 \cdot DS_2 + DS_3)/4$ and $(Y_1 + 2 \cdot Y_2 + Y_3)/4$. After applying these calculations, the average DS was still significantly correlated to average yield over 1979/81 and to total yield from 1972 to 1981 ($r = 0.44$ and 0.58, respectively) (Table 3).

The analysis of variance for the average disease score indicated significant differences between the 99 lines, with scale values ranging from 6.2 to 8.2. The differences between the lines, arranged per mother tree selection, are shown in Table 4. Heritibility, as calculated from the line and residual mean square values, was 0.31. The significant correlation of disease score with total yield ($r = 0.58$, Table 3) indicates that $(0.58)^2 \times 100 = 34\%$ of the variance for disease score is due to the yielding capacity of the line. Therefore, and because of the relatively low genetic variation in 'Catuai' and 'Mundo Novo', selection for low disease scores within these cultivars does not seem rewarding.

Crosses between 'Ibaarê' and other Brazilian cultivars. Table 5 shows disease scores in the field, observed in March 1977, of 'Ibaarê', 'Mundo Novo', 'Bourbon', 'Arabica', and of four F_1 and F_2 populations from crosses between these cultivars. In 1977, yield of these coffee populations was low and no significant correlation between disease score and yield was observed. Table 5 shows that 'Mundo Novo' was least diseased and 'Ibaarê' most. The 'Ibaarê' plants showed intense defoliation on the date of observation. The average disease scores of the F_1 and F_2 populations were generally intermediate between both parents and no transgressive segregation was observed for any cross.

Table 5. Frequency distribution for coffee leaf rust disease score in the field (0 - 9 scale) in March 1977 on four cultivars of C. arabica and their F_1 and F_2 progenies.

Parents and progenies	Number of plants per class										Mean score
	0	1	2	3	4	5	6	7	8	9	
P_1 = 'Ibaarê'									2	13	8.9
P_2 = 'Mundo Novo'				8	6	1					3.5
P_3 = 'Bourbon'			1		3	8	4				4.9
P_4 = 'Arabica'						2	11	2	1		6.1
F_1 ($P_1 \times P_2$)							2				6.0
F_2 ($P_1 \times P_2$)				1	4	5	9	5	3	5	6.3
F_1 ($P_1 \times P_3$)								4			7.0
F_2 ($P_1 \times P_3$)			1	2	3	4	5	7	5	5	6.4
F_1 ($P_1 \times P_4$)								1	1		7.5
F_2 ($P_1 \times P_4$)						2	7	8	10	3	7.2
F_1 ($P_2 \times P_3$)			1	5	4	2					3.6
F_2 ($P_2 \times P_3$)				4	10	13	4				4.6

Heritabilities were calculated based on the variance of the F_2 and the average variance of the parents. The variance of 'Ibaarê' was very small, probably due to a scaling effect. When this parent was excluded from the calculations, heritabilities were 0.86, 0.73 and 0.60, for the $P_1 \times P_2$, $P_1 \times P_3$ and $P_1 \times P_4$ crosses, respectively. This suggests that relatively few genes are involved. The skewness of the F_2 distributions indicates the action of partially dominant genes of 'Ibaare'. For the cross $P_2 \times P_3$ the heritability was 0.08. This low value can be explained by the genetic similarity of 'Mundo Novo' and 'Bourbon' (Carvalho, 1967).

Laboratory and greenhouse tests were carried out on 'Ibaarê', 'Mundo Novo', the F_1 between both cvs, 10 selected F_2 plants and their F_3 progenies. For the laboratory test leaf disks from the field plants were used and for the greenhouse test seedlings, 20 per progeny, obtained by controlled handpollination or selfing. Table 6 shows the results of both tests and the disease score in the field of the respective mother plants. In the greenhouse test, the parents differed significantly for all components of resistance. LP of 'Ibaarê' was 18 days shorter than of 'Mundo Novo', LD was much higher and LRP about two times longer. In the laboratory test, significant differences were observed for LP, not for LD. LP of 'Mundo Novo' was 4.5 days longer than of 'Ibaarê', which is a much smaller difference than in the greenhouse test. For LP and LD intermediate values were observed in the F_1 and F_2 and parental values were recovered in some F_3 populations. The long LRP of 'Ibaarê' inherited in a more dominant way. For the F_3 populations no LRP values below 60 days were observed. The coefficients of correlation (r) between disease score in the field and the components of resistance were -.65, -0.61, 0.47 and 0.78 for LP in the laboratory, and LP, LD and LRP in the greenhouse, respectively. Only the r value for LD was non-significant at the 5% level. Multiple R, calculated from LP, LD and LRP in the greenhouse was significant (0.84) at $P \leqq 0.01$.

Table 6. Coffee leaf rust disease score in the field and components of resistance in laboratory and greenhouse tests of 'Ibaarê' (C1133-2-7), 'Mundo Novo' (CMP386-2-1), BC (P_1), BC (P_2), and of 10 F_2 field plants and their F_3 progenies (greenhouse test), inoculated with rust race II (v_5).

	Field	Components of resistance[2]			
Cultivar or progeny	Disease score[1] (0 - 9 scale)	Laboratory	Greenhouse		
		LP	LP	LD	LRP
$\bar{P}_1$ ('Ibaarê')	8.0	19.3	44	22	80
$\bar{P}_2$ ('Mundo Novo')	3.3	23.8	62	12	39
$\bar{F}_1$	5.3	22.1	57	13	71
$\bar{F}_2$	5.6	-	53	17	71
$\overline{BC}$ (P_1)	-	-	50	17	73
$\overline{BC}$ (P_2)	-	-	54	15	69
F_2-1	3.7	23.9	62	6	61
F_2-2	4.3	23.7	53	16	60
F_2-3	4.3	21.6	48	18	67
F_2-4	4.3	21.2	53	13	69
F_2-5	4.3	22.6	50	23	67
F_2-6	4.7	21.2	53	15	69
F_2-7	5.1	22.8	52	11	69
F_2-8	8.0	22.4	52	17	71
F_2-9	8.0	21.7	45	24	77
F_2-10	8.5	20.3	52	14	84

[1] Average of two readings made in March 1977 and March 1978

[2] LP = latency period, in days, LD = lesion density (number of lesions per leaf), and LRP = leaf retention period, in days, measured as the time between the day that LP is completed and leaf abscission.

The results indicate that LRP is the component which best explains the differences in disease level in the field. Consistently, 'Ibaarê' showed a much longer LRP than 'Mundo Novo' (see also Table 1).

Differences in LP appear to have some importance, although results varied between tests. LD was the least consistent component. Inconsistent results for LP and LD of 'Mundo Novo' and 'Ibaarê' have been reported earlier (Moraes et al., 1976, Scali et al., 1974).

The significant correlation between LRP and disease score in the field can be easily understood. A long LRP increases the quantity of inoculum at any time of the year and is of special importance for the carry-over of inoculum during the dry season, when leaf fall is highest.

Relative tolerance of 'Ibaarê'. 'Ibaarê' has a high yield potential in Campinas, even higher than 'Mundo Novo' (Monaco, 1977). During the first four years of rust occurrence in Campinas (1971 to 1975), 'Ibaarê' could be classified as 'tolerant' because it yielded well, notwithstanding its high susceptibility. However, after 1975, yields of 'Ibaarê' have dropped drastically in relation to 'Mundo Novo'. This can be illustrated by the results of two comparable yield experiments carried out in Campinas between 1961 and 1970 (exp. 1) and between 1971 and 1980 (exp. 2). In the absence of rust (exp. 1), 'Ibaarê' yielded 159 and 119% of 'Mundo Novo' during the first 4 and last 6 years of the experiment, respectively. In the presence of rust (exp. 2) these figures were 137 and 45%, respectively. The collapse of the productivity of 'Ibaarê' is apparently due to the exhaustive effect of continuous high rust attacks in this cultivar.

Segregation for resistance in F_3 populations from the Agaro C1164-19 × 'Catuai' cross. Progenies from the Agaro C1164 accession have shown low disease levels in the field, despite of the presence of the compatible race XV ($v_{4,5}$) in Campinas (Table 7). The low levels of disease may be partly explained by the low productivity of these progenies but could also indicate a high level of incomplete resistance. Great variation in disease level in the field was observed for 3 year old F_2 plants of the C1164-19 × 'Catuai' cross. In 1978, 70 F_3 populations were tested with race XV in the laboratory, together with both parents, the F_2 and 'Ibaarê'. Figure 1 shows that transgressive segregation for latency period (LP) towards higher resistance and higher susceptibility occurred. In the same year, 37 of these 70 F_3 populations were tested in the greenhouse, using 12 seedlings per population. Transgressive segregation was again observed for LP, and also for sporulating lesion density (SLD). The coefficient of correlation between LP observed in the laboratory and in the greenhouse was 0.80, significant at $P \leq 0.01$.

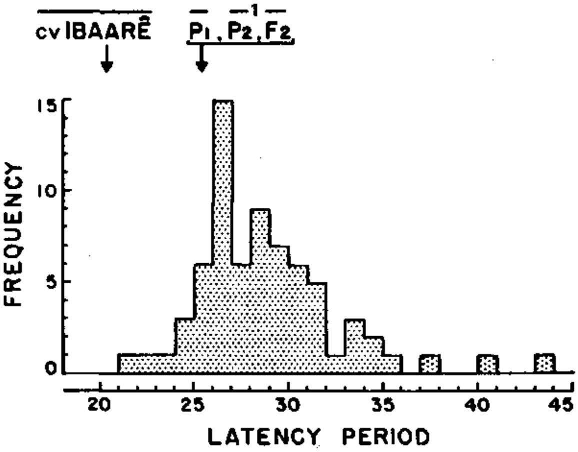
Table 7. Coffee leaf rust disease score in the field and yield, from 1972 to 1982, of two lines of the Agaro introduction, two F_1 hybrids between Agaro and Brazilian cultivars and of a susceptible control line of <u>C. arabica</u>.

Line or hybrid	Resistance genotype	Disease score[1] (0 - 9 scale)	Yield (kg fresh berries)
Agaro LC1164-19	S_H4S_H4	3.1^{a2}	3.6
Agaro LC1164-4	S_H4S_H4	3.4^{a}	11.3
H7480 (C1164-19 × 'Mundo Novo')	S_H4s_H4	4.8^{b}	20.4
H7317 (C1164-19 × 'Catuai')	S_H4s_H4	4.9^{b}	20.3
Control H3437-5	s_H4s_H4	8.0^{c}	17.0

[1] Average of two observations made in July 1979 and July 1980

[2] Different letters indicate significant differences according to the Tuckey test at $P \leq 0.05$.

Figure 1. Variation for latency period (in days) in leaf disks of 70 F_3 populations from the Agaro C1164-19 × 'Catuai' cross, of both parents, the F_2, and of 'Ibaarê', inoculated with rust race XV ($v_{4,5}$). Abscissa: frequency of F_3 populations.



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[1] The C1164-19 parent has been represented by its sister line C1164-4.

Ten of the more resistant F_3 populations were retested in the greenhouse in 1980. In 1978, nine of these populations had shown a significantly longer LP than 'Catuai' and 6 a significantly shorter SLD. In 1980, the differences with 'Catuai' for LP and SLD were much smaller, being significant only with two F_3 populations. The coefficients of correlation between the two tests were significant for SLD (0.69) but insignificant for LP (0.54). The analysis of variance showed interaction between tests and genotypes for LP ($P < 0.01$), but not for SLD ($P = 0.08$). Therefore, the resistance of the populations is, to a certain degree, affected by the test conditions.

