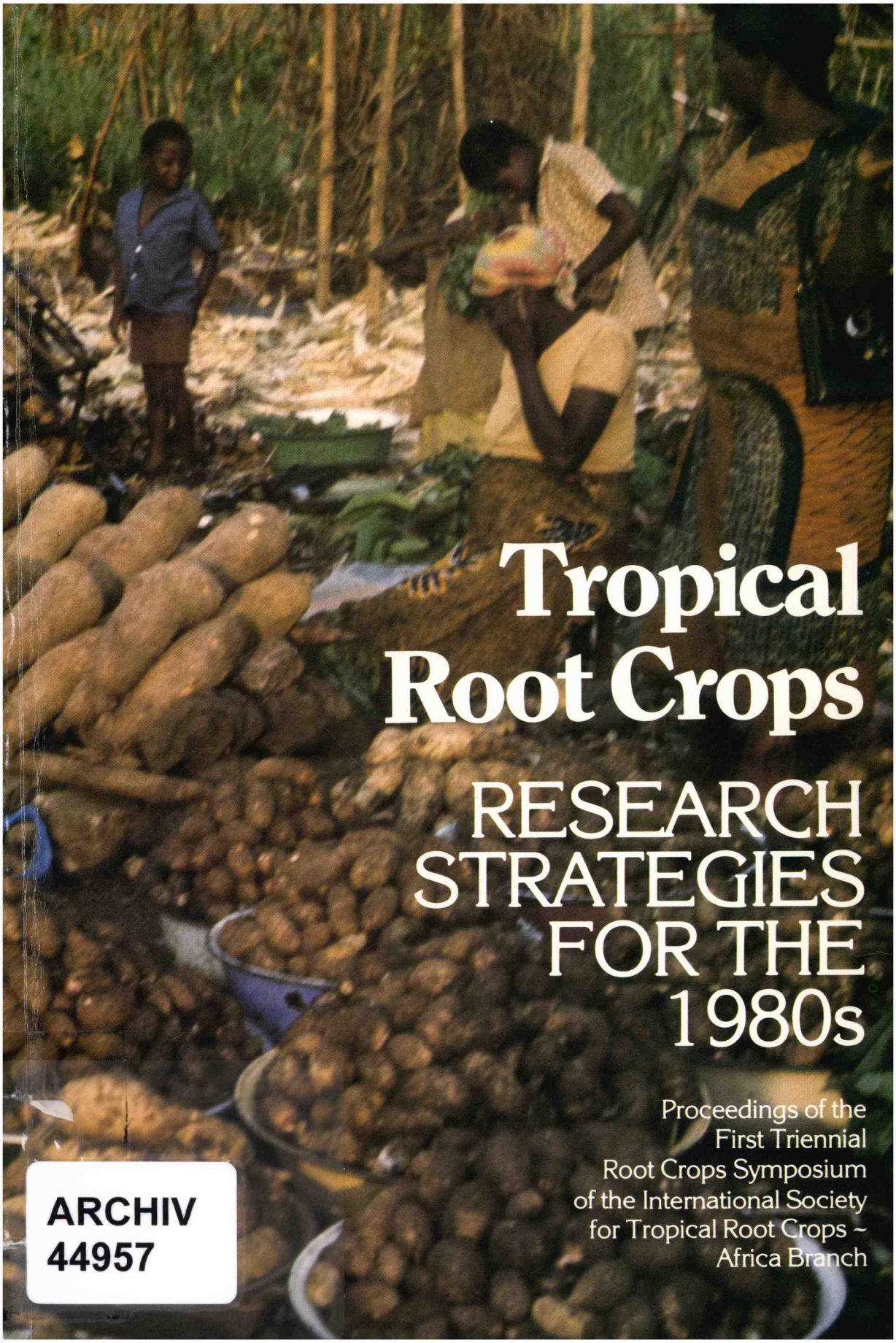

Tropical Root Crops

RESEARCH STRATEGIES FOR THE 1980s

Proceedings of the
First Triennial
Root Crops Symposium
of the International Society
for Tropical Root Crops ~
Africa Branch

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TROPICAL ROOT CROPS: RESEARCH STRATEGIES FOR THE 1980S

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EDITORS: E.R. TERRY, K.A. ODURO, AND F. CAVENESS

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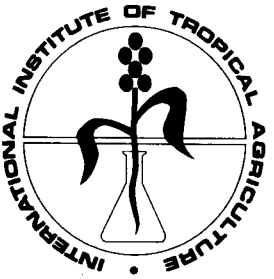

