Emphasis this year was on a study of disease presence, changes in severity and performance of cassava clones in each of five different environments. The identification of wide-type resistance to existing biotic problems as well as its durability in these ecosystems is being investigated. The relationship between plant reaction to cassava bacterial blight (CBB) in the greenhouse and field, and its stability through several continuous cycles was investigated. Field studies were begun to examine differences in the severity of CBB, superelongation and anthracnose in monoclonal and multiclonal plots at high and low inoculum levels. The sexual stage of the superelongation causal agent was found and its implication on pathogen-host interactions is being investigated.

Diplodia root-stem rot was identified at CIAT-Palmira. This disease, CBB, the frog skin and other viral diseases constitute the most threatening diseases of cassava in the production and distribution of vegetative planting material. Etiological studies were undertaken on the characterization of the frog skin disease causal agent.

Cassava Bacterial Blight

Screening for durable resistance. Plant reactions to cassava bacterial blight (CBB) infection under controlled conditions (by the clip inoculation technique, CIAT Ann. Rept. 1975) and in the first cycle of field testing were very similar. The pathogen was found invading the stem 5 cm above ground in cassava genotypes rated susceptible, intermediate-resistant and resistant, but the rate of pathogen recovery from susceptible genotypes was greater than from both the latter ones. Although bacterial invasion throughout the vascular system was positively correlated with the susceptible types as evaluated by external symptoms ($r = 0.914$ for greenhouse reaction and 0.927 for field reaction, both significant at the 0.1% level), several exceptions were observed.

Since the pathogen has poor pectinase activity, mature stem tissue may appear symptomless. Bacteria surviving in invaded xylem vessels of mature stems used as planting material spread systemically through young plants, which then serve as sources of inoculum in the next cycle. Consequently, the proportion of infected cuttings increases after several cycles of continuous cultivation of apparently intermediate-resistant or resistant genotypes when ratings are assigned after only one cycle of greenhouse or field evaluation. Using such "resistant" material continuously could result in a progressive decline in stand density due to lack of germination, decreased plant vigor due to bacterial root rotting, and earlier onset of more severe epidemics.

This was corroborated with results obtained after planting several genotypes at Carimagua for four cycles using planting material from that site (Fig. 1). Due to poor soil fertility in this region, production of planting material decreased about 60% compared with CIAT-Palmira. However, resistant genotypes (group I, Fig. 1) in which bacterial infection in the stem was very low or absent produced a constant number of cuttings during a four-cycle period, while other resistant or intermediate genotypes only survived for two or three cycles (groups II and III, Fig. 1). Susceptible genotypes were eliminated during the first or second cycles.

The data indicate the great importance of the sanitary condition of planting material on genotype stability, the existence of durable CBB-resistant genotypes in *Manihot esculenta,* and the need to evaluate testing material in the field for several continuous cycles in CBB-endemic areas, to identify accurately durable CBB-resistant genotypes. Planting material for each successive cycle must be produced in an endemic test area. Final resistance evaluation should result from integrating data on plant reaction, cutting production and quality of propagating material.