Two other aspects of resistance in the F_3 populations were observed. Many F_3 populations showed a slightly higher percentage of non-sporulating lesions (flecks, tumefactions) than 'Catuai'. This percentage varied from 5 to 27 in the 1980 test, being 7% for 'Catuai'. Also, early necrosis of apparently susceptible-type lesions was observed with a few populations, reaching a maximum of 31% of the total number of lesions with one population in the 1980 test. Early necrosis of lesions has also been observed with other genotypes from the Agaro accession (Eskes, 1983[a]). Early necrosis appeared to be a highly unstable characteristic, being pronounced in some tests and non-apparent in others.

The most promising F_3 populations have been transplanted to the field for further selection and evaluation of resistance. It might be possible to find genotypes in these F_3 populations with high levels of incomplete resistance and good yielding capacity.

GENERAL BREEDING ASPECTS

Screening in the laboratory or greenhouse. In the laboratory and greenhouse tests significant variation for latency period (LP), lesion density (LD) and leaf retention period (LRP) was observed in *C. arabica*. The coefficient of variation (C.V.) was relatively low for LP, high for LRP and extremely high for LD. Interactions between genotypes and tests were observed for LP and LD. Furthermore, LP and LD were not always correlated with disease level in the field. Therefore, selection for a long LP or a low LD in the laboratory or greenhouse may be of low efficiency in obtaining lower levels of disease in the field.

Incomplete resistance to coffee leaf rust is affected by leaf age and light intensity (Eskes, 1982[b], Eskes and Toma-Braghini, 1983). Also soil humidity may affect resistance (J.C.G. Hoogstraten, personal communication). Therefore, in screening for incomplete resistance the test conditions before and after inoculation should be as uniform as possible for all plants.

Heritabilities for LP and LD, calculated from the variances in parental and F_2 populations of the crosses between 'Ibaarê' and 'Mundo Novo' and between 'Catuai' and 'Agaro C1164-19' were extremely low (0.41 and 0.26 for LP, and 0.00 and 0.24 for LD, with the two crosses respectively). Hence, individual plant selection for LP or LD in segregating populations in the greenhouse is not expected to be successful. Screening in the greenhouse might only have some success if many replications are used per genotype (clones or progenies obtained by selfing).

It is not possible to indicate one component of resistance as the most suitable selection criterion for all *C. arabica* populations. The importance of each component varied according to the population. LRP appeared to be important in the progenies from the 'Ibaarê' × 'Mundo Novo' cross, with LP and LD successively as next in importance. In the progenies from the Agaro C1164-19 × 'Catuai' cross, LP and SLD were important components, but also early necrosis of lesions may have importance.

In general, LP and LD were well correlated. With the progenies from 'Ibaarê' × 'Mundo Novo' (Table 6) the coefficient of correlation between LP and LD was -0.82. With the F_3 populations from the Agaro C1164-19 × 'Catuai' cross this value was -0.85. Since C.V. values were lower for LP than for LD, it is recommendable to use LP rather than LD as a selection criterion.

If LRP is to be used as a selection criterion, genotypes with either very long or very short LRP's should be avoided. A long LRP may be related to high disease levels in the field (Table 6). Short LRP's might be related to low productivity, as indicated by studies of van der Vossen and Browning (1978). These authors studied the effect of ethylene on leaf abscission in *C. arabica*. They concluded that the ability to retain leaves under stress conditions may be vital for successful commercial cultivation of *C. arabica* in Kenya. Rust-induced leaf abscission may be similar to ethylene-induced leaf abscission, hence, short LRP's induced by coffee leaf rust infections might relate to a low production capacity of the coffee genotype.

Screening in the field. Heritabilities for disease level in the field were low for different lines from 'Catuai' and 'Mundo Novo'. Higher heritabilities were found in the F_2 populations from crosses between 'Ibaarê' and other Brazilian coffee cultivars (Table 5), which is due to the greater genetic variation for disease level in these progenies. This indicates that individual tree selection for low disease level in the field can be successful, provided that a certain amount of genetic variation is present.

Disease level in the field was positively correlated with yield (Table 3). Therefore, when screening for low disease levels in the field, the yielding capacity should be taken into account. Field observations should be carried out during more than one year to account for differences in bearing pattern between the coffee trees.

The relationship between disease level and yield in the same year is a known feature of rust epidemics in Brazil (Monaco, 1977). In years of high productivity the disease level is generally higher than in years of low productivity. It is a related but new aspect that the production capacity of a coffee line may also significantly influence the disease level (Tables 3 and 4). However, the coefficients of correlation are not very high and, therefore, selection of productive cultivars with increased levels of resistance might be possible. Whether such cultivars can be selected will probably depend more on the genetic variation for incomplete resistance present in the population than on the relationship between yield and disease level.

Conclusions. Breeding for increased levels of incomplete resistance to coffee leaf rust may be important for obtaining durable resistance. The present research indicates some perspectives for selection in *C. arabica*. In progenies from varietal crosses, such as Agaro C1164-19 × 'Catuai', genotypes may be selected with increased levels of resistance and good production capacity. For selection success on the long term, it seems desirable to create a variable population with good production capacity and without the presence of major genes. The crosses realized between the accessions from Ethiopia and 'Catuai' are a first step in this direction.

The present research also indicates factors which may hamper selection success: a) the positive correlation between yield and disease level in the field, b) the relatively low variation for incomplete resistance among *C. arabica* cultivars and productive breeding lines, and c) inconsistency of results between resistance tests.

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7 CHARACTERIZATION OF INCOMPLETE RESISTANCE TO *HEMILEIA VASTATRIX* IN *COFFEA CANEPHORA* CV. KOUILLOU

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INDEX WORDS
Coffee Leaf Rust, components of resistance, heterogeneous reaction type, durable resistance.

SUMMARY

Incomplete resistance to coffee leaf rust *(Hemileia vastatrix)* varied among genotypes of *Coffea canephora* cv. Kouillou from near 'immunity' to a higher susceptibility than that of *C. arabica* cv. Mundo Novo. In 4 series of laboratory tests resistance components and reaction type (RT) were observed. Low disease levels in the field were mainly associated with low, or heterogeneous, RT and/or a low lesion density (LD). Among the components of resistance sporulating lesion density (SLD) showed the highest coefficient of correlation with disease level in the field ($r = 0.83$). Components of resistance were intercorrelated and were also correlated with RT. Interactions between inoculation series and genotypes were significant for all parameters of resistance, but especially so for LD.

A small part of the Kouillou population showed complete resistance to race II of *H. vastatrix*. Two rust genotypes isolated from 'Kouillou' interacted with some genotypes with complete or incomplete resistance. The complete and race-specific resistance of one genotype was governed by a dominant gene.

High levels of incomplete resistance appeared to inherit either monogenically or in a more complex way. Five genotypes with high levels of incomplete resistance, expressed by a low LD and a relatively high RT, were crossed with *C. arabica* cv. Catuai. The triploid F_1 plants showed a variable but susceptible RT. These crosses may have value for obtaining coffee cultivars with durable resistance to coffee leaf rust.

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INTRODUCTION

Durable resistance to coffee leaf rust *(Hemileia vastatrix* Berk. et Br.) is considered as a main objective in perennial coffee crop breeding. 'Kouillou' is a cultivar of *Coffea canephora* Pierre, which is a diploid allogamous species. 'Kouillou' is responsible for 50% of the coffee production in the State of Espírito Santo, Brazil. Individual genotypes of 'Kouillou' show wide variation for resistance to coffee leaf rust. In the State of Espírito Santo all trees show some susceptibility to *H. vastatrix* but only a very small part of the population becomes highly diseased. Chemical control of coffee leaf rust in 'Kouillou' is reportedly ineffective (Paulino, 1981). The incomplete resistance of 'Kouillou' might provide a more durable type of resistance than is provided by complete or major gene resistance.

Cadena G. and Buriticá C. (1980) studied the epidemic development of coffee leaf rust during one year in a population of 'Kouillou' in Campinas, Brazil. Disease levels and leaf fall due to the disease were much lower in 'Kouillou' than in 'Mundo Novo' *(C. arabica* L.). In greenhouse tests, Scali et al. (1974) found a lower number of lesions per leaf for 'Kouillou' than for *C. arabica* cvs Catuai and Ibaarê. Cadena G. (1978) detected significant differences in resistance components between 'Kouillou' genotypes tested in the nursery and laboratory. Eskes (1982^a) considered a laboratory test, using coffee leaf disks, suitable for assessing resistance to coffee leaf rust. The present study further characterizes resistance of 'Kouillou' in the field and laboratory. Segregation for resistance was studied in F_1 populations in the nursery.

MATERIALS AND METHODS

'Incomplete' resistance is defined here as a form of resistance which does not completely inhibit reproduction of the pathogen. Incomplete resistance may be governed by minor genes, but also by major genes (Parlevliet, 1979).

The genotypes of 'Kouillou' used in this study belong to a population of 64 trees, raised from seed introduced in 1933 from Castello, Espírito Santo. This population consists of 5 families, named C66 to C70. Trees of each family are probably half-sibs. They are part of the coffee collection of the Instituto Agronômico of Campinas (I.A.C.). One 'unidentified' genotype (Table 2) refers to an unnamed 'Kouillou' tree present in the same collection. Six trees of *C. arabica* cv. Mundo Novo planted in the same lot as the 'Kouillou' genotypes have been used as a control.

Unless stated otherwise, race II of *H. vastatrix* has been used. Race II is the most common race in Brazil. Inoculation methods and methods used for measuring resistance components are as described by Eskes (1982[a] and 1982[b]). Four laboratory tests, with leaf disks of 23 'Kouillou' genotypes, were carried out in December 1978, and in March, May and October 1979. Per genotype 80 to 120 leaf disks from 40 randomly chosen mature leaves, distributed over 4 to 5 replications, were used for each test (series). Resistance components observed are: latency period (LP = number of days from inoculation to sporulation of 50% of the total number of sporulating disks), lesion density (LD = % of disks with visible lesions), sporulating lesion density (SLD = % of disks with sporulating lesions) and relative sporulating lesion efficiency (RSLE = 100. SLD/LD). A field test was carried out in March 1981. In this test, LD refers to the number of visible lesions per 10 cm^2 leaf surface. Reaction type (RT) was scored by a 0 to 9 scale (Eskes and Toma-Braghini, 1981). Values 0 to 3 indicate a resistant RT, 4 and 5 a moderately resistant RT, 6 and 7 a moderately susceptible RT, and 8 and 9 a susceptible RT. RT's 4 to 7 are generally heterogeneous, with resistant-type lesions and sporulating lesions occurring together.

The disease level in the field was scored by a 1 to 5 scale (disease score, DS). Value 1 indicates absence of sporulating lesions, values 2 to 5 increasing numbers of sporulating lesions associated with increasing sporulating intensity of the lesions. The percentage of leaves with sporulating or necrotic lesions has been assessed by sampling 200 leaves per genotype.

For statistical elaboration of the results computer programs from the Statistical Package for the Social Sciences (SPSS) have been used. Percentage data have been transformed into the arcsine of the square root of the percentages.

RESULTS

Field observations. Disease scores (natural infection) were made 11 times between 1976 and 1981 on 64 'Kouillou' genotypes. A continuous variation from complete resistance to high susceptibility was observed. Most trees gave intermediate scores and a few were more severely affected than *C. arabica* cv. Mundo Novo. The variance for disease scores was highest for genotypes with intermediate scores. The 95% confidence interval for average disease scores between 2.5

and 3.9 was about 0.5 scale units.

The epidemic development of coffee leaf rust in 'Kouillou' differed from that in *C. arabica*. For the 'Kouillou' population as a whole, epidemics started quite early in the season, but subsequent development was slower than in *C. arabica* cv. Mundo Novo (Table 1). In 'Kouillou' the occurrence of necrotic lesions, besides sporulating ones, was commonly observed in the field (Tables 1 and 2). Necrosis started before or after sporulation, causing small and large necrotic lesions, respectively. Some genotypes showed a remarkable increase in the % of necrotic lesions during the season, indicating that their resistance was changing. Necrosis of lesions in 'Mundo Novo' was only observed under extremely dry or hot weather conditions.

