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# Plant Pathology

## Introduction

The major aims of the Plant Pathology Section continued to be: a) To SCREEN all new germplasm for resistance to diseases in major evaluation sites; b) to detect, identify and assess diseases of germplasm under forage evaluation; c) to evaluate and develop control measures for damaging diseases of promising forage species.

Evaluation of germplasm continued as a major activity at Carimagua and Quilichao, Colombia, and CPAC, Brazil. Further information was collected on disease distribution. Studies continued on anthracnose and blight of *Stylosanthes* spp., Rhynchosporium leaf spot of *Andropogon gayanus*, biological control of spittlebug with fungus *Metarrhizium* in close collaboration with Entomology, and on seed pathology. Studies were initiated on several bacterial diseases and Rhizoctonia foliar blight of *Centrosema brasilianum*. Several new diseases were detected.

## Disease Survey

The following additions were made to the table of distribution with respect to ecosystem of forage diseases (Table 1).

1. Sooty blotch (*Polythrincium* sp.), affecting some of the more prostrate *Aeschynomene* sp., was detected only at Carimagua;

2. Bacterial wilt (*Corynebacterium flaccumfaciens*) affecting *Zornia* 7847, and *Zornia brasiliensis*, was found both at Quilichao and Carimagua;

3. Alternaria leaf spot, another disease of *Centrosema* spp., was detected at Carimagua and in regional trials of the Llanos.

During the three years of disease survey, 25 diseases have been detected in the Tropical Iso-hyperthermic Savanna Ecosystem, 18 in the Tropical Isothermic Savanna Ecosystem, 11 in the Tropical Semi-Evergreen Seasonal Forest Ecosystem and 24 in the Tropical Rainforest Ecosystem (Table 1). It should be noted that the table does not include genus host range. Some diseases, e.g., anthracnose, affect 10 or more genera.

## Diseases of Stylosanthes spp.

Field screening continued as a major activity. New and old germplasm was evaluated at 4-6 week intervals in Carimagua and Quilichao and once at CPAC, Brazil. Results are presented in Table 2.

a) Stylosanthes capitata. As for the last three years, most accessions were susceptible at CPAC, Brazil, and resistant in Carimagua.

b) Stylosanthes guianensis "common". Although less accessions have been evaluated at CPAC, Brazil, the Carimagua environment appears to have greater anthracnose pressure than the CPAC environment (Table 2).

c) Stylosanthes guianensis "tardío". Although few have been screened in the two major evaluation sites, CPAC-Brazil and Carimagua, these two sites have greater anthracnose pressure than Quilichao. The total collection should be evaluated next year at both sites.

A comparison among accessions of S. guianensis "tardío" in reaction to anthracnose at Quilichao, Carimagua and CPAC was made. The similarity between CPAC and Carimagua was considerably higher than between Quilichao and those two sites, again stressing the importance of screening the whole collection in the major screening sites. It was interesting to note that in both, the Venezuelan "tardíos" are more susceptible to anthracnose than the Brazilian "tardíos". In Quilichao recuperation of anthracnosed accessions of S. guianensis "tardío" was common in the dry seasons.

d) S. macrocephala. It remained resistant to anthracnose in both major screening sites. Also, the large CIAT-EPAMIG trial in Sete Lagoas, Minas Gerais, Brazil, was free of anthracnose. This species is worthy of further collection and study.

e) S. leiocarpa. In Carimagua most accessions were susceptible to anthracnose.

f) S. scabra. In CPAC many accessions are susceptible to anthracnose. In addition, plants are stressed by another problem, tentatively described as an insect-virus complex, which probably increases their susceptibility to anthracnose. This will be studied in Brazil.

g) S. viscosa. The planting in Quilichao had two levels of anthracnose during 1981.

Specific screening studies

Anthracnose of S. guianensis in the forest (e.g., Pucallpa, Perú). During the past ten years, S. guianensis cultivars Cook, Endeavour, Schofied and CIAT 136 and 184 have persisted in Pucallpa, Peru, and for several years in other forest environments in Bahía, Brazil and Leticia, Colombia, with only slight levels of anthracnose. At the same time, these ecotypes are severely affected by anthracnose in savanna ecosystems. Studies were therefore initiated to investigate why S. guianensis is only slightly affected by anthracnose in Pucallpa, Peru.

Table 1. Distribution of forage diseases in different ecosystems. Summary.

| | Forage disease | Ecosystems | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 9* | 1 | 4 | 1 | 9 | | 11 |
| | | Tropical savanna, Isohyperthermic ("Llanos") | Tropical savanna, isohyperthermic ("Llanos") Carimagua, Colombia | Tropical savanna, Isothermic ("Cerrado") | Tropical savanna, isothermic ("Cerrado") Brasilia, Brasil | Tropical semi-evergreen seasonal forest | Tropical sub-montane seasonal forest, Quilichao | Tropical rainforest |
| Grasses | 1. Anthracnose | + | + | + | + | + | + | + |
| Legumes | 2. Cercospora leaf spot (A) | + | + | + | | + | + | + |
| | 3. Cercospora leaf spot (B) | + | + | + | + | + | + | + |
| | 4. Root-knot nematode | | | +** | | | + | |
| | 5. Blight | + | + | + | | | | + |
| | 6. Sphaceloma scab | + | + | + | + | + | + | +** |
| | 7. Smut - Ustilago | | + | + | + | | + | + |
| | 8. Smut - Urocystis | | | +** | | | | |
| | 9. Camptomeris leaf spot | | + | | | | + | + |
| | 10. Rust - Uromyces | + | | + | + | + | + | + |
| | 11. Rust - Puccinia | | | | | +** | | + |
| | 12. False rust | | + | + | + | +** | | + |
| | 13. Rhizoctonia solani | + | + | +** | + | + | + | + |
| | 14. Rhynchosporium leaf spot | + | + | | | | + | +** |
| | 15. Drechslera leaf spot | +** | + | + | + | | + | + |
| | 16. Little leaf phyllody | + | + | + | + | | + | + |
| | 17. Ergot | | + | +** | | | + | +** |
| | 18. Giberella inflorescence blight | | | +** | | + | | +** |
| | 19. Botrytis inflorescence blight | | | | | | | +** |
| | 20. Black mold | | | +** | + | | | |
| | 21. Powdery mildew | + | + | | + | | + | +** |
| | 22. Slime mold | | | | | | + | |
| | 23. Bacterial blight | + | + | | | | + | +** |
| | 24. Bacterial pod blight | | | | | | + | + |
| | 25. Botryosphaeria canker | | + | | | | | + |
| | 26. Macrophomina phaseolina | | + | | | | | |
| | 27. Pokkah Boeng | + | + | | | | | +** |
| | 28. Cerebella inflorescence blight | | + | | | + | | +** |
| | 29. Viruses | +* | + | + | + | + | + | + |
| | 30. Rhizopus inflorescence blight | + | + | | | | | |
| | 31. Sooty blotch | | + | | | | | |
| | 32. Bacterial wilt | | + | | | | + | |
| | 33. Alternaria leaf spot | + | + | | | | | |

* Number of sites surveyed
** Only at one site

Table 2. Disease, evaluation, anthracnose, at CPAC, Carimagua, and Quilichao. 1979-1981.

| Sp./site | Evaluation[1] R | MR | MS | S | Total accessions |
|---|---|---|---|---|---|
| <u>Stylosanthes</u> <u>capitata</u> | | | | | |
| CPAC-LVE[2] | 6.3[3] | 11.8 | 35.4 | 46.5 | 119 |
| Carimagua | 83.3 | 10.6 | 6.1 | 0 | 132 |
| <u>Stylosanthes</u> <u>guianensis</u> "common" | | | | | |
| CPAC-LVE | 0 | 4.8 | 64.6 | 30.6 | 62 |
| Carimagua[4] | 1.6 | 6.8 | 20.4 | 71.2 | 545 |
| <u>Stylosanthes</u> <u>guianensis</u> "tardío" | | | | | |
| CPAC-LVE | 15.7 | 19.3 | 25.3 | 39.7 | 51 |
| CPAC-LVA[6] | 25.3 | 18.4 | 24.1 | 32.2 | 55 |
| Quilichao | 77.9 | 16.8 | 5.3 | 0 | 131 |
| Carimagua | 27.3 | 33.3 | 27.2 | 12.1 | 33 |
| <u>Stylosanthes</u> <u>macrocephala</u>[7] | | | | | |
| CPAC-LVE | 92.6 | 7.4 | 0 | 0 | 41 |
| Carimagua | 87.1 | 12.9 | 0 | 0 | 31 |
| <u>Stylosanthes</u> <u>leiocarpa</u>[8] | | | | | |
| Carimagua | 0 | 15.3 | 61.5 | 23.1 | 13 |
| Quilichao | 23.3 | 36.7 | 20.0 | 20.0 | 30 |
| <u>Stylosanthes</u> <u>scabra</u>[9] | | | | | |
| CPAC | 1.5 | 24.5 | 46.4 | 24.6 | 102 |
| <u>Stylosanthes</u> <u>viscosa</u>[10] | 72.6 | 17.9 | 5.1 | 4.4 | 117 |

[1] R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible.
[2] LVE = dark red latosol soil site.
[3] Percentage of accessions.
[4] Evaluation till September 1981.
[5] [7] [8] [9] Evaluation in 1980-1981.
[6] LVA = red-yellow latosol soil.
[10] Evaluation in 1981.