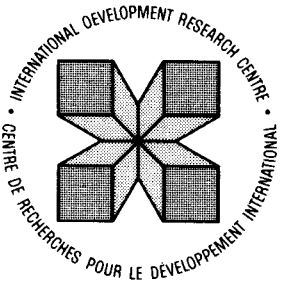

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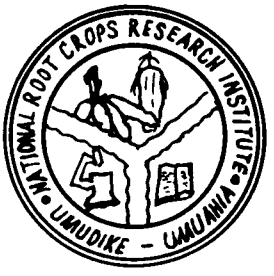

CONTENTS

FIELD SCREENING OF CASSAVA CLONES FOR RESISTANCE TO *CERCOSPORA HENNINGSII*

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Field screening of cassava clones for resistance to *Cercospora henningsii* was conducted by artificial inoculation and natural infection during October–November 1977 and June–July 1978, dry and wet seasons, respectively. During both screening seasons, clones K7709, K7713, K7717, and K7718 showed some resistance, whereas clones Isunikakiyan, TMS 711121, TMS 30395, K7707, and K7711 showed susceptibility. *Cercospora* leaf spot, which generally was more severe during the wet season, developed more reliably following artificial inoculation than from natural infection. Although none of the clones proved to be immune, the differences in responses to *C. henningsii* suggest that opportunities exist for breeding clones with brown leaf spot resistance.

La sélection sur le terrain de clônes de manioc pour la résistance à *Cercospora henningsii* a été effectuée par inoculation artificielle et infestation naturelle en octobre et novembre 1977 et en juin et juillet 1978, soit en saison sèche et humide. Pendant la période d'essais, les clônes K7709, K7713, K7717 et K7718 ont démontré une certaine résistance à l'anthracnose alors que Isunikakiyan, TMS 711121, TMS 30395, K7707 et K7711 faisaient plutôt preuve de tolérance. L'inoculation artificielle a, plus que l'infestation naturelle, favorisé le développement de *Cercospora*, généralement plus grave en saison humide. Bien qu'aucun clône n'ait résisté à l'attaque, les différentes réactions à l'anthracnose font envisager la possibilité de sélectionner des variétés en vue de la résistance à cette maladie.

Several *Cercospora* species have been reported to induce leaf spots on cassava (Ciferri 1933; Arene 1974; Lozano and Booth 1974; Maduewesi 1974; Teri et al. 1977; Kasirivu 1978). The severity and geographical distribution of *C. henningsii*, causing brown leaf spots on cassava, indicate that it is the most important species (Van Overeen 1952; Lozano and Booth 1974; Teri et al. 1977).

Recently, at Centro Internacional de Agricultura Tropical (CIAT) weekly spraying of fungicide to control brown leaf spot and leaf blight on a susceptible variety of cassava, Llanera, increased yields by 14% (Teri et al. 1977). Control measures against brown leaf spot alone resulted in an increase in fresh root yield from susceptible cultivars ranging from 10 to 23% (Teri et al. 1977).

Little attention has been paid to the development of control measures, as the disease has never been reported to be lethal; infected plants continue to yield (Ciferri 1933; Viegas 1941; Müller and Roberts 1951). It seems probable that the conditions of traditional cultivation of cassava in small backyard plots with other crops either have restricted the spread of the disease or have concealed epidemics (Lehman 1972). The disease may become important, however, when cassava is grown in intensive monocultures of one variety (Rorer 1915).

Eradication measures, such as frequent raking and burning of fallen cassava leaves during the dry season, cutting back the plants to 15 cm during the dry season and burning the debris, and rotating cassava with other crops have been suggested by Powell (1968). Several fungicides have shown promise for the control of brown leaf spot (Arene 1974; Lozano and Booth 1974; Teri et al. 1977), but fungicidal control of brown leaf spot, although feasible, is uneconomic except where multiplication of disease-free planting material is involved (Arene 1974).

Although other control measures have been attempted, breeding for resistance has received little attention (Powell 1968; Arene 1974; Lozano and Booth 1974). More research in evaluating resistance and its mechanisms is still required. Ciferri (1933) reported on the assessment of resistance to *C. henningsii* of cassava clones based on lesion numbers and distribution. CIAT (1975) and Teri et al. (1974) used the amount of leaf retention to grade disease severity.

Differences in the reaction of cassava clones to *Cercospora* spp. have been demonstrated (Ciferri 1933; CIAT 1974, 1975, 1976; Maduewesi 1974). Screening at CIAT of cassava germ plasm led to the identification of a number of clones with resistance and tolerance to *C. henningsii* (Teri et al. 1977).

Greenhouse screening has been difficult because fungal sporulation in culture is insufficient for artificial inoculation. Recently, however, CIAT (1975) and Kasirivu (1978) have reported satisfactory sporulation of *C. henningsii* in culture, and their technique should facilitate disease evaluation of artificially inoculated plants.

