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PROJECT IP-5

Tropical grasses and legumes: Optimizing genetic diversity for multipurpose use

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

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IP-5: Tropical grasses and legumes: Optimizing genetic diversity for multipurpose use

Objective: To identify superior gene pools of grasses and legumes for sustainable agricultural systems in sub-humid and humid tropics.

Outputs: Genetic diversity for quality attributes, for host-parasite-symbiont interactions, and for adaptation to edaphic and climatic constraints, not only for legumes but also for selected grass species. Selected grasses and a range of herbaceous, and shrubby legume evaluated with partners, available to farmers for ruminant production, soil conservation and improvement.

Gains: Defined genetic diversity in selected grass and legume species for key quality attributes, disease and pest resistance and environmental adaptation. Known utility in production systems of elite grass and legume germplasm. New grasses and legumes will contribute to increased milk for children and cash flow for small dairy farmers, while conserving and enhancing the natural resource base.

Milestones:

1998 New *Arachis pintoi* accessions with dry season tolerance, persistence in association with agressive grasses. Shrubby legumes with adaptation to subhumid (*Cratylia argentea*) and humid (*Codariocalyx gyroides*) ecoregions available to farmers.

1999 Gene pools of *Brachiaria* identified with resistance to spittlebug, adaptation to low soil fertility, and adaptation to poor drainage in soils. Methods developed to detect endophytes in *Brachiaria* and *Panicum* species.

2000 Genetic diversity of *Brachiaria, Arachis* and *Cratylia* germplasm characterized using biochemical and molecular techniques.

Users: Governmental, non-governmental, and producer organizations throughout the subhumid and humid tropics that need additional grass and legume genetic resources with enhanced potential to intensify and sustain productivity of agricultural and livestock systems.

Collaborators: National, governmental and nongovernmental agricultural research and/or development organizations. Specialized research organizations (U. Höhenheim; Cornell U., IGER, OFI, CSIRO).

CGIAR system linkages: Enhancement and Breeding (20%); Livestock Production Systems (15%); Protecting the Environment (15%); Biodiversity (40%); Strengthening NARS (10%). Participate in the Systemwide Livestock Initiative (ILRI).

CIAT Project linkages: Genetic resources conserved by SB-1 will be used to develop superior gene pools, using when necessary molecular techniques (SB-2). Selected grasses and legumes evaluated in production systems (PE-5) in collaboration with national partners (SN-2).

PROJECT WORK BREAKDOWN STRUCTURE

Project IP-5. Tropical grasses and legumes: Optimizing Genetic Diversity for Multipurpose Use

Project Purpose

To identify superior gene pools of tropical grasses and legumes based on characterization of genetic diversity in plant attributes that contribute to livestock and agricultural production and to protection of the environment in sub-humid and humid areas

OUTPUTS	ACTIVITIES
Grass and legume gene pools with known quality attributes	• Evaluate the role of anti-nutritional factors in grasses (e.g. saponins) and legumes (e.g. tannins) in digestion and metabolism of ruminant animals • Define genotype x environment interactions on forage quality in *Brachiaria, Arachis* and *Calliandra* germplasm • Define synergisms in quality attributes among contrasting forages • Measure potential milk yield of selected accessions of *Brachiaria, Arachis, Cratylia* and *Calliandra*
Grass and legume gene pools with known diversity in host/ parasite/symbiont interactions	• Study the bioecology of spittlebug species in contrasting environments • Identify host resistance for spittlebug in grasses • Elucidate the role of endophytes in tropical forage grasses • Define interactions between host and pathogen (fungus, bacterium, virus) in *Brachiaria, Arachis* , and *Stylosanthes* • Link information on genetic iversity with biotic constraints in *Brachiaria* and *Arachis*
Grass and legume gene pools with known adaptation to edaphic and climatic constraints	• Identify genotypes of *Brachiaria* and *Arachis* with adaptation to low fertility soils • Identify accessions of *Brachiaria*, *Arachis* and *Calliandra* with dry-season tolerance • Identify accessions of *Brachiaria* and *Paspalum* with adaptation to poorly drained soils • Identify accessions of shrubby legumes that tolerate cool temperature and drought • Define genotype x environment interactions on performance of *Brachiaria* and *Arachis* • Link information on genetic diversity with environmental adaptation of *Brachiaria* and *Arachis*
Superior and diverse grasses and legumes delivered to partners for evaluation	• Develop partnerships with IARC's and NARS in LAC, SE Asia and Africa to undertake evaluation of a range of grasses and legumes for multipurpose use • Selected grasses and legumes for specific ecological niches, based on genotypes x environment interactions • Identify partners to deploy superior grass and legume germplasm in contrasting production systems • Develop expert systems for legume biodiversity by linking geographic information with biological data • Facilitate communication through newsletters, journals and workshops

PROJECT WORKPLAN

Project IP-5. Tropical grasses and legumes: Optimizing genetic diversity for multiple purpose end uses

Project Purpose

To identify superior gene pools of tropical grasses and legumes based on characterization of genetic diversity in plant attributes that contribute to livestock and agriculture production and to protection of the environment in sub-humid and humid areas

	Sub-Project 1	Sub-Project 2	Sub-Project 3	Sub-Project 4
OUTPUTS	**Output 1: Grass and legume gen pools with known quality attributes**	**Output 2: Grass and legume gene pools with known diversity in host/ parasite/symbiont interactions**	**Output 3: Grass and legume gene pools with known adaptation to environmental constraints**	**Output 4: Superior and diverse grasses and legumes delivered to partners for evaluation**
SUB-OUTPUTS	1.1 Role of antinutritional factors in grasses and legumes in digestion and metabolism of ruminant animals identified (CEL) 1.2 Defined environmental 'niches' to grow herbaceous and shrub legumes with tannins as feed resources (CEL) 1.3 Identified synergism in quality parameters among contrasting forages (CEL) 1.4 Known quality and animal production potential of selected grasses and herbaceous and shrub legume species (CEL)	2.1 Host-plant relationships, ecology and population dynamics of spittlebug understood (DP) 2.2 Spittlebug resistance in *Brachiaria* genotypes assessed and characterized (CC) 2.3 Genotypes of *Brachiaria* resistant to spittlebug identified and reconfirmed 2.4 Genetic control and molecular markers identified for spittlebug resistance and apomixis in *Brachiaria* (JWM) 2.5 Role of endophytes in tropical grasses elucidated (SK) 2.6 Interactions between host and pathogen identified for key forage species (SK) 2.7 Information on genetic diversity of *Brachiaria* and *Arachis* linked with biotic constraints (JWM)	3.1 Genotypes of *Brachiaria*, *Panicum* and *Arachis* with adaptation to low fertility soils identified and characterized (IMR) 3.2 Genotypes of *Brachiaria*, *Arachis* and *Calliandra* with dry-season tolerance identified and characterized (IMR, JWM) 3.3 Genotypes of *Brachiaria* and *Paspalum* with adaptation to poorly drained soils identified and characterized (IMR, JWM) 3.4 Genotypes of shrubby legume with tolerance to cool temperatures and drought identified and characterized (IMR, JWM) 3.5 Defined genotype x environment interactions on performance of *Brachiaria* and *Arachis* (JWM) 3.6 Information on genetic diversity of *Brachiaria*, *Arachis* and selected shrub legumes linked with environmental adaptation (JWM)	4.1 Release and deployment to farmers in different production systems of successful grass and legume cultivars through partnership (CEL) 4.2 Defined niches for selected grass and legume cultivars based on analysis of G x E interacitons (JWM) 4.3 Expert systems on forage biodiversity by linking geographic information with biological data (JWM) 4.4 Effective communication of research results through newsletters, journals and workshops (CEL)

Research Highlights in 1997

OUTPUT 1: Grass and Legume gene pools with known quality attributes

- Differences in quality among legume species are not only related to tannins but also to potential degradability of cell wall constituents
- Condensed tannins bound to the forage have a greater effect on digestion than soluble condensed tannins
- Large differences in level and type of condensed tannins between provenance of *Calliandra calothyrsus* grown at contrasting sites.
- Variation in soluble and bound tannins among accession of *Cratylia argentea* and defined the type of tannins present.
- Soluble condensed tannins in leaves of *D. ovalifolium* increased linearly with plant growth up to 6 months and that tannin reactivity with protein also increased to a maximum of 5 months but then declined.
- Quality of *D. ovalifolium* genotypes varied due fertilizer application and environmental conditions defined by soil and climate.
- Level and frequency of feeding of legume-based supplements can significantly increase nitrogen retention in sheep on a low quality basal diet.
- Feeding legume-based energy supplements in the right combination can significantly increase milk yield of grazing cows.
- Acceptability ranking of shrub legumes with contrasting quality was the same with sheep and goats.

OUTPUT 2: Grass and Legume gene pools with known diversity in host/parasite/ symbiont interactions

- Development of a new rearing unit facilitated the initiation of studies on the biology and habits of three major lowland species of spittlebug.
- First-time studies were initiated on vibrational communication behavior among adult spittlebugs.
- Methodologies were established to initiate comparative studies on the development of eggs and the incidence of diapause in spittlebug.

- Six species of spittlebug were found to be associated with lowland pastures in different regions of Colombia.

- In seasonally dry sites in Colombia one species of spittlebug dominates and abundance coincides with the rainy season.

- In wet sites in Colombia three species of spittlebug are sympatric and abundance appears to decline with increased precipitation; the higher species diversity, abundance and rainfall corresponds with increased presence of natural enemies

- Abundance of spittlebug nymphs and adults does not vary between pure *Brachiaria* pastures and *Brachiaria/Arachis pintoi* associations.

- There is evidence that diverging habitat preferences contributes to habitat partitioning between two major sympatric species of spittlebug.

- A new, simpler, faster, and reliable glasshouse technique for screening *Brachiaria* genotypes for resistance to spittlebug was developed, tested, and implemented.

- *Brachiaria* genotypes with resistance to spittlebug were identified using the new glasshouse screening methodology.

- Significant progress was made in the development of a reliable, uniform method of artificial infestation with spittlebug for screening of *Brachiaria* genotypes under field conditions.

- Endophyte detection protocols within plant tissues and seeds using various stains have been further improved for tropical grasses.

- New endophytes, which resemble those reported in temperate grasses, were identified in species of *Brachiaria.*

- Inoculation methods to introduce endophytes in various *Brachiaria* species and accessions were initiated.

- Specific antisera for quick detection of endophytes in grass tissues are being developed.

- Alkaloid profile work is being conducted at Rutgers University on the endophytic fungi isolated from species of *Brachiaria.*

- *Stylosanthes* genotypes with resistance to a wide range of *Colletotrichum gloeosporioides* isolates were identified.

- Differences in host reaction to *Lasiodiplodia theobromae*, the causal agent of dieback disease of *Stylosanthes* spp., were detected.

- Inoculation methods for some foliar diseases of *Arachis pintoi* were developed.

OUTPUT 3: Grass and Legume gene pools with known adaptation to edaphic and climatic constraints

- Root apices of *B. decumbens* accumulate phosphorus in the presence of aluminum and low supply of nutrients in solution which could contribute to aluminum tolerance in low fertility acid soils.

- Two genetic recombinants (BRN093/3204, FM9201/1873) of *Brachiaria* were outstanding in their adaptation to low fertility acid soil conditions.

- *Arachis pintoi* CIAT 18748 was outstanding in it's adaptation to low soil nutrient supply as revealed by the extent of partitioning of N in leaves and in maintaining greater concentration of inorganic P in leaves.

- A number of plant attributes of *Brachiaria* were influenced by genotype and by P supply in the soil.

- Secretion of phytase from roots of *Brachiaria decumbens* and *Stylosanthes guianensis* was found to be an important mechanism for acquiring P from organic phosphorus sources in the soil.

- *S. capitata* is less adapted to a clay loam Oxisol because of thicker roots with fewer root tips which contribute to reduced uptake of calcium when compared to S. *guianensis* and *A. pintoi*.

- Establishment of *A. pintoi* was more rapid when associated with a tussock forming grass, *P. maximum* than with a stoloniferous grass, *B. dictyoneura*.

- Two genetic recombinants of *Brachiaria* were found to have better dry season performance than the commercial *B. decumbens* cultivar.

- *B. brizantha* CIAT 26110 survived in waterlogged soils by producing adventitious roots, stratifying roots along the surface of the water table and by developing aerenchyma tissue in root cortex.

- *B. brizantha* cv. Marandú (CIAT 6780) also survived to waterlogged conditions by developing aerenchyma tissue and the stratification of the roots along the surface of the water table.

- *B. brizantha* CIAT 26110 and *B. brizantha* cv. Marandú survived better under waterlogged conditions than on water-stressed soils, while *B. dictyoneura* cv. Llanero

adapts to either condition by modifying cortical cells into intercellular spaces and by a strong lignification of the vascular cylinder.

- Collections of new *Paspalum* accessions released from quarantine and established in small-plot field trial in CIAT Quilichao to evaluate under waterlogged conditions.

- Recorded differences in DM yields, regrowth capacity, dry season leaf retention, and tolerance to the Psyllid insect of new species and lines of *Leucaena.*

- Found shrub legume species (*Rhynchosia, Calliandra* and *Flemingia*) that perform well in mid altitude hillsides.

OUTPUT 4: Superior and diverse grasses and legumes delivered to partners for evaluation

- Delivered selected forage species to partners through existing Consortia like TROPILECHE and livestock/forage development projects in the region.

- Official release of a blend of *A. pintoi* accessions (CIAT 17434 and 18744) as cv. Maní Forrajero in Panama.

- Release of *A. pintoi* 18744 as cv. Porvenir is planned for May 1998 by MAG in Costa Rica.

- Contracted multiplication of selected grasses and legumes to support a livestock development project in Colombia coordinated by CORPOICA.

- Contracted the multiplication of selected ecotypes of *Arachis pintoi* for on-farm evaluation and to support release in Cost Rica.

- Basic and experimental seed of highly promising ecotypes of *Arachis pintoi, Cratylia argentea* and *Brachiaria brizantha* was multiplied for distribution in Central America.

- Publication of a Newsletter by the Forage Project Team and continued publication of the Journal Pasturas Tropicales.

Progress towards achieving Output Milestones

OUTPUT 1: Grass and Legume gene pools with known quality attributes

List of legume species and genotypes with known quality attributes. Results obtained during 1997 are allowing us to make progress towards being able to produce a list of herbaceous and shrub legumes species on the basis of forage quality attributes. It is now clear that protocols for the assessment of quality of tropical legumes have to consider proper sampling schemes, tannin levels, tannin types, cell wall composition and degradability of cell wall constituents by rumen microorganisms.

Parameters on soil fertility and climate associated with quality attributes. The initial analysis of the G x E study with *D. ovalifolium*, used as a model species, has allowed us to broadly define the effects of environment (soil and climate) on quality attributes. Subsequent analysis of the data collected will allow us to better define the environmental factors associated with differences in forage quality and palatability among genotypes and thus we will be able to recommend specific genotypes of *Desmodium* and possibly other legumes with tannins for a given location.

Regression models to predict milk yield of cows with alternative feed resources. A major objective of our work is to adjust existing feed models to predict milk yield of cows with alternative feed resources. Results obtained during 1997 provide baseline information on interactions between animal responses (N retention, milk yield) and quantity and quality of tropical feed resources, which will be useful for adjusting existing nutrition/feed models.

Level of milk yield increment in cows fed improved grasses and legumes. One of our goals is to be able to define the potential of different legume species to improve performance of ruminant animals in different production systems. Through simple methodologies, such as the short-term intake method, we can now facilitate the selection of legume species to be included in more detailed and costly animal production studies.

OUTPUT 2: Grass and Legume gene pools with known diversity in host/ parasite/symbiont interactions

Genera and species of spittlebug fully identified. Progress has been made in describing genera and species of spittlebug in contrasting environment in Colombia .

Biology of major spittlebug species described. We are gathering information on pest status such as when eggs hatch, when nymphs peak in abundance and when females lay eggs.. Bioecological studies will provide the information necessary to confirm resistance with spittlebug species other than *A. varia*. The best hope for raising the efficiency of cultural, chemical and biocontrol alternatives lies with a detailed understanding of the variation as well as patterns in the spittlebug biology, behavior and ecology.

Reliable glasshouse method for screening *Brachiaria* for spittlebug resistance developed. A new glasshouse method that is 50% faster and requires 70-80% less material resources was developed. With the current level of resources, annual capacity has increased from 200 to 2,000 test genotypes evaluated per year, an extremely significant development for the *Brachiaria* genetic enhancement program. The most important aspect of the new screening method is the improvement in the quantity and quality of the information generated, which not only produces more precise estimates of host-plant genetic resistance, but also allows both tolerance as well as true antibiotic resistance to be assessed simultaneously.

Reliable sampling and screening methods for spittlebug in the field developed. A major challenge, which is already being successfully addressed, is to accurately sample spittlebug and develop improved mass spittlebug infestation methods for field conditions to ensure a more realistic evaluation of host- plant resistance. Progress has been made on the characterization of the spittlebug complex, which is critical for field evaluation of resistance of new *Brachiaria* genotypes.

Model to explain patterns of spittlebug infestation developed. The information on pest status being gathered in different environments will eventually be useful to establish correlations between the arrival, abundance and synchronization of life stages of spittlebug with meteorological data.

Genotypes of *Brachiaria* resistant to spittlebug identified. With the development of a faster and reliable glasshouse screening method, new *Brachiaria* genetic recombinants with antibiotic resistance have been identified.

Heritability estimates of spitttlebug resistance in *Brachiaria* from half-sib family determined. A suitable sexual breeding population now has been assembled. Recent advances in screening methodology for spittlebug make the proposed genetic studies feasible. Appropriate half-sib families will be formed during 1998 for evaluation in 1999.

Known potential to use markers assisted selection (MAS) for spittlebug resistance. This milestone will remain outside of our capacity until significant new funding becomes available. Serious contacts were made during 1997 with the FEDEGAN(Colombia) and with NESTLE Research and Development to fund this line of research.

Detection methods of endophytes in tropical grasses and protocols developed. Detection protocols of endophytes within plant tissues and seed have been further improved. Development of an inoculation method to introduce endophytes in various *Brachiaria* genotypes is the current priority.

Endophytes of tropical origin identified. New endophytes, which resemble those reported in temperate grasses have been identified in species of *Brachiaria.* This

discovery will allow to test the effect of endophytes on adaptation of grasses to environmental stresses, resistance to pest and diseases and animal production.

Isoyzme characterization data integrated with biotic constraint information on a geographical basis. The necessary raw data exist, and await analysis (not a trivial exercise). We are hopeful that a new Senior Research Fellow will be able to dedicate attention to this activity.

OUTPUT 3: Grass and Legume gene pools with known adaptation to edaphic and climatic constraints

Improved methodology to screen forage grasses and legumes for adaptation to low fertility soils. Using a simulated acid soil stress solution, we have found that the outstanding adaptation of *B. decumbens* to infertile acid soils could be due to its ability to accumulate phosphorus in root apices. Further evaluation of this phenomenon will help us to develop simple screening methodology to assess acid soil adaptation differences among genetic recombinants and germplasm accessions of *Brachiaria*.

In collaboration with Hokkaido University in Japan, we also found that the extent of exudation of phytase from roots is an important plant attribute that contributes to adaptation of forage grasses and legumes to low supply of phosphorus. We now need to evaluate genotypic differences in this plant attribute so that we can develop a screening methodology to identify grass and legume genotypes with superior adaptation to low P soils.

Glasshouse and field studies have shown that two plant attributes are critical for adaptation to low fertility acid soils. These are leaf area production and root system development. We are testing nondestructive methods to estimate leaf area production under field conditions. We need to test the usefulness of minirhizotrons to identify genotypic differences in root development, particularly in forage legumes.

List of accessions of *Brachiaria* and *Arachis* for specific environments. A small number of elite *Brachiaria* accessions has been identified from multilocational trial data. Following complete data analysis from multilocational trials, definitive lists of elite *Arachis* accessions can be prepared. However, based on preliminary observations a list of accessions of *Arachis* accessions has been elaborated for seed multiplication and further evaluation.

List of accessions of *Brachiaria*, *Arachis* and *Calliandra* with tolerance to dry season. Agronomic trials of *Brachiaria* (11 sites) have been established, two series of trials of *Arachis* accessions have been completed and a *Calliandra* collection from OFI is being evaluated in the Qulichao station. Analysis of existing data from multilocational trials will help us identify accessions with particularly outstanding performance during periods of moisture stress.

List of accessions of *Brachiaria* and *Paspalum* selected for poorly drained soils. A new collection of *Paspalum* was established at the Quilichao station and *Brachiaria* accessions were included in multilocational trials in Colombia for initial observations on performance under poorly drained conditions.

List of accessions of shrub legumes selected for tolerance to cool temperatures. Results to date from two trial sites in hillsides of Cauca suggest several shrub legumes, which warrant seed multiplication for further on-farm testing.

OUTPUT 4: Superior and diverse grasses and legumes delivered to partners for evaluation

Number of collaborative projects with NARS on evaluation and utilization of new grasses. Through the establishment of linkages with on-going on- farm projects like TROPILECHE (Costa Rica, Peru, Nicaragua and Honduras), NESTLE (Caquetá, Colombia) and Plan de la Modernización de la Ganaderia (Colombia), the elite forage germplasm selected by our project has now reached or is in the process of reaching farmers fields. In addition, *A. pintoi* was released in Panama and there are plans for release of a selected genotype of *Arachis pintoi* in Costa Rica during 1998.

List of forage accessions for different agroecologies. We have now completed multilocational trials with *Desmodium ovalifolium* and *Arachis pintoi* and results are being analyzed to determine GxE interactions.

Number of institutions and individuals receiving/contributing to publications on forage research. Through a Newsletter published by the Forage Project and through the continued publication of the Journal Pasturas Tropicales we are accomplishing the objective of informing a large number of partners in the region the progress achieved in forage research carried at CIAT and other institutions.

Output 1: Grass and legume gene pools with known quality attributes

Suboutput 1.1 Role of anti-nutritional factors in grasses and legume in digestion and metabolism of ruminants identified

A large number of tropical herbaceous and woody legumes species have high levels of condensed tannins (CT), which are known to negatively affect intake, digestion and nitrogen utilization by ruminants. To develop screening procedures and to better define strategies for utilization of these legumes in feeding systems we need to understand how CT and other antiquality factors affect the nutrition of ruminants.

Over the past two years we have collaborated with IGER (Institute of Grassland and Environmental Research) in the UK through a project (Amelioration of antinutritional factors in Tropical Legumes) funded by DFID (Department for International Development) and carried out by a Ph.D. student (R. Barahona) in the U of Reading. In addition, during 1997 we initiated collaboration with OFI (Oxford Forestry Institute) in the UK through a project to investigate factors affecting the nutritive value of *Calliandra calothyrsus* leaf as browse for ruminants which is also funded by DFID.

To carry out the research, a range of contrasting legume species (*Desmodium ovalifolium, Flemingia macrophylla, Leucaena leucocephala, L. pallida, L. macrophylla, Calliandra calothyrsus, Clitoria fairchildiana* and *Cratylia argentea*) have been included in vitro experiments to study the effect of CT and cell wall composition on forage quality.

Our previous results had show differences among legume species in: a) proportion of soluble and bound tannins, b) extent of digestion and end products of fermentation by rumen microorganisms, and c) condensed tannin (proanthocyanidins) types as indicated by different ratios of cyanidin, delphinidin, pelargadinin and fisetinidin.

Activity 1.1.1: Studies on the influence of condensed tannins from a range of herbaceous and shrub legumes on forage quality

Highlights

- Differences in quality among legume species are not only related to tannins but also to potential degradability of cell wall constituents

- Condensed tannins bound to the forage have a greater effect on digestion than soluble condensed tannins

Non-starch polysaccharide (NSP) composition in legumes and losses after fermentation (R. Barahona, M. Theodorou, P. Morris, E. Owen and C. Lascano)

Rationale: Previous research at CIAT with tropical tanniniferous legumes had suggested that in

understanding factors related to nutritive value of such legumes there was the need to study not only tannin composition, but fiber composition as well. It has also been suggested that gravimetric methods for determining dietary fiber such NDF and ADF can result in variable losses of one or more of the NSP components and lead to underestimation of the dietary fiber present in a given feed. Techniques that measure the individual constituent monomers of the NSP fraction do so without losses of those constituent groups.

Methods: Content of NSP was measured in mature and immature leaf samples of *Desmodium ovalifolium, Flemingia macrophylla, Leucaena leucocephala, Leucaena pallida, Leucaena macrophylla, Calliandra calothyrsus* and *Clitoria fairchildiana* both pre- and post-incubation with rumen microorganisms using the pressure transducer technique (PTT) developed at IGER. Neutral sugars in NSP were measured using a Varian 3400 chromatograph fitted with an automatic sampler (Varian 8000) and a flame ionization detector, linked to a Dell PC with Dionex A1-450 integration software. Uronic acids in the hydrolysates were determined by a calorimetric method.

Results: Results obtained in the determination of NSP composition are shown in Table 1. The range of NSP content varied from 109 mg/g in immature *L. pallida* to 269 mg/g in immature *D. ovalifolium*. Composition of the NSP differed greatly among legume species, although glucose and uronica acids were the most abundant cell wall carbohydrate constituents in all samples, their

Table 1. Non-starch polysaccharide (NSP) content and composition of mature and immature (terminal) leaves from seven tropical legumes (mg/g dry matter).

Sample	Maturity	NSP component							Total
		Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose	UAC	NSP_L
D. ovalifolium	Immature	0.0	7.0	26.2	14.6	7.7	174.2	39.3	268.9
	Mature	0.0	6.9	25.3	11.6	6.5	154.0	33.7	238.1
F. macrophylla	Immature	0.0	9.9	24.4	5.7	9.8	123.4	34.3	207.5
	Mature	0.0	9.8	26.4	6.2	9.4	122.2	32.9	206.9
L. leucocephala	Immature	6.1	10.4	6.4	3.5	11.4	40.0	45.5	123.3
	Mature	6.5	10.3	5.5	2.8	11.6	37.8	45.6	120.1
L. pallida	Immature	1.2	10.2	3.6	2.8	11.5	35.8	44.1	109.1
	Mature	3.9	9.5	4.6	2.9	11.2	45.9	47.5	125.5
L. macrophylla	Immature	0.0	17.3	29.4	4.2	10.8	98.7	40.4	200.7
	Mature	0.0	17.2	29.2	3.4	10.6	86.9	35.7	183.0
C. calothyrsus	Immature	3.6	7.5	4.4	4.6	9.8	55.0	34.7	119.6
	Mature	5.5	5.4	4.7	2.9	7.3	45.5	37.5	108.7
C. fairchildiana	Immature	0.0	15.0	29.1	4.9	13.8	126.1	28.5	217.2
	Mature	0.0	14.3	27.3	4.3	10.0	122.1	35.1	213.1

NSP components (neutral sugars) were determined by gas chromatography as described by Englyst and Cummings (1984). Uronic acids (UAC) were determined by the colorimetric method of Scott (1979). Total NSP were calculated by summing neutral sugars and uronic acids.

combinantion accounting for ca 67-79% of the NSP. Content and composition of the NSP was similar between *L. leucocephala, L. pallida* and *C. calothyrsus*. In contrast, content and composition of NSP from *L. macrophylla* differed greatly from that of the other *Leucaena* species, especially in what refers to glucose, xylose and arabinose content. There appeared to be no major differences among legumes in the content and composition of NSP due to maturity, except in the case of *D. ovalifolium*.

The loss of total NSP (NSP_L; mg/g of NSP) after incubation with rumen microorganisms for 144 h varied among legumes, ranging from 206 in mature *F. macrophylla* to 711 in immature *L. leucocephala* (Table 2). There was a positive relationship between NSP_L and gas pool at 144 h ($r = 0.73$) as determined by the pressure transducer technique. Losses of NSP from *L. leucocephala* and *L. pallida* did not differ greatly. Conversely, NSP_L from *L. macrophylla* was much lower than that of the other *Leucaena* species. Losses of uronic acids tended to be higher than that of the other NSP constituents. On the other hand, xylose was, on average, the least digestible NSP fraction. However, great variability in the digestibility of NSP constituents was observed among legumes. For example, losses of rhamnose were much greater in the *Leucaena* species than in *C. calothyrsus*. Comparison of the NSP_L on the basis of leaf maturity, showed that immature leaves tended to have greater NPS_L that their mature counterparts in three of the seven legumes studied.

Table 2. Loss of non-starch polysaccharide (NSP_L) components from mature and immature (terminal) leaves of seven tropical legumes (mg/g NSP or NSP constituents).

Sample	Maturity	NSP component							Total
		Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose	UAC	NSP_L
D. ovalifolium	Immature	-----	579	475	564	568	574	504	553
	Mature	-----	588	524	476	543	546	500	535
F. macrophylla	Immature	-----	97	222	8	117	289	432	280
	Mature	-----	-5	147	10	11	196	446	206
L. leucocephala	Immature	866	612	424	617	637	635	846	711
	Mature	717	558	257	485	547	584	855	671
L. pallida	Immature	900	720	522	542	621	617	648	638
	Mature	733	558	502	422	519	649	639	618
L. macrophylla	Immature	-----	291	159	240	282	361	616	368
	Mature	-----	132	130	32	236	338	630	404
C. calothyrsus	Immature	-49	495	-139	147	280	378	468	363
	Mature	26	510	120	285	219	547	522	445
C. fairchildiana	Immature	-----	443	311	310	512	475	522	456
	Mature	-----	299	317	198	260	334	513	353

NSP components (neutral sugars) were determined in samples pre- and post-incubation with rumen micro-organisms by gas chromatography and uronic acids (UAC) were determined by the colorimetric method. Total NSP losses and NSP constituent losses were determined by subtracting the amounts remaining in the residue after 144 h of incubation from those initially present prior to incubation.

***Discussion*:** Greater variability on the loss of NSP and its constituents was observed among legume species than it could be predicted in terms of NSP composition. For example, losses of NSP and NSP constituents (Table 2) from *C. calothyrsus* were much lower than the corresponding losses from either *L. leucocephala* or *L. pallida*, despite the great similarity in NSP content and composition between these three legumes (Table 1). Similar conclusions can be drawn when examining NSP_L from *F. macrophylla* and *C. fairchildiana.* These differences could be related to factors such as side-chain substitution and binding of CT to cell wall components. These findings suggest that the lack of CT in tropical legumes is not guarantee for a superior digestibility of plant cell wall, as demonstrated by the case of *L. macrophylla* which contains no measurable tannins, but has low digestibility.

Fermentation kinetics in tanniniferous tropical legumes (R. Barahona, M. Theodorou, P. Morris, E. Owen and C. Lascano)

Rationale: To better predict nutrient availability to ruminants fed tanniniferous legumes there is a need to determine not only the end-points of digestion (i.e. extent) but also the kinetics (i.e. rates) of microbial degradation of those forages in the rumen. The pressure transducer technique developed at IGER can provide reasonable estimates of rates of forage degradation and it is a technique with tremendous potential as a tool to investigate the key mechanistic processes of forage degradation by rumen microbes.

Methods: In each of two experiments, 1.0-g samples of 7 legumes species were fermented for 144 h in three 165-ml capacity serum bottles according to the pressure transducer technique. Rumen-digesta grab samples were taken from wethers fed grass hay at 8.00 h before the morning feeding, and transported to the laboratory in a pre-warmed (39°C) vacuum flask. Each serum bottle received 10 ml of microbial inoculum, 85 ml of buffer and 4 ml of reducing agent. Gas production data were fitted to the model of France. The equation is in the form, $Y = A\{1 - e^{[-b(t-T) - c(\sqrt{t}-\sqrt{T})]}\}$ where Y is the cumulative gas production (ml), A is the asymptote (i.e. gas pool), T is lag time, b (h^{-1}) and c ($h^{-0.5}$) are decay rate constants. A combined fractional rate (h^{-1}) of gas production (μ) was calculated as, $\mu = b + c/2\sqrt{t}$, where t is incubation time (h).

Results: There were considerable differences among legumes in gas accumulation profiles (Figure 1) with leaves of *L. leucocephala* producing the most gas and leaves of *F. macrophylla* producing the least gas. In only three legumes *(F. macrophylla, L. leucocephala* and *L. macrophylla*), ≥ 50% of the gas pool was produced within the first 24 h of fermentation. There were no clear-cut effects of legume maturity in gas accumulation profiles. In one legume gas pool at 144 h decreased and in another two legumes gas production increased as sample maturity increased. However, gas accumulation within the first 25 h as a proportion of gas pool at 144 h was greater in matures than in immature samples in three of the seven legumes. Moreover, compared to immature samples, gas volumes (ml) were at least 15% greater by 25 h in mature
samples in four of the seven legumes studied.

***Discussion*:** Comparison of gas accumulation data with levels of condensed tannin (see 1996 Annual Report, CIAT) and NSP content (See Table 1) in the legumes studied, suggest that tannins

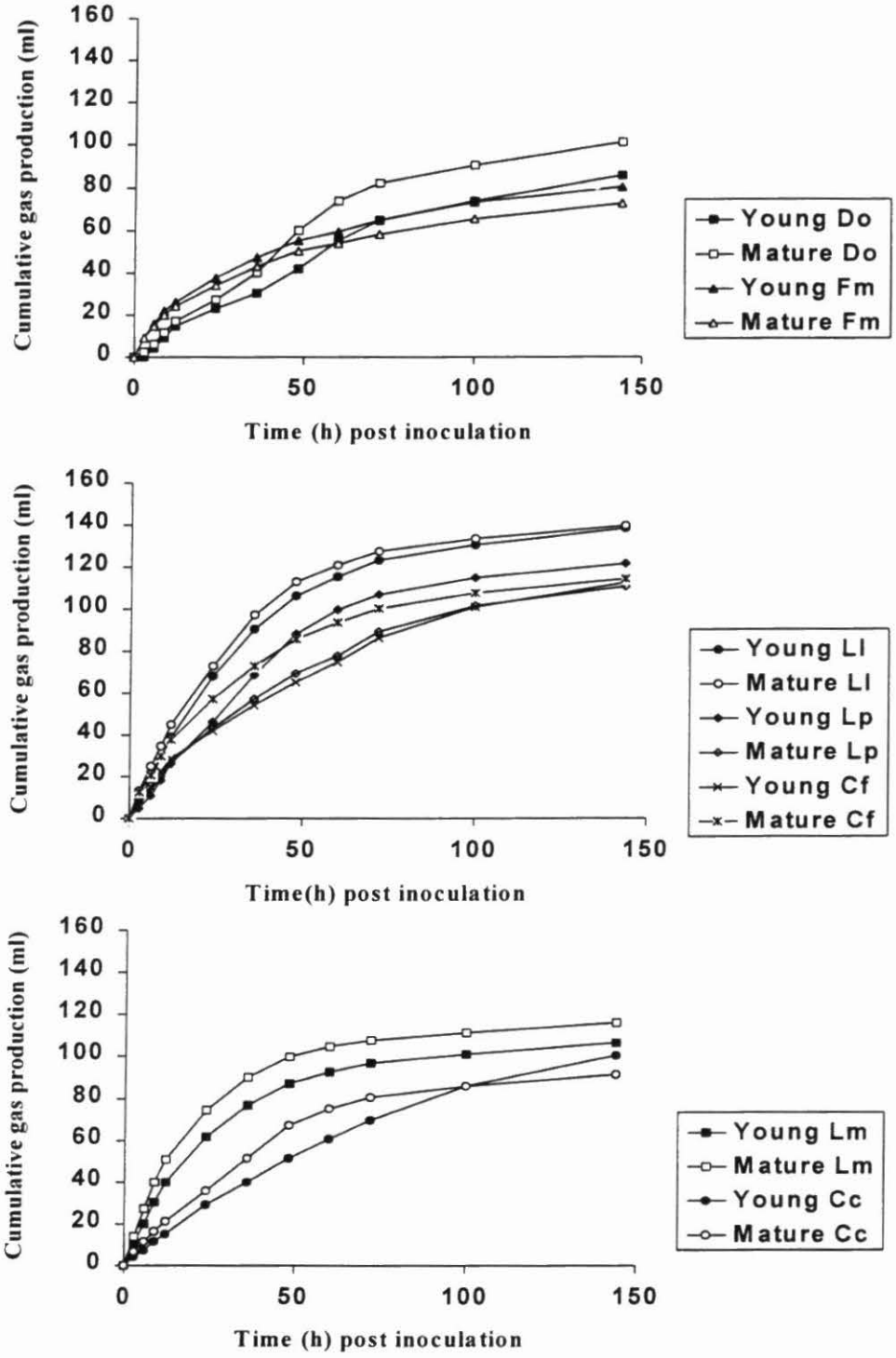

Figure 1. Cumulative gas production profiles from the fermentation of mature and immature samples of -seven tropical legumes (Do= *D. ovalifolium*; Fm= *F. macrophylla*; Ll= *Leucaena leucocephala*; Lp= *L. pallida*; Cf= *C. fairchildiana*; Lm=*L. macrophylla*; Cc= *C. calothyrsus*).

from different tropical legumes have different nutritional effects. For example, the in vitro fermentation of immature *L. leucocephala* and *L. pallida* differed greatly (gas pool at 144 h: 139 vs. 127 ml; in vitro DMD: 62 vs. 44 % of DM), despite similar NSP composition (123 vs. 109 g/ kg) and CT (96 vs. 88 g /kg) content. Likewise, a higher percentage of CT in *L. pallida* was bound to cell wall than it was the case with *L. leucocephala*.

Determination of the kinetics of binding condensed tannins to grass cell wall (R. Barahona, M. Theodorou, P. Morris, E. Owen and C. Lascano)

Rationale: Tannins are known for their capacity to bind to protein, fiber and other molecules. Tannins in plant tissue are found both in soluble (solvent extractable) and bound forms and this distribution changes according to factors such as plant species, plant maturity, plant growth temperature, etc. Traditional methods for determining the biological activity of tannins include a number of protein precipitation assays. Valuable information can be gained by the use of such techniques, although these techniques are mostly limited to what refers to the possible nutritional effects of dietary soluble tannins. Knowledge about the capacity of tannins to bind to grass cell walls would be of great benefit in understanding the nutritional role of CT in ruminants.

Methods: An experiment was carried out to determine the kinetics of binding of condensed tannins (CT) to grass cells from *Festuca arundinacea*. Tannins used had been previously isolated from the legume species included in our studies. Twelve different sources of CT were added to triplicate 25-mg samples of grass cells in five different levels (1, 2, 4, 7 and 10 % of plant cell DM). Cell walls were previously rehydrated in 1 ml of distilled water and CT were added (1 ml) dissolved in a 25% aqueous methanol solution. After vigorous shaking, the mixture was allowed to react for 2 h at room temperature. Then, a further 4 ml of distilled water was added with additional shaking and the resulting mixture was left to stand overnight at 4 °C. The following morning, after shaking and centrifuging at 2500 rpm for 5 minutes, a 2 ml aliquot of the supernatant was collected. The resulting cell wall-CT (CWCT) complexes were recovered by vacuum filtration through 1 layer of Mira cloth and washed generously with distilled water. Recovered CWCT were then frozen to -20 °C and subsequently lyophilized to constant weight. Tannin content was determined in duplicate aliquots of the supernatant (0.5 ml) and of the CWCT complexes (5 mg) using the Butanol- HCl procedure.

Results: We only report the result obtained with CT from *D. ovalifolium* and *F. macrophylla* (Figure 2). Large differences were observed in the binding affinity of CT to cell wall among different tannin types. The highest levels of binding (% of cell wall DM) were observed with CT extracted from young *D. ovalifolium* (range of binding from 0.92 to 4.44 for 1 to 10 of addition, respectively). On other hand, the lowest levels of binding occurred with CT extracted from young *F. macrophylla* (range of binding from 0.19 to 2.12). There were also differences in the binding of CT to the cell walls due to the maturity of the sample. In 4 of the 6 legume species, CT extracted from mature leaves had higher level of binding than CT extracted from their immature counterparts. The opposite was the true in the case of tannins extracted from *D. ovalifolium* and *C. fairchildiana*.