Various hypotheses were set up: 1) less pathogenic isolates in the Pucallpa environment; 2) lack of inoculum in the forest environment; 3) reduced inoculum spread; 4) favorable environmental conditions; 5) biological control agents.

The first hypothesis was tested by seedling inoculation studies in the greenhouse with isolates of <u>C. gloesporioides</u> from <u>Stylosanthes</u> spp. collected in Pucallpa, Peru, Colombia and Brazil. Isolates from <u>S. guianensis</u> CIAT 17 and 184 from Pucallpa were just as pathogenic to <u>S. guianensis</u> as isolates from CIAT 136, 184 and 13 in Colombia (Table 3).

At the same time, it was found that four isolates from <u>S. guianensis</u> "common" (CIAT 13, 17 and 184) were pathogenic to <u>S. guianensis</u> "tardío" 1283. Previously it had been found that isolates from "common" types do not attack "tardíos". It was also found for the first time that isolates from <u>S. capitata</u> affected <u>S. guianensis</u> (Table 3).

Table 3. Reaction of 10 *Stylosanthes* spp. accessions to eight isolates of *Colletotrichum gloeosporioides* from Brazil, Colombia and Perú.

| *Stylosanthes* spp. CIAT No. | Species | Anthracnose reaction 1019[1] B[2] | 2310 C | 1097 P | 136 C | 17 P | 13 C | 184 P | 184 C |
|---|---|---|---|---|---|---|---|---|---|
| 147 | *S. hamata* | ++ | - | + | - | - | - | - | - |
| 1283 | *S. guianensis* | + | + | - | + | + | + | + | ++ |
| 136 | *S. guianensis* | + | + | +++ | +++ | +++ | +++ | ++ | +++ |
| 184 | *S. guianensis* | + | + | ++ | +++ | +++ | +++ | +++ | +++ |
| 1019 | *S. capitata* | ++ | - | - | - | - | - | - | - |
| 1405 | *S. capitata* | ++ | - | - | - | - | - | - | - |
| 1315 | *S. capitata* | ++ | - | - | - | - | - | - | - |
| 1078 | *S. capitata* | + | - | - | - | - | - | - | - |
| 1074 | *S. viscosa* | - | - | - | - | - | - | - | - |
| 1047 | *S. scabra* | + | - | - | - | - | - | - | - |

[1] CIAT accession numbers
[2] B = Brazil, C = Colombia, P = Peru

Other hypotheses are presently being tested in Pucallpa particularly to determine whether biological control agents of *C. gloeosporioides* exist on leaves and stems of *S. guianensis*.

Studies on *S. guianensis* in Colombia. During the past two years, 545 accessions of *S. guianensis* "common" (all accessions of which seed was available) have been evaluated in Carimagua (Table 2). Almost all accessions were susceptible to anthracnose and died within one wet season. One moderately resistant accession, CIAT 1875 from Panama, is under further evaluation in a larger planting.

Isolates are being collected from all anthracnosed accessions in Carimagua. Pathogenic variation studies are in progress, and isolates are being grouped according to their reactions. To date, many isolates were found not pathogenic. Of the pathogenic ones, eight groups have so far been identified (Table 4). One group affected all accessions tested of *S. guianensis* common, others affected certain accessions, while Group 8 affected both *S. guianensis* common and *S. guianensis* tardío. Isolate collection and screening is continuing.

Table 4. Reactions of *Colletotrichum gloeosporioides* isolates from *Stylosanthes guianensis*, Carimagua, to seedlings of *Stylosanthes* spp.

| *Stylosanthes* spp. | Reactions | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| *S. capitata* 1019 | | | | | | | | | |
| *S. capitata* 1405 | | | | | | | | | |
| *S. capitata* 1315 | | | | | | | | | |
| *S. guianensis* 136 | + | + | | + | + | + | | | |
| *S. guianensis* 184 | + | | | + | | + | + | + | |
| *S. guianensis* 77 | + | + | + | + | | + | | | |
| *S. guianensis* 1003 | + | | | | | + | + | + | |
| *S. scabra* 1047 | | | | | | | | | |
| *S. viscosa* 1074 A | | | | | | | | | |
| *S. hamata* 147 | | | | | | + | | | |
| *S. macrocephala* 1281 | | | | | | | | | |
| *S. guianensis* T 1283 | | | | | | | | + | |
| No. of isolates | 15 | 1 | 2 | 2 | 1 | 3 | 2 | 2 | 40 |

Screening studies with *S. capitata*. Seedling screening of the whole *S. capitata* collection of which seed was available was completed with isolates from *S. capitata* CIAT 1019, 1405 and 1315 from CPAC. Isolates from CIAT 1019 and 1405 were more pathogenic than those from 1315 (Table 5). It appears more likely that differences in reaction among isolates are due to strain differences, rather than pathogenic variation or race differences. All Venezuelan accessions were resistant to all isolates. The data is being analyzed for pathogenic variation. In addition, studies are in progress with isolates collected from *S. capitata* in Minas Gerais. Results show that isolates from Minas Gerais are generally less pathogenic than those from CPAC.

To date, studies of pathogenic variation among isolates of *C. gloeosporioides* from *Stylosanthes* spp. have recognized seven groups of isolates:

Group 1 - *Stylosanthes guianensis* "common"

Group 2 - *Stylosanthes guianensis* "tardío"

Group 3 - *Stylosanthes guianensis* "common" and "tardío"

Group 4 - *Stylosanthes capitata* and *Stylosanthes scabra*

Group 5 - *Stylosanthes capitata* and *Stylosanthes hamata*

Group 6 - *Stylosanthes capitata* late flowering accessions

Group 7 - *Stylosanthes capitata* and *Stylosanthes guianensis* "common"

Groups 1, 2, 3 and 6 have been found in Colombia, while Groups 4, 5 and 7 have been found only in Brazil; Groups 1 and 2 are present in both countries; Groups 1 and 6 appear to be closely related.

These studies are showing that *C. gloeosporioides* is an extremely variable pathogen, and considerable work will be needed to fully classify its variation.

Table 5. Reaction of *Stylosanthes capitata* seedlings to isolates of *Colletotrichum gloeosporioides* from *S. capitata* at CPAC.

| Isolate | Reaction | | |
|---|---|---|---|
| | Resistant (%) | | Susceptible (%) |
| 1019 I[1] | 40 | (40)[2] | 60 |
| 1019 II | 25 | (64) | 75 |
| 1315 | 59 | (27) | 41 |
| 1405 I | 34 | (47) | 66 |
| 1405 II | 37 | (43) | 63 |

1 Roman numerals indicate different isolates from the same host
2 16 accessions of Venezuelan *S. capitata*

## Multilocational screening trials

The first multilocational S. capitata screening trial was planted in El Tigre, Venezuela in August. This comprised 86 accessions of S. capitata, including 14 from Venezuela. The first evaluation will be made in October. The second trial will be planted in Acaua in northern Minas Gerais in November. This comprises 100 accessions of S. capitata, 24 accessions from Minas Gerais, 29 accessions from Bahía, 13 from Venezuela, and the remainder from various sites in Brazil. Several of the Minas Gerais accessions were collected at the trial site. The third trial is planned for Bahía in collaboration with EMBRAPA; however, alternative sites will be considered in Maranhão and Pernambuco.

## Host plant resistance studies

Studies were continued on the physical and chemical characters of anthracnose-susceptible and-resistant accessions of S. guianensis.

Physical studies. During the past year they showed that neither removing the trichome secretions nor removing the trichomes of S. guianensis "tardío" 1283 had any effect on the reaction of this legume to slightly pathogenic isolates of C. gloeosporioides. Further work was planned with isolates of higher pathogenicity.

Recent tests with pathogenic isolates have shown that seedlings of several accessions of S. guianensis "tardío" were susceptible to anthracnose while adult plants were resistant. Trichomes may form a physical barrier to penetration by Colletotrichum gloeosporioides.

The effect of age on trichome density was therefore evaluated in stems of S. guianensis "common" CIAT 136, and S. guianensis "tardío" CIAT 1283 (Figure 1). Trichome density reached a maximum in CIAT 136 at 17 weeks of age at 160 trichomes/$cm^2$. In CIAT 1283, however, trichome density increased rapidly when plants reached 18-19 weeks of age. Counts are continuing on older plants, and further work is planned to study trichome density on susceptible and resistant accessions of S. guianensis "tardío".

Determination of phenols in Stylosanthes guianensis. In the past, various plant phenols have been noted as toxins to fungi. These include tannins, polyphenols and glucosides. Plants possessing phenols have been shown to be resistant to plant pathogenic fungi. In particular, it has been shown in many tropical fruits that the phenomenon of anthracnose latent infection caused by Colletotrichum spp. is due to immature fruit containing high levels of phenolic compounds which prevent development of anthracnose. As fruit ripens, levels of tannins decreased markedly, and anthracnose ripe rot occurs. Also, it has been shown that onions resistant to rot by Colletotrichum dematium possess phenolic compounds in their skins. Preliminary analyses of two S. guianensis "tardíos" showed glucosides and dihydro-monophenols, both members of the phenol group.