Yield affected slightly the level of disease of Kouillou genotypes with incomplete resistance. A tree with normally low disease levels would become moderately diseased in years of heavy bearing. However, the coefficient of correlation between average yield and disease score was insignificant (r = -0.21).

Table 1. Average percentage of leaves with sporulating rust lesions and percentage of necrotic lesions of 17 trees of C. canephora cv. Kouillou and of 6 trees of C. arabica cv. Mundo Novo, observed in March and May 1979 and 1981.

Cultivars	Leaves with sporulating lesions (%)				Necrotic lesions (%)	
	1979		1981		1981	
	March	May	March	May	March	May
Kouillou	17	30	17	15	29	53
Mundo Novo	20	57	11	39	0	0

Components of resistance and reaction type. Table 2 shows results of field observations, of laboratory tests (average of 4 tests) and of a field test. Two genotypes showed complete resistance; C68-11 did not show any disease symptoms and C67-3 showed only a hypersensitive type of lesions. High levels of incomplete resistance were related to a low, or heterogeneous, reaction type (RT) and/or to a low lesion density (LD). The degree of heterogeneity of the reaction is expressed by the relative sporulating lesion efficiency (RSLE). Two 'Kouillou' genotypes showed higher susceptibility than 'Mundo Novo'. The 'unidentified' genotype was so much diseased in the field that this tree did not produce any fruit during the observation period.

Table 2. Disease level of coffee leaf rust in the field and components of resistance observed in the laboratory (averages of 4 tests) of 15 'Kouillou' genotypes and of 'Mundo Novo'.

Genotype	Field			Components of resistance[4]					
	Disease score[1] (1-5 scale)	% Leaves with sporulating lesions[2]	% Necrotic lesions[3]	Laboratory tests				Field test	
				LP	LD	SLD	RSLE	LD	RT
'Kouillou'									
C68-11	1.0	0	-	-	0	0	-	0	0
C67-3	1.0	0	100	-	49	0	0	-	-
C66-1	1.6	3	70	45	27	10	39	2.0	3.8
C69-7	1.7	6	73	39	50	31	63	1.3	3.9
C70-11	1.8	5	27	37	44	36	82	0.6	3.7
C69-14	2.0	3	25	34	36	32	77	0.2	5.0
C70-14	2.1	2	23	36	28	26	91	0.7	4.5
C66-3	2.2	9	78	38	50	34	62	0.8	5.4
C69-15	2.5	12	48	36	44	40	86	-	-
C68-4	3.1	14	47	38	55	46	85	4.0	5.9
C68-10	3.6	18	24	38	52	46	87	4.0	7.7
C70-3	4.0	17	50	36	49	43	88	-	-
C69-10	4.4	38	58	35	58	49	81	1.5	4.5
C67-15	4.6	45	25	32	69	65	92	-	-
C 'unidentified'	5.0	61	0	24	69	68	100	-	-
'Mundo Novo'	4.4	27	0	27	56	55	99	11.5	8.5

[1] Average of 11 observations made from 1976 to 1981

[2] Average of 4 observations made in 1979 and 1981

[3] Average of 2 observations made in 1981

[4] LP = latency period, in days
LD = lesion density; in laboratory test: % disks with lesions, in field test: number of visible lesions per 10 cm^2 leaf surface
SLD = sporulating lesion density (% disks with sporulating lesions)
RSLE = relative sporulating lesions efficiency (100.SLD/LD)
RT = average reaction type (0 - 9 scale)

The field test showed lower levels of disease of most 'Kouillou' genotypes, in comparison to 'Mundo Novo', than expected from the laboratory tests or from the average disease scores in the field. The dry and hot weather prevailing during the field test may have induced the relatively high resistance of 'Kouillou'.

Rank correlation coefficients (r_s) were calculated between resistance parameters of 20 'Kouillou' genotypes, with varying degrees of incomplete resistance (Table 3). In the laboratory tests, the sporulating lesion density (SLD) was best correlated to the disease score in the field ($r_s = 0.83$). The components of resistance were correlated with each other. SLD was highly correlated with LD and RSLE. LD and RSLE were less correlated to each other. This shows that SLD is based on lesion number and reaction type. RSLE was also highly correlated with latency period (LP), indicating a relationship between LP and reaction type. The % of necrotic lesions in the field (%NL) was related to RSLE, a relationship which can be expected since both parameters are an indication for reaction type.

Table 3. Rank correlation coefficients of Spearman (r_s) between components of resistance observed in the laboratory and disease score (DS, 1 - 5 scale) and percentage of necrotic lesions (%NL) in the field of 20 plants of 'Kouillou', with varying degrees of incomplete resistance to coffee leaf rust. Significance of r_s is ± 0.44 at P = 0.05.

	Components of resistance[1]			Field observations	
	LD	SLD	RSLE	DS	%NL
LP	-.49	-.70	-.74	-.68	.67
LD		.89	.54	.80	-.17
SLD			.81	.83	-.38
RSLE				.69	-.74
DS					-.46

[1] For explanation of abbreviations see Table 3

In the laboratory test of December 1978, sporulation of 13 'Kouillou' genotypes was measured by counting the number of urediospores produced per disk with sporulating lesions. The counts were made 10 days after the day that 50% of the disks sporulated. Between genotypes, spore production varied from 0.09 to 2.53×10^4 per disk. A significant correlation was found with RSLE ($r = 0.81$), indicating that spore production is narrowly related to reaction type.

Interaction between inoculation series and genotypes. The four series of leaf disk inoculations were carried out with 4 or 5 replications each. The pooled analysis of variance detected significant effects of genotypes, series and of interaction between series and genotypes for all resistance components at a level of significance lower than 0.005. The relative variance due to series (K^2_S), genotypes (K^2_G), interaction between series and genotypes ($K^2_{S\times G}$) was estimated according to a fixed model analysis (Snedecor and Cochran, 1967). The variance ratio of $K^2_{S\times G}/K^2_G$ was highest for LD, intermediate for SLD and relatively low for LP and RSLE (Table 4). Therefore, LD appears to be a less reliable characteristic of resistance than is LP or RSLE. Hence, selection for a low lesion density might be less successful than selection for a low reaction type.

Table 4. Estimation of the percentage of variance due to series (K^2_S), genotypes (K^2_G), interaction between series and genotypes ($K^2_{S\times G}$) and experimental error (σ^2_e) calculated from 4 series of leaf disk tests for 20 'Kouillou' genotypes with incomplete resistance to coffee leaf rust.

Variance component or ratio	Component of resistance[1]			
	LP	LD	SLD	RSLE
K^2_S	9	52	38	6
K^2_G	40	14	29	48
$K^2_{S\times G}$	9	15	15	7
σ^2_e	42	19	18	40
$K^2_{S\times G}/K^2_G$	0.23	1.07	0.52	0.15

[1] For explanation of abbreviations see Table 3

Specificity of resistance. Table 5 shows representative results of a laboratory test with 29 'Kouillou' genotypes inoculated with four rust isolates. More detailed results of this test have been published elsewhere (Eskes, 1983). Three 'Kouillou' genotypes with complete resistance to race II (e.g. C68-11, Table 5) were susceptible to isolate 10 (Is. 10). Several genotypes, with varying degrees of incomplete resistance to race II (e.g. C67-1, C69-7 and C69-15), were more susceptible to isolate 11 (Is. 11) than to race II. Other genotypes, some of which have low disease levels in the field (e.g. C69-14 and C70-14), did not show higher susceptibility with the 'Kouillou' isolates than with race II.

Table 5. Average sporulating lesion density (SLD = % of leaf disks with sporulating lesions) and reaction types (RT, 0 to 9 scale) of six 'Kouillou' genotypes and 'Mundo Novo' inoculated with coffee leaf rust race II, isolate 11 (Is. 11) and isolate 10 (Is. 10). Is. 10 and Is. 11 were obtained from 'Kouillou' C66-13 and C67-1, respectively.

Coffee genotype	SLD			RT		
	Race II	Is. 11	Is. 10	Race II	Is. 11	Is. 10
'Kouillou':						
C68-11	0^{a}[1]	0^{a}	80^{b}	0^{a}	0^{a}	7.7^{b}
C67-1	24^{b}	62^{c}	0^{a}	5.0^{b}	7.0^{c}	2.0^{a}
C69-7	51^{b}	78^{c}	22^{a}	5.3^{a}	6.7^{b}	4.0^{a}
C69-15	60^{b}	91^{c}	13^{a}	6.0^{b}	6.7^{b}	4.0^{a}
C69-14	71^{b}	27^{a}	24^{a}	6.3^{a}	6.0^{a}	5.3^{a}
C70-14	24^{a}	29^{a}	22^{a}	6.3^{a}	6.3^{a}	5.3^{a}
'Mundo Novo'	62^{a}	65^{a}	59^{a}	8.9^{a}	8.8^{a}	8.8^{a}

[1] Different letters indicate significant differences, between rust races within genotypes at LSD 0.05.

Furthermore, Table 5 shows that some 'Kouillou' genotypes were more resistant to Is. 10 than to race II (e.g. C67-1, C69-7 and C69-15). This may indicate that Is. 10 and race II differ for more than one virulence gene or that interaction occurs between virulence genes in the rust genotype (Eskes, 1983).

Segregation for resistance. Crosses between 'Kouillou' genotypes with different levels of resistance were made by controlled hand pollinations. The evaluation of resistance of two-year-old F_1 plants was done in a nursery test. Scoring was done by a 1 to 9 scale which expresses variation in reaction type and lesion density. Table 6 shows the resistance scores of plants from 9 F_1 populations. The populations have been grouped together when the following common parents have been used: C66-1, C68-11 and C69-9.

The resistance of C66-1 is high but incomplete (Table 2). The bimodal segregation in the F_1's with C66-1 as a parent suggests mono- or oligogenic inheritance, although the results are not conclusive due to the low number of F_1 plants scored. The segregation in the F_1's with C68-11 as a parent indicate the presence of a dominant major gene, in heterozygous condition, controlling complete resistance. This resistance is race-specific, it is overcome by isolate 10 (Table 5). Furthermore, continuous variation for incomplete resistance was observed in the F_1's from C68-11 suggesting also the presence of minor genes for resistance. The incomplete resistance of C70-11 and C70-14 appeared to be based on minor genes too, as continuous variation was observed in the F_1 populations derived from these genotypes. A few plants with complete resistance (classes 1, 2

Table 6. F_1 plants of nine crosses between 'Kouillou' genotypes classified over 9 classes for coffee leaf rust resistance.

Cross or cultivar	Disease score (1-5 scale) of parents in the field	Number of F_1 plants								
		Resistance class[1]								
		1	2	3	4	5	6	7	8	9
'Kouillou'										
C66-1 × C67-6	1.6 × 4.5			2	5			1	1	
C66-1 × C70-13	1.6 × 4.2		2	1	1		1	3	2	
Total			2	3	6		1	4	3	
C68-11 × C67-6	1.2 × 4.5	4	15		2	3	4	5	2	2
C68-11 × C69-9	1.2 × 3.5	3	14		1	4	7	4	4	1
Total		7	29		3	7	11	9	6	3
C70-11 × C67-6	1.8 × 4.5			1	1	5	3	5	9	3
C70-11 × C69-9	1.8 × 3.5		1			3	6	4	3	
Total			1	1	1	8	9	9	12	3
C67-6 × C69-9	4.5 × 3.5						2	8	19	5
C68-4 × C69-9	3.1 × 3.5		1		2	3	5	16	7	1
C69-9 × C70-14	3.5 × 2.1		2		9	7	8	8	4	1
'*Mundo Novo*'	4.4						4	10		

[1] Value 1 indicates absence of disease symptomes, values 2 and 3 indicate presence of small flecks and large chlorotic areas without sporulation, respectively, and values 4 to 9 indicate increasing numbers of sporulating lesions per leaf area unit. Values 4 and 5 were often heterogeneous in reaction, showing sporulating and non-sporulating lesions simultaneously.

and 3, Table 6) were observed in the F_1's of these parents. These are possibly due to a very high level of resistance to infection and to an occasional outcross.

