ANNUAL REPORT
1999

PROJECT IP-5

Tropical grasses and legumes: Optimizing genetic diversity for multipurpose use

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

Centro Internacional de Agricultura Tropical (CIAT)
Apartado Aéreo 6713
Cali, Colombia, S.A.

Tropical grasses and legumes: Optimizing genetic diversity for multipurpose use (Project IP5)

Project Manager: Carlos E. Lascano
Fax: (57-2) 4450073
Email: C.Lascano@CGIAR.ORG

Tropical grasses and legumes: Optimizing genetic diversity for multipurpose use (Project IP5). 1999. Annual Report 1999. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.

TABLE OF CONTENTS

PROJECT OVERVIEW

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 and persistence in association with agressive grasses.
Shrub legumes with adaptation to subhumid (*Cratylia*) and humid (*Codariocalyx*) ecoregions available to farmers.

1999 Gene pools of *Brachiaria* identified with resistance to spittlebug, dry season tolerance and adaptation to low soil fertility.
Gene pools of *Paspalum* identified with resistance to poorly drained soils.
Methods developed to detect endophytes in *Brachiaria* and other tropical grasses.

2000 Gene pools of *Brachiaria,* identified with resistance to poorly drained soils.
Genetic diversity of *Brachiaria* and *Arachis* using molecular techniques.

2001 Molecular map of *Brachiaria* developed for marker assisted selection.
Defined interaction of endophytes in *Brachiaria* with pest and disease resistance.

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. Hohenheim; 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 (SB2). Selected grasses and legumes evaluated in production systems (PE5) in collaboration with national partners (SN2).

PROJECT WORK BREAKDOWN STRUCTURE

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	Grass and legume genotypes with high qualilty attributes are developed	Grass and legume genotypes with known reaction to pests and diseases and to interaction with symbiont organisms are developed	Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed	Superior and diverse grasses and legumes delivered to NARS partners are evaluated and released to farmers
ACTIVITIES	• Evaluate the role of anti-nutritional factors in legumes (e.g. tannins) in digestion and metabolism of ruminant animals • Define effect of genotype and environment on quality parameters of selected grasses and legumes • Define synergisms in quality attributes among contrasting forages • Measure potential milk yield of selected accessions of grasses and legumes	• Study the bioecology of spittlebug species in contrasting environments • Diagnosis of spittlebug for elaborating IPM components • Develop *Brachiaria* hybrid populations to screen for spittlebug resistance, edaphic adaptation and quality • Identify host mechanisms for spittlebug resistance in *Brachiaria* • Identify *Brachiaria* genotypes resistant to spittlebug • Identify genetic control and molecular markers for spittlebug resistance and apomixis in *Brachiaria* • Elucidate the role of endophytes in tropical forage grasses • Define interactions between host and pathogen (fungus, bacterium, virus) in *Brachiaria, Arachis,* and *Stylosanthes*	• Identify genotypes of *Brachiaria,* Panicum and *Arachis* with adaptation to low fertility soils • Identify genotypes of grasses and legumes with dry season tolerance • Identify accessions of *Brachiaria* and *Paspalum* with adaptation to poorly drained soils • Identify accessions of shrub legumes with adaptation to different environments • Identify accessions of grasses and legumes adapted to environmental constraints in systems • Identify genotypes of *Brachiaria, Desmodium,* and *Arachis,* with broad edaphic and climatic adaptation • Link information on genetic diversity and environmental adaptation of *Brachiaria* and *Arachis* • Study the genetics of selected grass and legume species to facilitate conservation and improvement	• 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 • Evaluate and select with farmer participation multipurpose forages for crop/livestock systems • Develop expert systems for legume biodiversity by linking geographic information with biological data • Facilitate communication through newsletters, journals, workshops and internet

Revised Project Log-Frame

CIAT
Area: Genetic Resources Research
Project: **IP5-Tropical Grasses and Legumes: Optimizing Genetic Diversity for Multipurpose Use**
Manager: Carlos Lascano

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To contribute to the improved welfare of small farmers and urban poor by increasing milk and beef production while conserving and enhancing the natural resource base	New cultivars of grasses and legumes used by farmers and raise productivity of livestock/crops while protecting biodiversity and land in savannas, forest margins and hillsides	Statistics on income and natural resource conservation in smallholder livestock farms in LAC and SE Asia	Policies are put in place by governments to favor sustainable livestock/forage development in marginal areas occupied by small farmers

Purpose
NARS use superior grasses and legumes to develop improved and sustainable livestock/ crop production systems in humid and sub-humid areas.
	Demonstrated economical and ecological benefits of multipurpose grasses and legumes to livestock/ crop farmers in savannas, forest margins, and hillsides agroecosystems	• Range of variation in desirable traits
		• Performance of forage components in systems
			• Support from traditional and non-traditional donors
			• Effective collaboration:
			  • CIAT's Projects
			  • AROs, NARS, NGOs

Outputs
1. Grass and legume genotypes with high quality attributes are developed.
	• New *Brachiarias* and *Calliandras* with superior forage quality are accessible to NARS for improved animal performance by 2000	• On-farm demonstrations
		• Scientific publications
		• Annual reports
		• Theses
			• Effective collaboration with:
			  • CIAT Projects (PE2)
			  • AROs, NARS, and Farmer Groups

2. Grass and legume genotypes with known reaction to pests and diseases and to interaction with symbiont organisms are developed.	• Molecular map of *Brachiaria* developed for marker assisted selection by 2001
	• *Brachiaria* genetic recombinants with resistance to spittlebug are available to NARS by 2002
	• Known diversity of *Colletotrichum gloeosporioides* are used by NARS to develop/ select resistant genotypes of *Stylosanthes* by 2001
	• Benefit of endophytes biotic (pest diseases) and abiotic (drought) to overcome constraints are demonstrated by 2001
		• On-farm demonstrations
		• Scientific publications
		• Annual reports
		• Theses
			• Effective collaboration with:
			  • CIAT Projects (SB1, SB2)
			  • AROs, NARS and Farmer Groups

3. Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed.	• New *Brachiaria, Paspalum, Leucaena, Calliandra* and *Arachis* with adaptation to major abiotic constraints (infertile soils, drought, poor drainage and cool temperatures) are accessible to NARS by 2000	• On-farm demonstrations
		• Scientific publications
		• Annual reports
		• Theses
			• Effective collaboration with:
			  • CIAT Projects (SB1, PE2, PE4, PE5)
			  • AROs, NARS, NGOs, Farmer Groups

4. Superior and diverse grasses and legumes delivered to NARS partners are evaluated and released to farmers.	• New grass and legume cultivars released by NARS are accessible to farmers by 2001
	• Improved multipurpose grasses and legumes result in increased on-farm milk, beef and crop production in benchmark sites (hillsides and forest margins) by 2001
		Surveys on adoption of new grasses and legumes:
		• Seed sold
		• Area planted
		• Production parameters
		• Environmental/socio-economic indicators
			• Effective collaboration with:
			  • CIAT Projects (PE2, PE5, SN2, SN3, BP1 and Ecoregional Program)
			  • NARS, NGOs and Farmer Groups

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Activities	**Milestones (1999-2000)**		
1.1 Evaluate the role of anti-nutritional factors in legumes (e.g. tannins) in digestion and metabolism of ruminant animals (CL)	• Demonstrated effects of soluble and bound condensed tannin in protein degradation and digestibility in a range of legume species	• PhD thesis, scientific publications and annual report	• Continued collaboration with IGER, UK
1.2 Define effect of genotype and environment on quality parameters of selected grasses and legumes (CL, JWM)	• Defined range of variability in digestibility of *Brachiaria* hybrids	• Pre-graduate and Ph.D. theses, scientific publications, annual report	• Access to forage samples from the *Brachiaria* collection of the GRU in Popayán • Genetic variability exist for digestibility in *Brachiaria*
1.3 Define synergisms in quality attributes among contrasting forages (CL)	• Known intake and milk production of animal supplemented with provenances of *Calliandra calothyrsus*	• Pre-graduate thesis, and annual report	• Continued availability of funds from TROPILECHE (ILRI, IDB) housed in PE5
1.4 Measure potential milk yield of selected accessions of grasses and legumes (CL)	• Known level of milk yield increment with new *Brachiaria* ecotypes and hybrids	• MS thesis and annual report	• Adequate amount of seed of selected *Brachiaria* genotypes available
2.1 Study the bioecology of spittlebug species in contrasting environments (DCP)	• Defined variation in the biology and abundance of spittlebug species in Colombia	• Papers on biology/ecology of spittlebug submitted for publication • Annual report, and undergraduate theses	• CORPOICA delivers remaining funds for spittlebug bioecology studies
2.2 Diagnosis of spittlebug for elaborating IPM components (DCP)	• IPM components relevant to spittlebug species in Colombia better understood	• Technical guide on grassland cercopids • New project proposal on biological control of spittlebug submitted to PRONATTA • Annual report	• Funding obtained to support development of technical/training package on spittlebug • Additional funds are obtained to retain entomopathogen personnel
2.3 Develop *Brachiaria* hybrid populations to screen for spittlebug resistance, edaphic adaptation and quality (JWM)	• Tetraploid sexual parental clones of *Brachiaria* selected for spittlebug resistance, edaphic adaptation and forage quality	• List of *Brachiaria* hybrids with resistance to spittlebug and adaptation to acid soil complex • Annual report	• Additional funds to support the *Brachiaria* improvement program are identified
2.4 Identify host mechanisms for spittlebug resistance in *Brachiaria* (CC)	• Mechanisms of resistance to the spittlebug complex better understood	• Publications on screening methodology for spittlebug resistance and on antibiosis in *Brachiaria* • Annual report	• New funds are available for testing the field screening method for spittlebug resistance • Additional funds from FEDEGAN and other donors are available to support work on antibiotic effects of *Brachiaria* to spittlebug
2.5 Identify *Brachiaria* genotypes resistant to spittlebug (CC, JWM)	• Apomictic genotypes of *Brachiaria* resistant to spittlebug under glasshouse and field conditions available for multisite evaluation	• List of *Brachiaria* genotypes with resistance to spittlebug are available for evaluation with NARS partners • Annual report	• Effective flow of *Brachiaria* genetic recombinants for screening for spittlebug resistance

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
2.6 Identify genetic control and molecular markers for spittlebug resistance and apomixis in *Brachiaria* (JT, JWM, CC)	• Known potential to use marker assisted selection (MAS) for apomixis and spittlebug resistance in *Brachiaria*	• Annual report and scientific publications	• *Brachiaria* genotypes for marking are available • Special funds from ACIAR are available for comparative mapping with pearl millet • Continued and effective collaboration with SB2

2.7 Elucidate the role of endophytes in tropical forage grasses (SK, IMR, CC)	• Alkaloid profiles of endophytes isolated from *Brachiaria* are determined
	• Effects of endophytes in *Brachiaria* on:
	  • spittlebug and rhizoctonia resistance
	  • drought tolerance
		• Scientific publications and annual report	• Effective collaboration with a US institution for determining alkaloids
			• Effective collaboration with the Entomologist and Plant Nutritionist of IP5

2.8 Define interactions between host and pathogen (fungus, bacterium, virus) in *Brachiaria, Arachis*, and *Stylosanthes* (SK)	• Isolates (100) of *C. gloeosporioides* are isolated from *S. guianensis* using PCR and differential host • Benefit of chitinase gene on conferring resistance to anthracnose in transformed *Stylosanthes* is demonstrated	• Scientific publications and annual report	• Funds from ACIAR channeled through CSIRO for anthracnose research are made available to CIAT
3.1 Identify genotypes of *Brachiaria, Panicum* and *Arachis* with adaptation to low fertility soils (IMR, JWM)	• Selected *Brachiaria* and *Arachis* genotypes with superior adaptation to low soils fertility available to partners for evaluation	• Kilograms of seed of selected *Brachiaria* and *Arachis* genotypes distributed to NARS • Scientific publications submitted • Annual report	• Continued collaboration with Hokkaido University, Japan and University of Göttingen, Germany • Funds are available to support PhD student in Germany
3.2 Identify genotypes of grasses and legumes with dry season tolerance (IMR, MP, PJA)	• Plant attributes of *Brachiaria, Arachis* and *Calliandra* for adaptation to dry season stress are defined • New genotypes of *Brachiaria, Arachis, Cratylia* and *Calliandra* with dry season tolerance are available for evaluation with NARS partners	• Field trials with selected accessions of *Brachiaria, Arachis, Cratylia* and *Calliandra* in the Llanos of Colombia, and Costa Rica • Annual report, publications	• Continued support from forage agronomist in Costa Rica
3.3 Identify accessions of *Brachiaria* and *Paspalum* with adaptation to poorly drained soils (PJA, MP)	• New accessions of *Brachiaria* and *Paspalum* selected for poorly drained soils are available to partners for evaluation	• Field trials with selected accessions of *Brachiaria* and *Paspalum* in Costa Rica with NARS partners • Annual report	• Continued support from Forage Agronomist in Costa Rica
3.4 Identify accessions of shrub legumes with adaptation to different environments (PJA, MP)	• New accessions of *Leucaena, Calliandra, Cratylia,* and *Rhynchosia* selected for tolerance to a wide range of environments are available to partners for evaluation	• Field trials with shrub legumes in Costa Rica with NARS partners • Annual report	• Continued support from Forage Agronomist in Costa Rica • Effective collaboration with OFI, UK, MAG, Costa Rica and CIAT Projects (PE2, PE5)

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
3.5 Identify accessions of grasses and legumes adapted to environmental constraints in systems (MP, PJA)	• New accessions of *Pueraria, Centrosema,* cowpea, *Arachis, Desmodium,* and *Calliandra,* are available for evaluation with NARS partners as cover crops and green manures in savannas and hillsides	• Field trials with selected accessions of legumes for cover crops and green manures with NARS partners in Colombia and Central America • Annual report, publications	• Continue to have support from Forage Agronomist in Costa Rica • Seed of legumes are available to plant in the Llanos, Colombia and Atenas, Costa Rica
3.6 Identify genotypes of *Brachiaria, Desmodium,* and *Arachis,* with broad edaphic and climatic adaptation (JWM, MP, PJA)	• New accessions of *Brachiaria, Desmodium,* and *Arachis* with adaptation to soil and climatic conditions are evaluated with NARS partners and farmer groups	• On-farm trials with selected accessions of *Brachiaria, Desmodium,* and *Arachis* with NARS partners • Publications on performance of *Arachis* and *Brachiaria* in contrasting locations of Colombia	• Favorable climatic conditions to harvest seed of *Arachis, Desmodium,* and of *Brachiaria* in Costa Rica and Colombia
3.7 Link information on genetic diversity and environmental adaptation of *Brachiaria,* and *Arachis* (MP)	• Passport, agronomic performance and isozyme marker data for *Brachiaria* and *Arachis* is linked to the FLORA-MAP	• Annual report	• Effective collaboration with PE4 for use of the FLORAMAP Model
3.8 Study the genetics of selected grass and legume species to facilitate conservation and improvement (JWM, MP)	• Genetic markers characterized and level of outcrossing known in *Arachis*	• Annual reports	• Effective collaboration with CIAT Projects SB1 and PE4
4.1 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 (IP5 Team)	• Selected *Brachiaria* and *Cratylia* cultivars are released in Central America and Colombia	• Technical bulletins on grasses and legumes released by NARS • Annual report	• Effective collaboration with NARS that form part of TROPILECHE • New NARS partners interested in joining TROPILECHE
4.2 Evaluate and select with farmer participation multipurpose forages for crop/ livestock systems (MP, PJA, JWM)	• Multipurpose grasses and legumes are tested by farmers in hillsides of Honduras and Nicaragua • *Cratylia argentea* is used as a dry season feed by farmers outside the Tropileche benchmark sites by farmers in Costa Rica	• Kilograms of seed of selected accessions distributed to NARS partners • On-farm trials in Colombia and Costa Rica • Annual report	• Effective collaboration with: • CIAT Projects PE2, PE3, PE4, PE5 and Ecoregional Program • NARS, NGO and Farmer Groups
4.3 Develop expert systems for legume biodiversity by linking geographic information with biological data (MP)	• Forage Data base in CD-ROM is available to NARS and CIAT Projects • Decision support system is available for targeting forage germplasm for agronomic performance in agroecosystems	• Diskettes with Forage Database • Annual report	• Effective collaboration from PE-4 and PE5 on linking forage database to GIS
4.4 Facilitate communication through newsletters, journals, workshops and internet (IP5 Team)	• Institutions and individuals receive and contribute to publications • Web Page of the Forage Project is developed • Collaborative work with EMBRAPA forage scientist is defined	• Number of recipients of Newsletters • Number of published articles in Pasturas Tropicales • Proceedings of joint workshop of IP5 members with EMBRAPA	• Effective contributions of IP-5 members to the Newsletter • IP-5 members and NARS partners continue to submit papers for publication in Pasturas Tropicales • Funds are identified to organize workshop with EMBRAPA forage researchers

Summary of Research Progress

In December 1998, the Forage Team met to discuss progress made and to define priority activities and milestones for 1999 and 2000. A major outcome of this meeting was a revised Project Logical Framework, which served as guide to prepare the present Annual Report.

The objective of the Forage Project continues to be the identification of superior gene pools of grasses and legumes that contribute to livestock and agriculture production and to protection of the environment in sub-humid and humid tropical regions. To accomplish this objective we emphasize selection of superior genotypes within key grass and legume species with adaptation to biotic and abiotic constraints prevailing in savanna, forest margins and hillside agroecosystems. Establishment of partnerships with other CIAT Projects, IARC, NARS continues to be an important mechanism of diffusion to farmers of superior forage genotypes. However, to accelerate adoption of improved forages by farmers, we initiated collaborative research efforts with CIAT and NARS partners in hillsides of Honduras to use participatory research methods for the evaluation and selection of multipurpose forage species.

In what follows we summarize research progress made this year for each Outputs of the Project.

Output 1: Grass and legume genotypes with high quality attributes are developed

Condensed tannins and quality of tropical legumes: This year we finalized a 4 -year collaborative project with IGER, UK. The main objective of the work was to develop improved methods to assess quality of legumes with tannins through understanding of the chemistry and biological effects of these secondary compounds in digestion processes in ruminant animals. Our results demonstrate that substrate-associated tannins (bound) in tropical legumes are more effective in preventing digestion of forages by rumen microorganisms than soluble tannins. Furthermore, results showed variation among legume species in tannin structures (molecular weights and monomer composition), that explain differences in inhibition caused by tannins of forage digestibility and protein degradability by rumen microbes.

We now have an improved understanding of the role of tannins on quality of tropical legumes and have standardized laboratory protocols to determine structure-activity effects of condensed tannins. Future research should explore the potential usefulness of tannins in tropical legumes on reducing protein losses in silage and on parasite control in ruminants.

Quality parameters of selected grasses and legumes as affected by genotype and environment: Our results from this year showed that environmental factors had more effect on tannins than legume genotype. Both the molecular weight and monomer composition of tannin in *Desmodium heterocarpon* ssp *ovalifolium* changed more with soil fertility than with genotype, which has implications in the feed value of the legume. This information will be useful to define environmental niches to grow legumes with tannin as a feed resource.

During 1999 we calibrated NIRS (Near-infrared spectrometer) for in vitro digestibility (IVDMD) and demonstrated high variability in digestibility in a hybrid population produced in the Brachiaria breeding program, indicating that large genetic gains can be obtained by selecting for this quality trait. Thus selection for IVDMD in the Brachiaria breeding program is now an objective of the Forage Project. We propose to use NIRS to measure IVDMD in hybrid progenies selected in the field on the basis of yield and plant architecture and to use this quality trait as selection criteria in the ongoing improvement program.

Results from this year also indicated large variation in crude protein and in vitro digestibility among genotypes of the shrub legume *Cratylia argentea*, suggesting that there is scope for

selecting genotypes with superior quality as compared to genotypes that have been advanced to on- farm trials, but possibly sacrificing yield.

Synergism in quality attributes among contrasting forages: In livestock farms new forages are most likely to be fed in different combinations with existing feed resources. Thus it is important that we define how introduced forage species might be best matched with available forages to overcome nutrient deficiencies. This year we used an in vitro system to assess the effects of different legume species in combination with sugarcane and found that digestibility and ammonia- N production were highly affected by legume species and proportion of legume in the mixture, which has practical implications in developing improved supplementation strategies for the dry season. In a subsequent in vivo trial we found that supplementing cows with *Calliandra calothyrsus* (high tannin content) in combination with sugarcane had no effect on milk yield. It is urgent that we define the utility of *C. calothyrsus* as protein supplement given the positive agronomic attributes of this woody legume.

Milk yield with selected accessions of grasses and legumes: The agronomic evaluation of *Brachiaria* genotypes in Colombia and Costa Rica has resulted in the selection of accessions with better performance than commercial cultivars. Such is the case of *B. brizantha* CIAT 26110 selected for high forage yield and drought tolerance. However, first results on animal production with this genotype indicate that individual cow milk yield under grazing is lower than with commercial cultivars (*B. decumbens* cv Basilisk and *B. brizantha* cv Marandu), possibly associated with low protein in the edible forage. Further evaluations will be carried out in 2000 to verify these results.

Output 2: Grass and legume genotypes with know reaction to pests and diseases and to interactions with symbiont organisms are developed

Bioecology of spittlebug species in contrasting environments: Despite a high pest status and long history in the neotropics, an effective and coordinated pest management program for grassland spittlebugs does not yet exist due to poor understanding of the basic biology and behavior of the family. Thus high priority has been given to the development of methodologies to facilitate biological studies and to defining the bioecology of spittlebugs in contrasting environments. An outcome of this work has been the development of small scale rearing unit and an improved mass rearing methodology. Comparative biological studies of Colombian spittlebugs (*A. reducta, A. lepidor, Mahanarva* sp.) revealed differences among species in aspects such as size, duration of the life stages and reproductive biology. In addition, new research on substrate communication showed that adult spittlebugs use host plants as a media to transmit calls for communication (males seek females) and that this behavior is a critical component of reproductive behavior and might serve as a taxonomic tool to distinguish species.

Two years of population surveys were completed in four lowland sites in Colombia with contrasting rainfall (amount and distribution) and results showed differences among sites in the dominant spittlebug species present and in seasonal population dynamics. In sites with well-defined dry seasons there was complete disappearance of the insect during the dry months with extreme population peaks and synchronous population development during the wet season. In contrast, the site without a defined dry season experienced little population synchrony with nymphs and adults present all year round. It is therefore clear that depending on climatic conditions of a region, different strategies will be necessary for the integrated management of spittlebug.

IPM components for spittlebug: Advances in the elaboration of IPM components for spittlebug were limited during 1999 due lack of funding. However, we continued gathering information on the identity and distribution of spittlebugs in graminaceous crops of Colombia and Ecuador.

Thirteen species have been reported, three of these new to Colombia. Studies were continued on the determinants of egg diapause to advance understanding of early season population synchrony in sites with well-defined dry and wet seasons as a key element of spittlebug IPM. Results with *A. varia* indicated that plant age, drought stress and their combination did not influence egg diapause. Thus we still lack an understanding of what climatic factors induce initial outbreaks of spittlebugs in subhumid environments. Photoperiod and temperature are possible factors associated with egg diapause and should be studied in the future.

Studies were continued on fungal entomopathogens, the most promising natural enemy for spittlebug biological control. Major results so far have been the establishment of new ceparium to isolate and house fungal entomopathogens plus the development of a methodology to screen for virulence of entomopathogens against spittlebug adults. Applying this methodology, results showed an efficiency of up to 77% in the control of adults of *A. varia* by selected isolates of fungal entomopathogens. A future priority is to adapt the methodology developed for adults to eggs and nymph of *A. varia* in order to identify the most susceptible life stages to selected entomopathogens.

***Brachiaria* hybrids resistant to spittlebug and host mechanisms associated with resistance:** Over 3000 open pollinated progenies were produced, propagated and established in field nurseries at two sites during 1999. By the end of the year, it is expected that this population would be culled down to 500 entries for inclusion in the spittlebug glasshouse screening in 2000. In addition, 10 elite sexual clones of *Brachiaria* with resistance to spittlebug were set out in seed multiplication plots to be exposed to pollen of the most promising apomictic accessions and one apomictic hybrid in order to generate apomictic genotypes with high levels of resistance to spittlebug, acid soil tolerance and high forage digestibility.

Studies on the resistance of *Brachiaria* genotypes to different species of spittlebug were continued in 1999. Results indicated differences in terms of visual damage scores and % nymphal survival. Lower nymph survival of *Mahanarva* sp. and *A. varia* was detected in resistant genotypes due to high levels of antibiosis. When resistant genotypes were exposed to *Z. pubescens* and *Z. colombiana* damage was not great, but there was no indication of antibiosis as indicated by similar emergence of *Zulia* species in resistant and susceptible genotypes. Thus the low level of damage caused by *Zulia* spp on resistant genotypes could be due to tolerance or to another mechanism that remains to be elucidated.

Results from greenhouse screening of *Brachiaria* accessions (65) from CIAT's collection resulted in the selection of 7 accessions with resistance to spittlebug. From a set of 92 hybrids used for molecular marking of genes associated with apomixis and spittlebug resistance, 9 were resistant to *A. varia.* Two of the resistant hybrids resulted in considerably lower nymphal survival as compared to the most resistant checks, indicating that great progress has been made in the Brachiaria improvement program.

Given that assessment spittlebug resistance under natural infestation in the field is very difficult due to focal, unpredictable occurrence of the insect, a field screening methodology was developed and described in the 1998 AR. This year the methodology was evaluated in the CORPOICA research station in the amazon piedmont of Caquetá, Colombia and results indicated that with few exceptions, ratings for resistance under field conditions matched previous greenhouse ratings.