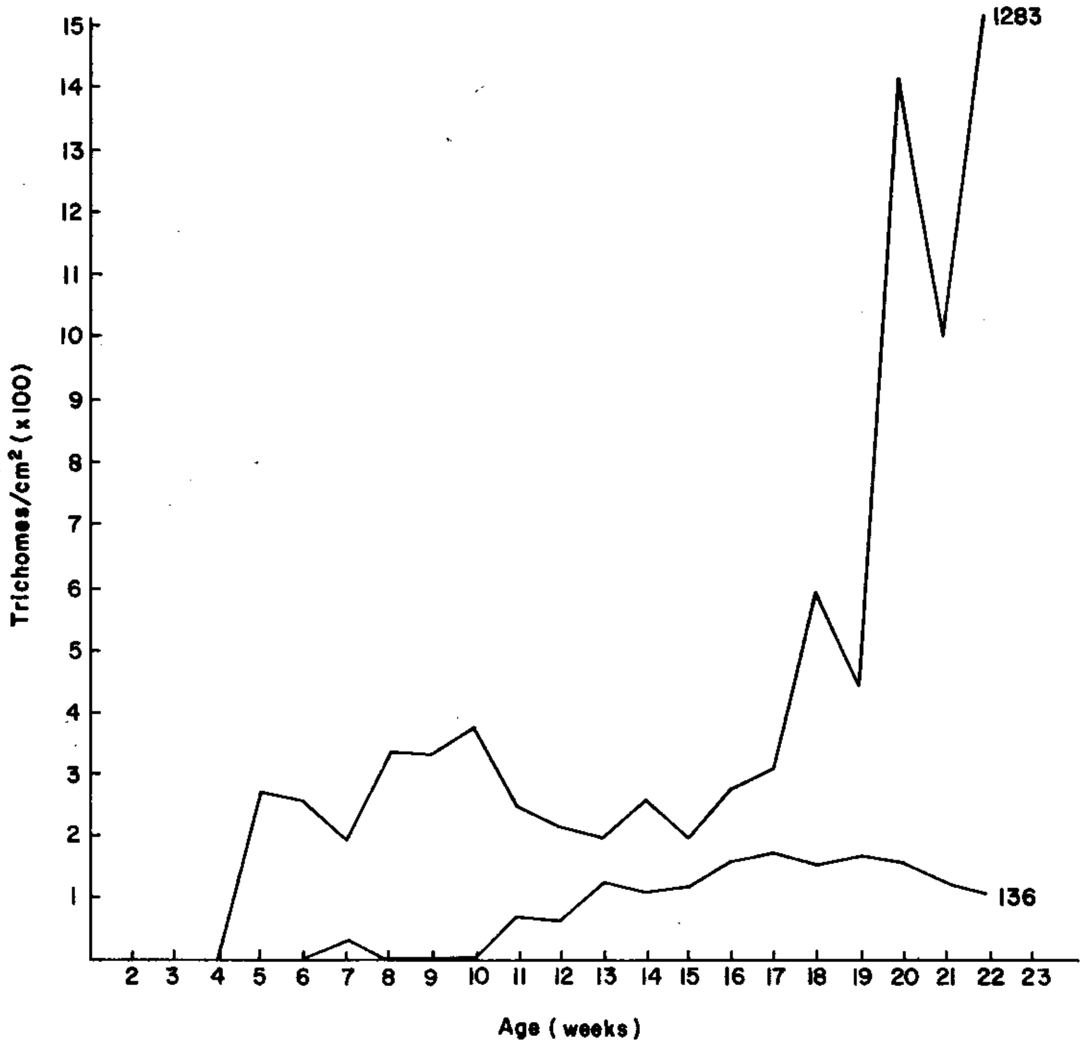


Figure 1. Changes in time in the density of trichomes on stems of S. guianensis CIAT 136 and CIAT 1283.

A project in collaboration with the Animal Nutrition Section was therefore initiated to survey S. guianensis for phenols using the Folin-Dennis method to determine if there is any correlation between possession of phenols and resistance to anthracnose.

Small samples of adult plants of S. guianensis "tardío" were taken from field plots and greenhouse at the same time. Percentage tannic acid was determined. Results were classified according to adult plant reaction to anthracnose at Carimagua (Table 6). For greenhouse samples, there was a decrease in percent tannic acid from 2.98 to 1.15 as the level of anthracnose increased from 1 to 5. For field samples, although percent tannic acid decreased from 2.25 to 1.37 as the level of anthracnose increased from 1 to 3, the mean percent tannic acid in accessions rating 4 and 5 was, however, higher than the level in accessions rating 3. Preliminary results suggest that a relationship may exist between plants possessing higher levels of phenols and resistance to anthracnose. Further studies are planned to identify phenols in resistant and susceptible accessions of S. guianensis "tardío".

Table 6. Analysis of tannins in accessions of Stylosanthes guianensis "tardío".

| Sample | No. of samples | Reactions to anthracnose in Carimagua | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Greenhouse | 23 | 2.50[1] | 2.98 | 1.81 | 1.74 | 1.15 |
| Field | 51 | 2.25 | 1.72 | 1.37 | 1.58 | 1.70 |

[1] % Tannic acid

Cross-protection. It has been shown that plants inoculated by non-pathogenic isolates of pathogens are protected against disease caused by subsequent infections of pathogenic isolates. Also, that primary infection by pathogenic isolates, followed by recuperation and subsequent infection by the same isolates results in less damage. Activation of chemical defense mechanisms appears to be part of the plants' resistance to some diseases, including anthracnose.

Various preliminary studies were therefore made to investigate cross-protection against anthracnose in Stylosanthes spp. The effect of different concentrations of inoculum on the reaction of S. guianensis CIAT 136 and S. capitata 1019 to anthracnose was studied. In each treatment the first inoculation was made with a non-pathogenic isolate and subsequent inoculations with a pathogenic isolate. In CIAT 136, no protection by the non-pathogenic isolate was found; in CIAT 1019, protection was found for the first inoculation with the pathogenic isolate; however, all other inoculations cause infection.

The effect of different concentrations of inoculum and time of recuperation on the reaction of S. guianensis CIAT 136 and S. capitata CIAT 1019 to anthracnose was also studied. In CIAT 136, no protection by either the non-pathogenic or pathogenic isolates was observed. In CIAT 1019, although no protection by the non-pathogenic isolate was observed, protection by the pathogenic isolate occurred for four weeks and six weeks after the inoculation. By 12 weeks after inoculation with the pathogenic isolate, however, plants were susceptible to the subsequent inoculation with the pathogenic isolate. Possibly there is activation of chemical defense mechanisms to anthracnose in S. capitata 1019, but the effect appears to be short-term and thus of little value for anthracnose resistance in S. capitata.

Effect of anthracnose on yield and quality of S. guianensis[1]. Although it has been known since the early 70's that S. guianensis is severely affected by anthracnose, no attempts have been made to quantify losses in yield and quality. A study was made during the past year of the effect of anthracnose on yield and quality of S. guianensis CIAT 136 and CIAT 184.

Level of disease followed the rainfall pattern closely (Figure 2). Yield reduction in dry matter over one year was 62.8% in CIAT 136 and 64.4% in CIAT 184 in relation to the fungicide protected control (Figure 3). Losses in crude protein (Figure 4), phosphorus, potassium and digestibility were of the same level.