A vital prerequisite of successful breeding for resistance is a reliable screening technique. Our research was undertaken to determine the usefulness of the assessment scale of disease-severity ranking as well as the effect of environmental conditions on disease development on inoculated cassava clones. We hoped to find out how reliable and comparable were the results of artificial inoculation from year to year and how they compared with natural infection.

MATERIALS AND METHODS

The cassava clones used in the screening experiments included Isunikakiyan, 60444, 58308, 711121, TMS 30017 (TMS = tropical manihot), TMS 30211, TMS 30337, TMS 30395, TMS 30555, TMS 30572, and 17 other clones designated K7701 to K7719 (K77 = Kasirivu 77 standing for the clones screened in 1977 by Kasirivu); they were selected from the cassava seedling nursery at IITA. Only 27 clones were used in the field experiments for efficient experimental management and evaluation of the developed scale.

PURE CULTURE AND CRUDE MACERATE AS INOCULUM

Crude macerate and pure culture were used for inoculation. Naturally infected leaves of cassava were collected from IITA cassava fields, and disease lesions cut out with a pair of scissors. Approximately 500 g of the diseased tissue discs were macerated in a blender at low speed in 500 ml of distilled water for 30 seconds and then filtered through cheesecloth. Also, 10 pure culture plates on bean pod agar (BPA), which had been flooded with pure culture preparations and incubated for 14 days, were blended in 300 ml of distilled water and sieved through cheesecloth.

The inoculum suspensions were adjusted at 50 000 spores/ml, unless otherwise stated, by use of a hemocytometer (Kasirivu 1978). Tween 80 (a sticker) was added at a rate of one drop for every 100 ml to reduce runoff of the inoculum suspension on the treated foliage. The foliage of the plants was sprayed to the runoff point with the inoculum suspension. To assess the differences in crude macerate and pure inoculum, we used 8–10-week-old potted plants of Isunikakiyan cultivar raised in the greenhouse, inoculating them with either crude macerate or pure culture suspension (25 000 spores/ml). Treated plants were maintained in a humidity chambre (90–100% relative humidity) for 48 hours and then transferred to the greenhouse bench. Four plants were used per treatment and replicated three times. The assessment was carried out in terms of incubation period.

EFFECTS OF HUMIDITY

Isunikakiyan, 60444, 58308, and TMS 30211 cassava clones raised in the greenhouse to age 8–10 weeks were inoculated with the crude macerate and given one of four treatments after inoculation:

- Some plants were maintained outside throughout the assessment where the monthly average temperature ranges were 20.4–31.9°C for November–December 1977 and 22.9–31°C for April–May 1978;
- Some plants were maintained on a greenhouse bench throughout the observation period;
- Some plants were transferred to a humidity chambre for 48 hours at 90–100% relative humidity (RH) and then transferred outside where they were maintained throughout the observation period; and
- The remainder were placed in a humidity chambre (90–100% RH) for 48 hours and then transferred to the greenhouse bench for the rest of the observation period.

The experiment was replicated twice. The assessment method was to determine the incubation period of the disease and leaf spot count per plant at 15-day intervals from the 30th to the 60th day after inoculation.

EFFECT OF MOISTURE STRESS

Potted plants of Isunikakiyan cultivar raised in a greenhouse to age 8–10 weeks were inoculated with the crude macerate and subjected to one of four watering regimens:

- Watered daily (control);
- Watered every other day;
- Watered once every 3 days; and
- Watered once every 4 days throughout the observation period.

The treatment results are presented in terms of incubation period of the disease.

HOST VARIETAL RESPONSE

Potted plants of clones Isunikakiyan, 60444, 58308, and TMS 30211 raised in the greenhouse to age 8–10 weeks were artificially inoculated with