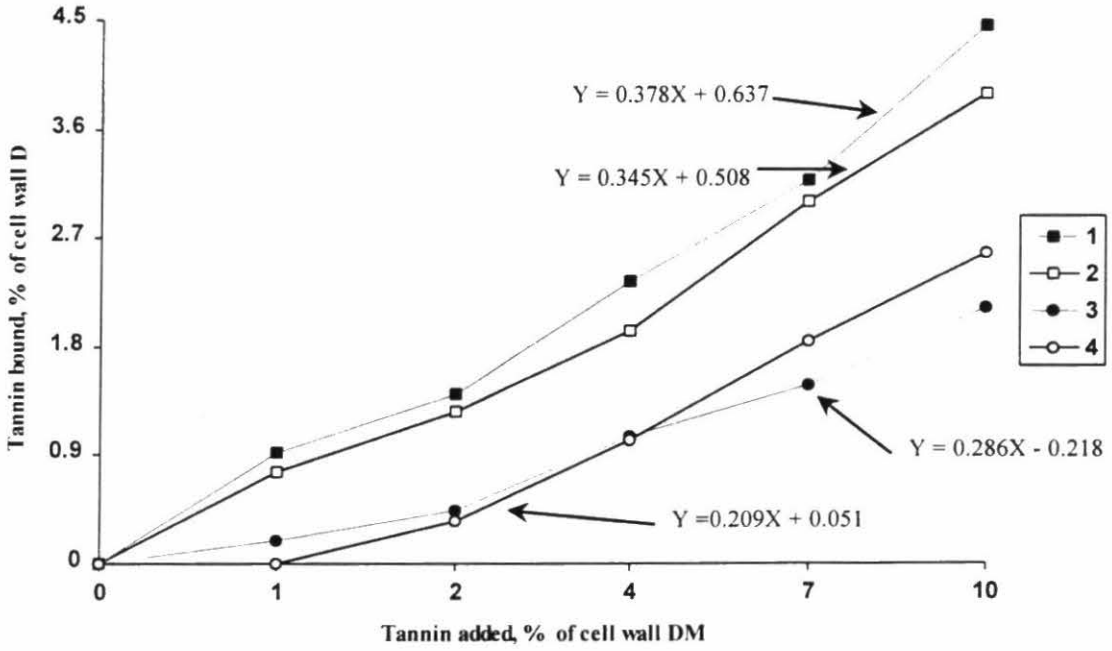

Figure 2. Kinetics binding of condensed tannins (CT) to grass cells from *Festuca arundinacea*. Sources of CT were 1= young *Desmodium ovalifolium*, 2= mature *D. ovalifolium*, 3= young *Flemingia macrophylla*, 4= mature *F. macrophylla*

Discussion: These results corroborate previous findings that indicated that CT from *D. ovalifolium* are more biologically active than CT from *F. macrophylla*, which could be associated with a higher cyanidin: delphinidin ratio in the chemical structure of the tannin fraction. However, it should be pointed out that in temperate legumes the reactivity of CT with protein has been associated with increasing delphinidin: cyanidin ratios and/or molecular weight of tannins (i.e. the higher the MW the lees reactive). The interactions between tannin types and molecular weights with tannin reactivity need to be elucidated for tropical legumes.

Differences in the binding of CT to cell walls were not related to the actual levels of bound CT observed in lyophilized leaves. For example, the level of bound CT in leaves is higher in *F. macrophylla* than in *D. ovalifolium*. Two reasons can be cited in explanation for this disagreement: First, extractability of tannins in 70% aqueous acetone might not reflect the actual solubility of CT in other, more natural solvents. Second it has been suggested that CT in intact plant cells are contained in vacuoles. Hence, tannin extractability in acetone might only reflect this compartmentalization and not be related to CT binding ability.

Effect of soluble and bound condensed tannins on fermentation by rumen microorganisms
(R. Barahona, M. Theodorou, P.Morris, E. Owen and C. Lascano)

Rationale: Although the boundary between extractable and bound CT fractions might appear to be arbitrary, it is evident that both CT fractions do exist in plant tissue. The fact that in most

legumes there is a tannin fraction that remains associated with either the protein and/or fiber components of the cell wall after extraction with 70% aqueous acetone is indicative of such occurrence. It can be hypothesized that by virtue of differences in positioning and of flexibility for interaction with other molecules, soluble and bound CT differ in their mode of action and in their biological significance. However, relatively little is known about the relative nutritional impact of these CT fractions.

Methods: Three different gas production experiments were conducted to model the nutritional effects of different concentrations of extractable and bound CT. All PTT runs were carried out in triplicate in 75-ml capacity serum bottles, each receiving 42.5 of digestion medium, 2 ml of reducing solution and 5 ml of rumen fluid inoculum. The purified CT used in all PTT runs reported in this section were obtained from *D. ovalifolium*

Experiment 1: *Soluble condensed tannins and D-glucose fermentation*
Two PTT runs were carried out to examine the effects of different additions of CT on the fermentation of D-glucose by rumen micro-organisms. In the second run, triplicate treatment flasks received 0 (control), 200, 400, 800, 1400 and 2000 g of CT/kg of D-glucose and the flasks were incubated at 39°C period of 30 h. The main carbon source present in the digestion medium was D-glucose. Media were prepared so that, upon mixing of all constituent solutions, the final concentration of D-glucose was 0.25 g per liter of medium.

Experiment 2: *Soluble condensed tannins and grass cells fermentation*

In this experiment, 0.5 ml of solutions containing CT dissolved in 50% aqueous methanol were added to serum bottles containing intact (zero bound CT) *Festuca arundinacea* cells. Final concentrations of CT obtained were 0 (control), 6.25, 12.5, 25, 50, 87.5 and 125 g/ kg of plant cells DM. In this PTT run, samples were allowed to ferment for 144 h.

Experiment 3: *Bound condensed tannins and grass cell fermentation*
In this experiment, we examined the fermentation kinetics of the different CWCT complexes obtained in previous studies. Weight of the sample incubated ranged between 100 to 200 mg per serum bottle and content of bound CT ranged between 0 (control) to 60 g kg^{-1} of plant cells DM. The fermentation period was of 120 h.

Results: For ease of comparison, we express gas accumulation in each pressure transducer run as a percent of their respective control treatments. Such comparison of percent gas accumulation by 24 h post-inoculation is depicted in Figure 3. Results show that by far bound CT were more effective in inhibiting fermentation by rumen microorganisms than soluble CT. At a maximum concentration of 60 g /kg of cell wall DM, bound CT inhibited fermentation (gas accumulation) of grass cell wall by about 35%. On the hand, soluble CT at concentrations of 125 g /kg of cell wall DM did not produce any inhibiting effect in the fermentation of grass cells. Much greater ratios of soluble CT to D-glucose (2:1) were required to inhibit D-glucose fermentation by about 40% of control. Results also showed that the inhibition in the fermentation of grass cells caused by bound CT decreased with time (Figure 4).

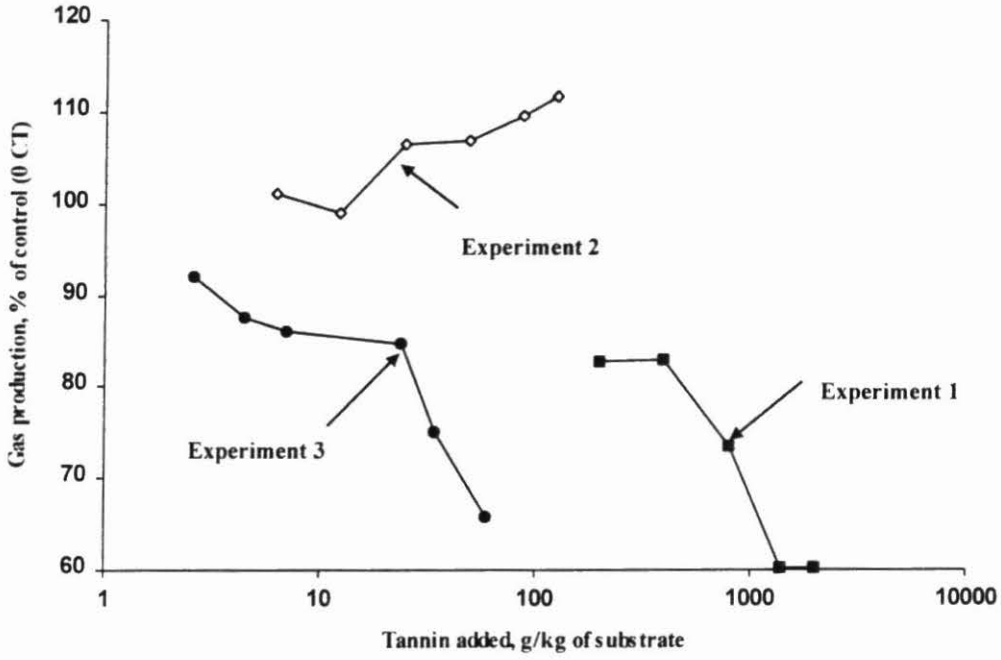

Figure 3. Comparison of the relative effect of soluble vs. bound condensed tannins (CT) from mature *D. ovalifolium*. Experiment 1=Soluble CT, from 0 to 2000 g /kg of D-glucose; Experiment 2=Soluble CT from 0 to 125 g/kg of *Festuca arundinacea* cell walls; Experiment 3=Bound CT from 0 to 60 g/kg of *F. arundinacea* cell walls. Please note the logarithmic scale of the X-axis.

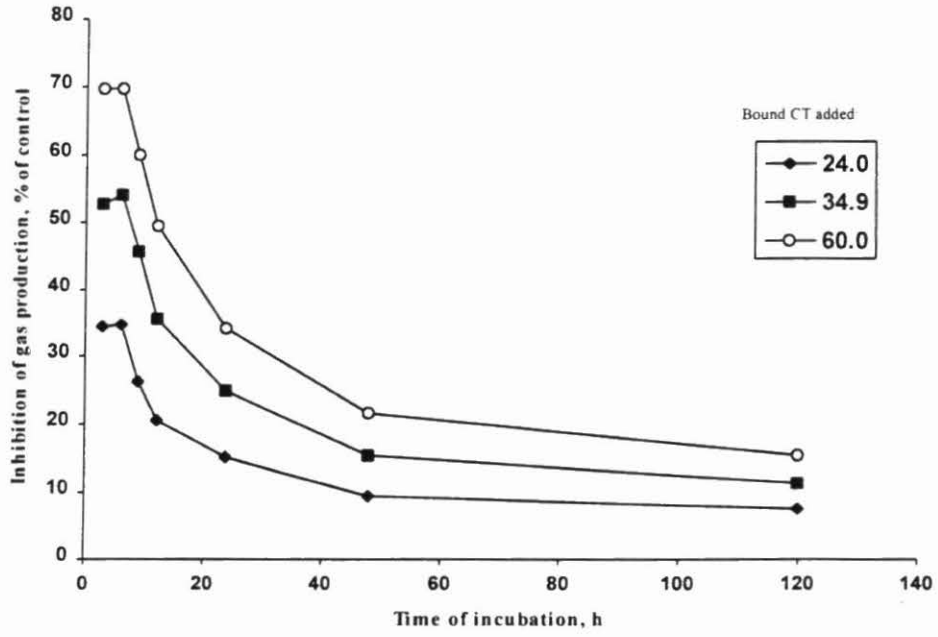

Figure 4. Effect of different amounts of bound tannins from *Desmodium ovalifolium* on the fermentation of whole *Festuca arundinacea* cells. Data is expressed relative to grass cells with no tannin bound to them. Binding of tannins to grass cells was carried out prior to fermentation.

***Discussion*:** Condensed tannins can act as inhibitors of the microbial degradation of DM either by precipitating microbial enzymes, by direct toxicity to rumen microorganisms (membrane integrity) or by simply acting as a barrier against microbial activity (substrate deprivation). Soluble CT, being free to interact randomly with other molecules would be more likely to act in the two first ways. Bound CT, on the other hand, would probably act by simply preventing access to the rumen microbes or their enzymes to the forage DM by forming a "protective" surface. Within that reasoning, our data suggest that by far, substrate deprivation is a more effective way to inhibit DM degradation by rumen microbes. The observed decreases in the inhibitory effect of bound CT as incubation time increased could probably be explained by detachment of CT from the cell wall as incubation time increased. Such a response would lend support to our suggestion of the superior efficiency of bound CT in preventing DM degradation as compared to soluble CT.

In related research, we have evaluated the effect of soluble CT on the activity of microbial enzyme and results shown that there is a significant decrease in enzyme activity in response to the presence of soluble CT. However, higher reductions of enzyme activity were recorded when CT were bound to the substrate than when similar amounts of soluble CT were added to the reaction media.

Activity 1.1.2: Identification of tannins present in selected shrub legumes

Highlights

- Differences in level and type of condensed tannins between provenance of *Calliandra calothyrsus* grown at different sites.

- Variation in soluble and bound tannins among accession of *Cratylia argentea* and defined the type of tannins present.

Quality attributes in provenance of *Calliandra calothyrsus* (N. Narváez, C. Lascano and J. Stewart)

Rationale: *Calliandra calothyrsus* is a tree legume native in Mexico and Central America which is of interest throughout the humid and subhumid tropics owing to its fast growth and its tolerance of soils with low pH. However, reports of its feeding values vary widely apparently due to interactions of post-harvest management of the forage with condensed tannins. Several workers have reported a large reduction in digestibility and intake when the forage is fed dried as compared to fresh. The mechanism of this change is probably related to changes in polymerization of tannins present. On the other hand, very little is known about the environmental effects on Calliandra quality. Thus a collaborative OFI/CIAT project is underway to study the effect of provenance, environment and management on quality of Calliandra forage.

Methods: Two provenance from Guatemala (Patulul-CIAT 22316) and Nicaragua (San Ramon-CIAT 22310) of *Calliandra calothyrsus* selected in OFI on the basis of different forage quality

(i.e. digestibility and tannins) were planted during 1996 in Palmira and Quilichao. Forage from each provenance harvested at the two sites will be used in 1998 for feeding trials with sheep at the Quilichao Research Station. For preliminary chemical characterization we harvested and freeze dried immature leaves from the two provenance and results are reported bellow.

Results: Results indicate that soluble CT in *Calliandra* were very high and varied more between sites than between provenance (Table 3). Leaves harvested at Quilichao had almost half the amount of soluble CT than leaves from Palmira. Differences between provenance in soluble CT were observed at Quilichao but not at Palmira. However, it was interesting to note that differences between provenance in vitro digestibility were not related to soluble CT but rather to cell wall (NDF and ADF) content.

Table 3. Quality of young leaves of two provenances of *Calliandra calothyrsus* grown at two

Provenance (CIAT No.)	CP	IVDMD	NDF	ADF	SCT	ICT
			(%)			
			Quilichao Site			
San Ramón (22310)	21.3	36.8	25.7	20.3	15.8	12.2
Patulul (22316)	17.2	22.2	35.6	29.3	10.5	10.9
			Palmira Site			
San Ramón (22310)	16.8	36.1	25.1	20.7	27.9	15.1
Patulul (22316)	18.2	38.7	22.8	20.2	31.9	13.4

We also examined the types of soluble CT present (i.e. proanthocyanidin ratios) in the two *Calliandra* provenance and found that cyanidin was in higher proportion than delfinidin, with the exception of CIAT 22316 grown at Quilichao (Table 4).

Table 4. Proanthocyanidin ratios in purified condensed tannins from two provenances of *Calliandra calothyrsus* grown at two sites.

Provenances (CIAT No.)	Proanthocyanidin ratios	
	Cyanidin	Delfinidin
	Quilichao Site	
San Ramón (22310)	89.8	10.2
Patulul (22316)	24.9	75.1
	Palmira Site	
San Ramón (22310)	88.7	10.0
Patulul (22316)	74.4	25.6

Discussion: These initial results confirm the idea that tannin production in legumes is closely associated with growing conditions. For reason not clear, the two provenance of *Calliandra* established faster and grew better at Quilichao than at Palmira. On the other hand, it is evident that tannins in *Calliandra* have a high cyanidin : delphnidin ratio, which as indicated could mean that tannins in *Calliandra* are very reactive with protein. However, it is not clear of why the shift in cyandin: delphinidin ratios in the provenance grown at Quilichao as compared to Palmira.

Levels and types of condensed tannins in *Cratylia argentea* (N.Narvaez and C. Lascano)

Rationale: *Cratylia argentea* is a shrub legume with tremendous potential as a dry season protein source in livestock systems in subhumid areas. Over the past years we have been studying quality attributes in different accessions of *Cratylia* and had come to the conclusion that immature leaves were poorly consumed due to some unknown antiquality factor. However, recent evidence suggests that low intake of immature *Cratylia* could be associated with condensed tannins. Our previous screening for tannins in *Cratylia* had failed to show presence of these secondary compounds because we were using aqueous methanol as a solvent rather than aqueous acetone. This latter solvent seems to be more appropriate when dealing with plants that have low levels of tannins as is the case with *Cratylia*. Given the presence of tannins in *Cratylia*, we were interested in determining what levels and types of tannins were present in different accessions.

Methods: Immature leaves from eight accessions of *Cratylia argentea* were harvested from plots in Quilichao and freeze -dried for tannin analysis. Purified tannins were obtained using sephadex columns and tannins from samples were extracted using a 70%aqueous acetone solution. Tannin level and type (proanthocyanidin ratio) were determined using Butanol-HCl and HPLC, respectively.

Results: There was considerable variation among accession in soluble and bound CT levels. Soluble CT ranged from 0.5 to 3.5 % of DM, while bound CT ranged from 4.1 to 7.8% of DM. On the other hand, the predominant tannin type in *Cratylia* accessions was delphinidin followed by cyanidin (Table 5).

Table 5. Level and type of soluble condensed tannins in immature leaves of different accessions of *Cratylia argentea* grown at Quilichao.

Accession	Condensed tannins (%)*		Proanthocyanidin ratio		
(CIAT No.)	Soluble*	Bound	Cyanidin	Delfinidin	Unknown
18676	0.5	3.9	15.1	84.9	---
18516[1]	0.6	7.8	13.7	81.5	4.8
18667	1.1	4.1	19.7	76.9	3.4
18675	1.2	4.2	24.8	75.2	---
18674	1.4	4.7	27.4	68.5	4.1
18666	1.7	7.0	14.4	81.9	3.7
18557	3.3	4.3	18.9	74.5	6.6
18671	3.5	5.9	15.5	77.0	7.5

*Tannin extraction using 70% aqueous acetone solution.

Discussion: Our results show that levels of CT in *Cratylia* are low, but that there is scope for selecting accessions based on level of soluble and bound tannins. On the other hand, it is clear that highest proportion of CT in *Cratylia* are bound (73% of the total), which could be associated with the medium digestibility (45 to 55%) levels commonly found in leaves. It is also interesting to note that the mayor tannin fraction found in *Cratylia* was delphinidin, which is believed to be less reactive with proteins than cyanidin. The low levels of CT and the high delphinidin : cyanidin ratio found in soluble CT present in *Cratylia* leaves is consistent with previous results that indicated that the protein fraction of *Cratylia* is highly degradable in the rumen and consequently results in high levels of rumen ammonia.

Activity 1.1.3: Studies on the effect of plant development on production of condensed tannins in *Desmodium ovalifolium* (J. Martinez, A. Schmidt, N. Narváez, C. Lascano and R. Schultze-Kraft)

Highlight

- Soluble condensed tannins in leaves of *D. ovalifolium* increased linearly with plant growth up to 6 months and that tannin reactivity with protein also increased to a maximum of 5 months but then declined.

Rationale: Even though condensed tannins are found in many tropical legumes, there is limited knowledge on how plant development and growth affects tannin production. This understanding is key for the development of sampling methods for screening tropical legumes with tannins, which is major objective in our work. Thus studies were initiated to determine quality parameters in *D. ovalifolium* at different stages of growth.

Methods: Five ecotypes of *D. ovalifolium* (CIAT 350, 3788, 13110, 13305, 33058) were selected for a greenhouse trial with three replicates per accession. Seeds were pre-germinated and young seedlings transplanted into 40- kg containers, filled with soil from Quilichao. A high level of fertilizer (100 P, 100 K, 500 Ca, 20 S, 2 Zn and 0.5 B, kg/ha) was applied to stimulate plant growth. Throughout the experiment plants were watered frequently to avoid any stress. Sampling (6) began 30 days after seedling emergence and subsequently was carried out every 30 days over a total of 6 months. To determine leaf age, stolons from individual plants were marked in the last emerged leaf 30 days prior to sampling. At each sampling period, young (< 30 days) and stems were separated, freeze-dried and ground for chemical analysis. Variables measured included: plant height, diameter, LAI, specific leaf weight, crude protein, IVDMD, P, S, condensed tannins (soluble and bound), tannin astringency and cell wall (NDF and ADF) content.

Results: Since there were small and non- significant differences among ecotypes in the response variables measured, results presented represent an average of the 5 ecotypes included in the experiment. It was observed that in young leaves soluble CT increased linearly over the 6 month growing period, starting below 1% of DM and reaching up to 6.7%of DM at 6 months (Figure 5). In contrast, bound CT fraction varied little (range: 2.6 and 2.4% DM) with plant development. Consequently, the proportion of soluble CT relative to total CT increased with plant age while the proportion of bound CT decreased.

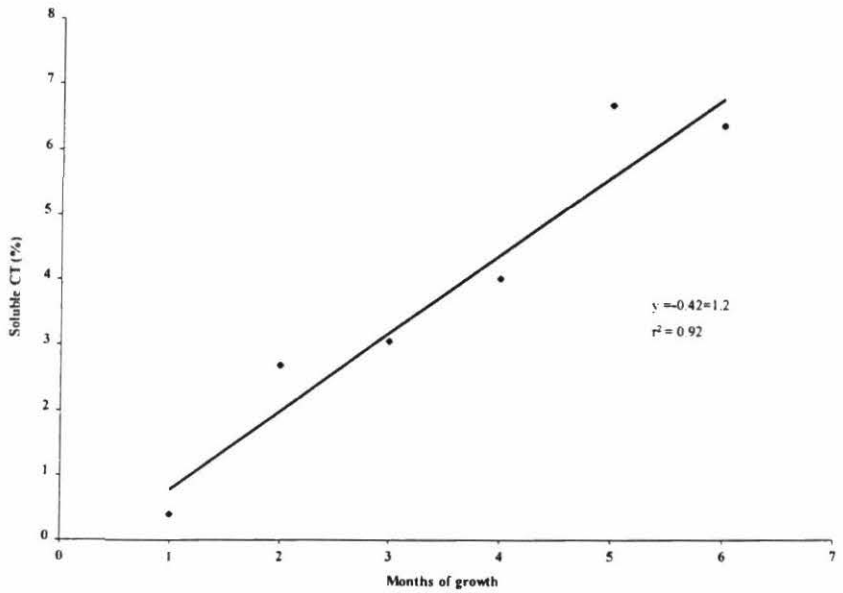

Figure 5. Soluble condensed tannin concentration in young leaves of Desmodium ovalifolium as affected by plant growth after seedling emergence

Tannin astringency also increased during the early stages of growth, but then reached a maximum in 5 month-old leaves and started to decline as indicated by the data fitted with a reciprocal quadratic model (Figure 6).

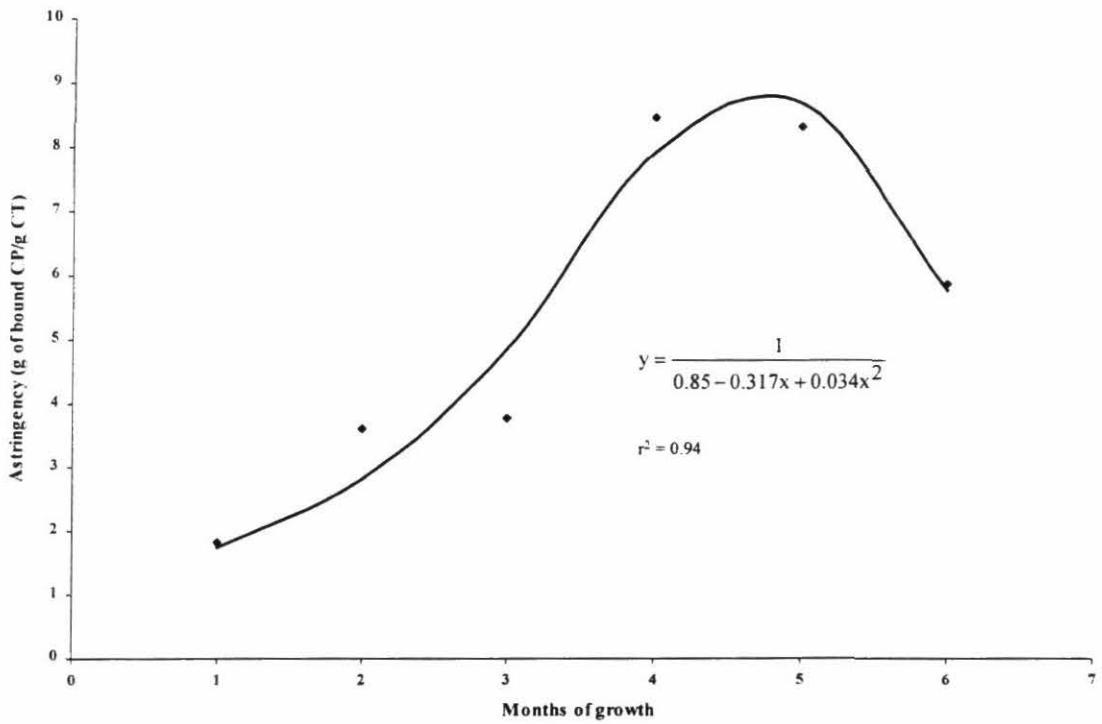

Figure 6. Astringency (reactivity with [protein) of soluble condensed tannins in young leaves of *Desmodium ovalifolium* as affected by plant growth after seedling emergence.

Other quality parameters such as CP and IVDMD decreased as expected with age and IVDMD was found to be negatively correlated (r= -0.8) with level of soluble CT in the leaf tissue. Correlation analysis did not show any strong association between quality parameters and specific leaf weight (thickness) or LAI.

Discussion: Results clearly indicate that *D ovalifolium* starts synthesizing tannins very early in its development, probably as a mechanism to protect young growing tissue from pest and disease. It was interesting to note that tannin astringency, a reflection of tannin reactivity with protein, in young leaves also increased rapidly during the first five months of active plant growth, but then started to drop. This suggests that as plants develop there is changes not only in tannin level but also in tannin chemical structure. More detailed chemical studies, including molecular weights and tannin composition should help understand observed changes in tannin astringency.

The relatively constant level of bound CT in leaves of different age has also been observed in D *ovalifolium* harvested in the field at different ages. However, from our results it is not clear what significance these bound CT have on forage quality.

A conclusion from these results is that considerable care needs to be taken when sampling legumes for tannin analysis. The fact that tannin level rapidly change with plant growth, dictates that sampling needs to be done in leaf tissue of well defined physiological age in order to make valid conclusions about the amount of tannins present in a given legume species.

Suboutput 1.2 Defined environmental "niches' to grow herbaceous and shrub legumes with tannins as feed resources

It is well documented that tannin production in tropical legumes can be affected by growing conditions. Plants grown under stress (i.e. soil nutrient deficiencies, water deficit) have higher tannin levels than when grown under no stress. Thus to be able to define "niches" for particular legume species, it is important to quantify the effect of soil and climatic stress on tannin production and forage quality in general. To accomplish these objectives, a special Project funded by BMZE/GTZ, Germany is being executed by Axel Schmidt (Ph. D student from the U. of Höhenheim). In this Project which has completed its third year, we have been studying G x E interactions on productivity and quality of *D ovalifolium*. A total of 18 genotypes of *D. ovalifolium* were planted in 6 contrasting sites in Colombia, which represent major ecosystems (subhumid and humid hillsides, savannas, and forest margins) and soil texture and fertility. Expected outputs from the Project includes (1) quantification of the effects of changes in soil fertility and climate (rainfall and temperature) on quality parameters of different *Desmodium* genotypes and (2) selection of appropriate Desmodium genotypes for different ecological "niches".

Activity 1.2.1: Studies on the effect of environment on quality of legumes

Highlights

- Gas pressure transducer and NIRS techniques are useful to study changes in quality attributes of legumes due to genotype and environment.

- Quality of *D. ovalifolium* genotype varied due fertilizer application and environmental conditions defined by soil and climate.

Use of the pressure transducer and NIRS techniques in G x E studies (R. Barahona, A. Schmidt, N. Narváez and C. Lascano)

Rationale: Given the large number of samples generated in the U.Honheim/CIAT Desmodium - GxE study, we were interested in evaluating the use of the pressure transducer technique and near infrared reflectance spectroscopy (NIRS) as tools for measuring the nutritional quality of *D.ovalifolium* grown under different environments.

Methods: Two gas production experiments were carried out to examine the nutritive quality of five selected accessions of *D. ovalifolium* (CIAT 350, 3788, 13125, 23618 and 33058) planted in four sites at two fertility levels and harvested during the dry season. The near infrared reflectance spectra of those same 39 samples were recorded with a NIRS systems 6500 scanning monochromator. Predictions equations were developed relating obtained reflectance and gas accumulation data.

Results: Great differences in gas accumulation profiles, which are correlated with digestibility were observed among accessions due to changes in environment, with accession planted in La Rueda (acid soils in forest margins) showing the lowest gas accumulation profiles (Table 6).

Table 6. End-point (144h) cumulative gas production of five *Desmodium ovalifolium* accessions grown in four contrasting sites at two fertility levels.

Accession	Fertilization	Sites			
		Chinchina[1]	Macagual[3]	La Rueda[3]	Alcancia[2]
		Cumulative gas production after 144 h, ml			
350	*Low*	137.9	138.9	118.7	128.2
3788		146.7	139.3	115.7	109.7
13125		136.0	139.1	95.6	107.2
23618		119.7	137.5	92.1	105.3
33058		173.3	167.3	93.9	122.9
Average		*142.7*	*144.4*	*103.2*	*114.6*
350	*High*	150.4	157.9	124.8	180.3
3788		Missing	167.3	112.2	177.1
13125		147.4	160.2	120.7	170.8
23618		138.1	147.2	121.9	175.9
33058		164.9	165.9	102.5	187.0
Average		*150.2*	*159.7*	*116.4*	*178.2*

[1]Hillsides-wet
[2]Well drained savanna
[3]Forest margins

In terms of fertilization, the most dramatic effect on gas production was observed in those accessions planted in Alcancia (well-drained savanna site). In Table 7 we show the parameters describing the fit of the NIRS equations to gas accumulation at different times. Except accumulation by 6 h of incubation, the r^2 for the predicted and observed values was ≥ 0.85.

Discussion: These results point towards the feasibility of using both the pressure transducer and NIRS techniques as tools for measuring the nutritional quality of tanniniferous legumes of different genotype grown in contrasting environments. The implications are enormous in terms of costs and time, particularly for screening large legume collections for forage quality.

Table 7. Statistics for the prediction equations generated by comparison of the near infrared spectra and gas production parameters of samples from 5 accessions of *Desmodium ovalifolium* planted at two fertility levels in four contrasting sites in Colombia, South America.

Parameter [c]	SEC	R^2	F value	SECV
Gas 6 h [a]	1.484	0.699	9.31	1.741
Gas 12 h [a]	2.324	0.853	25.86	3.001
Gas 24 h [a]	5.388	0.911	8.20	7.117
Gas 48 h [a]	8.116	0.928	18.61	9.933
Gas 144 h [a]	6.359	0.942	8.41	8.542
DMD 144 h [b]	2.289	0.898	9.07	3.206

[a] Cumulative gas production at different sampling points.
[b] End-point dry matter disappearance after 144 h.
[c] SEC = Standard Error of the Calibration; SECV = Standard Error of the Cross Validation.

Studies on the effects of environment (soils, climate) on quality of *D. ovalifolium* (A. Schmidt, C. Lascano, N. Narváez and R. Schultze-Kraft)

Rationale: Tannin concentration in plant tissue has been shown to vary with plant species, plant part, plant maturity and soil fertility. The forage quality and acceptability *of D. ovalifolium* by grazing ruminants has been observed to change from one environment to the other and this could be related to changes in CT content. Having defined the effect of soil and climate on production and quality of Desmodium, then it will be possible to better select genotypes for given regions in the tropics.

Methods: A large experiment was set up to evaluate the agronomic performance and quality of a core collection of *D. ovalifolium* in contrasting environments represented by 6 sites in Colombia (Table 8). From April to June, 1995 field trials were established at each site in 108 plots (6 x 5 m each), which represented 18 genotypes, 2 levels of fertility and 3 replications.

Following the evaluation of seedling emergence 15 weeks after planting, the following measurements were made every 6 weeks: plant height and lateral extension, soil cover, plant vigor, pest and disease incidence and flowering pattern.

Table 8. Description of sites in Colombia included in the G x E studies with *Desmodium ovalifolium*.

Site	Lattitude	Longitud	Elevation	Dry season	Soil		
			(m)	stress	Acidity	Al saturation	Fertility
El Melcho –Cauca	02°44'23"N	76°33'34"W	1555	H	M	L	M
La Romelia –Chinchiná	04°58'20"N	75°39'58"W	1360	L	M	L	M
Macagual –Caquetá	01°29'59"N	75°39'33"W	190	L	H	H	L
La Rueda –Caquetá	01°26'10"N	75°25'47"W	180	L	H	H	L
Alcancía –Carimagua	04°34'37"N	71°21'09"W	150	H	H	H	L
Maquenque –Carimagua	04°31'17"N	71°15'41"W	150	H	H	H	L

H= high M= moderate L= low

Throughout 1996 a total of 1224 leaf samples were obtained from the 6 sites in order to perform quality analysis. Following an uniformization cut, samples from 6-8 weeks regrowth were taken during a period of low and high rainfall. Sampling consisted on handpicking 50 g of fresh leaves, which were immediately frozen for subsequent freeze-drying, grinding and laboratory analysis. All samples were analyzed for quality using NIRS with calibration equations derived using traditional laboratory methods.

Results: The data collected in the study is still being analyzed and what is presented is a preliminary assessment of the effect of fertilizer application and site on quality of *D. ovalifolium* averaged across accession. With the application of a high fertilizer level there were increase in IVDMD, CP, P and S in leaf tissue of *D.ovalifolium* but decreases in soluble and bound CT (Table 9).

Table 9. Differences in forage quality of *D. ovalifolium* in relation to low (L) and high (H) fertilizer application (averaged across sites).

Fertility level	ADF	IVDMD	CP	SCT*	BCT**	Tannin astringency	P	S
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
L	35.2 a	40 b	13.9 b	7.7 a	2.7 a	8.8 a	0.14 b	0.27 b
H	34.7 b	45.4 a	15.4 a	6.8 b	2.6 b	7.2 b	0.17 a	0.29 a

a,b Means followed by the same letter are not significantly different ($p < 0.01$) according to Duncan test

*Soluble condensed tannins

**Bound condensed tannins

The overall effect of sites on quality parameters of *D.ovalifolium* is presented in Table 10. At the Chinchina site (fertile soils in humid hillsides) IVDMD values were highest (45.4%), whereas IVDMD in la Rueda site (acid infertile soils in forest margins) were lowest (39.4%). Differences in IVDMD of *D. ovalifolium* leaves were not related to cell wall content. Crude protein also varied among sites, with highest levels recorded in the Macagual site (moderately fertile soils in forest margins) and lowest in the Cauca site (infertile soils in dry hillsides).

Contrary to what was expected, the level of soluble CT was found to change little (range: 6.8 to 7.5% of DM) among sites (Table 10). In contrast, tannin astringency or tannin reactivity with proteins varied considerable among sites, being highest at the Cauca site and lowest at the Macagual site. P and S and levels in leaf tissue also varied among environments.

Table 10. Differences in forage quality of *D. ovalifolium* in relation to sites (average across fertilizer levels).

Sites	IVDMD (%)	CP (%)	SCT[1] (%)	BCT[2] (%)	Astringency (%)	P (%)	S (%)	ADF (%)
Chinchiná	45.4 a	14.7 c	6.8 b	2.56 c	9.2 b	0.16 b	0.21 e	33.6 c
Cauca	40.7 d	13.5 d	6.8 b	3.49 a	10.7 a	0.15 bc	0.26 d	31.7 d
Alcancía	44.1 ab	15.5 b	7.3 a	2.44 d	7.2 d	0.14 c	0.30 b	35.6 b
Maquenque	42.3 c	14.2 c	7.4 a	2.55 c	8.7 c	0.19 a	0.27 cd	34.1 c
Macagual	43.6 bc	16.6 a	7.5 a	2.56 c	6.3 e	0.13 d	0.33 a	35.4 b
La Rueda	39.4 d	13.4 d	7.4 a	2.69 b	6.6 e	0.15 bc	0.28 c	38.5 a

[1]Soluble condensed tannin

[2]Bound condensed tannin

a,b,c,d Means followed by the same letter are not significantly different ($p < 0.01$) according to Duncan test

Discussion: Preliminary results indicate a reduction in tannin content and an increase in IVDMD and CP in *D. ovalifolium* leaves due to fertilizer application. These findings confirm previous results from CIAT in which tannins in *D. ovalifolium* were shown to increase when grown in acid soils deficient in sulfur. On the other hand, it is evident that quality of *D. ovalifolium* varied among sites in terms of forage IVDMD, CP, mineral content and tannin astringency, but not in terms of soluble CT as had been expected. Thus it will be interesting to determine if the observed changes in quality parameters due to site are correlated with palatability of the different *D. ovalifolium* genotypes included in the study.

Suboutput 1.3 Identified synergism in quality parameters among contrasting forages

In livestock production systems new forages are most likely fed in different combinations with existing forage resources. Thus it is important that we understand how introduced forages might best be matched with available forages to overcome nutrient deficiencies in different livestock groups.

To derive some principles on how best to match feed resources a number of experiments have been carried out in the Quilichao research station with sheep in metabolic cages and grazing milking cows. The studies with sheep have received financial support from COLCIENCIAS through a scholarship to Wilson Quiñonez who was responsible for the feeding trials.

Activity 1.3.1: In vivo studies on complementarity among different forage basal diets and supplements

Highlights

- Level and frequency of feeding of forage-based supplements can significantly increase nitrogen retention in sheep on a low quality basal diet.

- Milk yield of grazing cows can be significantly increased by feeding forage-based energy supplements in the right combination.

Studies with sheep in metabolism cages (W. Quiñonez, P. Avila and C. Lascano)

Rationale: Previous results from confined feeding trials suggested that in supplementing available forage sources to overcome nutrient deficiencies in ruminants, it is important to synchronize feeding of the higher quality forage supplement with the basal forage so that energy and protein are available concurrently. On the other hand, synergism among forages may vary not only with the types of forages fed but also with how they are fed (level and frequency).

Methods: Eight African type wethers (24 kg LW) fed a low quality grass basal diet were randomly allocated to 4 treatments of supplementation with sugar cane (60%) mixed with *Cratylia argentea* (40%). Treatments arranged in 4x4 Latin Square design were:

T1- Low level of supplementation (0.5% of BW) fed once a day (AM),
T2- High level of supplementation (1.0%% of BW) fed once a day (AM),
T3- Low level of supplementation (0.5% of BW) fed twice a day (AM+PM) and
T4- High level of supplementation (1% of BW) fed twice a day (AM+PM).

Measurements included quality of the basal diet and supplements offered, intake, digestibility and N balance.

Results: The low quality grass basal diet was low in CP (4.8%) and high in cell wall content (NDF 79% and ADF 44%). On the other hand, shopped sugar cane fed as an energy supplement was low in CP (3.1%) but had low cell wall content (39% NDF and 24%ADF). The legume fed (leaves of *Cratylia*) had high CP (21%) and high levels of cell wall (67%NDF and 37% ADF). Thus the supplement fed was high in energy and medium in protein (10% of DM). Intake of the basal diet did not differ among treatments, but there were differences in supplement intake due to treatments (Table 11).

As expected intake of sugar cane and *Cratylia* tended to be higher when fed at the highest level. However, it is interesting to note that when supplements were offered at the high level, intake of sugar cane and *Cratylia* increased with twice a day feeding relative to once a day feeding (Table 11).

These differences in intake of supplements were not reflected in significant changes in DM or cell wall digestibility. Nevertheless, there was a tendency for higher digestibility with a twice a day feeding at both levels of supplementation.

Table 11. Effect of level and frequency of forage-based supplementation on intake and digestion by sheep fed a low quality grass.

Item	Frequency and level of supplementation*				
	AM** 0.5% BW	AM 1% BW	AM + PM*** 0.5% BW	AM + PM 1% BW	SE
Intake (gDM/kgBW/d)					
Basal diet	25.7	25.2	25.2	25.7	0.8
Sugar cane	2.5 b	3.3 ab	2.8 b	4.0 a	0.4
Cratylia	2.0 c	3.1 b	2.0 c	3.6 a	0.1
Digestibility (%)					
DM	53.0	53.0	55.4	56.6	1.7
NDF	54.1	54.3	57.7	57.1	1.8
ADF	51.6	50.7	54.3	53.1	1.9

*60% sugar cane + 40% *Cratylia argentea* (leaves)
**Supplement fed once a day at 0.5 or 1% of BW
***Supplement fed twice a day at 0.5 or 1% of BW
a,b,c Means different (P<0.05)

As expected, N intake was highest with increased level of supplementation (Table 12); however at the high level of supplementation N intake was higher with twice a day feeding. Since fecal and urinary N did not change with treatment, N retention was greater when sheep were given the forage-based supplements at the high level and twice a day (Table 12).

Table 12. Effect of frequency and level of forage-based supplementation on nitrogen utilization by sheeps fed a low quality grass.