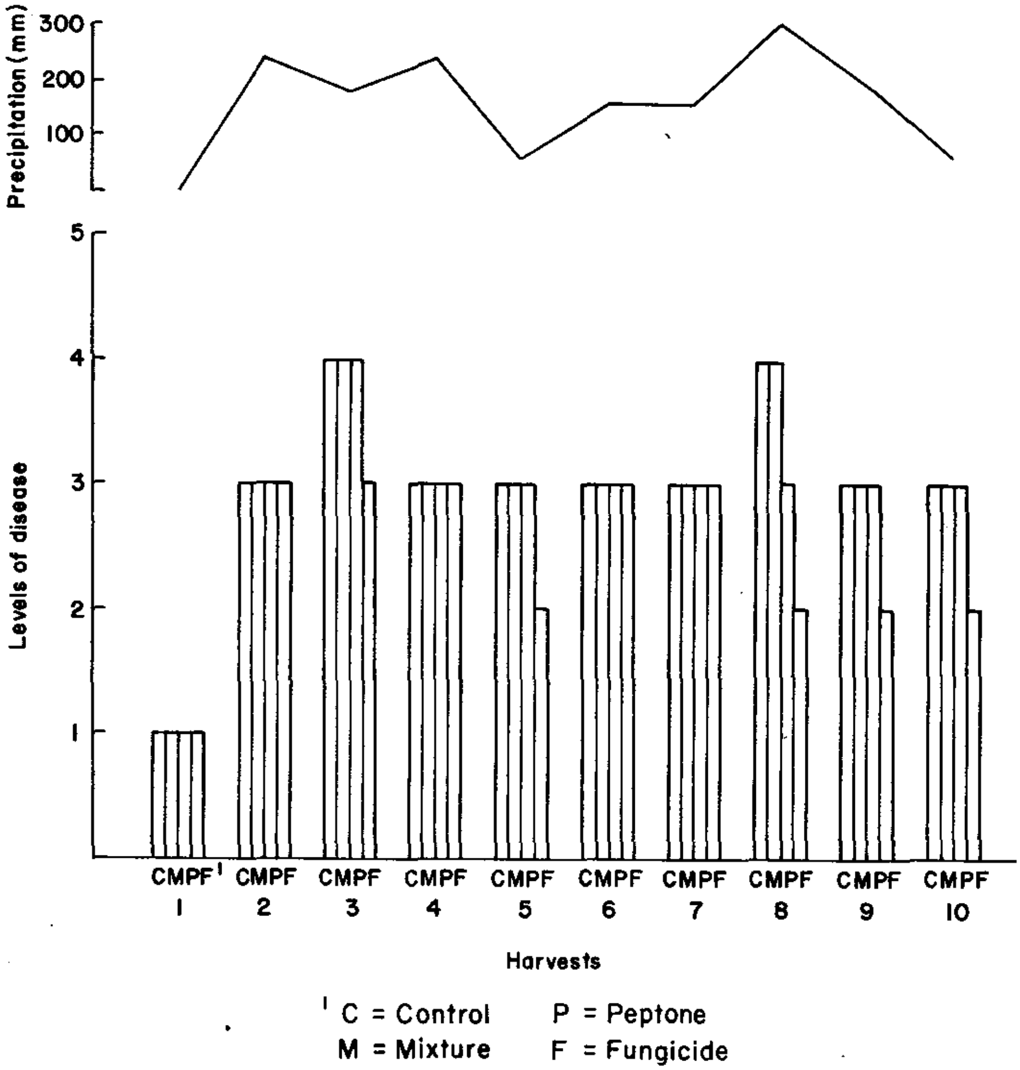


Figure 2. Levels of disease during the trial.

[1] Student thesis project by Jorge Gutiérrez and Carlos Cardozo.

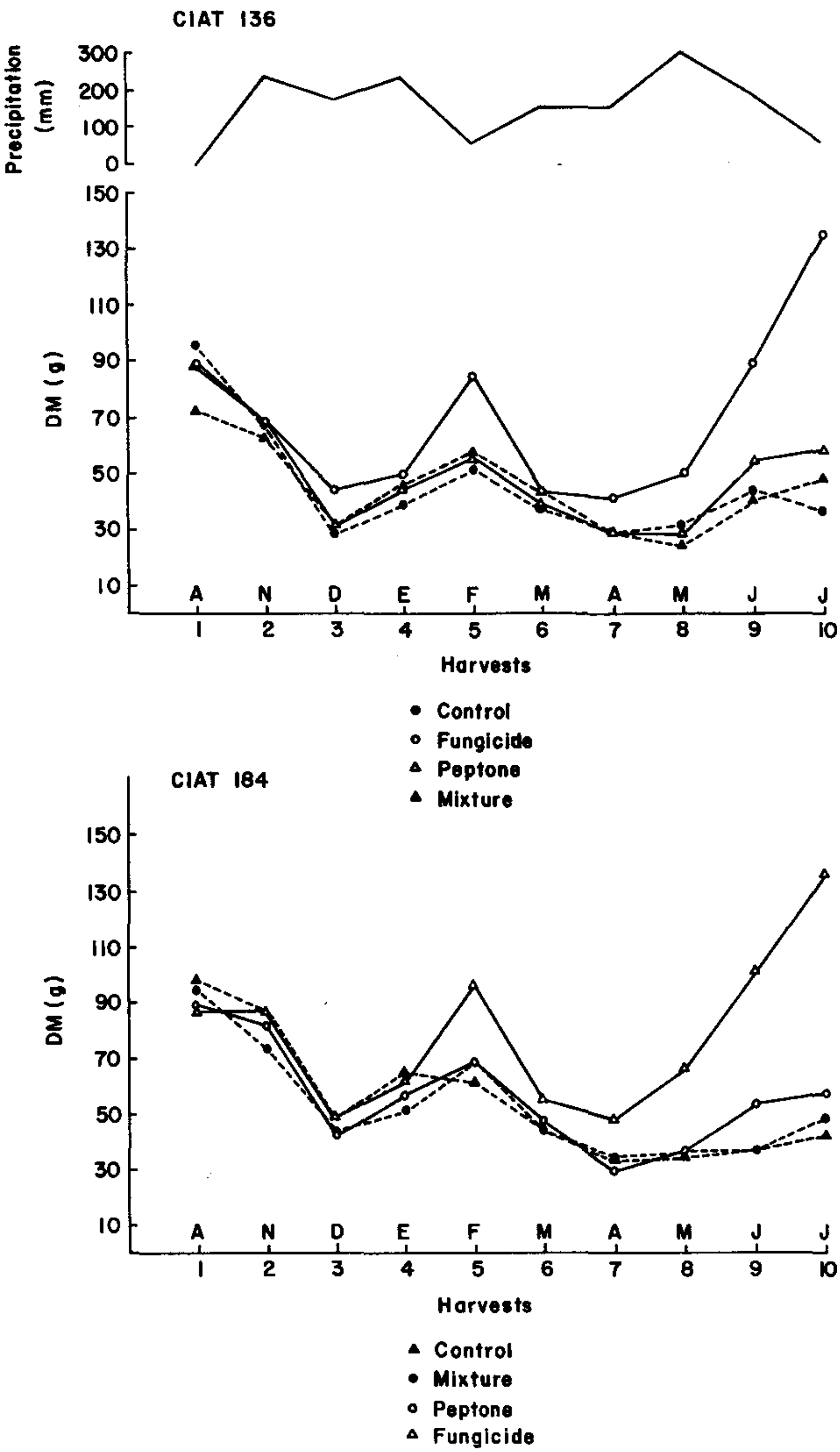


Figure 3. Yield of S. guianensis by treatment and harvest.

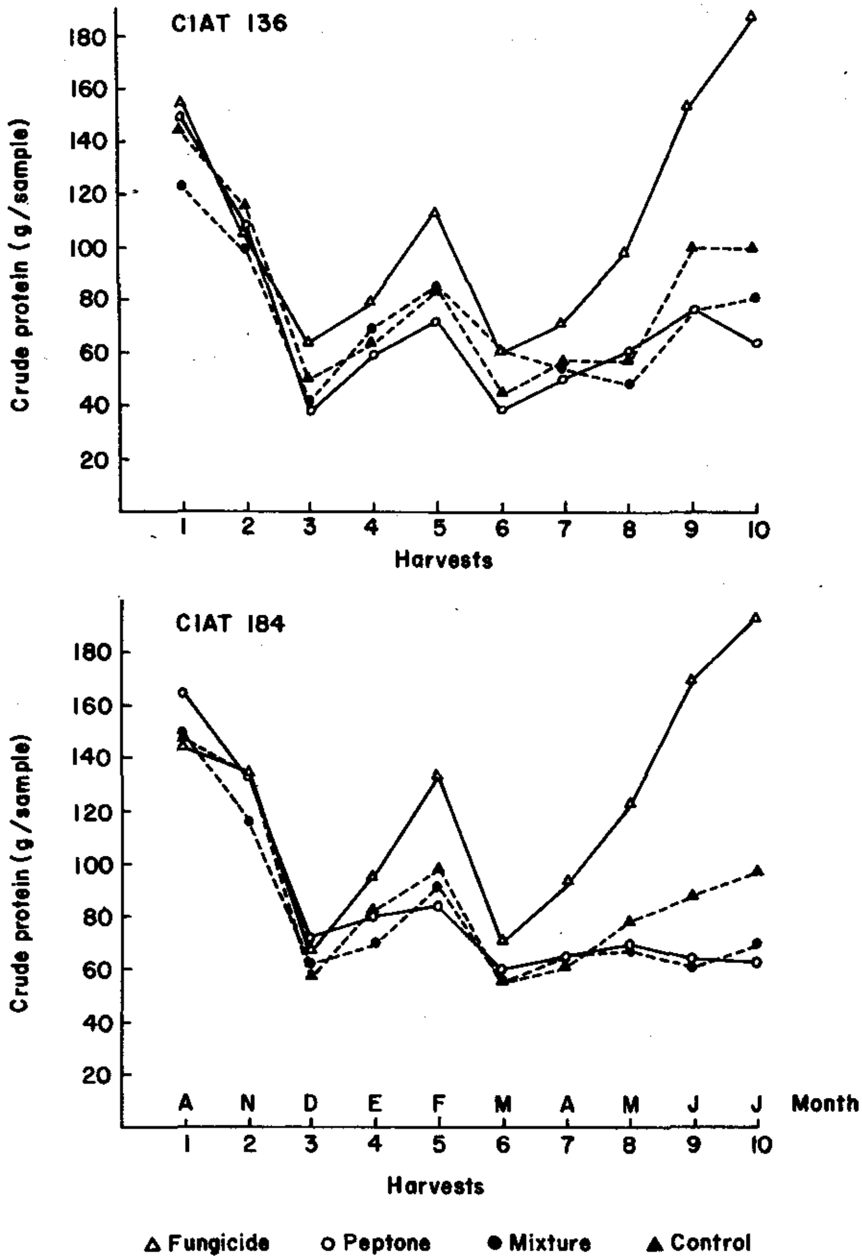


Figure 4. Production of crude protein of S. guianensis by treatment and harvest.