Genetic control and molecular markers for spittlebug resistance in *Brachiaria*: It has been hypothesized that molecular markers linked to QTL's conditioning resistance to spittlebug will improve selection efficiency for this trait in the Brachiaria breeding program. A hybrid population was generated by crossing the spittlebug susceptible tetraploid sexual *B. ruziziensis* with the spittlebug-resistant, natural apomictic *B. brizantha* CIAT 6294 to obtain marker and phenotypic

data. These hybrids clones propagated vegetatively were evaluated for spittlebug reaction and are currently being analyzed for QTL's. The frequency distribution of genotypes (226) based on damage scores did not suggest monogenic resistance. However, the distribution of genotypes showing resistance and susceptibility did show that the hybrid population used for marking covered the range of damage of the resistant and susceptible parents, which is possibly an indication of a relatively simple inheritance.

Role of endophytes in tropical grasses: Additional progress was made during 1999 on elucidating the role of endophytes in tropical grasses. To develop quick and specific methods of endophyte detection, antiserum was developed capable of differentiating isolates of endophytes in way consistent with DNA data. Three endophytic isolates from *Brachiaria* species were effective in inhibiting fungal pathogens *in vitro*, whereas another isolate was only effective in inhibiting bacteria. However, the effect of endophytes on fungal pathogens *in planta* were not consistent across experiments possibly due to variations in endophyte concentration.

This year we also tested the role of endophytes on spittlebug resistance in *Brachiaria*. Results from two consecutive trials using plants treated and not treated with fungicide and genotypes of *Brachiaria* known to be resistant and susceptible to the insect indicated no measurable effect of endophytes on reaction to spittlebug. In contrast, tests with *Brachiaria arrecta* with and without endophytes showed that infected plants were more tolerant to severe drought as indicated by greater leaf expansion and leaf biomass production as compared to plants free of endophytes.

Interactions between host and pathogens in *Stylosanthes* and *Arachis*: Although sources of resistance to anthracnose in accessions of *Stylosanthes guianensis* (Stylo) are available high level of genetic and pathogenic variability in isolates of *Colletotrichum gloeosporioides* makes long-term stable resistance difficult to attain. Thus additional, cost-effective strategies are needed to deal with this host-pathogen system. One approach tested this year was the introduction in *S. guianensis* CIAT 184 of a chitinase (protein expressed by plants in response to infection) derived from rice. The gene was successfully introduced in Stylo and transgenic plants showed high levels of anthracnose resistance in both laboratory and glasshouse tests.

The herbaceous legume *Arachis pintoi* is now being used as pasture legume and as a cover crop in several tropical regions. Anthracnose caused by *C. gloeosporioides* is an increasing threat to *A. pintoi*. This year we carried out a study to examine the genetic diversity among isolates of *C. gloeosporioides* of *A. pintoi* collected in 4 regions of Colombia. Results indicated differential reactions, which suggest the existence of pathogenic specialization of the fungus on *A. pintoi*. The genetic variability among isolates was also measured using RAPD and southern blot analysis and results revealed at lest 5 groups with the isolate from one region (amazon piedmont, Caquetá, Colombia) distributed in all groups. Thus isolate from Palmira were probably introduced from Caquetá through vegetative cuttings. It was also found that isolates of *C. gloeosporioides* isolated from infected *A. pintoi* were pathogenic to Stylo and that some originating from Stylo can infect *A. pintoi*.

Output 3: Grass and legume genotypes with superior adaptation to edaphic and climatic constraints

Genotypes of *Brachiaria*, *Panicum* and *Arachis* with adaptation to low fertility soils: Previous research had demonstrated that *Brachiaria* species when cultivated under nutrient-limiting conditions, exhibited significant interspecific variation in Al tolerance, with *B. decumbens* cv Basilisk being the most tolerant. This year we report results on specific physiological mechanisms contributing to the high level of Al tolerance of *B. decumbens*. Results confirmed that *B. decumbens* has an outstanding level of Al tolerance when compared with *B. ruziziensis* and food crops such as maize and wheat. Both *Brachiaria* species detoxify Al in all

parts of the root system by chelation with citrate, but in the case of *B. decumbens* detoxification of Al in root apices is with malate in addition to citrate, which seems to enhance its tolerance to high levels of Al. In addition, results showed that both species of *Brachiaria* retain Al within the root system. However, oxalic and malic acids in *B. decumbens* detoxify the relatively small amount of Al that is translocated into shoots, which is not the case for *B. ruziziensis*. Finally, results showed that increases in pH around the root apices also contributed to Al tolerance in the two *Brachiaria* species evaluated. Thus it would seem that the very high level of Al tolerance in *B. decumbens* may not only be due to internal chelation of Al by organic acids, but to a combination of mechanisms.

The identification of plant attributes associated with Al tolerance in *Brachiaria* species was key for the development of a greenhouse screening procedure for Al tolerance applicable to the Brachiaria improvement program. In 1998 a stepwise screening procedure based on root and shoot measurements was proposed to evaluate seedlings of genetic recombinants of *Brachiaria* for their degree of adaptation to acid soils. However, availability of adequate amounts of sexual seed for evaluation of newly generated recombinants was a major limitation for applying the methodology in the improvement program. This year we tried to adapt the root elongation method to vegetative cuttings in order to screen genetic recombinants to Al tolerance. Results indicated that root elongation of cuttings (rooted in a solution containing 200 μ M Ca Cl_2 (pH 4.2), of the three species of *Brachiaria* was greatly influenced by level of Al in the nutrient solution and that 200 μ Al Cl_3 was the most suitable level to distinguish tolerance to Al in genotypes of *Brachiaria*. This screening methodology will now be incorporated in the on going Brachiaria improvement program to allow early elimination of hybrids with poor tolerance to Al.

It is now well documented that *B. decumbens* cv. Basilisk is extremely well adapted to the "acid soil syndrome" (Al toxicity, N and P deficiencies). Thus *B. decumbens* could be a useful model species to understand how different mechanisms operate to allow plants to acquire and utilize efficiently soil nutrients under high levels of Al. We also propose that identification of the genes that contribute to the adaptation of *B. decumbens* to Al tolerance will open the possibility of improving acid soil adaptation of other important crops.

Introduction of *Arachis pintoi* in degraded pastures in regions with low fertility acid soils has not always been successful due to low Ca and P availability in the soil. Given that amelioration of P deficiency with fertilizers is not always a viable option to resource poor farmers, we have been investigating the extent of genetic variability in *A. pintoi* for adaptation to low soil P. A series of greenhouse and field trials were completed during 1999 and results indicated that with all genotypes (10) of *A. pintoi* tested, the association with VAM (vesicular-arbuscular mycorrhizae) significantly improved shoot biomass production with no P and with applications of rock phosphate, but less with soluble P sources (triple super phosphate). However, one genotype (CIAT 18747) was outstanding in acquiring P from rock phosphate when in association with VAM and also exhibited rapid establishment in the field. Other genotypes (CIAT 22160 and 22172) were found to be less dependent on VMA when rock phosphate was applied and thus could be more appropriate for degraded soils where native inoculum of VMA may be low. Field results also showed that P supply was more important than tillage for establishing *A. pintoi* in acid soils.

The African grass *Panicum maximum* is well known for having high quality and to sustain high levels of animal production. However, the commercial cultivars available are not well adapted to the acid low fertility soils that prevail in many cattle growing areas. Thus we investigated the extent of genetic variability for acid soil adaptation in a collection of *P. maximum* acquired by CIAT some years ago. Results from two greenhouse studies involving commercial cultivars (2) and CIAT accessions (5) indicated that CIAT 36000 produced more tillers at lower supply of N and P as compared to other genotypes. The low external and internal requirements for N and P

make this genotype suitable for intensive milk and beef production systems in savanna environments.

Grasses and legumes with dry season tolerance: A major constraint for livestock production in subhumid areas is the lack of forage during the dry season. The evaluation of different accessions of *Brachiaria* in multilocational trials in Colombia allowed collaborators to select three accessions of *B. brizantha* (CIAT 6387, 16467 and 26110) for superior performance in the dry season. Work conducted in Atenas, Costa Rica also identified *B. brizantha* CIAT 16488 and 26110 as being well adapted to drought and this was associated with low Ca in the tissue. Seed of these selected accessions has been now multiplied and on-station and on-farm grazing trials with some of the accessions established in Colombia and Costa Rica.

Work carried out in Atenas, Costa Rica has also allowed us to evaluate genetic variability to drought tolerance in *Arachis pintoi.* Results indicate that of 19 accessions evaluated, CIAT 22148 and 22160 had the best performance in the dry season and this was associated with lower levels of Ca, K and total non-structural carbohydrates in the tissue. Seed of these accessions has been multiplied for on -farm evaluation in Colombia and Costa Rica. Future work will concentrate in better defining plant attributes in *A. pintoi* associated with drought tolerance in order to develop indicators to facilitate selection.

Grasses with adaptation to poorly drained soils: Widely used commercial cultivars of grasses are not well adapted to large areas of poorly drained soils in savannas and certain regions of Central America. To provide grass alternatives for these areas, we have been evaluating a collection of *Paspalum* spp in a poorly drained site in CIAT-Quilichao. Out of 8 accessions planted *P. plicatulum* CIAT 26989, *P. arundinellum* CIAT 26987 and *P. atratum* CIAT 26989 were selected due to superior performance. Seed of these accessions was multiplied and are currently being evaluated together with accessions of *Brachiaria* spp with partners in well-drained and poorly drained sites in Costa Rica.

Shrub legumes with adaptation to different environments: Woody or shrub legumes can be an important component of smallholder livestock systems given their potential use a protein supplements during the dry season. Well known woody legumes like *Leucaena leucochepala* and *Gliricidia sepium* are not well adapted to acid low fertility soils or to prolonged drought. The shrub legume *Cratylia argentea* being promoted by CIAT is well adapted to low fertility soils and to drought but does not perform well in cool environments found in higher- altitude hillsides. This year we completed a multilocational trial with different species of *Leucaena* and found that some species (*trichandra, pallida, leucocephala* subs *glabrata*, and *macrophylla* subs *nelsonii*) had good adaptation to a range of soils in subhumid and humid environments. A future priority is to multiply seed of selected *Leucaena* species for on-farm evaluation and to determine their feeding value.

We have now also identified accessions of shrub legume species with good adaptation to acid-low fertility soils in mid-altitude hillsides (*Calliandra calothyrsus* CIAT 22310 and 22320) and for higher altitude hillsides (*Rhynchosia schomburgkii* CIAT 918, 17918 and 19235). Seed of these accessions is being multiplied for on-farm evaluation and for assessing their feeding value.

Grasses and legumes with adaptation to environmental constraints in systems: A major emphasis of CIAT's Forage Project is to select grass and legume species for different uses in production systems, such as green manures, covers and erosion barriers. This year we planted a small collection of *Vigna unguiculata* (cowpea) received from IITA to evaluate its utility as a green manure. In addition, we initiated the evaluation of different legume species as covers in oil palm and rubber plantations in the Llanos of Colombia.

***Brachiaria, Desmodium* and *Arachis* with broad adaptation**: A major objective of the Forage work at CIAT is to select genotypes among key grass and legume species with broad adaptation to biotic and abiotic constraints in tropical regions. To accomplish this objective we have relied on multilocational trial carried out with partners in the regions. We have now completed multilocational evaluations of core collections of *Brachiaria* spp, *Desmodium heterocarpon* ssp *ovalifolium* and *Arachis pintoi*.

Accessions of *B. brizantha* (CIAT 6387, 1647 and 26110) and a hybrid from the breeding program CIAT 36061 (FM9201/1873) were selected by partners in the Brachiaria Network in Colombia due to good performance in the wet and dry seasons. In evaluations carried out in Costa Rica, we have documented that B. *brizantha* CIAT 26110 is resistant to foliar blight and to *Fusarium* sp, which is not the case with the commercial cultivar *B. brizantha* cv Marandu.

Analysis of results from the multilocational evaluation in Colombia of a core collection of *A. pintoi* is still underway. However, results from two site with contrasting rainfall (Palmira and Chinchiná, coffee zone) and fertile soils indicated that the accessions CIAT 18744 (released in Costa Rica) and CIAT 22268 performed better than the commercial cultivar (CIAT 17434). Genotypes of *D. heterocarpon* ssp. *ovalifolium* selected last year from a multilocational trial are now under evaluation in on-farm grazing trials in the Llanos of Colombia.

Delivery of seed of selected *Brachiaria*, *Desmodium* and *Arachis*: An on going activity in the Forage Project is the multiplication of selected grassed and legumes for advanced evaluation and for distribution to partners for regional evaluation. During 1999 we harvested in CIAT-Popayan a total of 1,176 kg of seed of accessions of *Brachiaria* spp (14) and hybrids (3) that were selected by partners in regional trials in Colombia. In addition, the Seed Unit in Palmira multiplied 162 kg of nine accessions of *D. heterocarpon* ssp. *ovalifolium* selected in a multilocational trial for on-farm testing. In Central America, the Seed Unit in Atenas delivered seed of *Cratylia argentea* (100 kg) and of *Brachiaria brizantha* CIAT 26110 (90 kg) to NARS partners for on-farm evaluation. Selected accessions of *Arachis pintoi* were multiplied in Bolivia by a private seed company and the seed has been used for on–farm evaluation in the Llanos and Caquetá and to sell to other seed companies in Colombia and Costa Rica.

Genetics of selected grasses and legumes species: No rigorous, quantitative data are available on reproductive biology (i.e. rates of natural outcrossing) in *Arachis pintoi*. This information is important for germplasm maintenance, seed production and plant breeding procedures. A small collection of *A. pintoi* was assembled to characterize one or more single- locus genetic markers. So far we have identified two genetic markers: a) one co-dominant isozyme and b) one morphological (flower color). In addition, we established F1 hybrids to generate F2 seed for subsequent genetic characterization. We expect from these studies to be able to define quantitative estimates of natural outcrossing in *Arachis pintoi*.

Linking environmental adaptation of key forage species with GIS: For conserving biodiversity and for planning future collections of key forage germplasm it is necessary to determine sites of high genetic diversity. In addition we would like to define collection sites from which accessions of agronomic interest have originated. To accomplish this objective we are using the GIS-based FLORAMAP developed at CIAT. Ten forage genera were selected as test species and available passport data was checked for mismatches. Next year we expect to be able to provide recommendations for in situ conservation of selected legumes species.

Output 4: Superior and diverse grasses and legumes delivered to NARS partners are evaluated and released to farmers.

Development of partnerships with IARC's and NARS in LAC, SE Asia and Africa to undertake evaluation of range of grasses and legumes for multipurpose use: Elite forage germplasm form CIAT is currently being evaluated with farmer participation in SE Asia through the Forages for Smallholders Project funded by ACIAR and trough the Tropileche Consortia, which forms part of the Systemwide Livestock Program led by ILRI. During 1999 we continued collaboration with the Systems Project of CIAT (PE-5), with ILRI and NARS partners in Colombia, Peru, Costa Rica, Nicaragua and Honduras. WE also initiated contacts with NARS in Ecuador to establish collaborative work on spittlebug though WB funds in the coast and amazon rainforest.

A project funded by NESTLE in the amazon piedmont of Colombia ended this year. A major accomplishment of this collaborative project was to demonstrate that milk yield of cows could be increased by 0.5 liters/day with the introduction of *A. pintoi* in degraded *Brachiaria* pastures. At the end of the 4-year project a total of 3,000 ha had been planted with *A. pintoi* by over 100 farmers, of which 87% were in association with grasses and the remainder as pure legume stands.

Collaborative work in Tropileche, Costa Rica has continued to demonstrate the value of *Cratylia argentea* as protein supplement to replace expensive concentrates in the dry season and for making silage during the wet season, which has been a farmer - led initiative. Spontaneous adoption of *Cratylia* is occurring outside the Tropileche benchmark site in Costa Rica and commercial seed producers are now multiplying seed.

Results obtained by Tropileche in Costa Rica have also demonstrated that *Brachiaria-Arachis pintoi* associations increase milk yield by 8% over the straight grass in cows receiving concentrate supplementation. The use of *Stylosanthes guianensis* in farms of the forest margins of Colombia and Peru for feeding pre-weaned calves has resulted in 20% more milk for sale, and 30% higher weight gain of calves.

The *Brachiaria brizantha* accession CIAT 26110 selected on the basis of high forage yield and drought tolerance is now under evaluation in farms of the Llanos of Colombia and Costa Rica. A commercial seed producer in Costa Rica is multiplying seed of this accession and plans are being made for its release next year. In addition, the *Brachiaria* hybrid CIAT 36062 (BR93-NO/1371) with high spittlebug resistance was included for the first time in grazing trial in the amazon piedmont of Colombia.

Selection of multipurpose forage for crop/livestock systems with farmer participation: Novel forage germplasm cannot be evaluated with partners and farmers or adopted into commercial production systems without some propagation vehicle, normally seed. Thus we continue to maintain active Forage Seed Units in CIAT-Palmira and Atenas, Costa Rica. During 1999 the main seed multiplication focus in CIAT-Palmira was on *Brachiaria* and *Desmodium* accessions as indicated before. In addition, we harvested seed of wide range of germplasm including a total of 25 accessions representing at least six different species. Seeds were dispatched to a total of 6 countries, and 5 institutional types, including two private individuals. In the Seed Unit of Atenas in Costa Rica a total of 812 kg of seed was either produced or procured. A total of 61 requests were processed in 1999 and 1750 kg of experimental and basic seed distributed to Costa Rica, Nicaragua, Guatemala, Honduras, Panama, Puerto Rico, Colombia, Belize, Jamaica and Nepal.

The concept of involving farmers in the evaluation and selection of forage germplasm to facilitate adoption is being tested in hillsides of Nicaragua and Honduras in collaboration with CIAT's

Hillsides Team and NARS. During 1999 a range of grasses was subject to the assessment and selection by farmers at two sites in Honduras. In both sites, *B. brizantha* CIAT 26110, *Panicum maximum* CIAT 16031 (cv Tanzania) and the *Brachiaria* hybrid CIAT 36061 (FM9201/1873) were the most preferred lines. Main selection criteria used by farmers were productivity, rooting intensity, color of leaves, and ability to cover the soil. The *Brachiaria* hybrid was mainly selected due to soft leaves and green color of leaves, which were seen as indicators of high forage quality. Seed requests of selected grasses were made by participating farmers to plant large plot in their farms.

In the case of herbaceous legumes, farmers in Honduras participated in the selection of a range of accessions and results indicated that they preferred two *Arachis pintoi* accessions (CIAT 17434 and 22160) for their ability to cover the soil and high leaf proportion and *Stylosanthes guianensis* CIAT 184 for its ability to retain green leaf during the dry season.

The participatory evaluation of grasses and legumes, now expanded to Nicaragua, will be further strengthened in the next three years through a recently approved Special Project funded by BMZ, Germany.

User friendly forage database and expert system for targeting forage germplasm: The Forage work at CIAT has generated over the years a large database on adaptation of a large number of grass and legume species and ecotypes to different environments in tropical America and Africa. A large proportion of the information is in ORACLE and only available to CIAT researchers. In order to make the forage database more widely available to partners through the Internet and via CD-ROM we are in the process of converting the database to a user-friendly graphical platform. A trial version was developed this year and the final version is expected to be ready in 2000 in the Internet and in CD-ROM.

Targeting forage germplasm to agro-ecological and socioeconomic niches is a major challenge of the Forage Project. A working group was formed to device a strategy and procedures for the development of a GIS- based expert system. Parameters for the description of ecological adaptation of forage accessions across environments were developed. Currently the group is defining a classification of agro-ecosystems for use in the GIS tool. It is expected that the system being developed will assist in targeting forage germplasm to farmers' conditions and demands. We are currently seeking funds to support the continuation of this work.

Communication with partners through newsletters, journals, workshops and Internet: The Forage project has placed high priority on communicating research results to partners in the region not only through peer reviewed journals (see attached list of publications during 1999), but through newsletters and workshops. Due to budgetary constraints, in 1999 we only produced one newsletter in which we reported advances in the endophyte work. If the Project is not successful in attracting additional funds the newsletter will be suspended and emphasis placed in communication of research results via Internet .The design of a Web page is now in progress.

Together with the Systems Project (PE5) we continued to publish the journal Pasturas Tropicales. The two volumes published in 1999, included 16 contributions from researchers of different countries and covered a wide range of topics such as forage germplasm evaluation, N fixation in Stylo, response of legumes and grasses to fertilizer, use of shrub legume to supplement milking cows, nutritional indicators, legume seed quality and adoption of *Arachis*.

Project Highlights

- Developed and adapted laboratory protocols to assess structure-activity effects of condensed tannins in tropical legumes.
- Demonstrated that endophytes had a positive effect on growth of *Brachiaria arrecta* subject to severe drought.
- Transformed *Stylosanthes guianensis* CIAT 184 by introducing chitinase gene from rice showed high level of anthracnose resistance.
- Demonstrated high level of control of spittlebug adults by selected isolates of fungal entomopathogens.
- Demonstrated that with few exceptions, ratings of resistance to spittlebug of *Brachiaria* genotypes screened with a new field method matched ratings obtained with the greenhouse method.
- Developed new *Brachiaria* hybrids with more antibiotic resistance to spittlebug than resistant checks.
- Advanced for the first time to evaluation under grazing a spittlebug resistant *Brachiaria* hybrid.
- Elucidated physiological mechanisms associated with Al tolerance in *Brachiaria decumbens* and developed a glasshouse methodology to assess Al tolerance in genetic recombinants of *Brachiaria* using vegetative cuttings.
- Selected a genotype of *Panicum maximum* with adaptation to low fertility acid soils and new *Leucaena* species with adaptation to a wide range of environments in subhumid and humid areas.
- Initiated evaluation and selection of multipurpose forages in hillsides of Honduras using participatory methods.

Progress Towards Achieving Output Milestones (1999-2000)

Output 1: Grass and Legume genotypes with high quality attributes are developed

- **Demonstrated effects of soluble and bound condensed tannins in protein degradation and digestibility in a range of legumes**

Results from the collaborative IGER-CIAT work clearly demonstrate that substrate-associated condensed tannins (bound) in tropical legumes are more effective in preventing digestion of forages by rumen microorganisms than soluble tannins. Furthermore, we have shown variation among legume species in tannin structures (molecular weights), which explain differences in the inhibition of forage digestibility by tannins. As a result, we now have improved understanding of the role of tannins on quality of tropical legumes and have standardized laboratory procedures to determine structure-activity effects of condensed tannins.

- **Defined range of variability in digestibility of *Brachiaria* hybrids**

During 1999 we calibrated NIRS for in vitro digestibility (IVDMD) and demonstrated high variability in digestibility in a hybrid population produced in the *Brachiaria* breeding program, indicating that large genetic gains can be obtained by selecting for this quality trait. Thus selection for IVDMD in the Brachiaria breeding program is now an objective of the Forage Project. We propose to use NIRS to measure IVDMD in hybrid progenies selected in the field on the basis of yield and plant architecture and to use this quality trait as selection criteria in the ongoing improvement program.

- **Known intake and milk production of animals supplemented with provenance of *Calliandra calothyrsus***

Results from this year indicated that supplementing cows with provenance of *Calliandra calothyrsus* of contrasting quality and in combination with sugar cane had no effect on milk yield relative to the use of only sugarcane. However, further studies are needed before concluding that *Calliandra* forage has no utility as protein supplement for milking cows fed low quality grasses. It is urgent that we obtain conclusive evidence on the forage value of *Calliandra* given its positive agronomic attributes and vigorous promotion as a protein source for dairy cows in smallholder farms in tropical regions.

- **Known levels of milk yield increment with new *Brachiaria* ecotypes and hybrids**

A new ecotype *B. brizantha* CIAT 26110 were evaluated for its capacity to produce milk as compared to commercial cultivars. Results indicated that cows grazing this ecotype produced less milk than *B. decumbens* cv Basilisk and *B. brizantha* cv. Marandu, apparently due to lower protein level in the leaf tissue. This initial finding will be confirmed in subsequent grazing trials and detailed forage quality measurements will be carried out to better define cause: effect relationships.

Output 2: Grass and legume genotypes with known reaction to pests and diseases and to interaction with symbiont organisms are developed

- **Defined variation in the biology and abundance of spittlebug species in Colombia**

New methodologies for biological studies, population ecology studies, analysis of substrate communication and small and large scale rearing colonies have been established, evaluated and

implemented. These tools must now be transferred to partners throughout the neotropics to support and foment quality research of high impact in other regions where spittlebugs are pests. Contrasting sites in Colombia have been successfully developed as model systems for linking bioecological studies with improvements in pest management as well as to broaden our understanding of the bioecological variation exhibited by this particular group of pests and by the family Cercopidae in general.

- **IPM components relevant to spittlebug species in Colombia better understood**

Advances in the elaboration of IPM components were limited due to lack of new funding sources. The strategic research fund of CIAT, however, supported a highly successful program to study fungal entomopathogens. Through those studies we have acquired the facilities, expertise and methodologies to seriously assess fungal entomopathogens for biological control potential. Isolates of high virulence were identified and shown to affect adults of *A. varia*. High priority now is the confirmation of virulence of selected strains of entomopathogens against other life stages and species, and employment in the field through new studies that test formulation, application and field evaluation technologies.

- **Tetraploid sexual parental clones of *Brachiaria* selected for spittlebug resistance, edaphic adaptation and forage quality**

Evaluation nurseries containing >3,000 progenies were successfully established at two contrasting sites (Quilichao and Matazul). Visual scoring and initial culling began and on the order of 20-50 final selections will be identified by mid-2000 to screen for spittlebug resistance, tolerance to high levels of Al in the soil and in vitro digestibility.

- **Mechanisms of resistance to spittlebug complex better understood**

We have now confirmed that the high levels of antibiotic resistance of some *Brachiaria* hybrids to *Aeneolamia varia* and *Mahanarva* do not operate with *Zulia* species. Biochemical studies are urgently needed to better understand the mechanisms of antibiotic resistance in selected hybrids.