Item	Frequency and level of supplementation*				
	AM** 0.5% BW	AM 1% BW	AM + PM*** 0.5% BW	AM + PM 1% BW	SE
N Intake (g/d)	5.6 a	6.2 b	5.6 c	6.7 a	0.01
Fecal N (g/d)	3.5	3.5	3.3	3.5	0.1
Fecal N, % N intake	62.5 a	57.1 a,b	59.9 a	52.0 b	2.0
Urine N (g/d)	1.4	1.5	1.5	1.6	0.2
Urine N, % N intake	25.4	25.8	28.6	24.7	3.7
Retained N (g/d)	0.8e	1.2 d,e	0.8 e	1.6 a	0.2

*60% sugar cane + 40% *Cratylia argentea* (leaves)
**Supplement fed once a day at 0.5 or 1% of BW
***Supplement fed twice a day at 0.5 or 1% of BW
a,b,c,d,e Means different (P<0.05)

Discussion: With the forage-based supplement fed it was evident that level and frequency of feeding had an effect on nitrogen utilization by growing sheep. However, the results indicate that feeding twice a day would only be justified when high levels of forage-based supplements are offered. When the high level (1% of BW) of sugarcane/*Cratylia* was fed twice a day there was a 33% increase in N retention relative to feeding the same amount of supplement once a day. This was not the case when the low level (0.5% of BW) was offered.

Studies with milking cows under grazing (P. Avila and C. Lascano)

Rationale: In the seasonally dry hillsides of Central America, the major constraint to increasing milk production is lack of suitable forages from the naturalized pastures during the dry season. Cattle are turned loose into remaining natural forest on steep areas of the farms with the result that attempts at re-afforestation have proved difficult. The introduction of leguminous fodder shrubs for use as dry season supplements would assist in overcoming the feed shortage and the reduce overgrazing in steep slopes. Thus a major goal of our work is to determine the degree of complementarity or synergism of forage-based energy and protein supplements with other feed resources on milk yield of cows under grazing conditions and to generate management guidelines for feeding forage-based energy and protein supplements.

Methods: An experiment was carried out in the Quilichao Research Station to study the effect of increasing levels of shrub legume (*Cratylia argentea*) in combination with sugar cane on milk production of cows grazing a low quality pasture. High grade Holstein crosses (4) and low grade Zebu crosses (4)were allowed to graze a *Brachiaria decumbens* pasture using a high stocking rate (2.7AU/ha) and subject to the following supplementation treatments arranged in a 4x4 Latin Square design:

T1- 100% sugarcane
T2- 75% sugarcane and 25% *Cratylia*
T3- 50% sugarcane and 50% *Cratylia*
T4- 25% sugarcane and 75% *Cratylia*

All supplements were offered at 1.5% DM of BW and fed twice a day in the barn after milking Milk yield was recorded for 7 consecutive days in each of the 4 experimental periods, following a 7 day adjustment phase.

Results: A strong interaction of cow genotype x supplementation was observed for milk yield (Table 13). With low grade Zebu crosses there was no response to the inclusion of different levels of Cratylia leaves in the supplement. In contrast, with the high grade Holstein crosses there was a linear response to increasing levels of *Cratylia* in combination with sugarcane. With the combination of 25% sugarcane and 75% *Cratylia* there was a 25% increase (1.6 liters/cow/d) in milk yield relative to feeding only sugar cane.

Discussion: We confirm once again that milk yield responses due to improved feed resources is dependent on the potential of the cows to produce milk. The implications are very significant, since in most dual -purpose cattle farms in the Latin American tropic farmers have cows of low genetic potential for milk production. On the other hand, our results also indicate that there is a strong synergism between energy and protein rich forage supplements, which needs to be considered when giving guidelines to farmers on how to best use supplements for dairy cattle.

Suboutput 1.4. Know quality and animal production with selected herbaceous and shrub legumes

Legume species can significantly contribute to more sustainable land use, since they help

Table 13. Effect of different levels of *Cratylia argentea* (Cratylia) and sugarcane (sc) feeding on milk yield of high grade Holstein and low grade Zebu cows grazing *Brachiaria decumbens* (CIAT's experiment station in Quilichao, Colombia).

Treatment	Intake of forage supplement (kg DM/cow/d)	Milk yield (kg cow/d)
High grade Holstein[1]		
- 100% SC	5.0	6.6 b,c
- 75% SC-25% Cratylia	4.5	7.4 a, b
- 50% SC-50% Cratylia	4.0	7.8 a
- 25% SC-75% Cratylia	5.1	8.2 a
Mean	4.6 a	
Low grade Zebu[1]		
- 100% SC	3.8	5.9 c,d
- 75% SC-25% Cratylia	3.1	5.8 c,d
- 50% SC-50% Cratylia	3.5	5.5 d
- 25% SC-75% Cratylia	4.3	5.4 d
Mean	3.7 b	

a,b,c,d (P<.05); [1]Stocking Rate= 2.7 AU/ha

regenerate degraded soils and add N to the system. However, it is postulated that adoption by farmers of multipurpose herbaceous and shrub legumes will be to a great extent dependent on how they impact livestock production in the farm. Thus for selecting new legume species we are interested in defining factors associated with quality attributes such as: voluntary intake, live-weight gain and milk production.

Activity1.4.1 Studies on intake by sheep and goats of selected shrub legumes (Yi Kexian, P. Avila and C. Lascano)

RF 21

Highlight

- Acceptability ranking of shrub legumes with contrasting quality was the same with sheep and goats.

Rationale: In smallholder systems, particularly in Asia and Africa, small ruminants are important in the household economy. It is well known that sheep and goats have high protein requirements per unit of body weight and that they are highly selective.

In CIAT we have selected a number of shrub legumes of contrasting quality and with variable adaptation to acid soils. Thus we were interested in determining differences in intake between selected shrub legumes fed to sheep and goats.

Methods: A short -term intake experiment was carried out in the Quilichao Research Station using growing sheep and goats. Three sheep and three goats on a grass basal diet were fed short periods of time the following shrub legume species arranged in a 3x3 Latin Square design:

T1: *Cratylia argentea*
T2: *Desmodium velutinum*
T3: *Flemingia macrophylla*

Short-term intake was recorded on each experimental period for 4 consecutive day in the morning (1/2 h) and afternoon (½ h), prior a 7day adjustment period. Samples of the forage (mostly leaf and fine stems) offered was frozen-freeze-dried and ground for subsequent laboratory analysis.

Results: In table 14 we show quality parameters of the three legume species fed to sheep and goats. Large differences were observed among legume species in IVDMD and NDF and ADF, but not in CP. *Desmodium velutinum* had the highest IVDMD which was associated with low cell wall content and with no condensed tannins. Lowest IVDMD was recorded in *F. macrophylla*, which had intermediate cell wall content, but high tannin content. *C. argentea* had intermediate IVDMD, which associated with high levels of cell wall.

Table 14. Quality parameters of contrasting shrub legumes fed to sheep and goats

Quality parameters	Shrub legumes		
	D. velutinum	*C. argentea*	*F. macrophylla*
IVDMD (%)	54.4	48.4	22.9
CP (%)	20.2	21.1	17.9
NDF (%)	38.8	58.9	48.6
ADF (%)	29.4	36,7	36.8
N-FAD (%)	0.53	1.32	1.26
Tannins Extractable (%)	0.53	1.32	1.26

Short-term intake varied among shrub legume species, being highest for *D. velutinum* and *C. argentea* and lowest for *F. macrophylla*. On average goats consumed 28% more legumes than sheep, even though the ranking of legumes was the same with both animal species (Table 15).

Table 15. Short-term intake of contrasting shrub legumes by sheep and goats

Animal species	Shrub legumes			
	D. velutinum	*C. argentea*	*F. macrophylla*	Mean
	(g DM/kg ^{75} BW/h)*			
Sheep	24.9	31.8	17.4	24.7 b
Goats	35.1	34.3	25.7	31.7 a
Mean	30.0 c	33.1 c	21.5 d	

a,b Means in the same column are different (P<.05)
c,d Mean in the same row are different (P<.05)

Discussion: Short-term intake measured in this study mainly reflects palatability of the legumes fed, which is not necessarily related to long-term intake and overall animal production.

Nevertheless, the lower short-term intake of *F. macrophylla* by sheep and goats as compared to other legumes species fed is consistent with its high level of condensed tannins and low digestibility. Thus low animal performance can be expected when *F. macrophylla* is fed alone or in combination with other forages to either sheep or goats. Conversely, high animal performance can be expected when feeding *D. velutinum*.

Output 2: Grass and legume gene pools with known diversity in host/parasite/symbiont interactions

Suboutput 2.1 Host plant relationships, ecology and population dynamics of spittlebugs are understood

Grassland spittlebugs (Homoptera: Cercopidae) pose the greatest biological limit to forage grass production in the neotropics. Outbreaks of these native xylem-sucking insects dramatically reduce forage quality causing declines in pasture productivity and persistence and limiting the establishment of improved forage species such as *Brachiaria decumbens*. There is still no program for their integrated management. Part of the problem stems from the tendency to overgeneralize similarities among the diverse species and genera. This is aggravated by a deficient understanding of the basic biology and behavior, a scarcity of detailed site-specific studies of their ecology, and the fact that spittlebugs attack pastures across a wide range of ecological zones. Despite the similarities in bioecology and pasture habitat, the nature of their impact on forages varies fundamentally among regions given the taxonomic diversity of the group superimposed on this range of environments. The present research addresses the components of bioecology most relevant to an interpretation of pest status through a detailed elaboration of taxonomic, seasonal, local and regional determinants of abundance and distribution.

A research program on the comparative biology and ecology of Colombia's grassland spittlebugs was initiated 16 September 1996 with the arrival of Dr. Daniel Peck. D. Peck was contracted as a post-doctoral fellow to lead bioecology studies as part of a special project funded by the Colombian Fondo Nacional de Ganado awarded to CORPOICA Regional 2 (C.I. Turipaná, Montería, Córdoba) and led by Nora Jiménez. CIAT and the Organization of American States funded one half of the position and further operational expenses were awarded through Nestlé to ensure detailed comparative studies in four of the departments most impacted by spittlebugs.

The bioecology research group has grown to 16 participants. The comparative population dynamics studies, which form the foundation for most other activities, take place in Montería (Córdoba), Corozal (Sucre), Villavicencio (Meta) and Florencia (Caquetá) with the participation and collaboration of CORPOICA and departmental universities.

Activity 2.1.1: Studies on the basic biology and behavior of major spittlebug species

Highlights

- First-time studies, facilitated by a new rearing unit, were initiated on the biology and habits of three major lowland species.
- First-time studies were initiated on vibrational communication behavior among adults.

Rearing unit that facilitates biological and life cycle studies of spittlebug (U. Castro, D. Peck, R. Pareja)

Rationale: Geographical differences in species ranges prevent us from establishing colonies of certain Colombian SPB species in CIAT because of the risk of introduction to a region where they do not currently occur. Studies on the biology and life cycle of these species must therefore take place *in situ*. Difficulties that accompany this need are the costs and labor associated with the establishment of a colony in the same style as CIAT's that ensures access to all three life stages throughout the year, especially during the dry season when SPB essentially disappears from the field for a few months. In addition, reproductive biology studies require access to large groups of newly emerged adults (tenerals), which are much too difficult to obtain in the field.

Methods: We sought to develop a rearing unit that facilitates *in situ* biological studies of SPB by being adaptable for different species and genera and requiring little space, materials, labor or specialized training to maintain a successful colony. It was also desirable to search for a unit that eliminated one major bottleneck in the CIAT's mass rearing colony: the individual capture of newly emerged adults from nymphal rearing pots for release into oviposition chambers. The prototype is under evaluation in three sites with three species: *A. reducta* in Montería, *Z. pubescens* in Villavicencio, M. *fimbriolata* in Florencia and *A. varia* in CIAT. A series of trials measuring efficiency of *A. varia* adult and egg production are underway to allow comparison of this system with other new rearing strategies under evaluation for CIAT's massive rearing program.

Results: The rearing unit's key feature is the combination of chambers for nymph rearing and egg laying. A tray of with host plant roots for nymphal development fits into the top of a rectangular aluminum frame (61.6 x 31.4 x 32.3 cm) elevated by four feet (15.5 cm). A tray that contains oviposition substrate slides in at the bottom of the frame that is wrapped with a sheet of black nylon mesh to form walls that reduce light, raise humidity and prevent escape.

Need for the rearing unit spurred establishment of a new method to raise nymphs. Young stems of *Brachiaria*, when planted in a 3 cm layer of soil on top in a plant tray (60.7 x 30.5 x 3.6 cm) with a network of holes in the bottom, produce roots that are ready for infestation with eggs in approximately three weeks. The roots form a mat between the planted tray and a second tray fitting below, and are infested by replacing the upper tray back onto the lower tray that holds pieces of filter paper with eggs ready to hatch.

Nymphs form spittle masses and feed on roots throughout development of the five instars. If the soil layer is too thin, spittlebugs escape by climbing to the upper soil surface through fissures in the soil. Ten days before emergence of adults the root tray is placed on a 10 cm tall wooden frame that allows the roots to descend. When adults are ready to emerge, the root tray is placed at the top of the chamber, a tray with oviposition substrate is placed below at the bottom, and leaves for adult feeding enter through openings in the sides. New adults feed, copulate and lay eggs for later collection.

Initial data from *A. varia* indicate successful development of adults from nymphs restricted to

feeding exclusively on the descending roots, however adult emergence is prolonged by about one week. Up to 4100 eggs were obtained from the oviposition substrate under one nymph tray from an unrecorded initial number of eggs and resultant adults. In three other trials the ratio of eggs retrieved to eggs used in infestation was 1.08, 2.01 and 2.04, demonstrating an efficiency promising for continuity of the colony and use of excess individuals for other studies.

Discussion: Although this rearing unit is under further evaluation with three other species, initial trials with *A. varia* have shown it to be promising for the year-round maintenance of SPB for biology and life cycle studies. It is collapsible, occupies little space, requires small volumes of soil and reduced labor, and combines the breeding and oviposition chambers into one unit that eliminates the need to collect and transfer all newly emerged adults. The major disadvantage is the prolongation of the nymphal stage. Efficiency will increase with adjustments designed to reduce the escape of adults and with better understanding of when to infest the roots. Current studies will measure the efficiency of the unit for *A. varia* and thereby its potential for use in CIAT's mass rearing program for resistance evaluation.

Biology and habits of three major species of spittlebug (D. Peck, W. Medina, B. León, C. Gallego and Y. Ballesteros)

Rationale: An inadequate understanding of the basic biology and behavior of most SPB species contributes to their ineffective management. For four of the six major lowland species in Colombia we lack information on all aspects of biology that are relevant to their control, such as duration of the life stages, fecundity, oviposition sites, and preoviposition period. *A. varia* has been well-studied given its pest status in sugar cane; while studies of *Z. colombiana* have been carried out previously at CIAT.

Methods: Studies to characterize the biology, habits and morphology of SPB life stages were initiated for *A. reducta* in Montería, *Z. pubescens* in Villavicencio and *M. fimbriolata* in Florencia, with comparative data collected at CIAT for *A. varia* and *Z. pubescens*. Observations on the developmental stages of the eggs, instars of the nymphs and adult sexes are accompanied by measurements of duration under laboratory conditions and measures of size and other distinguishing morphological features. Groups of newly emerged adults will be studied to assess reproductive biology including precopulation and preoviposition periods, fecundity, duration and frequency of copulation, and longevity. Selection of oviposition sites will be studied with adults on potted plants within sleeve cages.

***Results*:** The proposed studies have just been initiated and will proceed rapidly once laboratory colonies are securely established. Measurements of the head capsule width in *A. reducta* nymphs (Figure 7) verified the existence of five instars that are morphologically distinguishable when size is accompanied by features of the wing pads, antennae and eyes.

Discussion: These studies will lead to a description of the aspects of biology and behavior that are most relevant to an accurate interpretation of SPB pest status and their association with the pasture habitat. The results of the four previously undescribed species will be

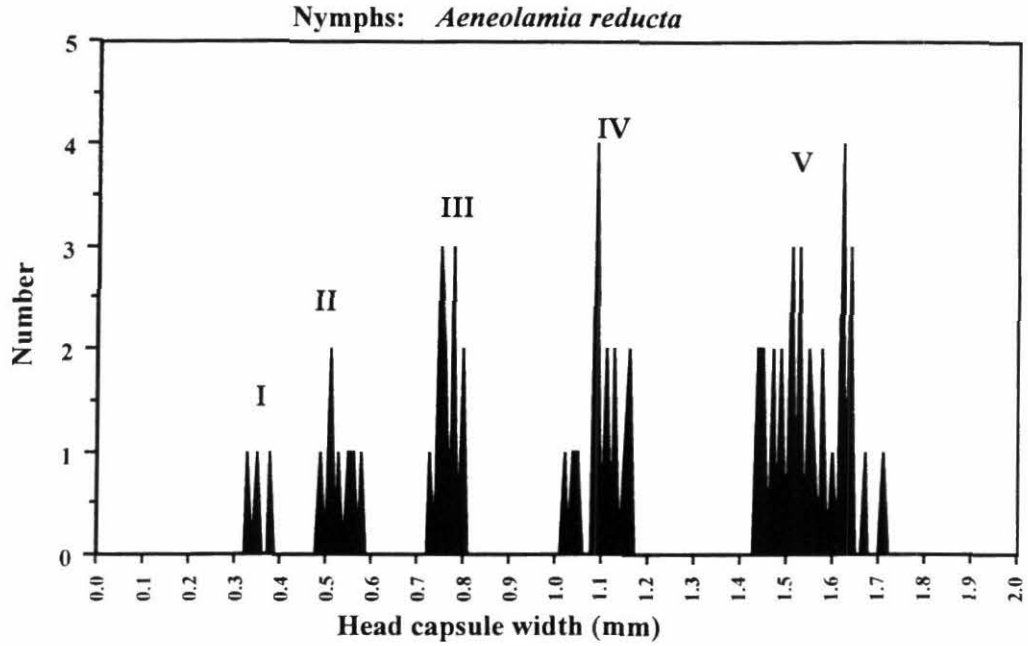

Figure 7. Determination of *A. reducta* ninfal instars by measurements of the head capsule.

compared with *A. varia* and *Z. pubescens* to widen our view on the degree of variation within this pest complex.

Biological aspects of substrate communication in adult *Z. colombiana* (F. López, D. Peck and P. Calatayud)

Rationale: Vibrational communication is one fundamental aspect of SPB behavior that has never been examined in detail beyond sound recordings and personal observations that verify its existence. Among related insects, bioaccoustics is known to play a role in reproductive biology and species differentiation, both of which are poorly understood and critical aspects of our basic understanding of SPB.

Methods: Apparatus for producing electrical penetration graphs (EPG) will be adapted to record and visualize vibrations produced by *Z. colombiana.* It will be determined whether this species communicates primarily via air or substrate borne vibrations. The physical characteristics of these calls will be described and categorized, and their role in precopulation behavior will be assessed.

Results/Discussion: Results from these initial studies will constitute the first description of this behavior among froghoppers. Detailed information on substrate communication in *Z. colombiana* will launch additional studies on species differentiation that compare the behavior among various species and genera.

Activity 2.1.2: Effects of environmental conditions on spittlebug egg development

Highlight

- Methodologies were established to initiate comparative studies on the development of eggs and the incidence of diapause

Comparative egg morphology and development of spittlebug (D. Peck and U. Castro)

Rationale: Behavior of the egg stage links prediction of the phenology and synchronization of the early season SPB populations with meteorological data. Therefore the interpretation of comparative population performance (2.1.3.1) is complemented by basic studies on the egg morphology and development of the relevant SPB species. This information is also absent from our understanding of the basic biology and behavior of many species.

Methods: Studies are underway to describe and compare egg development stages among major species of SPB under controlled conditions of temperature and humidity. The objectives are to measure the duration, size and external morphology of developmental egg stages in six species, determine the stage that is prolonged in diapause, and compare the variation in development within and between three genera of SPB. Pilot studies were also performed to test a methodology for measuring the response of diapause eggs to varying lengths of drought conditions.

Results: Data have been obtained thus far from *A. reducta*. Of 3248 eggs collected in November 1996 from field populations and maintained under continually moist conditions, 88.2 % were immediately developing, hatching on average 20.7 days after oviposition (Figure 8).

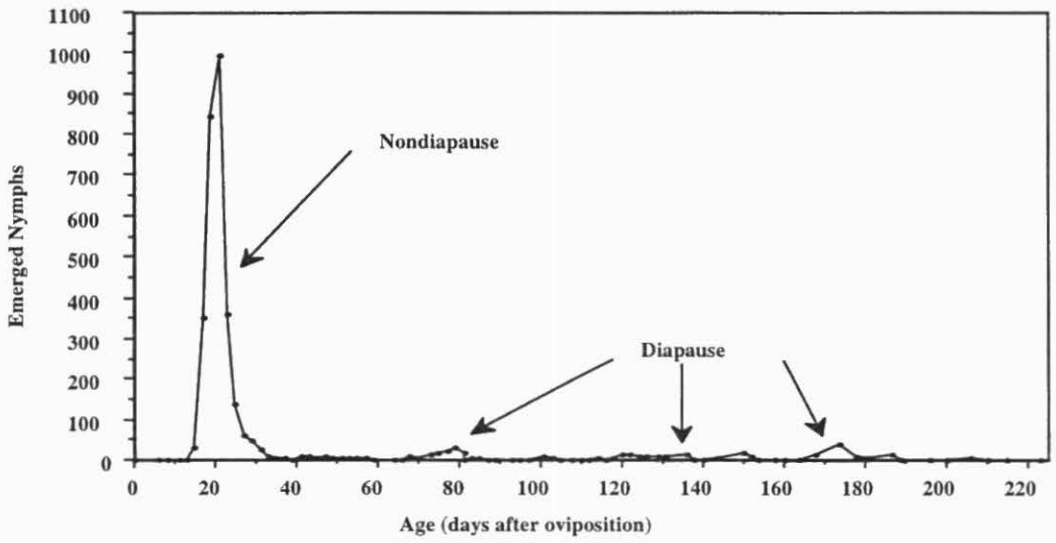

Figure 8. Pattern of eclosion of *A. reducta* eggs under humid conditions.

The remainder of eggs entered diapause and hatched sporadically 39 - 206 days later. Morphological changes that accompanied development were similar to other species. Eggs swelled in size especially after rupture of the chorion at the start of stage S3 (Figure 9).

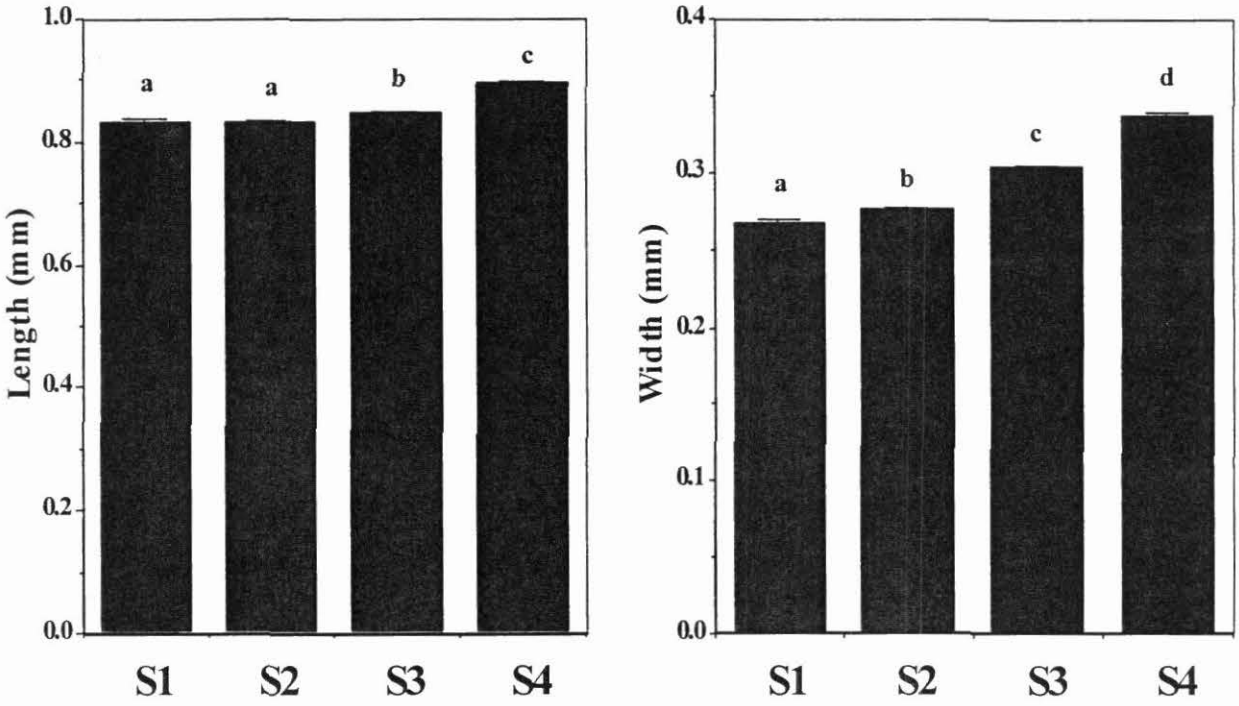

Figure 9. Changes in the size of *A. reducta* eggs during four development stages.

A group of 235 diapause *A. reducta* eggs (35 days old) were divided among five unreplicated treatments of drought conditions (0, 5, 10, 25, 50 day) followed by continual moisture. Mortality appeared to increase with dryness (20.8, 34.4, 28.3, 56.6 and 61.2 %), whereas mean time to development varied little (110, 103, 111, 116, 119 days).

Discussion: Initial studies with *A. reducta* show it feasible to compare certain taxonomically variable characteristics of egg development and morphology among the species under study. These measures include size and physical description, externally visible changes that accompany development, the stage extended in diapause, and the duration of stages under controlled conditions, in addition to the impact of drought on diapause egg mortality and duration. Along with parallel studies on oviposition behavior and reproductive biology, this new information will offer the detailed basic understanding currently lacking in many SPB species.

Seasonal variation in the incidence of egg diapause of spittlebug (U. Castro and D. Peck)

***Rationale*:** Egg diapause permits the synchronization of SPB populations with the wet season during which growth and development are possible. It is known that the incidence of diapause (i.e. the percentage of eggs that are not immediately developing) in some species is lowest among females at the start of the wet season and highest among those in later generations before the start of the dry season. Therefore a description of seasonal changes in diapause incidence - and how it varies among species - is an essential element in understanding and predicting the synchronization and timing of early season nymph populations.

***Methods*:** Collection and incubation methodologies were tested in one of three sites where detailed studies will be carried out in 1998. Eggs under study were obtained in July, September and November from groups of females collected in the field at CORPOICA C. I. La Libertad near Villavicencio (Dept. Meta), site of population surveys. One group of 15 - 25 females of *A. varia* were collected from each half of three observation plots and allowed three days to oviposit into moist filter paper lining the bottom of large petri dishes that housed each of the six groups (repetitions). After express delivery to CIAT, eggs were maintained moist in petri dishes under ambient lab conditions (July) or incubation (September) and scored for empty chorions (nymph eclosion) twice weekly. Given that field populations initiated in March (see 2.1.3.1), these adults only represented the second half of the SPB season.

***Results*:** Females laid 43 - 399 eggs per group. Incidence of diapause was low for each date of collection, 0.86 ± 0.53 and 0.37 ± 0.17 % (mean ± S.E.) in July and September respectively, and did not differ significantly (P = 0.199). Mean days to egg eclosion was 18.12 ± .23 and 16.72 ± 0.19 respectively, but the difference (P = 0.0004) was probably due to different incubation regimes.

Discussion: The methodology is appropriate for collection of eggs from females in the field and their successful development and observation in the laboratory. In 1998 this experiment will include two additional sites (Florencia and Montería) and three additional species (*A. reducta*, *M. fimbriolata* and *Z. pubescens*). Collections will be made bimonthly throughout the SPB season. This methodology will permit a measure of diapause incidence at different periods of the wet season, correlation of changes with the progression of the wet season, description of the different categories of egg (nondiapusing, short and long term diapause) and comparison of diapause behavior among four SPB species and between populations of *A. varia* in three sites.

Activity 2.1.3: Comparative population ecology of spittlebug in four lowland regions

Highlights

- Six species of spittlebug were found to be associated with lowland pastures in Colombia.
- In three seasonally dry sites one species of spittlebug dominates and abundance coincides with the rainy season.

- In wet sites three species of spittlebug are sympatric and abundance appears to decline with increased precipitation; the higher species diversity, abundance and rainfall corresponds with increased presence of natural enemies

Comparative population dynamics of spittlebugs: local and regional *variation* in species composition, abundance, phenology and synchronization (D. Peck, W. Medina, A. Pérez, F. Gamboa, J. Hincapie, Y. Ballesteros, C. Gallego and C. Mantilla)

Rationale: There are very few detailed site-specific studies on SPB population dynamics. An accurate interpretation of what occurs at a particular locale depends on frequent and long-term surveys that emphasize the discrimination of all five nymphal instars, adult sex and species. Identical survey methodologies employed at different sites can also allow a comparison of population performance measures including abundance, phenology, synchronization and species composition. An understanding of the patterns and variation in these measures is critical to assess and predict pest status.

Methods: The population dynamics of SPB was studied in four lowland sites in departments most affected by this insect. We chose farms with a history of SPB attack that comprised *Bothriochloa pertusa* ("Colosuana") in Córdoba and Sucre and *Brachiaria decumbens* in Meta and Caquetá (Table 16). Three 0.5 ha plots were marked in different pastures on each of the four farms and subdivided into four sections to facilitate subsampling. Nymph surveys comprised counts in two 0.25 m^2 frames per section; spittle mass occupants were removed and determined to instar. Adult surveys comprised 50 sweeps of an insect net per section; adults were determined to sex and species. Both surveys were performed approximately twice weekly during the wet season and less frequently during the dry season when populations had diminished.

Table 16. Description of population survey sites.

Site	Farm	Predominant Forage	Initiation Date
Córdoba: Montería	Bella Luz and El Olivo	*Bothriochloa pertusa*	1 Oct 96 and 1 Jan 97
Sucre: Corozal	Tarapacá	*Bothriochloa pertusa*	26 Sept 96
Meta: Villavicencio	C.I. La Libertad	*Brachiaria decumbens*	31 Jan 97
Caquetá: Florencia	C.I. Macagual	*Brachiaria decumbens*	24 Jan 97

Results: Five species were encountered at these three sites: *Aeneolamia lepidior*, *Aeneolamia reducta*, *Aeneolamia varia*, *Mahanarva* sp. and *Zulia pubescens* (Table 17). *Zulia colombiana* was found in the vicinity of Florencia but not at the survey site. One major species was accompanied by the presence of one or two other species in all sites but Florencia where three species occurred sympatrically, two in high abundance. *Mahanarva* sp. is still undetermined but appears to be a distinct species related to *M. fimbriolata* that occurs in southern Brazil.

In the three seasonally dry sites, Montería, Corozal and Villavicencio, SPB abundance coincided with the rainy season, and adult abundance followed closely after nymphs. Both life stages disappeared at the end of the wet season and reappeared at the start of the rainy season

Table 17. Species composition at population survey sites.

Species	% Adult abundance			
	Montería	Corozal	Villavicencio	Florencia
A. lepidior	1	<1*		
A. reducta	99	100	1	
A. varia			98	71
M. nr *fimbriolata*				1
Z. colombiana				< 1 *
Z. pubescens			1	21

* Species found in the vicinity but not encountered at survey site.

(Figure 10). Low population densities in the North Coast during 1997 are probably related to the dry rainy season associated with El Niño (Table 18); the high density of *A. reducta* in Montería relates to November 1996. On farm variation in abundance can be measured by the area bounded by the population curve; nymph and adult abundance varied by 3.7 and 3.5 times, respectively, among the three Corozal plots.

Table 18. Maximum abundance of nymphs (per 2 m^2) and adults (per 200 sweeps) at each survey site.

Nymph/Adult spp.	Montería	Corozal	Villavicencio	Florencia
Total nymphs	14	14	75	158
A. lepidior	6	0	0	0
A. reducta	597	189	9	0
A. varia	0	0	370	88
M. nr *fimbriolata*	0	0	0	2
Z. pubescens	0	0	4	25

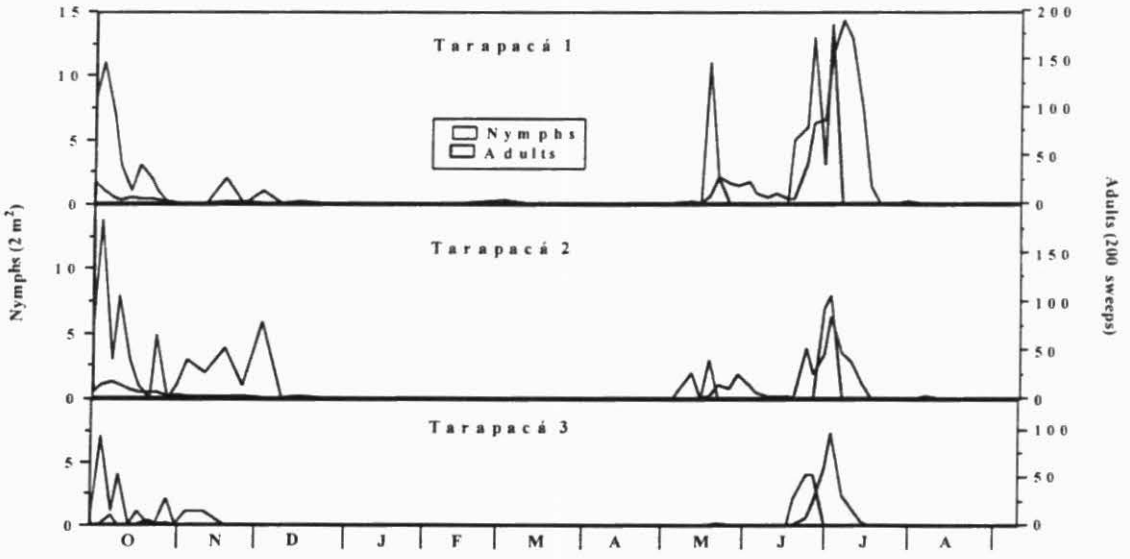

Figure 10. Abundance of *A. reducta* nymphs and adults in three plots at the farm Tarapacá, Corozal (Dept. Sucre), 1996-1997.

Discrimination of instars permit a much more detailed view of local population dynamics than analyzing total nymphs. Differences in phenology, population synchrony and number of generations between plots become apparent. In Villavicencio, for instance, Plot La Loma experienced a large initial generation between March and April (Figure 11).

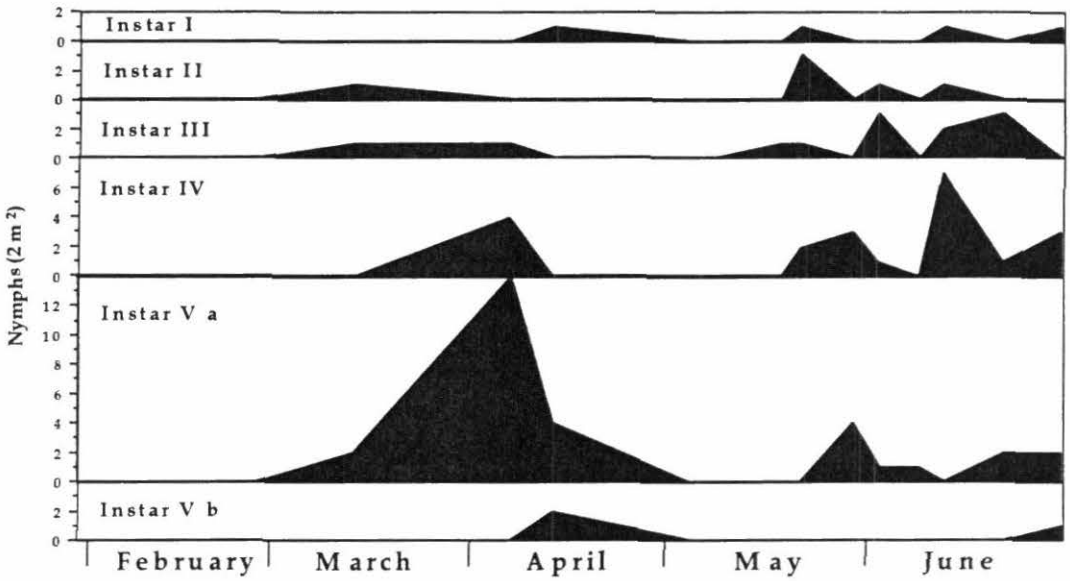

Figure 11. Abundance of nymphs and adults of *A. varia* in the plot La Loma at C.I. La Libertad, Villavicencio (Meta), 1997.

The progression of abundance from early to late instars is evidence for the population's high synchrony relative to nymphs appearing after May. Plot El Retoño experienced a small initial generation February to March and a second from April to May (Figure 12).

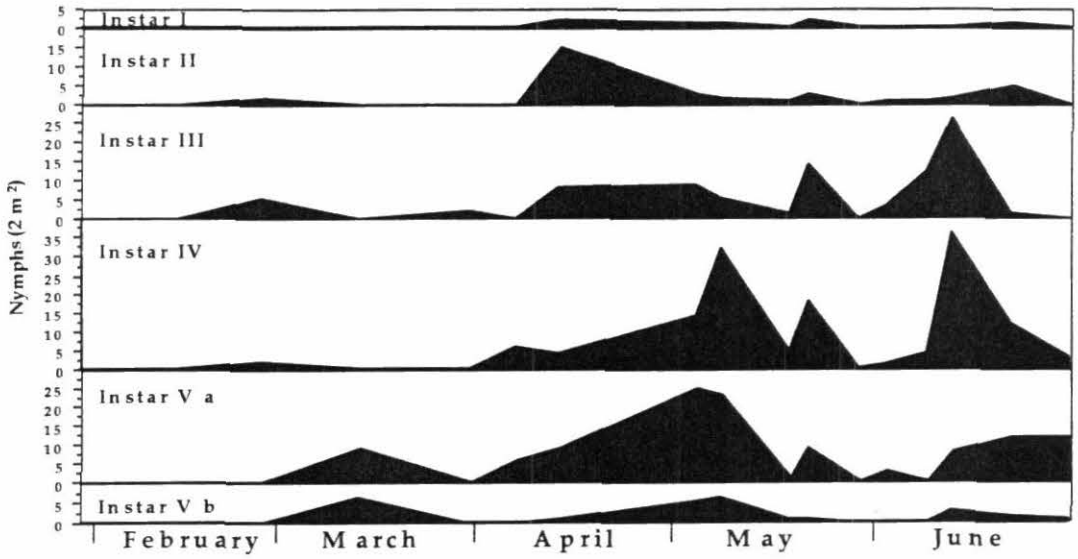

Figure 12. Abundance of nymphs and adults of *A. varia* in the plot El Retoño at C.I. La Libertad, Villavicencio (Meta), 1997.

Adult populations of three species in Florencia all showed a similar pattern of decline in February and resurgence in April (Figure 13). In contrast to the other sites, the decline of SPB populations was not associated with dry conditions. Florencia receives an average of 3500 mm rainfall a year and has periods of less precipitation but not a distinct dry season like the other sites. Rainfall in February (252.5 mm) and March (274.7 mm) was much higher than in January (79.8 mm); decline of SPB therefore coincided with periods of increased precipitation.

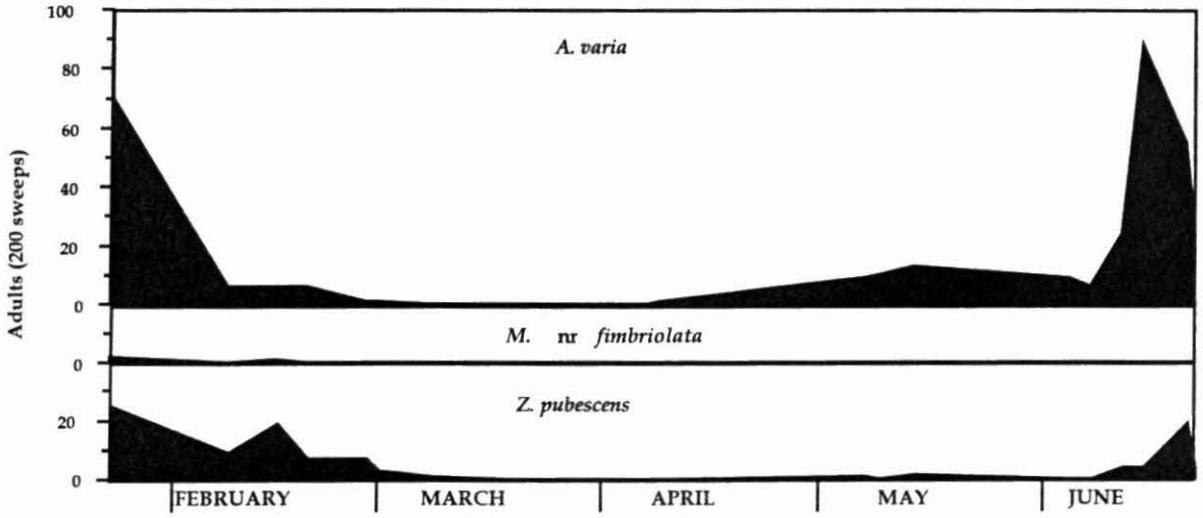

Figure 13. Abundance of adult *A. varia*, M. nr *fimbriolata* and *Z. pubescens* in the plot La Loma at C. I. Macagual, Florencia (Caquetá), 1997.