## Blight

Counts of S. capitata plants killed by S. rolfsii continued in Carimagua this year. Only 2-4% of plants were killed (Table 7). Viable sclerotia were again monitored at different sites in Carimagua. Levels have been considerably lower during the past two years than in 1979 (Table 8). Blight has now been classed as a minor disease of S. capitata, and no further studies of this disease are planned.

Table 7. Counts of S. capitata plants killed by Sclerotium rolfsii in Carimagua (mean of various sites).

| CIAT No. | Dead plants (%) | | |
|---|---|---|---|
| | 1979 | 1980 | 1981 |
| 1019 | 5 | 5 | 4 |
| 1315 | 9 | 6 | 2 |
| 1405 | 6 | 3 | 2 |

Table 8. Viable sclerotia of Sclerotium rolfsii in the soil at different sites in Carimagua.

| Sites[1] | Sclerotia/100 g soil (No.) | | |
|---|---|---|---|
| | 1979 | 1980[2] | 1981[3] |
| 1 | 9.8 | 2.3 | 0.3 |
| 2 | 6.8 | 1.0 | 0.8 |
| 3 | 2.7 | 0.9 | 0.4 |

[1] Sites: 1 = 3 years; 2 = 2 years; 3 = 1 year
[2] Mean of May and December samplings
[3] May sample only

Rhizopus head blight

This disease was first detected in Carimagua in 1980 causing severe damage to several accessions of S. capitata. It was detected again in July, August and September at low levels in S. capitata in Carimagua and also in the regional trials in the Llanos. It is very similar to Rhizopus head rot of sunflower, an important disease in Asia and the Americas. Three species of Rhizopus are thought to be involved: R. arrhizus, R. oryzae and R. stolonifer. Surveys of the occurrence of this disease will continue.

Diseases of Desmodium spp.

Germplasm surveys. Germplasm surveys of Desmodium spp. continued in Quilichao and Carimagua (Table 9). Of the more important species, D. heterocarpon has most disease problems, including anthracnose, Cercospora leaf spot, little leaf mycoplasm and root knot nematodes sporadically at Quilichao. Desmodium ovalifolium and D. heterophyllum have few disease problems.

Root knot nematode. No further studies were made on this disease due to low populations of nematodes in Quilichao.

Table 9. Disease evaluations of Desmodium spp., 1980-1981.

| Disease | D. ovalifolium C[2] | D. ovalifolium Q | D. heterocarpon C | D. heterocarpon Q | D. heterophyllum C | Others[1] C |
|---|---|---|---|---|---|---|
| Anthracnose | - | - | + | ++ | - | + |
| Cercospora leaf spot | -/+ | -/+ | + | + | + | + |
| Little leaf mycoplasma | - | - | ++ | ++ | - | ++ |
| False Rust -Synchytrium | -/++ | - | - | - | - | - |
| Root knot nematode | - | ++ | - | ++ | - | - |

[1] Others include: D. barbatum, D. tortuosum, D. scorpiurus, D. adscendens
[2] C = Carimagua; Q = Quilichao

False rust. A new disease of Desmodium ovalifolium was detected in El Tomo, Carimagua, in July. It caused distortion of young leaves, shortening of internodes and, subsequently, stunting of plants. Fruiting bodies or sori of the fungus filled with orange sporangia were produced in large quantities on the undersurface of leaves, on petioles and young stems. Galls were formed on old stems. The seed for this planting was a mixture made up of lots imported from Sri Lanka and India via Singapore. The disease was identified as Synchytrium desmodii, first described on D. ovalifolium in Sri Lanka in 1955. Because of the importance of D. ovalifolium, the El Tomo planting was destroyed and resown with Brachiaria dictyoneura. All plantings of D. ovalifolium will be surveyed periodically over the next year for false rust.

## Diseases of Leucaena sp.

Bacterial pod rot. In February 1980, pod rot was observed for the first time on Leucaena leucocephala in southern Mexico and at forage evaluation sites in Belize and Panama. In 1981, the same disease was observed in Colombia, Brazil, and Perú on cultivars of L. leucocephala Cunningham. A bacterium was consistently isolated from rotted pods and seeds, and on the basis of morphology, cultural characters, biochemical and physiological properties, it was identified as Pseudomonas flourescens Biotype 2.

Symptoms were manifest firstly as water-soaked lesions surrounding insect feeding holes. Lesions expanded and became necrotic as seeds began to rot. Under humid conditions, there was general pod rotting and bacteria oozed from insect feeding holes. Pods often fell prematurely, and few seeds were recovered from affected pods.

The bacterium caused pod rot of L. leucocephala when inoculated by injection, and bacteria were readily reisolated from affected pods. Cross-inoculation studies with four other species of Leucaena showed that all were susceptible to pod rot; however, *L. diversifolia* and *L. shannoni* were more resistant than *L. esculenta* and *L. pulverulenta*.

The development and association of lesions with insect-feeding holes under natural conditions indicated that the bacterium may be insect-borne by a Heteroptera of the family Pentatomidae (Mario Calderón, personal communication). However, a survey of seed harvested from healthy pods of 14 accessions of *L. leucocephala* and one each of *L. macrocephala* and *L. pulverulenta* found 48-95% of seed infected with the bacteria (Table 10). It is possible that *Pseudomonas flourescens* Biotype 2 is a natural component of the microflora of *Leucaena* pods and seed and usually does not cause disease. However, when insects feed on pods, the wounds caused enable the bacteria to enter and cause rotting of seeds. This will be further investigated.

Table 10. Survey of *Leucaena leucocephala* seed for *Pseudomonas flourescens* Biotype 2.

| Accession of *L. leucocephala* | Seeds with bacteria (%) |
|---|---|
| 78-36 | 60 |
| K 4 | 66 |
| K 8 | 88 |
| K 9 | 76 |
| K 29 | 76 |
| CIAT 734 | 80 |
| K 72 | 80 |
| 78-165 | 95 |
| 78-19 | 68 |
| K 132 | 76 |
| 78-50 | 58 |
| 78-85 | 78 |
| K 341 | 48 |
| 78-24 C | 86 |
| K 340* | 50 |
| 78-65** | 64 |

* *L. pulverulenta*
** *L. macrocephala*

## Diseases of Zornia spp.

*Sphaceloma scab*. Evaluations of germplasm continued in major screening sites (Table 11). Most accessions in Carimagua were susceptible. Resistant accessions included *Z. brasiliensis*, *Z. myriadena* and other four-leafed types. Although Sphaceloma scab pressure was not as great in CPAC, the virus-fungus complex affected many accessions. Evaluations of regional trials in the Llanos of Colombia also showed that Sphaceloma scab is the most important disease of Z. latifolia. CIAT 9199, however, was resistant at all sites.

Table 11. Disease evaluations of Zornia spp. 1980-1981.

| Site | Sphaceloma scab evaluation | | | | Total |
|---|---|---|---|---|---|
| | R | MR | MS | S | accessions |
| Carimagua[1] | 17.0 | 6.6 | 43.9 | 32.5 | 212 |
| CPAC-LVE[1] | 25.0 | 19.3 | 52.7 | 3.0 | 72 |
| Quilichao | 44.4 | 28.9 | 0 | 16.7 | 54 |
| | Virus-fungus complex evaluation | | | | Total |
| | R | MR | MS | S | accessions |
| CPAC-LVE[1] | 31.9 | 19.4 | 33.3 | 15.4 | 72 |

[1] Collections contain a high percentage of Z. latifolia and related spp.

Bacterial wilt. Over the past year, young plants and mature plants after cutting of accessions of Z. brasiliensis and Zornia sp. CIAT 7847 wilted and often died at Quilichao and Carimagua. Of 73 accessions of Zornia sp. at Quilichao, 13 were found affected in the field. At Carimagua, however, only CIAT 7847 was affected. Cross-sections of lower stems and taproots showed brown coloration of the outer vascular tissue. The bacterium consistently isolated from affected tissues was identified as Corynebacterium flaccumfaciens on the basis of its morphological, cultural, biochemical and physiological characters.

All isolates of the bacterium caused chlorosis, wilting, dieback, and death of young plants of CIAT 7847. In addition, it caused chlorosis and severe wilting of young plants of Phaseolus vulgaris P 635. Cornyebacterium flaccumfaciens is an important pathogen of beans in the U.S.

The bacterium was readily isolated from seed of CIAT 7847 at levels ranging from 75-100% of seed infected. One hundred percent of seed from diseased plants carried the bacterium while 75% of seed from apparently healthy plants in the same plot were affected. The bacterium is able to colonize plants in a symptomless manner. It is also present in the Quilichao soil.