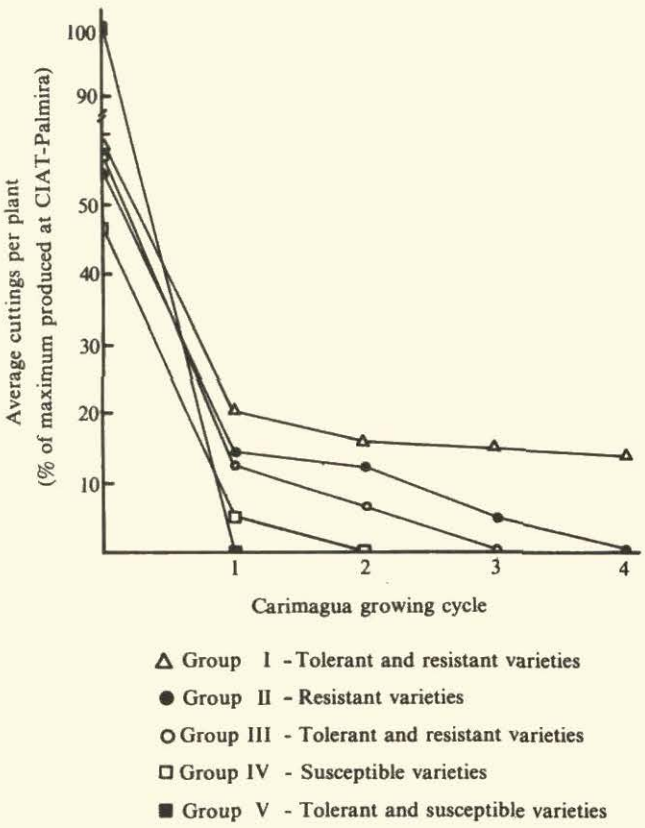

Figure 1. *Survival of cassava genotypes having differing reactions to cassava bacterial blight after cycles of continuous cultivation in the Carimagua environment, when utilizing locally grown planting material.*

Epidemiology. Considerable yearly fluctuations in CBB attack have been found in Carimagua and Media Luna. This suggests that continuous evaluations over a period of years may be necessary so as to identify sufficiently good levels of durable resistance.

Superelongation Disease

Causal agent. The sexual stage of the causal agent, *Sphaceloma manihoticola,* has been discovered and found to be abundant at a number of sites. Based on morphological studies of the fungus collected in Carimagua, CIAT-Quilichao, Media Luna and Mexico, it has been tentatively identified as a species of *Elsinoe,* a loculoascomycete. With one exception, all the known sexual stages of species of *Sphaceloma* are *Elsinoe.* A preliminary literature survey indicates that this may be an undescribed species. Growth stages of the fungus are shown in Figure 2.

Single ascospore isolations produced colonies typical of *S. manihoticola* and similar to those described for other members of the genus. Susceptible cultivars inoculated with these colonies showed characteristic superelongation symptoms and reisolation yielded *S. manihoticola.* That this sexual stage is so common suggests that the pathogen may be pathogenically variable. Preliminary field observations and previous laboratory experiments support the existence of physiological races (see below and CIAT Ann. Rept. 1977), and a thorough investigation is being undertaken.

Epidemiology. A multiclonal experiment was begun in Carimagua to test the influence of mixing resistant varieties on disease epidemic development and yield (root and stake production). Eight varieties were planted nonrandomly so that no two plants of the same variety were adjacent. Both next to the multiclonal plots and 2 km away each variety was planted in pure plots for comparison. Since the data are preliminary and incomplete only a few of the most interesting results will be presented.

Figure 3 shows that the varieties in the multiclonal plot had somewhat more superelongation disease than the same varieties had in pure plots. From field observations during the disease season, CMC 40 plants appeared to act as foci for sub-epidemics in the multiclonal plots. Thus interspacing a susceptible variety with more resistant varieties seemed to increase the total level of disease for all varieties rather than appreciably protecting the susceptible variety. This is contrary to what was expected based on epidemiological research with cereals. Investigations to clarify these results are being planned for the coming year.

It is interesting to compare the disease levels in CMC 40 and other varieties within the multiclonal site, between that site and the site 2 km distant where there was considerably more superelongation disease. At the multiclonal site, all varieties, which are considered resistant or intermediate-resistant based on data from recent years, showed more disease than they did in the remote site. This was extreme for CMC 40 (Fig. 3). Considering the level of disease in susceptible varieties planted adjacent to CMC 40 and the other resistant varieties, the low disease levels in these plots could not be due to escape. This difference in susceptibility within a clone under more or less uniform environmental conditions is consistent with the existence of physiological races within the pathogen.