The average level of resistance of the F_1 populations was fairly well related to the average disease scores of the parents in the field. This is clearly so with F_1's which have C69-9 as a common parent (Table 6).

Crosses between 'Kouillou' and 'Catuai'. With the aim of introducing minor genes for resistance to coffee leaf rust from 'Kouillou' into *C. arabica* cultivars, crosses were made in 1980 between 5 'Kouillou' genotypes, used as male parents, and 'Yellow Catuai'. The 'Kouillou' genotypes (C69-14, C70-9, C70-11, C70-12 and C70-14) were selected on the following criteria: 1) low levels of disease in the field despite of a quite susceptible reaction type, 2) absence of interaction with the rust isolates shown in Table 6, 3) apparently complex inheritance of resistance, and 4) fairly good yielding capacity. C70-9 and C70-12 were the most resistant genotypes in the field, showing only sporadically sporulating lesions but no hypersensitive type of lesions. About 250 F_1 seedlings were raised in the nursery in 1981/1982, all showing a triploid appearance. Screening of the triploids was done in 1982 by scoring reaction type and number of lesions per plant resulting from natural infection.

All triploids from C69-14, C70-11 and C70-14 showed susceptible reaction types, but the number of lesions varied between plants. About 80% of the triploids from C70-9 and C70-12 also showed susceptible reaction types. The rest showed a range of intermediate, heterogeneous, reaction types and great variation for the number of lesions per plant. No plants were found with complete resistance. This type of segregation would exclude the presence of dominant major resistance genes in these populations.

DISCUSSION

Some 'Kouillou' genotypes showed complete resistance to the rust population of Campinas. However, in the State of Espírito Santo no rust-free trees of 'Kouillou' can be found (Paulino, 1981). Therefore, rust races may be present in the State of Espírito Santo which do not occur in Campinas.

The susceptibility of most 'Kouillou' genotypes to the common race II of *H. vastatrix* has been taken as an indication that its resistance might be 'horizontal resistance' (Cadena G. and Buritica C., 1980). However, the present results show that race-specific resistance, at various degrees, can be found in 'Kouillou' (Table 5). Some genotypes appeared to have monogenic dominant resistance (Table 6). Therefore, certainly not all resistance in 'Kouillou' is durable resistance. Great care should be taken in the selection of 'Kouillou' genotypes to be used in breeding programmes for resistance.

Polygenic incomplete resistance to plant pathogens is considered to be more durable than monogenic resistance of the hypersensitive type (e.g. Vanderplank, 1968, Simons, 1972, Parlevliet and Zadoks, 1977). Parlevliet (1979) suggested that selection for polygenic resistance to cereal rusts can be achieved by selecting genotypes with a low disease incidence, despite of a high (susceptible) reaction type. This so called 'partial' resistance can be measured by the components of resistance as latency period, lesion density and sporulation intensity. Selection of genotypes of 'Kouillou' for breeding purposes has followed these criteria of Parlevliet. The five selected genotypes showed generally a high reaction type, but in some tests (e.g. Table 3, field test) intermediate or low reaction types were scored. A clear distinction between resistance of a high or low reaction type was not possible. It seems likely that polygenically inherited resistance to coffee leaf rust can be expressed by a low or heterogeneous reaction type, depending on the test conditions.

The components of resistance in 'Kouillou' were correlated with reaction type (RT) (Table 3). A similar relationship between resistance components and RT was found f.i. with slow leaf rusting wheat cultivars (Milus and Line, 1980). It seems important that RT is observed in studies on incomplete resistance, because it may give a clue to possible resistance mechanisms.

Incomplete resistance to *H. vastatrix* in 'Kouillou' varies according to leaf age and light intensity (Eskes, 1982^b, Eskes and Toma-Braghini, 1982). These effects may partly explain the seasonal variation in resistance, the interaction between inoculation series and genotypes (Table 3) and the variation in reaction types observed within trees. Effects of environment and development stage of host tissues on resistance are common features for many rust diseases (Hooker, 1967, Sharma et al, 1980, Milus and Line, 1980, Stubbs, 1980). Brown and Sharp (1969) reported that minor genes for resistance to yellow rust of wheat vary greatly in effectiveness depending on temperature. In Kouillou similar environment dependable minor genes for resistance to *H. vastatrix* may be present.

Minor genes which are affected by plant and environmental factors are not continuously operative in the resistance process. Hence, selection pressure for increased virulence in the pathogen is not continuous. Mutants for virulence may initially have a low fitness (Parlevliet, 1981). If selection pressure is not constant, such mutants are likely to be less competitive than other rust genotypes. Therefore, the influence of plant and environment factors on resistance as occurs in Kouillou may enhance the durability of resistance.

The crosses realized between 'Kouillou' and 'Catuai' are a first step to introducing minor resistance genes from 'Kouillou' into *C. arabica*. In the first backcross generation (BC_1) it will be necessary to work with a great number of plants because resistance will be diluted. Selfing and intercrossing among selected BC_1 genotypes should be carried out to allow for accumulation of the resistance genes from 'Kouillou'. The success of such a breeding programme will depend on the number of resistance genes involved and on the expression of the 'Kouillou' resistance in the *C. arabica* genetic background.

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8 CHARACTERIZATION OF INCOMPLETE RESISTANCE TO *HEMILEIA VASTATRIX* IN THE ICATU COFFEE POPULATION

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INDEX WORDS
Coffea spp., Coffee Leaf Rust, heterogeneous reaction type, durable resistance

SUMMARY

Incomplete resistance of the hybrid coffee population Icatu to race II of coffee leaf rust (*Hemileia vastatrix*) was studied in the field, greenhouse and laboratory. The resistance components observed were: latency period (LP), lesion density (LD), sporulating lesion density (SLD) and relative sporulation lesion efficiency (RSLE = 100 SLD/LD). RSLE is an indicator for the heterogeneity of the reaction.

Disease score in the field was highly correlated with RSLE, less with LP and SLD, and not with LD. LP was highly correlated with RSLE.

A new rust race (Is. 2), isolated in the field from Icatu in 1979, was more virulent than race II on some resistant, moderately resistant or moderately susceptible genotypes. This indicates that incomplete resistance, at different levels, can be race specific.

Resistance was affected by leaf age and light intensity. Inheritance studies suggest that incomplete resistance in Icatu might be related to major genes, the effectiveness of which may depend on gene dose and genetic background.

It is concluded that selection for incomplete resistance to coffee leaf rust in Icatu may not lead to durable resistance.

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INTRODUCTION

The two main commercial coffee species are *Coffea arabica* L. (autogamous, allotetraploid) and *C. canephora* Pierre (allogamous, diploid). The first species is responsible for 80% of the world coffee production and is growing best in tropical highlands; the second species is best adapted to the tropical lowlands (Carvalho et al., 1969).

In *C. arabica* five major genes, S_H1 to S_H5, for resistance to coffee leaf rust (*Hemileia vastatrix* Berk. et Br.) exist. These genes have not been efficient in controlling coffee leaf rust. In Brazil, after ten years of rust presence, only the S_H3 gene is still effective (Eskes, 1983[a]). All combinations of the other 4 genes have been matched by virulent races. Most breeding efforts are now focussed on two tetraploid hybrid populations, called 'Icatu' and 'Catimor', wich are derived from crosses between *C. canephora* and *C. arabica*. These populations contain several resistance genes from *C. canephora*, which differ from S_H1 to S_H5, and provide resistance to some or to all known rust races (Rodrigues and Bettencourt, 1975).

Icatu shows wide variation for agronomic traits, resistance to coffee leaf rust, to coffee berry disease and to coffee root knot nematodes (Carvalho et al. 1976, Costa, 1978, Mônaco, 1977). In relation to the coffee leaf rust resistance, Marques and Bettencourt (1979) observed inconsistency in reaction between subsequent inoculations. Field observations in Brazil of Costa and Ribeiro (1975) indicated that the majority of Icatu genotypes is highly resistant to the common race II (v_5) of *H. vastatrix*, but that also many genotypes with intermediate resistance are present. Costa et al. (1978) showed a high correlation between resistance scores in the greenhouse and field.

The existence of quantitative variation for resistance in Icatu may suggest the presence of minor genes or polygenes. Polygenic resistance may be more durable than monogenic resistance (eg. Vanderplank, 1968, Simons, 1972, Parlevliet, 1978). The present work investigates characteristics of incomplete resistance in Icatu in order to assess its value in obtaining durable resistance to coffee leaf rust. The term 'incomplete resistance' is used to indicate all types of resistance which allow for, at least, some reproduction of the pathogen.

MATERIALS AND METHODS

Icatu genotypes: Icatu is a tetraploid coffee population derived from a cross between a *C. canephora* genotype, with a doubled number of chromosomes ($2n = 4x = 44$), and *C. arabica* cv. Bourbon Vermelho ($2n = 4x = 44$). This cross was made in the 1950's at the Instituto Agronômico of Campinas (I.A.C.), SP, Brazil. The F_1 has been backcrossed mostly to *C. arabica* cvs Mundo Novo and Caturra Vermelho. Progenies derived from these backcrosses show great vigour. Aneuploidy occurs in a number of progenies (Mônaco et al., 1974). The Icatu genotypes used in the present work belong to a field experiment, planted in 1971, including 2400 plants derived by open pollination from some of the best BC1 and BC2 plants. Codes used to indicate genotypes include hybrid numbers and plant numbers, separated by dashes (generations). The hybrid numbers H 3849 and H 3851 are first backcrosses of the F1 with 'Caturra Vermelho' and 'Mundo Novo', respectively. H 4782 is a second backcross, for which 'Mundo Novo' has been used twice.

Inoculation methods. Information on standard inoculation methods and on the suitability of the laboratory test in assessing resistance is given by Eskes, 1982[a]. For the greenhouse tests (Tables 1 and 2) two-year-old grafted plants were used. These grafts were made on rootstocks with similar genotype and resistant to race II of *H. vastatrix*. The 24 Icatu genotypes used were selected because of good yielding capacity and great variation in resistance. Three plants of *C. arabica* cv. Mundo Novo were used as a control. Of each plant two branches, with 6 leaves each, were inoculated. For the field test (Tables 1 and 2) three branches were used from the same 26 genotypes with 6 leaves per branch. Only branches growing in the shade were inoculated, to avoid interference with high insolation. For the laboratory test (Table 3), 6 replicates were used with 20 leaf disks each. For the field tests (Table 4), one branch with 8 to 12 leaves was inoculated per genotype/isolate combination and for the laboratory test (Table 4) 15 leaf disks were used. Inoculations were carried out in December 1976 (Tables 1 and 2), December 1977 (Table 3), November 1980 and March 1981 (field tests, Table 4), March 1981 (laboratory test, Table 4), and January 1977 (Table 5).

Except when mentioned otherwise, for all tests *H. vastatrix* race II was used. Isolate ICT (Table 1) was obtained in 1976 from several Icatu field plants, derivatives from H 3851-2. Isolate 2 (Table 4) was obtained in 1979 from H 3851-4-40, a genotype which is incompatible to race II.