Further studies are in progress to determine the host range of the pathogen, especially among tropical forage legumes, to determine the survival of the bacterium in soil, and to produce clean seed. Survival was studied by locating nylon discs impregnated with bacterial suspensions in nylon bags of soil on the soil surface and at 10 cm depth. Disc samples were taken each week to determine survival by the dilution plate method. Preliminary results suggest that *C. flaccumfaciens* does not survive for long in soil without the presence of plant roots. After three weeks in the field, percent survival at the soil surface was as low as 13.8% and at 10 cm depth was 10.8%. After five weeks in the field, no colonies of *C. flaccumfaciens* were found associated with the nylon discs. Sampling is continuing; however, it appears that this bacterium can survive only in association with plants.

## Diseases of Centrosema spp.

*Germplasm surveys*. Due to increased planting of *Centrosema* spp. in Carimagua and Quilichao during the past year, detailed disease evaluations were made. Diseases detected were Cercospora leaf spot, Anthracnose, Rhizoctonia foliar blight, bacterial blight and Alternaria leaf spot. However, the importance of disease in *Centrosema* depends on the species.

In Carimagua it was found that Cercospora leaf spot has a wide host range affecting all species especially *C. pubescens* (Table 12). Rhizoctonia foliar blight affects *C. brasilianum* severely and *C. pubescéns* slightly while bacterial blight affects *Centrosema* sp. CIAT 5112, 5118 and 5278 moderately (Table 12). Anthracnose and Alternaria leaf spot are presently regarded as minor diseases. *Centrosema macrocarpum* has less disease problems than other species. A similar species-disease pattern was found in Quilichao; however, Bacterial blight was severe on CIAT 5112, 5118 and 5278 and also affected *C. virginianum* and *C. brasilianum*. Due to their severity on particular species of *Centrosema*, the two diseases are being studied further.

Table 12. Diseases commonly associated with species of *Centrosema* in Carimagua.

| Species | CLS[1] | A | RFB | BB | ALS |
|---|---|---|---|---|---|
| *C. brasilianum* | + | + | +++ | | + |
| *C. macrocarpum* | + | | | | |
| *C. plumieri* | ++ | + | | | + |
| *C. pubescens* | +++ | + | + | + | ++ |
| *Centrosema* sp.[2] | ++ | | | ++ | |

[1] CLS = Cercospora leaf spot; A = anthracnose; RFB = Rhizoctonia foliar blight; BB = bacterial blight; ALS = Alternaria leaf spot.
[2] CIAT 5112, 5118, 5278

Bacterial leaf spot and dieback of Centrosema spp. In 1980 and 1981, a previously unreported leaf spot and dieback of young growth was detected on accessions of C. brasilianum, C. plumieri, C. pubescens, C. virginianum and Centrosema spp. at Quilichao and Cariamgua. At Quilichao, leaf spotting and dieback appeared to greatly reduce yield of promising accessions of Centrosema sp. CIAT 5112, 5118 and 5278. This disease, however, was less severe in Carimagua. A bacterium was consistently isolated from affected plants and was identified as a species of Pseudomonas.

The first symptoms expressed were wilting of young leaves and terminals and chlorotic spotting of mature leaves. Young leaves and terminals became partially or completely necrotic and dieback developed. On mature leaves, chlorotic spots became necrotic and were of varying size and shape. Leaves were often crinkled or distorted.

All isolates of the bacterium caused wilting, dieback and necrotic spotting of four-week old plants of Centrosema spp. in pathogenicity tests. The bacterium was also found associated with seed of CIAT 5112 and 5118 at levels of infection ranging from 8 to 32%.

High susceptibility to bacterial leaf spot and dieback appears to be restricted to accessions of Centrosema sp. CIAT 5112, 5118, 5277 and 5278 which are similar morphologically. Although it has been isolated from accessions of five other Centrosema spp., few accessions are more than slightly affected.

Rhizoctonia foliar blight. In the past, Rhizoctonia foliar blight (RFB) was considered a minor disease in Carimagua occasionally attacking Pueraria and Macroptilium. Since 1980, however, RFB has been observed as an important disease of Centrosema brasilianum in Carimagua.

Observations and rating of RFB in two Centrosema brasilianum plantings were made each month during 1981. Levels were found generally high early in the wet season but declined as the wet season progressed, with lowest levels of damage being recorded to date in September. Exceptions were 5173 where damage increased and 5367 where damage remained at a low level. Because rainfall and, probably, relative humidity increased, as disease level decreased, and because RFB is favored by high humidity, climatic conditions failed to explain the decrease in RFB.

One possible explanation is an increase in the population of antagonists of R. solani in soil and on foliage which reduced the population of the fungus. A study of natural antagonists of Rhizoctonia has been initiated. High populations of Trichoderma spp., known antagonists of R. solani, have been found associated with soil and on leaves from plots of C. brasilianum with reduced RFB. In addition, various fungi, bacteria and actinomycetes have been isolated. Antagonism tests with these micro-organisms and R. solani are planned.

Studies are also being made on the pathogenicity of various isolates of R. solani from C. brasilianum, Desmodium ovalifolium and from Phaseolus vulgaris to Centrosema spp. All four isolates of R. solani from C. brasilianum were pathogenic to 4-week-old seedlings of C. brasilianum, C. pubescens 438 and C. macrocarpum 5065 (Table 13). They were also pathogenic to five cultivars of Phaseolus vulgaris. Isolates from D. ovalifolium were slightly pathogenic to Centrosema spp. and Phaseolus vulgaris. Four isolates from Phaseolus vulgaris, however, were variable in pathogenicity.

Table 13. Reaction of Centrosema spp. to isolates of Rhizoctonia solani from Centrosema brasilianum, Desmodium ovalifolium and Phaseolus vulgaris.

| Centrosema spp. | Reaction to Rhizoctonia solani | | |
|---|---|---|---|
| Accession No. | Isolates from C. brasilianum[1] | Isolates from D. ovalifolium | Isolates from P. vulgaris[2] |
| 438 | +++ | +++ | + → +++ |
| 5055 | +++ | ++ | + → +++ |
| 5062 | +++ | ++ | + → +++ |
| 5065 | ++ | ++ | + → +++ |
| 5173 | ++++ | ++ | - → +++ |
| 5178 | ++++ | ++ | + → ++++ |
| 5184 | ++++ | ++ | - → +++ |
| 5234 | ++++ | ++ | - → +++ |
| 5247 | +++ | ++ | - → ++ |
| 5369 | ++++ | ++ | - → +++ |
| 5372 | +++ | ++ | + → +++ |

[1] Isolates from Centrosema brasilianum CIAT 5178, 5211, 5369, 5372, Carimagua

[2] Isolates from foliage of P. vulgaris Restrepo 1981, Huila 486, 1980. Isolates from roots of P. vulgaris I, II

## Diseases of Andropogon gayanus

Rhynchosporium leaf spot. Studies on the effect of Rhynchosporium leaf spot (RLS) on yield of A. gayanus both with and without grazing continued this year at La Libertad, Villavicencio. Results from the 1980 harvest showed no effect of RLS on yield of A. gayanus (Annual Report, 1980). The second harvest was taken in August this year (Table 14). In the medium stocking rate plot, no significant differences were found among the four treatments. Although the highest level of RLS lesions was found in the treatment without grazing and fungicide, it was as low as 8.3 lesions per 100 leaves. As RLS was present as foci of infection, further evaluations during the year may show higher levels of infection. In the high stocking rate plot, the ungrazed treatments significantly outyielded the grazed treatments; however, there were no differences between treatments with and without fungicide. The RLS level in this plot was extremely low.

Table 14. Effect of Rhynchosporium leaf spot on *Andropogon gayanus*. Harvest August 4, 1981.

| Treatment | Dry weight ($g/m^2$) | Moisture content (%) | Lesion No. |
|---|---|---|---|
| **Medium stocking rate** | | | |
| A. Without anim. & fung. | 361 a | 46.9 | 8.3 |
| C. Without anim. & with fung. | 425 a | 53.8 | 2.0 |
| B. With anim. without fung. | 432 a | 43.9 | 3.0 |
| D. With anim. & fung. | 379 a | 38.9 | 2.0 |
| **High stocking rate** | | | |
| G. Without anim. & fung. | 238 b | 53.3 | 1.3 |
| E. Without anim. with fung. | 273 ab | 58.4 | 0 |
| H. With anim. without fung. | 135 c | 55.0 | 0 |
| F. With anim. & fung. | 88 c | 52.7 | 0 |

Harvests will continue every two months. Samples are also being taken to determine forage quality. At present, it appears that RLS is a minor disease of *A. gayanus*. It has been detected on *A. gayanus* at other sites including Carimagua, Quilichao and regional trials in various countries. In all cases, only a few spots have been found. In addition, studies are continuing on the identity of the *Rhynchosporium* which attacks *A. gayanus* and its relation to *Rhynchosporium oryzae* which attacks rice. Isolates of the fungi are being collected from both. Cross-inoculation studies will be made later this year.