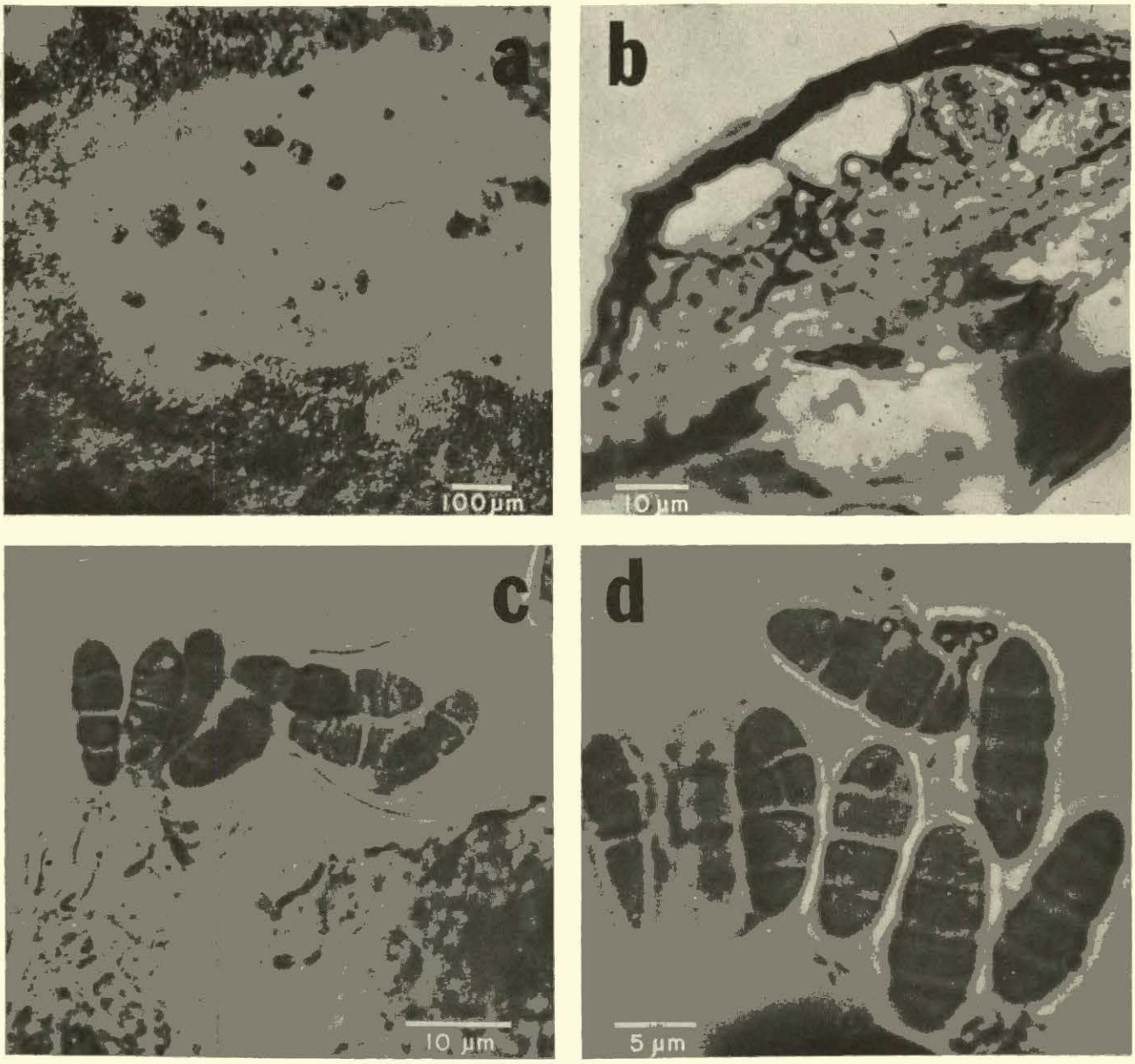

Figure 2. *Ascostroma, asci and spores of the sexual stage of* **Sphaceloma manihoticola,** *tentatively identified as a species of* **Elsinoe.** *a) Ascostroma on surface of a stem lesion; b) Section through an ascostroma showing well-defined locules with only one globose ascus per locule; c) Ruptured bitunicate ascus showing eight ascospores; c) Ascospores showing muriform character.*

