Neth. J. Pl. Path. 88 (1982) 127-141

The use of leaf disk inoculations in assessing resistance to coffee leaf rust (Hemileia vastatrix)

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Accepted 24 May 1982

Astract

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: Coffea, 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 occasionally been used to studie 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.

Material 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 over 30 years old and the Icatu plants were planted in the field in 1970. Icatu is derived by back-crossing an artificial hybrid between *C. arabica* (4x = 44) and *C. canephora* (2x = 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 fullgrown leaves were obtained from field, nursery or greenhouse plants. The use of old and damaged leaves was 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 then 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 edges of the disks 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. After-

wards, 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).

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 entry 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' 66-13	?	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 iedere waarneming gebaseerd op 15 à 30 geïnoculeerde bladschijven.

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

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.

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

far as qualitative differences in resistance are concerned, observations based on 15 leaf disks will provide a fair estimate.

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 ell., 1976).

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.

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 the genotype × treatment interaction was obtained for PGU and PAF. 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

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.

Table 5. Latency period (LP, in days), number of lesions per disk (NLD), and number of urediospores produced per lesion (SP, $\times 10^3$) 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)	Uredisspore 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, $\times 10^3$) van twee koffiegenotypen geïnoculeerd op twee manieren met twee urediosporedichtheden. Het kiempercentage van de urediosporen was 24. Ieder getal berust op 166 geïnoculeerde bladschijven.

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.

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 experiments 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 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, sporula-

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 h. 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 $LSD_{0.05}$ value.

Treatment			PDS of coffee genotype		Mean
number	collection of leaves (hour)	cutting of disks (hour)	cv. Mundo Novo	cv. Kouillou C70-11	
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 $LSD_{0.05}$ waarde.

tion 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.

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

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.

per plant) and the opposite leaves were inoculated in the greenhouse. The coefficients or 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 C1164-19 × 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 diseage 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 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 correlation between the

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	−0.66	−0.67	−0.64	−0.58	−0.76
	LP	−0.69	−0.72	−0.54	−0.67	−0.77
	NLD	0.48	0.55	0.54	0.71	0.78
	PDL	0.51	0.61	0.57	0.60	0.79
	PDS	0.66	0.70	0.63	0.69	0.82
	PDSDL	0.63	0.55	0.52	0.53	0.61
Multiple R		0.84	0.78	0.76	0.81	0.89
R^2		0.70	0.61	0.58	0.66	0.79

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 mest sporulatie betrokken op PDL (PDSDL = 100 · PDS/PDL). Enkelvoudige r waarden zijn significant bij $P \leq 0.05$ als $r \geq 0.46$.

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	0.95	−0.77	−0.73	−0.92	−0.86
LP		−0.69	−0.63	−0.85	−0.83
NLD			0.95	0.87	0.54
PDL				0.86	0.53
PDS					0.87

Tabel 9. Coëfficiënten van lineaire correlatie tussen de gemiddelden van zes resistantiecomponenten 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.

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.

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 al 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).

Acknowledgements

The research was supported by the Food and Agricultural Organization of the United Nations (FAO), the Instituto Agronômico of Campinas (IAC), SP, Brazil, and the Agricultural University, Wageningen, the Netherlands. The author wishes to thank all staff members and personnel of the Genetics Department of the IAC for the kind cooperation received. Special thanks go to Drs R.A. Robinson, L. Chiarappa and N.A. van der Graaff of FAO and to Dr Alcides Carvalho of the IAC for the stimulus received. The critical reviewing of the text by Professors J.C. Zadoks and J. Sneep is acknowledged. Mrs Masako Toma-Braghini, Mr Jaap Hoogstraten and Mr Andrés Rivera Fernandez are thanked for their participation in the experimental work.

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 resistantie 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, in 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 roestresistantie en bij het onderzoek naar de relatie tussen koffie en *H. vastatrix.*

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