Discussion: Detailed surveys that distinguish among all life stages, sexes and species, offer the resolution necessary for an interpretation of local population dynamics and for the measure of farm-level and regional variation in abundance, phenology, synchrony, number of generations and species composition. This information will assist in the prediction of the magnitude and timing of initial outbreaks based on meteorological data and new understanding of the determinants of egg diapause.

Identification, abundance and phenology of natural enemies associated with spittlebug (D. Peck, W. Medina, A. Pérez, F. Gamboa, J. Hincapie, Y. Ballesteros, C. Gallego, C. Mantilla and A. Morales)

Rationale: The identification, abundance, distribution and impact of SPB natural enemies have not been studied in Colombia. Intensive population surveys allow the simultaneous collection of data on the occurrence of natural enemies including species identification, life stage attacked, abundance, seasonality and potential for future consideration as biological control agents. Up to now, the only promising contenders for SPB biological control belong to a narrow diversity of fungal entomopathogens, particularly *Metarrizhium anisopliae*.

Methods: Natural enemies were collected as part of spittle mass and sweep net surveys in population dynamics studies at four lowland sites. Predaceous larvae of *Salpingogaster nigra* (Diptera: Syrphidae) were removed from spittle masses where they attacked nymphal and teneral adult inhabitants. Adult flies were recorded from sweep net surveys. Nymphal and

adult cadavers, especially adults collected from sweep net surveys, were stored dry in microvials and shipped to CIAT for the isolation and identification of associated fungal entomopathogens. The presence of parasitic mites and entomopathogenic nematodes in samples was also recorded.

Results: Four varieties of natural enemies were encountered: predaceous syrphid fly larvae, entomopathogenic nematodes, entomopathogenic fungi and parasitic mites (Table 19). Florencia recorded the highest abundance and diversity of enemies which could be attributed to any or all of the region's distinctions: continual presence of SPB, high SPB diversity and high humidity with lack of a severe dry season. Although all fungal entomopathogens isolated thus far belong to Florencia, cadavers from Villavicencio are currently under examination. The genera *Fusarium*, *Metarrizhium* and *Paecilomices* have been isolated thus far.

Table 19. Incidence of natural enemies associated with SPB at four sites.

Site	Enemy	SPB species attacked	Life stage attacked	Months recorded
Corozal	Nematode	*A. reducta*	adult	June
	Parasitic mite	*A. reducta*	adult	July
Villavicencio	Nematode	*A. varia*	nymph, adult	June
	Parasitic mite	*A. varia*	adult	June, July
	Salpingogaster nigra	*A. varia*	nymph	June
Florencia	Nematode	unknown	nymph	Feb
	Parasitic mite	*A. varia*	adult	May, June
	Salpingogaster nigra	unknown	nymph	Jan, June
	Fusarium sp.	*A. varia*	adult	Feb
	Metarrizhium anisopliae	unknown	nymph	Feb
	Metarrizhium sp.	unknown	nymph	Jan
	Paecilomices sp.	*Z. pubescens*	adult	Jan
	Paecilomices sp.	unknown	nymph	Feb

The presence of *S. nigra* is not surprising given its pan-neotropical distribution and use of a wide variety of grassland spittlebug species. It has not yet been found on the North Coast probably due to the unusually dry rainy season in 1997. Adults, pupae and larvae have all been recorded from other sites but abundance is extremely low. Life stages of this species have been encountered only eight times in all surveys thus far.

Nematodes were recorded from all four sites but species identification has not yet been made. These enemies may reach high abundance; in an unrelated study in which 90 *A. varia* females from Villavicencio were held for three days in captivity, 12 nematodes were recovered.

Discussion: The diversity of fungal entomopathogens collected thus far is promising for future studies on biological control. The high level of contact between field staff at four sites with six species of SPB will reward us with a collection of diverse species and isolates. This material is valuable in the light of most biocontrol studies that emphasize a very narrow diversity of species and isolates.

Activity 2.1.4: Effect of certain management practices on spittlebug abundance

Highlights

- Abundance of spittlebug nymphs and adults does not vary between pure *Brachiaria* pastures and *Brachiaria/Arachis pintoi* associations.
- There is evidence that diverging habitat preferences contributes to habitat partitioning between two major sympatric species of spittlebug.
- Release of adult spittlebug females merits further consideration for artificial infestation in field resistance trials.

Response of spittlebug to grass/legume associations (W. Puentes, C. Ramírez, G. Ruiz, D. Peck and C. Troncoso)

Rationale: Despite the benefits of grass/legume forage associations, adoption of this cultural practice will partially depend on how it influences SPB abundance and impact. Habitat changes that might accompany diversification of the pasture, such as higher densities of natural enemies and dilution of SPB host plants, suggest a lower abundance of SPB in response to grass/legume associations. Equally possible, however, is that increased nitrogen availability might make grasses more attractive to SPB, but also more tolerant to attack. This study evaluates changes in the abundance (rather than impact) of SPB in associated and non-associated pastures.

Methods: Differences in SPB abundance were examined at six on-farm trials in Caquetá that each featured paired plots of *Brachiaria* and *Brachiaria/Arachis pintoi* established earlier as part of the Nestle Project (Table 20). Plots of 0.5 ha were marked in each treatment and divided into 10 to facilitate subsampling of nymphs and adults. Approximately once a month, spittle mass and sweep net surveys were carried out at all farms over a two day period.

Table 20. Description of experimental sites and species composition.

Site	Date estab.	Forage	Adult species composition (%)			
			A. varia	*M.* nr *fimbriolata*	*Z. colombiana*	*Z. pubescens*
South of Florencia:						
Diamante	1995	*B. decumbens*	30.7	3.2	0	66.2
Norglandia	1995	*B. decumbens*, *B. humidicola*	29.4	3.3	0	67.3
Villa Clarita	1995	*B. brizantha*, *B. decumbens*	18.1	0	0	81.9
North of Florencia:						
Caña Brava	1995	*B. humidicola*	100	0	0	0
Higuerón	1989	*B. decumbens*	79	0.3	19.4	1.2
Primavera	1995	*B. decumbens*	34.3	0	0	65.7

Surveys were similar to population studies except that nymphs counts were made in a total of 20 0.0625 m^2 frames and adults were collected in 10 series of 20 sweeps per treatment.

Results: *Arachis* associations caused no significant changes in any measure of SPB nymph and adult abundance (Table 21). In any given farm, however, there tended to be seasonally consistent differences in abundance such that one treatment was preferred month after month over its paired treatment irrespective of treatment type (Figure 14). This indicates that characteristics of the habitat other than *Arachis* association were more important in driving SPB abundance.

Table 21. Mean density of SPB life stages in associated and non-associated pastures.

Abundance measure (combined months)	*Brachiaria* Mean ± SE	*Brachiaria/Arachis* Mean ± SE	Prob > |t| Paired t-test
Total nymphs (per 1.25 m^2)	27.3 ± 6.64	36.2 ± 8.99	0.427
Total adults (per 200 sweeps)	59.0 ± 13.46	53.6 ± 11.21	0.866
Total *A. varia* adults	20.5 ± 7.75	21.4 ± 4.21	0.102
Total *M.* nr *fimbriolata* adults	1.0 ± 0.45	1.3 ± 0.48	0.608
Total *Z. pubescens* adults	36.5 ± 10.64	28.1 ± 9.37	0.555

Separation of the adult species showed that the two dominant species, *A. varia* and *Z. pubescens,* appeared to partition themselves spatially, preferring opposite treatments at the three farms where they were most abundant (Figure 14). This curious pattern probably relates to diverging habitat preferences.

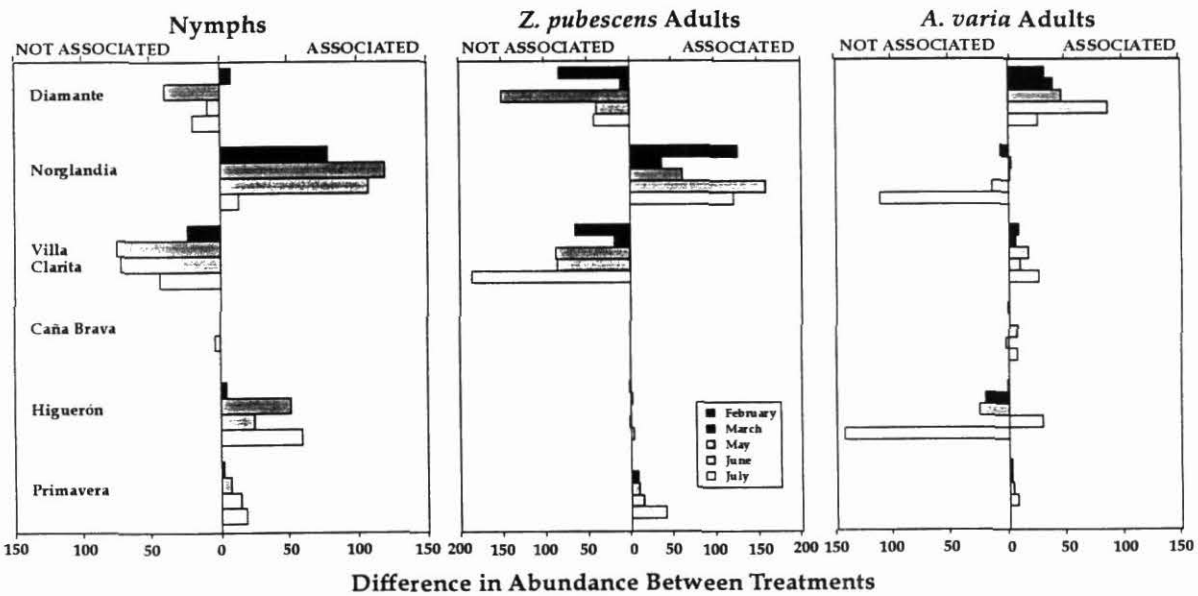

Figure 14. Abundance of nymphs, adult *Z. pubescens* and adult *A. varia* in paired pastures of *Brachiaria* and *Brachiaria/Arachis pintoi* in 6 farms over several months in 1997. Data are the difference in total abundance between paired treatments per sampling date.

The region south of Florencia suffered greater SPB pressure. Over the months of the experiment, mean number of nymphs (per 1.25 m^2) and adults (per 200 sweeps) was 49.3 and 88.5 in the south and 14.1 and 24.1 in the north. Overall species composition of adults varied with no clear pattern between south and north and treatments (See Tables 20 and 21). *Z.*

colombiana was encountered at only one site; *M.* nr *fimbriolata* at three. All four classes of natural enemies were recorded but numbers were insufficient to evaluate abundance differences between treatments.

Discussion: Surveys will continue monthly through January 1998. Thus far it appears that abundance of nymphs and adults does not depend on pasture association. Although establishment of grass/legume mixes does not increase SPB numbers it may increase impact given that the same number of insects is attacking a reduced grass component. Future analyses and studies will begin to assess the effect of grass/legume associations on SPB impact, keeping in mind the opposing factors of enhanced attractiveness and tolerance of grass when in the company of nitrogen fixers.

Evaluation of control alternatives of spittlebug in Caquetá (M. Milena, B. Giraldo and J. Barreto)

Rationale: Although host plant resistance should ultimately serve as the foundation for improved management of SPB, there will be a continued need to manage susceptible varieties. In addition, until such improved varieties become available producers require new alternative management tactics and the validation of those currently in use. Despite the importance of forage production and spittlebug impact in Caquetá, alternatives for SPB management in that area have never been evaluated.

Methods: An experiment was established to determine the effect of fertilizers, soil correctives and entomopathogenic fungi on SPB abundance and forage production. At two plots established on a farm near Florencia (Dept. Caquetá) soil analyses were carried out to determine the recommended amount of correctives and/or fertilizers. Four treatments will be replicated at each site in 10 x 10 m plots: application of lime according to soil analyses, application of N:P:K fertilizer according to soil analyses, application of a commercial fungal biocontrol product according to laboratory recommendations, and a control without applications. Monthly evaluations will determine nymph and adult density, plant composition and forage production.

Results/Discussion: This experiment was established at the end of 1997 and will be carried out for 12 months.

Artificial field infestation with spittlebug through liberation of adult females (D. Peck and W. Medina)

Rationale: It has been difficult to evaluate the resistance of new *Brachiaria* germplasm to SPB in the field given the insect's sporadic spatial and seasonal occurrence and variable abundance. Adequate evaluation requires a uniform dispersion and high density of SPB. Although such conditions cannot yet be predicted they could be created artificially. Previous attempts to augment field populations with eggs have failed due to arthropod predation; and it is considered that augmentation with nymphs may be too laborious. Liberation of adult females therefore remains an option worth examining given evidence for site fidelity of gravid females and their ability to lay eggs in sites best protected from predators and adverse climate.

Methods: An adult augmentation methodology was tested on one farm in the municipality of Ciénaga de Oro (Dept. Córdoba). We hoped to cause an increase in early season nymph abundance in experimental plots by releasing late fall females and early season females whose complement of diapausing and nondiapausing eggs, respectively, would contribute to a larger initial nymph outbreak upon the return of the rainy season in May. A grid of 49 2 x 2 m plots were staked for seven repetitions of four treatment combinations and a control: adult density (40 or 80 adults) and season of release (end and start of wet season; November and May). Because of the unusually dry wet season imposed by El Niño, no adults were available for the May release. November *A. reducta* adults (approximately 1:1 sex ratio) were collected from the surroundings and released at the center of each appropriate plot on 1 November. Nymphs were counted in each plot on two dates in the spring.

Results: The new rainy season was extremely dry due to El Niño. SPB populations were barely detectable in a nearby population survey site. Nymphs were counted on two dates but because only a few individuals were encountered it was impossible to compare densities between the control and release plots.

Discussion: Dry rainy season conditions made it impossible to test the effectiveness of adult release in augmenting field populations of nymphs. Nevertheless, collecting adults from surrounding outbreak areas and releasing them in evaluation sites appears a simpler option for artificial field augmentation than liberation of nymphs or eggs. Gravid females are less capable fliers and may lay eggs in a uniform manner near their sites of release and in microsites most appropriate for protection against dry season drought and predation. And use of locally collected adults obviates the need to massively rear nymphs under laboratory conditions and finesse their release given their special microhabitat requirements. Diapausing eggs laid at the end of the wet season, plus immediately developing eggs added by early season adults collected from areas where they appear sooner, will be poised to hatch at the start of the new growing season. As drawbacks, adult release requires scouting of the region to find collectable populations, and may require irrigation of evaluation plots to ensure the best conditions for egg eclosion and nymph survival.

Activity 2.1.5 Training events

Workshop on the bioecology and management of grassland spittlebugs (D. Peck)

Rationale: Despite the pest status of SPB in Colombia, there is little national expertise on their biology and management outside of CIAT. Access to information is also extremely limited because there is no text that summarizes the status of our knowledge of the family Cercopidae and existing guides to pasture cercopids are outdated, inaccurate, and ignore family level bioecology. Establishment of a new research group on SPB bioecology therefore needed a means to offer collaborators a common foundation of knowledge on the study organism.

Methods: The first "Workshop on the Bioecology and Management of Pasture Spittlebugs" was held in CIAT 20 - 26 April, 1997. The objective of the workshop was to unite all participants involved in the spittlebug bioecology project for a week of intensive lectures, labs and discussions with the goal of establishing population survey methods, team building and

giving a solid foundation on the insect, its habitat and management. It was also the opportunity to advance student thesis project proposals, become familiar with related CIAT research activities and acquire literature relevant to particular projects.

Results: Eighteen people from five institutions participated, including ten people directly involved with Suboutput 2.1 on bioecological studies of SPB in Corozal, Montería, Villavicencio and Florencia. The other eight participants attended because of personal interest or involvement in related projects. Detailed notes, diagrams and reference lists accompanied the three core lectures on Diversity, Biology and Behavior, and Ecology. Invited speakers gave lectures on Host Plant Resistance (C. Cardona) and *Brachiaria* Improvement (M. Escandón). Notes were also prepared for the three core labs on Taxonomy I, Taxonomy II, and Biology and Behavior, which were complimented by other activities including a field trip to Santander de Quilichao, visits to the entomological collection, bean entomology and library, and discussions on spittlebug mass rearing, resistance evaluation, egg manipulation and integrated pest management. Participants were also provided a package of 20 key readings on basic and applied themes of the family Cercopidae.

Discussion: The workshop will be repeated in 1998 and subsequent years because it was invaluable for team building. The notes will be published as a guide to the ecology and biology of grassland spittlebugs so that they will be available for students and researchers who request information. Finally, this one-week Taller will serve as a platform for a five week "International Course on Methods for Evaluating Genotypes of *Brachiaria* for Spittlebug Resistance" to be held at CIAT in 1998.

Suboutput 2.2 Spittlebug resistance in *Brachiaria* genotypes assessed and characterized

Highlights

- A new, simpler, faster, more reliable glasshouse technique for screening of *Brachiaria* genotypess for resistance to spittlebug was developed, tested, and implemented.

- Significant progress was made in the development of a reliable, uniform method of artificial infestation with spittlebug for screening of *Brachiaria* genotypes under field conditions.

Activity 2.2.1 Development of a new, improved greenhouse technique for screening *Brachiaria* genotypes for spittlebug resistance

Rationale: Assessment of resistance to spittlebug under field conditions is extremely difficult due to the focal, unpredictable occurrence of the insect. Greenhouse techniques previously developed at CIAT were dependable but inefficient, time consuming and cumbersome. At best, 250 genotypes could be assessed for resistance in a given year, an output that was obviously far from the needs of current breeding activities. Consequently, the main purpose of our research in 1997 was to develop better alternatives for mass screening of resistance to spittlebug under greenhouse conditions.

Methods: The first step was to develop a smaller, simpler experimental unit. The second step was to study the possibility of detecting resistance to *Aeneolamia varia* in single, young plants of *Brachiaria*. For this purpose, research was conducted in two main areas: (1) The effect of plant age and levels of infestation with adults on resistance expression; and (2) The effect of plant age and levels of infestation with nymphs on resistance expression. The third step was to study the role of nonpreference (antixenosis) in resistance to spittlebug. The fourth step was to study the reliability of the new system in detecting antibiosis and tolerance as fundamental mechanisms of resistance to spittlebug. The fifth step was to study the effect of fertilization and screening technique on resistance expression. The sixth step was to implement the new technique in routine massive screening of genotypes originating from breeding activities.

Results and Discussion

A new experimental unit for evaluation of resistance to spittlebug in the greenhouse (C. Cardona and G. Sotelo)

As indicated in the 1996 Annual Report, several alternatives were compared. The one chosen for all future work can be called the "single-tube" assay. It consists of a 6-cm wide x 10-cm long PVC tube topped with a cap with a 2.5 cm hole through which a single plant stem is placed (the unit can also be used to promote superficial root growth in the plants by inverting them for 15-20 days prior to infestation with eggs). This methodology proved to be successful in promoting proliferation of superficial roots and in providing adequate humidity and shade conditions necessary for nymphal development. On susceptible genotypes, nymphal survival can be as high as 100%, while on resistant genotypes, antibiosis is fully expressed. The most important favorable characteristic of the new methodology is that it allows the measurement of nymphal damage with precision, something that was virtually impossible with the previously used standard pot assay.

Effect of plant age and level of infestation with adult spittlebugs on resistance expression (C. Cardona and G. Sotelo)

Since the new screening technique implies the evaluation of single, young plants of *Brachiaria*, it became necessary to study the effect of plant age (15 vs 30 days old plants) and levels of infestation (ranging from 0-12 adults per plant) on resistance expression. A susceptible (CIAT 0654, *B. ruziziensis*) and a resistant (CIAT 6294, *B. brizantha*) genotype were compared. There was not a significant difference between plant ages. We chose 30-day old plants because root proliferation and overall plant performance at this age was best. The regression of adult infestation levels on visual damage scores for the susceptible genotype was $y = 1.05 + 0.348x$ ($r = 0.879$; $P<0.01$). This equation was used to calculate the level of infestation needed to obtain a visual damage score of 3, discriminatory between susceptibility and resistance. The optimal level of infestation was thus set at 6 adults per plant.

Effect of plant age and level of infestation with nymphs of spittlebug *on* resistance expression (C. Cardona and G. Sotelo)

To determine the optimal levels of infestation with nymphs, 30-day old plants of a susceptible

(CIAT 0654) and a resistant (CIAT 6294) genotype were infested with increasing levels of infestation ranging from 0-15 nymphs per plant. Damage scores and nymphal survival were recorded. The regression of nymph infestation levels on visual damage scores (Figure 15) for the susceptible variety was $y = 1.824 + 0.179x$ ($r = 0.984$; $P<0.01$) which means that in order to obtain a damage score of 3.5 in a 1-5 visual scale, each plant would have to be infested with 9.4 nymphs. A standard level of 10 nymphs per plant was chosen. The tests also showed that the new screening methodology permits a precise measurement of antibiosis in terms of percentage survival of nymphs (Figure 15).

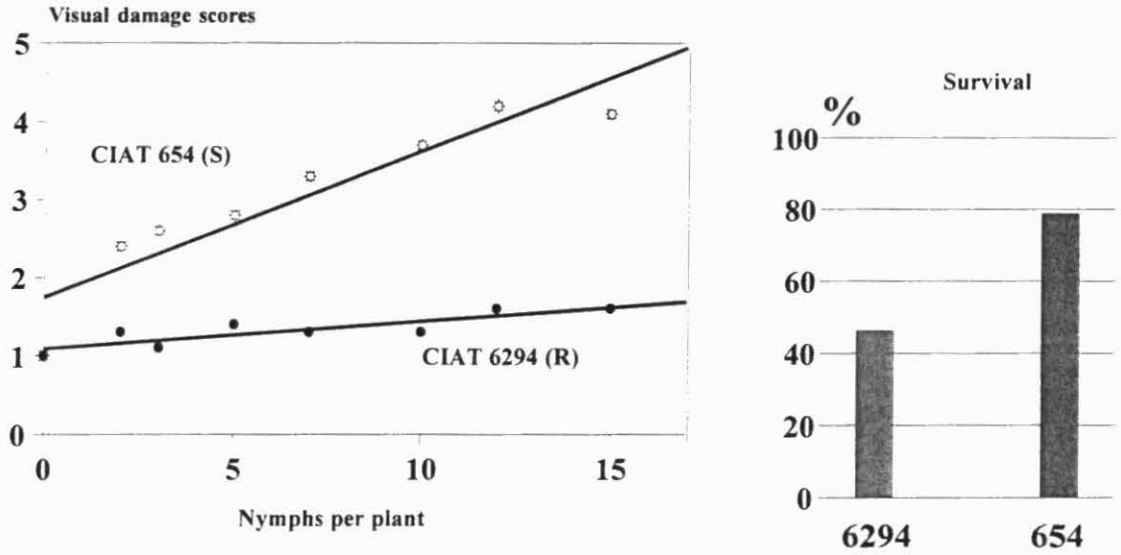

Figure 15. The relationship between levels of infestation and with spittlebug nymphs and visual damage scores in two *Brachiaria* genotypes.

The role of antixenosis on resistance to spittlebug (M. Cruz, C. Cardona, G. Sotelo)

The role of antixenotic or nonpreference mechanisms in resistance to spittlebug was studied in 1997 for the first time. Four genotypes selected for varying levels of resistance were used to study adult feeding and ovipositional preferences using infestation levels of 6 adults per plant. The results (Table 22) of three feeding tests indicated that the insect did not show a marked

Table 22. Free choice tests for feeding and ovipositional preferences of *Aeneolamia varia* on *Brachiaria* spp. Means of three feeding tests and two oviposition tests.

Genotype	Species	Resistance record	No. of adults/plant	No. of eggs/plant
CIAT 6294	*B. brizantha*	Resistant	5.8a	155.3a
CIAT 6133	*B. dictyoneura*	Intermediate	6.4a	168.8a
CIAT 0679	*B. humidicola*	Intermediate	5.3a	108.6b
CIAT 0606	*B. decumbens*	Susceptible	6.3a	135.7a

Means within a column followed by the same letter are not significantly different (P = 0.05, Duncan's Multiple Range Test).

preference for feeding on any of the varieties tested. Interestingly, a reduced level of oviposition occurred on the intermediate accession *B. humidicola* (CIAT 0679). This suggests that ovipositional antixenosis may play a role in the resistance of certain genotypes, an aspect that deserves future attention.

Reliability of the new system in detecting antibiosis and tolerance as fundamental mechanisms of resistance to spittlebug (C. Cardona, G. Sotelo and J. W. Miles)

Once the levels of infestation with adults and nymphs were established, we proceeded to conduct a large scale test of the new methodology in order to determine the viability of the system for resistance detection and for further characterization of the mechanisms underlying resistance to spittlebug. Based on previous results, 40 genotypes with known levels of resistance were chosen. These included germplasm accessions as well as hybrids bred for resistance to spittlebug and agronomic performance. In replicated "blind" tests (the identity of test materials was known to the breeder alone), two sets of the genotypes were exposed simultaneously to either adult or nymphal attack. Those surviving the test with adults were then exposed to nymphal attack and those surviving the test with nymphs were subjected to adult attack. The main purposes of the study were to identify genotypes possessing resistance to adults (tolerance) or nymphs (antibiosis) or both, to see the relationship between mechanisms and to verify to what extent the new methodology would allow us to discriminate with precision among different levels of resistance and or susceptibility. Some of the highlights of this study can be summarized as follows. The correlation between adult and nymphal damage scores was 0.248 (ns) which can be explained in terms of independence of mechanisms of resistance. The correlation between nymphal damage scores and percentage nymphal survival ($r=0.567$, $P<0.05$) may be biased by the predominance of susceptible materials among those genotypes included in the trial. Nevertheless, it is an indication of the importance of antibiosis in the reduction of nymphal damage to the plant. There was a significant negative correlation ($r=-0.774$, $P<0.05$) between nymphal damage scores and percentage of poorly developed nymphs. This could be useful in future large-scale screenings because it suggests that all susceptible materials could be discarded on the basis of nymphal damage scores. Nymphal counts to measure antibiosis could then be performed only on those materials showing little damage. This would save considerable work and time spent in nymphal counts. More important is the fact that the system does indeed discriminate clearly between resistant and susceptible genotypes. As shown in Figure 16, materials can be separated in four categories: those in the lower left quadrant of the figure are resistant, showing low nymphal survival (antibiosis) and little damage due to adult feeding (tolerance). That is the case of the resistant check 'Marandú' and a new hybrid (BR93NO/1371).

Genotypes possessing some tolerance to adult damage and no antibiosis occupy the upper left quadrant of the figure. Susceptible materials occupy the two other quadrants. Lower nymphal survival in some of these genotypes can be explained in terms of their extreme susceptibility to adult damage which renders the plant unsuitable for nymphal development due to depletion of food. When nymphal damage and percentage nymphal survival are compared (Figure 17), again the lower left quadrant of the figure contains those materials that combine tolerance and/or antibiosis as a response to nymphal attack. Experience throughout the year suggests that a safe approach to select for high levels of resistance to spittlebug would be to choose those materials showing damage scores of 2 or less and nymphal survival values of 40% or less (Figure 17).

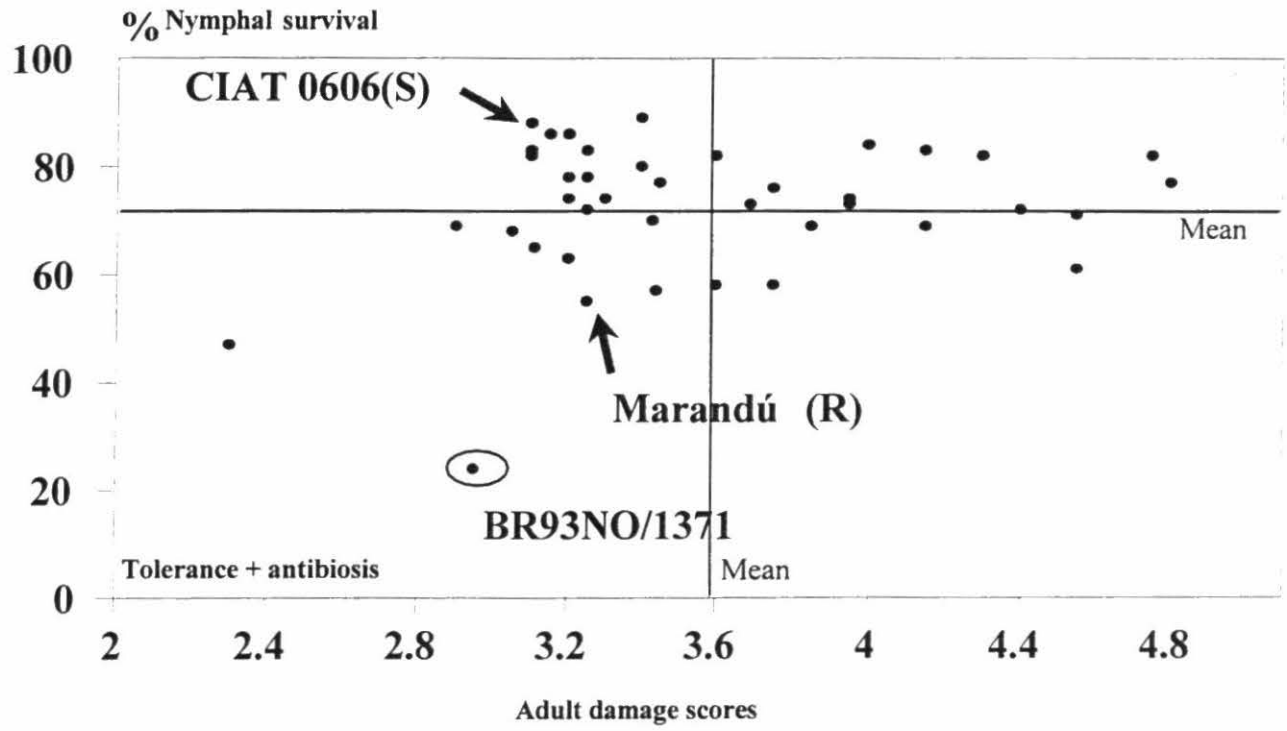

Figure 16. The relationship between adult damage scores and percentage survival of nymphs in 40 *Brachiaria* genotypes evaluated for resistance to spittlebug.

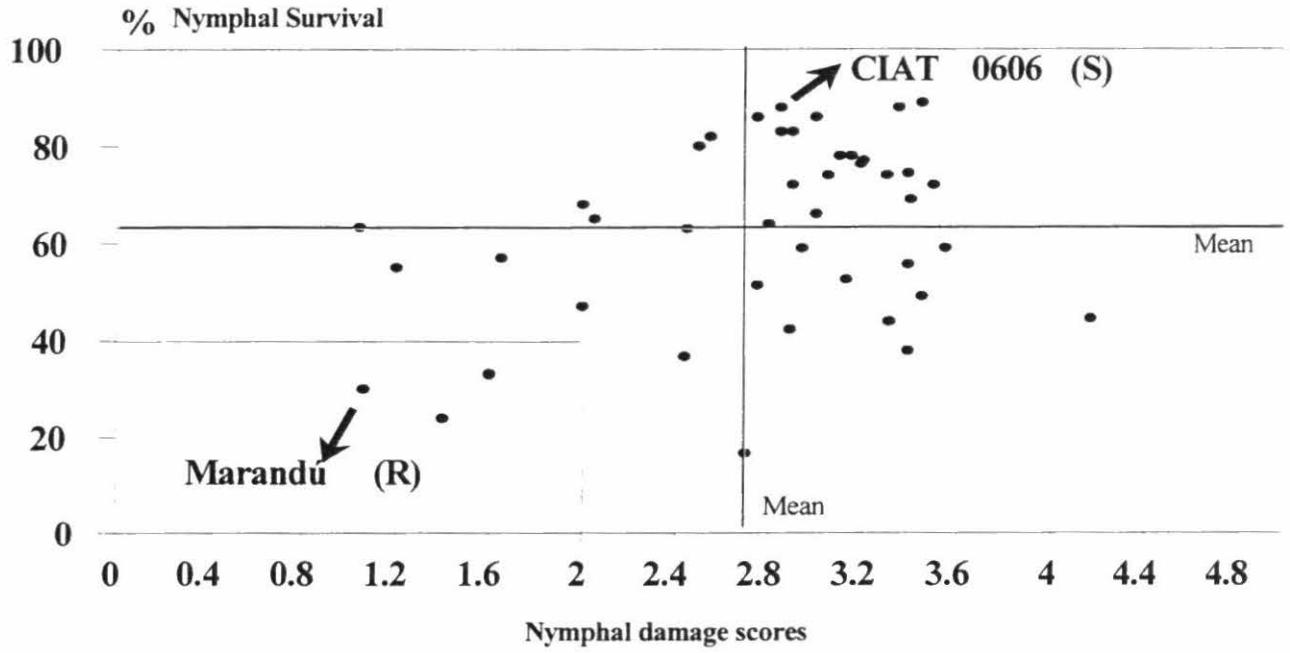

Figure 17. The relationship between adult damage scores and percentage survival of nymphs in 40 *Brachiaria* genotypes evaluated for resistance to spittlebug.

Resistance ratings obtained in the trial were compared with those obtained in previous years using the traditional pot assay (Table 23). All susceptible materials matched. Of those rated resistant using the traditional method, two were rated intermediate and one was found susceptible using the new methodology. This suggests that the new technique may be more astringent in the discrimination of genotypes.

Table 23. A comparison of resistance ratings for 40 *Brachiaria* spp. genotypes tested for resistance to *Aeneolamia varia* using two screening methodologies.

Genotypes	Resistance[1] record using 'traditional' screening method	Resistance ratings according to new screening method
BP-BRCUC1016-0009	R	I
BP-BRCUC1027-0110	R	S
CIAT 6294 ('Marandú')	R	I
BR93NO/1371	R	R
4 Hybrids	I	S
32 Hybrids	S	S

[1]R = Resistant; I = Intermediate; S = Susceptible.

The advantages of the new methodology can be summarized as follows:

a) It is faster than the previous one (60 vs 120 days between infestation with nymphs and final scoring) (Figure 18).

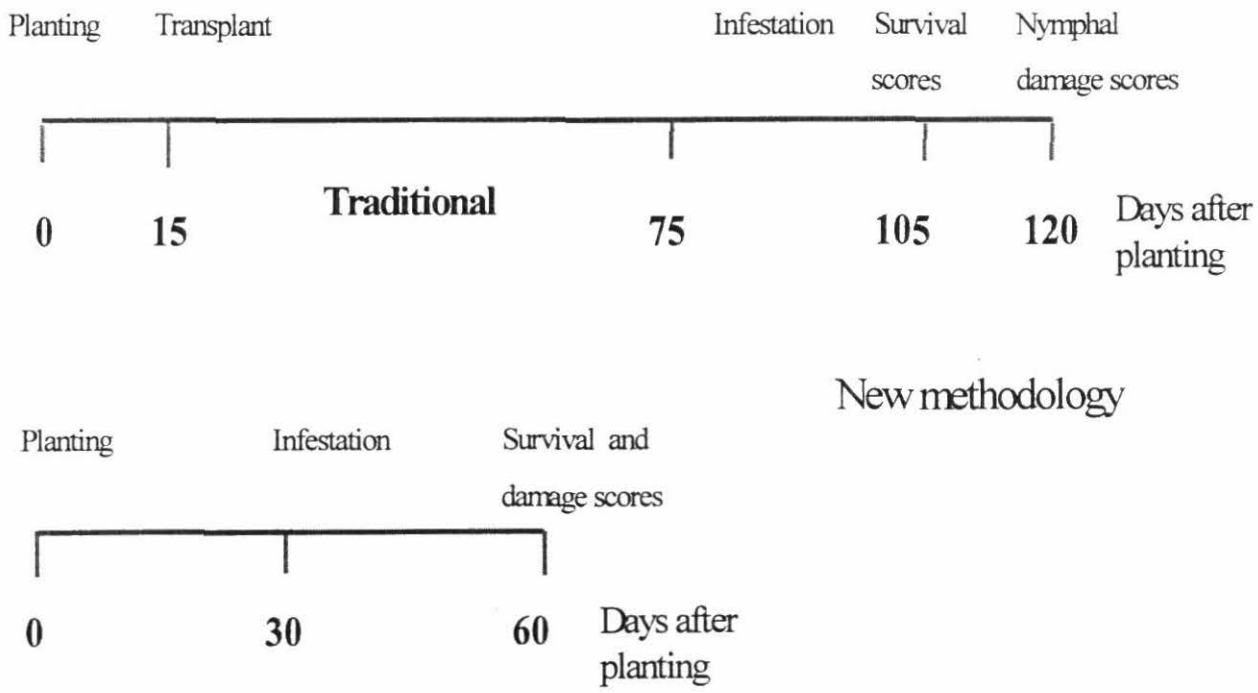

Figure 18. Sequence of events and time (days) needed to evaluate *Brachiaria* spp. for resistance to spittlebug using two methodologies.

Table 24. Effect of fertilization and screening methodology on survival of nymphs in three *Brachiaria* genotypes tested for resistance to *Aeneolamia varia*.

Screening methodology	% nymphal survival[1]								
	CIAT 0606 (S)[2]			BR93 NO/1371 (R)			CIAT 16531 (R)		
	With fertilizer	Without fertilizer	Difference	With fertilizer	Without fertilizer	Difference	With fertilizer	Without fertilizer	Difference
Traditional	71.5	55.0	16.5*	11.5	2.5	9.0 ns	13/5	16/5	-3.0 ns
Improved	60.0	31.0	29.0*	10.0	5.0	5.0 ns	8.0	10.0	-2.0 ns
Difference	11.5 ns	24.0*		1.5 ns	-2.5 ns		5.5 ns	6.5 ns	

[1]Means of 10 replications: *Significant at 5% level; ns=Not significant

[2]Resistance ratings: (S)=Suscepible; (R)=Resistant

Activity 2.2.2: Development of artificial infestation methods with sipttlebug for field screening (C. Cardona, G. Sotelo and J. W. Miles)

Rationale: As stated, assessment of resistance to spittlebug under field conditions has been impossible due to the focal, unpredictable occurrence of the insect. However, intensive work on the development of an artificial infestation technique to be used in future field evaluations was conducted in 1997 with promising results.

Methods: In trying to develop a methodology for uniform field infestation with spittlebug, three main aspects were considered: (1) The source of infestation; (2) The host plant and the creation of a microenvironment in its base that is suitable for adequate development of nymphs (root proliferation, shade and high humidity); (3) A "vehicle" to transfer thousands of live early instars to the test materials. All of these aspects were studied in 1997 at CIAT and at Macagual, Corpoica's Research Station in Caquetá.

Results and Discussion: The source of infestation has not been a problem. Mass rearing techniques developed in previous years at CIAT allow us to produce as many insects as needed. Through simple improvements and streamlining of procedures, we have developed the capability to attend future needs for mass screening infestation in the field. A highlight of this work in 1997 was the establishment of a mass rearing facility in Caquetá.

Through trial and error we found that sections of bamboo placed around the base of the plant are adequate to create the necessary conditions for full development of spittlebug nymphs. Development of a "vehicle" to transfer early instar nymphs to the field has been more difficult. Rice, sorghum, maize, and wheat seedlings were tested as food substrates for early instars. It was found that maize seedlings can sustain infestation levels of up to 100 nymphs per seedling (mean: 87.8). When the different components were tested under simulated field conditions, we found that nymphs readily moved on from the maize seedlings to the *Brachiaria* roots and colonized the plants. However, the mean percentage of survival of the nymphs to the fourth or fifth instar was 36, not enough to induce symptoms in test plants. It is not clear at this point whether the low rate of nymphal survival after transfer to the field is due to interference by ants and millipedes that could be acting as predators of early instars, or if there is a nutritional problem that affects the ability of the nymphs to develop. The presence of poorly developed nymphs in final counts suggests that this might be the case. Trials are underway to clarify this critical issue.

Suboutput 2.3 Genotypes of *Brachiaria* resistant to spittlebug identified and reconfirmed (J.W. Miles, C. Cardona and G. Sotelo)

Highlight

- Using the new screening methodology, genotypes of *Brachiaria* with antibiotic resistance to spittlebug were identified.

Rationale: Breeding for resistance to spittlebug is one of the main objectives of breeding efforts within the Project. The Entomology section provided continuous support through screening of materials for resistance to the insect.

Methods: Using the methodologies outlined above, 77 hybrids received from the breeder and three checks were screened in replicated tests.

Results and Discussion: In terms of nymphal survival, hybrid FM9503/SO15/010 was more resistant than 'Marandú', the standard resistant check. Hybrids FM9503/SO57/014, FM9503/SO94/011, and FM9503/SO75/028 were intermediate for nymphal survival and resistant in terms of nymphal damage scores. The rest were susceptible.