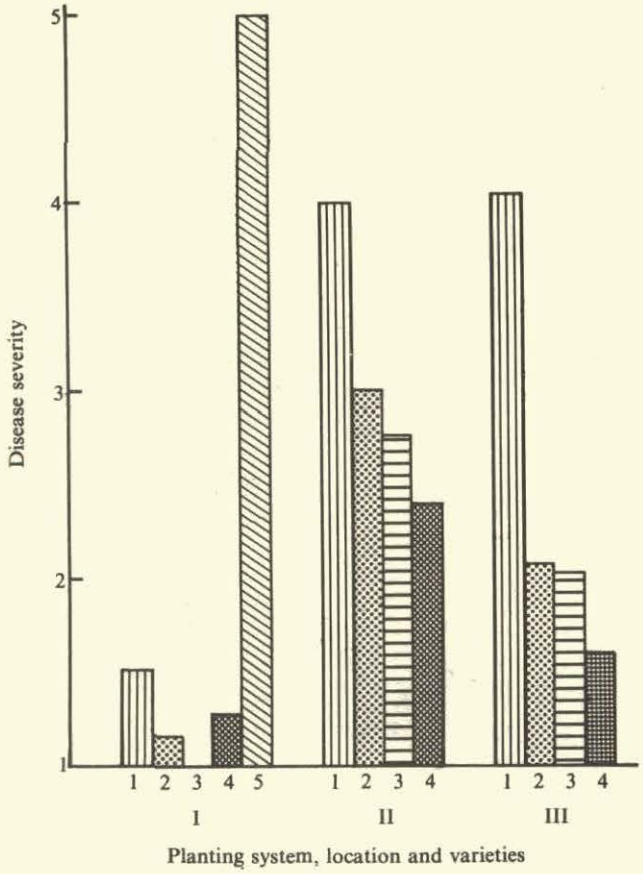

Figure 3. *Comparison of superelongation disease among varieties in a multiclonal experiment. Group I: varieties planted remotely (2 km from main plantings). Group II: varieties in a multiclonal pattern (see text) in an area of low inoculum pressure. Group III: varieties in multiclonal (pure) plots adjacent to multiclonal plots. Varieties: 1= CMC 40; 2=M Ecu 82, M Ven 77, M Pan 12B and M Pan 19; 3=M Col 1914 and M Col 1916; 4 = M Col 638; S = several susceptible varieties in the remote site all killed by superelongation and included to demonstrate inoculum pressure at that site.*

located just beneath epidermal tissues of necrosed roots and stems. Immature picniospores are hyaline, but mature ones are dark, two-celled with thick walls. They are released through the picnidium, opening mostly during rainy periods. Penetration, fungal establishment and invasion are being investigated.

Epidemiology. There are two disease phases. The first is a root rot initiated by infection from infested soil or by using diseased cuttings taken from diseased plants. In this case, the fungus, which is a facultative parasite, could infest the soil and remain indefinitely as a saprophyte. Infected plants show root deterioration, sudden wilt and death. Symptoms are similar to those induced by other root-rot pathogens.

The second phase, a stem rot, is generally induced by systemic invasion from roots or picniospore infection of the stem. The fungus invades most of the stem tissues producing gumosis, sudden wilting, dieback and phloem and xylem rotting. Picnidia are produced readily on the stem epidermis of infected stems. During this phase, roots and mature stems may remain symptomless. Systemically infected stems may exhibit no external symptoms and appear to be suitable planting material. Symptoms can be confused with those caused by anthracnose, *Phoma* and superelongation dieback, CBB, drought stress, salinity, insects and spider mites. However, the fungus can be readily identified by the features of picnidia and picniospores produced.

The fungus is disseminated over long distances by infected cuttings taken as planting material. Within a plantation, wind and rain-splash dissemination of picniospores is probably most important, while land preparation machinery and irrigation water may also sometimes be important.

Diplodia Root and Stem Rot

Causal agent. Severe outbreaks of diplodia root and stem rot were observed in Colombia's Cauca Valley. This disease has been reported as one of the most serious ones in Brazilian cassava plantations, and in Africa, India and Cuba.

Its causal agent was isolated and identified as *Diplodia manihotis* Sacc., which could be synonymous with *Botryodiplodia manihoticola* Petr. Confirmation of this synonymy awaits further taxonomic study. The fungus produces clusters of picnidia on stromatal structures

Root "Smallpox" Disease

This disease has been found in Colombia associated with a subterranean sucking insect (Cydnidae) which causes the initial injury (other agents like nematodes can cause similar injuries and might initiate the same symptom development). This Cydnidae is described in the Entomology section of this report.