- **Apomictic genotypes of *Brachiaria* resistant to spittlebug under glasshouse and field conditions available for multisite evaluation**

We have results which indicate that with few exceptions, ratings for resistance to spittlebug under field conditions match ratings obtained in the glasshouse. Seed multiplication of a spittlebug-resistant hybrid CIAT 36062 (BR93-NO/1371) was initiated and we should have sufficient seed for grazing trials in 2000. Using vegetative cuttings the resistant hybrid CIAT 36062 (BR93-NO/1371) was advanced for the first time to a grazing trial in the forest margins in Caquetá, Colombia.

- **Known potential to use marker assisted selection (MAS) for apomixis and spittlebug resistance in *Brachiaria***

The *Brachiaria* mapping population has now been developed and spittlebug resistance phenotyping was completed.

- **Alkaloid profiles of endophytes isolated from *Brachiaria* are determined**
- **Effects of endophytes in *Brachiaria* on spittlebug and rhizoctonia resistance, and a drought tolerance defined**

The identification of alkaloids induced by endophytes isolated from tropical grasses is in progress in collaboration with a US based laboratory.

Antisera developed interacted with isolates of endophytes consistent with DNA data and work is in progress to develop specific DNA probes for quick endophyte detection. Our first results indicate that endophytes had no effect on conferring resistance to spittlebug in *Brachiaria*, but did have an effect on conferring resistance to severe drought, at least in *B. arrecta*. Isolates of endophytes from *Brachiaria* inhibited in vitro growth of bacterial pathogens, but the effect was variable in planta possibly due to differences in concentration of endophytes in the tissue.

- **Isolates (100 of *C. gloeosporioides* are isolated from *S. guianensis* using PCR and differential host**
- **Benefit of chitinase gene on conferring resistance to anthracnose in transformed *Stylosanthes* is demonstrated**

A paper describing a comprehensive anthracnose pathogen population study was published in the European Journal of Plant Pathology. This paper analyses the pathogen population in various *Stylosanthes* growing locations.

Transgenic *Stylosanthes guianensis* plants containing a rice chitinase encoding gene were generated and these plants showed high level of anthracnose resistance in the laboratory and glasshouse tests performed. Our results from this year also indicate that anthracnose disease may be a serious threat to *Arachis pintoi*.

Output 3: Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed

- **Selected *Brachiaria, Arachis* and *Panicum* genotypes with superior adaptation to low soils fertility available to partners for evaluation**

We were successful in developing a rapid and reliable screening method to evaluate aluminum tolerance of *Brachiaria* genetic recombinants. This method uses relative root elongation as a simple measure to identify aluminum sensitive genotypes. We adapted this method for vegetative stem cuttings so that we can evaluate large numbers of *Brachiaria* hybrids for their aluminum tolerance. We plan to use this method to evaluate a hybrid population of *B. decumbens* x *B. ruziziensis*.

Promising *Brachiaria* accessions have been identified from agronomic trials and seed has been multiplied for evaluation under grazing. However, only a limited number of grazing trials have been established owing to lack of financial support.

This year we identified accessions of *Arachis pintoi* with variable dependence on VAM association when rock P is applied. Genotypes of *A. pintoi* (CIAT 22160 and 22172) which are less dependent on VAM association when rock phosphate is applied maybe a good option to introduce in degraded pastures where native VAM inoculum could be low.

- **Plant attributes of *Brachiaria, Arachis* and *Calliandra* for adaptation to dry season stress are defined**
- **New genotypes of *Brachiaria, Arachis, Cratylia* and *Calliandra* with dry season tolerance are available for evaluation with NARS partners**

We continued our efforts at Atenas site in Costa Rica to identify plant attributes that confer tolerance to dry season. Results obtained so far indicate that lower levels of Ca and higher levels of total nonstructural carbohydrates in green leaves and a greater proportion of green leaves compared to dry leaves during dry season may serve as indicators of dry season tolerance in *Brachiaria*, and possibly *Arachis*.

Two accessions of *B. brizantha* (CIAT 16488 and 26110) were identified as promising materials for areas with prolonged drought and seed has been multiplied for distribution to partners for regional evaluation. Among the *Arachis* accessions tested, two accessions of *A. pintoi* (CIAT 22148 and 22160) were identified as having some drought tolerance. Further testing is needed to identify plant indicators for dry season tolerance.

- **New accessions of *Brachiaria* and *Paspalum* selected for poorly drained soils are available to partners for evaluation**

A list of accessions of *Paspalum* spp. well adapted to poorly drained soils is available. Seed production of these accessions has been poor in Quilichao and Popayán but not in Costa Rica. A small plot trial with different genotypes of *Brachiaria* and *Paspalum* was established this year in well-drained and poorly drained soils in Costa Rica in collaboration with partners.

- **New accessions of *Leucaena, Calliandra, Cratylia* and *Rhynchosia* selected for tolerance to a wide range of environments are available to partners for evaluation**

We have now selected accessions of *Calliandra calothyrsus* and *Rhynchosia schomburgkii* with adaptation to mid-altitude hillsides and cool temperatures, respectively. In addition, we have identified species of *Leucaena* (*trichandra, pallida, leucocephala* subs *glabrata, and macrophylla* subs *nelsonii*) with good adaptation in subhumid and humid environments. Seed of selected accessions within species is being multiplied for multilocational evaluation with partners.

- **New accessions of *Pueraria, Centrosema,* cowpea, *Arachis, Desmodium,* and *Calliandra,* are available for evaluation with NARS partners as cover crops and green manures in savannas and hillsides**

The acquisition this year and planting of a collection of cowpea from IITA will allow us to select lines that are suitable as green manures in different production systems. In addition, we initiated the evaluation of different legume species as cover crops in rubber and oil palm plantations in the Llanos of Colombia.

- **New accessions of *Brachiaria, Desmodium,* and *Arachis* with adaptation to soil and climatic conditions are evaluated with NARS partners and farmer groups**

Agronomic evaluation of accessions of *Brachiaria* and *Desmodium* in different sites was completed and seed of promising accessions multiplied to facilitate the establishment of grazing trials with partners. Two accessions of *Arachis pintoi* (CIAT 18744 and CIAT 22268) were found to outperform the commercial cultivar in sites with good soil fertility in Colombia.

- **Passport, agronomic performance and isozyme marker data for *Brachiaria* and *Arachis* is linked to the FLORAMAP**

Passport data from accessions representing 10 legume species was checked for mismatches and is now ready to link to FLORAMAP. The classification of agro-ecosystems is being revised for better targeting of forage germplasm to climatic and edaphic conditions in a given site.

- **Genetic markers characterized and level of outcrossing known in *Arachis***

Two, probably single-gene markers have been identified in *Arachis pintoi*. F_2 seed is being produced for complete genetic characterization. Open pollinated seed of two parental genotypes of *A. pintoi* are being produced for estimation of natural outcrossing and quantitative estimates of outcrossing should be available by mid-2000.

Output 4: Superior and diverse grasses and legumes delivered to NARS partners are evaluated and released to farmers

- **Selected *Brachiaria* and *Cratylia* cultivars are released in Central America and Colombia**

In collaboration with partners we identified new accessions of *Brachiaria* with broad adaptation and superior agronomic attributes relative to commercial cultivars. Seed of these accessions was multiplied and on station and on –farm grazing trials initiated in Colombia and Costa Rica. Commercial Seed Companies in Costa Rica are already multiplying seed of *B. brizantha* CIAT 26110 and this together with results from on-station and on-farm grazing trials should help NARS decide on future releases.

The *Brachiaria* hybrid CIAT 36062 (BR93-NO/1371) selected for high resistance to spittlebug was included for the first time in a grazing trial in the amazon piedmont, Caquetá.

- **Multipurpose grasses and legumes are tested by farmers in hillsides of Honduras and Nicaragua**
- ***Cratylia argentea* is used as a dry season feed by farmers outside the Tropileche benchmark sites by farmers in Costa Rica**

Substantial progress was made this year in the participatory selection by farmers of grasses in Honduras; there is rising interest among participating farmers in some of the herbaceous legumes, in particular *Arachis pintoi*. Efforts are being made to enhance and support artisanal seed production. During the following year it is anticipated that some farmers in Nicaragua and Honduras will establish selected grasses on their farms – seed requests were made – and have selected some legumes. Farmers and NGO's from neighboring communities have shown interest in the work being carried out and an extension of the approach is planned for the next planting season.

Spontaneous adoption of *Cratylia argentea* is occurring in Costa Rica. During the last 12 months 100 kg of experimental seed was sold to 28 farmers located in four different sites. The early adoption of *Cratylia* has stimulated private seed growers in Costa Rica to multiply seed, which will in turn facilitate the official release of this legume by MAG (Ministry of Agriculture and Livestock) in Costa Rica.

The shrub legume *C. argentea* has not been included up to now in the farmer participatory evaluation in hillsides of Honduras and Nicaragua. However, it is being evaluated by farmers in the llanos piedmont in Colombia.

- **Forage database in CD-ROM is available to NARS and CIAT Projects**
- **Decision support system is available for targeting forage germplasm for agronomic performance in agroecosystems**

A preliminary version of the graphically and user-friendly forage database is available for selected scientists through the CIAT's Intranet. During 2000 this database will be refined for use

via the Internet and a CD-ROM. This database will also be utilized in developing the Decision Support Tool for the targeting of forage germplasm.

- **Institutions and individuals receive and contribute to publications**
- **Web Page of the Forage Project is developed**

Through the Forage Newsletter and the Journal Pasturas Tropicales we continue to reach a wide audience of researchers in LAC. Both publications are being distributed to 350 international subscribers including libraries. As a consequence, we are able to communicate not only our research results but also that of our partners. The challenge that we still have is to identify funds to continue publishing the Forage Newsletter and Pasturas Tropicales.

The communication group of CIAT trained this year a member of the IP-5 Team on design of Web pages for Projects in CIAT. We are now working on the design of the Web page and hope to have a first version by mid 2000.

Output 1: Grass and legume genotypes with high quality attributes are developed

Activity 1.1 Evaluate the role of antinutritional (tannins) factors in legumes in digestion and metabolism of ruminant animals

Highlights

- Developed and adapted laboratory protocols to assess structure-activity effects of condensed tannins in tropical legumes.
- Showed that substrate-associated condensed tannins were more effective in preventing digestion of forages by rumen microorganisms than tannins in solution.
- Showed that the negative effect of condensed tannins present in tropical legumes on forage digestion was related to their molecular weight.
- Found that rate of digestion of tropical legumes had an inverse relationship with the content of cyanidin in condensed tannins.

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.

During 1999 we finalized the collaborative project with IGER on the effect of tannins in quality attributes of tropical forage legumes and we continued the collaboration with OFI on standardization of protocols to measure CT concentration on legumes and on the evaluation of *Calliandra* provenance as protein supplement for ruminants fed a low quality grasses. Finally, we examined two protein-binding assays to measure astringency of CT's in tropical legumes.

1.1.1 Summary of major findings on the effect of condensed tannins (CT) on quality of tropical legumes (R. Barahona, M. Theodorou, P. Morris and C.E. Lascano)

Over the past four years we 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 Reading University. To carry out the research, a range of contrasting legume species (*Desmodium heterocarpon* ssp. *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. In what follows we present a summary of major findings and highlight future research needs.

Effect of CT on digestibility of tropical legumes

Effect of concentration of CT on digestibility. Results showed that concentration of CT's in tropical forage legumes have an overall negative relationship with their in vitro degradability, but that rate of degradation seemed to be more affected than the extent of degradation. In the majority of the cases, the impact of concentration of CT on rate of forage degradation (as indicated by the r^2 of the appropriate linear regression relationship) reached its maximum during the early stages of fermentation (usually within the first 20 hours post-inoculation) (Figure 1).

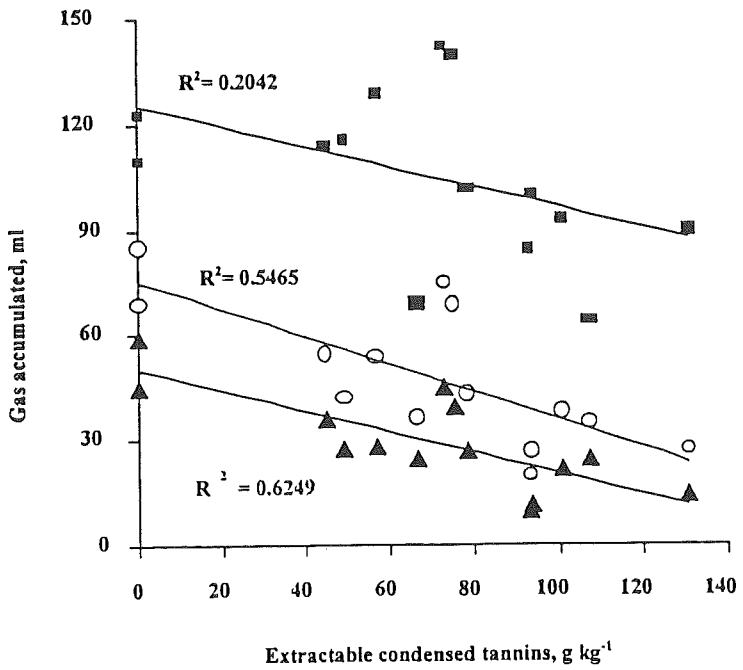


Figure 1. Relationships between volume of gas accumulation (ml) at different times of incubation and extractable condensed tannin content of mature and immature leaves from seven tropical legumes. ▲ = gas at 12 h; ○ = gas at 24 h; ■ = gas at 144 h. With the exception of the relationship between gas at 144 h and extractable tannins, all other relationships are significant ($P < 0.007$).

Although it is evident that the these results must be corroborated in vivo, our observations are in agreement with results obtained with temperate legumes (*Lotus pedunculatus*) that have high levels of CT.

In the experiments where D-glucose was used as substrate, there was evidence that at a high tannin-substrate ratio, a point was reached where further increases in this ratio were not associated with more reductions in gas accumulation. Similarly, when the activity of different fibrolytic enzymes in the presence of CT was determined, the observed relationship between increasing concentration and resulting enzyme activity was curvilinear and best described by polynomial equations. This is in agreement with previous work that showed that the formation of insoluble tannin-protein complexes is dependent upon the relative concentration of tannin and protein and on the stoicheiometry of the resulting precipitates.

Effect of form of CT on digestibility: When comparisons of the effect of CT on digestibility were made on the basis of tannin form, substrate-associated tannins were consistently shown to be more effective in preventing the degradation of forages by rumen microorganisms and in inhibiting the activity of fungal fibrolytic enzymes than soluble tannins. Much lower levels of substrate-associated CT's were required to obtain a given level of inhibition on gas production than with soluble CT (Figures 2 and 3).

On the other hand, the concentration of CT non-extractable in 70% aqueous acetone was not related to forage digestibility, which might be partly explained on the basis of structure-activity relationships.

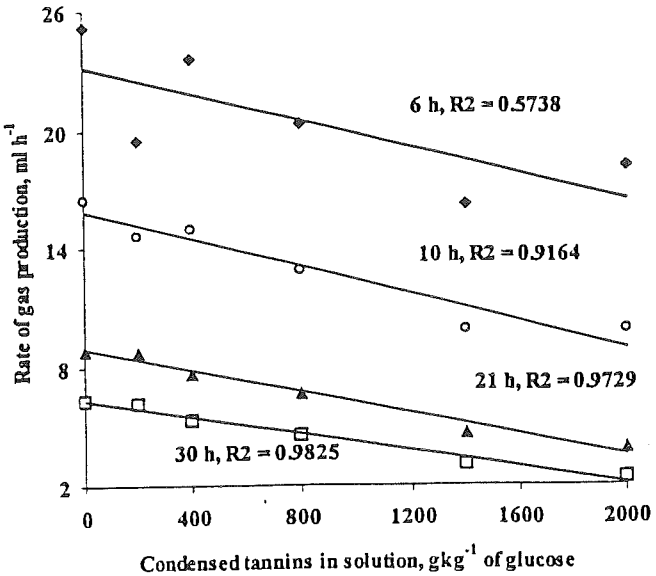


Figure 2. The relationship between the rate of gas production and the concentration of condensed tannins from *Desmodium heterocarpon* ssp. *ovalifolium* in solution at different times during the fermentation of D-glucose with rumen microorganisms.

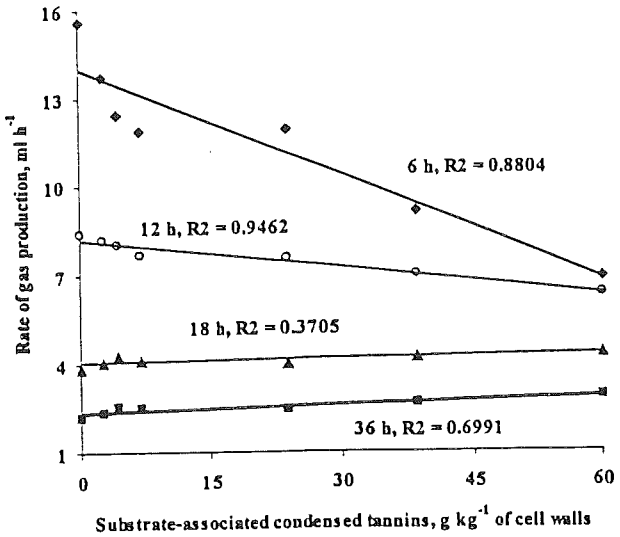


Figure 3. The relationship between the rate of gas production (ml h^{-1}) and the concentration of substrate-associated condensed tannins from *Desmodium heterocarpon* ssp. *ovalifolium* at different times during the fermentation of *Festuca arundinacea* cell walls with rumen microorganisms.

Effect of molecular weight of CT on digestibility: The impact of CT structure (i.e. molecular weight) upon forage degradability was demonstrated by our research. Combination of the results obtained in several experiments allowed us to observe that the negative impact of condensed tannins upon forage degradability was maximized when their number-average molecular was around 4900 Dalton (Figure 4). Other researchers suggested that there is an optimum molecular weight below and above which there is a reduction in the ability of CT to precipitate proteins and other molecules. The use of gel permeation chromatography to determine molecular weight of CT showed that these compounds are polydisperse molecules. The coexistence of tannin molecules of different molecular weight within the same plant creates the possibility of having specialized tannins.

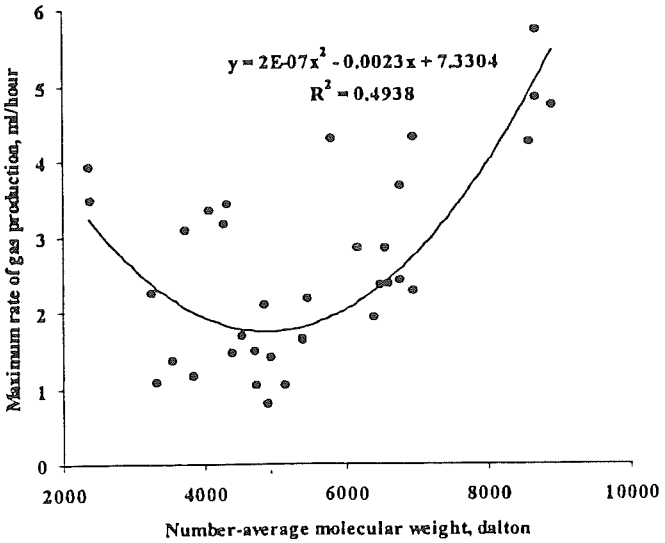


Figure 4. Relationship between the maximum rate of gas production during fermentation with rumen microorganisms and the number average molecular weight of condensed tannins extracted from immature and matures leaves of six tropical legumes.

This is because as their molecular weight changes, the ability of these tannins to bind and precipitate other molecules will also change. Finally, there were also indications that the monomer composition of acetone-extractable tannins would differ from that of non-extractable tannins. Because all these changes in structure will result in changes in CT activity, it is not possible to continue to study the nutritional impact of CT on the sole basis of their concentration in plant tissue.

Effect monomer composition of CT on digestibility: Results from our experiments provided preliminary evidence of the influence that monomer composition of CT can have on quality of tropical legumes. For example, the rate of gas accumulation was found to have an inverse relationship with the content of cyanidin (Figure 5). Previous work had shown that increases in delphinidin content were associated with increases in astringency of CT (IP5 ,1998 AR).

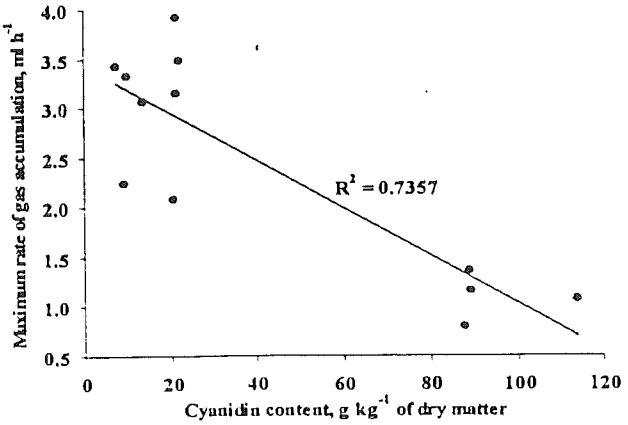


Figure 5. Relationship between maximum rate of gas production during fermentation with rumen microorganisms and total content of cyanidin in condensed tannins in immature and mature leaves of six tropical legumes.

However, care must be taken when interpreting these results. First of all, as evidenced by our experiments, the potential variability in monomer composition in CT from different sources is likely to be much greater than encountered in the legume species included in our experiments.

Thus a greater number of tanniniferous legume species than evaluated in the current study is needed to unequivocally establish the impact of monomer composition of CT on the nutritional value of tropical legumes. Likewise, because the influence of tannin molecular weight appears to be so dominant on the biological activity of tannins it would be ideal to study the role played by different levels of hydroxylation in CT of similar molecular weight. Finally, it is possible that the nutritional impact of CT would be not only a function of their monomer composition, but also of the sequential arrangement of these monomers within the complex tannin molecule.

Other factors affecting digestibility of tropical legumes

Although our results showed that CT negatively affect the degradation of dry matter, they also demonstrated that in vitro degradability of the tropical legumes studied was a function of their cell wall content. The level of neutral and acid detergent fiber was found to have a negative impact on forage digestibility, as reported in the IP5, 1997 and 1998 AR's. It was also demonstrated that fiber degradability is a function of fiber composition, as non-starch polysaccharide constituents such as xylose were found to be highly undegradable, whereas the uronic acids were highly degradable. Protein, on the other hand, was positively related to in vitro degradability in the legumes studied, which is agreement with the findings of other researchers.

Variation on the effect of CT in tropical legumes on enzyme activity

Our results indicate that at a given tannin-enzyme ratio, the inhibition of enzymatic activity observed in vitro is a function of the characteristics of both the protein and the CT. For example, by virtue of their low molecular weight, CT from *Leucaena leucocephala* were consistently shown to be the least effective in inhibiting the activity of enzymes from the anaerobic fungus *Neocallimastix hurleyensis*. In contrast, the larger CT from *D. heterocarpon* ssp. *ovalifolium* and *Flemingia macrophylla* were generally shown to be the most effective in inhibiting these enzymes. In turn, the xylanases were shown to be more susceptible to inhibition by CT that the CMCases , which is agreement with the observations of other researchers who measured the activity of these enzymes in the presence of an extract from *Calliandra calothyrsus*. Interestingly, the glucosidases and xylosidases appeared to be even less susceptible to inhibition by CT than the CMCases. It is also possible that these differences in susceptibility to inhibition by CT are related to characteristics of the substrate hydrolyzed by these enzymes.

Future research needs

The presence of CT in tropical forage legumes results in overall reduction in forage digestibility as supported by the results obtained in several in vitro gas production and enzymatic activity experiments carried out in this study. However, we would like to suggest that this does not necessarily mean that CT's in tropical legumes could not be viewed as having some positive effects in ruminant production. For example, reduction of ruminal protein degradation in response to the presence of CT is considered as beneficial in meat and milk production, provided that the rumen-undegraded protein is later made available to the ruminant. Likewise, there have been reports of decreased carcass fatness in animals consuming tannin-containing diets. These observations could be associated with the changes in the molar proportion of volatile fatty acid produced during the fermentation of tanniniferous diets such as those observed in gas accumulation experiments. Thus future research should explore the potential usefulness of CT on ruminant feeding systems in the tropics as outlined below.

Techniques to measure tannins and their biological activity. In the future research on the nutritional impact of CT on animal nutrition must not only emphasize concentration in plant tissue, but must also include structure-activity considerations. Unfortunately, the techniques required for the structural study of CT involve some degree of sophistication, especially in what refers to instrumentation (i.e. chromatography systems, specialized detectors, nuclear magnetic resonance). However, if this obstacle is removed, any laboratory can easily adopt the techniques used in the current study to measure molecular weight and monomer composition in CT. Both techniques are simple and reliable and do not require extensive sample preparation. The technique used to measure monomer composition has the advantage of having a short run time (20 minutes). Depending on the chromatography system, the run time for this technique can even be shortened to 15 minutes. Likewise, by the use of a column oven, the run time of the technique used to measure condensed tannin molecular weight can also be significantly reduced.

Relationship between tannin and energy availability. Little attention has been given to the possible interaction between energy availability (both rate and extent) and the nutritional impact of CT. Our results indicated that the presence of CT could result in reduced energy availability to ruminants, especially during the early stages of fermentation. In addition, in ruminants fed forage-based diets, energy availability is highly dependent upon fiber degradability, which relates to fiber composition. Since the rate and extent of energy availability to ruminants is affected by factors other than the presence of CT, energy availability could determine to a great extent the nutritional impact of tannins, which is contrary to the general belief that energy availability is a function of the of CT. Concepts relevant to this question include the relationships between voluntary intake and retention time of feeds in the rumen, the synchrony between the release of protein and energy and microbial growth efficiency and the specific inhibition of fibrolytic enzymes in the presence of tannins.