Suboutput 2.4 Genetic control and molecular markers identified for spittlebug resistance and apomixis in *Brachiaria*

Highlight

- New screening methodology for resistance to spittlebug in *Brachiaria has* made genetic studies feasible.

Activity 2.4.1: Estimates of heritability for spittlebug resistance (J.W. Miles, M.L. Escandón, C. Cardona)

Rationale: Data have been obtained (Miles et al. 1995) showing a strong genetic component in spittlebug resistance. We propose a detailed study to quantify more rigorously the heritability of resistance in our breeding populations. Estimation of variance components from half-sib families will be an appropriate approach, given the sexual breeding population available.

Results: We have no advance to report for this activity in 1997. Appropriate half-sib families will not likely be available for study until 1999.

Activity 2.4.2: Molecular apomixis locus (P. Rocha, J. Vargas, A. Bernal, M. Escandón, J. Miles, and J. Tohme)

Rationale: A molecular marker tightly linked to a single, dominant gene conferring apomictic reproduction would be an extremely useful in assessing reproductive mode in segregating, hybrid *Brachiaria* populations.

Methods: Individual hybrid progeny individuals are phenotyped by embryo sac analysis. Molecular markers [RFLP (including 45 cDNA rice probes, 2 cDNA maize probes, and 4 cDNA oat probes) RAPD, SCAR, and AFLP] associated with the apomictic phenotype are sought.

Results: Linkage analysis revealed a region of the *Brachiaria* genome, corresponding to chromosome 1 in rice, bearing the apomixis gene linked to RFLP, AFLP and a SCAR marker. The apomixis gene was flanked by two RFLP markers RZ 413 and RZ 995, both markers on chromosome 1 of rice. Both RFLP markers were at a distance of approx. 5 cM from the apomixis gene. A third RFLP marker, again, from rice chromosome 1 mapped approximately 10 cM away from the apomixis gene. Two other markers, SCAR marker SN14 and AFLP marker PdMg4, were located at 6 and 7 cM from the apomixis gene respectively. One other AFLP marker EeMf28 mapped 22 cM away from the apomixis gene. Figure 19 shows a tentative map of the relevant chromosome region in *B. brizantha* and *in B. decumbens.*

Discussion: Results so far reveal linkage of RFLP, AFLP and SCAR markers to the apomixis gene corresponding to chromosome 1 of rice.

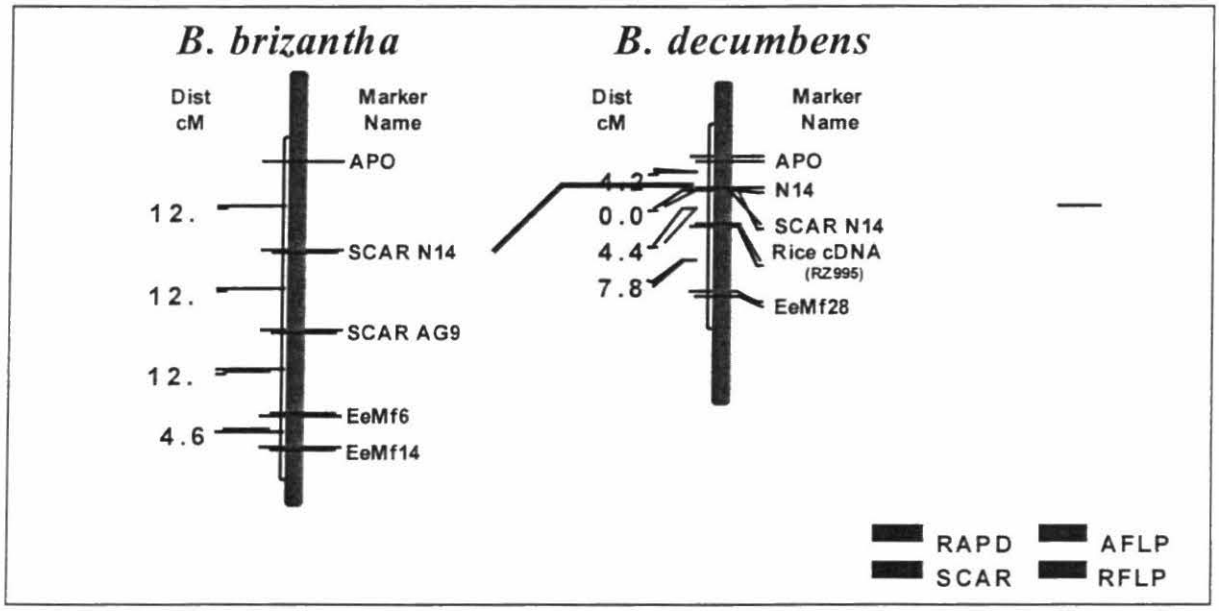

Figure 19. Preliminary linkage map of apomixis in *Brachiaria*

Suboutput 2.5 Role of endophytes in tropical grasses elucidated

Highlights

- Endophyte detection protocols within plant tissues and seeds using various stains were further improved.
- New endophytes which resemble those reported in temperate grasses were identified in species of *Brachiaria.*

- Inoculation methods to introduce these endophytes in various *Brachiaria* species and accessions commenced.

- Specific antisera for quick detection of endophytes in grass tissues are being developed.

- A graduate thesis work was intiated on the biology of an endophyte isolated from *Brachiaria arrecta.*

- Two students from the Universidad Tolima in Ibague completed an intensive two months training in endophyte detection and characterization methods in the forage pathology lab of CIAT.

- Alkaloid profile work is being conducted at Rutgers University on the endophytic fungi isolated from species of *Brachiaria.*

- Contacts were made and research collaboration initiated with specialized laboratories in New Zealand and USA.

Activity 2.5.1: Field Surveys for endophytes in Colombia (S. Kelemu, A. C. Bolaños, C. E. Posso, M. X. Rodriguez)

Rationale: Field surveys of native grasses and improved cultivated grasses are needed to document the extent of endophyte distribution in nature in the tropics. These surveys provide a diverse group of endophytes for further research work.

Methods: Tissue samples were collected in parts of Colombia (Quilichao, Popayan, Caquetá, Carimagua) and in greenhouses of the forage breeding program in Palmira. These plant tissue samples were stored in Carnoy's solution (6:3:1 ethyl alcohol: chloroform: 85% glacial acetic acid) for up to 12 weeks if not examined immediately in the laboratory using tissue staining protocols for detection of endophytes. Those samples which showed any presence of endophytic fungi were plated on various culture media for isolations of pure fungi. Seeds obtained from the forage germplasm collections held at the Genetic Resource Unit of CIAT, forage seed operation of the forage project, and fields in Carimagua, and Santander de Quilichao were also examined for the presence of endophytes.

Results and Discussion. Materials examined for endophyte presence are shown in Table 25. Few materials (eg. *B. brizantha* CIAT 6780, *B. brizantha* CIAT 6294, *B. arrecta* 16845) showed the presence of endophytic fungi either in leaf tissues or seeds. Figure 20 shows a scanning electron microscope (SEM) photograph of an endophyte in *B. brizantha* (CIAT 6780). This is an indication that endophytes exist in both warm season and temperate grasses. The tropics may provide new strains of endophytes for beneficial agricultural use, but a great deal of research is needed before this can be a reality.

Table 25. List of grass species surveyed and locations or sources.

Host and CIAT #	Location or Source[1]
Brachiaria brizantha 6294	Seed section, tropical forages
B. brizantha 6780	Seed section, tropical forages
B. brizantha 16827	Seed section, torpical forages
B. brizantha 16338	Seed section, tropical forages
B. brizantha 6297	GRU, CIAT
B. brizantha 16767	GRU, CIAT
B. brizantha 16829	GRU, CIAT
B. jubata 16203	GRU, CIAT
B. jubata 16531	GRU, CIAT
B. humidicola 6705	Seed section, tropical forages
B. humidicola 6133	Seed section, tropical forages
B. humidicola 675	GRU, CIAT
B. ruziziensis 26156	Santander de Quilichao
B. decumbens 606	Seed section, tropical forages
B. arrecta 16843	Santander de Quilichao
B. arrecta 16845	Santander de Quilichao
B. arrecta 16846	Santander de Quilichao
B. arrecta 6020	Santander de Quilichao
B. arrecta 16844	Santander de Quilichao
B. brizantha 16327	Carimagua
B. brizantha 16328	Carimagua
B. brizantha 16767	Santander de Quilichao
B. nigropedata 16902	Santander de Quilichao
B. dictyoneura 16508	Santander de Quilichao
B. dictyoneura 16509	Santander de Quilichao
B. dura 26638	Santander de Quilichao
Andropogon bicornis	Carimagua
Panicum rudgei	Carimagua
Lolium multiflorum	Commercial, Colombia
B. brizantha 26110	Carimagua
B. decumbens 606	Carimagua

[1]Seeds obtained from GRU or seed section were from various locations in Colombia such as Popayán, Quilichao and Carimagua.

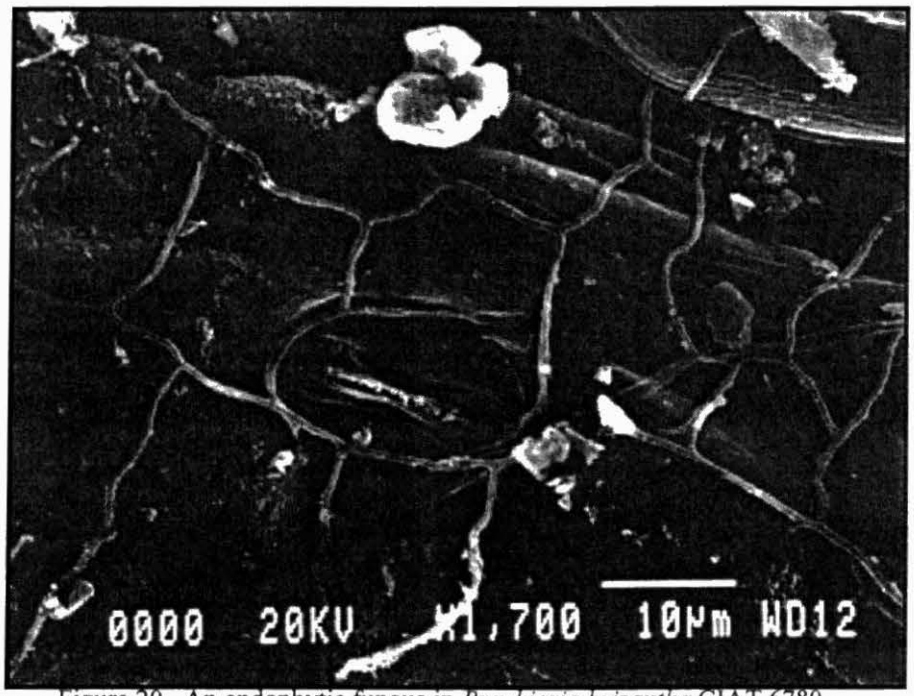

Figure 20. An endophytic fungus in *Brachiaria brizantha* CIAT 6780.

Activity 2.5.2: *Histological studies on* grass leaf tissue (S. Kelemu, A. C. Bolaños, C. E. Posso, M. X. Rodriguez)

Rationale: Histological studies provide insights into host-parasite interactions. These studies may help us in better understanding of these complex fungi and their coexistence with their host.

Methods: Samples were processed and examined under SEM. Various methods of tissue staining were evaluated for histological studies. The tissue staining method used for samples of *Brachiaria* is as follows:

A. Place small pieces of plant tissues in Carnoy's solution (6:3:1 ethyl alcohol: chloroform: 85% glacial acetic acid) for a minimum of 24 hours at room temperature.

B. Tissues are transferred to 70% aqueous ethyl alcohol twice, each for 24 hours to remove chlorophyll.

C. Tissues are cut to small pieces of 0.5- to 1.0-cm lengths and stained with aniline blue stain (2:1 aniline blue in 70% aqueous ethyl alcohol : 85% lactic acid). Time for staining is 18 hours for old tissue samples, 8 hours for young plant tissues, and 5 hours if the tissues originated from *in vitro* culture.

D. The stained tissues are cleared by sequential transferring of tissues in the following solutions:

- 100% ethyl alcohol for 60 min
- 100% ethyl alcohol for 60 min
- 1:3 methyl salicylate:ethyl alcohol for 60 min
- 1:1 methyl salicylate:ethyl alcohol for 60 min
- 3:1 methyl salicylate:ethyl alcohol for 60 min

Results: Electron microscopy studies showed the growth endophytic fungi in tissues of *Brachiaria* spp. (Figure 21). Although the efficiency of the methods developed varies from *Brachiaria* species to species, they generally work well. Both conidia and mycelial growth were observed in tissues of *Brachiaria*. The diameter of hyphae was variable (see Figure 22).

Activity 2.5.3: Isolation and identification of endophytes in grass species (S. Kelemu, A. C. Bolaños, C. E. Posso, M. X. Rodríguez)

Rationale: In order to examine and identify endophytic fungi and subsequently inoculate plants, isolation in pure cultures is essential. Because endophytic fungi are slow growing, good sterile techniques which prevent the growth of all other microbes are needed. The isolation of endophytic fungi in pure cultures is crucial for all subsequent studies in host-endophyte interactions and in the role of endophytes on biotic and abiotic stresses.

Methods: To isolate endophytes in pure culture, 5 mm long pieces of leaf sheaths. culms and

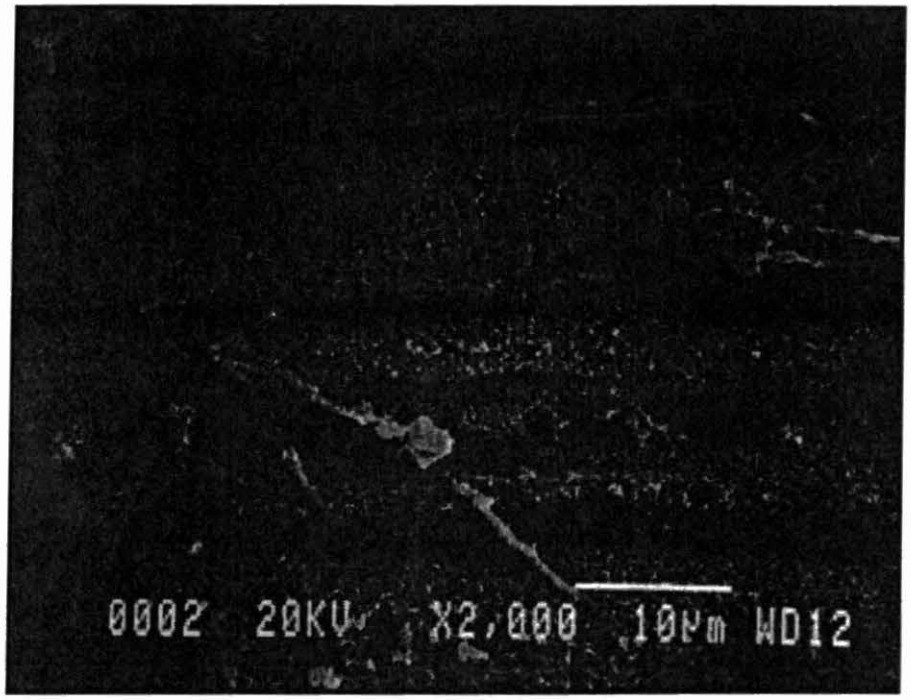

Figure 21. Scanning electron microscopy of endophytic growth in the leaf sheath of *Brachiaria*.

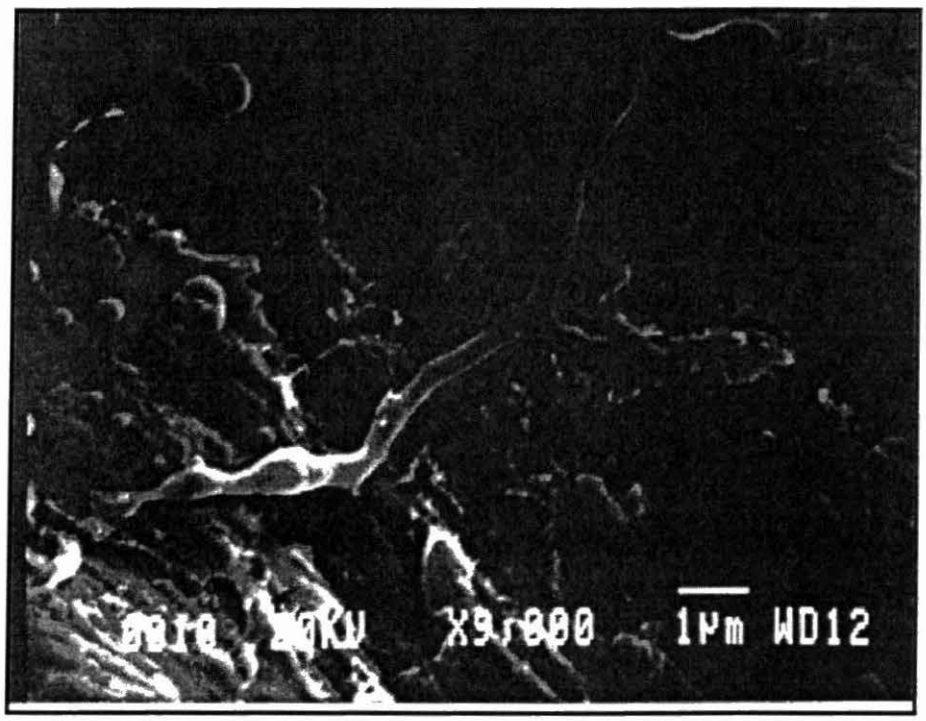

Figure 22. Variable diameter of the hyphae of the endophyte in tissues of *Brachiaria*.

leaf blades were disinfected in sodium hypochlorite (3.25%) for variable time depending on the specie (e.g 10 min for *B. arrecta* and 15-17 min for *B. brizantha*) and in ethanol (70%) for 1 min. Samples were then rinsed in sterile distilled water three times, and excess water removed using sterile filter paper. Tissue pieces were then plated on six different media, potato dextrose agar, glucose citrate agar, starch milk agar, tryptone sugar agar, yeast extract glucose agar and corn meal malt extract agar, and incubated for 4-6 weeks at 28°C.

Results: Of the materials examined to date, endophytic fungi were isolated in *B. arrecta* (CIAT 16845), *B. brizantha* (CIAT 6780), *B. brizantha* (CIAT 26110). Endophytic fungi were detected in tissues and seeds of *B. brizantha* (CIAT 6294). Colonies were slow growing and generally took 4-6 weeks to grow, white and cottony (Figure 23). Conidia are one-celled and hyaline as shown in Figure 24.

Discussion: Endophytic fungi are probably more widely distributed in warm-season grasses than previously thought. It remains to be seen if these endophytes are similar or profoundly different from those described in temperate grasses in alkaloid profiles, plant protection, their biology and other characteristics.

Activity 2.5.4: Detection Methods (ELISA, PCR) of endophytes (S. Kelemu, A. C. Bolaños, C. E. Posso, M. X. Rodríguez)

Rationale: The detection methods currently is use are time consuming and cumbersome. If rapid and reliable detection methods are developed, a large number of samples can be processed and endophytes identified. Effective techniques are fundamental for in vitro and *in planta* studies of grass-endophyte symbiosis. Endophyte antisera can be useful to study taxonomic relations among endophtes as well as detections in plant tissues. Polymerase chain reaction (PCR) methods may provide a quick way of detection if specific primers are designed.

Methods: To date there are no specific DNA-based probes which can be used to detect endophytes in temperate or warm-season grasses. Specific antisera can be developed inorder to detect endophytes using ELISA. Bioworld Laboratory Services in USA have agreed to develop antisera specific to the endophytes isolated in *Brachiaria.*

Activity 2.5.5: Development of inoculation methods (S. Kelemu, A. C. Bolaños, C. E. Posso, M. X. Rodríguez)

Rationale: Endophyte-free and uniformly endophyte-infected plants of the same genetic background are crucial to study physiological and morphological responses of these plants and to study the effect of endophytes on plant responses to biotic and abiotic stresses. Although there are various methods of inoculations developed for temperate grasses and their respective endophytes, not inoculation method is available for *Brachiaria* and its endophytes.

Methods: In temperate grasses, the most widely used inoculation method involves applying mycelia from pure endophyte cultures into the meristematic region through a small incision in young seedlings. Other methods of inoculations include the use of callus cultures, somatic.

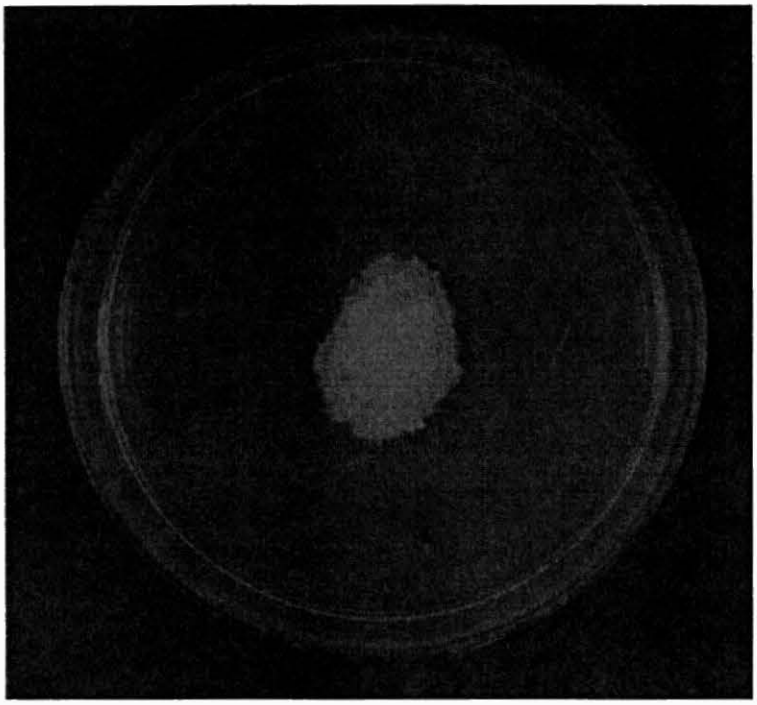

Figure 23. Pure culture of an endophytic fungus isolated from *Brachiaria arrecta* 16845 on potato dextrose agar after 27 days of incubation at 28°C.

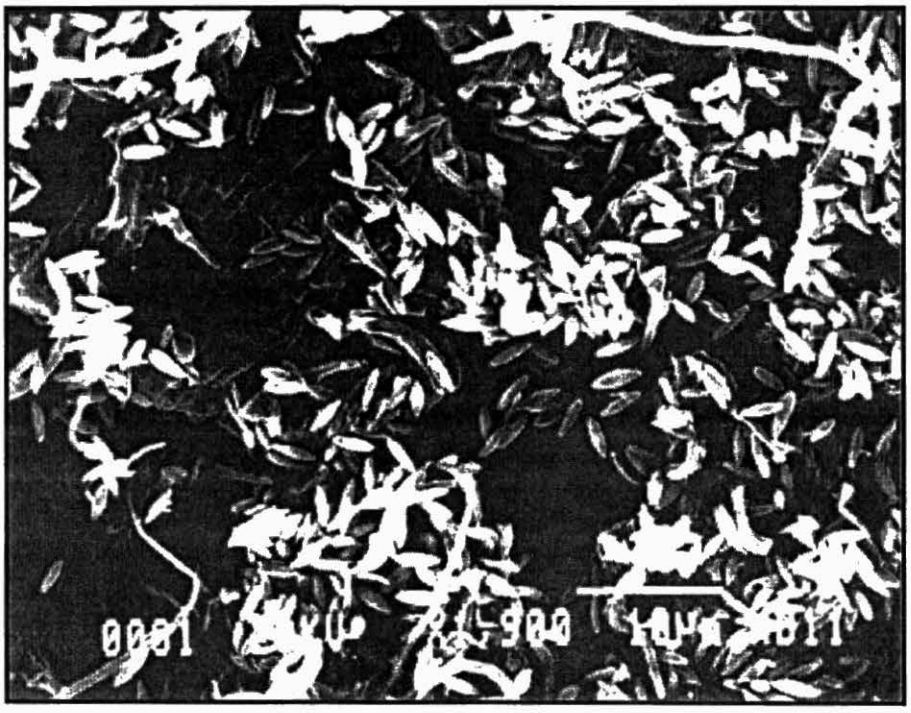

Figure 24. Conidia of an endophytic fungus isolated from *Brachiaria arrecta* (CIAT 16845).

embryos, or plantlets derived from meristem cultures. Infection rate is variable in all these methods. However, no one knows if any of these methods work in *Brachiaria* and its endophytes. We are currently examining the seedling inoculation methods and various modifications of it in order to introduce endophytes in endophyte-free *Brachiaria* spp.

Seeds of *B. brizantha* cv. Marandu which originated from various seed lots (Popayan 1995, 1996; Carimagua 1989; Brazil 1985, 1988) were disinfected and placed in water agar and Murashige & Skoog media to obtain young seedlings for inoculations. Three different experiments involving seedlings of slightly different ages were conducted using published seedling inoculation protocols.

Results and discussion: Over 350 plants were inoculated. More than 50% of the inoculated plants turned yellowish and eventually died. A little more than 100 inoculated plants are available for further evaluation of the success of the method. Published seed treatment protocols for generating endophyte-free temperate grasses severely affected the viability of *B. brizantha* cv. Marandú seeds. We are currently in the process of doing experiments using systemic fungicides (propiconazole, benlate, folicur), which have successfully been used to eradicate endophytes in temperate grasses.

Suboutput 2.6 Interactions between host and pathogen studied for key forage species

Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Sacc., is an important disease of *Stylosanthes guianensis* with a world-wide distribution. The host, *Stylosanthes guianensis,* is a diverse tropical and subtropical forage legume of great potential naturally distributed in Central and South America.

Dry matter losses of up to 100% as well as reduction in nutritive value have been reported in Colombia. *C. gloeosporioides* is a heterogeneous and complex species consisting of various host-specific populations that exhibits extreme variability in both morphology and pathogenicity. Information on pathogenic and genetic variability among isolates of the pathogen is a key element in effective breeding programs for anthracnose resistance and deployment of resistance. One of the limiting factors in the study of pathogenic variability in the South American pathogen population was the lack of appropriate differential hosts. A new set of differentials to classify pathotypes was recently described (Kelemu *et al.*, 1996).

Highlights

- *Stylosanthes* genotypes with resistance to a wide range of isolates identified.
- Differences in host reaction to *Lasiodiplodia theobromae*, the causal agent of dieback disease of *Stylosanthes* spp., detected.
- Inoculation methods developed for some foliar diseases of *Arachis*.

Activity 2.6.1: Evaluation of *Stylosanthes* populations for their reactions to anthracnose (J. W. Miles, J. L. Badel and S. Kelemu)

Rationale: Damage due to anthracnose is a major limitation in persistence and production of forage *Stylosanthes*. Host resistance is the cheapest and cleanest method of disease control. Due to pathogen variability and evolution of new strains over time, resistance may "breakdown". Identification of various sources of resistance, in areas where pathogen diversity is great, is an ideal way for having reliable resistance. Our studies show that the pathogen population in Carimagua is extremely diverse indicating that this location is ideal for evaluation for anthracnose resistance.

Methods: Ten parental accessions (FM-037, -028, -054, -053, -056, -032 –052, -036, -089, one missing due to lack of seed) and products of genetic recombination including some form of selection (Avanmas/01-06, ranging from very early maturity to very late maturity were included with checks for anthracnose evaluation (Table 26). Pedigree-derived hybrids (CIAT 11833, CIAT 11844) were also included in the evaluation.

The ten parental accessions were selected on the basis of field performance at Carimagua in 1979. All 45 possible biparental crosses were made and F1's grown. The two pedigree-derived lines were the result of single plant selections in the F2 and F3 generations. Selected F4 lines have since been maintained as bulks. A bulk of F2 seed was planted at Carimagua at the onset of the rainy season (late April or early May) in 1983, and sub-plots were bulk harvested on 12 different dates ranging from late September to early March the following year to form 12 bulk sub-populations.

These populations have been maintained over successive years by planting and bulk harvest on the date corresponding to each sub-population. Six populations were lost over the years owing to extremely low seed yields, so that only the earliest three and latest three populations have survived. The three checks were selected for known susceptibility (FM-104 and FM-103) or resistance (FM-23) to anthracnose. Disease ratings were done as described earlier (Kelemu *et al.*, 1996).

Results and Discussion: Table 26 shows the mean disease ratings of each genotype. Both pedigree-derived hybrids CIAT 11833 and CIAT11844 showed no anthracnose symptoms. Populations "Avanmas/04" and "Avanmas/05" did as well as the most resistant parent used in the cross whereas the other populations generally did better than the most susceptible parent. This is an indication that both pedigree and recurrent selection methods work in breeding for anthracnose resistance in *Stylosanthes*. It would be interesting to know whether other agronomic traits were also improved in these materials.

Activity 2.6.2: Spatial diversity in resistance of anthracnose in Stylosanthes (J. Badel, M. J. Charchar (EMBRAPA/CPAC), J. W. Miles and S. Kelemu)

Rationale: Due to differences in pathogen population compositions in different locations, resistance developed in one location may not necessarily be global. In this study, we tested some *S. guianensis* advanced materials in three field locations.

Table 26. Mean anthracnose ratings in *Stylosanthes guianensis* genotypes in field experiments in Carimagua.

Host ID	Rating[1]
FM-023	0.0
FM-037	7.9
FM-028	0.0
FM-054	3.6
FM-053	8.7
FM-056	0.0
FM-032	0.0
FM-052	5.5
FM-036	0.0
FM-089	0.0
FM-104	2.0
FM-103	7.0
CIAT 11833	0.0
CIAT 11844	0.0
AVANMAS/01	4.1
AVANMAS/02	6.2
AVANMAS/03	5.3
AVANMAS/04	0.0
AVANMAS/05	0.0
AVANMAS/06	7.9

[1] Disease reaction based on visual leaf necrosis rated by the Horsfall-Barratt rating scale in which 0 = 0%, 9 = 95-100%.

Methods: Materials developed and provided by the breeding provided were evaluated for their anthracnose reactions at three locations (Carimagua and Caqueta in Colombia and Planaltina in Brazil). Disease evaluations were done as descibed earlier.

Results and Discussion: Table 27 shows the reaction of *S. guianensis* genotypes to anthracnose at three locations. The highest disease rating for most of the Stylos tested was observed at the experiment station in Planaltina, Brazil. All the materials were developed and selected in Carimagua. Anthracnose intensity was least in Caqueta. The field plots in Planaltina have been continuously under *Stylosanthes* evaluations for many years. It is possible that there was heavy inoculum build-up, great pathogen diversity and optimum environmental conditions for high anthracnose intensity.

On the other hand, the plots in Caquetá were newly planted with *Stylosanthes*. In general, with the exception of few cases, the reactions of plants in Carimagua and Planaltina are similar.

Activity 2.6.3: Molecular analysis of anthracnose pathogen diversity (C. X. Moreno, M. X. Rodriguez and S. Kelemu)

Rationale: Randomly amplified polymorphic DNA (RAPD) using the polymerase chain reaction (PCR) has found various applications such as pathogen detection, race or species differentiation, and population genetics. The technique uses random short oligonucleotide

primers to amplify DNA fragments from small amounts of template DNA with the polymerase chain reaction, producing DNA fingerprints. We used this method to assess the extent of genetic diversity among isolates of *C. gloeosporioides* in South America.

Table 27. Anthracnose reactions of *Stylosanthes* genotypes at three locations[1]

Host ID	Carimagua	Caquetá	Planaltina
FM 10E	0.0	0.0	ND
FM 12E	8.5	3.0	4.8
FM 13D	0.0	0.0	ND
FM 2E	0.0	0.0	6.7
FM 42G	3.8	4.5	5.8
FM 4E	1.3	2.3	4.3
FM 6E	0.0	0.0	4.9
FM 7D	2.5	2.5	5.1
FM 8E	2.4	0.0	ND
FM 9205 Parcela 1	5.4	5.3	6.5
FM 9205 Parcela 2	6.2	6.0	5.8
FM 9205 Parcela 3	6.0	5.0	6.4
FM 9205 Parcela 5	4.6	1.0	4.3
FM 9205 Parcela 6	3.0	1.0	4.6
FM 9D	0.0	0.0	6.7

[1] Disease ratings were based on visual leaf necrosis where 0 = 0%, 9 = 95-100%
ND = Not determined

DNA restriction fragment length polymorphisms detect variations in DNA sequences among homologous sections of chromosomes. Several studies of pathgenic fungi have been reported on RFLPs in nuclear DNA. A collaborative project with the University of Queensland, Australia resulted in the isolation of a repetitive element, termed CgT1 (*Colletotrichum gloeosporioides* Transposon 1), which is dispersed in the genome of *C. gloeosporioides* and present in about 30 copies. The CgT1 DNA probe detected polymorphism in anthracnose-causing isolates of the fungus specific to *S. guianensis*. In this study, we used CgT1 DNA probe to analyze the complexity of the pathogen population primarily in South America.

The objectives of this study were to determine:

a) the variability in pathogenicity of South American isolates of *C. gloeosporioides*,
b) to measure the amount of genetic diversity by polymerase chain reaction (PCR) amplification of DNA and RFLP analysis, and
c) to evaluate possible correlations between genetic diversity as measured by random amplified polymorphic DNA (RAPD) or RFLP and race as defined by pathogenicity pattern on differential host genotypes.

Some of the data related to this work has been reported (Kelemu et al. 1995, 1996,1997).

Methods: All isolates (with the exception of 3 Australian isolates) of *C. gloeosporioides* used in this study were collected in various regions of South America during the period 1980-1994 from naturally infected *S. guianensis* plants. Monoconidial cultures of each isolate were

derived as described previously (Kelemu et al., 1996). All culture incubations were done at 28° C.

For DNA isolations, fungal cultures were grown in fresh V-8 juice liquid medium for 3 days at 28°C with shaking at 200 rpm. Genomic DNA (3 to5 μg) from each isolate was digested to completion with *Eco*RI at 37 C as recommended by the enzyme manufacturer (Gibco BRL). DNA fragments were separated by electrophoresis in 0.8% agarose in 1x TBE buffer at 33 V for 48 h and blotted onto Hybond N+ (Amersham) by alkali transfer as described by the manufacturer.

Detection of hybridized bands, and labeling of pCHB1 probe containing a repetitive element were done using ECL direct nucleic acid detection systems kit (Amersham) as described by the manufacturer.

Nine arbitrary 10-base oligonucleotide primers from Operon Technologies were used for PCR amplification work.These primers were 5'-TGCCGAGCTG-3', 5'-AGTCAGCCAC-3', 5'-GTCGGAGTGG-3', 5'-ACGGCACGCA-3', 5'-AGGGTCGGTC-3', 5'-AGGTCGGCGT-3', 5'-TCGCAGCGAG-3', 5'-GTGAGGCGTC-3', and 5'-GTCGCCGTCA-3'. The amplification products were resolved on electrophoresis in a 1.2% agarose gel (Bio-Rad) and stained with ethidium bromide and photographed under UV lights.

Comparison of each banding profile for each primer was conducted on the basis of the presence or absence (1/0) of RAPD products of the same size. Bands of the same size were scored as identical. An analysis of the genetic variation over the entire data set was undertaken to assess the probable number of genetic lineages present. First, coefficients of dissimilarity (1-Jaccard's coefficient) were calculated for all combinations of isolates using programming steps in written in PROC IML. A multiple correspondence analysis (MCA) was carried out on the distance data using PROC CORRESP.

A cluster analysis was carried out using PROC CLUSTER with several clustering methods and parameters. The clustering statistics; cubic clustering criterion (CCC), pseudo F, and pseudo t^2, were graphed. A consensus of the three statistics(local maxima of CCC and pseudo F concomitant with a local minimum of pseudo t^2 [SAS, 1989#11] page 98]) was sought as an indication of the number of modal clusters present in the data set. Consensus of the various clustering methods on the number of clusters present was accepted as an indication of the number of distinct genetic lineages represented in the data set.

A three-dimensional graph of the first three dimensions of the MCA analysis was constructed using the "spin" platform of JMP software (SAS, 1994) to provide a visual representation of the associations (Figure 25).

The next step, to test the statistical reproducibility of the associations found, was carried out by combining cluster analyses with the resampling ("bootstrapping") technique (Efron; Williamson and Young). The resampling analysis consisted of constructing 130 X 80 data sets by random sampling, with replacement, from the original data set; then carrying out the cluster

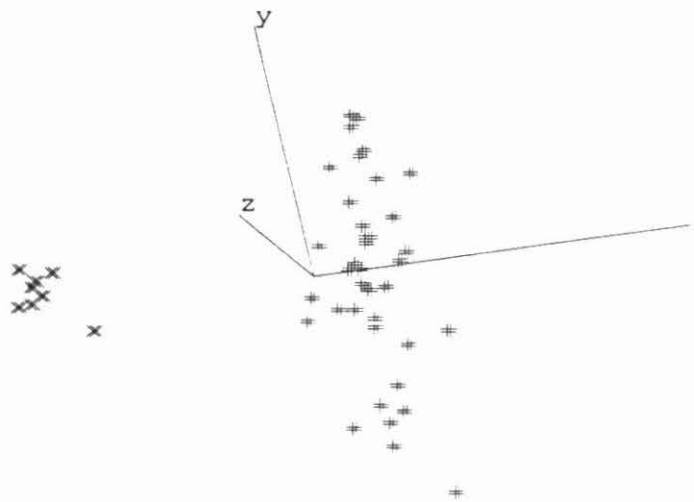

Figure 25. Isolates of *Colletotrichum gloeosporioides* clustered together by their geographic origin.

analysis using the TWOSTAGE method with K=4 on the new data set. The cluster assignments of each isolate were tabulated; the process was repeated 1000 times. Isolates which were placed in the same cluster in more than 90% of the 1000 resampling and cluster analysis iterations were considered to represent a distinctive genetic lineage. The total number of lineages represented was estimated from the number of unique groups formed by this 90% criterion. This criterion assumes that RAPD fragments judged as being of the same size had a single historical origin, and that the clustering method used is sufficient to detect unique lineages.

Results: The amount of genetic diversity in isolates of *C. gloeosporioides* originated from *S. guianensis* genotypes was measured at molecular level by polymerase chain reaction (PCR) amplifications of DNA using arbitrary primers of 10 bases, and by restriction fragment length polymorphism (RFLP) with a non-LTR retrotransposon DNA sequence. Generally, isolates were clustered together by their geographic origin (Figure 25), probably indicating a single introduction event. In clusters where isolates of various regions are clustered together, most of the isolates had identical host genotype origin. The pathogen population sampled from Carimagua, Colombia, a savanna ecosystem and a long time *Stylosanthes* breeding and selection site, was highly diverse, with very few isolates related at more than 40% similarity in RAPD fragment profiles. Southern blot analysis of the isolates revealed a fairly conservative variation. A total of 23 hybridizing fragments were found resulting in 41 morphotypes among 130 isolates.

Of the isolates tested, 63 comprised 13 distinctive lineages by the 90% criterion. Four isolates were assigned to two lineages implying that one had diverged from the other. We define a lineage as a group of isolates that are assigned to the same cluster in more than or equal to 90% of the resampling iterations. None of the remaining isolates clustered with any other isolate in more than 90% of the resampling iterations suggesting that the isolates were either quite unique, or were intermediate between clusters in RAPD fragment profiles and could have

been part of more than one cluster with more-or-less equal likelihood.

From these results we concluded that *C. gloeosporioides* isolates collected from some geographic area are quite similar, probably indicating a single introduction event. On the other hand, some clusters of isolates consist of collections from very diverse geographic areas and/or very different collection dates, indicating that the RAPD fragment profiles used (Figure 26) were capable of revealing genotypes that have become widely dispersed, and/or persist in nature for extended periods of time.

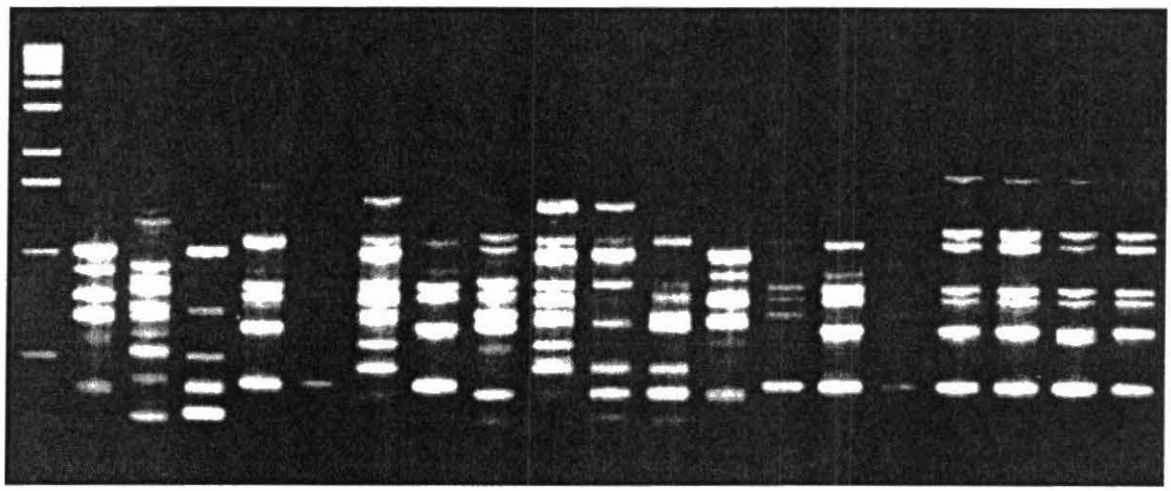

Figure 26. RAPD fragment profiles of some representative isolates of *Colletotrichum gloeosporioides*.