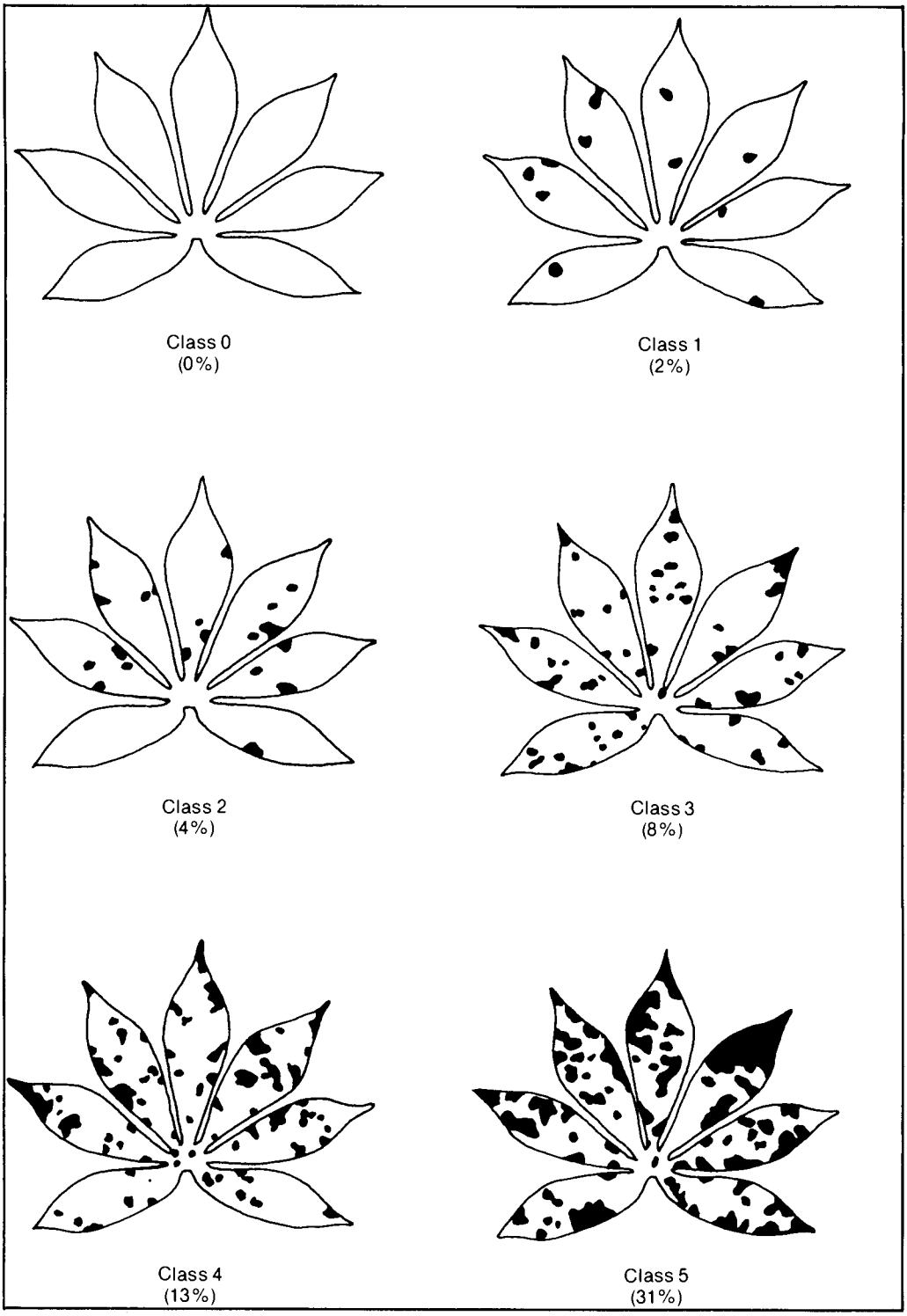

Fig. 1. *A standard scale for brown leaf spot disease rating with percentage of diseased leaf.*

crude macerate and maintained outside throughout the observation period. Plants sprayed with distilled water were used as controls. The observations included leaf spot count per plant and the amount of defoliation at 30, 45, and 60 days after treatment.

Planting, Cultural Practices, and Inoculation

Six stems of each of the 27 clones were planted a metre apart on ridges also a metre apart. There were 27 rows in a block (replicate), and 3 metres between each block. Two replicates were established for each screening experiment. The weeding and irrigation were carried out when necessary. The experiment established during the dry season (February 1978) was sprayed twice with Gammalin 20 at weekly intervals to protect the plants from the variegated grasshopper, *Zonocerus variegatus*.

The foliage of the test plants was inoculated when the plants were 3 months old. Inoculation was done in the evening. Two of the four blocks received the inoculation treatment, and the other two were uninoculated controls.

Standard Disease Assessment Scale

Equal-sized mature leaves representing all possible levels of infection were collected from the field, taken to the laboratory, and categorized into six arbitrary classes of disease severity based on lesion numbers and proportion of leaf areas diseased. The representative leaves of each class were selected and traced on translucent paper for an assessment scale (Fig.1). The leaf area affected by the disease was assessed by square counting. Resistance–susceptibility grading of screened clones was on a scale of 0–5: immune (0), highly resistant (1), resistant (2), moderately susceptible (3), susceptible (4), and highly susceptible (5).