Impact of form of CT on forage digestion. Our results suggested that substrate-associated tannins are more efficient in inhibiting the fermentation of substrates by rumen microbes and substrate degradation by microbial enzymes than soluble CT. Although we speculated about the mechanisms underlying this response, we are still far from understanding the basis of this phenomenon. However, our current knowledge suggests that in future in vivo nutritional studies emphasis must be placed in establishing whether during transit through the gastrointestinal tract, CT act as substrate-associated or as soluble tannins.

Impact of monomer composition of CT on forage digestion. Our results allowed clear delimitation of the influence of tannin molecular weight upon in vitro degradation of dry matter. In contrast, from our observations it is not possible to make final conclusions on the concomitant role of monomer composition of CT on forage quality. Therefore, we suggest that a greater number of observations than used in the present study are needed to clarify the nutritional impact of monomer composition of CT in tropical legumes. Furthermore, there is a strong likelihood that the spatial arrangement of those monomers within the tannin molecule could have a significant role in determining the nutritional impact of CT. Thus a goal of CT research must be the determination of the sequential order of monomers in the tannin molecule and their concomitant impact on the nutritional value of tropical legumes.

Interaction of proteins and tannins. As observed in our experiments with fungal enzymes, the interaction between proteins and CT can be quite specific. Indeed, both tannin and protein characteristics can influence the strength of the interaction. An implication from this observation relates to the idea of co-feeding tanniniferous and non-tanniniferous forages, in order to protect dietary protein (from the non-tanniniferous component) from excessive degradation in the rumen. For this idea to be successful, the specificity of binding between tannins and the protein to be protected must be very strong. Otherwise, CT might end up binding with other molecules and not providing the intended protein protection. In a related idea, it would also be interesting to

determine what is the specificity of binding between CT and protein produced by the same plant. These determinations would have practical application to other processes such as protein preservation during the production of silage from tanniniferous species. Additionally, these observations might shed some light on the subject of CT function in plants and their evolutionary significance.

1.1.2 Standardization of protocols to measure condensed tannins in tropical legumes (J. Stewart, N. Narvaez and C.E. Lascano)

Rationale: As part of the collaborative project OFI-CIAT to determine the nutritive value of two provenance of *Calliandra calothyrsus* grown in contrasting sites, we were interested in standardizing laboratory protocols used in the UK and CIAT to measure concentration of soluble and insoluble condensed tannins (CT) in tropical legumes.

Methods: The Butanol-HCl is the standard method used in both laboratories to determine concentration of CT in legumes. Blanks (Butanol-HCl) and standard curves from purified tannins of individual provenance are used with both methods. However, when examining in detail the analytical procedures from both laboratories it was found that CIAT used ether for removing plant pigments, whereas DCM (dicloromethane) was used in the UK (Reading). Thus a number of test were carried out to determine: (a) correspondence in standard curves used in both laboratories and (b) effect of using DCM in the extracting solution instead of ether to remove plant pigments.

Results and Discussion: Results indicated that standard curves obtained for CT's of *Calliandra* provenance purified in CIAT ($y = 0.06 + 0.97x$ for CIAT 22310 and $y = 0.01 + 0.71x$ for CIAT 22316) were very similar to corresponding curves obtained with CT purified in the UK ($y = 0.07 + 0.97x$ for CIAT 22310 and $y = 0.02 + 0.72x$ for CIAT 22316). In contrast, the concentration of soluble and insoluble CT was consistently higher in *Calliandra* samples analyzed using ether (CIAT) as compared with DCM (UK) regardless of accession (Table 1). To further confirm these results six legume species with variable concentrations of tannins were analyzed for CT with either ether or DCM as pigment removers in the extraction solution and results (not shown) also indicated higher values for soluble and insoluble CT with ether. However, It should be noted that there was a very high and positive correlation ($r = 0.98$; $P < 0.001$) between soluble CT values obtained with the use of ether and DCM. The correlation ($r = 0.55$; $P < 0.0016$) for insoluble CT was also positive and significant, but lower.

Table 1. Effect of laboratory method in the concentration of soluble and insoluble condensed tannins in immature edible forage of two accessions of *Calliandra calothyrsus.*

Accession (CIAT No)	Method	Soluble CT (%)	Insoluble CT (%)
22310	A[1]	17.3	5.6
22316	A	40.8	4.2
22310	B[2]	12.6	3.2
22316	B	28.3	2.3

[1]Method A: Extraction of tannins with aqueous acetone/ether/H_2O-CIAT
[2]Method B: Extraction of tannins with aqueous acetone/DCM/H_2O-UK

It is clear from these results that for reasons not understood, the absolute concentration of soluble or insoluble CT in tropical legumes obtained with the Butanol- HCL method changed significantly depending on the reagent used in the tannin extraction solution to remove plant

pigments. However, the relative values of soluble and insoluble CT are similar with both methods.

1.1.3 Standardization of method to measure astringency in tannins in tropical legumes (N. Narvaez and C. E. Lascano)

Rationale: Different species of forage plants synthesize tannins that have the ability of binding with proteins, which is commonly known as tannin astringency. Thus astringency of tannins has important implications on the nutrition of livestock fed tropical legumes with tannins. Because the affinity of tannins for different proteins varies with the physicochemical conditions, it is important to study these interactions using conditions that are similar to those under study, in this case the rumen.

Methods: A method known a radial diffusion measures the astringency of tannins present in plant extracts in an indirect manner given the ability of the tannins to precipitate bovine serum albumine (BSA). In this assay tannins diffuse through a protein –containing gel and a tannin-protein precipitate ring is formed. The area of the ring is assumed to be proportional to the amount of tannins in the extract. Given that different proteins may not be precipitated in the same manner by tannins from different plant species, it is important to use in the radial diffusion assay a protein source that is similar to those found in forages fed to animals. This is particularly true if the objective is to evaluate whether tannin-protein biding protects protein ingested by animals from degradation by rumen microorganisms.

Researchers at Cornell University with the collaboration of CIAT adapted the well known radial diffusion assay to work with protein extracted from alfalfa leaves using conditions similar to those found in the rumen (pH 6.8 and incubation temperature of 39^0C). Their results indicated that most condensed tannins extracted from different legumes (*Arachis pintoi, Desmodium heterocarpon ssp. ovalifolium* and *Gliricidia sepium*) precipitated more alfalfa leaf protein than BSA protein when protein was included at the same level. It was also found that tannins from different plant species precipitated different amounts of BSA and alfalfa leaf protein.

During 1999 we compared the astringency of purified condensed tannins in two accessions of *Calliandra calothyrsus* using BSA protein and rubisco extracted from spinach, using the same level of protein (100mg) in both assays

Results and Discussion: Results presented in Table 2 indicated that with all legumes the astringency of purified tannins was considerably greater with rubisco than with BSA, even though there was high correlation ($r = 0.93$, $P<0.0001$) between methods.

Results on astringency with the two radial diffusion methods confirm previous findings (IP5 1998, AR), which indicated that CT from immature edible forage of C. *calothyrsus* CIAT 22310 were more reactive with proteins than CT from CIAT 22316. This difference in tannin reactivity has been associated with different monomer composition in the tannins of the two genotypes. In the case of *C. calothyrsus* CIAT 22130 there is a higher delphinidin: cyanidin ratio than in CIAT 22316. It should be noted that the concentration of soluble or extractable CT is considerably higher in CIAT 22316 than in CIAT 22310 (see Table 1), but as indicated the soluble CT in 2310 are more reactive with proteins.

We conclude from this study that astringency values of CT in tropical legumes using Rubisco are highly correlated to values obtained using BSA as protein source in the radial diffusion assay. However, Rubisco would seem to be a better protein source for the radial diffusion assay to measure astringency of CT in tropical legumes, given that with this protein the pH used (6.8) is similar to pH usually found in the rumen of animals fed forage-based diets.

Table 2. Astringency of condensed tannins (CT) from contrasting accessions of *Calliandra calothyrsus* using two sources of protein (Bovine Serum Albumin and Rubisco).

Accession CIAT No	Site	BSA (pH 5.0) CT-Astringency (mg protein bound/mg of CT)	Rubisco (pH 6.8) CT-Astringency (mg protein bound/mg of CT)
22310	Palmira	0.97	8.32
22316	"	0.57	6.05
22310	Quilichao	0.90	7.40
22316	"	0.59	6.21

Progress towards achieving output milestone

- Demonstrated effects of soluble and bound condensed tannins in protein degradation and digestibility in a range of legumes

Results from the collaborative IGER-CIAT work clearly demonstrate that substrate-associated condensed tannins (bound) in tropical legumes are more effective in preventing digestion of forages by rumen microorganisms than soluble tannins. Furthermore, we have shown variation among legume species in tannin structures (molecular weights), which explain differences in the inhibition of forage digestibility by tannins. As a result, we now have improved understanding of the role of tannins on quality of tropical legumes and have standardized laboratory procedures to determine structure-activity effects of condensed tannins.

Activity 1.2 Defined effect of genotype and environment on quality parameters of selected grasses and legumes

Highlights

- Showed that level and effects of condensed tannins in a tropical legume were more affected by environment than by genotype.
- Found high genetic variability in digestibility of *Brachiaria* hybrids indicating possibility of selecting for this quality trait.
- Selected the *Cratylia argentea* CIAT 18666 for superior forage quality relative to the accession being tested on-farm.

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 with tannins, it is important to quantify the effect of soil and climate on tannin production and other quality parameters.

During 1999 we completed the analysis of G x E interactions on tannins in *Desmodium heterocarpon* ssp. *ovalifolium* planted in contrasting sites in Colombia and assessed genetic variability in quality attributes in a small collection of *Cratylia argentea* and a hybrid *Brachiaria* population.

1.2.1 Variation in chemical structure of tannins in tropical legumes as influenced by environmental factors (R. Barahona, A. Schmidt, C. E. Lascano, and R. Schultze-Kraft)

Rationale: 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.

Methods: To accomplish these objectives, a special project funded by BMZ/GTZ, Germany was executed by Axel Schmidt (Ph.D student from the U. of Hohenheim) to study G x E interactions on productivity and quality of *D. heterocarpon* ssp. *ovalifolium.* A total of 18 genotypes of *D. heterocarpon* ssp. *ovalifolium* were planted with two fertilizer levels in 6 contrasting sites in Colombia, which represent major ecosystems (subhumid and humid hillsides, savannas, and forest margins) and soil texture and fertility. Samples of selected ecotypes of *D. heterocarpon* ssp. *ovalifolium* grown in the different sites were used by R. Barahona in collaborative IGER-U. Hohenheim –CIAT project to study in detail the effects of environmental conditions on tannin structure.

Results and Discussion: Results indicated that there was variation in monomer composition of CT in *Desmodium heterocarpon* ssp. *ovalifolium* accessions grown under different environmental conditions in Colombia (Figure 6). Examination of the results from the perspective of fertilization showed that CT from plants grown under low fertilization in a savanna site (Alcancia) tended to have higher cyanidin and pelargonidin content than their higher fertilization counterparts. As result, CT from plants grown under low fertilization had a lower proportion of unidentified peaks than CT extracted from plants grown under high fertilization.

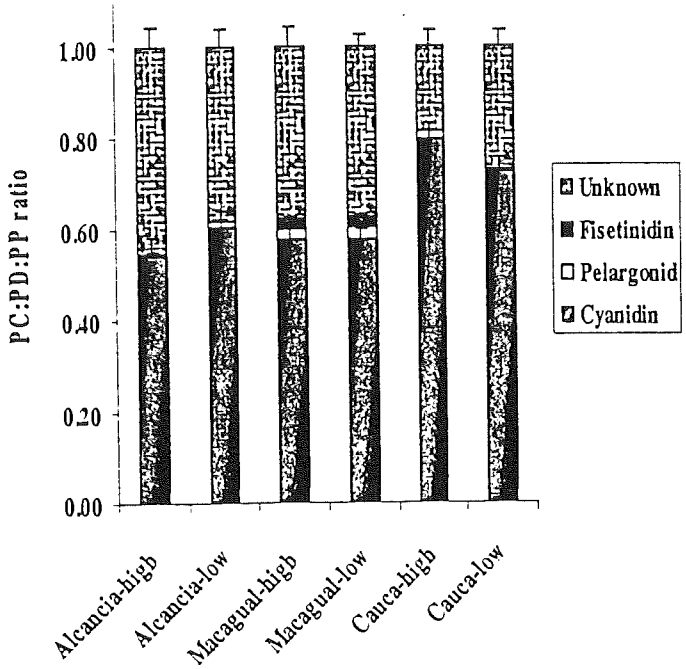


Figure 6. Environment-related variation in the procyanidin-prodelphinidin-propelargonidin ratio of purified condensed tannins from five *Desmodium heterocarpon* ssp. *ovalifolium* accessions planted at two fertilization levels (high and low) in three contrasting sites at Colombia and harvested during the rainy season. Experimental sites comprised Cauca (dry hillsides), Macagual (humid tropics) and Alcancia (well-drained savanna). Bars represent SEM where n=5.

On the other hand, plants of *D. heterocarpon* ssp. *ovalifolium* grow under low fertilization in a hillside site (Cauca) had lower contents of cyanidin and pelargonidin and higher proportions of unidentified peaks (Figure 6). We also found variation in molecular weight of CT extracted from plants of *D. heterocarpon* ssp. *ovalifolium* grown in contrasting environments, as illustrated in Figure 7. It is evident that not only did the average molecular weight of CT change across sites, but also that the mass distribution profiles were different. For example, CT from the accession CIAT 3788 grown in the savanna site (Alcancia) had distinctive peak at about 20 minutes (>2,500 Dalton). This peak was also present, although less prominently, in CT from plants grown at a forest margin site (Macagual), but was absent from those plants grown in the hillside site (Cauca).

All estimates of molecular weight of CT's were positively correlated with rate of gas production during the early stages of fermentation. For example, the "number average" molecular weight had its highest correlation with rate of gas production after 12 h fermentation (Rate $_{12h}$ = 0.0049x – 0.88, r = 0.55, P<0.05).

Important conclusions from this G x E study are that:

a) CT's in tropical legumes are not a uniform chemical entity, as evidenced by variation in molecular weight and monomeric composition of tannins extracted from plants grown in different environments and
b) Impact of environment upon structure of CT of *D. heterocarpon ssp. ovalifolium* was more pronounced than the effect due to genotype.

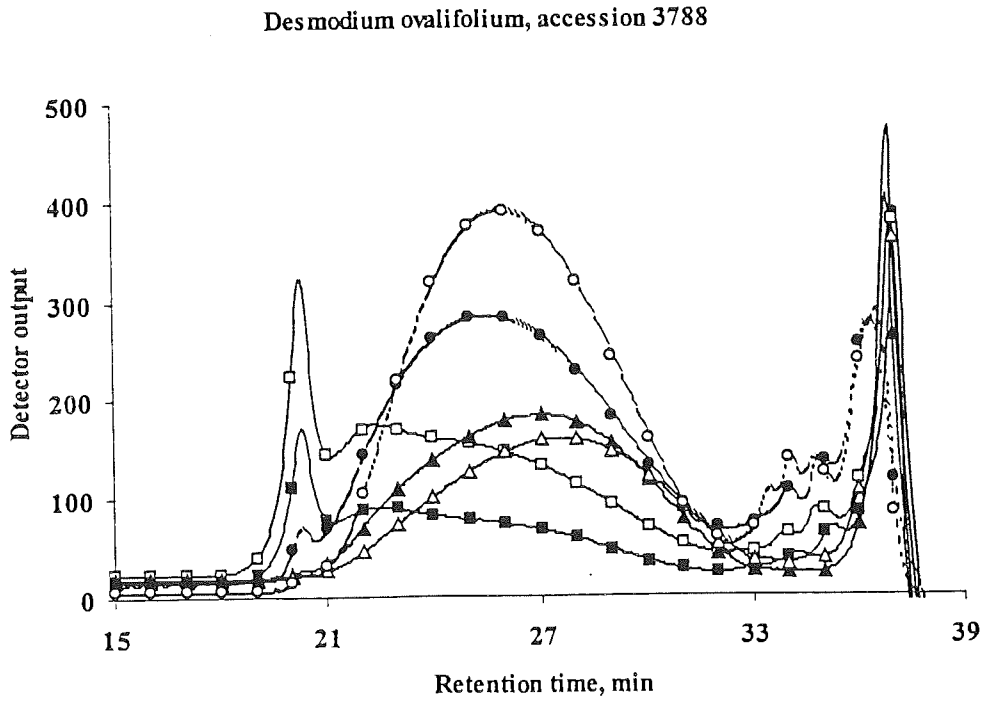


Figure 7. Variation in the molecular weight of condensed tannins from *Desmodium heterocarpon* ssp. *ovalifolium* accession CIAT 3788 grown under two fertilization levels (high and low) in three contrasting sites at Colombia and harvested during the rainy season. Experimental sites comprised Cauca (dry hillsides, high = ▲, low = △), Macagual (humid tropics, high = ●, low = O) and Alcancia (well-drained savanna, high = ■, low = □). Bars represent SEM where n=5.

Our results have demonstrated that the structure of CT from *Desmodium heterocarpon* ssp. *ovalifolium* changed when this tropical legume was grown under different environments. The plant mechanisms and environmental factors bringing about these changes still remain unknown and future research should be devoted to their identification. Furthermore, identification the nutritional impact of these changes is also a high priority researchable issue. Another aspect of these observations that deserves some attention is the determination of the evolutionary advantage gained by plants as a result of the production of CT of different structure. If anything, our observations suggest the existence of molecular mechanisms that allow the alteration of CT structure and with that, the possible amelioration or increment of their nutritional effects.

1.2.2 Calibration of NIRS and variability in digestibility in a *Brachiaria* hybrid population

(N. Narvaez, J. Miles, F. Feijoo and C. E. Lascano)

Rationale: Selection for improved forage quality is clearly justified if genetic variance for digestibility or crude protein is greater than the variance resulting from the interaction of genotype with environment (G x E). Previous work at CIAT with accessions of *B. brizantha* and *B. decumbens* had shown that the variance in vitro dry matter digestibility (IVDMD) caused by genotype was four times greater than the variance from G x E.

In the on going *Brachiaria* improvement the main objective has been to breed for spittlebug resistance and for adaptation to acid-low fertility soils. In terms of quality attributes, such as IVDMD and crude protein, our approach has been to maintain the quality of *Brachiaria* bred lines at least as equal to that of *B. decumbens* cv Basilisk, which is the most widely planted cultivar in tropical America.

A justification for this strategy had been that with the current in vitro system in the Forage Quality Laboratory it was not possible to handle the large number of genotypes (over 3,000) generated annually by the breeding program. However, with the acquisition of a Near-Infrared Spectroscopy (NIRS) it is now possible to analyze large number of samples in the Forage Quality Laboratory provided good calibration curves are available.

Methods: Three main activities were carried out during 1999 to implement selection scheme for IVDMD in the Brachiaria breeding program:

a) calibration of NIRS for IVDMD using leaf samples from 50 *Brachiaria* accessions harvested from the collection held by the GRU in the Popayan station,
b) validation of NIRS to estimate IVDMD in genotypes of *Brachiaria* and
c) assessment of the variability in IVDMD in a hybrid population (tetraploide sexual biotype of the naturally diploid *B. ruziziensis x B. brizantha* cv. Marandu) used for the development of molecular markers for spittlebug.

For the calibration of NIRS for assessing forage quality there are two options:

a) broad-based equations (calibration with large number of samples representing different species, maturities etc) are useful for routine analysis of forage samples for a particular quality attribute (IVDMD) with predictions having little bias but high random variation;

b) narrow- based equations (calibration is done with samples similar to the unknowns) would be the choice for plant breeders in order to give high precision and high degree of discriminatory potential, but with little regard to for the accuracy or bias of the prediction.

Results and Discussion

Calibration of NIRS: To calibrate NIRS for IVDMD we used a narrow – based equation and resulting parameters are shown in Table 3. It is evident that the NIRS curve obtained for IVDMD has a very high level of precision as indicated by the low SE of calibration.

Table 3. Parameters of the calibration of NIRS for IVDMD in *Brachiaria* genotypes

Variable	No of samples	Range	Mean	R^2	SEC[1]
IVDMD	185	57.8 - 82.9	72.4	0.95	0.98

[1] Standard error of calibration

Validation of NIRS: For the validation of NIRS to estimate IVDMD we used leaf samples of uniform maturity from 18 genotypes randomly selected from a hybrid population of *Brachiaria* grown in the greenhouse. These samples were analyzed for in vitro digestibility and results indicated a narrow range in IVDMD values (68.2 to 74.8%). However, results form the validation of NIRS indicated a high correlation (r = 0.89) between observed and predicted values of IVDMD (Table 4). The low SE of predicted values gives confidence on the use of NIRS to screen large number of samples for IVDMD in the *Brachiaria* improvement program.

Table 4. Parameters of the validation of NIRS to estimate IVDMD in *Brachiaria* hybrids

Variable	No of samples	R^2	SEP[1]
IVDMD	18	0.79	0.75

[1]Standard error of prediction

Variability in IVDMD in *Brachiaria* hybrids: In order to examine the extent of variability in IVDMD in *Brachiaria* hybrids produced in the breeding programs vegetative propagules of 224 hybrid clones and parental genotypes were established in 15-cm (6-in) plastic pots in the greenhouse. We harvested whole plants of similar maturity and all samples were free-dried and then separated into leaf and stem fractions. Leaf samples were then analyzed for IVDMD using the conventional two stage in vitro system.

Results presented in Figure 8 show that within this hybrid population sampled the range in IVDMD was from 61.2 to 77.6 % following a normal distribution. From these results it is evident that high animal productions gains could be achieved if progenies from the breeding program are selected for digestibility. It is well documented from work in Tifton, Georgia that increases in IVDMD in the order of 7 percentage units (54 to 61%) in Coastal Bermuda grass resulted in 50% increase on individual liveweight gain of steers.

Based on the results from this year and with the help of NIRS we propose in 2000 to incorporate IVDMD as an additional selection criterion in the *Brachiaria* breeding program. All progenies selected in the field on the basis of plant yield and morphology and which go for spittlebug screening will be sampled and analyzed for IVDMD. With these strategy we should be able to breed *Brachiaria* genotypes that combine resistance to spittlebug, adaptation to acid-low fertility soils and high quality.

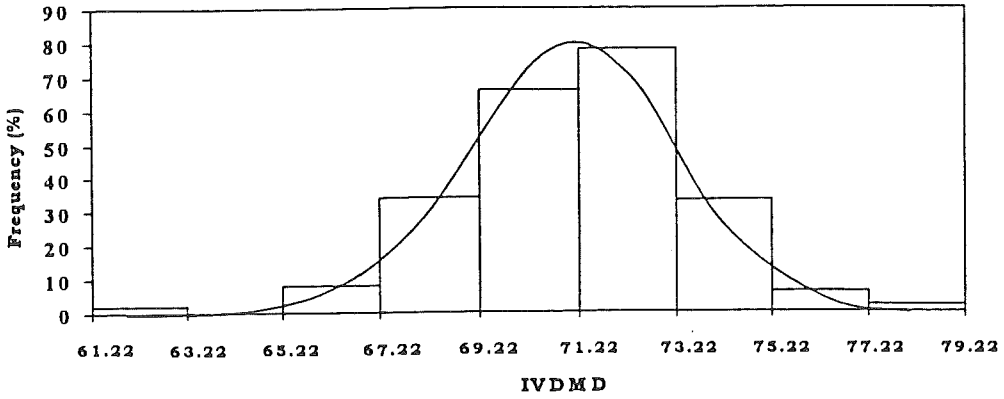


Figure 8. Frequency distribution of in vitro digestibility (IVDMD) values recorded in a population of *Brachiaria* hybrids used for developing molecular markers for spittlebug resistance (N = 224; Mean = 71.2 %; SE Mean = 0.149)

1.2.3 Variability in quality attributes in *Cratylia argentea*

Rationale: The Forage Project has placed high priority to the development of shrub legume alternatives for acid-low fertility soils in subhumid areas. Among the shrub legumes evaluated, *Cratylia argentea* has been the most successful due to high biomass production, good seed yield and high tolerance to drought.

Methods: Agronomic evaluation of the original CIAT collection (11 accessions) of *C. argentea* was carried out in sites representing acid-infertile soils (San Isidro - Costa Rica, CPAC- Brazil, and Quilichao-Colombia) and subhumid environments (Atenas- Costa Rica and CPAC- Brazil) with prolonged dry seasons (5- 6 months). In these evaluations the main selection criteria was seasonal dry matter yield and as a result the accessions CIAT 18668 and 18676 were selected for feeding experiments and for on –farm evaluation. Some preliminary observations indicated that within the small collection of *C. argentea* there was scope for selection on the basis of forage quality. Thus to confirm these initial findings, we screened for forage quality of the 11 original accessions established in Quilichao by harvesting immature and mature leaves.

Results and Discussion: Results shown in Table 5 indicate as expected a large maturity effect on quality parameters, but also large variation among accessions within maturity. Forage crude protein ranged from 22.3 to 30.3% in immature leaves and from 14.9 to 20.3 % in mature leaves. On the other hand, in vitro digestibility values ranged from 56.9 to 69.0 % in the immature leaves, but only from 42.9 to 48.9% in the mature leaves. Digestibility of the accessions was negatively correlated to NDF ($r = -0.76$, $P < 0.0001$) and ADF ($r = -0.89$, $P < 0.0001$) in the immature leaves, but not in the mature leaves.

It was interesting to observe that the high yielding accessions CIAT 18868 and 18676 had similar IVDMD values in immature (63.5 vs. 61.6%) and mature (42.9 vs. 43.3%) forage. However, the IVDMD of these two high yielding accessions was lower than what was recorded with immature (69.0%) and to a lesser extent mature (46.3%) leaves of the lower yielding accession CIAT 18666.