Evaluation of resistance. Reaction type was scored by a 0 to 9 scale (Eskes and Toma-Braghini, 1981). Value 0 indicates absence of visible symptoms, 1 to 3 variation within a resistant (R) reaction type, 4 to 7 heterogeneous reaction types with increasing percentage of sporulating lesions and increasing sporulation intensity. Values 4 and 5 are classified as moderately resistant (MR), 6 and 7 as moderately susceptible (MS). Values 8 and 9 indicate a susceptible (S) reaction type with moderate (8) to high (9) sporulation intensity. The resistance components were measured according to Eskes (1982^a). Latency period (LP) is the time, in days, between inoculation and sporulation of 50% of the lesions that come to sporulate; lesion density (LD) is the number of visible lesions (resistant and susceptible types) per leaf area unit; sporulating lesion density (SLD) is the number of sporulating lesions per leaf area unit; and relative sporulating lesion efficiency (RSLE) is the percentage of sporulating lesions (100.SLD/LD). RSLE is a measure of the heterogeneity of the reaction.

In the field the disease level has been scored yearly by a 1 to 5 scale (disease score). The scale has been adapted from a 1 to 6 scale described by Costa (1978). Value 1 indicates absence of symptoms or presence of only resistant-type lesions, 2 to 5 indicate increasing numbers of sporulating lesions associated with an increase in sporulation intensity. Values 2 and 3 generally indicate heterogeneous reaction types with slight (2) and moderate (3) sporulation intensity. Furthermore in May 1977, the percentage of leaves with sporulating lesions and the percentage of necrotic lesions was observed by sampling 100 leaves per genotype.

RESULTS

Expression of resistance. Twenty four genotypes of Icatu were tested in the field and greenhouse. Table 1 shows the results of ten representative genotypes and of the control 'Mundo Novo' of *C. arabica*. In the field, one genotype showed a higher disease score than 'Mundo Novo', two were similar to 'Mundo Novo', five showed intermediate or low disease levels and three were nearly completely resistant. Increasing levels of resistance were related to a higher percentage of necrotic lesions in the field. 'Mundo Novo' showed 11% necrotic lesions, which is probably not due to a resistance reaction but to overheating by the sun (Eskes, 1982^b). The inoculation test in the field resulted in sporulation on 5 genotypes; 6 showed resistant type lesions.

Table 1. Disease level of coffee leaf rust in the field and components of resistance of ten Icatu genotypes and of 'Mundo Novo' tested in the field and greenhouse with H. vastatrix, race II and an isolate from Icatu (Is. ICT).

				Components of resistance[4]					
	FIELD			Field	Greenhouse				
Genotype	Disease score[1]	Leaves with sporulating lesions (%)[2]	Necrotic lesions (%)[2]	LP	LP	LD	SLD	RSLE	
				Race II	Race II	Race II	Race II	Race II	Is. ICT
Icatu:									
H3851-2-291	5.0	92	24	33	34	82	82	100	100
H3851-2-513	4.6	62	43	-[3]	55	45	6	13	57
H4782-7-960	4.4	78	18	38	34	41	40	91	97
H4782-13-72	4.2	10	58	41	39	17	14	85	86
H4782-13-152	3.8	24	68	44	39	63	33	53	58
H3851-4-41	3.4	32	65	-	53	58	1	1	15
H3849-9-27	3.4	8	61	-	69	15	2	13	27
H3851-2-437	2.0	1	98	-	59	30	4	14	23
H4782-7-624	1.2	2	95	-	-	28	0	0	0
H3851-2-457	1.0	0	100	-	-	10	0	0	0
Control:									
'Mundo Novo'	4.5	83	11	33	35	25	23	82	87

[1] Average of 5 yearly observations from 1975 to 1980.

[2] Observed in May, 1977, as a result of natural infection. Necrotic lesions indicate resistant-type lesions or lesions necrosed by sunburning.

[3] A dash (-) indicates a resistant reaction type.

[4] LP = latenty period, in days
LD = lesion density (number of visible lesions per 10 cm^2 leaf surface)
SLD = sporulating lesion density (number of sporulating lesions per 10 cm^2 leaf surface)
RSLE = relative sporulating lesion efficiency (100·SLD/SLD).

In the greenhouse, sporulation occurred on 8 genotypes, the other two showed only resistant type lesions. It is not expected that the rootstock has influenced the reaction of the grafts in the greenhouse test, because the resistance of the rootstocks has not passed to grafts from susceptible Icatu genotypes.

Significant variation between genotypes was observed for all components of resistance observed in the field and greenhouse test (Table 1). For the analysis, each leaf has been taken as a replicate. The coefficient of variation (C.V.) was low for latency period (LP) in the field (8%) and greenhouse (11%), but high for lesion density (LD) and sporulating lesion density (SLD) (93 and 90%, respectively). The C.V. for the relative sporulating lesion efficiency (RSLE) was about 100% for intermediate RSLE values (between 20 and 80), and lower for RSLE values near to 0 or 100. A few genotypes with intermediate resistance showed significant differences in LD, SLD and RSLE between grafts and also between branches, within grafts. However, in general the variance due to leaves within branches was as great as the variance due to branches and grafts.

Table 2 shows the relationship between resistance parameters for 18 Icatu genotypes which had at least some disease in the field.

Table 2. Coefficients of correlation between resistance parameters observed in the field and greenhouse of 18 Icatu genotypes with incomplete resistance to *H. vastatrix* race II

Parameter	Field observations[1]		Components of resistance[1]			
	Disease score[2]	Necrotic lesions (%)	Field LP	Greenhouse LP	Greenhouse LD	SLD
Field:						
Necrotic lesions (%)	-.75*[3]					
LP	-.71*	.75*				
Greenhouse:						
LP	-.80*	.55*	.65*			
LD	.39	-.33	-.23	-.53*		
SLD	.61*	-.46	-.39	-.65*	.93*	
RSLE	.94*	-.71*	-.76*	-.91*	.48*	.64*

[1] For explanation of abbreviations see Table 1

[2] Average score for 1975 to 1977

[3] An asterisk (*) indicates significance at $P \leq 0.05$

For the correlations with disease score in the field genotype H 3851-2-513 was excluded because of its interaction with the rust isolate from Icatu (Is. ICT, Table 1). The highest coefficient of correlation was observed for RSLE (0.94), followed by LP (0.80) and SLD (0.61). The correlation between disease score in the field and LD was not significant. RSLE was correlated with the % necrotic lesions in the field, which can be expected since both are indicators for reaction type. Furthermore, RSLE was significantly correlated with LP and SLD, but not with LD. It is concluded that the variation for incomplete resistance of the Icatu genotypes is basically related to the variation in heterogeneity of the reaction type.

Effect of leaf age and light intensity. Table 3 shows the results of a laboratory test in which leaf disks were taken from 'young' and 'old' leaves growing in the shade or exposed to sunlight. Resistance was highest for young leaves growing in the shade, and lowest for old leaves exposed to sunlight. For the analysis of variance SLD was transformed into arcsin$\sqrt{\text{SLD}}$. Significant effects were detected of genotypes, leaf age (leaf position) and light intensity (shaded or sun exposed leaves). The effects of interaction between genotypes and leaf age and between genotypes and light intensity were also significant, although the same tendency was present for all geno-

Table 3. Sporulating lesion density (SLD = % of leaf disks with sporulation) and *reaction type* (RT, 0 to 9 scale) for leaves from different positions on the branch and different light conditions in the field of 3 Icatu genotypes inoculated with H. vastatrix race II in the laboratory.

		Leaf position[1]			
	Parameter	1,2		4,5	
Genotype	of resistance	Shade	Sun	Shade	Sun
H 3851-4-41	SLD	1^{a}[2]	8^{a}	30^{b}	43^{c}
	RT	4.5	4.0	5.5	6.5
H 3851-2-689	SLD	18^{a}	39^{b}	17^{a}	67^{c}
	RT	4.0	5.0	4.5	6.5
H 4782-13-152	SLD	49^{a}	68^{b}	67^{b}	71^{b}
	RT	5.5	7.0	6.5	7.5
Mean	SLD	23^{a}	38^{b}	38^{b}	60^{c}
	RT	4.7	5.3	5.5	6.8

[1] Leaf positions 1 and 2 indicate the youngest and second fullgrown leaf on the branch. Leaves of positions 4 and 5 were old leaves, grown in the foregoing growing season.

[2] Different letters indicate differences for SLD between treatments within rows according to the LSD 0.01 value.

types. Differences in resistance were also expressed by differences in reaction type which was heterogeneous for most combinations.

Specificity of resistance. A first indication of physiologic specialization of *H. vastatrix* in the Icatu population was obtained in the greenhouse test, carried out in January/February 1977 (Table 1). The rust isolate from Icatu field plants (Is. ICT) was slightly more pathogenic than race II on four genotypes. However, attempts to obtain a new race from Is. ICT failed. Reinoculation of the 4 genotypes with spores of Is. ICT taken from well sporulating lesions was done in the winter of 1977. Almost only resistant-type lesions were obtained, whereas on 'Mundo Novo' 100% sporulating lesions was observed. This indicates that the resistance of the Icatu genotypes had changed between the two tests. Also in other tests, Icatu genotypes have shown a more resistant reaction in the winter than in the summer.

Between 1977 and 1980 about 3% formerly resistant Icatu genotypes became diseased in the field. In 1979 a new race was isolated from H 3851-4-40, a genotype with complete resistance to race II of *H. vastatrix*. Table 4 shows the average reaction type of 6 Icatu genotypes inoculated twice in the field and once in the laboratory with race II and Is. 2. The 6 genotypes varied in resistance to race II, from moderately susceptible to highly resistant, but all showed increased susceptibility to Is. 2. Therefore, intermediate resistance in Icatu can be race-specific. Variation in reaction types was observed between tests. The reaction to Is. 2 of the more resistant genotypes (H 3851-4-40 and H 3851-4-41) varied from moderately resistant to susceptible, indicating that additional resistance factors may be present. 'Mundo Novo' has some resistance to Is. 2 (Table 4). This has been confirmed in the greenhouse, where multiplication of Is.2 on 'Mundo Novo' is difficult in the winter months. Is. 2 has been sent to the Coffee Rusts Research Center in Oeiras, Portugal (C.I.F.C.) for further identification.

Table 4. Disease score in the field (1 to 5 scale) and average reaction type (0 to 9 scale) and range for reaction type of 6 Icatu genotypes and of 'Mundo Novo' inoculated twice in the field and once in the laboratory with H. vastatrix, race II and isolate 2 (Is. 2., obtained from H 3851-4-40).

Genotype	Disease score		Reaction type			
			Average		Range	
	1975	1980	Race II	Is. 2	Race II	Is. 2.
Icatu:						
H 3851-2-689	3	5	7.3	9.0	7.0-7.5	9.0
H 3851-2-513	4	5	7.0	7.7	7.0	7.0-9.0
H 4782-10-203	2	5	5.5	7.7	5.0-6.0	7.0-8.0
H 3849-7-104	2	5	3.5	8.0	2.0-5.0	7.5-8.5
H 3851-4-41	2	4	3.0	7.0	2.0-4.0	5.0-8.0
H 3851-4-40	1	4	2.5	6.5	1.0-3.0	5.5-8.0
'Mundo Novo'	5	5	9.0	7.7	9.0	6.5-9.0

Segregation for resistance in progenies from resistant genotypes. One-year-old seedlings, obtained by self-fertilization, from resistant and moderately resistant genotypes were tested in the greenhouse. All leaves of each seedling were inoculated with race II of *H. vastatrix*. Segregation for resistance occurred in the progenies of 7 genotypes (Table 5). Within each reaction type (R, MR, MS and S) a variation in the expression of resistance existed. The variation among plants of the same progeny was rather quantitative than qualitative. A few resistant-type seedlings (R) showed no disease symptoms. Others varied in reaction from tiny flecks to large chlorotic areas, which often occurred together. Seedlings with intermediate reaction types (MR, MS) showed, in general, a heterogeneous reaction with resistant and susceptible-type lesions occurring on the same leaf or on the same seedling. Several seedlings showed a resistant reaction on young leaves but were susceptible on old leaves, confirming the results of Table 3. Genetic analysis of the segregation ratio is difficult because of the quantitative variation in reaction type observed and because meiotic irregularities may occur in some Icatu progenies (Mônaco et al. 1974). However, polygenic inheritance seems unlikely, because the distribution of the seedlings over the resistance classes was generally not normal. It seems more plausible that mono- or oligogenic resistance is involved, which may be affected by gene dose, genetic background, and/or environment.