The disease severity of both inoculated and uninoculated treatments was assessed at 30, 45, and 60 days after inoculation (Kasirivu 1977, 1978). A mean reaction score was calculated for each clone. An assessment scale based on percentage of diseased leaf area was used for evaluation by a quantifying scale (Thurston 1971; van der Plank 1976). Personnel rated disease by looking at the lowest quarter of the test plant's foliage and assigning a score according to the most severely diseased leaf of those examined on the inner four plants in each row.

Results

Crude Macerate Versus Pure Culture Inoculum

Crude macerate and pure culture suspensions produced similar disease symptoms on treated plants; the incubation ranged from 20 to 23 days for crude macerate and 20 to 25 days for pure culture. Fungi were isolated from the infected plants of both treatments, and the cultures were identical with those of the original isolates.

Humidity and Disease Development

Although the second aim was to find out the role of humidity in disease establishment and subsequent progress, varietal response to the humidity treatments was observed also. Isunikakiyan, 60444, and TMS 30211 transferred to the greenhouse bench immediately after inoculation showed a delay in symptom appearance and no disease symptoms developed on clone 58308 in either experiment. Inoculated plants maintained under high humidity and then on the greenhouse bench showed a delay in symptom appearance on

Table 1. Clonal response to inoculation with *C. henningsii* followed by different humidity treatments. Data expressed as leaf spots per plant 45 days after inoculation. Incubation period (days) is given in parentheses.

Treatment soon after inoculation	Experiment[a]	Isunikakiyan (1)	Isunikakiyan (2)	60444 (1)	60444 (2)	TMS 30211 (1)	TMS 30211 (2)	58308 (1)	58308 (2)
Maintained outside		45.75 (22)	24.34 (19)	48.25 (21)	31.67 (23)	35.00 (23)	25.50 (19)	3.75 (40)	18.50 (27)
Maintained on greenhouse bench		3.50 (24)	4.00 (32)	— [b]	2.50 (41)	—	0.50 (32)	—	—
Maintained under high humidity; then outside		39.33 (23)	42.75 (21)	34.75 (23)	54.65 (19)	5.50 (26)	65.75 (19)	2.50 (32)	9.75 (29)
Maintained under high humidity; then greenhouse bench		8.25 (24)	53.75 (22)	2.00 (31)	2.50 (31)	1.75 (35)	4.50 (23)	—	1.00 (31)

[a]Experiment (1) November–December 1977; experiment (2) April–May 1978.
[b]A dash indicates no disease symptom development.

Table 2. Moisture stress and brown leaf spot disease establishment on Isunikakiyan cultivar, expressed as incubation period (days).

Watering treatment	Exp. 1	Exp. 2
Once a day	21	29
Every 2 days	26	34
Every 3 days	30	30
Every 4 days	52	35

all clones in both experiments. Lowest disease severity was observed on plants inoculated and maintained on the greenhouse bench. The highest disease level on all clones was observed on plants inoculated and maintained outside in the first experiment (November–December 1977), although in the second experiment (April–May 1978) the clones exhibited different responses (Table 1).

MOISTURE STRESS

The appearance of disease symptoms was delayed as the frequency of watering was reduced. The brown leaf spots were observed first on plants watered everyday and last on plants watered once in 4 days (Table 2).

VARIETAL RESPONSE

The lesion numbers on inoculated plants were highest on Isunikakiyan clone and lowest on 58308 clone, with clones 60444 and TMS 30211 exhibiting intermediate reactions at 30 and 45 days after inoculation. After symptom appearance, disease progress on clones Isunikakiyan, 60444, and TMS 30211 was rapid between the 30th and 45th day after inoculation; the disease level decreased rapidly between the 45th and 60th day to almost the same level as the control treatment. Clone 58308 exhibited the least reaction and its lesions per plant were still increasing at the end of the assessment (Table 3).

The defoliation of both inoculated and uninoculated controls was assessed. The clones of Isunikakiyan, 60444, and TMS 30211 that exhibited a high number of lesions and then a sudden decrease showed 80–97% loss of inoculated leaves. Clone 58308 showed a defoliation of about 59% of the inoculated leaves. Defoliation due to the disease was determined by subtraction of the defoliation on control plants. At 45 days after inoculation, defoliation was highest on clone 60444 and least on clone 58308; the other clones were intermediate; and, at 60 days after treatment, clones 60444 and TMS 30211 showed the highest defoliation, clone 58308 the least, and clone Isunikakiyan remained intermediate (Table 4).

FIELD SCREENING FOR RESISTANCE

A third aim was to identify the resistant and susceptible clones to artificial inoculation and natural infection during both dry and wet seasons. In the 27 clones screened, no immunity was observed; all clones became infected even without inoculation. Inoculated clones during both seasons showed a higher disease level than the uninoculated controls except for clones TMS 30337 and TMS 30017, which had the same disease severity score on both treatments during the dry-season screening, as did clone K7717 during the wet-season screening.