## Effect of Different Levels of Various Fertilizers on the Reaction of Tropical Forages to Insect Pests and Diseases

The effects of fertility on disease development and resistance in crops has received much attention in the past. Diseases caused by bacteria, fungi, nematodes and viruses have been shown to be affected by fertilization. For various pathogens, evidence has accumulated that increasing levels of potassium reduced disease levels, while increasing levels of nitrogen increased them.

An experiment was set up in Carimagua in May to determine the effect of different levels of various fertilizers on the reaction of tropical forages to diseases and insect pests, in collaboration with the Entomology Section. With the exception of *Centrosema pubescens* 438, disease levels are low, and trends in the reaction of diseases to different fertilizers are not yet obvious. For *C. pubescens* 438, the highest levels of Cercospora leaf spot were found in zero Mg and high Ca treatments while the highest levels of Rhizoctonia foliar blight were observed in the zero S treatment. It is also planned to assess the most important problem, either disease or pest, in each forage at each fertilizer treatment in collaboration with the Entomology Section.

## Surveys of Diseases and Pests of Native and Naturalized Legumes and Grasses

Periodic surveys of diseases of native and naturalized legumes and grasses are being made in various sites in Colombia and other countries to gain more information about the range and types of pathogens that may affect tropical forages. In Central and South America collections are being made for pathogenicity tests of isolates of *Colletotrichum* spp. mainly from *Stylosanthes* spp. in Colombia.

## Classification of Diseases of Tropical Forage Plants According to Host and Country

During the past year information was collected and collated on diseases of tropical forage plants. The major sources of information were the Commonwealth Mycological Institute, Mycological and Phytopathological Papers, Host-Disease lists from as many tropical countries as possible, including Asia, Africa, Australia, Caribbean, Central America and South America, and miscellaneous papers on the microflora of various countries. This information is being developed into a manual that should provide useful information to plant pathologists and agronomists working with tropical forages.

## Seed Pathology

Surveys of changes occurring in the microflora of *S. capitata* seed in Carimagua and CIAT-Quilichao continued during 1981. Surveys concentrated on *Aspergillus* spp. which are noted for aflatoxin production in seed. After a two-year survey in Carimagua it was found that the percentage of both green and dry seed infected with *Aspergillus* spp. increased toward the end of the wet season and reached a maximum in the dry season before declining as the wet season progressed (Figure 5). Cattle are therefore eating more seed at the time when most seed is infected with *Aspergillus* spp.

Ten different species of *Aspergillus*, including four potential toxin producers, have been isolated from seed since May 1979 (Table 15). *Aspergillus favus* and *A. ochraceous*, both potential toxin producers, were most commonly associated with seed in Carimagua (Table 15). Analysis of toxin production by isolates of both *A. flavus* and *A. ochraceous* associated with *S. capitata* seed is in progress in collaboration with Universidad del Valle.

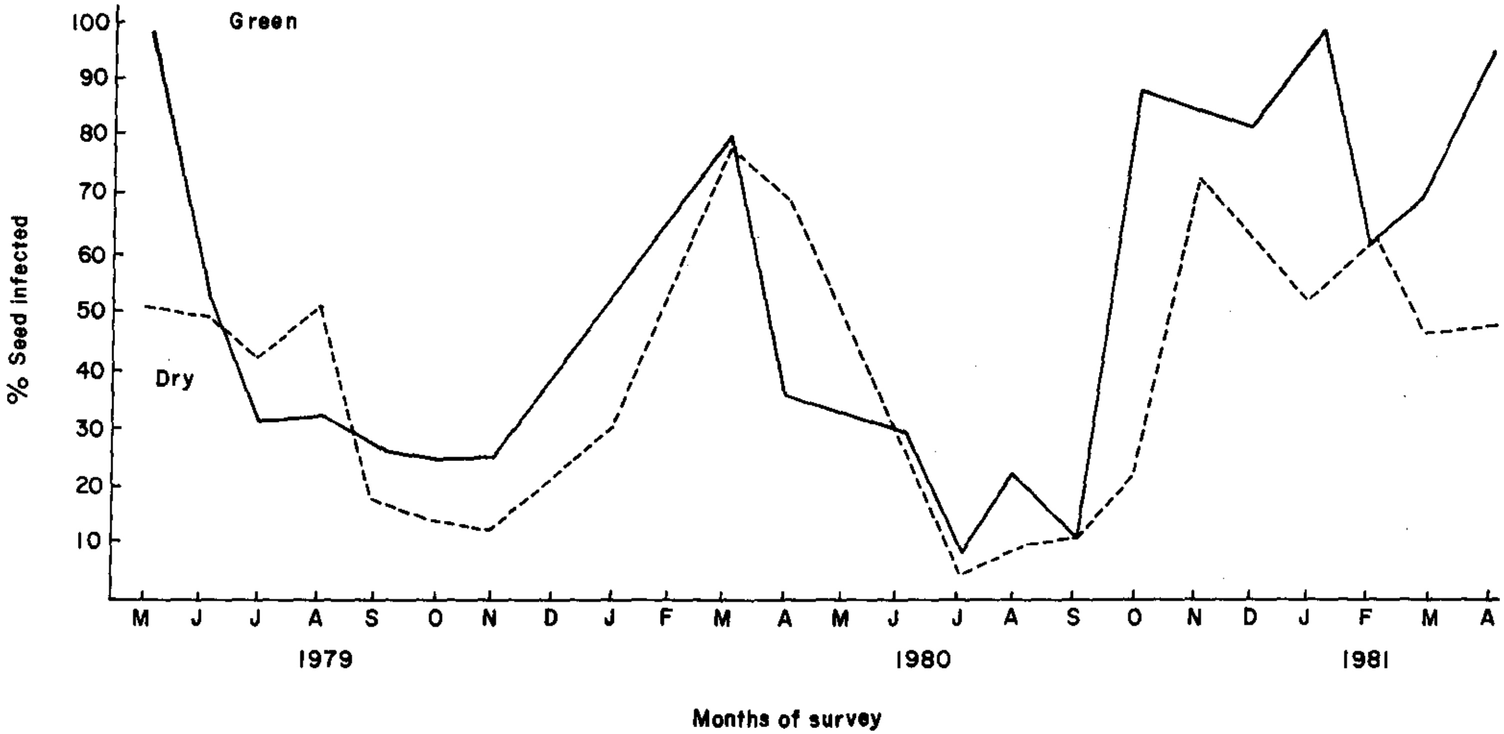


Figure 5. Percentage of dry and green seed of _S. capitata_ infected with _Aspergillus_ spp. in Carimagua.

Table 15. Species of *Aspergillus* associated with seed of *S. capitata* in Carimagua.

| Species | Frequency of isolation (%) | Potential toxin producers |
|---|---|---|
| *A. flavus* | 74 | T |
| *A. ochraceous* | 17 | T |
| *A. niger* | 4 | |
| *A. fumigatus* | 3 | T |
| *A. terreus* | 1 | |
| *A. versicolor* | 0.5 | |
| *A. sydowii* | 0.2 | |
| *A. nidulans* | 0.1 | T |
| *A. chevalieri* | 0.1 | |
| *A. tamarii* | 0.1 | |

## Biological Control of Spittlebug with Entomogenous Fungi

In 1980, a project in collaboration with the Entomology Section was begun on biological control of spittlebug with entomogenous fungi, in particular, *Metarrhizium anisopliae*. Indigenous entomogenous fungi were collected from various pasture evaluation sites in Colombia, including Carimagua, Quilichao, Popayán, Espinal, from infected nymphs and adults of spittlebug and from soil (Table 16). A selective medium was developed to readily isolate *M. anisopliae* from soil. In addition, 35 isolates were obtained from various countries either by collection or request (Table 15). These isolates are being evaluated in pathogenicity and soil survival studies.

Pathogenicity studies were made with 45 isolates of *Metarrhizium* spp. on nymphs and adults of the spittlebug *Zulia colombiana* Lallemand on *Brachiaria decumbens* in pots placed in cages in the greenhouse. Data was taken on death of nymphs and adults, reisolation of fungi and development of adults from inoculated nymphs. Among isolates there was a wide range in percent pathogenicity (Table 15). Of 45 isolates, 25 were rated as pathogenic. Two isolates CAR 7 from Carimagua, Colombia, and FL 11 from Australia, were 100% pathogenic on both nymphs and adults (Table 16).

Studies were also begun on the effect of soil infested with the most pathogenic isolates of *Metarrhizium* spp. on nymphs emerging from soil. Plants of *B. decumbens* were established in trays of fungus-infested soil with four isolates of *M. anisopliae* - CAR 1, CAR 7, FL 11 and FL 12 (Table 17). Emergence of nymphs was irregular and low. This may be due to the methods of placing eggs in the soil and will be further investigated. Pathogenicity to emerging nymphs of infested soil was high for CAR 1 and FL 11.