Table 5. Percentage of leaves with sporulating lesions (% LSL) and reaction type (RT) in the field of 9 Icatu genotypes and RT of seedlings from these genotypes, obtained by self pollination, tested with H. vastatrix race II in the greenhouse.

Genotype	Field[1]		Number of seedlings RT[2]			
	% LSL	RT[2]	R	MR	MS	S
H 4782-7-549	0	R	25	7	3	5
H 4782-7-918	1	MR	22	1		
H 4782-13-206	2	MR	7	4	6	3
H 3851-2-363	3	MR	32	2	7	2
H 4782-7-724	3	MR	3	10	4	6
H 3849-14-3-22	5	MR	35	3	4	
H 4782-7-896	6	MR	14	2	4	4
H 3849-7-104	8	MR	5	6	4	4
Control:						
H 4782-7-27	69	S			2	19

[1] Observations made in May 1977.

[2] 'R' indicates presence of non-sporulating lesions, 'MR' a heterogeneous reaction type with a low % of sporulating lesions and a low sporulation intensity, 'MS' a heterogeneous reaction type with a high % of sporulating lesions and intermediate sporulation intensity, 'S' a susceptible reaction type with sporulating lesions only.

Quantitative variation, similar to what was observed in the Icatu progenies, is found in F_2 populations segregating for the resistance gene S_H4. Depending on environment and leaf age this gene shows partial or complete dominance (Eskes, 1982[b], Eskes and Toma-Braghini, 1983). In Icatu major genes with similar expression may be present. For instance the segregation in the progenies from H 4782-13-206, H 4782-7-724, and H 3849-7-104 suggests the presence of a major gene which, in heterozygous condition, gives an MR of MS reaction type. The resistance of H 3847-7-104 to race II is race-specific (Table 4), which is also an indication that a major gene is involved.

DISCUSSION

Incomplete resistance in Icatu is mainly characterized by the degree of heterogeneity of the reaction type (Table 2). Heterogeneous reaction types have been related to race-specific major genes (Parlevliet, 1979). The expression of such genes can be affected f.i. by temperature (Vanderplank, 1978), light intensity (Stubbs, 1967) and/or development stage of the plant (Milus and Line, 1980). Equally, the heterogeneous reaction type of coffee leaf rust in Icatu might be related to race-specific genes which have a labile expression.

Marques and Bettencourt (1979) reported inconsistent results between inoculation tests in Icatu, which makes classification of Icatu genotypes in resistance groups difficult. Such inconsistency is partly explainable by the present results, which indicate that resistance in Icatu can be affected by light intensity and leaf age (Table 3) or by season. To obtain consistent results in resistance tests with Icatu it will be necessary to confine the test conditions as much as possible.

Most studies on incomplete resistance to plant pathogens report only on resistance components, without observing reaction type. Milus and Line (1980) showed that components of slow leaf rusting resistance in wheat were correlated to reaction type. Similar results were observed in Icatu (Table 2). This suggests that there may be no basic difference in the mechanisms of resistance expressed by resistance components or by reaction type. The relationship between resistance components and reaction type has been discussed in more detail by Eskes (1981).

The occurrence of race-specific resistance at varying levels (Table 4) indicates that selection for incomplete resistance in Icatu may not lead to durable resistance. It is possible that durable, polygenically inherited, incomplete resistance is present in Icatu. However, it will be extremely difficult to separate such resistance from monogenic incomplete resistance, unless accurate inheritance studies are made. Similar difficulties are apparently faced with breeding for incomplete resistance to certain cereal rusts (Parlevliet, 1978 and 1979). Alternative breeding strategies for durable resistance to coffee leaf rust in Icatu, based on accumulation of several resistance genes in one genotype or on combination of certain resistance factors from Icatu with others from Catimor, are discussed by Eskes (1983^a).

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GENERAL DISCUSSION

COFFEE RESEARCH

The scales proposed for assessing the disease level of coffee leaf rust (Chapter 1) may be a help to coffee breeders and epidemiologists. The 0 to 9 scale for reaction type is considered to be important because it includes heterogeneous reaction types which are common with coffee leaf rust.

When screening for incomplete resistance to coffee leaf rust it is recommended to measure both the rust incidence and the reaction type. Assessment of resistance components, such as latency period and sporulation intensity, is only recommended if very detailed observations need to be made because, in general, RT is correlated to latency period and sporulation intensity.

The predisposing effect of high light intensity on resistance to coffee leaf rust (Chapter 3) indicates that screening for resistance should preferably be done under low light intensity. Part of the variation for incomplete resistance may not come to expression under high light intensities.

The effect of leaf age (Chapter 4) indicates that leaves of different age need to be inoculated for accurate assessment of the level of resistance. The screening method applied at the Coffee Rusts Research Center (C.I.F.C.) in Oeiras, Portugal, uses young tender leaves only. The present results indicate that the C.I.F.C. method may under- or over-estimate the real level of resistance of certain coffee genotypes.

The laboratory inoculation method proved to be a versatile tool in assessing resistance to coffee leaf rust (Chapter 2). It can be recommended e.g. for race identification, for resistance screening of field plants and nursery populations, for studies on quantitative host-pathogen interactions, and for studies on the effect of environment on resistance. An advantage of the method is that the residual error in experiments can be reduced to a minimum by distributing disks from the same leaf over different treatments. Other advantages are that the method is cheap, efficient in time and space, and that all necessary controls can be easily incorporated into one experiment.

Till 1975, most information on resistance to coffee leaf rust was related to major genes for resistance present in coffee differentials emphasizing the qualitative relationship between host and pathogen. The present research gives information on the quantitative relationship between coffee and *H. vastatrix*. It is an interesting finding that incomplete resistance to *H. vastatrix*, at various levels, can be race-specific. This suggests that selection for incomplete resistance to coffee leaf rust may not necessarily lead to durable resistance. A better indication of the durability of resistance may be obtained by inheritance studies. Selection should be for incomplete resistance which inherits polygenically (Parlevliet, 1978^b, Parlevliet and Zadoks, 1977).

Some genotypes of *C. arabica*, Icatu, and 'Kouillou' were more susceptible to coffee leaf rust than the Brazilian cultivars Mundo Novo and Catuai. The low level of resistance of 'Mundo Novo' and 'Catuai' is likely to be durable resistance. It is expressed by a lower lesion density and by a relatively short leaf retention period.

Till 1981, eleven qualitatively distinct rust races were detected in the State of São Paulo (Chapter 5). Most of these races were found in the coffee breeding plots at Campinas. This shows that new races may appear even when the corresponding resistance factor is present in only a few coffee trees. Monitoring of race formation in breeding plots is of importance for the early detection of these races and should therefore receive full attention.

Some of the new races neutralized the resistance of high yielding breeding lines. The residual resistance of these lines varied. In several cases the residual resistance of these lines was considerably lower than that of 'Mundo Novo' or 'Catuai'. This shows that the 'Vertifolia' effect (Vanderplank, 1968) may occur with breeding for resistance to coffee leaf rust and emphasizes the need for durable resistance.

Different breeding strategies to acquire durable resistance are discussed in Chapter 5. The use of single genes in cultivars cannot be recommended. The use of multilines may favour, as observed in the breeding plots, the formation of complex races. The accumulation of several new resistance genes in one cultivar could be valuable, but is difficult to realize with the available breeding techniques. The use of new technology for rapid vegetative multiplication may in future help to produce hybrid cultivars carrying several major genes for resistance.

The present results offer a first evaluation of incomplete resistance for obtaining durable resistance to coffee leaf rust. Some promising selections were made of genotypes which seem to possess polygenic incomplete resistance. A breeding programme based on crosses between 'Kouillou' genotypes and *C. arabica* cv. Catuai was started. It is realized, however, that selection for polygenic resistance is not so easy. Progress may be hampered by the following observations:

a) incomplete resistance can be race-specific (Chapters 5 and 8),

b) results between resistance tests are sometimes inconsistent (Chapter 6), and

c) disease level in the field is positively correlated with the yielding capacity (Chapter 6).

Indications were obtained that still another approach for durable resistance could be worthwhile. Two new races of coffee leaf rust showed increased virulence to some coffee genotypes and decreased virulence to others (Chapter 5). This finding may point to a difficulty for *H. vastatrix* to combine certain virulence factors. Therefore, it is recommended to combine the resistance genes of the corresponding coffee genotypes into one cultivar. Selection for this combined resistance may be relatively easy because probably major genes are involved.

RELATIONSHIP TO RESISTANCE CONCEPTS

The present results show parallels with results reported from other pathosystems. They can be explained with current resistance theories, as discussed below.

The 'gene-for-gene' concept. According to the gene-for-gene concept there is for each resistance gene in the host a corresponding gene for virulence in the pathogen. Generally, the resistance (R) and the avirulence (A) alleles are dominant. Interactions between host and pathogen are supposedly based on recognition between the dominant R and A alleles, resulting in incompatibility. Non-recognized alleles are expected to behave as though they were a single recessive gene (Ellingboe, 1979). Following these postulates, two levels of compatibility can be expected for each corresponding R/A gene combination.

In the past, these postulates have created too much the impression that resistance governed by the gene-for-gene relationship is exclusively a qualitative trait. This generalization appears to be based on the fact that R-genes with a great effect on resistance were used in the studies underlying the gene-for-gene hypothesis. There is much evidence that quantitative resistance can also be governed by the gene-for-gene relationship. This is supported in part by the research on coffee leaf rust. Some of the evidence is listed below.

1) Resistance and avirulence genes may not be completely dominant. If partial dominance occurs with the R-gene and intermediate inheritance with the A-gene, then up to five levels of compatibility can be expected between a diploid host and a dikaryotic pathogen (Table 1).

Table 1. Levels of compatibility (0 = completely incompatible, 100 = completely compatible) assuming intermediate inheritance for the avirulence gene (A) and partial dominance for the resistance gene (R) in a gene-for-gene relationship. The effects of both virulence and resistance alleles are thought to be additive.

Pathogen	Host genotype		
genotype	RR	Rr	rr
AA	0	20	100
Aa	50	70	100
aa	100	100	100

In coffee, resistance genes were found which showed intermediate inheritance or partial dominance (Chapters 3, 4 and 8). The intermediate virulence found with 3 genotypes of *H. vastatrix* (Chapter 5) is most simply explained by assuming an intermediate phenotype of the heterokaryon carrying one avirulence and one virulence allele. Several other examples in literature indicate incomplete dominance of resistance genes (e.g. Biffen, 1905) and of avirulence genes (e.g. Statler and Jones, 1981).

2) Many major genes for resistance are known which do not completely inhibit the reproduction of the pathogen. Even host differentials, chosen because of their qualitative reactions to different strains of the pathogen, are often not completely resistant to avirulent races (e.g. Roelfs and McVey, 1979). Furthermore, the expression of resistance genes may be influenced by test conditions such as temperature, light intensity and plant age (Hooker, 1967, Lewellen and Sharp, 1968, Stubbs, 1967, Vanderplank, 1978, Zadoks, 1961). In a similar way, part of the race-specific resistance to coffee leaf rust was affected by the test conditions (Chapters 3, 4, 6 and 8).