The insect introduces its stylet through the root epidermis and cortex injuring the root tissues and inoculating them with soilborne microorganisms (mostly fungi). Several fungal species have been isolated from these

lesions. Artificial inoculations simulating the insect damage have induced similar symptoms (Fig. 4). These microorganisms degrade the infected root tissues causing initial localized rots which can invade the entire root along the vascular system. Young lesions are pale to dark brown spots which show tissue degradations. Symptoms are most striking and lesions are most frequent in swollen roots and during harvest periods. A zone which fluoresces light blue under UV light occurs adjacent to the lesions, suggesting that the mechanism of discoloration could be related to that occurring in post-harvest physiological deterioration.

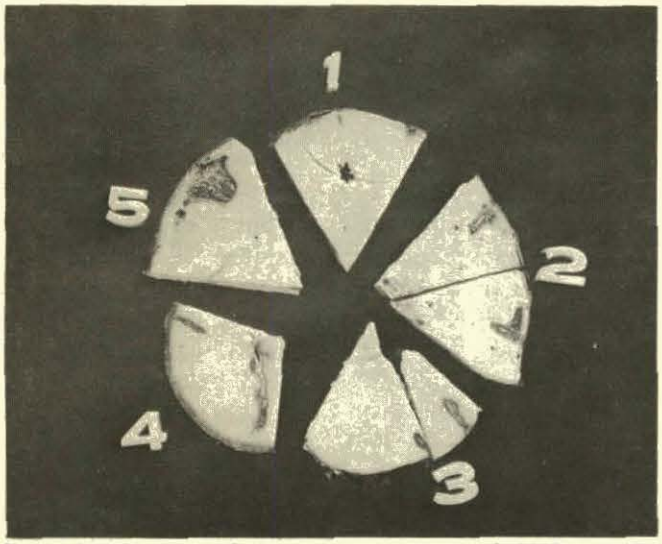

Figure 4. *Smallpox disease of cassava induced 24 hours after inoculating through the peel with an infested needle with spore suspensions of 2* = **Genicularia,** *3* = **Aspergillus,** *4*= **Fusarium,** *5* = **Pythium;** *1* = *control.*

Frog Skin Disease

In 1980, characterization of the frog skin disease causal agent continued with emphasis on detection, transmission and isolation studies.

A cytological study revealed considerable degeneration of the phloem of young roots showing frog skin symptoms as well as the presence of massive inclusions blocking the phloem parenchyma in these tissues. Similar inclusions were occasionally detected in the phloem of petioles and midribs of diseased plants. No such inclusions were detected in the phloem tissues of healthy plants. Based on these observations, mycoplasma-like organisms or a phloem-restricted virus have been suggested as probable causal agents.

The possibility of a phloem-restricted virus such as those in the closterovirus class is currently being investigated. Preliminary results have shown the presence of long filamentous particles, similar to those of closteroviruses, in partially purified preparations from roots of frog skin-affected cassava plants. Whether these particles are indeed virions or artifacts is still unknown. The possibility of a mycoplasma-like organism as the causal agent of frog skin disease will be reinvestigated despite negative results in earlier tests.

Preliminary field results suggest that the disease is efficiently transmitted (up to 62%) by natural root grafts whenever stakes are planted closer than 1 m. Natural root injury or the existence of soil vectors, however, have not been discarded as possible transmission mechanisms.

No transmission via sexual seed was observed in 9-month-old plants grown from seed produced by 46 different cassava clones affected with the frog skin disease. So far, an alternative host has not been found among 50 other plant species following conventional mechanical inoculation experiments.

Fertilization with 200-200-100 kg/ha of N-P-K increased symptom expression, especially in cultivar M Col 22.

Environmental Studies

Screening for durable resistance to biotic problems. Germplasm evaluation in the past has shown that durable resistance to biotic problems of each environment exists although frequency is relatively low. Evaluations by Pathology and Entomology sections consist of growing accessions in each environment utilizing planting material produced in the same environment. Only those varieties which survive the season and produce adequate healthy planting material are carried over to the next year.

In Popayan, stable varieties could be identified after the fourth cycle, and in Carimagua, after the third. Up to the third cycle, the stability of the varieties has not been confirmed in Media Luna(Table 1). It is important to stress that resistance identified in these evaluations appears to include durable resistance to all biotic problems existing in the evaluation site and may also integrate resistance to abiotic problems.