Carimagua had a surprisingly diverse collection of isolates. The most similar isolates were related with a genetic distance of 0.34. All other pairs of *C. gloeosporioides* isolates differed by genetic distances of 0.47 to 0.94, with an overall mean of 0.77. Of the isolates collected from the same host accession, five isolates,from CIAT 184 were genetically distinct with a minimum genetic distance of 0.47, and a maximum of 0.94; the mean was 0.73. Thus there did not appear to be any influence of host genotype on the genetic constitution of those five isolates. It is difficult to judge how many distinct genetic lineages were represented in the collection of 36 isolates from Carimagua. It seems unlikely that there had been genetic diversfication to the extent seen from a single introduction of the fungus. It seems much more likely that many unique *C. gloeosporioides* genotypes are endemic to the area.

In summary, the genetic situation with *C. gloeosporioides* populations capable of causing anthracnose disease of *S. guianensis* appears to be that most sites are populated by fairly homogeneous endemic populations that have undergone diversification at some level, usually evidenced as less than 30% difference in RAPD fragment profiles. There are also a substantial number, about 20% of the population, that are very different from the bulk of the population suggesting that multiple introductions into a site usually occurred. This kind of pattern was evident in the Campo Grande, Quilichao, and Caqueta sites.

In the case of the Carimagua site, the population sampled was highly diverse, with very few isolates related at more than 40% similarity in RAPD fragment profiles. It may be because the Carimagua site has been under *S. guianensis* cultivation longer than the other sites sampled, allowing time for more *C. gloeosporioides* diversification. If that is not the reason, then it seems likely that numerous *C. gloeosporioides* introductions occurred into that area.

Our results on RFLP analysis of the isolates with pCHB1 as a probe, revealed conservative variations. A total of 23 hybridizing fragments were found resulting in 41 morphotypes among the isolates. Twenty three of the 130 isolates had no fragments that hybridized to the probe (Figure 27), indicating a lack of the repetitve element. Of these 23 isolates, six were from a variety of locations, and 17 were isolated from Carimagua collected over a period of 14 years from 1980 to 1994. This result indicated that a population of the fungus that lacked the repetitive element persisted in the area for years. Five of these isolates were collected on June 16, 1994. However, another 6 isolates from Carimagua collected on that same day, all carried the pCHB1 element, hybridizing to identical patterns consisting of 19 of the 23 fragments. This is clear evidence of a second introduction of the fungus into the Carimagua area. Using RFLP analysis, Carimagua again appeared to consist of a wide variety of morphotypes, 14 of them.

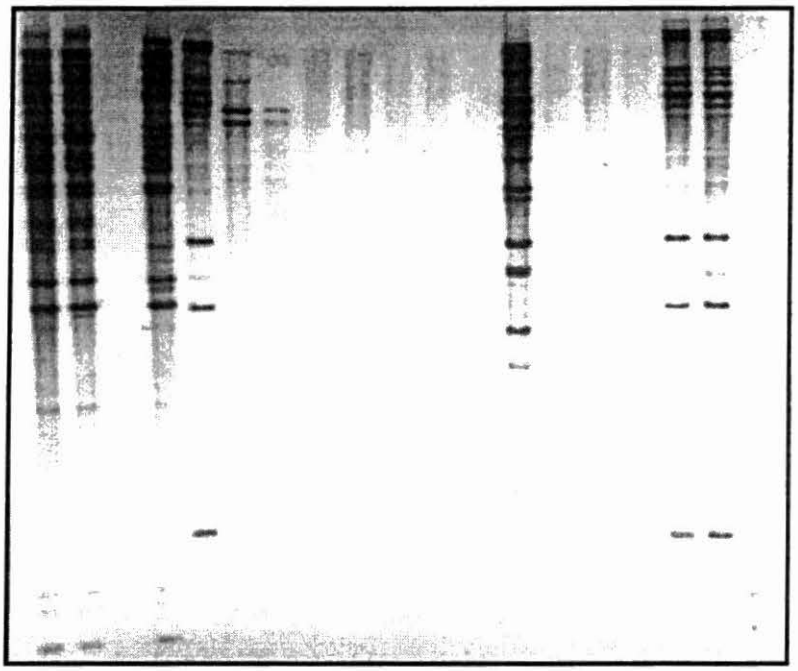

Figure 27. Southern hybridization of probe pCHB1 to a gel blot of EcoRI-digested DNA of some representative isolates of *Colletotrichum gloeosporioides*.

Discussion: In this study, we examined pathotypes, RFLP and PCR polymorphisms to evaluate genetic relatedness and diversity in an attempt to understand the South American *Colletotrichum gloeosporioides* population. We have shown that South American isolates of *C. gloeosporioides* infecting *S. guianensis* exhibit a wide range of genetic diversity. Pathogenic variation based on *S. guianensis* differntials, revealed a wide range of pathotypes

to exist among the isolates tested. New virulence patterns were detected among these isolates. The results of this study show that the population of *C. gloeosporioides* in South America is highly heterogeneous. This further confirms previous observations that South America contains a diverse population of *C. gloeosporioides* (Kelemu et. al. 1995, 1996, 1997). A general correspondence between the isolate groups and their geographic origin was observed

Activity 2.6.4: Biochemical analysis of anthracnose pathogen (M. S. Sanchez, M. X. Rodriguez and S. Kelemu)

Rationale: Cell wall degrading enzymes that degrade the non-cellulosic components of the plant cell wall play an important role in diseases caused by nectrotrophic bacteria and fungi. Enzymes predominantly produced by pathogens in dicots are polygalacturonases, pectate lyases and pectin lyases which degrade cell walls and kill protoplasts. Pectolytic enzymes contribute to fungus penetration and the initial stages of plant infection. The pectic-degrading enzymes produced by a number of pathogens macerate plant tissue, cause loss in electrolytes, and result in cell death. Although cell wall degrading enzymes have been reported in species of the genus Colletotrichum, there are no reports of the production of pectate lyase in *C. gloeosporioides* infecting *Stylosanthes* spp. which may contribute to pathogenesis and increased virulence.

Methods: To produce axenically grown plants, seeds of *Stylosanthes* were surface-sterilized by first suspending in 70% ethanol with mild agitation for 7 minutes. The seeds were then transferred to a solution of 30% chlorox and 0.025% Triton X-100 for 15 minutes with shaking , then washed 3 times with sterile distilled water. Seeds were then transferred to sterile filter papers to remove excess water. Five to ten seeds were planted in Magenta vessels containing 50 ml of MS medium. The vessels were incubated in a growth chamber at 24°C with a photo period of 12 hours and fluorescent lighting. Plants were inoculated with fungal conidial suspensions as described earlier (Kelemu et al. 1996).

Enzyme samples from infected *Stylosanthes* tissues were obtained by collecting infected plant tissues into sterile tubes with an approximately equal volume of sterile water, grinding using sterile glass rods, and centrifuging at 13,000 x g. For extractions from culture fluids, fungal parts were removed from *C. gloeosporioides* V-8 juice culture (12-day-old, incubated with shaking at 200 rpm at 28 C) fluids by centrifugation at 5,000 x g for 10 minutes. Residual fungal fragments were removed by passage of the supernatant through 0.22-μm-pore-size cellulose acetate membranes. All enzyme preparations were then cleared of low-molecular-weight solutes and concentrated several fold (at least 10-fold) by ultrafiltration with Centricon-10 membrane tubes (10,000-molecular-weight cutoff) for further analysis. For enzyme assays, enzyme activity was monitored by A_{232} assays spectrophotometrically. Isozyme profiles were determined by isoelectric focusing gels in ultra-thin-layer polyacrylamide gels activity stained with 0.4-mm pectate-agarose overlays.

Results: Production of pectolytic enzymes by *C. gloeosporioides* was explored in liquid cultures. All cultures were harvested after 8 days of incubations. Preliminary experiments have shown that at the culture conditions used, the highest enzyme activity was detected at 8 days after inoculation. Extracts from artificially infected *Stylosanthes* tissues showed much higher

level of activities than culture fluid extracts. Using a buffer for substrate overlay optimum for pectate lyase (Pel) activity (Tris-HCl at pH 8.5), several Pel isozymes ranging from acidic to alkaline pIs were revealed. Many of the isolates used with few exceptions produced isozymes with identical patterns (Figure 28). Extracts from healthy control plant tissues showed no isozymes. The enzyme preparations had nearly fourfold lower activity on 93% methlesterified pectin than on polygalacturonic acid. No major differences in the level of activity was observed on either pectin with 31% or 68% methylesterifications. When sterile enzyme preparations extracted from infected tissues were infiltrated into the leaves of healthy *Stylosanthes* plants, dark blighted lesions appeared within three days.

Figure 28. Pectate lyase isozyme profiles in *Colletotrichum gloeosporioides* infecting *Stylosanthes* spp.

Discussion: The Pels have been suggested to have a role in the virulence of soft-rotting bacteria such as Erwinias. In *Erwinia* spp pathogenesis, it has been shown that certain isoenzymes are more crucial for disease development than others. It is possible that some or all of the isoenzymes we detected in *C. gloeosporioides* are essential for virulence of the pathogen. All pathogenic isolates we examined to date produced Pel isozymes. However, the role of these isoenzymes in virulence can accurately be studied by creating targeted mutations into the corresponding *pel* genes.

Activity 2.6.5: Occurrence of diseases in forage *Arachis* (M. X. Rodriguez and S. Kelemu)

Rationale: Limited disease surveys were done in order to document diseases of *Arachis* and to observe the severity of some of these diseases. The results of these surveys are used to help us prioritize our research needs on diseases of *Arachis*.

Methods: Surveys were done in plots of *Arachis* in Popayán, Carimagua, Palmira and Caqueta. Samples were collected from a number of accessions in these locations and processed

in the laboratory using standard isolation protocols.

Results: The most prevalent disease of Arachis in 1997 in the locations surveyed was anthracnose caused by *Colletotrichum gloeosporioides*. Few other foliar diseases occurred. No severe damage occurred in any of the accessions examined.

Activity 2.6.6: Development of inoculation methods for commonly occurring diseases in *Arachis* (M. X. Rodriguez and S. Kelemu)

Rationale: We have already reported that we developed a successful inoculation method for foliar blight of forage *Arachis* caused by *Rhizoctonia solani*. In the past, we attempted several inoculation methods (including the method being used for *Stylosanthes* spp.) for isolates of *C. gloeosporioides* on *Arachis*. Because reliable artificial inoculation methods are essential for screening for sources of resistance, we continued the work of developing inoculation methods for the anthracnose pathogen in *Arachis*.

Methods: Matured and healthy leaves of *Arachis* were collected and surface-sterlized with followed by 1 % NaOCl for 2 min. followed by 70% ethanol for I min, and rinsed three times in sterile water. Three trifoliates of each accession were placed in pots containing sterile sand with Hoagland nutrient solution (which contains N, P, K, Ca, Mg, Fe and microelements). Each accession was replicated three times. Pots were transferred to a growth chamber with a photoperiod of 12 hours, temperature 25 C and 90% relative humidity for 24 hours. After this period, the leaves were inoculated with conidial suspension (2×10^6 conidia/ml of sterile distilled water). The pots were covered with transparent plastic and placed back in the growth chamber. This method of inoculation was adopted from a mthod developed for other foliar diseases of peanuts.

Results and Discussion: Evaluations of disease reactions were done two weeks after inoculations. Symptoms usually appeared in the form of brown to black leaf spots. In some cases, spots lighter color centers with brown borders appeared. The method allowed large number of materials to be processed in a relatively small area. Disease reactions were reproducible for each host genotype isolate interactions. This method may probably be useful for other foliar diseases of *Arachis*.

Activity 2.6.7: Screening of *Arachis* accessions for reaction to certain pathogens
(F. Muñoz, M. X. Rodriguez and S. Kelemu)

Rationale: Although no disease of epidemic proportion has been reported in forage *Arachis* to date, anthracnose caused by *Colletotrichum gloeosporioides* is becoming predominant in field plots in Carimagua, Caqueta and Popayan. In earlier studies, we have shown that isolates of the pathogen isolated from *Arachis* were virulent on *Stylosanthes* spp. and vice versa. Anthracnose is the most important disease in *Stylosanthes*. The complexity and great diversity shown in *Stylosanthes*-anthracnose studies makes the pathogen to be a candidate for a potential disease epidemic cause in *Arachis*. In this study, we examined the reaction of few accessions of *Arachis* to isolates of *C. gloeosporioides*.

Methods: One hundred eighty three isolates of *Colletotrichum gloeosporioides* collected from various naturally infected accessions of *Arachis* were used to inoculate 5 accessions of *Arachis pintoi* (CIAT 17434, CIAT 18744, CIAT 18748, CIAT 22160 and the original accession from which the isolate came from). Plants were inoculated as described in inoculation methods for *Arachis*. Isolations and culture maintenance were as described in methods in *Stylosanthes-C.gloeosporioides* interactions.

Results and Discussion: Of the 183 isolates used, 74 isolates produced anthracnose symptoms on some or all of the accessions tested (see symptoms in Figure 29). The reactions of the accessions tested to 25 of the 74 pathogenic isolates is shown in Table 28. It is important to note that differential reaction are expressed in plant accession-isolate interactions. Differential host can thus be assembled to assess race composition in the pathogen population. Using this information, improvement strategies may be designed to create *Arachis pintoi* populations resistant to anthracnose.

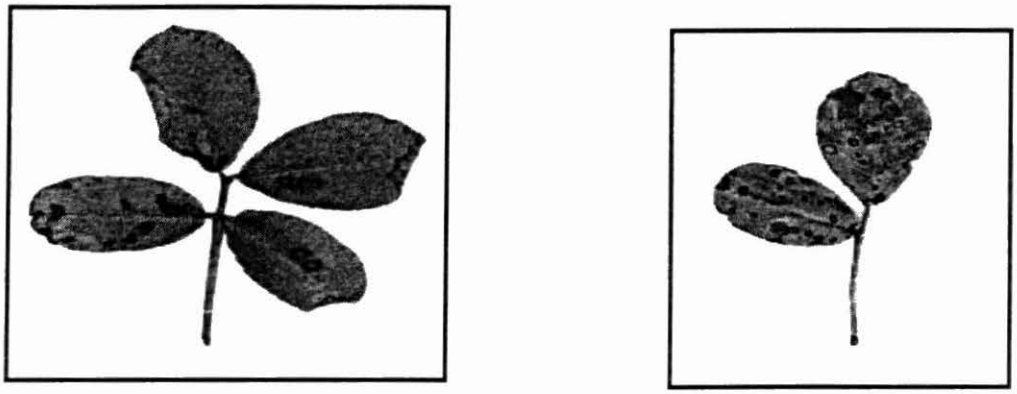

Figure 29. Anthracnose symptoms (naturally infected leaves, right; artificially inoculated leaves, left) in forage *Arachis*

Activity 2.6.8: Development of inoculation methods of *Stylosanthes* dieback disease (J. Badel, C. Fernandes (EMBRAPA/CNPGC, Brazil) and S. Kelemu)

Rationale: A reliable and fast method of artificial inoculation is essential for reliable identification of sources of resistance. In our earlier studies, we developed an artificial inoculation method for the dieback pathogen *Lasiodiplodia theobromae* and its host *Stylosanthes*. This method has been further improved in order to increase its efficiency and consistency.

Methods: Seven weeks (16 weeks for adult plant inoculations) after transplanting five-day-old seedlings, each plant was wounded with a needle at the base of the stem just above the soil line. A sterile toothpick was used to inoculate the plant with a 14-mm-diameter mycelial disk, taken from a 12-day-old colony of the fungus cultured on oatmeal agar. Control plants were inoculated with 14-mm-diameter sterile oatmeal agar disks. Inoculation points were then wrapped in parafilm. The plants were kept in the greenhouse under natural daylight and at temperatures between 19 and 30 C until symptoms were expressed.

Table 28. Reaction of *Arachis pintoi* accessions to isolates of *Colletotrichum gloeosporioides*.

Isolate #	Host accession CIAT #					
	17434	18744	18748	22160	Original host	Original host accession
16240	+	-	+	-	ND	ND
16457	-	-	+	+	-	17434
16568	-	-	-	+	-	18744
16321	-	+	+	-	+	18748
16572	+	-	-	+	+	20826
16448	+	+	-	+	+	22153
16476	-	-	+	+	-	22155
16481	+	+	+	+	+	22160
16330	-	-	+	-	+	22163
16576	+	-	+	-	+	22175
16495	+	+	-	-	-	22176
16578	+	-	-	+	+	22232
16504	-	-	+	-	+	22233
16508	-	-	+	-	+	22234
16513	+	-	+	-	+	22235
16518	-	-	-	-	+	22236
16519	+	-	+	-	+	22238
16525	-	-	-	-	+	22239
16586	+	-	-	-	+	22240
16590	+	-	+	+	+	22241
16541	-	+	+	+	+	22259
16542	-	+	-	+	-	22262
16544	+	+	+	-	+	22264
16560	-	+	-	-	-	22269
16555	+	-	+	-	+	22270

+ = anthracnose disease; - = no disease; ND = not determined

Results: Symptoms appeared 8-10 days after inoculations in seedling plant tests and up to a month later in adult plants. Variability in reaction among plants of the same genotype was reduced greatly when fresh fungal inoculum was used and the same amount of inoculum was applied. The virulence of the inoculum was reduced if the fungus was transferred several times on culture media and kept on media for prolonged time. A typical thick-walled and striate conidium of the fungus *L. theobromae* is shown in Figure 30.

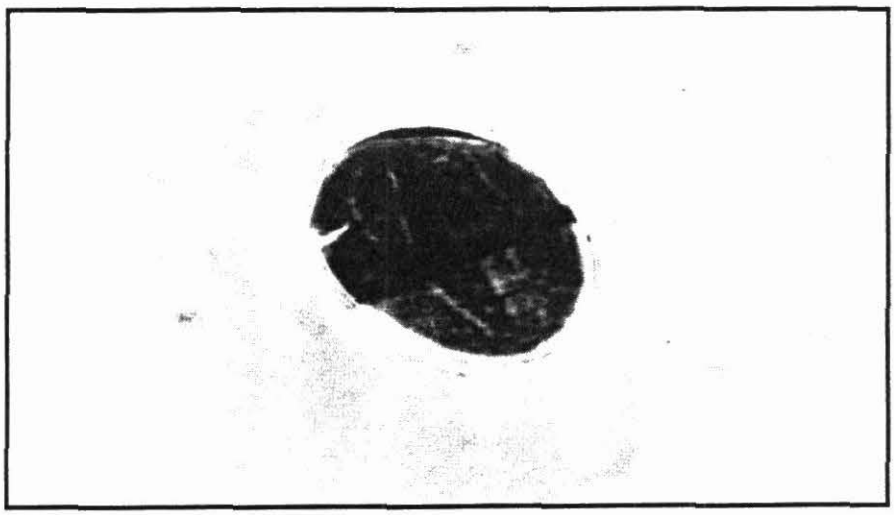

Figure 30. Conidium of *Lasiodiplodia theobromae* (phase contrast 2500X).

Activity 2.6.9: Screening of *Stylosanthes* accessions for reaction to dieback disease (M. Satizabal and S. Kelemu)

Rationale: We earlier identified the causal agent of the dieback disease of *Stylosanthes* as *Lasiodiplodia theobromae*. The pathogen is a virulent, unspecialized, facultative wound pathogen with worldwide distribution and more than 500 different host plants. The disease has become prominent in recent years. This study was conducted to search for sources of resistance among commonly grown genotypes and improved and advanced populations.

Methods: Plant inoculations were conducted as described above. Tests were arranged in a completely randomized design with three replications. Each replication contained 10 plants. Pathogenicity tests were repeated at least once for verification. The length of stem lesions on each plant was measured 8-10 days after inoculations. Longitudinal sections were carefully made on all plants to measure the length of vascular lesions. To measure root dry weight, above ground parts were cut from the roots at the soil level with a surgical blade. The roots were washed free of soil, placed on paper towels for initial drying, and subsequently placed in paper bags. Roots were dried for 65 h at 60 C. The weight of the dried roots was then determined. All statistical analyses were done using Duncan's multiple range test.

Results: Symptoms include wilting of seedlings, complete dieback of branches in adult plants, and vascular brown discolorations in both adult and seedling plants. In most susceptible genotypes, the entire plant eventually dies. Inoculated adult plants expressed dieback symptoms 25-30 days after inoculations, whereas seedlings of the same genotypes showed wilt symptoms 8-10 days after inoculation. The *S. guianensis* genotypes tested in this study varied in their reactions to infections by the pathogen (Table 29).

Table 29. Reaction of seedlings of selected *Stylosanthes guianensis* genotypes to *Lasiodiplodia theobromae* in the greenhouse[1].

Host ID	Stem lesion height (cm)	Vascular discoloration (cm)
Mineirao	3.84 a	4.02 a
FM 53	2.79 b	3.10 b
FM 56	1.18 cd	1.31 c
FM 37	0.67 de	0.83 cd
FM 36	0.65 de	0.79 cd
FM 123	0.15 e	0.45 d
FM 54	0.09 e	0.33 d
FM 52	0.04 e	0.32 d

[1]Each value is the mean of three replicates. Data in columns followed by the same letter are not significantly different (P=0.01), according to Duncan's multiple range test.

Discussion: As a wound pathogen, the fungus may have ready access to the host through cattle grazing and trampling, or cutting with machines. Symptoms of the advanced stages of the dieback disease can be confused with certain abiotic stresses such as drought or damage caused by stem borers. The distinguishing symptom of this disease, however, is vascular discoloration. Although we do not know what the mechanisms of resistance to the pathogen

are, we have shown that sources of resistance do exist in *the S. guianensis* populations. Stem lesion measurements correlated well with those of vascular lesion. Resistance to *L. theobromae* can, thus, be quickly assessed using stem lesion measurements in *S. guianensis*.

Suboutput 2.7 Information on genetic diversity of *Brachiaria* and *Arachis* linked with biotic constraints

Highlight

- Isozyme data exist for both *Brachiaria* and *Arachis* and molecular marker data exits for *Brachiaria*.

Activity 2.7.1: Genetic diversity through isozymes in *Brachiaria* and *Arachis* (B.L. Maass, J.W. Miles).

Rationale: An understanding of the organization of genetic diversity within the genera *Brachiaria* and *Arachis* will assist, for example, in choosing parental materials for a hybridization program and for selecting a core germplasm collection for regional testing.

Methods: Izozyme analyses of collections of both *Brachiaria* and *Arachis* have been completed. These data are awaiting appropriate analysis.

Results: No advance in this activity was made during 1997.

Discussion: A proper rigorous analysis of existing data will require additional human and financial resources.

Output 3: Grass and legume gene pools with known adaptation to edaphic and climatic constraints

Suboutput 3.1: Genotypes of *Brachiaria*, *Panicum* and *Arachis* with adaptation to low fertility soils identified and characterized

Low nutrient supply is a major limitation of forage adaptation and production in low fertility, acid soils of the tropics. Widespread adoption of forage cultivars depends on efficient acquisition of nutrients from the soil and for utilization in growth. Adapted plants have attributes that are linked to strategies to acquire nutrients in a low pH and high aluminum (Al) environment. Understanding these strategies is fundamental to developing more efficient screening procedures. Improving adaptation of forages to infertile soils without loss of forage yield or quality will contribute to lower input requirements, lower animal production costs, and fewer environmental problems from soil degradation.

Previous research had indicated that : a) adaptation of *Brachiaria* genotypes to low fertility acid soils is related to three plant attributes - leaf area production, total root length and phosphorus (P) uptake efficiency per unit root length; b) the poor persistence of *Brachiaria ruziziensis* on infertile acid soils may be due to its inability to partition greater amounts of dry matter to root growth compared to shoot growth; c) genotypic variation in leaf area production and nitrogen (N) partitioning to leaves in *Brachiaria* was greater than the variation in N acquisition from the soil; d) nutrient uptake efficiency of forage legumes, particularly P, was markedly greater than that of the forage grasses while the efficiency in utilization of acquired nutrients for growth was greater in forage grasses; and e) acquisition of calcium (Ca) by forage legumes from infertile acid soils was related to root architecture, particularly the number of root tips.

Activity 3.1.1: Studies on mechanisms of acid soil tolerance in *Brachiaria*

Highlight

- Root apices of *B. decumbens* accumulate phosphorus in the presence of aluminum and low supply of nutrients in solution which could contribute to aluminum tolerance mechanism in low fertility acid soils.

Rationale: A graduate student thesis (Ph.D.) project funded by the Austrian Academy of Sciences was continued in collaboration with the Biotechnology Research Unit at CIAT, the National Accelerator Centre in Faure, South Africa, and the University of Vienna, Austria. The aim of this project was to get insights into mechanisms of acid soil tolerance of three *Brachiaria* species which are being used as parents in a breeding program at CIAT: *B. decumbens* cv. Basilisk (well adapted), *B. brizantha* cv. Marandú (less adapted), and *B. ruziziensis* cv. Common (poorly adapted).

Methods: Two important facts determined the research approach chosen. First, differences with respect to acid soil adaptation between *Brachiaria* cultivars become apparent only after one to several years of pasture utilization, when growth of less adapted species is increasingly

reduced. This suggests small differences in edaphic adaptation that result in a cumulative effect. Second, the relative importance of the simultaneous stress-factors that constitute the so-called "acid soil syndrome" (Al-toxicity and low supply of P, N, Ca, Mg, K) for *Brachiaria* species is still a matter of discussion. Thus, a multiple-stress nutrient solution was designed based on nutrient concentrations measured in soil solutions of two low-fertility acid soils from the Colombian savanna region. This solution was confirmed to simulate acid soil conditions by comparing growth in nutrient solutions with field performance.

In a second phase, the nutrient solution was employed as a standard treatment to investigate a number of traits that might contribute to differential acid soil adaptation of *Brachiaria* cultivars, which is expected to be a multigenic aggregate trait. Simultaneously, a study on differences in adaptation to P- and N-deficiency (the two main factors commonly assumed to be involved in pasture degradation) was conducted with special emphasis on root architecture. As a complementary approach, a cDNA library enriched for acid soil stress-induced genes was constructed from roots of the best adapted cultivar (*B. decumbens*), with the aim of isolating genes involved in differential acid soil adaptation in a near future, using results from physiological experiments as a base line.

Results and Discussion

A multiple-stress nutrient solution to simulate the "acid soil syndrome" (P. Wenzl, L. I. Mancilla, I. M. Rao, J. E. Mayer and R. Albert)

The soil solution is considered as the closest approximation of the medium that is in contact with plant roots that determines nutrient uptake. Analyses indicated that nutrient concentration of a commonly used low-ionic strength nutrient solution (*control* solution in Figure 31-left

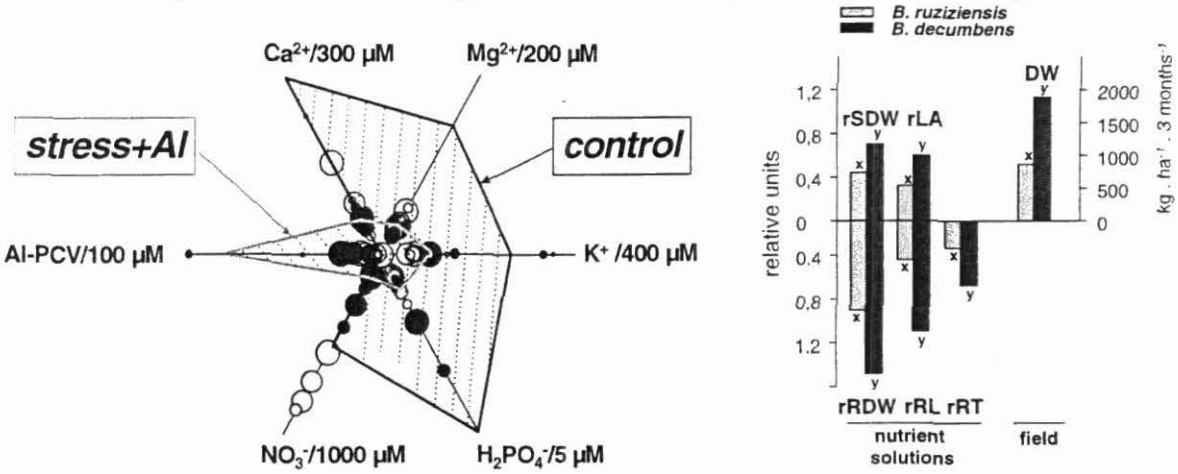

Figure 31. **Left half.** Comparison of nutrient concentrations in soil solutions (symbols) with nutrient concentrations of the designed nutrient solutions (lines). Nutrients concentrations are depicted on six separate axes with the maximum values given at the end of each. ***Soil solutions.*** Gray symbols: clay loam Oxisol, black symbols: sandy loam Oxisol. The symbol size is inversely proportional to incubation time before soil solution extraction. ***Nutrient solutions.*** The black line connects values used for the *control* solutions and the gray line connects values used for the *stress+Al* solution. **Right half.** Relative yield of two contrasting *Brachiaria* cultivars in the designed nutrient solutions (stress+Al/control) compared to above-ground dry matter production one year after pasture establishment, averaged over several sites in Latin America. ***Upper half (shoots).*** rSDW, rLA: relative shoot dry weight and leaf area. ***Lower half (roots).*** rRDW, rRL, rRT: relative root dry weight, root system length and total number of root tips, respectively.

half) were higher than those found in soil solutions of Colombian savanna soils. Thus, we lowered the nutrient concentrations (*stress+Al* solution in Figure 31, left half) and evaluated the short-term relative growth of *Brachiaria* cultivars. Growth differences between the well and poorly adapted cultivar were comparable with field data (Figure 31, right half). We concluded that the designed treatment was useful to simulate acid soil stress and applied it for subsequent experiments.

A comparison of the toxic effect of Al with nutrient-sufficient conditions (traditional nutrient solution) and with nutrient-limited conditions (designed solution) revealed that the poorly adapted cultivar, *B. ruziziensis* was significantly less Al-tolerant (in the presence of low supply of nutrients) than the best adapted cultivar *B. decumbens* (Figure 32). Consequently, the investigation of putative Al-tolerance mechanisms was one main focus of this project.

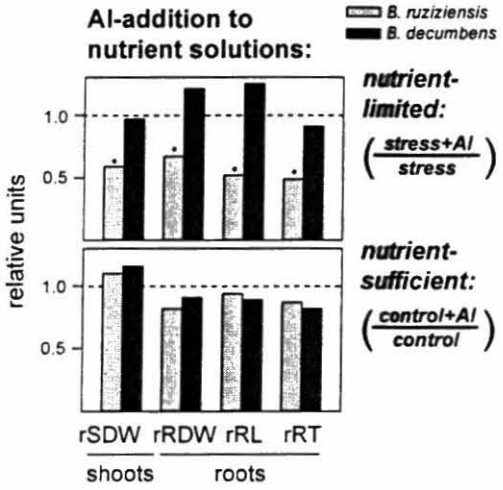

Figure 32. Interspecific difference in Al-tolerance under nutrient-limited conditions (upper half) in contrast to the lack of difference under nutrient-sufficient conditions (lower half). **Abbreviations.** rSDW, rRDW, rRL: relative shoot and root dry weight, root system length and root tip number, respectively.

Citric acid is involvement in Al-tolerance in *Brachiaria* (P. Wenzl, A. L. Chaves, J. E. Mayer, I. M. Rao and R. Albert)

The exudation of organic acids, such as malic acid and citric acid, by root apices excludes toxic Al^{3+} ions from the symplasm by chelation in the rhizosphere. Alternatively, organic acids seem to be employed to detoxify Al in the symplasm, probably by sequestration in the vacuole. An analysis of organic acids in root tissue of *Brachiaria* cultivars clearly demonstrated that Al stimulates a several-fold increase of the concentration of citric acid, especially under nutrient-limited conditions (Figure 33).

It is currently not clear whether citric acid is also exuded. However, the large amount of citric acid found in entire root systems suggests that it does not accumulate exclusively in root apices. Accumulation of citric acid in mature portions of the root apices could play a role in internal Al-detoxification. In fact that no significant differences in citric acid-accumulation under simultaneous Al- and nutrient-stress could be detected among the cultivars tested indicates that alternative Al-tolerance mechanisms may underlie the interspecific growth differences observed above (See Figure 32).

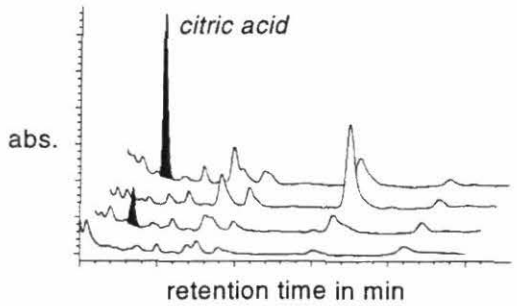

Figure 33. Organic acids in entire root systems of *B. decumbens*. Plant were grown in a *control*, *control+Al*, *stress* and *stress+Al* nutrient solution (from bottom to top).

Aluminum-phosphorus interactions in root apices (P. Wenzl, C. A. Pineda, I. M. Rao and J. E. Mayer)

When plants were grown in the presence of Al, roots tended to increase their P-content, with the notable exception of *B. ruziziensis* grown in the presence of Al under nutrient-limited conditions (*stress+Al* solution; Figure 34-left halt).

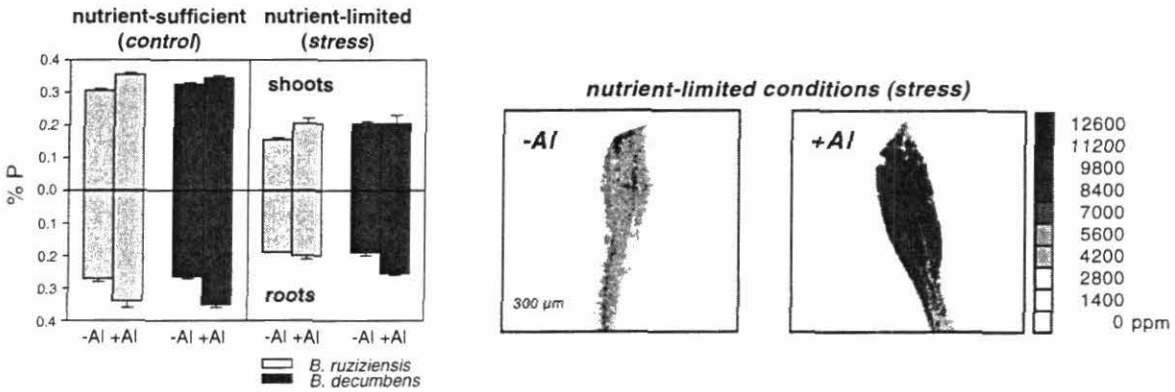

Figure 34. **Left half.** Root and shoot P-content of two contrasting *Brachiaria* cultivars grown under nutrient-sufficient and -limited conditions with and without Al. **Right half.** Distribution of P in root apices of *B. decumbens* grown under nutrient-limited conditions with and without Al, visualized by PIXE.

Recently, exudation of phosphate by root apices has been suggested to act as an Al-exclusion mechanism in wheat and maize. Thus, we were interested whether the Al-stimulated accumulation of P was located in root apices, where it could eventually be exuded as inorganic phosphate. Proton-induced X-ray emission (PIXE) was applied to map the distribution of nutrients in root apices. Preliminary results indicate that - at least in the case of *B. decumbens* - this seems to be the case (See Figure 34, right half). Experiments are underway to determine the extent of phosphate exudation as a function of the distance from the root tip.

A possible link between Al-toxicity and nutrient-uptake capacity (P. Wenzl, L. I. Mancilla, A. L. Chaves, I. M. Rao and J. E. Mayer)

When Al was applied under nutrient-sufficient conditions it did not affect growth. However, when applied under nutrient-limited conditions, it significantly inhibited growth of *B. ruziziensis* but not of *B. decumbens* (Figure 35). The causal relationship underlying this negative synergism in *B. ruziziensis* is not obvious, because either Al-toxicity as such or an impaired nutrient uptake-capacity under Al-stress might have reduced growth. To address the latter hypothesis, we investigated a possible link between both stress components: the plasma membrane (PM) H^+-ATPase. This enzyme creates the proton gradient which is used to drive nutrient uptake, and is also a possible target site of toxic Al^{3+} ions.

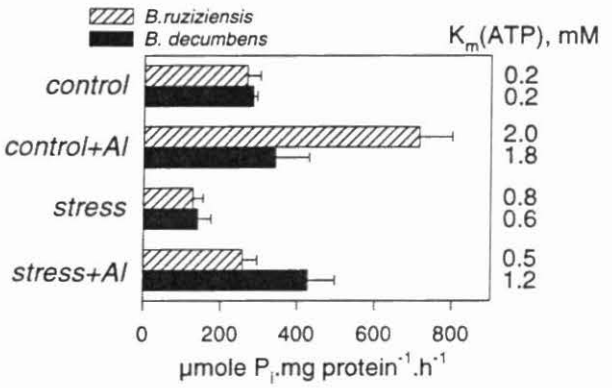

Figure 35. Plasma membrane H^+-ATPase activity of two contrasting *Brachiaria* cultivars grown under nutrient-sufficient and nutrient-limited conditions with and without Al. Corresponding K_m values for ATP are given at the right side of the figure.

Measurements of the PM H^+-ATPase activity in purified plasma membranes indicated that (i) Al in the growth medium stimulated an increase in activity of the enzyme, (ii) in *B. decumbens* the activity-increase was associated with a higher value of K_m for ATP, (iii) a similar increase in K_m under Al-stress occurred in *B. ruziziensis* only under adequate nutrient supply (control+Al; Figure 35). The apparent association between maintenance of root growth under Al-stress and an increasing K_m (ATP) value of the root PM H^+-ATPase suggests an involvement of the PM H^+-ATPase in differential Al-tolerance between *B. decumbens* and *B. ruziziensis* under limiting nutrient supply. A differential expression of isoforms with different kinetic characteristics under Al-stress could be involved.

Furthermore, the increasing activity of the PM H^+-ATPase under Al-stress indicates that Al-tolerance mechanisms in *Brachiaria* may consume the electrochemical proton gradient at the plasma membrane. Exudation of Al-chelating compounds, including organic acids and/or phosphate would be in line with this finding.

The role of root cation exchange capacity in Al-tolerance (P. Wenzl, A. Hernández, I. M. Rao and J. E. Mayer)

Cation exchange capacity (CEC) of roots has been suggested to be involved in differential Al-tolerance within some crop species. It is probably an important factor for cation-uptake in the presence of Al^{3+} ions and might thus play a role in the poor performance of *B. ruziziensis* under simultaneous Al- and nutrient-stress. Purified root cell walls of plants grown in the four basic nutrient solutions were equilibrated with a *stress+Al* nutrient solution in order to evaluate their relative affinities to Al^{3+} and other cations such as Ca^{2+}, Mg^{2+}, and K^{+}. A detailed analysis using absorption isotherms in conjunction with a thermodynamic analysis based on mass-action descriptions of ion exchange processes is in progress.

Identification of aromatic metabolites synthesized under acid soil stress (P. Wenzl, A. L. Chaves, I. M. Rao and J. E. Mayer)

Besides Al-toxicity, symbioses relevant for uptake of certain nutrients are also likely to play a role in edaphic adaptation of *Brachiaria* species. For example, P-acquisition of certain *Brachiaria* species is known to be highly dependent on vesicular-arbuscular mycorrhizae (VAM) and some species are excellent hosts for nitrogen-fixing bacteria in the rhizosphere. In both symbioses aromatic secondary compounds, e.g. derived from the phenylpropanoid metabolism, might be involved in signal exchange between plants and microorganisms. This motivated us to investigate aromatic metabolites synthesized in roots under acid soil stress.

An initial screening using multiple-stress nutrient solutions demonstrated that two probably aromatic compounds accumulated several-fold in roots of *B. ruziziensis*, but not in the other two investigated cultivars. Growth in single-stress nutrient solutions revealed that accumulation of both compounds is stimulated by P-deficiency and that one of them is also produced under N-deficient conditions (Figure 36). Interestingly, all cultivars synthesized these metabolites when grown in a P-deficient soil, with *B. ruziziensis* exhibiting the highest concentrations. If these compounds are part of the plant's response to P-deficiency, this might imply that *B. ruziziensis* triggers P-stress response at P-concentrations that are still sufficient for the other cultivars or that other cultivars need a longer P-starvation period to react in this sense.