3) Major genes for resistance may be modified by the genetic background of the host (Parlevliet and Kuiper, 1977, Dyck and Sambarski, 1968, Athwal and Watson, 1954). Various levels of incomplete

resistance in different coffee genotypes were matched by one and the same new race of *H. vastatrix* (Chapter 5), which also suggests an effect of the genetic background on the expression of major genes. Also, virulence genes might be modified by the genetic background of the pathogen. Wide variation in virulence to one major resistance gene has been observed, which was explained by allelism for virulence or by interallelic interactions in the pathogen (Schwartzbach, 1979, Statler and Jones, 1981, Watson and Luig, 1968). With coffee leaf rust wide variation in virulence was observed in relation to the S_H3 gene (Chapter 5).

It seems desirable to complement the common postulates for the gene-for-gene hypothesis (Ellingboe, 1981) with the following postulate which describes quantitative effects of R- and A-genes:

'The expression, i.e. the mode of action, of R- and A-genes may be influenced by gene dose, genetic background, and test conditions (environmental factors, condition of the plant tissue, and the precision with which differences in resistances are measured).'

The 'horizontal resistance' concept. This concept suggests that besides resistance governed by the gene-for-gene relationship (vertical resistance, VR) there is also race-non-specific resistance (horizontal resistance, HR). HR is considered to be long lasting resistance (Vanderplank, 1968). Its expression would generally be quantitative and its inheritance based on minor genes (polygenes). Robinson (1976) has interpreted the possible ecological value of HR and VR in terms of the eso- and exodemic, respectively. His interpretation is based on the supposition that VR-genes give a complete protection to allo-infection. This supposition seems incorrect because VR can be quantitative (see discussion gene-for-gene concept).

The HR concept has stimulated studies, such as the present one, on the quantitative relationship between host and parasite. One of the main criticisms to the HR concept has been the discovery of differential interactions between host and parasites in polygenic systems (e.g. Parlevliet, 1979, Parlevliet and Zadoks, 1977). Robinson (1979) argues that the occurrence of quantitative VR is no proof that there is only one kind of resistance. The main difficulty with the HR concept is that the existence of HR can never be established experimentally. There can be historical evidence on the durability of resistance, but durable resistance need not be race-non-specific and can be based on major genes (Eenink, 1976, Johnson and Taylor, 1976, Parlevliet and Zadoks, 1977). Furthermore, race-non-specific resistance can theoretically be overcome by gene non-specific pathogenicity or 'horizontal pathogenicity'. Therefore, the definition of HR seems inadequate. As already pointed out by Caldwell (1968) there is also no need for the term HR. Hence, the use of this term should be avoided.

The 'durable resistance' concept. Although the word 'concept' has been used in relation to durable resistance (Johnson, 1979), there is in fact no concept but only a definition of the term durable resistance (long lasting resistance) and a recommendation to use proven durable resistance in breeding programmes. Durable resistance should never be used as a term synonymous to HR.

With coffee some historical evidence exists for durable resistance to *H. vastatrix* in *C. arabica* (in Ethiopia) and *C. canephora* (in Indonesia) (Eskes, 1981[a]). It is also likely that the low level of incomplete resistance of 'Mundo Novo', in comparison to more susceptible coffee genotypes (Chapters 6 and 7), is durable resistance. This resistance is expressed by a relatively short leaf retention period (LRP) and low lesion density. Variation in LRP might be largely race-non-specific. Histological evidence has indicated that resistance expressed by a low lesion density is probably based on a mechanism different from hypersensitive cell death (Niks and Kuiper, in press). Such resistance, at low levels in 'Mundo Novo' and at high levels in 'Kouillou' (Chapter 7), may therefore be quite durable.

The 'integrated resistance' concept and 'partial' resistance. Parlevliet and Zadoks (1977) postulate that the gene-for-gene relationship applies also to polygenic systems. In this concept all true resistance genes have matching pathogenicity genes. Hence, race-non-specific true resistance or cultivar-non-specific pathogenicity would not occur. In has been argued, however, that there is evidence for the occurrence of non-specific pathogenicity (Robinson, 1976, Eskes, 1981[b]). This can be concluded for instance from pathogenic variation occurring on hosts which do not possess any resistance genes, as reported by Parlevliet (1976 and 1978[a]) and by Nelson et al. (1970). Facultative parasites especially may vary in level of cultivar-non-specific pathogenicity. The occurrence of cultivar-non-specific pathogenicity is however no proof for the occurrence of race-non-specific resistance.

Polygenically inherited 'partial' resistance is likely to be durable (Parlevliet and Zadoks, 1977, Parlevliet, 1978[b]). Parlevliet defines 'partial' resistance as resistance of a susceptible or a high reaction type which is expressed by resistance components like latency period, lesion density and spore production. With coffee leaf rust, it has been difficult to distinguish between 'partial' resistance and other types of incomplete resistance. Reaction type was affected by the test conditions and by leaf age (Chapters 3, 4, 7 and 8). Generally, reaction type was related to the resistance components. Some 'Kouillou' genotypes, which resistance appeared to be complex, showed high levels of 'partial' resistance in most tests, but in a few tests low or heterogeneous reaction types were observed. This suggests that high levels of polygenic resistance might also be expressed by a low or hetergeneous reaction type.

'Horizontal resistance' based on defeated major genes for resistance. Defeated major genes for resistance may have a 'residual' effect (e.g. Martin and Ellingboe, 1976). According to Nelson (1978), a combination of defeated major genes could be responsible for 'horizontal resistance'. A restriction of this concept appears to be that not all major genes may have residual effects (Nass et al., 1981) and that the degree of the residual effect may depend on the pathogen genotype (e.g. Martin and Ellingboe, 1976). The research with coffee leaf rust indicates the same. No apparent residual effects were observed for the genes S_H1, S_H2 and S_H4. With other resistance genes varying levels of virulence were observed for different matching rust genotypes (Chapter 5) indicating that the level of residual resistance may depend on the rust genotype. Residual resistance might contribute to durable resistance only if the residual effect is based on interallelic interactions for virulence or on a negative effect of virulence genes on the fitness of the pathogen.

Host-pathogen-environment interactions. Complex interactions between host, pathogen and environment have been observed in several pathosystems (e.g. Eskes, 1981^b). Such interactions may help to stabilize pathogen populations (Chandrashekar and Heather, 1981), because pathogen races may have a selective advantage under certain conditions but no or a negative selective advantage under other conditions.

Incomplete resistance is generally more affected by environment or plant factors than complete or major gene resistance. Individual minor genes, contributing to polygenic incomplete resistance, might be affected in a different way by environment or plant factors. This means that not all minor genes may be effective under all conditions.

Then, selection pressure for increased virulence to minor genes would not be constant. This may help to explain durability of polygenic incomplete resistances.

The above considerations may also apply to the coffee/*H. vastatrix* relationship, because incomplete resistance to *H. vastatrix* is considerably affected by the test conditions (Chapters 3 and 4). The effectiveness of resistance to coffee leaf rust varied from leaf to leaf and even within leaves, as indicated by the frequent occurrence of heterogeneous reaction types. Such a variable resistance reaction to coffee leaf rust might compensate for the relative shortage of genetic variability in time of the perennial coffee crop.

Dissociation and association of virulence. Vanderplank (1982) suggested that virulence tends to either dissociate or associate. With other words, virulence to one resistance gene would promote virulence or avirulence to other resistance genes. Stabilizing selection (Vanderplank, 1968), in the context of the new hypothesis, could result from dissociation of virulence factors in the exodemic. The hypothesis is based on observed discrepancies between expected and found frequencies of combinations of virulence factors matching wheat stem rust resistance genes in America and Canada.

One possible argument against Vanderplank's hypothesis is that discrepancies between expected and found virulence frequencies might also be explained by absence of random mating in the pathogen population. Clearly, more research will be needed also with other pathosystems to come to a conclusion about the value of Vanderplank's hypothesis.

A consequence of dissociation of virulence factors would be that the combination of certain major resistance genes can be valuable for obtaining durable resistance. With resistance to coffee leaf rust there is some evidence that such combinations might be found. The strongest evidence comes from the rust race classification work done at the Coffee Rusts Research Center (CIFC) in Portugal (Rodrigues et al., 1975). At CIFC seven rust races of different origins show virulence to the *C. congensis* 263/1 differential. All these races are only moderately virulent to *C. arabica* differentials and lack virulence to the S_H5 resistance gene, whereas virulence to S_H5 is commonly found in other rust races. These results could be explained by assuming dissociation of virulence in *H. vastatrix*.

The present results also suggest a difficulty for *H. vastatrix* to combine certain virulence factors. Two new rust races showed increased virulence to some genotypes and lowered virulence, or avirulence, to others. Assuming that these races arose by single gene mutations from the wild type race, then these results could also indicate dissociation of certain virulence genes in *H. vastatrix*.

Breeding for durable resistance. The breeding approach advised by the 'horizontal' resistance concept is clearly more complicated than initially suggested. Firstly it may be questioned whether race-non-specific resistance exists at all. Secondly, there is no way to discern such resistance in breeding programmes. The best suggestion would be to select for polygenic resistance (Parlevliet, 1978[b]). The strategy based on selection in populations derived from crosses between susceptible parents (Robinson, 1976) seems suitable for the accumulation of minor genes. Among others, Krupinsky and Sharp (1979) observed transgression towards high resistance in progenies from crosses between susceptible parents. One would expect such resistance to be durable if additive minor genes are involved. However, high levels of 'transgressive' resistance could also be based on minor genes enhancing the expression of race-specific major genes. Research which distinguishes between both possibilities seems desirable.

Parlevliet (1978[b]) suggests that polygenic resistance against some cereal rusts could be achieved by selecting for incomplete resistance of a high, susceptible reaction type ('partial' resistance). With leaf rust of barley this strategy appears to be relatively easy, but with other pathosystems the situation may be more complicated (Parlevliet, 1979). There appears to be a difficulty in clearly separating 'partial' resistance from other types of incomplete resistance (Eskes, 1981[b]).

Breeding based on resistance that has proved to be long lasting (Johnson, 1979) may also have practical difficulties. Durable resistance can be based on an effective combination of resistance genes which by themselves do not provide durable resistance. Such combinations may be lost in the breeding process. Based on the assumption that dissociation of virulence may occur in the pathogen, Vanderplank (1982) suggested that a search should be initiated for effective combinations of resistance genes. As for resistance to coffee leaf rust, it could be that there are such combinations (Chapter 5).

A final conclusion is that in breeding for durable resistance the probabilities of success of individual strategies should be weighted against the need for obtaining such resistance. Then, for different pathosystems different approaches will be recommended. Durable resistance to *Hemileia vastatrix* is of such importance for the coffee crop that the breeding approaches initiated as a result of the present research deserve continuation, even though success cannot be fully warrented.

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SAMENVATTING

Onderzoek naar incomplete resistentie tegen veroorzakers van planteziekten kan waardevol zijn voor het verkrijgen van duurzame resistentie. Bovendien levert het een bijdrage tot een beter begrip van waard-pathogeen relaties, omdat de kennis hieromtrent veelal beperkt is tot resistentie veroorzaakt door 'major' genen. Incomplete resistentie tegen koffieroest (*Hemileia vastatrix* Berk. et Br.) is bestudeerd bij drie koffiepopulaties: *Coffea arabica* L., *C. canephora* Pierre cv. Kouillou en de populatie 'Icatu'. Icatu stamt af van een kruising tussen *C. canephora* en *C. arabica*.

Methoden zijn ontwikkeld om het niveau van resistentie tegen koffieroest te bepalen (Hoofdstuk 1). Voor het bepalen van de ziektegraad in het veld, in de kas en in het laboratorium werden 4 geïllustreerde schalen ontworpen, die van 0 tot 9 lopen. Tevens wordt het gebruik van een 0 tot 9 schaal voorgesteld voor het bepalen van het reactietype van koffieroest. Deze schaal bevat een brede reeks van heterogene reactietypen die vaak voorkomen, vooral in koffiepopulaties met resistentiegenen van *C. canephora*. De doelmatigheid van deze schalen wordt aangetoond.