We conclude from this study that within accessions of *C. argentea* there is scope for selecting genotypes (i.e. CIAT 18666) with superior quality as compared to genotypes that have been advanced to on- farm trials, but possibly sacrificing plant yield. In addition, differences in quality among accessions, particularly digestibility, are more pronounced in immature than in mature

forage, which could have practical implications in the selection of accessions of *C. argentea* for silage production in the wet season.

Table 5. Variability in quality attributes in a small collection of *Cratylia argentea* planted in Quilichao

Accessions CIAT No	NDF (%)	ADF (%)	CP (%)	IVDMD (%)
		Immature leaves		
18516	42.2	28.2	28.3	65.4
18666	42.5	25.6	30.3	69.0
18667	47.1	33.1	27.0	62.4
18668	44.8	30.3	27.7	63.5
18671	48.9	28.1	28.3	64.6
18672	51.3	31.4	28.8	64.8
18673	55.8	36.4	23.6	56.9
18674	50.9	30.2	25.3	62.4
18675	55.2	35.5	22.3	57.5
18676	52.0	32.7	26.6	61.6
18957	49.8	29.4	25.7	64.9
Mean	49.1	31.0	26.7	63.0
LSD, P < 0.05	3.8	1.7		3.1
		Mature leaves		
18516	59.6	36.9	20.3	48.9
18666	52.6	36.2	16.3	46.3
18667	58.7	39.2	17.3	44.9
18668	60.0	38.1	17.5	42.9
18671	49.0	35.3	18.1	45.7
18672	50.8	33.6	17.6	45.2
18673	54.8	39.3	16.3	44.3
18674	54.7	36.9	17.6	47.5
18675	60.1	41.1	15.1	44.3
18676	55.0	36.8	18.6	43.3
18957	56.6	38.8	14.9	44.2
Mean	55.6	37.5	17.2	45.9
LSD; P <0.05	3.2	1.3		3.9

Progress towards achieving output milestone

- Defined range of variability in digestibility of *Brachiaria* hybrids

During 1999 we calibrated NIRS for in vitro digestibility (IVDMD) and demonstrated high variability in digestibility in a hybrid population produced in the Brachiaria breeding program, indicating that large genetic gains can be obtained by selecting for this quality trait. Thus selection for IVDMD in the *Brachiaria* breeding program is now an objective of the Forage Project. We propose to use NIRS to measure IVDMD in hybrid progenies selected in the field on the basis of yield and plant architecture and to use this quality trait as selection criteria in the ongoing improvement program.

1.3 Defined synergism in quality attributes among contrasting forages

Highlights

- Found that different legume species when in mixture with sugarcane resulted in different in vitro rumen digestibility and ammonia-N production.
- Found that inclusion of *Calliandra calothyrsus* in a sugarcane-based supplement had no effect on milk yield of cows grazing a low quality.

1.3.1 Effect of mixtures of high energy and high protein forages on fermentation by rumen microorganisms (D. Alvarez, N. Narváez and C. .E. Lascano)

Rationale: In livestock production systems new forages are most likely to be 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.

Our approach to study synergistic effects among forages has been to measure milk yield as a response to feeding cows different grass and legume combinations. Results from this work has shown increased milk increments with increased proportion of legume (*Cratylia argentea)* in the forage-based supplement fed to cows grazing pastures with adequate biomass but with marginal levels of protein (IP5 1998, AR). These results were interpreted to mean that protein coming from the legume could be more limiting than energy coming from the grass (sugarcane).

Methods: During 1999 a series of in vitro experiments were carried out in the Forage Quality Laboratory to assess the effect of different legume species in combination with sugarcane on rumen fermentation parameters. In these experiments we measured gas production and the amount of ammonia N produced after 48 and 24 hours, respectively for different sugarcane-legume combinations. Regression analysis was performed on ammonia-N (24 h) and digestibility (48 h) vs. sugarcane-legume proportion for each of 4 legume species evaluated.

Results and Discussion

Ammonia- N: Results indicate that as expected, the amount of ammonia-N produced during fermentation (24 h) with rumen microorganisms increased as the legume proportion in the mixtures with sugarcane increased (Figure 9). However, the rate of change of ammonia-N due to increasing proportions of legume in the mixture was different among legume species. Similar rates of changes in ammonia-N with increasing proportion of legume were obtained with *Cratylia argentea, Leucaena leucocephala* and *Gliricidia sepium,* which were 3 times greater than changes observed with *Calliandra calothyrsus*. The lower ammonia-N obtained with *C. calothyrsus* is the result of the high levels of condensed tannins in this legume, which is not the case for the other three species. A very high correlation ($r = 0.98$, $P<0.01$) was found between rates of increase of ammonia-N production (24 h) as legume increased in the mixture with sugarcane and in vitro degradation of protein of the mixture.

Digestibility: Contrary to what was observed with ammonia–N, digestibility decreased as the proportion of legume in the mixtures with sugarcane increased (Figure 10). However, rates of reduction in digestibility due to increased levels of legume in the mixture were different among legume species, being high with *Calliandra calothyrsus*, intermediate with *C. argentea and L. leucocephala* and low *G. sepium*. As expected, rates of decline in digestibility with increasing proportion of legumes in the mixture with sugarcane was highly correlated ($r= 0.95$, $P<0.04$) with in vitro digestibility of the legume species tested.

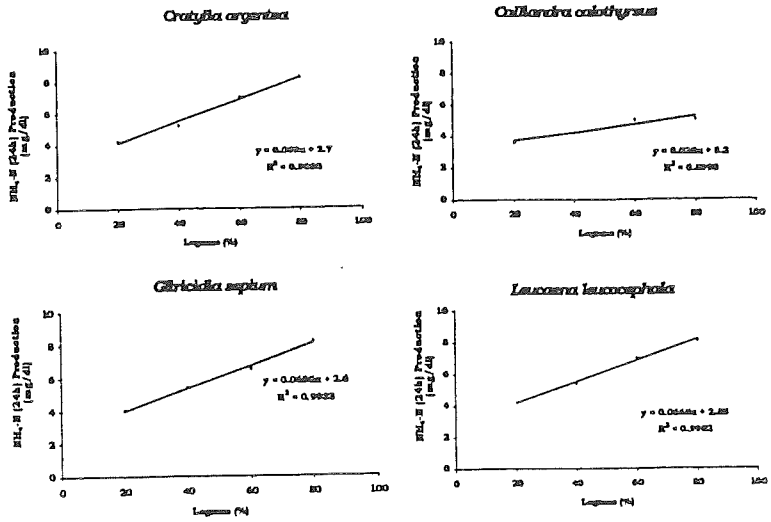


Figure 9. Effect of increasing the proportion of legume in a mixture with sugarcane on production of ammonia-N (24h) during in vitro fermentation with rumen microorganisms.

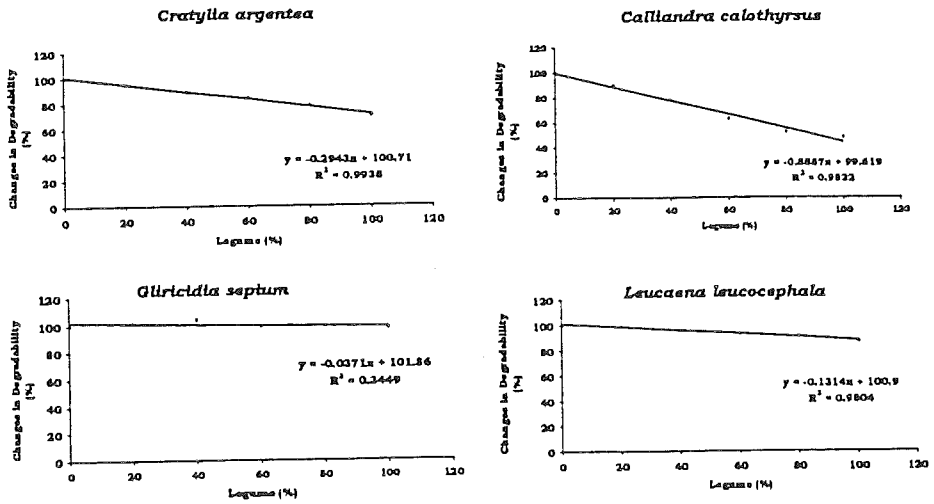


Figure 10. Effect of increasing the proportion of legume in a mixture with sugarcane on digestibility (48h) during in vitro fermentation with rumen microorganisms.

These results from in vitro experiments suggest that increments in milk yield as a response to energy: protein ratios in the rumen of milking cows will depend not only on the proportion of sugarcane and legume fed as supplement, but also on the digestibility and protein degradation potential of the legume species used. These in vitro results need to be validated in vivo in order to provide conclusive guidelines for the use by farmers of different legume species in combination with sugarcane or other cut/carry grasses.

1.3.2 Effect of provenance and drying of *Calliandra* on intake and digestion in sheep fed a low quality grass (P. Avila, J. Stewart and C. E. Lascano)

Rationale: Laboratory results on quality of *Calliandra calothyrsus* had shown differences in digestibility between two selected provenance (IP-5 1998 AR). Thus we were interested in determining to what extent the differences in digestibility between provenance found in the laboratory would be reflected when the material was fed to animals.

Methods: Eight hair sheep housed in metabolism crates and fed a basal low quality grass were allocated to the following treatments arranged in a 4 x 4 LS design:
T1: Dry *Calliandra* CIAT 22310
T2: Fresh *Calliandra* CIAT 22310
T3: Dry *Calliandra* CIAT 22316
T4: Fresh *Calliandra* CIAT 22316

Animals were fed 100 g of DM/BW $^{.75}$/d, of which the low quality grass comprised 60% and legume 40%. Each experimental period was carried out for 14 day of which 7 were for adjustment and 7 for measurements, which included intake, digestibility and N utilization.

Results and Discussion: Results shown in Table 6 indicate that Total (grass + legume) DM intake did not change with provenance or drying. However, DM digestibility was higher when *Calliandra* CIAT 22316 was fed either fresh or dried. Thus intake of digestible nutrients (data not shown) was higher when CIAT 22316 was fed as a supplement as compared to CIAT 22310. Edible forage of CIAT 22310 harvested in Palmira and Quilichao was shown to have lower digestibility than CIAT 22316. In contrast, the concentration of extractable condensed tannins was significantly higher in CIAT 22316. The lower digestibility of CIAT 22310 was correlated with fiber content and not with concentration of extractable tannins.

Table 6. Intake and digestibility by sheep fed a low quality grass and supplemented with two provenance of *Calliandra calothyrsus* dry and fresh[1]

Item	Dry *Calliandra* (22310)	Fresh *Calliandra* (22310)	Dry *Calliandra* (22316)	Fresh *Calliandra* (22316)	SE
DM Intake (g/kg of BW/day)	23.2	24.1	24.7	24.4	0.59
DM Digestibility (%)	57.1b	54.9b	60.9a	59.0a	1.41

[1] animals were fed a low quality grass basal diet (60%) and supplemented with 40 % legume
[a, b] means are different (P <0.05)

1.3.3 Effect of supplementing *Calliandra* on milk yield of grazing cows (P. Avila and C. E. Lascano)

Rationale: To further define the utility of *Calliandra calothyrsus* as a forage legume supplement, we conducted a feeding experiment with milking cows in the Quilichao Research Station.

Methods: During a wet period, six Holstein-crossbred cows grazing a very low quality *Brachiaria decumbens* pasture were assigned to the following supplementation treatments arranged in a 3 x 3 Latin Square design: T1: sugarcane; T2: 60% - sugarcane + 40%- *C. calothyrsus* (CIAT 22310); and T3: 60% - sugarcane + 40% *C. calothyrsus* (CIAT 22316).

In each of the three experimental periods cows were given 7 days of adjustment to the diets followed by 7 days of measurements, which included milk yield and composition.

Results and Discussion: Results presented in Table 7 show that milk yield tended to be lower (9%) when the two provenances of *C. calothyrsus* (40% of the supplement offered) were included in the sugarcane-based supplement, but there was no effect on milk fat. Contrary to what was expected, MUN did not increase with legume supplementation and values were extremely low. However, intake of the forage-based supplements (data not shown) was considerably higher 3.1 and 3. 6 g/kg of LW for sugar cane with *Calliandra* CIAT 22136 and 22310, respectively as compared with the intake of sugar cane alone (1 g/kg of BW).

Laboratory results discussed in the 1998 IP5-AR and results from a feeding trials with sheep reported this year (see Activity 1.3.2), had indicated differences in forage quality between provenance of *C. calothyrsus*. However, It is evident from the results with milking cows that differences in forage quality between provenance of *Calliandra* were not reflected in milk yield when the legume was included in sugarcane-based supplement.

The lack of response in milk yield, in spite high intake of the *Calliandra*-based supplements, could be due to the high level of tannins in both provenance evaluated, which resulted in the low MUN values shown in Table 7. High levels of tannins in the diet can result in low rumen ammonia-N for adequate function of rumen bugs and this is reflected in low MUN values. Further feeding trails, particularly during the dry season will be conducted to test the utility of *Calliandra* as protein supplement for milking cows grazing low quality grasses.

Table 7. Milk yield and composition of cows grazing *Brachiaria decumbens* pastures and supplemented with sugarcane alone or in combination with two provenance (CIAT 22310 and 22316) of *Calliandra calothyrsus*.

Treatment	Milk yield (l/cow/d)	Milk Fat (%)	MUN[1] (mg/dL)
Sugarcane	6.0	3.7	3.8
60% Sugarcane + 40% *C. calothyrsus* (CIAT 22310)	5.5	3.9	3.9
60% Sugarcane + 40% *C. calothyrsus* (CIAT 22316)	5.4	3.8	3.4
LSD (P<0.10)	0.41		0.22

[1] Milk Urea Nitrogen

Progress towards achieving output milestone

- Known intake and milk production of animals supplemented with provenance of *Calliandra calothyrsus*

Results from this year indicated that supplementing cows with provenance of *Calliandra calothyrsus* of contrasting quality and in combination with sugar cane had no effect on milk yield relative to the use of only sugarcane. However, further studies are needed before concluding that *Calliandra* forage has no utility as protein supplement for milking cows fed low quality grasses. It is urgent that we obtain conclusive evidence on the forage value of *Calliandra* given its positive agronomic attributes and vigorous promotion as a protein source for dairy cows in smallholder farms in tropical regions.

1.4 Measure potential milk yield of selected accessions of grasses and legumes

Highlight

- Found that *B. brizantha* CIAT 26110 selected on the basis of high forage yield and drought tolerance produced less milk that commercial cultivars

An assumption in CIAT's Forage Project is that adoption by farmers of improved multipurpose legumes and grasses will depend on how they impact livestock production in the farm. Thus for selecting grass and legume species we are interested in defining the potential of selected forages to increase live-weight gain and milk production.

During 1999 we concentrated our efforts in the evaluation of an improved genotype of *Brachiaria brizantha* that was selected in a multilocational evaluation of different *Brachiaria* accessions and hybrids in Colombia.

1.4.1 Milk yield with a new ecotype of *Brachiaria* relative to commercial cultivars (P. Avila and C. E. Lascano)

Rationale: The Brachiaria Network coordinated by CIAT included 13 evaluation sites in Colombia. The objective of this Network was to select accessions and hybrids of *Brachiaria* with superior agronomic traits relative to commercial cultivars. Among the accessions selected, *B. brizantha* CIAT 26110 ranked first in several sites, given its high forage yield, fast rate of establishment and growth following defoliation and tolerance to drought. This accession was also selected in the subhumid hillsides of Costa Rica as a promising grass.

As a follow-up to the initial agronomic evaluation, seed of selected *Brachiaria* accessions was multiplied in order to carry out on-station and on-farm grazing trials in different locations of Colombia.

Methods: Six Holstein-crossbred cows were assigned to the following treatments arranged in a 3 x 3 Latin Square design: T1: *B. decumbens* cv Basilisk, T2: *B. brizantha* cv. Marandu and T3: *B. brizantha* CIAT 26110. In each of the three experimental periods cows were given 7 days of adjustment to the diets followed by 7 days of measurements, which included milk yield and composition.

Results and Discussion: Milk yield was 11% higher in the two cultivars of *Brachiaria* as compared to yields recorded in the new *B. brizantha* ecotype CIAT 26110 (Table 8). The lower milk yield recorded in CIAT 26110 would appear to be related to a low protein level in the edible forage, given the lower values of MUN measured in cows grazing this grass. However, this interpretation will have to be confirmed through chemical analysis of forage samples obtained in the three experimental pastures.

These first results indicate that milk production per cow in *B. brizantha* CIAT 26110 is lower than in commercial cultivars such as *B. decumbens* cv Basilisk and *B. brizantha* cv. Marandu. However, the high forage yield and regrowth capacity of CIAT 26110 following defoliation would indicate that it is a grass with high carrying capacity and thus an alternative to increase milk production per unit of land. Intensification of dual-purpose cattle systems is of high priority in hillside ecosystems in order to facilitate reforestation in steep land.

Studies will be carried out next year to confirm our initial results on milk yield with *B. brizantha* CIAT 26110 and to clarify quality constraints that may be affecting animal production with this grass.

Table 8. Milk yield and composition of cows grazing two commercial cultivars of *Brachiaria* and a new selected genotype of *B. brizantha*.

Grass Treatment	Milk yield (l/ cow/d)	Fat (%)	MUN (mg/dL)
B. decumbens cv. Basilisk	8.8a	3.5	7.9c
B. brizantha cv.Marandu	8.9a	3.0	7.0c
B. brizantha CIAT 26110	8.0b	3.2	4.7d

a, b , means are different (P<0.08)
c, d means are different (P<0.01)

Progress towards achieving output milestone

- Known levels of milk yield increment with new *Brachiaria* ecotypes and hybrids

A new ecotype *B. brizantha* CIAT 26110 were evaluated for its capacity to produce milk as compared to commercial cultivars. Results indicated that cows grazing this ecotype produced less milk than *B. decumbens* cv Basilisk and *B. brizantha* cv. Marandú, apparently due to lower protein level in the leaf tissue. This initial finding will be confirmed in subsequent grazing trials and detailed forage quality measurements will be carried out to better define cause: effect relationships.

OUTPUT 2: Grass and legume genotypes with known reaction to pests and diseases and to interaction with symbiont organisms are developed

Activity 2.1 Study the bioecology of spittlebug species in contrasting environments

Highlights

- Developed a new small scale rearing unit and an improved mass rearing colony methodology.
- Completed comparative biological studies for two spittlebug species and initiated for four others.
- Obtained first substrate recordings of spittlebug communication and demonstrated that vibrational signals drive reproductive behavior, vary among species and could be used as characters of taxonomic importance.
- Demonstrated how fluctuations of spittlebug populations, phenology and management strategies vary on-farm and according to habitat rainfall seasonality.

Despite a high pest status and long history in the neotropics, an effective and coordinated integrated pest management program for grassland spittlebugs does not yet exist. Among the factors that contribute is a tendency to overgeneralize among the diverse species and genera (dozens of species from seven genera) despite a high degree of variation in the group's bioecology. Second, there is a poor understanding of the basic biology and behavior of the family. Third, the majority of economically important species has never been studied before and therefore lacks biological information that is relevant to an interpretation of their pest status. Finally, there is a scarcity of detailed site-specific studies on spittlebug ecology that offer the resolution necessary to guide advances in pest management.

In 1999 we continued studies to overcome these limitations, advancing in biological studies of additional species and analyzing two complete years of population data obtained from four contrasting sites. The methodologies established in 1997 and 1998 have been improved and implemented in new regions and on new species to further identify patterns and variation in this pest's association with forage grasses and to open opportunities for improved management.

Among the new methodologies are improved colony designs that offer more effective tools for studying spittlebug behavior and screening for fungal entomopathogen virulence as well as host plant resistance. Studies on vibrational communication among spittlebug adults has opened a new window to interpret pest behavior and resolve taxonomic difficulties.

2.1.1 Development of a new small-scale rearing unit for studies on spittlebug biology

(U. Castro and D. Peck)

Rationale: The biology and ecology of most economically important spittlebugs have never been studied. One limitation is the lack of appropriate methodologies for maintaining small-scale colonies in the greenhouse to support studies by small research groups. In addition, anecdotal evidence for the introduction of spittlebug species to new regions argues strongly against the transfer of species from one geographic zone to the next due to risks of new pest introductions. Biological studies should therefore be carried out in the insect's natural range.

Methods: Here we report the final results of a new rearing unit design described in 1997 and 1998 and evaluated with *A. varia*. We sought to develop a unit that met several requirements: adaptable for a variety of spittlebug species and genera, low labor, space and time requirements for operation by small research groups, and effective maintenance of all life stages year round to promote continued studies on biology despite the absence of the insect in the field during the dry season. A major component of our design strategy was to combine nymphal rearing and egg laying chambers thereby eliminating a major bottleneck in CIAT's traditional mass rearing colony.

Results: The oviposition component of the unit comprises a frame of aluminum posts (height 48 cm) (Figure 11). Plant trays (61.6 x 31.5 x 3 cm) were fitted at two levels (16 and 46 cm). The frame was wrapped with a sheet of black nylon mesh to form walls that reduce light, elevate humidity and prevent escape of the insects. Stems of potted host plant (*Brachiaria ruziziensis*) entered the unit through slits in the nylon at two sides to provide adults with food. A tray with specially prepared soil oviposition substrate was fitted in the bottom while the top was covered by either a tray serving as a lid or one with roots containing late instar nymphs.

The entire bottom of each plant tray is perforated with holes that allowed roots to descend. Trays were planted with 21 *Brachiaria zuziziensis* plants (5-7 stems each) in a 2 cm layer of soil . The best root growth was obtained by using young material with partially pruned roots and foliage and planted in contact with the bottom of the tray in soil fertilized with N:P:K at 2g/l water. Five days later this tray was stacked on top of another with a dusting of fertilized soil to stimulate root growth. The space between the trays filled with roots adequate for infestation with eggs about 3 weeks after transplantation. Trays were infested with 500 eggs 1-2 days from hatching. The eggs, in groups of 50 on pieces of cut filter paper, were put on the lower tray with the upper tray of roots replaced on top.

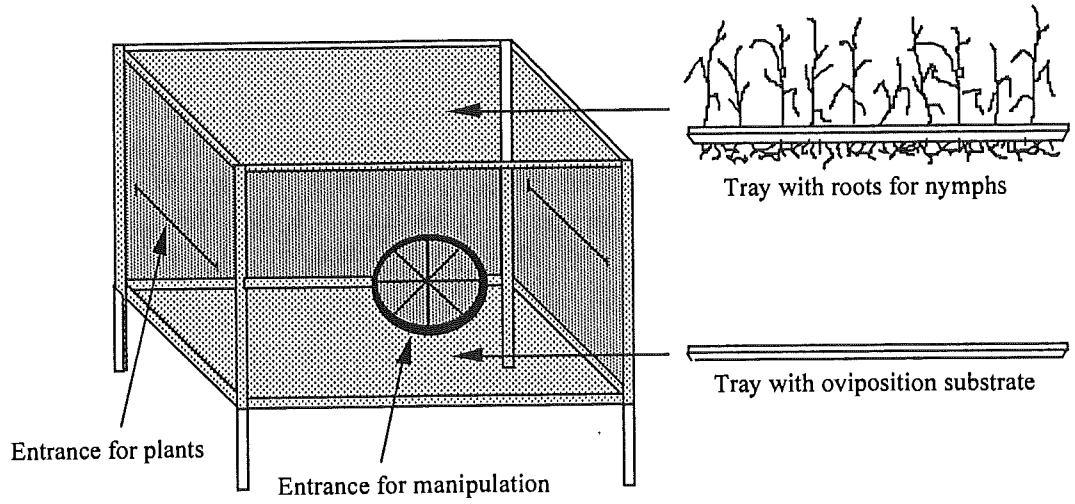


Figure 11. Oviposition component of the spittlebug rearing unit. A tray with roots and nymphs, or an empty tray serve as lids. At the bottom another tray slides in with oviposition substrate. Stems of potted adult host plants enter through lateral slits.

Three weeks after infestation the tray was mounted upon a wooden frame (height 10 cm) (Figure 12). This permitted the roots to extend down to give the adults more space to emerge. The dark and high humidity conditions helped to maintain root quality. This frame was connected to the aluminum oviposition chamber and adults moved toward the relative light of the oviposition chamber through a slit (1.5 x 56.0 cm) along one long side of the wooden frame.

Three versions of the rearing unit were evaluated for efficiency, or proportion of eggs that emerged as adults: 1) just the oviposition component (the root tray on top of the oviposition chamber without use of the wooden frame), 2) just the nymphal rearing unit [adults emerging from the wooden frame into an emergence cage (1 x 1 x 4 m)] and 3) the complete unit (adults recovered from the oviposition chamber after emerging from the wooden frame).