Cassava environment relationships. Environment studies begun in 1978 (CIAT Cassava Prog. 1979 Ann. Rept.) continued during the 1979-80 season, when plants from the first cycle were harvested. Cultivars which produced planting material were continued in a second

cycle. All diseases and pests identified during 1979 in each of the five target environment were present in 1980, with the exception of the ash disease which was first detected this year in Caribia and Media Luna (Table 2). However, anthracnose (caused by different *Colletotrichum* spp. in each environment) was more severe in Caribia and Carimagua than the other environment this year; brown leaf spot was also very severe in Caribia and Media Luna, as was white leaf spot in Caribia.

The lace bug (*Vatiga* spp.), which was unimportant during the rainy season of 1979, caused severe damage during the same season this year at Carimagua.

Thrips were important at CIAT-Palmira, gall midges in Carimagua and scale insects in Popayan.

The populations and severity of spider mites (*Mononychellus* and *Oligonychus* species) have increased considerably in the CIAT-Palmira environment.

In general fluctuations in population dynamics and severities of disease and pest damage were observed for each environment between 1979 and 1980.

Differences within and between the five environments were considerable for the characteristics shown in Table 3. Yield range at CIAT-Palmira was similar to that at Caribia but means in these environment were notably larger than in the other three environments. Stake number and starch production also resemble yield in those respects.

Differences in ranges and means for harvest index and HCN content were similar in all environments. A wide range of deterioration susceptibilities was found only in CIAT-Palmira and Popayan; in the other environments most cultivars were markedly resistant.

In general, yield and especially the harvest index were well-correlated between all environments excluding Popayán, although CIAT-Palmira correlated with Carimagua only for harvest index. HCN content correlations were generally low and susceptibility to physiological deterioration and stake number showed no relationships between environments, apart from between Carimagua and Media Luna.

Popayan was obviously different from the other environments. The cultivars which did best in Popayan, namely local regional varieties, produced poorly in all other environments and especially so on the Colombian North Coast (Caribia). The local cultivars from the North Coast fared equally poorly in Popayan.

On the basis of these relationships, CIAT-Palmira and Caribia have some similarities, as does Media Luna, with both Caribia and Carimagua, but the differences between the environments are still substantial, e.g., yield, starch content, stake number, general evaluation and root deterioration susceptibility in CIAT-Palmira showed no significant correlation with Carimagua.

This demonstrates the importance of carrying out varietal selection at the environment level at the earliest possible stage of any breeding program. This is particularly true for Popayan and Carimagua.

Selection must continue until stability of yield and other selected traits has been achieved using planting material produced on-site. Nevertheless, the high correlation between many characters in Media Luna and Carimagua suggests that similar material may be useful across these environments.

Table 1. Results of screening for durable resistance to biotic problems of cassava in three environments.

Environment	No. of genotypes per continuous cycle evaluation [1]					
	1	2	3	4	5	8
Popayan	1708 (100) [2]	217 (12.7)	67 (3.9)	10 (0.6)	10 (0.6)	10 (0.6)
Carimagua	1379 (100)	65 (4.7)	13 (0.9)	12 (0.9)		
Media-Luna	391 (100)	116 (29.7)	29 (7.4)			

[1] *1st cycle planting at* Carimagua and Media Luna: single rows with 5 plants/genotype; at Popayán: single rows with 11 plants/genotype. All replicated twice and using planting material from CIAT-Palmira. 2nd cycle: means of 15 plants/plot/genotype using locally produced stakes. 3rd and other cycles: mean of 30 plants/plot/genotype, replicated 3 times, using locally produced stakes.

[2] Values in parentheses are percentage of total number of genotypes planted in 1st cycle.

Table 2. Biotic negative production factors (BNPF) to cassava identified and evaluated in five environments in Colombia during 1979 (cycle 1) and 1980 (cycle 2).