Een laboratoriumtoets, waarbij gebruik gemaakt wordt van bladschijven met een diameter van 1,8 cm, bleek geschikt te zijn voor het bepalen van incomplete en complete resistentie (Hoofdstuk 2). Bij 20 genotypen van 'Kouillou', met een variërend niveau van incomplete resistentie, werd de laboratoriumtoets in viervoud toegepast. Met de resistentiecomponenten gemeten in deze toetsen bleek 79% van de waargenomen variantie voor veldaantasting van de genotypen te verklaren te zijn. Onderzoeksresultaten worden gegeven die geleid hebben tot de standaardisatie van de laboratoriumtoets.

Lichtintensiteit (LI) bleek de resistentie tegen koffieroest te beïnvloeden (Hoofdstuk 3). Over het algemeen waren bladeren die onder een lage LI groeien resistenter dan bladeren die onder een hoge LI groeien. Een hoge LI vóór inoculatie verhoogde de aantastingsgraad, terwijl een hoge LI ná inoculatie de aantastingsgraad verlaagde. Verschillen in resistentie als gevolg van de verschillende LI-behandelingen uitten zich vooral door verschillen in lesiedichtheid (LD) en reactietype (RT). Het resistentiegen S_H4 was volledig dominant in de kas (lage LI), maar was onvolledig dominant of zelfs bijna recessief in de kwekerij (hoge LI). Er kan niet geconcludeerd worden uit de resultaten dat beschaduwing van koffie-aanplanten automatisch zal leiden tot een verlaging van de ziektegraad in het veld. Of dit al dan niet gebeurt zal onder meer afhangen van de mate van beschaduwing en van het koffiegenotype.

In Hoofdstuk 4 wordt het effect van de bladleeftijd op resistentie beschreven. Dit effect varieerde per genotype. Bij de vatbare *C. arabica* rassen was er slechts een gering effect. Fysio-specifieke incomplete resistentie in *C. arabica* en in de Icatu populatie uitte zich voornamelijk in het jongere blad; oudere bladeren waren vatbaarder. Deze resistentieverschillen waren gerelateerd aan verschillen in RT. In het ras Kouillou werden drie fasen van bladleeftijd t.o.v. vatbaarheid waargenomen: volwassen bladeren waren resistenter dan jonge of oude bladeren. De resistentie van het volwassen blad uitte zich voornamelijk door een lagere LD, maar ook enigszins door een lager RT.

In Hoofdstuk 5 wordt de detectie en identificatie van 7 roestfysio's beschreven die gevonden zijn in de veredelingsproefvelden in Campinas. Er werden kwalitatieve en kwantitatieve verschillen in pathogeniteit waargenomen. Vier fysio's doorbreken combinaties van bekende resistentiegenen in *C. arabica*. Drie fysio's doorbreken de resistentie van nog onbekende genen uit *C. canephora*. Tevens werden 3 roestgenotypen gevonden met intermediaire virulentie. De mogelijke implicaties van de resultaten voor de veredeling op roestresistentie worden besproken.

In de Hoofdstukken 6, 7 en 8 wordt de incomplete resistentie tegen koffieroest van, respectievelijk *C. arabica*, *C. canephora* cv. Kouillou en Icatu gekarakteriseerd.

In *C. arabica* bleek genetische variatie aanwezig te zijn voor de periode van bladretentie nadat de sporulatie begonnen is (LRP). LRP bepaalt de duur van sporulatie. Lange LRP waaarden waren gecorreleerd met een hoge ziektegraad in het veld. Ook was er significante variatie aanwezig in *C. arabica* voor de latentieperiode (LP) en LD, maar de resultaten voor deze resistentie componenten waren enigszins inconsistent, vooral voor LD. De correlatiecoëfficiënten tussen LP of LD en de ziektegraad in het veld waren laag en over het algemeen niet significant. Een hoge produktiecapaciteit bleek positief gecorreleerd met een hoge ziektegraad in het veld. Dit werd waargenomen bij lijnen van *C. arabica* rassen en bij introducties uit Ethiopië. De conclusie is dat selectie voor verhoogde incomplete resistentie in *C. arabica* niet erg efficiënt zal zijn.

Er werd een grote variatie voor incomplete resistentie waargenomen tussen genotypen van 'Kouillou'. Een lage ziektegraad in het veld bleek gerelateerd te zijn aan een laag, of een heterogeen RT en/of aan een lage LD. Complete en incomplete fysio-specifieke resistenties werden aangetroffen bij sommige genotypen. De vererving van de resistentie van sommige andere genotypen met een lage ziektegraad in het veld bleek complex. Deze genotypen vertoonden geen interactie met de roestfysio's die geïsoleerd werden van 'Kouillou'. Hun resistentie uitte zich door een lage LD en een relatief hoog RT. Daarom hebben deze genotypen mogelijk waarde bij het verkrijgen van duurzame resistentie tegen koffieroest. Een veredelingsprogramma gebaseerd op kruisingen van deze genotypen met *C. arabica* werd gestart.

De incomplete resistentie in Icatu bleek vooral gerelateerd aan RT. Veelal was de resistentiereactie heterogeen. De resistentiecomponenten, waaronder LP, waren gecorreleerd met RT. Incomplete resistentie van verschillend niveau bleek fysio-specifiek. De conclusie is dat selectie voor incomplete resistentie in Icatu wellicht niet zal leiden tot het verkrijgen van duurzame resistentie.

In de algemene discussie, aan het eind van dit proefschrift, wordt het belang van de verkregen resultaten voor het koffieroestonderzoek bediscussieerd en wordt er verband gelegd met algemene resistentietheorieën.

RESUMO

Pesquisas sôbre resistência incompleta contra agentes patogénicos podem ter valor para a obtenção de resistência duradoura. Além disso, estas pesquisas contribuirão para um melhor conhecimento da releção entre patógeno e hospedeiro, o qual, muitas vezes, é restrito aos genes maiores de resistência. Nesta tese, a resistência incompleta contra a ferrugem do cafeeiro (*Hemileia vastatrix* Berk. et Br.) foi estudada em três popolações: *Coffea arabica* L, *C. canephora* Pierre cv. Kouillou e 'Icatu'. Icatu é uma população de café híbrida derivada de um cruzamento entre *C. arabica* e *C. canephora.*

Métodos foram desenvolvidos para determinar o grau de resistência em condições de campo, estufa e laboratório, com us o de escalas de 10 pontos (Capítulo no. 1). Cada valor nestas escalas de 0 a 9 recebeu uma descrição e foi ilustrado com desenhos estilizados, indicando um aumento exponencial da incidência du ferrugem. Descreveu-se também uma escala de 0 a 9 para a determinação do tipo de reação da ferrugem. Esta escala contem um amplo expectro de tipos de reação heterogenea, os quais ocorrem com frequencia em populações com resistência derivada de *C. canephora.* A utilidade prática das escalas de 0 a 9 foi demonstrada.

Desenvolveu-se um método de laboratório para medir resistência em discos de fôlha com diâmetro de 1,8 cm. Este método provou ser utíl na determinação de resistência completa e incompleta (Capítulo no. 2). O método foi aplicado quatro vezes em vinte genótipos de 'Kouillou', os quais tem um nível variável de resistência. O coeficiente de correlação múltipla (R) entre os valores médios de seis componentes de resistência, observados no laboratório e o grau de ataque no campo, foi altamente significativo (R = 0.89). Foram mostrados os resultados das pesquisas que levaram a uniformizar este método.

A intensidade da luz (LI) influencia na resistência contra *H. vastatrix* (Capitulo no. 3). Fôlhas do cafeeiro que crescem com LI baixa são em geral mais resistentes do que fôlhas que crescem com LI alta. LI alta antes da inoculação induziu um aumento no grau de ataque, enquanto LI alta após a inoculação teve um efeito oposto, provavelmente porque com a alta luminosidade o patógeno morre dentro da fôlha. Os diferentes tratamentos de LI induziram variações na densidade das lesões (LD) e no típo de reação (RT). O gene S_H4 comportou-se como um gene dominante em condições de estufa (LI baixa), mas foi quase recessivo em condições de viveiro (LI alta). Os resultados não permitem a conclusão de que o sombreamento irá

diminuir o grau de ataque da ferrugem em plantações suscetíveis. Se isto ocorre dependerá, entre outres fatores, da intensidade da sombra e do genótipo do cafeeiro.

No capítulo no. 4, é descrito o efeito da idade da fôlha sôbre a resistência. Este efeito depende do genótipo do cafeeiro. Com cultivares suscetíveis de *C. arabica* o efeito foi pequeno. A resistência incompleta e específica em *C. arabica* e Icatu salientou-se mais nas fôlhas novas do que nas fôlhas velhas. Houveram diferenças significatívos para RT. No 'Kouillou' distinguiu-se trés fases de suscetibilidade em relação à idade da fôlha. Fôlhas adultas tiveram menos lesões e, as vezes, um RT mais baixo do que fôlhas novas ou velhas.

Entre 1976 e 1981, foram detectadas sete raças novas de *H. vastatrix* na Estação Experimental do Instituto Agronômico em Campinas. Observaram-se diferenças qualitativas e quantitativas em virulência. Quatro raças quebraram resistências complexas no *C. arabica*, constituidas por combinações dos genes S_H1, S_H2 e S_H4. Trés raças quebraram a resistência de genes desconhecidos de *C. canephora*. Ainda foram detectados 3 genótipos de *H. vastatrix* com virulência intermediária. São discutidas as possíveis consequências dos resultados para o melhoramento visando resistência contra a ferrugem.

Nos capítulos 6, 7 e 8, caracterizou-se a resistência incompleta contra a ferrugem encontrada em *C. arabica*, *C. canephora* cv. Kouillou e Icatu, respectivamente. En *C. arabica* detectou-se variação genética para o período de retenção das fôlhas (LRP). LRP determina o período de esporulação. Valores altos de LRP tiveram relação com intensidades altos da ferrugem no campo. Detectou-se também variação significativa para o período de latência (LP) e para LD. Porém, houve certa inconsistência com estes componentes de resistência, especialmente para LD. Os coeficientes de correlação entre LP ou LD e o grau de ataque no campo, foram baixos e em geral não significativos. A produtividade mostrou ser um fator de importância. Obteve-se coeficientes de correlação positivos entre a produtividade e o grau de ataque no campo, nos linhagens de cultivares Brasileiras e nas introduções da Etiópia. Conclui-se que a seleção para resistência incompleta em *C. arabica* não terá uma alta eficiência.

En 'Kouillou' observou-se uma grande variação para resistência incompleta. Genótipos com pouco ataque da ferrugem no campo, mostraram um baixo RT ou RT heterogêneo, ou também uma LD baixa. Alguns genótipos com resistência incompleta mostraram interações diferenciais com duas raças novas de *H. vastatrix*, isolados de 'Kouillou'. A herança da resistência incompleta de alguns outros genótipos parece ser complexa. Estes genótipos não mostraram interação com as raças novas de *H. vastatrix*. Eles tinham em geral uma LD baixa e um RT alto. Por isso estima-se que estes genótipos podem ter valor na obtenção de resistência duradoura contra a ferrugem do cafeeiro. Foi iniciado um programa de melhoramento baseado em cruzamentos entre genótipos de 'Kouillou' e *C. arabica*.

A resistência incompleta em Icatu foi relacionada em grande parte com RT. Em geral o RT era heterogêneo. Os componentes de resistência, como LP, foram correlacionados com RT. Genótipos com níveis variáveis de resistência incompleta mostraram interação com uma raça nova de *H. vastatrix*, isolado de Icatu. Chegou-se a conclusão de que a seleção para resistência incompleta no Icatu, provavelmente não levara a uma resistência duradour.

Na discussão geral da tese, a importância dos resultados para o melhoramento do cafeeiro é avaliada e os resultados são comparados com teórias sôbre resistência.