During the dry-season screening (October–November 1977), only four clones (TMS 30211, K7701, K7704, K7709) exhibited high resistance, and only one (K7717) showed high resistance during the wet season (June–July 1978). Nine clones were resistant in the dry season, and four clones were resistant in the wet season, two of which (K7713, K7718) exhibited the same level of resistance in both seasons. Fourteen and 15 clones were rated moderately susceptible during the dry

Table 3. Leaf spot number per plant on inoculated and uninoculated cassava clones.

Clone	Treatment	Days after treatment		
		30	45	60
Isunikakiyan	Inoculated	68.50	221.75	25.25
	Control	6.75	23.00	21.25
TMS 30211	Inoculated	11.00	83.00	24.50
	Control	0.50	0.50	10.25
58308	Inoculated	1.33	26.33	36.00
	Control	1.00	3.00	2.00
60444	Inoculated	27.25	77.25	22.50
	Control	1.75	10.00	17.00

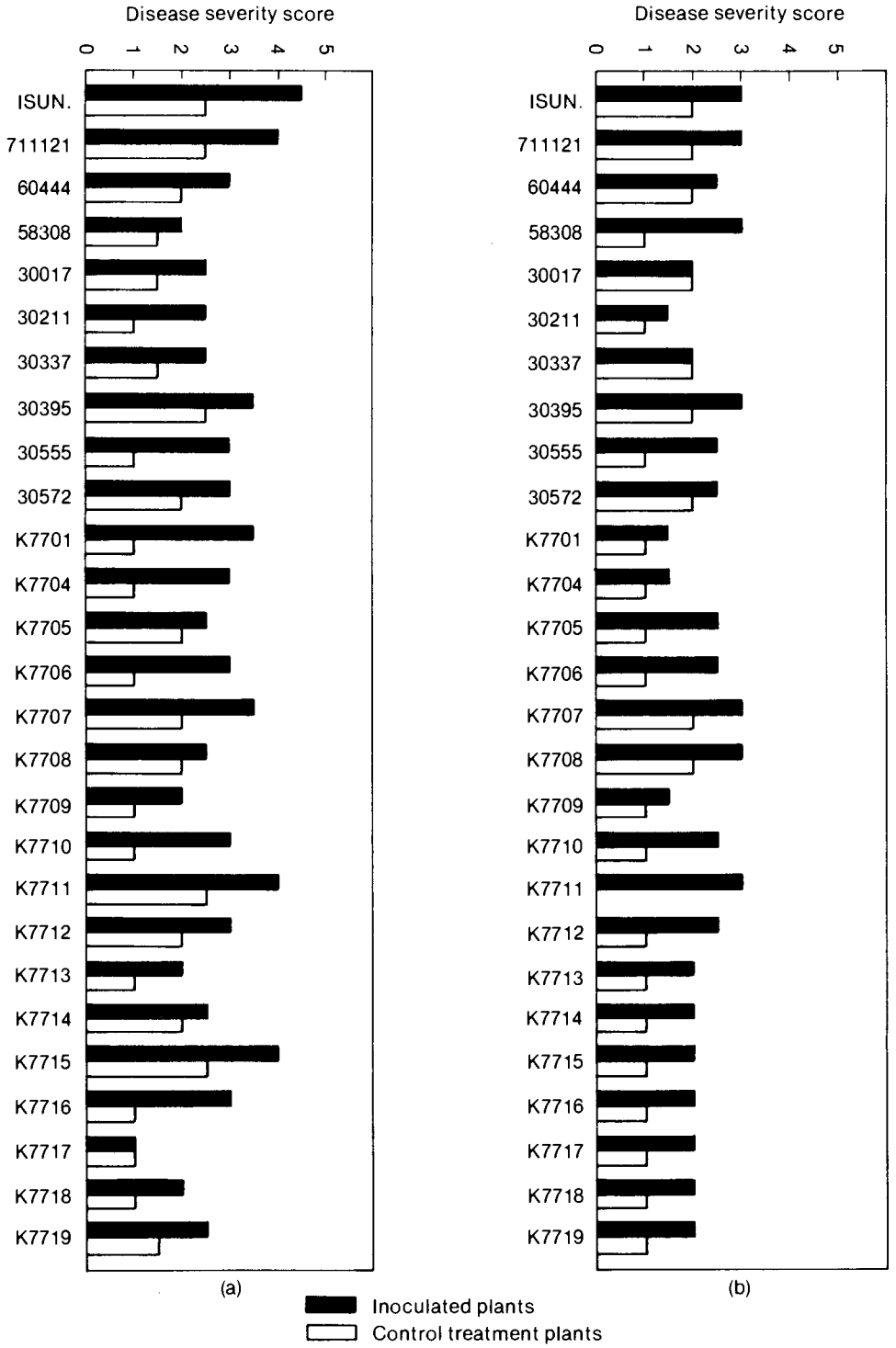

Fig. 2. *Cassava brown leaf spot disease severity score 30 days after treatment on 27 clones during the wet (a) and dry (b) seasons.*

Table 4. Defoliation percentage caused by brown leaf spots on four cassava clones.