BNPF	Environment									
	Caribia		Media Luna		Carimagua		CIAT-Palmira		Popayán	
	1979	1980	1979	1980	1979	1980	1979	1980	1979	1980
Diseases:										
Bacterial blight	M [1]	M	L	M	S	S	NP	NP	NP	NP
Superelongation	L	L	L	S	S	S	NP	NP	NP	NP
Concentric-ring leaf spot	NP	NP	NP	NP	NP	NP	NP	NP	S	M
Anthracnose	M	S	L	M	S	S	S	L	S	M
Brown leaf spot	M	S	S	S	M	L	L	M	NP	NP
Blight leaf spot	M	M	L	M	M	NP	L	M	NP	NP
White leaf spot	M	S	M	M	NP	NP	NP	NP	NP	L
Bacterial stem rot	L	L	L	L	L	L	M	M	NP	NP
Cassava ash	NP	L	NP	M	NP	NP	L	S	M	M
Cassava common mosaic	L	M	L	M	NP	NP	M	M	S	S
Frog skin	NE	L	NE	L	NE	NP	NE	M	NE	NP
"Smallpox" of root	NE	L	NE	M	NE	NP	NE	L	NE	NP
Root rots	NE	L	NE	L	NE	L	NE	M	NE	NP
Insects:										
Hornworm	L	L	L	L	S	L	L	L	L	L
Whitefly	M	L	M	L	L	L	L	L	L	L
Thrips	M	M	L	L	M	M	S	S	L	L
Lacebug	L	M	L	L	M	S	M	M	L	NP
Shoot fly	L	L	L	NP	L	L	M	L	NP	NP
Fruit fly	L	L	NP	NP	L	NP	L	L	L	NP
Leaf beetle	NP	NP	NP	NP	NP	NP	M	M	NP	NP
Gallmidge	L	L	L	L	M	S	L	NP	NP	NP
Termites	L	L	M	M	L	L	NP	NP	NP	NP
Stemborer	NP	NP	NP	NP	M	M	NP	NP	NP	NP
Leaf cutter ants	NP	NP	NP	NP	M	NP	NP	NP	NP	NP
Scales	NP	NP	L	NP	L	L	NP	NP	S	S
Mites:										
Mononychellus	L	L	M	M	L	L	M	S	L	L
Tetranychus	L	L	L	L	L	L	L	M	NP	NP
Oligonychus	L	M	L	L	M	NP	M	S	M	S

[1] Ratings. S = severe damage; M = moderate damage; L = light damage; NP = not present; NE = not evaluated.

Table 3. Ranges and average values for selected yield, quality and storage parameters of 31 cassava cultivars grown in five environments.

Environment	Yield (t/ha)		Root starch (t/ha)		Stake production per plant		Harvest index [1]		HCN content [2]		Root deterioration [3]	
	Range	Avg.	Range	Avg.	Range	Avg.	Range	Avg.	Range	Avg.	Range	Avg.
CIAT-Palmira	74.1 - 0.0	24.4	24.7 - 0.00	7.68	18 - 0.6	10.0	0.65 - 0.00	0.41	5 - 1	2.64	90.0 - 1.6	26.5
Media Luna	19.0 - 0.4	9.5	3.7 - 0.06	1.89	11 - 1.0	5.0	0.65 - 0.13	0.44	5 - 1	2.87	7.6 - 0.0	1.4
Carimagua	10.7 - 0.5	2.5	2.8 - 0.01	0.69	5 - 0.0	1.0	0.66 - 0.02	0.39	5 - 2	3.29	26.9 - 0.0	1.4
Caribia	55.9 - 2.4	23.2	16.3 - 0.33	6.43	16 - 2.0	8.2	0.61 - 0.12	0.40	5 - 1	2.99	28.1 - 0.0	3.6
Popayán	21.3 - 0.2	4.9	6.9 - 0.04	1.55	8 - 0.0	2.3	0.66 - 0.10	0.36	5 - 1	2.70	82.6 - 2.3	27.1

[1] Harvest index: [Root fresh weight/(Root fresh weight + Foliage weight)] x 100
[2] By picrate paper method: 5 = high and 0 = low HCN content.
[3] 100% = total and 0% = zero deterioration, three days after harvest.

Post-Harvest Deterioration

The two main areas of investigation this year have been the study of the variation encountered in the field within and among cultivars regarding their susceptibility to deterioration and the analysis of the biochemical processes which lead to the production of the blue-black pigments. Considerable progress was made in both areas.

Repeated evaluations of susceptibility to physiological deterioration showed that within one cultivar a wide range of susceptibilities can be encountered (*CIAT Cassava* Prog. 1979 Ann. Rept.). In the cultivar most studied, M Col 22, maximum and minimum deterioration values (Det. %, where 100% is total and 0% is no deterioration, after three days) were 98% and 18%, respectively at CIAT-Palmira, while even lower values were found at other sites (0% at Popayan and Carimagua). Values obtained from some other cultivars showed similar patterns.