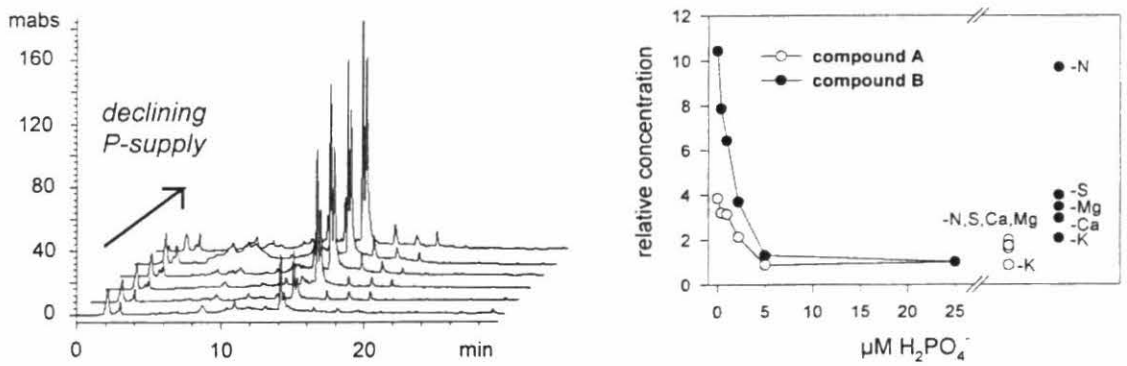

Figure 36. Synthesis of two probably aromatic secondary compounds in roots of *B. ruziziensis* under P-deficient conditions and other nutrient stresses. **Left**. Progressive accumulation under gradually declining P-supply (25 µM to 0 µM $H_2PO_4^-$). **Right**. Quantification of peak areas and comparison with other nutrient stresses.

Elucidation of their molecular structure is in progress in collaboration with Dr. Muralle Nair at Michigan State University and will enable us to develop hypotheses about their putative role in acid soil adaptation.

Root acid phosphatase as a measure of sensitivity to low P supply (P. Wenzl, I. M. Rao and J. E. Mayer)

The stimulation of root acid phosphatase activity is a typical component of the plant's response to P-deficiency and could thus be employed to estimate the level of P at which the plant becomes P-deficient. Analyses indicated that under nutrient-limited growth conditions (*stress* solution) *B. ruziziensis* was the only species that significantly increased its root acid phosphatase activity, confirming the above view that this species might trigger physiological responses to P-deficiency at higher P-levels than the other two species. Al-toxicity also stimulated root acid phosphatase activity. In the case of *B. decumbens*, an alternative isoenzymatic form was apparently expressed under Al-stress, which was more tolerant to Al *in-vitro*.

Recently, it has been demonstrated that certain *Brachiaria* species exude phytase under P-starvation. We will thus carry out experiments to study the differential induction and exudation of root acid phosphatase in greater detail and test whether the measured acid phosphatase activity is caused by phytase.

Adaptation to P- and N-deficiency: role of root morphology and architecture (P. Wenzl, N. L. Lasso, I. M. Rao and J. E. Mayer)

A decline of N-and P-availability in soils has frequently been proposed to be involved in pasture degradation over time. In general, plants allocate more biomass in root systems to explore soils more efficiently under these conditions. Therefore, we selected these two components of acid soils stress to analyze the putative contribution of root system shape and architecture. Preliminary results indicate that root growth of *B. decumbens* and *B. brizantha* was greater than that of *B. ruziziensis* under lower N-supply.

Isolation of acid soil stress-induced genes (P. Wenzl, L. I. Mancilla, J. E. Mayer, E. Heberle-Bors and I. M. Rao)

Once the physiological bases of mechanisms of acid soil adaptation become clearer, it will be desirable to isolate the underlying genes for two principal reasons. First, on the base of gene or promotor sequences, molecular markers could be designed for screening purposes. Second, specific genes of interest could be employed for direct gene transfer between *Brachiaria* cultivars via genetic transformation to circumvent the obstacle of apomictic propagation. In view of this strategy, we prepared a subtractive cDNA library enriched in genes that are induced by acid-soil stress in the well adapted cultivar (*B. decumbens*) but not in the poorly adapted cultivar (*B. ruziziensis*).

PCR-amplification of cDNAs remaining after subtraction, revealed several stress-induced genes, which emerge as distinct bands from the diffuse background smear of non-subtracted medium- to low copy-cDNAs (Figure 37). Initially, we will choose four genes corresponding to clearly visible bands for sequence analysis with the aim to get insights into their putative

function. However, we should be able to design more specific strategies to select genes from this library when the physiologically oriented experiments will be concluded.

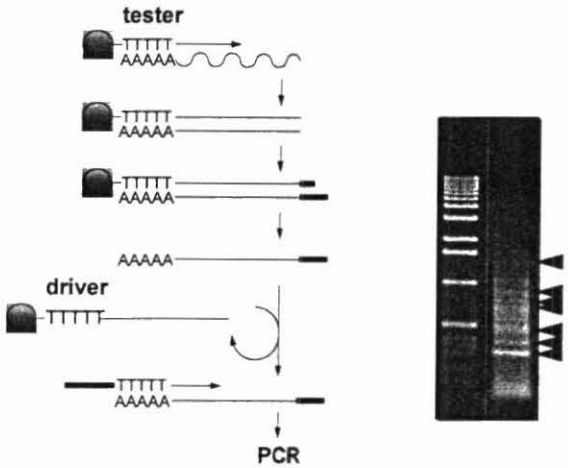

Figure 37. **Left half**. PCR-based subtractive hybridization used to enrich acid soil stress-induced genes. **Right half**. Subtracted cDNAs amplified by PCR and separated on an agarose gel. Arrows point to individual high-copy enriched genes.

Activity 3.1.2: Identification of plant attributes of *Brachiaria* genotypes for tolerance to low nutrient supply (I. M. Rao, J. W. Miles, C. Plazas and J. Jaumer)

Highlight

- Two genetic recombinants (BRN093/3204, FM9201/1873) of *Brachiaria* were outstanding in their adaptation to low fertility acid soil conditions.

Rationale: A number of promising genetic recombinants have been generated through the *Brachiaria* breeding efforts. However, tolerance of genetic recombinants to low fertility acid soil conditions has not been evaluated under field conditions. Evaluation of plant performance under field conditions will help to determine genetic variation in adaptation to low fertility, acid soil stress and to identify specific plant attributes that may be utilized as selection criteria in a genetic enhancement program. A field study is in progress at Carimagua to evaluate differences in edaphic adaptation and persistence of *Brachiaria* genotypes and to identify key attributes of edaphic adaptation.

Methods: The trial comprises 17 entries, including nine natural accessions (four parents) and eight genetic recombinants. The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots. The trial is submitted to periodic mob grazing according to forage on offer.

Results and Discussion: Measurements of forage yield and leaf area index during wet season (6.5 and 15 months after planting) indicated marked genotypic variation (Figures 8 and 9). With low initial fertilizer application, two genetic recombinants, BRN093/3204 and FM9201/1873, were found to be outstanding in forage yield and leaf area production when compared to other entries. One of the genetic recombinants (BRN093/1371) which is very resistant to spittlebug infestation (C. Cardona, unpublished data) was found to be responsive to initial fertilizer application in terms of leaf area production and forage yield. At 15 months after planting, three genotypes (BRN093/3204, CIAT 16467 and CIAT 6780) showed greater leaf area production with low initial fertilizer than that of high initial fertilizer application (Figure 9B). This may due to greater utilization of forage by animals from plots that received high initial fertilizer application. We need to monitor this trial for 2 more years to evaluate plant attributes that contribute to persistence of *Brachiaria* genotypes.

Activity 3.1.3: Genotypic variation in tropical forage legumes for tolerance to low nutrient supply (I. M. Rao, J. Ricaurte and R. García)

Highlight

- *A. pintoi*, CIAT 18748 was outstanding in it's adaptation to low nutrient supply to soil as revealed by the extent of partitioning of N in leaves and maintaining greater concentration of inorganic P in leaves.

Rationale: Intergeneric and interspecific variation in tolerance to low nutrient supply has been identified among a number of tropical forage legumes when grown in soils of contrasting texture. However, intraspecific variation in tolerance of legumes to low nutrient supply has not been determined.

Methods: A glasshouse experiment examined genotypic differences in tolerance to low nutrient supply among 24 genotypes of 6 legume species (4 genotypes for each species: *Arachis pintoi*, *Stylosanthes guianensis*, *Stylosanthes capitata*, *Centrosema macrocarpum*, *Centrosema brasilianum* and *Centrosema pubescens*). Shoot and root attributes were measured in order to evaluate their low fertility tolerance. The selection of genotypes was based on agronomic evaluation in the field (commonly used, very productive and less productive genotypes for each species). A sandy loam oxisol from Carimagua was used to grow the plants (4 kg of soil/pot). Nutrients were supplied before planting at three levels (nil, low and high). Low nutrient supply (kg/ha) included 20 P, 20 K, 33 Ca, 14 Mg and 10 S while the high nutrient supply included 80 N, 50 P, 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo. At the time of harvest (80 days of growth), the following shoot attributes were determined: (a) forage yield, leaf/stem ratio, (b) leaf area, (c) leaf chlorophyll and soluble leaf protein, (d) photochemical efficiency of photosystem II, (e) leaf and stem nutrient composition, (f) leaf nutrient partitioning index (leaf nutrient/shoot nutrient x 100), (g) nodule number and (h) nodule weight.

Results and Discussion: Plant attributes were influenced by nutrient supply and also by genotype (Table 30). The extent of leaf area production, with no external supply of nutrients, exhibited greater genotypic variation than the other plant attributes. As expected, increase in nutrient supply improved forage yield as a result of stimulation of leaf area production.

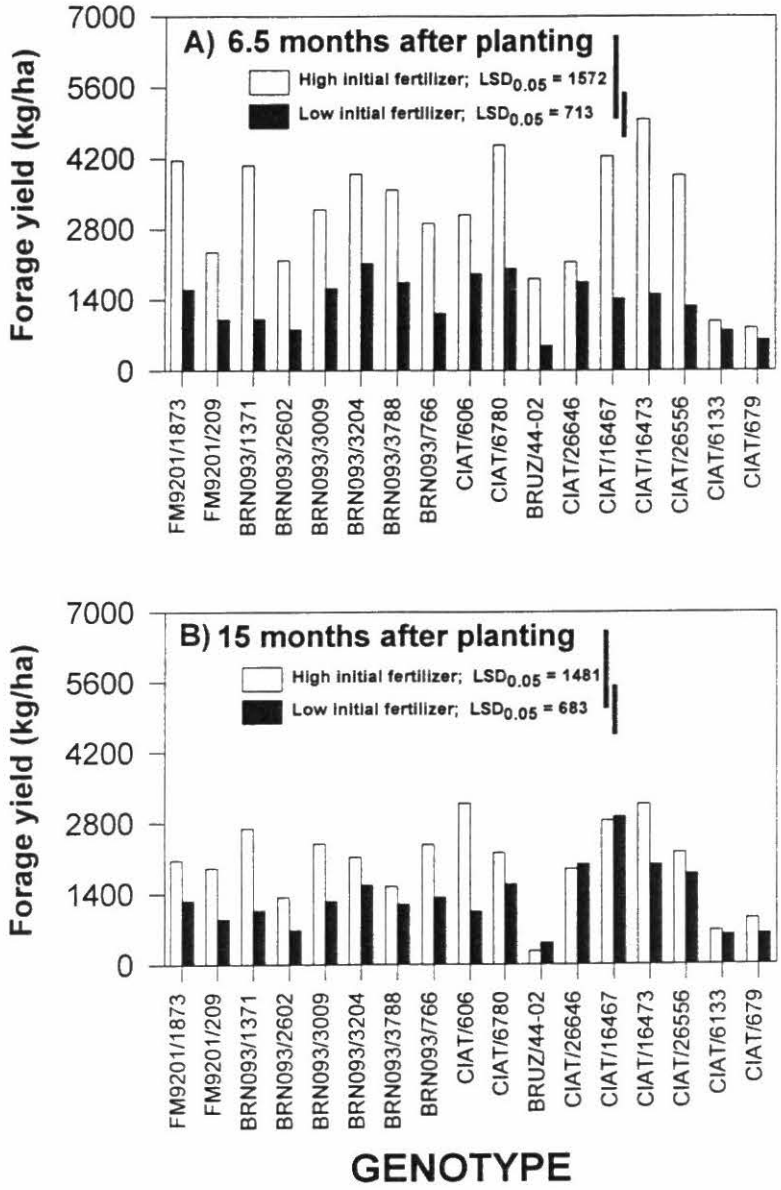

Figure 38. Influence of initial fertilizer application on genotypic variation in forage yield of genetic recombinants, parents and other germplasm accessions of Brachiaria at 6.5 and 15 months after planting in a sandy loam Oxisol site at Carimagua, Colombia.

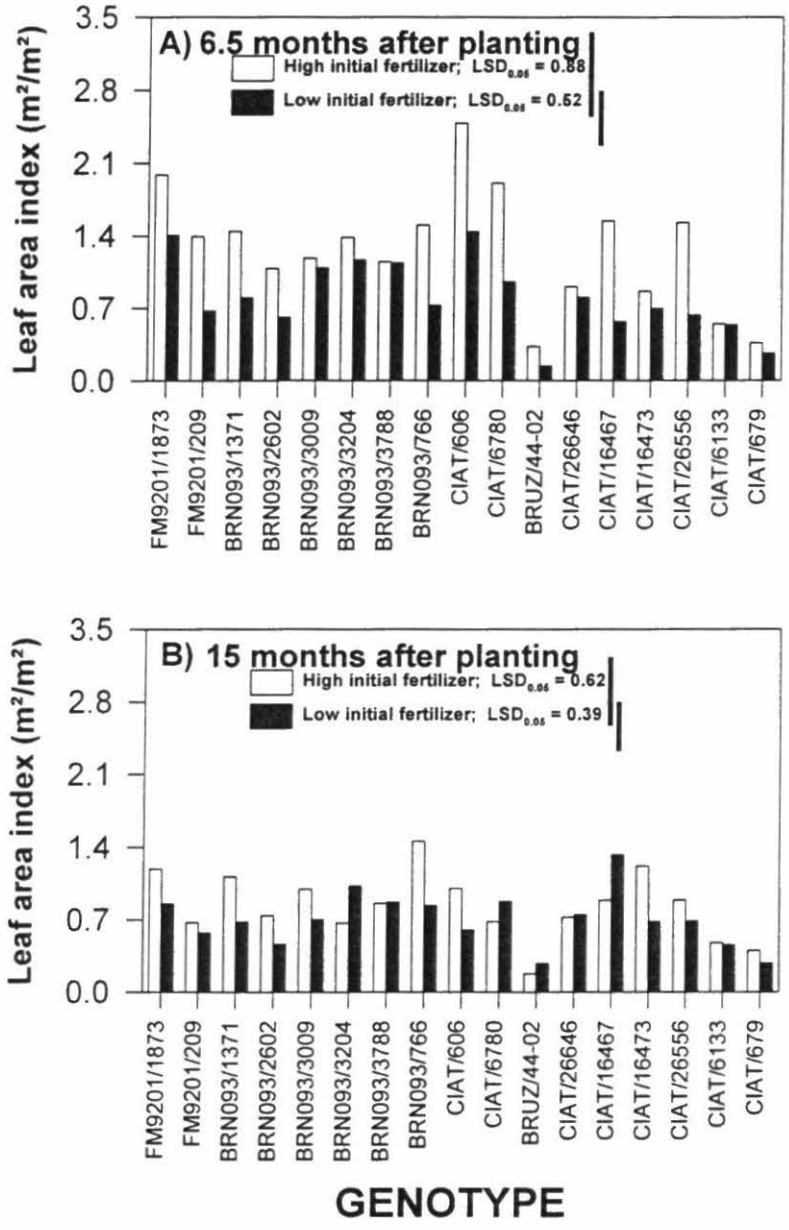

Figure 39. Influence of initial fertilizer application on genotypic variation in leaf area index of genetic recombinants, parents and other germplasm accessions of *Brachiaria* at 6.5 and 15 months after planting in a sandy loam Oxisol site at Carimagua, Colombia.

In general, our results show that genotypic variation in several plant attributes was greater than the variation induced by level of nutrient supply.

Table 30. Influence of nutrient supply on the range of genotypic variation in plant attributes of six tropical forage legume species (24 genotypes) when grown in pots (4 kg soil) in a sandy loam Oxisol from Carimagua.

Plant attributes	Nutrient supply		
	Nil	Low[1]	High[2]
Forage yield (g/pot)	0.11 - 0.91	2.38 - 6.06	6.99 - 16.7
Leaf area (cm^2/pot)	5.82 - 113	266 - 774	531 - 2085
Leaf chlorophyll (mg/m^2)	188 - 606	154 - 364	212 - 401
Inorganic P in leaves (mg/m^2)	10.8 - 62.5	15.5 - 57.9	15.5 - 46.7
Leaf N partitioning index (%)	50.8 - 77.9	44.9 - 79.9	45.6 - 76.1

[1]20P, 20 K, 33 Ca, 14 Mg, and 10.5 (kg/ha)
[2]40 N, 50 P, 100 K, 66 Ca, 28.5 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B, and 0.1 Mo (kg/ha)

Genotypic variation in plant attributes of *A. pintoi* and *S. guianensis* when grown at low nutrient supply is shown in Table 31. Among the four genotypes of *A. pintoi*, CIAT 18748 was outstanding in maintaining greater concentration of inorganic P in leaves and in partitioning of greater proportion of nitrogen to leaves. Among the four accessions of *S. guianensis*, CIAT 11844 maintained greater concentration of inorganic P in leaves.

Table 31. Genotypic differences in plant attributes for tolerance to low nutrient supply in six tropical forage legume species (24 genotypes) grown in pots (4 kg soil) in a sandy loam Oxisol from Carimagua.

Species	CIAT accession number	Forage yield (g/pot)	Leaf area (cm^2/pot)	Leaf chlorophyll (mg/m^2)	Inorganic P in leaves (mg/m^2)	Leaf N partitioning index (%)
A. pintoi	17434	5.01	504	231	15.5	52.7
	18744	4.97	438	346	17.6	44.9
	18748	4.64	501	238	24.4	64.6
	22160	5.06	492	301	17.9	60.5
$LSD_{0.05}$		NS	NS	95	NS	NS
S. guianensis	21	3.93	356	345	35.4	70.6
	184	3.01	295	290	32.4	74.8
	2950	3.72	375	300	33.1	79.9
	11844	3.27	315	274	44.6	78.0
$LSD_{0.05}$		0.56	51	NS	NS	4.7

Low nutrient supply = 20 P, 20 K, 33 Ca, 14 Mg, and 10 S (kg/ha).

Activity 3.1.4: Genotypic variation in nutrient acquisition and utilization by grasses (I. M. Rao, J. Ricaurte, and R. Garcia)

Highlight

- A number of plant attributes of *Brachiaria* were influenced by genotype and by P supply in soil.

Rationale: There is a need to explore the extent of genotypic variation between and within species in order to develop nutrient efficient genotypes which could meet the mineral nutrient

requirements of ruminants. A glasshouse experiment examined genotypic differences in acquisition and utilization of P among 15 genotypes of 5 species of *Brachiaria* (3 genotypes of each species: *B. decumbens*, *B. brizantha*, *B. ruziziensis*, *B. humidicola*, and *B. dictyoneura*). The selection of genotypes was based on prior agronomic evaluation in the field (commonly used, more productive and less productive genotypes).

Methods: A clay loam oxisol from Carimagua was used to grow the plants (4 kg of soil/pot). A basal nutrient mixture was applied to soil before planting (kg/ha: 80N, 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo). P was supplied at four levels (kg/ha: 0, 20, 50 and 200). At the time of harvest (53 days of growth), several shoot and root attributes such as forage yield, leaf area, root length, shoot P uptake, P uptake efficiency per unit root length and P use efficiency (kg of forage produced g of total P uptake) were determined.

Results and Discussion: Our results showed that shoot attributes were influenced by genotype and also by the level of P supply to soil (Table 32). Two plant attributes, P uptake efficiency and root length, exhibited greater genotypic variation than the other plant attributes.

Table 32. Influence of P supply on the range of genotypic variation in plant attributes of five species of Brachiaria (15 genotypes grown in pots (4 kg soil) in a clay loam oxisol from Carimagua. Measurements on plant attributes were made after 53 days of growth.

Plant attributes	Phosphorus supply (kg/ha)			
	0	20	50	200
Forage yield (g/pot)	0.92 – 2.74	10.4 – 22.9	17.7 – 35.9	16.4 – 43.2
Leaf area (cm^2/pot)	87 – 265	76.7 – 1674	86.4 – 2026	776 – 2293
Root length (m/pot)	15.6 – 81.9	137 – 334	158 – 381	178 – 584
Root length/leaf area (km/m^2)	1.70 – 7.78	1.22 – 4.25	1.26 – 2.75	1.31 – 2.96
Shoot P uptake (mg/pot)	0.15 – 0.57	4.38 – 8.59	7.56 – 15.5	11.6 – 24.9
P uptake efficiency (mg/m)	3.08 – 18.5	16.3 – 43.4	22.9 – 71.2	26.1 – 77.5
P use efficiency (kg/g)	1.48 – 5.17	2.05 – 3.88	1.52 – 2.77	1.14 – 1.92

As expected, increase in P supply improved forage yield as a result of stimulation of leaf area production.

Genotypic variation in plant attributes of 15 genotypes when grown at 20 kg/ha of P supply is shown in Table 33. Genotypic variation in root length, shoot P uptake and P uptake efficiency was greater with *B. humidicola and B. dictyoneura* than in the other three species. On the other hand, *B. humidicola* CIAT 679 was outstanding in root length and shoot P uptake, whereas P use efficiency by *B. ruziziensis* CIAT 26433 was greater than in the other genotypes.

Activity 3.1.5: Secretion of phytase from the roots under phosphorus-deficient conditions (M. Li, M. Osaki, I. M. Rao and T. Tadano)

Highlight

- Secretion of phytase from roots of *Brachiaria decumbens* and *Stylosanthes guianensis* is an important mechanism for acquiring phosphorus from organic phosphorus sources in soil.

Table 33. Genotypic differences in plant attributes related to P acquisition and utilization in five species of *Brachiaria* (15 genotypes) grown at 20 kg/ha of P supply in pots (4 kg soil) in a clay loam oxisol from Carimagua. Measurements on plant attributes were made after 53 days of growth.

Species	CIAT accession number	Forage yield (g/pot)	Leaf area (cm^2/pot)	Root length (m/pot)	Root length to leaf area (km/m^2)	Shoot P uptake (mg/pot)	P uptake efficiency (mg/m)	P use efficiency (kg/g)
B. decumbens	606	21.8	1649	279	1.70	6.61	24.0	3.00
	26180	22.5	1428	197	1.38	6.26	32.0	3.28
	16519	22.2	1225	227	1.85	7.59	33.5	2.80
B. brizantha	6780	14.9	1237	147	1.26	4.52	34.6	2.78
	26562	20.1	1072	157	1.45	6.32	43.4	2.93
	16431	20.1	1212	145	1.22	5.84	40.0	3.16
B. ruziziensis	655	22.6	1419	279	1.94	8.07	32.2	2.66
	16101	22.9	1674	245	1.46	5.87	24.3	3.57
	26433	22.3	1741	282	1.63	5.14	18.7	3.88
B. humidicola	679	22.6	804	334	4.25	8.59	25.7	2.46
	26425	22.4	1373	331	2.43	5.61	16.3	3.73
	16866	18.4	845	255	3.03	7.02	28.9	2.49
B. dictyoneura	6133	22.8	1109	230	2.08	7.60	33.2	2.85
	16506	17.9	942	168	1.77	5.68	35.5	2.82
	10508	10.4	768	137	1.67	4.38	38.6	2.05
Mean		20.2	1223	227	1.94	6.34	30.7	2.96
LSD (P=0.05)		6.5	346	89	0.60	1.85	15.3	0.63

Rationale: In a collaborative research program with the Hokkaido University, Japan, 6 tropical forage accessions were tested in comparison with 10 other crop and forage cultivars for their potential to secrete phytase from roots under P-deficient conditions. Phytase is secreted from plant roots as a mechanism of the mineralization of P from organic-P (inositol hexaphosphate) in soil. Since the substrates of phytases such as, phytin and other inositol phosphates, represent a major fraction of soil organic-P, the secretion of phytase from plant roots under P-deficient conditions may be of fundamental importance to provide an efficient mechanism for the utilization of organic phosphates in soil.

Methods: The secretory phytases were collected with a dialysis membrane tube for 24 h from roots of plants grown in a glasshouse with low or adequate supply of P in nutrient solutions. Phytase activity was assayed by measuring the amount of inorganic phosphate released by hydrolysis using sodium phytate as substrate.

Results and Discussion: The activity of not only secretory phytase, but also acid phosphatase, from roots increased with the the low-P treatment in all of the plant species examined. Secretion of phytase by the roots under P-deficient conditions was highest in *Brachiaria decumbens* CIAT 606 and *Stylosanthes guianensis* CIAT 184, moderate in *Brachiaria brizantha* CIAT 6780 and *Stylosanthes guianensis* CIAT 2950, and lowest in *Andropogon gayanus* CIAT 621 and *Stylosanthes capitata* CIAT 10280 (Table 34).

Immunoreactive protein band that reacted with a polyclonal antibody raised against wheat bran phytase, (corresponding to molecular weight 35-40 kD), could be detected in *Brachiaria brizantha* CIAT 6780 and *Stylosanthes guianensis* CIAT 2950. These results indicate that the secretory phytase may provide an efficient mechanism for *Brachiaria decumbens* CIAT 606 and *Stylosanthes guianensis* CIAT 184 to utilize organic-P in soil.

Table 34. Activity of phytase secreted from six tropical forage species grown with low (3 μM) or adequate (65 μM) supply of P in nutrient solutions.

Forage species	CIAT accession number	Activity of phytase secreted (Units/g dry wt.)		% increase with low P
		Adequate-P	Low-P	
Grasses:				
Brachiaria decumbens	606	0.32	1.25	390
Brachiaria brizantha	6780	0.56	0.79	140
Andropogon gayanus	621	0.18	0.36	200
Legumes:				
Stylosanthes guianensis	184	0.41	1.04	250
Stylosanthes guianensis	2950	0.53	0.74	140
Stylosanthes capitata	10280	0.11	0.21	190

Activity 3.1.6: Genotypic differences in root and shoot development among tropical forage legumes during seedling establishment (L. E. C. Martinez, J. Jaumer, R. García and I. M. Rao)

Highlight

- *S.capitata* is less adapted to a clay loam Oxisol because of thicker roots with fewer root tips which contribute to reduced uptake of calcium when compared to *S. guianensis* and *A. pintoi*.

Rationale: The wide adoption and utilization of tropical legumes in different production systems is dependent on the rapid establishment and extraction of nutrients from low fertility acid soils. There is a need to define if differences in establishment of legumes in acid soils are related to differences in: (a) partitioning of dry matter between roots and shoot; (b) morphological development of roots; or (c) efficiency in acquisition and utilization of nutrients.

Agronomic evaluation of *Arachis pintoi* accessions for dry season tolerance in the cerrados of Brazil had identified the accession CIAT 22160 as being superior to the commercial accession CIAT 17434. However it was not determined whether the superior adaptation to dry season could be related to better development of root system. Field studies conducted at Carimagua, Colombia, indicated that *Stylosanthes guianensis* CIAT 184 is better adapted to clay loam Oxisols than *Stylosanthes capitata* CIAT 1315. But the plant attributes that contribute to these differences in edaphic adaptation are not known.

Methods: Two glasshouse experiments were conducted to compare root growth and development and to test the relationship between root growth and shoot growth during seedling establishment in two genotypes of *Arachis pintoi* (CIAT 17434 and 22160) and two species of *Stylosanthes* (*S. guianensis* CIAT 184 and *S. capitata* CIAT 1315). Plants were grown in a clay loam Oxisol with low amounts of fertilizer application (kg/ha: 20P, 20K, 33Ca, 14Mg and 10S).

Results and Discussion: The use of minirhizotrons (plexiglass boxes) facilitated the visual assessment of differences in root development. Among the four legume accessions, *S. capitata* exhibited markedly smaller size of the root system (Figure 40). Root development was also markedly slower in *S. capitata* than in *S. guianensis*. The extent of root branching and total root length were also lower in *S. capitata* compared to the other three legumes. There were no significant differences in size of the root system between the two accessions of *A. pintoi* although CIAT 22160 showed greater ramification of the root system during seedling establishment.

Figure 40. Differences in size of the root system (minirhizotrons) and shoot growth (in pots) among four legumes grown at low nutrient supply in a clay loam Oxisol from Carimagua, Colombia. A= *Arachis pintoi* CIAT 17434; B=*Arachis pintoi* CIAT 22160; C=*Stylosanthes capitata* CIAT 1315; D=*Stylosanthes guianensis* CIAT 184.

Both accessions of *A. pintoi* established rapidly possibly due to larger seed size (158 mg compared to 2.3 mg of *Stylosanthes* species), which contributed to rapid shoot growth and root development (Table 35). This rapid development of root system facilitated greater uptake of nutrients from low fertility acid soils, particularly calcium (Ca). Among the two accessions of *A. pintoi*, CIAT 22160 appeared to establish more rapidly than the commercial accession, CIAT 17434. The slow establishment of *A. pintoi* in pastures could be mainly due to less number of plants per unit area of the pasture. It is conceivable that if seeding rates were increased, *A. pintoi* could overcome slow establishment problems. But this strategy will increase the costs of pasture establishment.

The superior performance of *S. guianensis* compared to *S. capitata* in clay loam Oxisols could be mainly attributed to it's vigorous root growth (abundance of fine roots and root tips) that contributed to greater acquisition of Ca from low fertility acid soil. Root tips are the main sites of Ca uptake along the root axis. The lower uptake of Ca by *S. capitata* compared with *S. guianensis* may be related to thickness of the roots (i.e., lower value of specific root length) together with reduced number of root tips. There is a need to test whether an increase in Ca supply in soil will improve the seedling vigor of *S. capitata*.

Table 35. Differences in root system development and calcium acquisition among two accessions of *Arachis pintoi* and two species of *Stylosanthes* when grown in pots (500 g of soil) in a clay loam oxisol from Carimagua, Colombia.

Plant age* (days)	Genotype (CIAT accession number)	Shoot biomass (mg/pot)	Root biomass (mg/pot)	Root length (m/pot)	Specific root length (m/g)	Number of root tips (No./pot)	Calcium uptake (mg/pot)
30	Ap17434	359a	220a	28.0a	28.1a	890	2.76
	Ap22160	295a	200a	25.5a	25.6a	669	2.60
	Sc1315	16b	7b	3.3b	3.33b	52	0.11
	Sg184	22b	8b	3.2b	3.25b	67	17.52
60	Ap17434	920ab	540a	32.5a	32.6a	1613	8.37
	Ap22160	1020a	500a	29.9a	29.9a	1305	7.49
	Sc1315	640c	120a	16.0b	16.0b	503	3.95
	Sg184	860b	240a	34.3a	34.4a	1335	6.30

*Days after transplanting

Means followed by the same letter are not significantly different ($P<0.05$) according to Duncan test.

Ap= *Arachis pintoi*; Sc= *Stylosanthes capitata*; Sg= *Stylosanthes guianensis*

Activity 3.1.7: Identification of plant attributes for compatibility and persistence of new legumes when associated with aggressive grasses (Y. Saito, P. Kerridge, I. M. Rao, C. Plazas and J. Ricaurte)

Highlight

- Establishment of *A. pintoi* was more rapid when associated with a tussock forming grass, *P. maximum* than with a stoloniferous grass, *B. dictyoneura*.

Rationale: A field study is in progress at Carimagua on a sandy loam oxisol to identify plant attributes that contribute to rapid establishment and persistence of new forage legumes in

association with aggressive grasses. The trial comprises three legumes (*A. pintoi* CIAT 22160, *S. guianensis* CIAT 11833, and *S. capitata* cv Capica) and stoloniferous (*B. dictyoneura* cv Llanero) and tussock (*P. maximum* CIAT 36000) grasses.

Methods: The trial was planted in June 1995 as a randomized complete block in a split plot arrangement (grasses as main plots and fertilizer x legumes as sub-plots). Initial application of fertilizer was at two levels (low: kg/ha, 10P, 10K, 33Ca, 14Mg, 10S and High: 40P, 40K, 66Ca, 28Mg, 20S and micronutrients). Pure grass treatments were also established to evaluate the effect of the legume on grass growth. Two fertilizer treatment levels have been included to allow a study of the interaction of nutrition on compatability. A number of plant attributes including botanical composition, shoot and root production, nutrient composition, and nutrient acquisition are being monitored with periodic mob grazing.

Results and Discussion: Measurements on pasture presentation yield and botanical composition at 4, 7.5 and 14.5 months after planting of grass alone and grass + legume associations indicated that the establishment of *A. pintoi* was more rapid when associated with *P. maximum* than with *B. dictyoneura* (Table 36). As expected, *A. pintoi* responded more than the the other two legumes to high level of initial fertilizer application. Among the three legumes, *S. guianensis* was more vigorous in growth and dominated the grasses, particularly *P. maximum* during the establishment phase with low initial fertilizer application.

Table 36. The influence of initial fertilizer application on pasture presentation yield (> 10 cm height; g dry weight/m^2) and botanical composition (% of legume in parenthesis) at 4, 7.5 and 14.5 months after establishment of grass alone and grass + legume associations in a sandy loam Oxisol at Carimagua, Colombia.

Initial Fertilizer	Pasture	Age of the pasture (months)		
		4	7.5[a]	14.5
Low	Bd	116	81.4	142
	Bd + Ap	148 (1.3)[b]	68.0 (4.3)	118 (4.1)
	Bd + Sg	111 (35)	136 (62)	291 (56)
	Bd + Sc	110 (22)	78.0 (28)	200 (35)
	Pm	111	75.2	147
	Pm + Ap	116 (0.5)	76.3 (8.1)	178 (21)
	Pm + Sg	192 (34)	122 (76)	214 (73)
	Pm + Sc	153 (30)	81.8 (31)	208 (58)
High	Bd	348	162	135
	Bd + Ap	270 (1.2)	144 (3.0)	131 (12)
	Bd + Sg	410 (37)	220 (59)	254 (53)
	Bd + Sc	311 (32)	161 (27)	215 (49)
	Pm	366	155	137
	Pm + Ap	241 (0.6)	96 (7.5)	195 (30)
	Pm + Sg	366 (22)	199 (28)	257 (52)
	Pm + Sc	388 (16)	159 (14)	271 (47)

Bd = *Brachiaria dictyoneura* CIAT 6133 (presently classified as *B. humidicola*); Pm = *Panicum maximum* CIAT 36000; Ap = *Arachis pintoi* CIAT 22160; Sg = *Stylosanthes guianensis* CIAT 11833; Sc = *Stylosanthes capitata* CIAT 10280

[a] Dry season

[b] Figures in parenthesis indicate % of legume content in the associciation

Suboutput 3.2: Genotypes of *Brachiaria*, *Arachis* and *Calliandra* with dry season tolerance identified and characterized (J. W. Miles, I. M. Rao, P. J. Argel, B. L. Maass)

Activity 3.2.1: Genotypic variation in dry season tolerance in *Brachiaria* and *Arachis*

Highlight

- Identified two genetic recombinants of *Brachiaria* with better dry season performance than the commercial *B. decumbens* cultivar of *Brachiaria decumbens* cultivar.

Rationale: Dry season feed shortage is among the important limitations to livestock productivity in tropical America. We are testing collections of elite accessions of *Brachiaria*, *Arachis* in multilocational trials.

Until recent years, CIAT's collection of *Arachis pintoi* germplasm was limited to not more than a dozen accessions. A major influx of *Arachis* germplasm was received by CIAT from Brazil (CENARGEN/EMBRAPA) in the early 1990's. A concerted effort was mounted to test this germplasm in order to identify any material with potential to replace the existing commercial cv. Amarillo (CIAT 17434). *A. pintoi* (CIAT 17434) is regarded as having good adaptation to humid environments with more than 1500 mm of annual rainfall, but often the legume is planted in less humid environments either as cover crop or as pasture. The persistence of the legume in drier environments is not well documented, particularly of new lines available in the germplasm collection.

The widely planted *B.decumbens* cv. Basilisk does not perform well under dry season conditions. Thus, a field study was conducted during two seasons (wet and dry) in a medium textured Oxisol at Carimagua, Colombia to evaluate differences in edaphic adaptation and dry season tolerance among 55 *Brachiaria* genotypes. We also tested the hypothesis that the tolerance to dry season in *Brachiaria* is greater in genotypes that accumulate greater amounts of nonstructural carbohydrates combined with less amounts of minerals (ash content) per unit dry weight of leaves and stems.

Methods:

***Studies with Arachis in Colombia*:** In Colombia, multilocational trials of elite accessions of *Arachis*, have been completed. Data from these trials will be examined to identify accessions with particularly good performance during dry periods.

Following initial multiplication, two sets of multilocational trials were established, one in 1994 (35 *Arachis* spp. accessions at six sites) and one in 1995 (39 *A. pintoi* accessions at seven sites) (See previous annual reports). Seven accessions were common to both sets of trials. Each set of trials was planted at six sites, ranging from hillside ecosystem in the Cauca Department of Colombia to the lowland Amazon piedmont of Caquetá. A seventh site (at CIAT-Palmira) was included in the 1995 trials. Twenty-square-meter plots were planted in association with *B. dictyoneura* or *B. decumbens*, and managed under periodic defoliation --

either by clipping or mob grazing (depending on the availability of grazing animals). Data on forage yield, botanical composition, and seed production was taken over two years.

Studies with Arachis in Costa Rica: In Costa Rica, plots of *A. pintoi* (CIAT 17434 and CIAT 18744) were planted in mid-1993 for seed multiplication at the Escuela Centroamericana de Ganadería (ECAG) located in Atenas, Costa Rica. This site is a subhumid tropical forest located at 200 masl, 1600 mm of annual rainfall from May to November (six months dry season), mean temperature of 23.7 C° and clay loam soils (inceptisols) of medium fertility. At this site *A. pintoi* defoliates severely during the dry season and a high proportion of stolons die, particularly with *A. pintoi* (CIAT 17434).

Beginning in 1994 (10 months after planting) seedling emergence and plant cover was monitored for four consecutive years at the commencement of the rains. Five replicates of 1 m^2 each were marked randomly and emerged seedlings were counted and removed at weekly intervals for about one month. Plant cover was also recorded in neighbor plots.

Studyy with Brachiaria in Colombia: *Brachiaria* genotypes, including 43 genetic recombinants selected from a breeding population, 4 parental accessions and 8 germplasm accessions were evaluated for drought tolerance in Carimagua. Low amounts of fertilizer (kg/ha: 40N, 20P, 20K, 14Ca, 12Mg and 12S) were applied during establishment. Forage yield, leaf area, specific leaf area, shoot nutrient uptake, nonstructural carbohydrates in leaves and stems, and ash content of leaves and stems were measured during both seasons.

Results and Discussion:

Studies with Arachis in Colombia: The second series of multilocational trials was terminated during 1997, and data are awaiting proper analysis. However, preliminary observations have identified a number of accessions, which perform better than the cv. Amarillo (CIAT 17434) and seed of these is being multiplied (Table 37). Several very vigorous accessions (CIAT 22233, 22236, 22238 and 22241) appear to have poor seed production (they hardly flower, at least at sites in Colombia), but may have utility where vegetative propagation is feasible.

Table 37. Seed yields of selected *Arachis pintoi* accessions, Armenia (Quindío), August 1997.

Species	Accession	Classified seed (kg)	Area (ha)	Yield (kg/ha)	Observations
A. pintoi	17434	643.0	0.25	2572.0	First harvest
A. pintoi	18748	818.0	0.48	1704.2	First harvest
A. pintoi	18744	727.0	0.49	1483.7	First harvest
A. pintoi	18744	120.0	0.09	1333.3	Second harvest
A. pintoi	22160	490.0	0.49	1000.0	First harvest
A. pintoi	22159	45.5	0.06	758.3	Vegetative material
A. pintoi	22155	2.1	0.01	210.0	Vegetative material
A. pintoi	18747	6.9	0.01	690.0	Vegetative material
A. pintoi	22172	11.5	0.01	1150.0	Vegetative material
A. pintoi	18751	10.3	0.01	1030.0	Vegetative material

Results so far indicate that there are *Arachis pintoi* accessions clearly superior (higher DM yield, and better persistence) than CIAT 1743. The priority now is for official release of these superior accessions and appropriate promotion to ensure wide adoption.