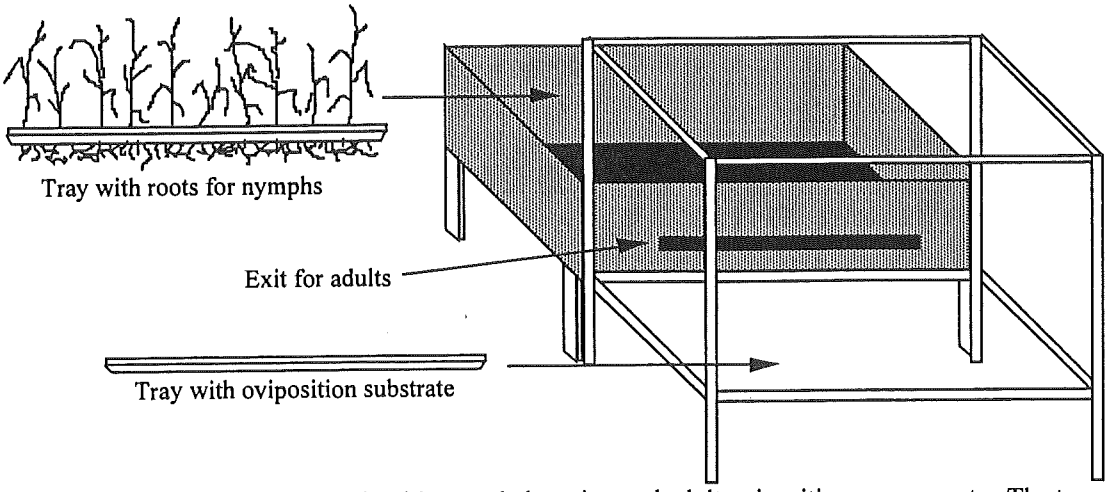


Figure 12. Spittlebug rearing unit with nymphal rearing and adult oviposition components. The tray with roots and nymphs is put on top of the wooden frame. New adults exit the frame and enter the oviposition chamber (wrapped with black nylon mesh).

Use of the wooden frame significantly increased nymph survivorship probably due to enhanced root vigor and quality given the higher humidity and darkness of the wooden box compared to the top of the oviposition chamber where the roots deteriorated rapidly (Table 9). A slit in the wooden frame was effective in allowing adults to exit towards the light of the emergence cage. Sixty percent of eggs successfully hatched, completed nymphal development and exited the wooden box. However, efficiency was significantly reduced when adults emerged into the oviposition chamber; the reduced light probably did not attract the adults as effectively as to the emergence cage. Modifications of this rearing unit were effectively employed to support biological studies of *A. reducta* on the North Coast, *Mahanarva* sp. in the Amazonia, and *Z. colombiana* and *Z. pubescens* in the Cauca Valley.

Table 9. Production of adults from 500 eggs of *A. varia* in three versions of the rearing unit.

Version	n	Mean adults recovered	Mean proportion male:female	Mean efficiency
Oviposition chamber	6	142	1.24	28.5% a
Wooden frame	6	300	1.06	60.0% b
Complete unit	4	124	0.97	24.9% a

Within columns, values with different letters are statistically different ($P<0.05$).

Discussion: This rearing unit offers an alternative for small research groups addressing the biology of different spittlebug species. It will promote in situ studies thereby reducing the risk of species invasions from ex situ studies carried out by larger institutions. With minor modifications the rearing unit is practical and effective for maintaining life stages of different grassland spittlebug species and genera year round. The unit is low cost (US$40-50), requires little space (0.5 m^2) and management, and produces an even sex ratio. Efficiency of 25% is

sufficient for this style of colony but advances in efficiency could be achieved through more effective manipulation of root quality, light conditions and perhaps the number of eggs infested. Evidence that adults can be attracted from nymphal sites to oviposition sites due to light gradients should be considered as a technique to integrate into mass rearing where a major bottleneck is the manual collection and transfer of new adults.

2.1.2 Establishment and evaluation of an improved methodology for mass-rearing spittlebugs (A. Morales and D. Peck)

Rationale: Mass rearing of spittlebugs is a necessary tool for evaluation of control techniques such as the pathogenicity of fungal entomopathogens and host plant resistance. Streamlining this methodology will reduce inputs of materials, labor and space as well as increase efficiency to ensure continuous and high-level production of eggs, nymphs and adults for screeing studies.

Methods: An alternative mass rearing facility was established to support studies on fungal entomopathogens. It was decided to implement and evaluate a new mass rearing technique that integrated recent advances, particularly new nymphal rearing techniques developed and tested at a small scale in 1997. The objective was to establish a reliable and more economical methodology for mass production of spittlebug eggs and adults.

Plants of *Brachiaria ruziziensis* were brought from the field, washed, partially pruned of roots and foliage and planted in pots. After one week they were moved to the nymphal rearing box that comprised a wooden frame (120 x 60 x 10 l/w/h) with a 4-5 cm soil layer on the bottom. Roots of the transplants (5-7 stems each) were placed on 16 small mounds of soil. Stems emerged from openings in a layer of cloth (black below and white above) that covered the box to provide dark and humid conditions that stimulated development of roots down the mounds of soil. One week later roots were sufficiently developed to support infestation with eggs of *A. varia* 1-2 days from hatching. Applications of urea (2%) were made at 2 and 4 weeks plant age. Eggs were applied in groups of 50 on pieces of filter paper placed near the roots. After 4-5 weeks or first adult emergence, an emergence cage was placed over the box to prevent escape. Adults were collected daily with an aspirator and those not required for screening tests were transferred to oviposition cages for the collection of eggs according to the traditional methodology.

To measure efficiency of adult production, 2 boxes were infested each of 32 consecutive weeks. Two egg infestation levels were evaluated: 100 per plant/1600 eggs per box (n=16) and 150 per plant/2400 per box (n=13). To measure the success of egg hatch under these conditions, a subsample of eggs (approx. 800) were placed on moist filter paper and divided among four petri dishes situated in the corners of the box. Hatching success was compared with another group kept under ideal incubation conditions (darkness, 27°C, 100% RH). Egg hatch was studied in 5 consecutive generations.

Results: Although adults from each generation emerged over a period of 2 weeks, 80% of those appeared within the first 8 days. Efficiency varied from 19-56% during the beginning of the study (weeks 1-21) as adjustments were made and the rhythm of management was established. In the last third of the study (weeks 22-32) efficiency stabilized to a mean of 44.1% (range 37.5-48.3%) During this last period, a mean of 980 adults/m^2 were produced (Figure 13).

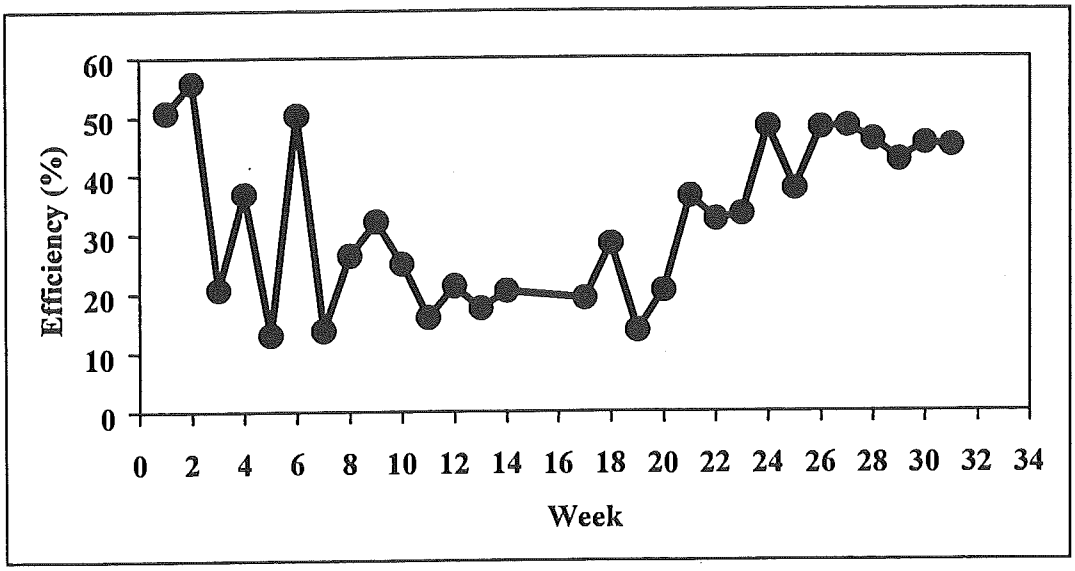


Figure 13. Efficiency (adults from eggs) of the new mass rearing colony for *A. varia* over 32 consecutive weekly cohorts.

For infestation levels of 100 and 150 eggs/plant, the mean number of adults produced per box was 576 and 648, respectively, but no significant difference was detected (Figure 14). Efficiency, however, did differ between infestation levels. Efficiency was 36.0% for 100 eggs/plant but only 27.0% for 150 eggs/plant. There was no difference in the eclosion success of eggs under conditions of the colony or incubation. Mean hatching success was 90.8% and 90.4% for the colony and incubation, respectively. The conditions of the colony were therefore optimal for the successful emergence of first instars.

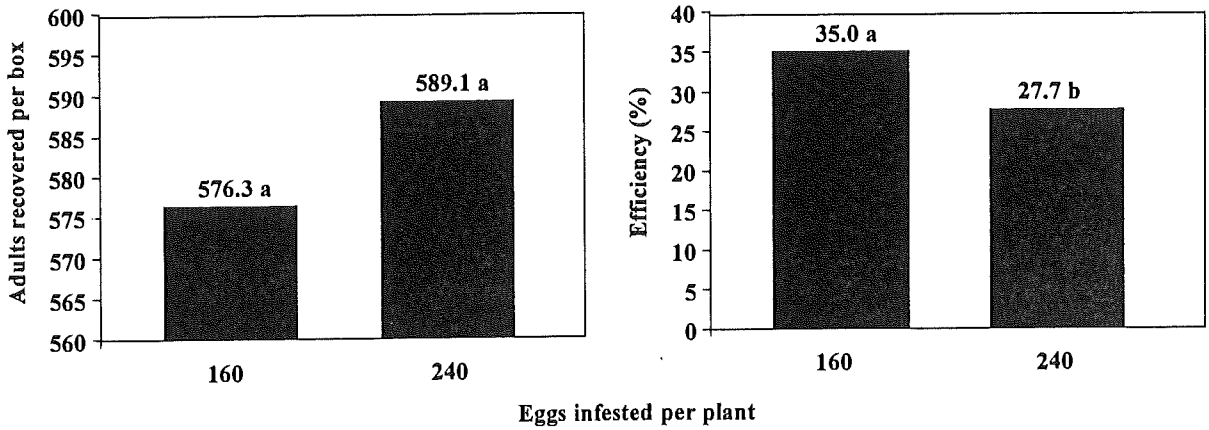


Figure 14. Efficiency of adult production from the colony at two egg infestation levels. Columns followed by different letters are statistically different ($P<0.05$).

Discussion: This alternative mass rearing colony is a more effective tool than the traditional CIAT colony for massive screening required in studies of host plant resistance and fungal entomopathogens. This methodology requires less space, soil and labor, yet maintains a similar efficiency. An additional advantage is the emergence cage that facilitates capture of new adults and decreases the risk of greenhouse escape. About 15 person-hours are required per week to maintain 2 boxes, but economy of scale would allow a doubling of adult production (to 2600) with 5 additional hours a week (largely dedicated to transplanting

material from the field). The variation in adult production from one cohort to the next is low, thereby ensuring a continuous and dependable supply of insects for evaluation. Increasing the efficiency of production (eggs to adults) depends on fine-tuning levels of egg infestation and adjusting fertilizer additions in consideration of host plant deterioration. Elevating egg infestation from 100 to 150 per plant did not increase overall adult production significantly but did decrease the efficiency of adult production. Adult emergence is sufficiently synchronous that in a single week period 80% emerge.

2.1.3 Biology and habits of Colombian spittlebugs (D. Peck, W. Medina, C. Gallego, Y. Ballesteros, A. Pérez)

Rationale: An inadequate understanding of the biology and behavior of most spittlebugs species, plus a tendency to overgeneralize in those same aspects among species, contribute to their ineffective management. For 7 of the 11 species found in graminaceous crops of Colombia we lack published information on all aspects of biology that are relevant to the refinement of management tactics. Only *Aeneolamia varia*, *Prosapia simulans* and *Zulia colombiana* have received attention.

Methods: Studies have been completed for *A. reducta* and *Mahanarva* sp., partially completed for *A. lepidior* and *A. varia*, and recently initiated for *Z. colombiana*, *Z. pubescens* and *Zulia* sp. The following results update the information presented in 1998. Comparative methodologies were established and employed to study morphological characterization and duration of the life stages, oviposition sites, and reproductive biology.

Morphological measurements were made with an ocular micrometer to characterize all nymphal and adult life stages including 5 instars and 2 adult sexes. The duration of nymphal life stages was quantified by following recently hatched nymphs confined to feeding sites in pots. Through a combination of direct daily observation of the nymphs and the presence of the molting exuviate we determined advancement to the next instar. Adult longevity was determined by daily assessing mortality of small groups of teneral adults confined over host plants under sleeve cages.

To determine oviposition sites, groups of females were confined under sleeve cages over plants with the presence of bare litter and soil substrates. After females died, eggs were recovered from 4 substrates: soil, litter, adhered to the plant surface or under leaf sheaths, and inserted into plant tissue. Reproductive biology was assessed by enclosing teneral adult females in large (15.0 diam, 2.5 cm tall) petri dishes lined at the bottom with moist filter paper for oviposition. Short *Brachiaria* host plant stems were maintained with their base in a vial with water and there was continual presence of one male. These arenas were observed continuously for 3 days and then daily until the female's death. We measured precopulation and preoviposition periods, copulation frequency and duration, daily oviposition rate and lifetime fecundity.

Results: These results update information reported in 1998. The spittlebug complex in Colombia represents a broad taxonomic, biological and habitat range and should therefore be illustrative of the variation in the association between spittlebugs and grasslands. For instance, morphological measures of *Mahanarva* sp., the largest species, were 1.2-1.9 times larger than *A. reducta*, the smallest species (Table 10). In addition, *A. reducta* occurs in the seasonally dry North Coast while *Mahanarva* sp. occurs in the continuously humid Amazonia.

Table 10. Morphological characterization of adults by sex (mm) (mean, range).

Species	Sex (n)	Body length	Body length with wings	Body width	Stylet length	Post. wing length
A. lepidior	Male	6.69	7.59	3.70	0.78	6.28
	(19)	5.43-8.14	7.00-8.07	3.43-4.00	0.70-0.85	5.86-6.64
	Female	7.18	7.91	4.05	0.79	6.52
	(20)	6.07-8.36	7.50-8.79	3.57-4.36	0.70-0.85	6.14-7.00
A. reducta	Male	5.54	6.62	3.18	0.67	5.36
	(40)	4.50-6.50	5.93-7.71	2.78-3.52	0.59-0.74	4.57-5.86
	Female	6.35	6.96	3.44	0.71	5.62
	(40)	4.29-7.79	6.21-7.57	3.11-3.85	0.63-0.78	5.07-6.07
Mahanarva sp.	Male	10.56		4.41	1.25	
	(20)	9.64-11.43		4.07-4.86	1.07-1.56	
	Female	11.43		4.96	1.49	
	(20)	10.71-12.43		4.57-5.36	1.16-2.67	

The instars of all species were distinguishable with high precision using morphological characters. The width of the head capsule was most diagnostic for distinguishing among nymphal and adult life stages within species (Table 11). This measure had very little overlap compared to other measures and so can be reliably used to determine instar in a single species population. In *A. reducta* and *Mahanarva* sp., stylet length, body length, and posterior wing pad length increased with instar. The level of schlerotization of the thorax, form and size of the wing pads were especially useful accompanying characters, but the number of antennal segments, tarsal segments and form of the eyes also varied among instars. These other characters are required to determine instar of nymphs from mixed species populations.

Table 11. Head capsule width for nymphal and adult life stages (mm).

	Instar (mean, range)						Adult sex	
Species (n)	1	2	3	4	5a	5b	Male	Female
A. lepidior							1.85	2.03
(19-20)							1.78 - 1.96	1.85 - 2.18
A. reducta	0.32 a	0.51 b	0.76 c	1.12 d	1.57 e	1.59 e	1.69 f	1.86 g
(40)	0.29-0.37	0.46-0.59	0.69-0.83	0.99-1.24	1.45-1.74	1.43-1.74	1.52-1.81	1.67-2.04
Mahanarva sp.	0.34 a	0.63 b	0.97 c	1.55 d	2.37 e	2.31 e	2.42 e	2.65 f
(12-20)	0.26-0.39	0.57-0.66	0.91-1.03	1.39-1.69	2.07-2.61	2.19-2.42	2.29-2.57	2.43-2.86

For species, means with different letters are significantly different ($P<0.5$).

Early (5a) and late (5b) instar 5 could be distinguished for all species despite showing no differences in morphological measures (Table 11). Instar 5b showed signs of the developing adult cuticle, particularly the black lateral spines and corona of spines on the metatibia (a family level adult character). Although evidence of the pigment of the adult wings could be seen on the wing pads, they are not always present or detectable especially in adults with indistinct color patterns. Mean time to nymph development among three species varied from 26– 44 days, increasing with species size (Table 12). There were no detectable trends between time to development and instar, i.e. the relative time in each instar. Duration of adults varied much less than nymphs, from 6.4–8.4 days for females and 6.2-7.4 for males in four species examined (Table 13). There were no detectable differences due to sex; in two species males outlived females while in the other two females outlived males.

Table 12. Duration of nymphs by instar (days).

	Instar (mean, range)					
Species (n)	1	2	3	4	5	1 - 5
A. lepidior	6.6	7.3	6.7	6.7	8.2	35.4
(60)	5-10	4-11	4-10	4-9	7-11	27-40
A. reducta	5.9	5.4	5.3	4.8	4.5	26.1
(43-72)	5-7	5-6	4-7	4-6	4-5	24-28
Mahanarva sp.	6.9	11.4	10.8	7.3	7.9	44.2
(21-30)	4-11	7-15	8-14	6-10	8-9	34-59

Table 13. Longevity of adults by sex (days).

Species (n)		Male	Female
A. lepidior	Mean	6.5	6.2
(17, 13)	Min-max	5-7	5-7
A. reducta	Mean	6.4	6.7
(33, 44)	Min-max	5-8	5-8
A. varia	Mean	6.9	7.4
(24, 24)	Min-max	2-17	2-17
Mahanarva sp.	Mean	8.4	6.7
(41, 19)	Min-max	3-11	3-11

The precopulation and preoviposition periods were very brief for both *A. reducta* and *Mahanarva* sp. (Tables 14 and 15). *Mahanarva* sp. copulated twice as much as *A. reducta* and stayed in copula much longer. Lifetime fecundity of *Mahanarva* was one third that of *A. reducta.*

Table 14. Copulatory behavior.

Species		Precopulation period (min)[1]	Number of copulations[2]	Duration of copula (min)		
				Copula 1	Copula 2	Copula 3
A. reducta	Mean	53	1.35	11.7	14.2	7.5
	Min-max	10-103	1-3	5-21	10-17	6-9
	N	20	20	20	4	2
Mahanarva sp.	Mean	39	2.85	400	103	61
	Min-max	30-47	2-3	120-570	36-198	12-138
	N	20	17	20	20	17

[1] Adults were 6-16 hours old at the start of observations.
[2] Number of copulations observed in first 72 hours after start of observations.

Table 15. Reproductive biology.

Species (n)		Preoviposition period (hours)[1]	Oviposition rate (eggs/ day)		Lifetime fecundity	Longevity (days)
			Days ovipositing	Days living		
A. reducta	Mean	16.4	12.4	7.5	42.2	4.2
(20)	Min-max	11.4-23.7	4.4-35.7	2.7-12.5	22-107	3-6
Mahanarva sp.	Mean	55.9	7.7	5.1	17.2	3.4
(20)	Min-max	43.4-68.2	4.0-10.0	2.7-8.0	8-24	3-4

[1] Adults were 6-16 hours old at the start of observations.

For each of the three species studied females preferred to lay eggs in the soil, yet eggs were also found to be adhered to litter and the plant surface (Table 16). In no case were eggs

found inserted into the plant tissue. *Mahanarva* sp. showed more flexibility than *A. reducta* and *A. varia* in laying in litter as well as soil.

Table 16. Oviposition site preferences (mean % eggs recovered).

Species	n	Soil	Litter	Plant surface	Plant tissue
A. reducta	10	90.4	8.2	1.4	0
A. varia	9	97.6	1.7	0.7	0
Mahanarva sp.	10	74.0	22.7	3.3	0

The life cycle of *A. varia, Mahanarva* sp. and *A. lepidior* based on these studies was 45.3, 64.6 and 52.6 days, respectively (Table 17).

Table 17. Life cycles summary for three spittlebug species.

Life stage		Duration (days)			
Egg	Sum	15.8	17.0	14.1	
	S1	5.7	5.9	5.7	
	S2	1.6	1.9	2.2	
	S3	4.1	4.5	2.6	
	S4	4.4	4.7	4.4	
Nymph	Sum	26.1	44.2	35.4	
	Instar 1	5.9	6.9	6.6	
	Instar 2	5.4	11.4	7.3	
	Instar 3	5.3	10.8	6.7	
	Instar 4	4.8	7.3	6.7	
	Instar 5	4.5	7.9	8.2	
Adult	Half longevity	3.4	3.4	3.1	
	Female	6.7	6.7	6.2	
Life cycle		45.3	64.6	52.6	

Discussion: We will continue to use this comparative methodology to obtain information on the biology of other species in Colombia such as *Z. pubescens* and *Zulia* sp. Studies on *A. varia* and *Z. colombiana* will be included so the results can be compared to information obtained by other researchers using different methodologies. Current results demonstrate this methodology to be effective at describing these fundamental aspects of biology and behavior. Future research will benefit from information such as the duration and determination of life stages that will promote higher resolution of population and life cycle studies. Such studies will further our understanding of the similarities and differences among the members of this pest group as well as broaden the known variation in the family cercopidae and its economically important subgroup.

2.1.4 Comparative egg morphology and development (D. Peck)

Rationale: Combat of spittlebug populations in seasonally dry regions will depend on identifying the first generation in space and time. An understanding of the biology of the egg stage is critical to link population data with climatic data for building predictive models of the phenology and synchrony of early season spittlebug populations. This information is also absent from our understanding of the basic biology and behavior of most species and will complement studies on the determinants of egg diapause. Comparative studies will broaden our understanding of the variation and generalities among this pest complex.

Methods: The objectives of this study were to measure the duration, size and external morphology of egg development stages, detect diapause, determine the stage that is

prolonged in diapause, and compare the variation in development within and between three genera of spittlebugs. Eggs were obtained from field-collected females enclosed in large petri dishes lined with filter paper that served as oviposition substrate. Duration of egg stages was determined by daily observing individual eggs under incubation (darkness, 27°C, 100% RH). At certain developmental stages the width and length was measured with an ocular micrometer to quantify morphological changes that accompanied development.

Results: Complete results have been obtained for *A. lepidior, A. reducta, Mahanarva* sp. and *Zulia colombiana* while identical studies have just been initiated for *Z. pubescens* and *Zulia* sp. The following results update the information presented in 1998.

Certain externally visible changes were common to all four species from three genera. All eggs passed through four generalized development stages S1, S2, S3 and S4 (Figure 15). S1 eggs were recently laid, pale yellow, spindle-shaped, and lacked evidence of the operculum (hatching lid). In S2 the operculum became evident below the anterior portion of the chorion, growing from an incipient gray streak to a dark gray oval. A pale red pigment spot could normally be seen under the operculum. In S3 the chorion split along the hatching line, exposing the dark black surface of the operculum, still with pale red pigment spot under the operculum near its midpoint. Finally, eggs in S4 revealed two pairs of red pigment spots associated with the eyes and abdominal color patches of the nymph. Immediately before hatch the lines of the stylet became visible along the side of the operculum. Variation in this process related to how visible the red pigment spots were and therefore how clearly the transitions from one stage to the next could be followed.

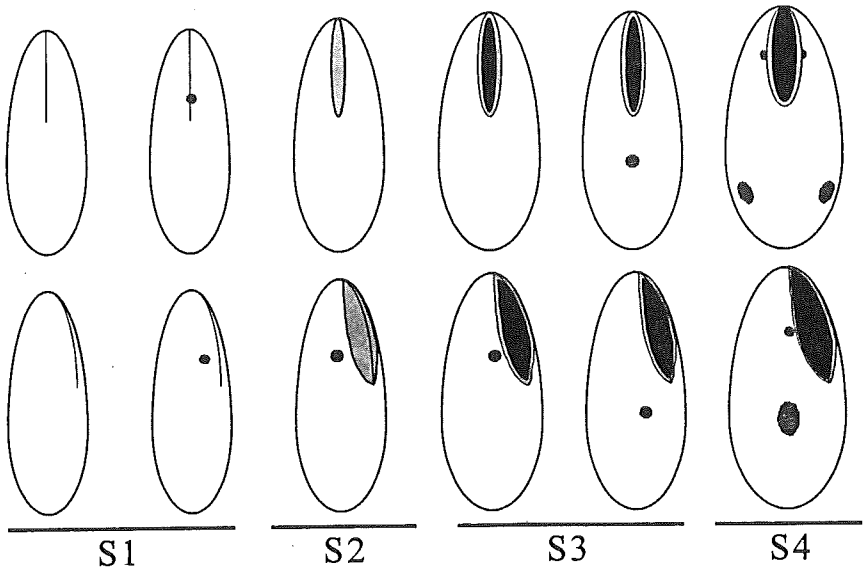


Figure 15. Generalized developmental phases of spittlebug eggs: S1 (recently laid), S2, S3, S4 (about to hatch). The ventral and lateral surfaces are shown.

Overall time of egg development differed among species and might be related to adult size (A.r.<A.l.=A.r.<Z.c.<M. sp. in size) (Table 18). Time to development in *A. lepidior* might have been underestimated due to difficulty in distinguishing nondiapause and diapause eggs. For nondiapause eggs in the five species, the mean proportion of time spent in each development stage was 33.6, 13.0, 23.0, 30.3% for S1-S4, respectively (Table 19). Although there were differences between species, all four species took longest to pass through S1, followed by S4, S3 and S2.

Table 18. Duration of egg development stages for nondiapause eggs (days).