Clone	Days after treatment		
	30	45	60
Isunikakiyan	7.50	24.32	35.69
TMS 30211	2.99	26.44	42.12
58308	7.58	13.51	20.83
60444	0.43	35.97	42.12

and wet seasons, respectively, and 8 of these (60444, TMS 30555, TMS 30572, K7705, K7706, K7708, K7710, K7712) exhibited the same disease score in both seasons. In the wet season, clones 711121, TMS 30395, K7701, K7707, K7711, and K7715 were graded susceptible, and Isunikakiyan clone, highly susceptible.

The inoculated treatments showed various clonal responses that followed a pattern similar to that of the control treatments except that the disease severity scores of the former were higher (Fig. 2).

The subsequent disease assessment of inoculated plants at 45 and 60 days after inoculation showed clonal response to the disease severity in the field (Table 5). Clone TMS 30395 maintained a moderately susceptible reaction throughout the assessment in the dry season, and clone TMS 30572 maintained a similar response during the wet season. Clones 58308 and K7718 were resistant, and clone K7717 had a highly resistant reaction during the wet season. Only clone K7708 showed a delayed disease development, its highest score for the wet season being observed 45 days after inoculation. Clonal response was most meaningful at 30 days after inoculation before second-generation lesions appeared on the treatments and before defoliation due to disease set in.

DISCUSSION AND CONCLUSIONS

The inoculation results showed that the disease organisms in pure culture and crude macerate are

Table 5. Brown leaf spot disease progress given as disease assessment score after 30, 45, and 60 days on inoculated cassava clones during the dry (October–November 1977) and wet seasons (June–July 1968).

Clone	Dry season			Wet season		
	30	45	60	30	45	60
Isunikakiyan[ab]	3	2	2	4.5	3	3
711121[a]	3	1.5	1.5	4	3	3
60444	2.5	1.5	1.5	3	2.5	1.5
58308	3	2.5	2.0	2	2	2
TMS 30017	2	1.5	1.5	2.5	2.5	2
TMS 30211	1.5	1.5	1	2.5	2	2
TMS 30337	2	1.5	1	2.5	2.5	2.5
TMS 30395[b]	3	3	3	3.5	2	2
TMS 30555[b]	2.5	2	1.5	3	2.5	1.5
TMS 30572	2.5	2	2	3	3	3
K7701	1.5	1	1	3.5	2.5	2
K7704	1.5	1	1	3	2	1.5
K7705[a]	2.5	1	1	2.5	2	1
K7706	2.5	1.5	1	3	2.5	2
K7707	3	2	2	3.5	2.5	2.5
K7708	3	2.5	1.5	2.5	3	2.5
K7709	1.5	1	1	2	1.5	1
K7710	2.5	1.5	1	3	2	1.5
K7711[ab]	3	1	1	4	3	3
K7712	2.5	1	1	3	2	2
K7713	2	1	1	2	2	1.5
K7714	2	1.5	1.5	2.5	2.5	2
K7715	2	1.5	1	4	3	3
K7716[b]	2	2	1	3	2.5	2
K7717	2	2	1	1	1	1
K7718	2	2	1.5	2	2	2
K7719	2	1	1	2.5	2.5	2

[a]High cassava mosaic disease level during dry season.
[b]Severe premature yellowing of leaves during wet season.

equally virulent on the tested clone, Isunikakiyan, although the spectrum of virulence of the pure culture was not tested.

The progress of infection was highly dependent on the environmental conditions to which the clones were exposed soon after inoculation and partially dependent on the clone. Clonal responses to humidity treatments are reflected in the length of incubation and lesion numbers. Drier environmental conditions in the greenhouse delayed or even circumvented symptom appearance, whereas natural conditions and graded humidity treatments induced the inherent response of the clones to the disease.

The high number of lesions on plants maintained outside indicates that there are many factors other than humidity involved in the process of infection, and these factors may include alternating high and low levels of relative humidity during the night and day, fluctuating temperature, and a combination of high humidity and low temperature at night.