Studies with Arachis in Costa Rica: In Table 38 we show the mean number of seedlings/m^2 recorded for the two accessions of *A. pintoi* (CIAT 17434 and CIAT 18744) for the years 1994, 1995, 1996, and 1997. Mean seedlings/m^2 were 23 or 12 respectively for CIAT 18744 or 17434 during the first year. These numbers increased from the second year and after; CIAT 17434 consistently produced more seedlings than CIAT 18744, reaching more than 1000 seedlings/m^2 for the years 1995 and 1997.

Table 38. Mean number of seedlings/m^2 and percent soil cover of two *Arachis pintoi* accessions at the beginning of the rains in a subhumid tropical site in Costa Rica.

Accession (CIAT No.)	Year of observation			
	1994	1995	1996	1997
	Mean number of seedlings/m^2			
18744	23	65	196	88
Range	(7-45)	(20-111)	(168-236)	(54-100)
17434	12	1013	473	1056
Range	(4-26)	(816-1270)	(350-550)	(558-1398)
Observation date	6/5-6/6	27/3-22/5	8/4-13/5	7/5-4/6
Soil plant cover at last date (%)	95	100	100	100

At the end of the observations, soil plant cover was 90 to 95% the first year and 100% cover for the years 1995, 1996 and 1997, namely one month after the commencement of the rains. Only a few stolons survived the dry season, particularly for CIAT 18744. Thus plant population originated mainly from underground seed.

In general, our results indicate that in a subhumid environment the cv. Maní Forrajero Perenne (CIAT 17434) produced more seedlings/m^2 during the years of observation. This higher seedling numbers was related to greater seed yields recorded at this site and elsewhere for this line. Accession CIAT 187440 is more stoloniferous and forms a thick mat of stolons that may hinder peg development during seed set, thus reducing seed yield and consequently number of seeds in the soil profile.

There were differences among years in the number of seedlings recorded for each accession of *A. pintoi* and this may be related to seed set, soil seed storage, crop management during the rainy season and sufficient regularity of the rains during the period of the observations to stimulate continuous germination. But in any case the number of seedlings observed plus some regrowth coming from surviving stolons was sufficient for a complete soil cover 30 days after the commencement of the rains.

In summary our results indicate that both accession of *A. pintoi* evaluated persist in subhumid conditions, although the forage contribution at the end of the dry season is negligible. As a forage plant the major contribution of the legume to the grazing animal occurs during the wet period.

Studies with Brachiaria in Colombia: Genetic variation among *Brachiaria* lines was observed in forage yield, and nonstructural carbohydrates and ash content of leaves and stems (Table 39). Among the promising genetic recombinants, BRN093/3009 showed greater levels of nonstructural carbohydrates and ash content in both leaves and stems in the dry season. But the differences among genetic recombinants and their parents either in nonstructural carbohydrate levels or in ash content were not always associated with differences in forage yield in dry season.

Table 39. Genotypic variation in nonstructural carbohydrates and ash content in leaves and stems and their relationship to forage yield among selected genetic recombinants, parents, and other germplasm accessions of *Brachiaria* grown during wet and dry seasons in a sandy loam Oxisol site at Carimagua, Colombia.

Genotype	Forage yield (g/plant)		Nonstructural carbohydrates (mg/g)				Ash content (%)			
			Leaves		Stem		Leaves		Stem	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Recombinants:										
BRN093/1371	175	84	131	156	127	117	13.1	10.3	8.5	8.4
FM920/1873	220	160	148	167	113	129	11.9	9.8	7.4	9.1
BRN093/3009	234	159	167	218	139	141	12.8	11.6	9.3	9.7
Parents:										
CIAT 606	246	85	156	176	105	130	9.4	6.9	8.5	9.5
CIAT 6780	343	174	183	155	102	116	11.1	9.3	11.9	9.3
BRUZ/44-02	59	4.7	155	166	128	134	11.1	6.6	9.6	4.9
CIAT 26646	273	139	166	161	107	114	5.3	7.9	5.8	7.4

These preliminary results indicate that under low fertility -acid soils the dry season tolerance may not be closely related to the levels of either nonstructural carbohydrates or minerals in leaves and stems as we had postulated, since the level of nutrient supply in soil may interact with dry season tolerance in *Brachiaria*. The utility of attributes such as nonstructural carbohydrate levels, ash content and specific leaf area to assess dry season tolerance is being tested further using field experiments (*Brachiaria* and *Arachis*) in Atenas, Costa Rica where soil fertility is not a major costraint for dry season tolerance.

Table 40. Tentative list of elite *Brachiaria* and *Arachis* germplasm identified in multilocational agronomic trials.

Arachis pintoi	*Brachiaria* spp.
18744	16322
18744	26110
22160	26124
22159	26318
22155	16113
18747	1873 & 1737 (hybrids)
22172	
18751	

Activity 3.2.2: Plant attributes in selected genotypes of *Brachiaria*, *Arachis*, and *Calliandra* for dry season tolerance (I. M. Rao, J.W. Miles)

Rationale: Identification of the attributes associated with dry season tolerance will assist in future germplasm screening in these, and perhaps in other, plant genera.

Results: No activity during 1997.

Suboutput 3.3 Genotypes of *Brachiaria* and *Paspalum* with adaptation to poorly drained soils identified and characterized

Highlight

- The accession *B. brizantha* (CIAT 26110) survived in waterlogged soils by producing adventitious roots, stratifying roots along the surface of the water table and by developing aerenchyma tissue in root cortex.

- The commercial cultivar *B. brizantha* cv. Marandú (CIAT 6780) also survived to waterlogged conditions by developing aerenchyma tissue and the stratification of the roots along the surface of the water table.

- Both *B. brizantha* (CIAT 26110) and *B. brizantha* cv. Marandú survived better under waterlogged conditions than on water-stressed soils, while *B. dictyoneura* cv. Llanero adapts to either condition by modifying cortical cells into intercellular spaces and by a strong lignification of the vascular cylinder.

- Collections of new *Paspalum* accessions released from quarantine and established in small-plot field trial, CIAT-Quilichao.

Activity 3.3.1: Field trials to determine genotypic variation in *Brachiaria* and *Paspalum* for adaptation to poorly drained soils (F. R. Casola, N. Vásquez, R. A. González, C. Henríquez, P. J. Argel, I. M. Rao, J. W. Miles)

Rationale: Large areas of poorly drained savannas occur in the Colombian/Venezuelan Llanos and elsewhere. Traditional forages such as *Brachiaria decumbens* and *B. brizantha* are poorly adapted to these conditions. Some species of *Paspalum* are well known for adaptation to poorly drained conditions. CIAT's germplasm collection has been deficient in *Paspalum* accessions until recent introductions of Brazilian materials.

The commercial availability of *Brachiaria* cultivars has increased during the last decade, particularly for the lowland tropics. Lines of this grass adapt to a wide range of environments including acid and poorly drained soils; however, *B. brizantha* cv. Marandú, known for its forage quality and resistance to spittlebug, is known to be susceptible to waterlogged soils exhibiting high plant mortality and loss of paddock productivity. New lines of *Brachiaria* are available either from plant collections or from breeding programs, thus it is necessary to study and characterized the tolerance to waterlogged conditions of the promising lines before more advanced evaluations are carried out.

Methods

***Studies in Colombia*:** Elite *Brachiaria* accessions are established in a network of multilocational trials throughout Colombia. While none of the sites was explicitly selected for poor drainage conditions, we expect to be able to make observations on adaptation at one or more sites at some time during the two-year lifespan of the trials. Eight accessions of *Paspalum* were released from quarantine and established in a small-plot trial on a poorly drained site at CIAT-Quilichao.

***Studies in Costa Rica*:** In Costa Rica, plants of *Brachiaria dictyoneura* cv. Llanero (CIAT 6133) and of *B. brizantha* CIAT 16322, 26110, and 6780 (cv. Marandú) were grown in 7 l pots filled with a sandy loam soil (pH of 5.3, 7.6% OM, 3.5 ppm of P and 0.12 ppm of K). The study was carried out in a glasshouse of the Centro Tropical de Investigación y Enseñanza (CATIE) located in Turrialba, at 602 masl and mean temperature of 21.7 °C.

The grasses were grown for 60 days and then the following treatments were imposed in a split-split plot design with three replications: (1) less than field capacity (water stress), (2) field capacity, and (3) in excess of field capacity (flooding). Measurements were taken at 0, 9, 18, and 27 days after beginning the treatments. Root anatomy, plant height, tiller number, leaf angle, stem insertion, DW of green leaves, stems, roots, and dead material were measured. During the period of the observations plants did not receive water for the water stress treatment, while flooding conditions were maintained by holding the wet soil in a plastic bag within the pot.

Results: In Costa Rica, moisture supply did not affect significantly plant height, tiller number, nor leaf angle stem insertion during the period of the observations. However, there was a tendency to more tillers under field capacity. After 20 days under water stress *B. brizantha* CIAT 26110, 16322, and cv. Marandú showed rolled leaves and signs of plant mortality, while *B. dictyoneura* cv. Llanero had better plant survival. The proportion of dead material, DW of roots, stems, and green leaves was significantly ($P< 0.05$) affected by moisture supply. *B. brizantha* CIAT 26110, cv. Marandú, and *B. dictyoneura* cv. Llanero produced similar proportions of green leaves at field capacity and under flooded conditions, while *B. brizantha* CIAT 16322 yielded green leaves only at field capacity. This accession tolerated neither drought or flooding and hence was not considered for the morphological and anatomical studies.

A high proportion of dead material was recorded for all species of *Brachiaria* under water stress. *B. brizantha* CIAT 26110 produced the lowest proportion of dead material under water saturation. This accession tolerated flooding conditions better than water stress. Stem and root DW was significantly reduced ($P< 0.05$) by both water stress and flooding, but no differences in root DW were observed among species of *Brachiaria*. The response to flooding was more conspicuous than that to water stress. Root anatomy of neither *B. brizantha* CIAT 26110 nor cv. Marandú was modified under water stress, and this may count for the poor survival of these genotypes. Meanwhile, cv. Llanero converted root cortex cells into aerial spaces that allowed gas interchange between the roots and the environment. This cultivar survived the 27 days of water stress treatment.

Under water saturation *B. brizantha* CIAT 26110, *B. brizantha* cv. Marandú, and *B. dictyoneura* cv. Llanero rapidly increased their intercellular spaces in the root cortex followed by the formation of abundant aerenchyma tissue (anatomical adaptation). Besides, *B. brizantha* CIAT 26110 began to produce adventitious roots from the lower nodes 9 days after imposing the treatments (see Figure 41). This accession and cv. Marandú stratified their roots along the surface of the watertable at the end of the observations, as an additional mechanism of adaptation to flooded conditions (morphological adaptation). However, at the end of the 27-day period of flooding, CIAT 26110 showed signs of general chlorosis.

Mechanisms of survival of soil water saturation of the *Brachiaria* species under study seem to be similar, and relate to anatomical and morphological adaptation, either the formation of aerenchyma tissue in the root cortex and/or the development of adventitious roots. The aerenchyma tissue enhances the diffusion of atmospheric oxygen from the shoots to the roots, while the adventitious roots allow absorption of water upon loss of the original root system, and contributes to rapid uptake of oxygen and nutrients from the water column. Adventitious roots are also sites of accelerated alcoholic fermentation that provide energy under anaerobic conditions.

Figure 41. Adventitious root formation in the lower nodes of *B. brizantha* CIAT 26110 under flooded conditions (Picture taken by Francisco Casasola, CATIE 1997).

Adventitious roots appear earlier in *B. brizantha* CIAT 26110 than for other *Brachiaria* species, and this may account for the adequate survival of the plant under the flooded conditions imposed. However, the chlorosis observed after 27 days of continuous flooding indicates that this *Brachiaria* may not tolerate prolonged periods of continuously saturated soil.

B. brizantha cv. Marandú also survived the flooded conditions imposed, although it did not form adventitious roots. Thus the high plant mortality reported for this cultivar in water saturated soils must be related to other causes, such as susceptibility to pathogens present in the soils. More studies are needed in this regard.

Changes in root anatomy, namely the formation of intercellular spaces under water deficit and a strong lignification of the vascular cylinder under water saturation, was the mechanism observed in this study in *B. dictyoneura* cv. Llanero to survive both flooded and drought conditions. This is interesting since flood-tolerant plants are generally drought sensitive. Perhaps other mechanisms not considered in this study are responsible for the good performance of this plant under stress conditions.

Activity 3.3.2: Core collection of *Brachiaria* and *Paspalum* with tolerance to poorly drained soils for seed multiplication and regional testing (P. J. Argel, J. W. Miles)

Rationale: A core collection of *Brachiaria* and *Paspalum* germplasm accessions with well documented tolerance to poorly drained soil conditions will be a valuable resource for large areas of the Colombian-Venezuelan Llanos and Varzea-type conditions in the Brazilian Cerrados.

Methods: Initial observations on small-plot trials to be confirmed by more rigorous assessment of performance under conditions of poor drainage.

Results: Several promising accessions are being identified from controlled studies in Costa Rica.

Discussion: Identification of the core collection awaits initial identification of desired germplasm and subsequent confirmation. We expect to identify one or more *Brachiaria* accessions with superior performance under poorly drained conditions. This result would require confirmation under more controlled conditions. Following initial seed multiplication, *Paspalum* accessions will be tested more rigorously for agronomic performance under poor drainage conditions, possibly at one or more sites in the north coast and in the Colombian Llanos (e.g. Casanare).

Suboutput 3.4 Genotypes of shrub legumes species with tolerance to cool temperatures and drought identified and characterized

Highlights

- Recorded differences in DM yields, regrowth capacity, dry season leaf retention, and tolerance to Psyllid insect of new species and lines of *Leucaena.*

- Found shrub legume species (*Rhynchosia, Calliandra* and *Flemingia*) that perform well in mid altitude hillsides.

Activity 3.4.1: Literature review on adaptation of tropical legumes to cool temperatures (B. L. Maass, J. W. Miles)

Rationale: With CIAT's special focus on higher elevation, hillside ecosystems, new forage germplasm adapted to lower temperatures is needed.
Methods: A first approach will be through a literature search.

Results: No activity during 1997.

Discussion: This task awaits expanded, targeted human and financial resources.

Activity 3.4.2: Access core collection of *Trifolium* from ILRI (B.L. Maass, C. Guevara)

Rationale: The genus *Trifolium* contains many perennial and annual species with excellent adaptation to cool, highland environments. ILRI has a large collection of *Trifolium* germplasm collected in the East African highlands, in environments, which should correspond well with highland Andean sites in South America. Much of this germplasm has been tested in Africa and promising accessions recommended.

Methods: Request set of "elite" accessions from ILRI collection, and process through quarantine to release for field trials at highland sites in Colombia and elsewhere in Latin America.

Results: No activity during 1997.

Discussion: A number of *Trifolium* accessions has been sent to CIAT (at the request, some time ago, of B.L. Maass), but these are unavailable for field trials owing to backlogs in quarantine processing.

Activity 3.4.3: Field trials to determine genotypic variation for tolerance to cool temperature of selected shrub legumes (B.L. Maass, E. Cárdenas, J.W. Miles)

Rationale: A wide range of shrub legumes has been tested for adaptation to tropical lowland conditions. Some of this germplasm may find a role at higher elevations.

Methods: Fifty-one accessions covering a wide range of species and genera are being tested in field trials established during 1996 at two sites in the Cauca Department, at 1,200 and at 1,600 masl. Plants were transplanted into single-row plots. These plots have been managed by periodic defoliation (with recording of dry matter yield). In addition to dry matter yield, plant persistence is an important attribute.

Results: Among the highest yielding materials were several accessions of *Rhynchosia shomburgkii*, *Calliandra houstoniana*, and *Flemingia macrophylla* (Table 41). We know that *Flemingia macrophylla* has palatability problems, such that its use as a forage plant is severely limited. However, it may find other, non-forage uses such as far fallow improvement and erosion barriers. The forage attributes of *Rhynchosia shomburgkii* and *Calliandra houstoniana* are being studied.

Discussion: Accessions of shrub legumes with promising adaptation to higher elevation sites are being identified. These need to be multiplied and distributed for wider, on-farm testing in selected hillside sites. The appropriateness for forage or other uses remains to be determined in detail.

Table 41. Dry matter yields (fine stemmed portion) of selected tree and shrub legumes at two mid-altitude sites in the Cauca Department

	CIAT Accession number	DM yield (g/plant)	Standard deviation
		San Vicente, 17 July 1997	
Trees species			
Flemingia macrophylla	17405	532	458
Calliandra houstoniana	20399	426	285
Pueraria wallichi	21076	327	145
Calliandra sp.	21420	326	208
Leucaena diversifolia	17271	305	186
Flemingia macrophylla	19457	302	168
Senna velutina	18704	291	170
Calliandra calothyrsus	21528	241	143
Senna siamea	20698	178	85
Clitoria fairchildiana	18721	173	86
Acacia farnesiana	21509	170	106
Clitoria fairchildiana	18724	145	137
Flemingia macrophylla	21089	71	99
Ateleia ovata	7362	26	44
Mean		254	545
Shrub species			
Rhynchosia shomburgkii	19235	483	340
Rhynchosia shomburgkii	20800	364	148
Flemingia macrophylla	21079	105	31
Flemingia macrophylla	18048	72	33
Mean		309	270
		El Melcho (31 July 1997)	
Trees species			
Calliandra houstoniana	20399	346	420
Flemingia macrophylla	19457	274	262
Mean		317	356
Shrub species			
Rhynchosia shomburgk	19235	496	260

Activity 3.4.4: Evaluation of new species of *Leucaena* in subhumid conditions (P. J. Argel, G. Pérez, and A. Pottinger)

Rationale: High forage and wood quality are well recognized in the species *Leucaena leucocephala.* However, the poor adaptation of the species to acid soils, lasck of tolerance to drought, and the susceptibility to psyllid insect (*Heteropsylla cubana*) are sufficient reasons to study a wide range of new species of the genus.

Recently the Oxford Forest Institute (OFI) of England has collected new species in Mexico and Central America. These lines are now available and merit study to characterize their range of adaptation and forage quality. Preliminary results of one trial established in Costa Rica are presented.

Methods: Nineteen lines of different species of *Leucaena* were planted for evaluation at the Escuela Centroamericana de Ganadería (ECAG), Costa Rica in 1996. The site is a subhumid tropical forest located at 200 masl, 1600 mm of annual rainfall, mean temperature of 23.7 °C and inceptisol soils of medium fertility. The rainy season normally starts in May, and the dry season extends for 5 to 6 months beginning in November.

The planting was made directly in rows placing 2 seeds per hill with 0.5 m between hills. Plants were later thinned to one per hill, leaving 10 plants per plot. Each species was replicated 4 times. Plant height and diameter were measured 3.5 months after planting, and leaf retention and plant mortality at 9.8 months from planting, at the end of the 6 months dry season. Plants were cut at 50 cm 11 months after planting, and DM yields measured after 8 weeks of regrowth. The incidence of Psyllid insects and of fungus diseases has been recorded at monthly intervals. In this trial the checks were a selected line of *L. leucocephala* (CIAT 17263), a *Calliandra calothyrsus* introduction from Australia (CPI 115690) and the new shrub *Cratylia argentea* (CIAT 18668).

Results: Results of the first evaluation cut are presented in Table 42. Ten different lines reached over one meter height during the first 9.8 months of growth; most species showed strong apical dominance with the exception of *L. pallida* 79/92, *L. leucocephala* (CIAT 17263) and *L. hybrid* 1/95 that showed weak apical dominance. Considerable variation has been observed with relation to plant growth, DM yields, and dry season tolerance between species of *Leucaena,* as shown in Table 42.

The regrowth capacity differed significantly among species of *Leucaena* following the uniformity cut. *L. leucocephala glabrata* 34/92 (K636), an introduction from Waimanalo (USA), produced the tallest regrowth (nearly 2 m in 8 weeks), followed by *L. collinsii* 52/88, *L. salvadorensis* 17/86, *L. macrophylla nelsonii* 47/85, and *L. diversifolia stenocarpa* 53/88. Regrowth was very poor in *L. multicapitulata* 81/87, *L. esculenta* 47/87, *L. trichodes* 61/88, *L. pulverulenta* 83/87, and the check *C. calothyrsus* DPI 115690.

During the six months dry period the species *L. pallida* and *L. esculenta esculenta* 47/87 defoliated severely, while that *L. leucocephala glabrata* 34/92 (K636), *L.* hybrid 1/95, *L. macrophylla nelsonii* 47/85, and *L. shannonii magnifica* 19/84, retained between 60 and 80%

of green foliage. However, in this regard the check *C. argentea* CIAT 18668 performed better and retained an even higher proportion of green leaf.

Table 42. Plant height, leaf retention, and dry matter (DM) yields of new species of *Leucaena* planted in Atenas, Costa Rica (Means of the first evaluation cut after 8 weeks of regrowth).

Species	(ID No.)	Plant height (cm)*	Leaf retention (%)*	DM yield (g/plant)	
				Total	Edible
L. collinsii	52/88	169	3.5	91 a**	76 (84)***
L. divers. stenocarpa	53/88	141	3.1	89 a	76 (85)
L.l. glabrata	34/92	254	4.4	89 a	72 (81)
C. argentea CIAT	18668	119	4.5	86 ab	83 (97)
L. pallida	79/92	119	2.9	74 abc	64 (86)
L. pallida	52/87	130	1.9	71 abc	59 (83)
L. salvadorensis	17/86	154	3.9	69 abc	62 (90)
L. diver.diversifolia	83/92	139	2.8	62 abc	51 (82)
L. pallida	14/96	124	3.1	59 abc	57 (97)
L. hybrid	1/95	128	4.0	58 abc	55 (95)
L. esculenta esculenta	47/87	74	1.9	53 abcd	49 (92)
L. lanceolata	43/85	115	3.5	48 abcde	45 (94)
L. macrophylla nelsonii	**47/85**	144	4.1	48 abcde	43 (90)
L. pulverulenta	**83/87**	87	3.4	44 bcde	44 (100)
L. collinsii zacapana	56/88	133	3.4	43 bcde	42 (98)
L. leucocephala	17263	91	3.8	43 bcde	42 (98)
C. calothyrsus DPI	115690	55	2.9	39 cde	36 (92)
L. shannonii magnifica	19/84	124	4.0	34 cde	32 (94)
L. lempirana	6/91	133	3.1	33 cde	33 (100)
L. trichodes	61/88	83	2.5	14 de	14 (100)
L. multicapitulata	81/87	56	2.5	9 e	9 (100)

* Measurements taken 9.8 months after planting and at the end of the 6 month dry season in 1997. (Leaf retention scale: 1= less than 20% leaf retention; 2= 20-40%; 3= 40-60%; 4= 60-80% and 5=> 80% leaf retention).

** $P < 0.05$

*** In parentheses the percentage of edible DM related to total DM.

Differences in Psyllid tolerance have been observed, although plant mortality has not been recorded so far. During the middle of the rains *L. multicapitulata* 81/87 presented the greatest defoliation caused by the insect (around 25% defoliation of young leaves). *L. collinsii zacapana* 56/88, *L.* hybrid 1/95, *L. lanceolata* 43/85, *L. lempirana* 6/91, *L. l. glabrata* 34/92 (K636), *L. salvadorensis* 17/86, and *L. trichodes* 61/88, have all shown tips of young leaves yellow and covered with sap. All other introductions have shown only presence of the insect.

Mild attacks of the fungus *Camptomeris leucaneae* were observed in *L. esculenta esculenta* 47/87, *L. pallida* 79/92, and 14/96.

Discussion: The new species of *Leucaena* under evaluation have diverse provenances that includes Hawaii, Mexico, Guatemala, Honduras, Panama, Ecuador. One composite line was introduced from Australia (*L. pallida* 14/96). The present trial is part of a wider sets of multilocational trials being established in humid and subhumid regions of LAC and supported by OFI.

These initial results show that there is variation among *Leucaena* lines in psyllid tolerance, DM yields, regrowth capacity after cutting, and leaf retention during the dry period. *L. collinsii* 52/88, *L. diversifolia stenocarpa* 53/88, and *L. leucocephala glabrata* 34/92 (K636) all combine good DM yields, adequate regrowth after cutting, and high leaf retention -- qualities that are desirable in any shrub with potential as a forage plant. However, future results of this trial and other similar trials planted in different sites need to be analysed before drawing final conclusions.

Activity 3.4.5: Seed multiplication and regional testing of core collection of shrub legumes for cool tolerance (J.W. Miles)

Rationale: Once promising shrub species and accessions are identified for adaptation at cool temperature sites, seed must be multiplied for release and promotion.

Methods: Proper, large-scale seed multiplication plots will be established at an appropriate site [e.g. CIAT-Popayán (1,800 masl)]

Results: Small-scale seed multiplication of a bulk of promising *Cajanus cajan* accessions was conducted in 1997. Several accessions (and individual plants within accessions) were selected from a field trial at the Melcho site in hillsides of Cauca, Colombia. These were allowed to open-pollinate following physical elimination of all remaining *Cajanus cajan* plants, and seed harvested.

Discussion: Large-scale seed multiplication efforts await identification of promising accessions for cool tolerance in a wider range of shrub legume species.

Suboutput 3.5 Defined genotype environment interactions of performance of *Brachiaria* and *Arachis*

Activity 3.5.1: Multilocational trials with core collections of *Brachiaria* and *Arachis* (J. W. Miles, B. L. Maass)

Highlight

- Multilocational *Brachiaria* trials established or in progress trials with *Arachis* terminated.

Rationale: A large collection of *Brachiaria* accessions was evaluated in Carimagua during 1991-94. Eighteen accessions were selected. Seed of these 18 accessions, two check accessions, and two hybrids was multiplied during 1995.

Methods: In order to assess adaptation over a range of environments, multilocational trials including 24 entries were established during 1996, in collaboration with Corpoica, universities, and private sector participants in a project funded by the Colombian Fondo Nacional del Ganado. Eleven trials have been established and are in progress. Sites range

from the north coast, to the middle Magdalena Valley, Llanos Orientales, and the Amazonian piedmont.

Results: Results of the establishment phase were reported during a meeting held in Cartagena in February, and individual site reports have been compiled in a written document. We expect to be able reliably to identify a small number (on the order of 5-6) of elite accessions by early 1998.

Discussion: As data are accumulated, it will be possible to identify promising accessions, in order to initiate large-scale seed multiplication for advance to large-plot, on-farm grazing trials to be established in 1999. Small quantities of seed of some accessions have been produced during 1996/97 and this can be used to establish perhaps two grazing trials as soon as 1998. These will probably be planted in the north coast region of Colombia owing to need there for new forage options and institutional collaboration (particularly financing) for this project.

Suboutput 3.6 Information on genetic diversity of *Brachiaria*, *Arachis* and selected shrub legumes linked with environmental adaptation

Highlight

- Isozyme data exist for both *Brachiaria* and *Arachis*.

Activity 3.6.1: Genetic diversity in *Brachiaria* and *Arachis* germplasm through isozymes

(B.L. Maass, J.W. Miles)

Rationale: An understanding of the organization of genetic diversity with the genera *Brachiaria* and *Arachis* will assist, for example, in choosing parental materials for a hybridization program and for selecting a core germplasm collection for regional testing.

Methods: Izozyme analyses of collections of both *Brachiaria* and *Arachis* have been completed. These data are awaiting appropriate analysis.

Results: No activity in this area was made during 1997.

Discussion: A rigorous analysis of existing data will require additional human and financial resources.

Output 4: Superior and diverse grasses and legumes delivered to partners for evaluation

Suboutput 4.1 Release and deployment to farmers in different production systems of successful grass and legume cultivars through partnerships

In the past the task of multilocational evaluation of superior gene pools of grasses and legumes was effectively accomplished through forage networks in LAC (RIEPT) and in West Africa (RABAOC), but due to lack of financial support these networks became non-operational. However, elite forage germplasm from CIAT is currently being evaluated with farmer participation in South East Asia through the Forages for Smallholder Project funded by ACIAR and housed in the CIAT's Systems Project (PE-5).

Thus an important objective of the Tropical Grasses and Legumes Project of CIAT is to identify research partners in LAC that have interest in evaluating selected grasses and legumes for multipurpose use in production systems. The identification of partners for forage evaluation is being accomplished through:

a) Periodic publication of lists of grasses and legumes for specific agro- ecosystems and uses,
b) Distribution of seed of selected grasses and legumes in response to requests,
c) Establishing linkages with CIAT's projects and consortia that form part of systemwide initiatives (i.e.TROPILECHE) and
d) Seeking opportunities for special projects.

Activity 4.1.1: Establishment of partnerships and linkages with existing consortia and networks to deliver new grasses and legumes for on-farm testing (C. Lascano, J. Miles, F. Holmann and P. Argel)

Highlights

- Delivery of selected forage species to partners through existing Consortia like TROPILECHE and livestock/forage development projects in the region.

- Official release of a blend of *A. pintoi* accessions (CIAT 18744 and 17434) as cv. Maní Forrajero in Panama.

- Release of *A. pintoi* 18744 as cv. Porvenir is planned for May 1998 by MAG in Costa Rica.

Colombia: During 1997 the evaluation of selected accessions and hybrids of *Brachiaria* in a range of environments in Colombia was continued through a Network financed by FEDEGAN (Federación de Ganaderos de Colombia). The *Brachiaria* Network comprises 12 sites and involves as partners forage researchers from CORPOICA (Corporación Colombiana de Investigación Agropecuaria), two Colombian Universities and a private producer. A progress report on the activities of the *Brachiaria* Network is presented in this report.

In 1996 CORPOICA launched a new initiative entitled "Plan de Modernización de la Ganaderia" with financial support from COLCIENCIAS and FEDEGAN. During the year we met in several occasions with the General and Technical Coordinators of the initiative to define areas of collaboration. The following activities were carried out during the year as part of the collaboration:

a) Training of CORPOICA researchers on methodologies for on-farm pasture evaluation through a workshop held in the Macagual-CORPOICA station in Caqueta,
b) Training of CORPOICA researchers on bio-economical simulation models for optimizing use of feed resources and
c) Seed multiplication of grasses (*Brachiaria brizantha* 26110), shrub legumes (*Cratylia argentea* 18516/18668), and herbaceous legumes (*Stylosanthes guianensis* 184 and *Arachis pintoi* 18744) for on-farm evaluation. The seed (50 kg/accession) is currently being multiplied in the Seed Unit of Atenas Costa Rica.

We are also collaborating with CORPOICA-Regional 2 in the North Coast of Colombia on the evaluation of herbaceous and shrub legumes for dual-purpose cattle systems in subhumid regions.

Peru: Our collaboration with Peruvian Institutions dates back to the mid 80's when Pucallpa was chosen by CIAT as major screening site for selecting forages adapted to forest margins. With the initiation of TROPILECHE in 1996, Pucallpa was chosen as a benchmark site representing the humid tropics. Through TROPILECHE we are now collaborating with CODESU, IVITA, INIA and the U. of Ucayali on the development of improved forage-based feeding systems for dual-purpose cattle.

During 1996 and 1997 activities were concentrated in the establishment in 5 farms of improved *Brachiaria/Arachis* pastures after different land uses: secondary forest, native pastures and degraded grass pastures. In addition, 5 farms were selected to evaluate the effect of using *S. guianensis* (Stylo184) on weight gain of grazing pre-weaned calves. On-farm results showed that by allowing 2.5 to 3.5 months old calves to graze Stylo (184) pastures, LWG were as good as with the traditional systems (calf with dams during part of the day), but saleable milk increased by 1 l/cow/d. Thus, this legume-based feeding system could have high potential adoption by farmers because it allows an improvement in income due to more milk without affecting negatively calve performance.

Costa Rica: Evaluation of new forage alternatives was initiated in 1996 within the TROPILECHE Project in collaboration with several institutions (MAG, ECAG, CATIE and the U. of Costa Rica). The Central Pacific region of Esparza in Costa Rica was selected as a benchmark site that represents the seasonally dry pacific coast of Central America. In 7 farms that collaborate with TROPILECHE we are evaluating:

a) *Cratylia argentea* and sugar cane as dry season fodder for milking cows,
b) Stylo (184) for pre-weaned calves and
c) *Brachiaria brizantha/Arachis* pastures for milking cows.

In 1997 it was necessary to replant *Cratylia* due to establishment problems (i.e. seed quality) and plans were made to introduce *Arachis* in the recently sown *Brachiaria* pastures. Thus it is expected that by the end of this year, all farms collaborating in TROPILECHE will have the new forage alternatives fully established and ready to generate results during 1998 and subsequent years.

Plans are being made by MAG in Costa Rica for the release in 1998 of *Arachis pintoi* (CIAT 18744) as cultivar Porvenir. We are currently multiplying seed to support the release of the new cultivar.

Nicaragua: Since 1996 we have been collaborating with Nicaraguan partners through a Dairy Development Project funded by the World Food Program. The work, which is linked to TROPILECHE focuses on the on-farm evaluation of new forage alternatives in sub-humid hillsides of Matagalpa in the Muy-Muy and Matiguas watersheds. Four farms were selected in 1996 to establish *B. brizantha* cv La Libertad in association with *A. pintoi* (18744) for grazing by milking cows. In addition, plots of *S. guianensis* (184) and *Cratylia argentea* (18516) were established in two farms for pre-weaned calves and for dry season supplementation of milking cows, respectively.

Results so far indicate that pre-weaned calves grazing Stylo (184) pastures gained 300 g/d in the dry season, which contrasts with losses in the order of 20% of BW by the end of the dry season in traditional systems.

Honduras: In attempt to find new partners for forage evaluation in the region we visited this year authorities of DICTA (Dirección de Ciencia y Tecnologia) in Honduras. The visit was timely since DICTA had just received financial aid from Japan to establish a revolving forage seed fund and was implementing a new model for technology transfer, which involved significant farmer participation. It was clear to DICTA and to us that an effective way of collaboration with CIAT was through TROPILECHE, given that a priority of Honduras was to improve milk production in the humid lowlands of the north coast and sub-humid hillsides represented by Yoro and Danli. Thus it was agreed with DICTA authorities that CIAT would collaborate with them through their on going PROPASTO (Proyecto de Pastos y Forrajes) Project in:

a) Supply of basic seed of selected grasses and legumes for on-farm evaluation and further seed multiplication
b) Direct supervision by Dr. P. Argel, adviser of IP-5 and PE-5 in CA, on establishment of improved pastures and legume fodder banks in selected farms,
c) Training on methodologies used in TROPILECHE for on-farm evaluation of new feed resources.

To initiate work this year, DICTA purchased 900 kg of *Arachis pintoi* seed and more than 2.5 tons of improved *Brachiaria* seed. In addition, Dr. P. Argel visited the study site in August of this year to:

a) Assist in the selection of collaborative farms within the already established farmer groups (SETS) operating under the supervision of DICTA and

b) Define forage components to be tested (*Brachiaria/Arachis* pastures, *Cratylia* fodder banks for dry season supplementation and Stylo 184 for pre-weaned calves) in selected farms.

Activity 4.1.2: Seed multiplication of selected forage species for on-farm testing (J. W. Miles and P. J. Argel)

Highlights

- Contracted multiplication of selected grasses and legumes to support a livestock development project in Colombia coordinated by CORPOICA
- Contracted the multiplication of selected ecotypes of *Arachis pintoi* for on-farm evaluation and to support release in Cost Rica
- Basic and experimental seed of highly promising ecotypes of *Arachis pintoi, Cratylia argentea* and *Brachiaria brizantha* was multiplied for distribution in Central America
- A total of 343 kg of experimental and basic seed of promising grasses and legumes delivered upon request to national partners during the period October 1996 to October 1997

CIAT-Palmira, Colombia: Seed multiplication of selected grasses and legumes to support on-farm evaluation and pre-release of cultivars remains an important activity of the Project. During 1997 the multiplication of selected *Arachis pintoi* ecotypes (CIAT 18744, 18748 and 22160) was contracted with SEFO-SAM in Bolivia to supply seed for the NESTLE Project in Caquetá and to provide basic seed for the release of Arachis (CIAT 18744) in Costa Rica. It is estimated that that 2 to 3 tons of each ecotype will be available by the end of the year.

In addition, a major effort was made in 1997 to multiply new *Arachis pintoi* accessions in Colombia and nearly 3 T in total was produced. More modest production of *Brachiaria* accessions was also conducted, at CIAT-Popayán and with Cenicafé (Chinchiná, Caldas, Colombia). Larger scale seed multiplication of three new accessions of *Arachis pintoi* and one of *Brachiaria brizantha* was contracted with SEFO-SAM (Cochabamba, Bolivia).

CIAT-Atenas, Costa Rica: Seed multiplication of promising forage species is a continouos activity of a Seed Unit located at the Escuela de Ganadería in Atenas, Costa Rica. The sites combines soils of medium fertility and a well distributed rainfall with a defined dry period that facilitates harvesting and processing of the seed. The basic and experimental seed produced is destined to support the regional evaluation and release process of new promising forages for multipurpose use.

A total of 200 kg of seed was produced during 1997, much of which was represented by *Cratylia argentea* CIAT 18516/18668 (67 kg), *Centrosema macrocarpum* CIAT 25522 - Ucayali (22 kg) and *Arachis pintoi* CIAT 18744 (78 kg). Small amounts of *Panicum maximum, Brachiaria* spp., *Desmodium velutinum, Stylosanthes guianensis* and several lines of *A. pintoi* were also produced.

The field multiplication areas have expanded during 1997 to meet additional demand of basic seed for CORPOICA (Colombia). The Seed Unit is committed to produce 70 kg of seed of *C. argentea,* and 150 kg of *A. pintoi* (CIAT 18744) and *B. brizantha* (CIAT 26110) to support CORPOICA. These forages are highly promising and have reached a prerelease status in Costa Rica; and are in advanced stage of evaluation in other countries of Central America.

Seed delivery: During the period October 1996-October 1997 the Seed Unit received 74 requests from 12 countries of experimental and basic seed of promising multipurpose forages. A total of 30 kg of grasses and 313 kg of legumes were delivered to National Institutions in the region. The seed was mainly destined to in-country seed multiplication, and for agronomic and on-farm evaluations.

Suboutput 4.2 Defined niches for selected grass and legume cultivars based on analysis of GXE interactions

Activity 4.2.1: Analysis of forage legume germplasm performance data from multilocational trials (A. Schmidt, B. L. Maass, B. Hincapié, L. H. Franco, G. Ramírez, J. W. Miles)

Highlight

- Preliminary selection of new *Arachis pintoi* accessions with superior attributes relative to the commercial cultivar

An important objective of the Forage Project is to be able to define environmental niches to grow selected grasses and legumes. To accomplish this objective multilocational trials with legumes *(Arachis pintoi* and *Desmodium ovalifolium*) have been completed and the analysis of results to define GXE interactions are currently underway. In addition, there is an ongoing multilocational trial with *Brachiaria* genotypes in 12 locations in Colombia.

Based on a preliminary assessment of the results from multilocational trials with *Arachis*, 10 promising accessions were selected for seed multiplication and subsequent on-farm evaluation.

The new accessions of *A. pintoi* have shown to be superior to the commercial cultivar (CIAT 17434) terms of faster establishment, and compatibility with aggressive grasses. However, results indicate large differences in seed yield among these *A. pintoi* accessions in the Colombian coffee zone.

Suboutput 4.4 Effective communication of research results through newsletters, journals and workshops

Highlight

- Publication of a Newsletter by the Forage Project team and continued publication of the Journal Pasturas Tropicales.

Activity 4.4.1: Newsletter (Forage Project Team)

A new initiative of the Forage Project during 1997 was to put out a quarterly Newsletter for partners in the region interested in forage research. The objective of the Newsletter is to inform our partners of recent developments in the Project, to update lists of forage species selected for different ecoregions and uses in production systems and to make announcements of international meeting dealing with forages. The first number of the Newsletter dealt with the objectives of the project, the research activities and expected outputs. In addition the Newsletter included a list of forage germplasm for different uses such as erosion barriers, covers, fallow improvement and livestock feed. In the second number we included a summary of the work on tannins in tropical legumes and a description of a new greenhouse methodology to screen *Brachiaria* genotypes for spittlebug resistance.

Activity 4.4.2: Pasturas Tropicales (C. Lascano)

During 1997 we continued publishing the Journal Pasturas Tropicales thanks to a one-time donation of COLCIENCIAS. Three volumes (18, No .3, 19, No. 1 and 19, No. 2) were published. The themes covered in these volumes were: forage agronomy, nutritive value, pathology, animal production, pasture reclamation, and seed production. Contributions to the three volumes came Brazil, Colombia, Mexico, and Argentina.

The demand for publication in Pasturas Tropicales is growing at a fast rate given that it is the only Journal in LAC dealing with forages that has a wide international distribution. Between December 1996 and August 1997, we have received 39 contributions, of which we have published 26.

Given the budgetary constraints we phase in the Forage Project we have the challenge of continuing the publications of Pasturas Tropicales in 1998 and subsequent years. To meet this challenge we are considering several options: increase the cost of subscription, accepting advertisements and/or charging a publication fee to contributors.

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