	Development stage (mean, range)				
Species (n)	S1	S2	S3	S4	S1 – S4
A. lepidior	5.7	2.2	2.6	4.4	14.1
(336)	4-10	1-60 [1]	1-8	1-8	13-18
A. reducta	5.7	1.6	4.1	4.4	15.8
(86-92)	5-8	0-8	0-5	3-7.5	15-22
A. varia	3.9	3.7	3.6	5.8	17.2
(96-100)	3.5-6	1-8	2-5	1-8	12-22.5
Mahanarva sp.	5.9	1.9	4.5	4.7	17.0
(100-104)	5-7	1-5	2-6	3-9	15-23
Z. colombiana	6.0	1.2	3.8	5.1	16.1
(114-120)	5-10.5	0-4	1.5-6.5	3.5-7	15.5-20

[1]For *A. lepidior* there was a continuum between nondiapause and diapause eggs based on the time in the diapausing stage S2. For these duration estimates, nondiapause was defined as 4 or less days in S2.

Table 19. Mean proportion of time in each development stage (%).

Species	S1	S2	S3	S4	S1 – S4 (days)
A. lepidior	36.8 c	15.2 c	17.3 a	30.7 b	14.1 a
A. reducta	36.2 bc	9.7 b	26.3 d	27.8 a	15.8 b
A. varia	22.7 a	21.5 d	20.9 b	33.7 d	17.2 c
Mahanarva sp.	35.0 b	11.2 b	26.5 d	27.3 a	17.0 c
Z. colombiana	37.1 c	7.2 a	23.8 c	31.9 c	16.1 b

For each development stage, means followed by different letters are significantly different ($P<0.05$).

Diapause was detected in *A. lepidior*, *A. reducta*, *A. varia* and *Z. colombiana* but not in *Mahanarva* sp. In all 3 species diapause was an extended period in S2, prolonging development time. Maximum time to development of the few diapause eggs observed was 75, 206, 33 and 51 days for *A. lepidior*, *A. reducta*. *A. varia* and *Z. colombiana*, respectively. For *A. lepidior* there was a continuum between nondiapause and diapause eggs based on the time in the diapausing stage S2 , i.e. there was no abrupt discontinuity showing short term and long term eggs in S2. There was a trend of increasing egg size with species size with the exception of *A. varia* that had eggs relatively small for its size (Table 20). For all species eggs swelled in size with development, particularly after rupture of the chorion between S2 and S3 that allowed the egg to take on water from the environment.

Table 20. Size of egg development stages (mm).

	Width (mean, range)				Length (mean, range)			
Species (n)	S1	S2	S3	S4	S1	S2	S3	S4
A. lepidior	0.29 a	0.31 b	0.35 c	0.39 d	0.91 a	0.94 b	0.98 c	1.00 c
(23-120)	0.24-0.35	0.29-0.35	0.30-0.40	0.33-0.45	0.84-1.00	0.90-1.00	0.90-1.05	0.95-1.05
A. reducta	0.27 a	0.27 b	0.29 c	0.33 d	0.82 a	0.82 a	0.85 b	0.89 c
(50-150)	0.23-0.36	0.26-0.30	0.26-0.33	0.29-0.37	0.71-0.90	0.76-0.91	0.77-0.96	0.80-0.96
A. varia	0.25 a	0.27 b	0.30 c	0.31 d	0.82 a	0.82 a	0.85 b	0.87 b
(100)	0.21-0.29	0.24-0.31	0.27-0.34	0.29-0.34	0.76-0.89	0.73-0.91	0.76-0.96	0.79-0.97
Mahanarva sp.	0.34 a	0.34 a	0.38 b	0.41 c	1.13 a	1.14 ab	1.15 b	1.18 c
(80-100)	0.30-0.37	0.30-0.37	0.33-0.41	0.37-0.44	1.07-1.21	1.07-1.24	1.07-1.27	1.10-1.27
Z. colombiana	0.33 a	0.34 b	0.38 c	0.40 d	1.08 a	1.07 a	1.11 b	1.15 c
(78-100)	0.29-0.37	0.30-0.37	0.33-0.43	0.37-0.44	1.01-1.20	0.99-1.20	1.01-1.21	1.07-1.27

For each species, means followed by different letters are significantly different ($P<0.05$).

Discussion: These methods are adequate for the comparison of certain taxonomically variable characteristics of spittlebug egg development and morphology. These measures include size and physical description, externally visible changes that accompany development, the detection of diapause, and the duration of stages under controlled conditions. Along with parallel studies on oviposition behavior and reproductive biology, this new information will offer the detailed basic understanding currently lacking in most spittlebug species.

2.1.5 Biological aspects of substrate communication in adult spittlebugs (F. López and D. Peck)

Rationale: Substrate communication in one fundamental aspect of adult spittlebug behavior that has never been examined. The existence of sound producing organs and microphone recordings is evidence that bioacoustic behavior occurs in this insect superfamily. Among related insects, bioacoustics in known to play a role in reproductive behavior and species differentiation, both of which are poorly understood and critical aspects of our basic understanding of spittlebugs. The objectives of this study are to develop a methodology that permits recordings directly from the substrate, confirm that vibrational communication occurs in adults, describe the different call patterns, understand their role in reproductive biology and gauge their utility as a taxonomic tool for species differentiation.

Methods: A ceramic crystal phonograph cartridge was used to convert vibrations into electrical pulses before amplification and entering a computer with specialized software for processing and analyzing sound (Cool Edit 96). *Z. colombiana* was used as the study species due to its large size and relative docility. Recordings were also made of *A. varia* for comparison between species. The phonograph cartridge was placed in contact with the base of a *Brachiaria* stem with three leaves giving sufficient architecture for feeding and walking. Different combinations of adults sexes and numbers were placed on the plant for simultaneous direct observations and recording to characterize the call repertoire.

Results: Although the technical development of the recording methodology was difficult, once in place the technique was simple to manage and did not alter the natural behavior of the insects in any perceivable way. Calls from *Z. colombiana* and *A. varia* were easy to elicit under these circumstances where they were free to feed and walk about the host plant. Three classes of calls were detected in both male and female *Z. colombiana*: common calls, alarm, and courtship. A fourth call, rivalry or aggregation, was recorded in males. Two call types (common call and courtship) were detected in initial recordings of *A. varia.*

In male *Z. colombiana*, frequency of common calls was 540.0 Hz with pulses lasting 85 msec (n=3). Alarm calls were 423.6 Hz with pulse duration 30 msec (n= 1) while rivalry/ aggregation calls were 445.2 Hz and a pulse duration of 65 msec with pulses every 800 msec (n=2). Courtship calls were studied in more detail for comparison with *A. varia* (Figures 16 and 17). These calls were 317.4 Hz and lasted 9.5 sec with a pulse duration of 50 msec, and pulse repetition frequency of 10.5 pulses per sec (Table 21). Female courtship (invitation) calls were 378.7 Hz with pulse duration of 60 msec (n=2).

Male courtship calls in *A. varia* were significantly different than *Z. colombiana* (Figures 16 and 17). Frequency and pulse repetition rate were higher at 425.2 Hz and 42.2 pulses per sec, respectively, while song length was shorter at 3.4 sec (Table 21).

Table 21. Summary of physical characteristics of male courtship calls in *Z. colombiana* and *A. varia*.

Call parameter	*Z. colombiana* (n = 11) (mean ± S.E.)	*A. varia* (n = 5) (mean ± S.E.)
Frequency (Hz)	317.39 ± 23.25 a	425.16 ± 37.27 b
Pulse repetition rate (pulses/second)	10.50 ± 0.37 a	42.16 ± 0.56 b
Call length (seconds)	9.50 ± 0.59 a	3.43 ± 0.08 b
Pulse length (milliseconds)	50	30

For each parameter, means followed by different letters are significantly different between species ($P<0.05$).

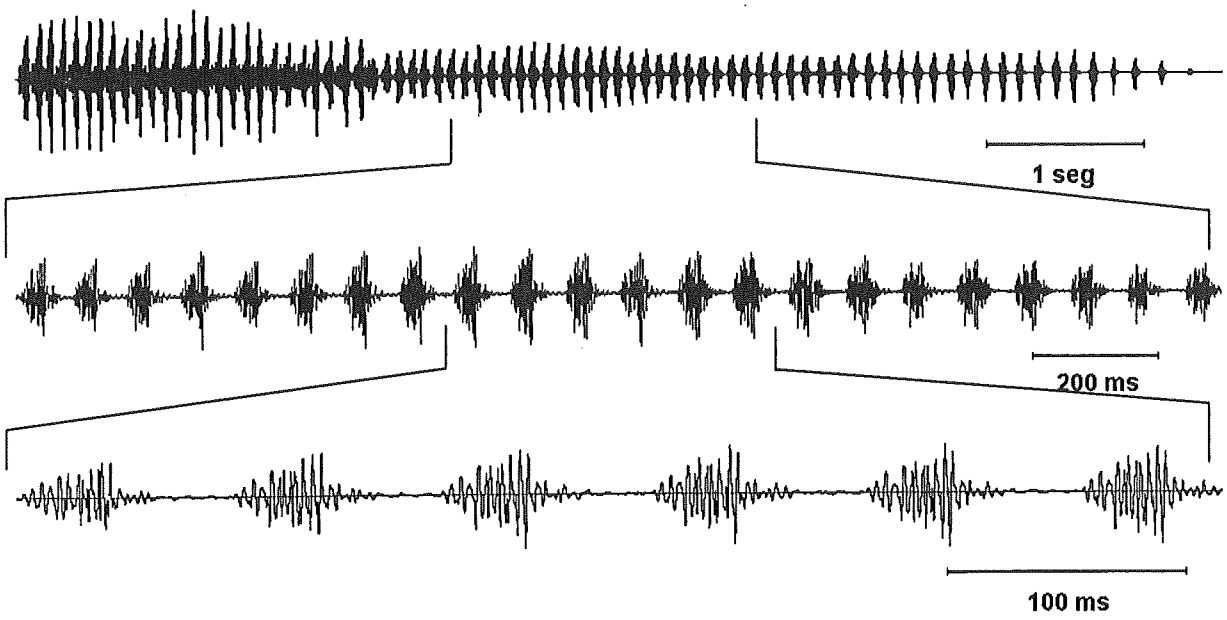


Figure 16. Oscilogram at three scales of the courtship call of male *Z. colombiana* inviting a response from receptive females.

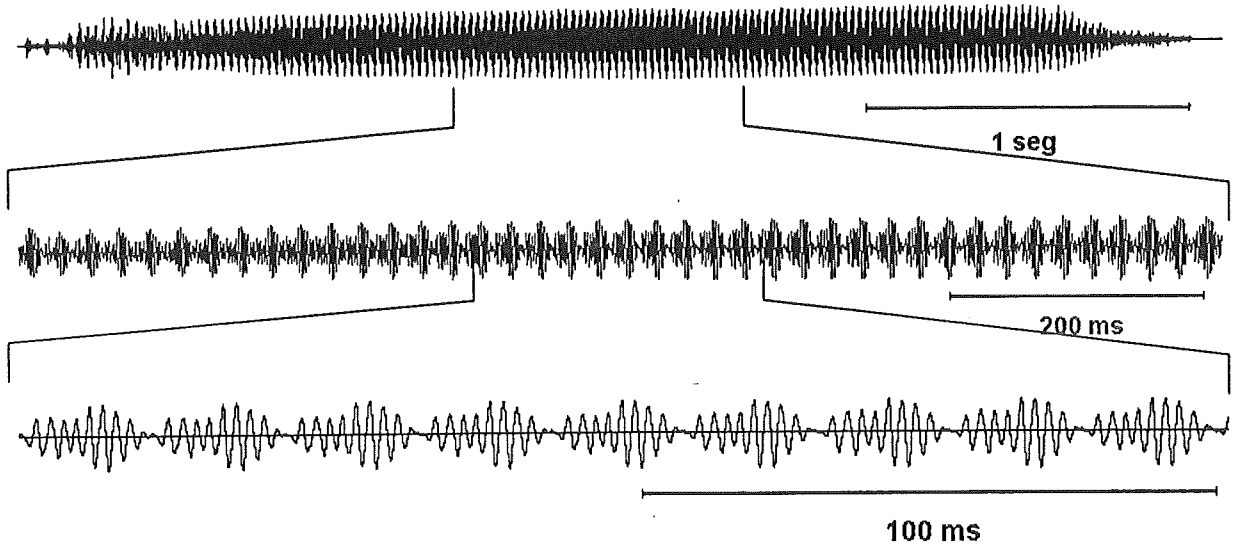


Figure 17. Oscilogram at three scales of the courtship call of male *A. varia* inviting a response from receptive females.

The reproductive behavior associated with courtship calls was similar between species. Once the male transmitted the courtship call the receptive female responded with her invitation call. The pair established a "dialogue" that served to orient the male toward the female who remained stationary at her feeding site. Once in contact the male moved to the female's side and both continued calling for a few seconds before initiating copulation. At that moment they stopped calling and remained silent during the time in copula.

Discussion. This methodology enabled us to confirm that grassland spittlebugs and the superfamily Cercopoidea use host plants as a media to transmit calls for communication. In small insects this form of communication has advantages over aerial transmission such as a larger range of perception and easier orientation between callers and receivers. Clearly substrate communication drives reproductive behavior. It is the male that seeks the female. Such information guides an interpretation of small scale spatial distribution in the field. The predominance of males in sweep net surveys, for instance, may be explained by local movement patterns of males moving in the upper canopy to emit calls and find mates.

Courtship calls should also play a role in recognition and localization of conspecifics thereby contributing to reproductive isolation of distinct species. Finally, differences in calls between the two species studied indicate the possibility that these calls can be used as tools to distinguish among closely related species and otherwise help overcome some of the taxonomic difficulties of this pest group.

2.1.6 Comparative population ecology of spittlebugs in four lowland regions (D. Peck, A. Pérez, W. Medina, Y. Ballesteros, C. Gallego, M. Barrios, J. Rojas, L. Rojas, J. Rubio, A. Hincapie, F. Gamboa)

Rationale: There are few detailed site-specific studies on spittlebug population dynamics. An accurate interpretation of what occurs in a particular site depends on frequent and long-term surveys that emphasize high resolution through determination of all life stages. Identical survey methodologies employed at contrasting sites allow the comparison of population performance measures such as abundance, phenology, population synchrony, voltinism and species composition. An understanding of the patterns and variation in these measures is critical for assessing and predicting pest status. Because of the diversity of habitats, Colombia serves as a model region for spittlebug population studies.

Methods: Two years (1997, 1998) of population surveys were completed in four lowland sites in departments of Colombia most affected by this pest (Table 22). As reported in 1997 and 1998, three 0.5 ha plots (P1, P2, P3) were established in different pastures on each of four farms representative of the North Coast (Córdoba and Sucre), the Eastern Llanos (Meta) and the Amazonia (Caquetá). Nymph surveys comprised counts in two 0.25 m^2 quadrats in each quarter plot while adult surveys comprised 50 sweeps of an insect net in each quarter plot.

Table 22. Description of population survey sites.

				Botanical composition of survey pastures (% green weight)				
				Forage grasses				
Department: Municipality	Farm	Elev (m)	Mean annual rainfall (mm)	*Brachiaria decumbens*	*Bothriochloa pertusa*	Others	Legumes	Weeds
Córdoba: Ciénago de Oro	Bella Luz/El Olivo	15	1421	0	87.1	0	0	12.9
Sucre: Corozal	Tarapacá	200	1006	0	82.1	3.1	3.0	11.7
Meta: Villavicencio	C.I. La Libertad	336	2829	89.9	0	5.2	1.5	3.4
Caquetá: Florencia	C.I. Macagual	280	3600	34.2	0	59.0	0.9	5.9

Surveys were performed approximately twice weekly during the spittlebug season but weekly or biweekly during the dry season. All nymphs were determined to instar and adults to sex and species. These results update partial analyses presented in 1998.

Results: During the course of this study 507 surveys were carried out across the four sites (Table 23). A total of 10,248 nymphs were collected and determined to instar/life stage (1, 2, 3, 4, 5a, 5b) while a total of 82,662 adults were collected and determined to sex and species.

Table 23. Material examined in population surveys.

	Total nymphs		Total adults		No. survey dates	
Site	1997	1998	1997	1998	1997	1998
Córdoba	313	734	291	2136	63	49
Sucre	194	2032	4261	53130	77	78
Meta	997	1855	5105	8240	30	71
Caquetá	1681	2442	3673	5826	56	83
Sum	10248		82662		507	

Five spittlebug species were recorded from survey sites while a sixth was encountered nearby one site but never detected in the survey pastures (Table 24). One species, *A. reducta*, dominated (100%) in the seasonally dry *Bothriochloa pertusa* pastures of the North Coast. *A. lepidior* was encountered at the survey site in 1996 but this species was largely limited to pastures of *Panicum maximum*.

The intermediate seasonal site of Meta, also exhibited one dominant species, *A. varia* (94.1%), but there was a strong presence of 2 other species, *A. reducta* (5.4%) and *Z. pubescens* (0.5%). Finally, in the continuously humid Amazonian site of Caquetá, 2 species dominated, *A. varia* (74.3%) and *Z. pubescens* (24.5%), with the presence of a third, *Mahanarva* sp. (1.2%).

Table 24. Adult species composition at survey sites (%).

	Aeneolamia			*Mahanarva*	*Zulia*	
	lepidior	*reducta*	*Varia*	sp.	*colombiana*	*pubescens*
Site	97/98	97/98	97/98	97/98	97/98	97 / 98
Córdoba	* / *	100/100				
Sucre	* / *	100/100				
Meta		1/8	98/92			1/<1
Caquetá			65/78	1/1	* / *	34/21

Spittlebug nymphs and adults occurred during the wet season, surviving the dry season as diapausing eggs. The greatest population fluctuations occurred in the two most seasonally dry sites, Córdoba and Sucre, based on the complete disappearance of the insect during the dry season months and the extreme population peaks during the wet season beginning with the return of the rains (Figure 18).

Populations fluctuations were less extreme in Meta, corresponding with the shorter and less severe dry season. The continuously humid site, Caquetá, demonstrated the presence of nymphs and adults throughout the entire year, corresponding to the lack of a distinct dry season.

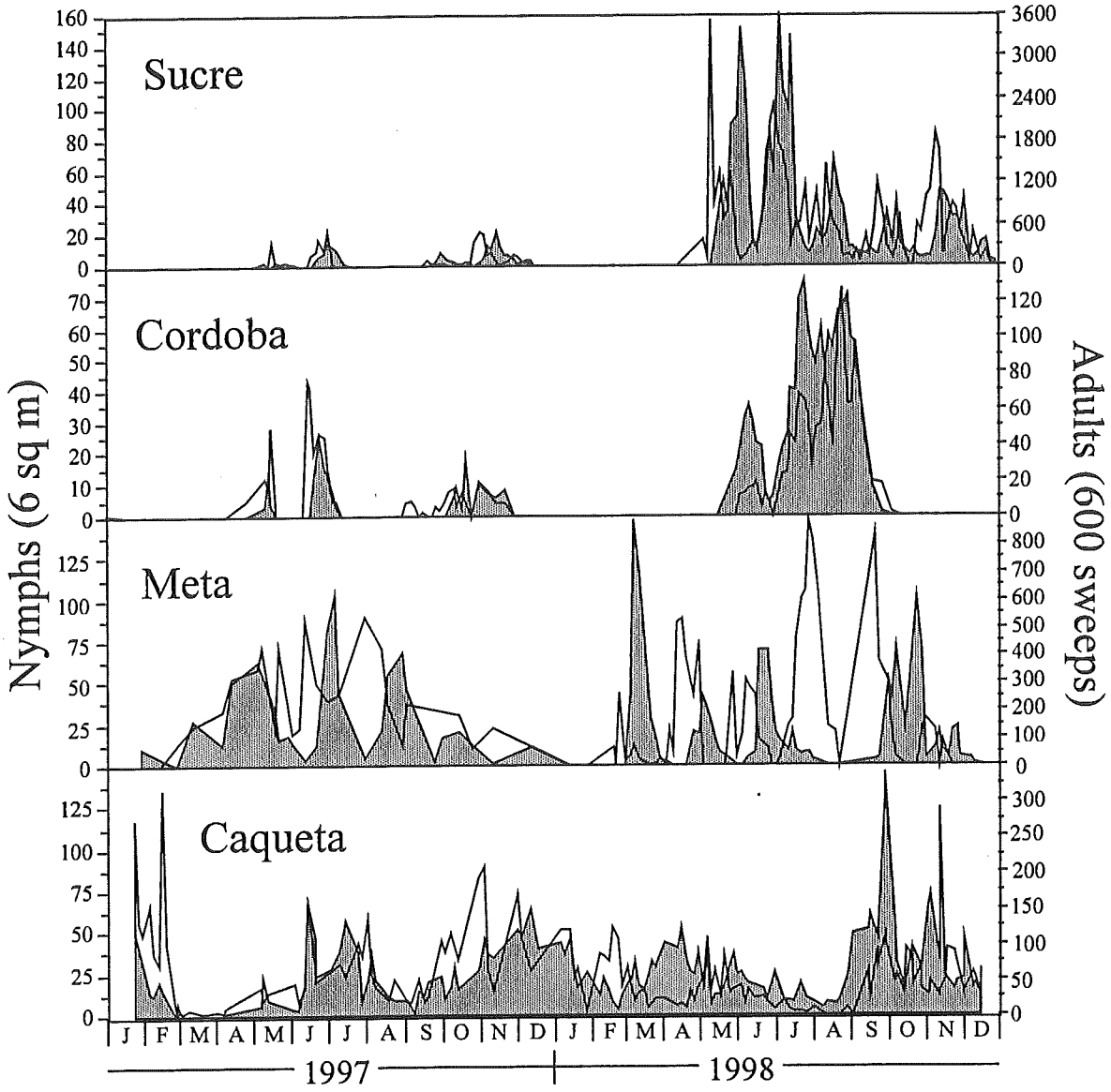


Figure 18. Population fluctuations of nymphs and adults during 2 years of study.

Sucre 1998 is used to illustrate how resolution of the population dynamics is greatly enhanced by considering separately all spittlebug life stages and all study pastures. Population curves could be assessed to gauge on-farm variation in abundance and phenology (Figure 19). P3 experienced 5-6 times more nymphs and 5.5 times more adults than P1 and P2. In terms of phenology, the first two population peaks of nymphs in P3 were not detected in P1 and P2.

The first adult peak in P1 and P2 occurred without a corresponding nymph generation, therefore those adults are considered immigrants into the study pastures. That the first adult peak in P1 and P2 corresponded to the first peak in P3 indicates that P3 could have been the source of these immigrants. These adults immigrants gave way to the subsequent nymph peaks in P1 and P2, corresponding with the second peak in P3. The differences among pastures is attributed to a fire that passed through P1 and P2 but not P3 and effectively killed all eggs in the soil.

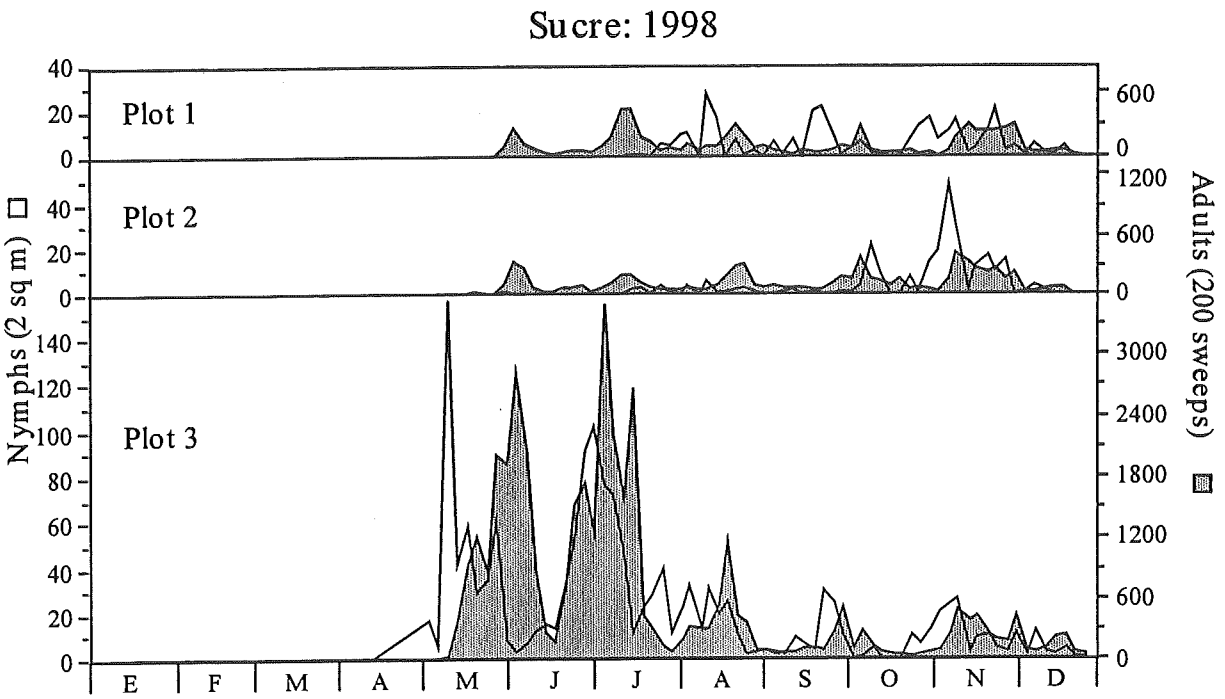


Figure 19. Population fluctuation curves of total nymphs and adults of *A. reducta* in three pastures of Sucre in 1998.

Due to the movement of adults out of areas such as P3, immigrants successfully reinvaded the burned areas of the farm and contributed to future generations still in synchrony with the rest of the farm.

An analysis based on nymphal life stages can confirm or resolve interpretations of the data based on total nymphs as well as describe the progression of the generations instead of population peaks. In addition, it is the progress of nymphal instars that describes population development while an analysis limited to total nymphs and total adults cannot detect movement events such as immigration.