Table 16. Pathogenicity of isolates of Metarrhizium spp. to nymphs and adults of the spittlebug Zulia colombiana.

| Isolate | Origin | Host | Pathogenicity[1] | |
|---|---|---|---|---|
| | | | Nymphs | Adults |
| BE 1 | Belize | Spittlebug - Adult | 50 | 100* |
| ES 9 | Brazil | Spittlebug - Adult | 50 | 83* |
| MET 1 | Japan | Bombyx mori | 25 | 16 |
| MET 4 | Japan | | - | 50 |
| MET 5 | Japan | Ornebius kanetataki | 75 | 83* |
| MET 6 | Japan | Popillia japonica | 25 | 16 |
| EU 1 | USA | Nemocestus incomputus | - | 50 |
| MET 3258 | New Zealand | Porina sp. | - | - |
| MET 3259 | New Zealand | Black beetle | - | 33 |
| MET 3095 | New Zealand | Costrelytra | 25 | 83 |
| MET 4560 | New Zealand | Rhinoceros beetle | - | 67 |
| CBS 130-22 | Holland | | 25 | 83 |
| CBS 285-59 | Holland | | 25 | 50 |
| CBS 431-64 | Holland | | 50 | 67 |
| CBS 248-64 | Holland | | 50 | 67 |
| CBS 218-56 | Holland | | 25 | 83 |
| CAR 1 | Colombia | Aeneolamia reducta - Adult | - | 100* |
| CAR 2 | Colombia | Soil | 16 | - |
| CAR 3 | Colombia | Aeneolamia reducta - Nymph | 33 | 83 |
| CAR 4 | Colombia | Aeneolamia reducta - Nymph | 83 | 33 |
| CAR 5 | Colombia | Aeneolamia reducta - Nymph | 33 | 50 |
| CAR 6 | Colombia | Mocis sp. - Larva | 16 | 67 |
| CAR 7 | Colombia | Mocis sp. - Larva | 100 | 100* |
| QUIL 1 | Colombia | Zulia colombiana - Adult | 33 | 16 |
| POP 1 | Colombia | Soil | 33 | 16 |
| ESP 1 | Colombia | Soil | 67 | 33 |
| FL 5 | Australia | Telegrillus commodus | 67 | 50 |
| FL 6 | Australia | Rhopaea verreauxi | 100 | 67 |
| FL 7 | Australia | Rhopaea verreauxi | - | 67 |
| FL 8 | Australia | Rhopaea verreauxi | 100 | 83 |
| FL 11 | Australia | Anoplognathus porosus | 100 | 100* |
| FL 12 | Australia | Rhopaea magnicornis | 100 | 83 |
| FL 13 | Australia | Sevicesthis geminata | 67 | - |
| FL 14 | Australia | Sevicesthis nigrolineata | 100 | 33 |
| FL 19 | Australia | Rhopaea verreauxi | 33 | 50 |
| RS 324 | Australia | Austraeris sp. | 83 | 83 |
| RS 435 | Australia | Cricket | - | 50 |
| RS 440 | Australia | Cricket | 16 | 16 |
| RS 445 | Australia | Cricket | 100 | 33 |
| RS 473 | Australia | Soil | 100 | 33 |
| RS 297 | West Samoa | Rhinoceros beetle | 100 | 50 |
| RS 455 | Philippines | Brown plant hopper | - | 16 |
| RS 457 | Philippines | Brown plant hopper | - | 16 |
| RS 485 | Philippines | Brown plant hopper | 100 | 67* |
| RS 487 | Philippines | Brown plant hopper | 50 | 50 |

[1] Pathogenicity determined with three replications of six nymphs and six adults

* Selected isolates for soil survival studies

Due to a lack of suitable sites with high populations of spittlebug during the past year, there was a delay in setting up pathogenicity tests in the field. However, an experiment was set up in Carimagua in September in an infested pasture of B. decumbens and P. phaseoloides. Seven isolates of M. anisopliae selected on the basis of greenhouse pathogenicity tests have been applied to determine their pathogenicity to spittlebug nymphs (Table 18).

Table 17. Pathogenicity to nymphs of spittlebug *Zulia colombiana* of soil infested with *Metarrhizium anisopliae*.

| Isolate | Country of origin | Emergence of nymphs (%) | Pathogenicity (%) |
|---|---|---|---|
| CAR 1 | Colombia | 22.5 | 67 |
| CAR 7 | Colombia | 22.5 | 0 |
| FL 11 | Australia | 30.0 | 75 |
| FL 12 | Australia | 27.5 | 36 |
| Control | | 10.0 | 0 |

Table 18. Survival of isolates of *Metarrhizium* sp. in wet* and dry soil from Quilichao under laboratory conditions.

| Isolate | Origin | Host | Survival after 3 months | |
|---|---|---|---|---|
| | | | Wet soil | Dry soil |
| BE 1 | Belize | Spittlebug - Adult | + | + |
| ES 9 | Brazil | Spittlebug - Adult | + | + |
| MET 1 | Japan | *Bombyx mori* | + | + |
| MET 4 | Japan | | + | - |
| MET 5 | Japan | *Ornebius kanetataki* | + | + |
| MET 6 | Japan | *Popillia japonica* | - | - |
| EU 1 | USA | *Nemocestus incomputos* | + | - |
| MET 3258 | New Zealand | *Porina* sp. | + | + |
| MET 3259 | New Zealand | Black beetle | + | + |
| MET 3095 | New Zealand | *Costrelytra* | - | - |
| MET 4560 | New Zealand | Rhinoceros beetle | + | + |
| CBS 130-22 | Holland | | + | + |
| CBS 285-59 | Holland | | - | - |
| CBS 431-64 | Holland | | - | - |
| CBS 248-64 | Holland | | - | - |
| CBS 218-56 | Holland | | - | + |
| CAR 1 | Colombia | *Aeneolamia reducta* - Adult | + | + |
| CAR 2 | Colombia | Soil | - | - |
| CAR 3 | Colombia | *Aeneolamia reducta* - Nymph | - | + |
| CAR 4 | Colombia | *Aeneolamia reducta* - Nymph | - | - |
| CAR 5 | Colombia | *Aeneolamia reducta* - Nymph | + | + |
| CAR 6 | Colombia | *Mocis* sp. - Larva | + | + |
| CAR 7 | Colombia | *Mocis* sp. - Larva | + | + |
| QUIL 1 | Colombia | *Zulia colombiana* - Adult | + | + |
| POP 1 | Colombia | Soil | - | - |
| ESP 1 | Colombia | Soil | + | + |
| FL 5 | Australia | *Telegrillus commodus* | - | - |
| FL 6 | Australia | *Rhopaea verreauxi* | - | + |
| FL 7 | Australia | *Rhopaea verreauxi* | - | + |
| FL 8 | Australia | *Rhopaea verreauxi* | - | + |
| FL 11 | Australia | *Anoplognathus porosus* | + | + |
| FL 12 | Australia | *Rhopaea magnicornis* | + | + |
| FL 13 | Australia | *Sevicesthis geminata* | + | + |
| FL 14 | Australia | *Sevicesthis nigrolineata* | - | - |
| FL 19 | Australia | *Rhopaea verreauxi* | - | - |
| RS 324 | Australia | *Austraeris* sp. | + | + |
| RS 435 | Australia | Cricket | + | + |
| RS 440 | Australia | Cricket | - | - |
| RS 445 | Australia | Cricket | - | + |
| RS 473 | Australia | Soil | - | - |
| RS 297 | West Samoa | Rhinoceros beetle | + | + |
| RS 455 | Philippines | Brown plant hopper | + | + |
| RS 457 | Philippines | Brown plant hopper | - | - |
| RS 485 | Philippines | Brown plant hopper | - | + |
| RS 487 | Philippines | Brown plant hopper | + | - |

*Soil was wet at the time of inoculation with fungi

The value of any fungus as a biological control agent depends not only on its pathogenicity but also on its survival and persistence in the environment. Studies were therefore begun on survival on _M. anisopliae_ in soil. Firstly, survival of isolates in wet and dry soil from Quilichao was studied under laboratory conditions in petri plates. After three months (Table 19) 28 isolates were readily reisolated from dry soil while 24 isolates could be reisolated from wet soil. Fourteen isolates did not survive in either soil including three isolates from Holland and five from Australia. Of the four isolates originally obtained from soil, three did not survive in Quilichao soil. It is becoming apparent that the ability of _M. anisopliae_ to survive in the soil environment is just as important as its pathogenicity to spittlebug. Of those pathogenic isolates selected for field studies, BE 1, ES 9, MET 5, CAR 1, CAR 7, FL 11 and RS 485, all survived in both soils with the exception of RS 485 in wet soil. Studies are continuing on survival in different soil types.

Secondly, studies were begun on survival of pathogenic isolates in the field in Quilichao and Carimagua. Seven pathogenic isolates (Table 16) were used in both sites, and in Quilichao the isolate Quil 1 was used as a control. Plots of _B. decumbens_ were selected and treatments included four pasture cuttings at heights of 2, 10, 20 and 40 cm, and application of the fungi as powder or in suspension with water. Rate of application was 100 kg/ha of rice/fungus mixture with a spore concentration averaging $10^7$ spores/g of mixture. Soil samples will be taken each month to assess survival of these fungi.