Plants inoculated and maintained outside developed more lesions per plant than did plants maintained under high humidity during November–December 1977. The explanation is that the humidity chambre reached temperatures as high as 38–40°C and reduced the number of successful infections, whereas, outside, the temperature was 20–32°C. The April–May 1978 experiment showed fewer lesions per plant on the plants inoculated and maintained outside than on plants inoculated and maintained under high humidity before being transferred outside. Perhaps the spores on the leaves outside were washed away by the rain.

The moisture stress experiment showed that vigorously growing plants with adequate moisture exhibit a high disease level by both short incubation and great numbers of lesions evenly distributed on the leaves. Inoculated leaves of plants subjected to moisture stress had delayed appearance of symptoms and fewer leaf spots.

The differences in clonal response to artificial inoculation may be attributable to the differences in the rate of infection following inoculation and to differences in resistance of invaded leaf tissues, which affected the rate at which infected leaves were destroyed (Thurston 1971; van der Plank 1963). As the test plants were artificially inoculated, other factors besides amount of inoculum per unit leaf area may have played a part.

Both inoculated and uninoculated controls of clone Isunikakiyan showed a high disease level, whereas both treatments of clone 58308 developed very few spots. Clones 60444 and TMS 30211 showed intermediate reactions. The peak reaction in terms of lesions per plant of clones Isunikakiyan, TMS 30211, and 60444 was observed at 45 days after inoculation, whereas clone 58308 showed a low lesion number and a low, steady increase of lesions per plant up to the end of the observation. Under the same disease pressure, i.e., inoculation, clone Isunikakiyan had about nine times — and clones 60444 and TMS 30211 had about three times — the number of lesions that clone 58308 had on the 45th day. The untreated control plants showed a similar response except for clone TMS 30211, which showed a more resistant reaction at lower disease pressure.

Of the four clones investigated, 60444 and TMS 30211 had the highest defoliation 60 days after inoculation, and clone 58308 had the least; clone Isunikakiyan had intermediate defoliation. When we compared disease severity with the defoliation due to disease, we observed that clones 60444 and TMS 30211 had the highest defoliation, although their disease severity was intermediate; in contrast, Isunikakiyan showed intermediate defoliation and the highest disease level. These findings suggest that clones 60444 and TMS 30211 have a more sensitive reaction to the disease.

In summary, clone Isunikakiyan showed susceptible reactions; clone 58308 resistant reactions; and clones 60444 and TMS 30211 intermediate reactions. The term susceptible denotes the total of qualities that make a plant a fit host for a pathogen, whereas resistant denotes the basic ability of the host to hinder the pathogen (Robinson 1969).

The field screening for resistance of 27 cassava clones was carried out twice during the dry season (October–November 1977) and the wet season (June–July 1978). The clonal response of both inoculated and uninoculated controls during both seasons exhibited the various disease levels, and disease levels were higher during the wet season than during the dry season. The responses agree with what was observed by Viennot Bourgin and Grimaldi (1950): cassava plants subjected to adverse growing conditions are more resistant to *Cercospora* sp. than those under favourable conditions. Whether the results indicate mere resistance or just lower disease scores is not clear. The results may indicate a pathogen–host–environment effect rather than a host–environment effect because humidity, moisture stress, and clonal differences affected disease level on artificially inoculated plants under controlled environmental conditions.

Of field-inoculated plants, 78% of the clones exhibited high disease scores during the wet season when compared with their levels during the dry season; 11% of the clones showed lower disease

scores during the wet season; and another 11% of the clones exhibited equal responses during the two seasons. These same clones when used as inoculated controls had different levels of disease: 33% showed higher disease levels during the wet season, 7% lower disease levels during the same season, and 60% an equal response during both seasons. Such clonal interaction with season and inoculum potential in these experiments confirms what Teri et al. (1977) pointed out that generally artificial infections are more reliable from year to year than natural infections and, therefore, more comparable.

The clonal responses of inoculated plants showed more or less a normal distribution during the wet season, whereas during the dry season the response was skewed from middle severity toward resistant. On the uninoculated controls, clonal response was skewed toward the resistant side of the assessment scale in both seasons with the exception of a few clones that exhibited moderate susceptibility during the wet season. Effective screening requires artificial inoculation during the wet season when the weather favours vigorous plant growth and is conducive to disease development. This approach would give reliable and more comparable results from year to year.

The differences in clonal responses to *C. henningsii* have been demonstrated in the experiments carried out, and these observations are similar to those of Arene (1974), CIAT (1974, 1975, 1976, 1977), and Maduewesi (1974). One may assume that there exists resistance at all possible levels in the cassava clones tested but no immunity. However, more greenhouse and field screening for resistance is needed to confirm the findings and the accuracy of the disease assessment scale.