The wide range of susceptibilities within one cultivar at one site makes the description of cultivars as "resistant" or "susceptible" to physiological deterioration difficult and of dubious validity.

Pruning studies. Pruning plants prior to harvest has been shown repeatedly to reduce susceptibility to physiological deterioration (CIAT Cassava Prog. 1979 Ann. Rept.). Experiments on M Col 22 plants have shown that this effect occurs regardless of the removal or supression of new regrowth from the pruned stump, although initially the loss of susceptibility is greatest in those treatments with less regrowth. The effect of pruning, regardless of regrowth removal, was also found to last considerably longer than previously reported. Plants still showed no signs of losing the resistance nine weeks or more after pruning. However, there is a loss of root quality (reduced starch content) associated with pruned roots which must be taken into account.

Environment studies. Evaluations of Det. % were done on 25 cultivars in the five sites of the environment experiment. Cultivar differenees between the sites were large, and environment correlations were not significant. In Carimagua, Media Luna and Caribia, a majority of cultivars showed marked resistance, regardless of their susceptibility in CIAT-Palmira or Popayan, the two sites which did produce the expected range of Det. % scores. The correlation of Det. % with the starch content of the roots (CIAT Cassava Prog. 1979 Ann. Rept.) was significant only at CIAT-Palmira ($r = 0.680$, $P<0.001$) and Popayan ($r = 0.558$ $P<0.01$). In the other environments there was a wide range of starch contents although Det. % values were all low.

Plant defoliation caused by insects, diseases or water stress in the months preceeding harvest could have the same effect as pre-harvest pruning, leading to an induction of resistance to physiological deterioration. In the three environments where low Det. % values were found, substantial defoliation due to both biotic and climatic factors had occurred prior to harvest. Controlled experiments are in progress to see if resistance in the field is related to the severity of water stress and other factors.

Biochemical Studies

Examination of roots under UV light 24-48 hrs after harvest revealed the presence of a brilliant blue fluorescence in the parenchymal tissue which was not visible in freshly harvested roots. These fluorescent areas were the first to develop the vessel pigmentation characteristic of physiological deterioration. Roots with deteriorated vessels showed this blue fluorescence in the advancing front of deterioration. Similar observations at CIAT and Tropical Products Institute, TPI (J. E. Rickard) have been made during a microscopical study of primary deterioration in cassava roots.

Chromatographic studies by J. E. Rickard at TPI have shown the blue fluorescing compound to have retention times identical to scopoletin, a coumarin derivative. Independent studies at CIAT have agreed with this identification.

Exogenous applications of high concentrations (500 µg ml^{-1}) of scopoletin to freshly harvested root tissue induce rapid and intense vessel and parenchymal discoloration, identical to that found in naturally deteriorating roots. A range of related phenolic compounds had no effect. Applications of scopoletin to tissue from pruned roots produced an identical reaction to that of unpruned root tissue suggesting that resistance in pruned roots is not due to an inability to respond to scopoletin.

Roots attacked by various fungi (*Aspergillus* sp., *Fusarium* spp., etc) also had areas of blue fluorescence and vessel discoloration around the infected area, suggesting that scopoletin accumulation is a general response to stress in the root tissue.

Errata

Page	Columm	Element	Printed:	Should be:
6	1	Figure 2	M Col 59	M Mex 59
6	2	Figure 3	M Col 59	M Mex 59
6	2	Figure 3	LSD (P<0.05)	LSD (P<0.05)
7	1	Figure 4	M Col 59	M Mex 59
60	2	Second para., line 8	more to growth	more top growth
61	2	Line 1	and K contents	and K concentrations
20	1	Figure 1	I - Tolerant III - Tolerant V - Tolerant	I - Intermediate-resistant III - Intermediate-resistant V - Intermediate-resistant
62	1	Figure 3	Stems □	Stems △
64	1	Figure 5	5.0 4.0 % N	5.0 4.0 % N 3.0
66	1	Figure 8	Figure 44	Figure 8
93	2	Footnote	*Left during 1979.	*Left during 1980.