In P3 of Sucre 1998, for instance, the clear recruitment of nymphs from one life stage up to teneral adults (still found in the spittle mass) was clear evidence for two large and synchronous initial generations (Figure 20).

These gave way to four other distinct generations but these were not as synchronized as the previous. In addition, the size of the generations decreased with the progression of the wet season. These generations matured approximately every 1.5 months: end of May, start of July, mid August, end of September, mid November and mid December.

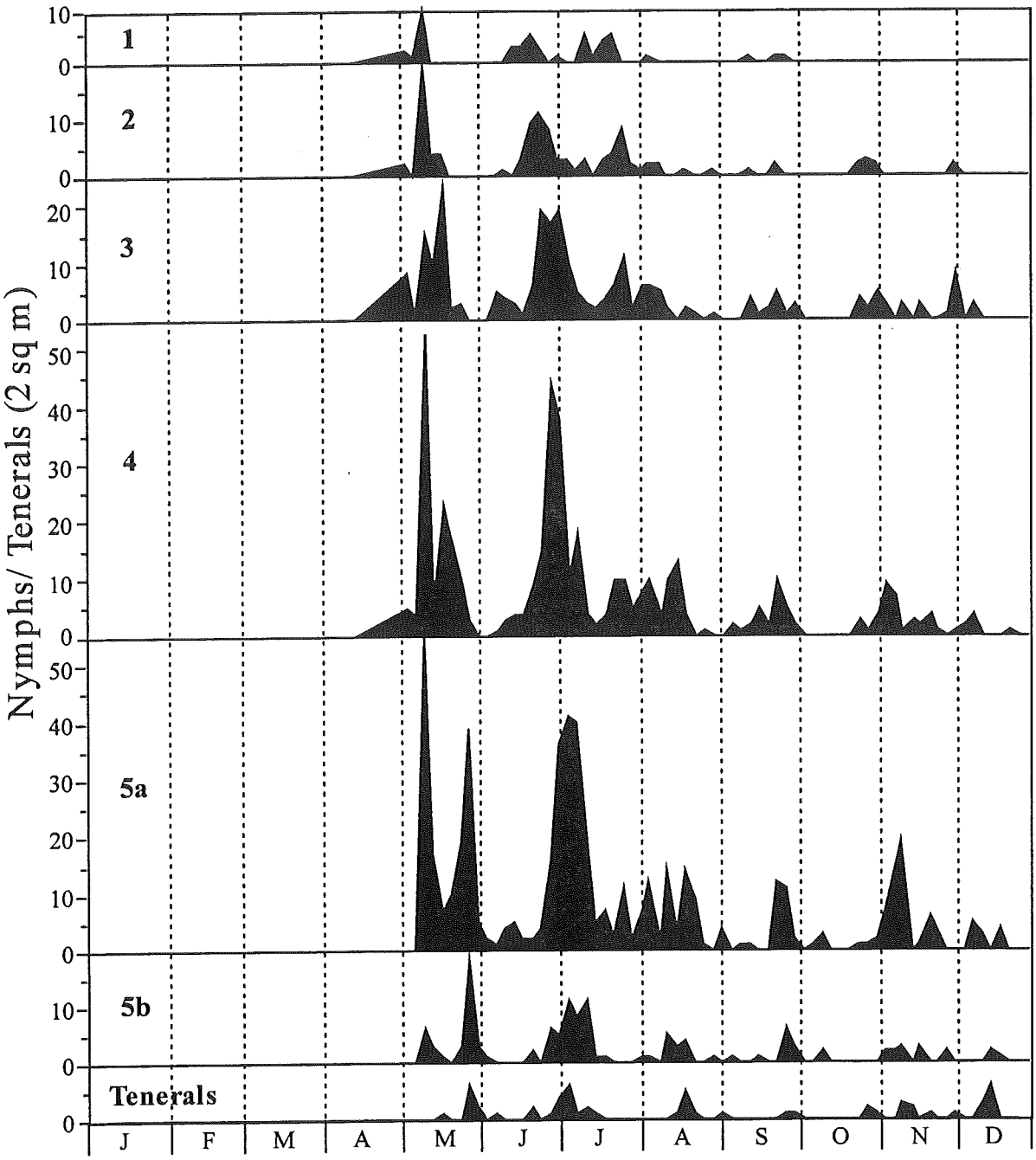


Figure 20. Population fluctuation curves of nymphal nymphal life stages and teneral adults of *A. reducta* in pasture P3 of Sucre in 1998.

This detailed analysis leads to phenograms that graphically depict population and generation development in the surveys sites (Figure 21). In Sucre 1998, for instance, the first generation of nymphs peaked 15 May, 27 days after the first heavy rains of the wet season. These nymphs gave way to an adult population peak 19 days later that colonized other areas of the farm where the first generation failed because of dry season fires. The second generation of nymphs and adults occurred 49 and 36 days after the first generation peaks of nymphs and adults, respectively. There was a strong third generation 35 and 44 days later, followed by a fourth generation in 51 and 42 days, fifth generation in 46 and 49 days and a sixth generation in 33 and 29 days. If the distinct population peaks in 1997 to 1998 in Sucre and Córdoba are considered, *A. reducta* completes its life cycle in the field in a mean of 43.1 days (n=16).

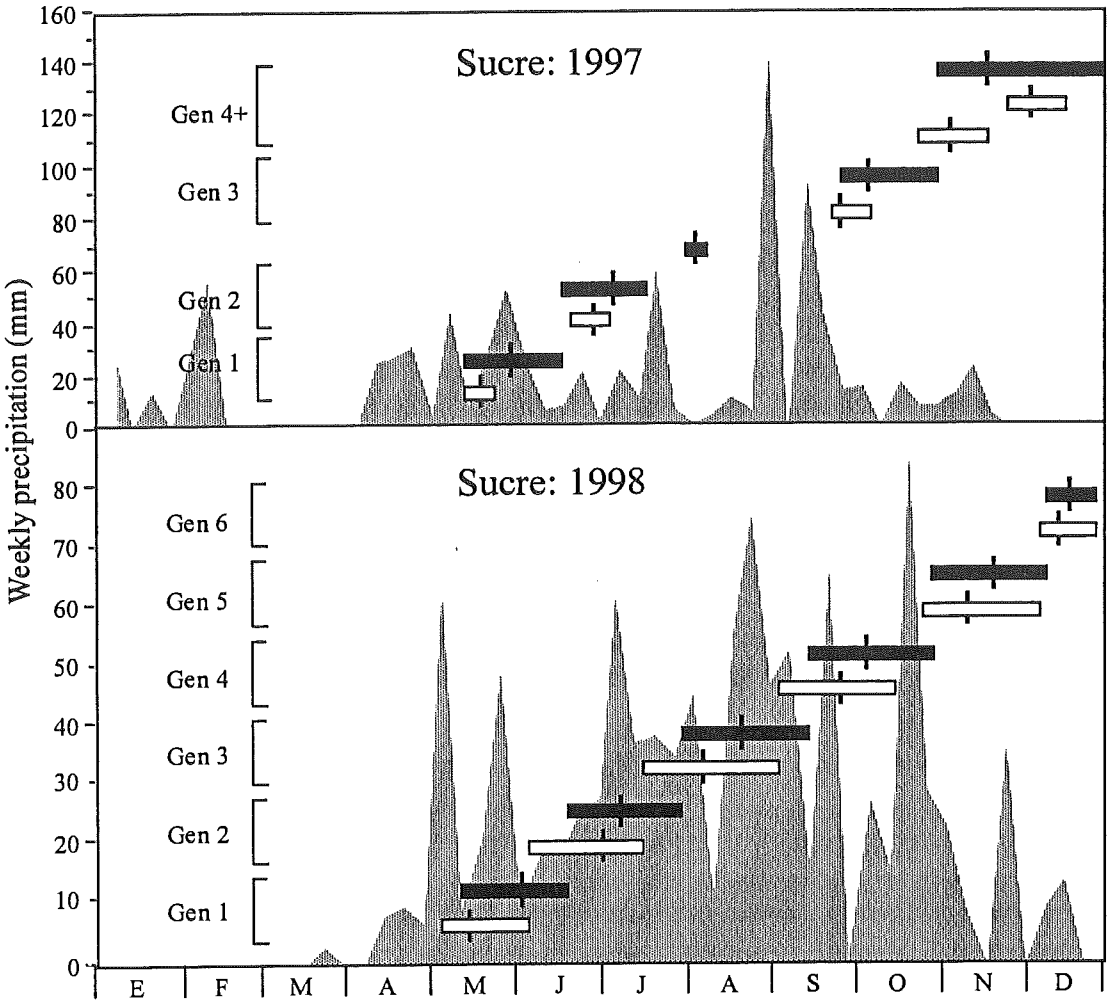


Figure 21. Weekly precipitation and phenograms of *A. reducta* populations on *Bothriochloa pertusa* in Sucre of the North Coast of Colombia over two years of study. Horizontal bars indicate the period of occurrence of the life stage while vertical lines indicate accumulation of 50% of the sampled individuals.

This corresponds very well with the results obtained from greenhouse studies that determined a life cycle of 45.3 days.

Discussion: Further analyses of these data will compare in detail the on-farm, regional and between year variation in abundance, phenology and population synchrony for the other regions in this study. These four sites represent a continuum of seasonality for lowland spittlebug populations. Analyses to date strongly suggest different strategies for management in the extremes of this range. In the highly seasonal sites, emphasis must be placed on the temporal and spatial identification of early season outbreaks. Scouting strategies must focus on the nymphs before the generation of mobile adults occurs. Control must be applied at these foci to suppress nymphal populations thereby decreasing colonization of other areas and reducing the size of subsequent generations. In continuously moist sites, the insect occurs all year round and presents little population synchrony. Therefore a control strategy should be based on cultural tactics to reduce habitat quality for reproduction and development. Habitat management such as grazing management, host plant selection and diversification should be investigated. In addition, because of the continual presence of the insect, continuously humid sites should be more amenable to application of biological control tactics such as use of fungal entomopathogens.

2.1.7 Phenology of spittlebugs in the Cauca Valley (U. Castro, D. Peck and A. Morales)

Rationale: Spittlebug pest problems have not yet been studied in the hillsides and the Interandean region of Colombia. This region has a bimodal seasonality and is therefore seasonally distinct from other lowland regions where population studies have been carried out (see Activity 2.1.6). We chose to implement a new field survey methodology modified from that used over two years in other regions. Our objective was to gather simultaneous information on population fluctuations of *Zulia colombiana* in improved *Brachiaria* pastures, egg diapause and development, incidence of natural enemies and climatic conditions.

Methodology: Surveys began 20 January 1999 and results were analyzed through June. Weekly surveys were carried out at a representative farm in the Cauca Valley featuring *B. dictyoneura* in association with the legume *Centrosema* sp. At this site at 1060 m only *Z. colombiana* occured. Three 0.5 ha areas in three separate pastures were marked and divided into 4 subplots each to facilitate sampling. In addition to nymph and adult surveys (see 2.1.6) parallel egg collections were made to document seasonal changes in the incidence and duration of diapause. When available, 5-10 adult females were collected once a week from around the area of each half paddock and allowed to oviposit over three days in large petri dished lined at the bottom with moist filter paper that served as oviposition substrate. Eggs were incubated (darkness, 27 °C, 100% RH) and evaluated every 5 days for presence of empty chorions (eclosed nymphs). Diapause eggs were considered those that hatched after 30 days.

Results: Over the period of study, fluctuation curves demonstrated 2 nymphal and 3 adult population peaks for each plot (Figures 22 and 23). The second and third adult peaks roughly followed from the first and second nymph peaks with the first adult peak representing the end of another population in January. Total abundance of nymphs and adults varied 8.3 and 3.3 times, respectively, between the plots of lowest and highest abundance (P1 and P2).

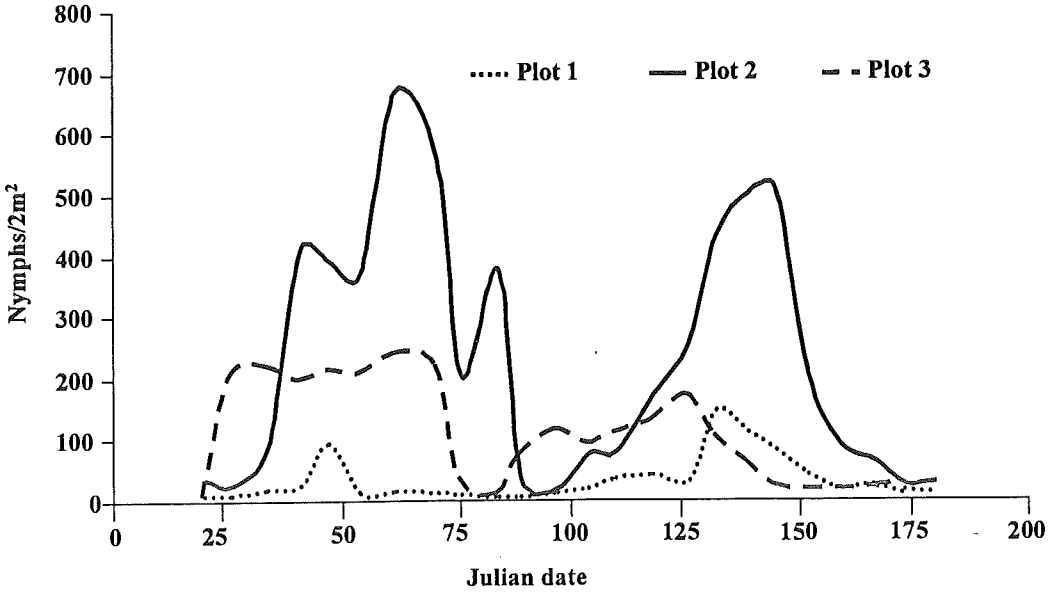


Figure 22. Total nymph population fluctuation curves for *Zulia colombiana* in pastures of *Brachiaria dictyoneura* in the Cauca Valley.

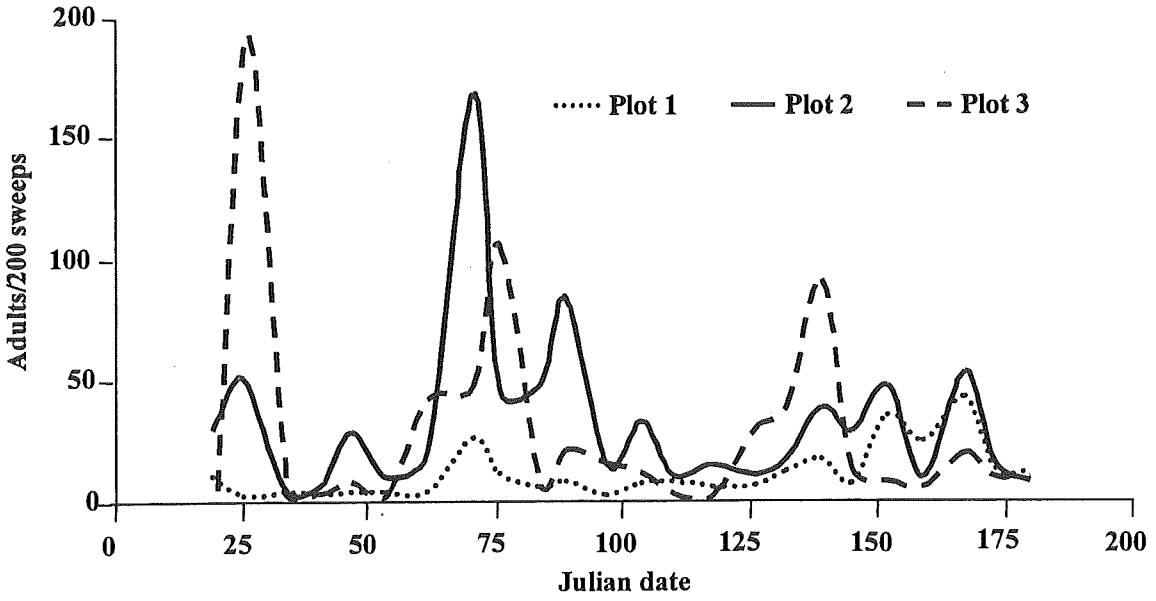


Figure 23. Total adult population fluctuation curves for *Zulia colombiana* in pastures of *Brachiaria dictyoneura* in the Cauca Valley.

Analysis of life stages permitted interpretation of fluctuation curves in terms of population development and generations (Figure 24) yielding phenograms based on cumulative insect-days (Figure 25). Two complete synchronous generations occurred on this farm over the sampling period, from January to April and April to June. This period was coincident with the rainy season.

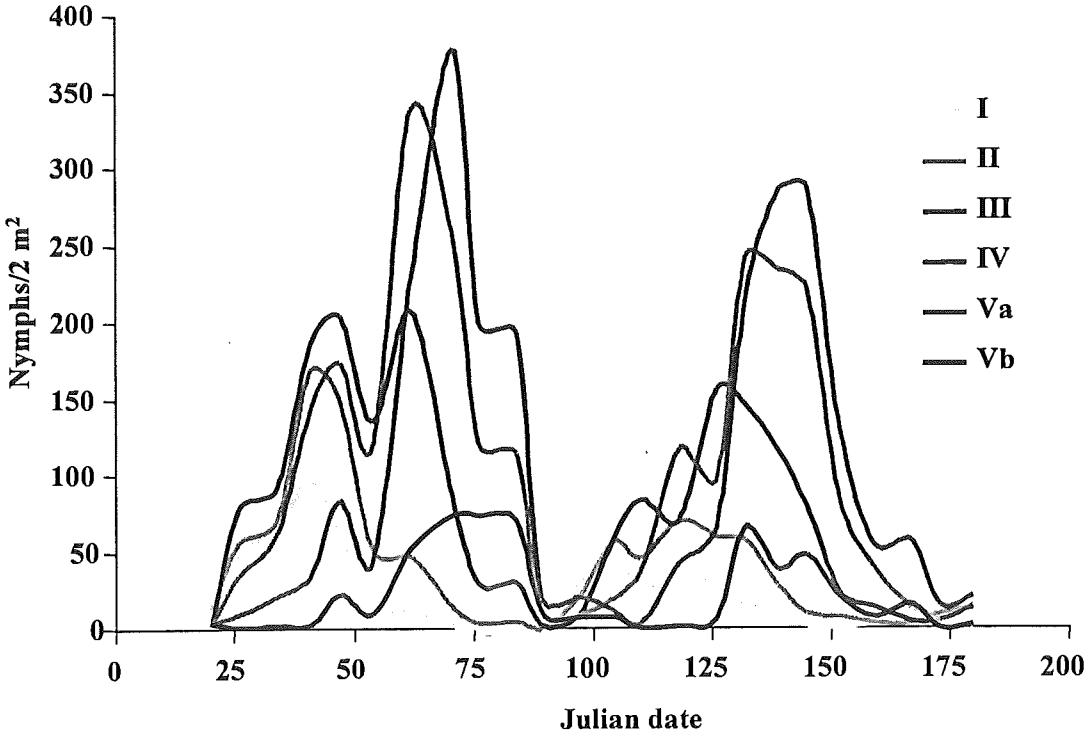


Figure 24. Fluctuation curves for all nymphal life stages of *Z. colombiana* in one survey paddock, P2.

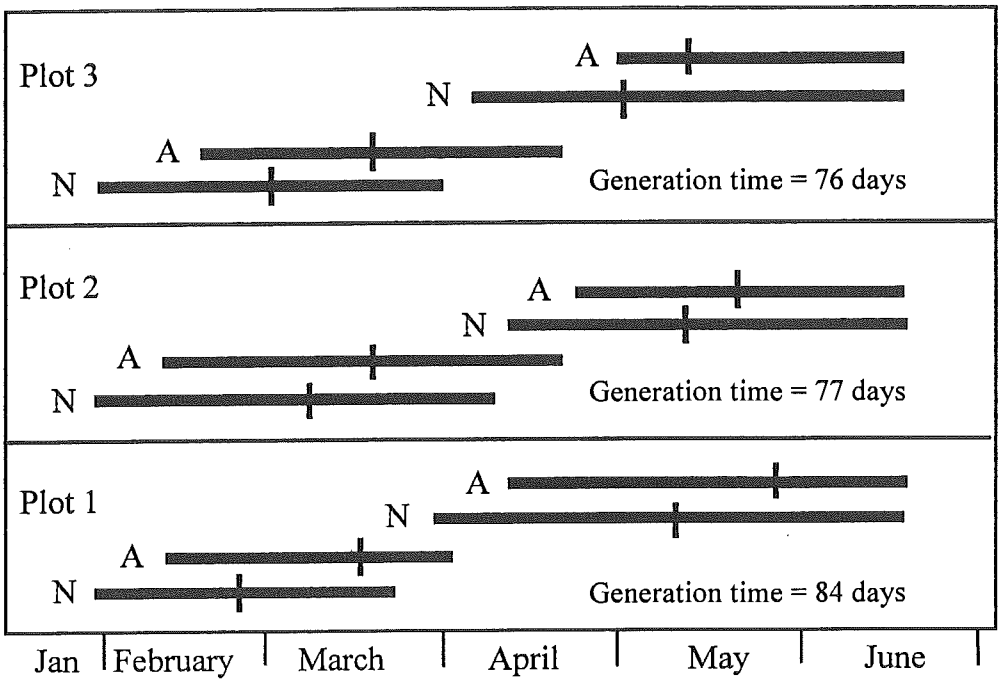


Figure 25. Phenograms of *Z. colombiana* populations on *Brachiaria dictyoneura* in 1999. Horizontal bars indicate the period of occurrence of the life stage while vertical lines indicate accumulation of 50% of the sampled individuals.

The generation time was calculated as the difference in nymph and adult peaks (50% accumulated insect-days). Mean generation time was 79 days (n=3 plots), 84, 77, 76 days for P1, P2 and P3, respectively. Only 0.6% of the eggs (n=7886) analyzed during these two generations were considered diapausing. Mean time to hatch was 18.0 days. Since spittlebugs endure the adverse conditions of the dry season only as diapausing eggs, it is expected that continuing egg studies will show an increase in the incidence of diapause among eggs laid by females at the end of the wet season.

The incidence of natural enemies was high at this site. Results are reported in section 2.2.3.

Discussion: Rains were present throughout the entire survey period offering adequate conditions for population development. It is expected that abundance will decline with the return of the dry season, heralded by rapid increase in the incidence of diapause in eggs. The methodology employed here will be effective for documenting and explaining spittlebug phenology. It is designed as a long-term study to link timing of population outbreaks with egg biology and rainfall conditions of the early rainy season. For comparisons with other study sites and for rate of information gathering, these studies should be particularly rewarding in this region where the bimodal precipitation offers two spittlebug seasons a year.

2.1.8 Impact of spittlebugs on grass/legume associations (J. Correa and D. Peck)

Rationale: Despite the benefits of grass/legume forage associations, adoption of this cultural practice will partially depend on the response of the major insect herbivore, grassland spittlebugs. Habitat changes that might accompany diversification of the pasture, such as higher densities of natural enemies and dilution of spittlebug host plants, suggest a decreased abundance of spittlebugs in response to grass/legume associations. Others, such as enhanced

nitrogen availability, however, might make associated grasses more attractive yet at the same time more tolerant to feeding damage. In 1998 we reported that spittlebug abundance did not differ between pure and mixed pastures, yet insect load (number of insects per unit grass) increased 23% in mixed pastures due to a reduced grass component. We now report results of a laboratory study to detect differences in spittlebug impact on grass grown with and without legumes.

Methods: An initial experiment was performed to measure differences in adult spittlebug impact on *Brachiaria decumbens* grown with and without the company of *Arachis pintoi.* A 2 x 2 factorial was designed with 10 replications of each treatment combination of *A. pintoi* (with, without) and fertilization (with, without). *A. pintoi* (CIAT 17434) was established in tubs with 5kg soil after ~15 cm long stolons were allowed to establish roots in water over 2 weeks. At the time of planting with *A. pintoi*, fertilized treatments received P, Ca and N in the dosis of 20, 50 and 50 kg/ha. *A. pintoi* was allowed 4 weeks to establish before the introduction of *B. decumbens*. New stems of *B. decumbens* (CIAT 0606) were transplanted from the field into small jiffy pots (No. 3) whose walls were rapidly penetrated by the growing roots. After one week of establishment, the pots were transplanted into the experimental tubs. Four additional weeks were allowed for the grass to grow and the roots of grass and legume to spread through the soil.

One *B. decumbens* plant tub was assigned for infestation, covered in a an acetate cylinder (8cm diam, 40cm tall) to house 10 adult 1-d old *Aeneolamia varia.* Adult mortality and plant impact were measured after 6 days of infestation. Visual damage was scored by recording the proportion of *B. decumbens* leaves with characteristic signs of chlorisis caused by adults. Grass was cut at the soil surface to determine dry weight. Replications for each treatment were combined to determine in vitro digestibility of dry matter, % neutral detergent fiber and % protein.

Results: Treatments had no effect on plant growth or insect mortality. Analysis of variance detected no differences in mean dry weight or percent mortality among treatments or their cross products. Overall mean plant dry weight was 2.78 g per plant and overall mean adult mortality was 38.8%. Visual damage of infested plants was also unaffected by treatments; analysis of variance detected no differences in mean percent damage among treatments or their cross product. The preplanned contrast between fertilized/associated and not fertilized/not associated also failed to detect differences.

In terms of plant quality, mean values for all treatment combinations were 10.43% protein, 64.57% in vitro digestibility and 72.10% neutral detergent fiber. For infested and non-infested plants, no differences were detected in the least squares means of these three quality measures. Fertilization treatments differed in % protein ($F = 42.22$; $df = 1, 4$; $P = 0.003$). Mean % protein for fertilized and unfertilized treatments were 11.78 and 9.08%, respectively. Association treatments also differed only in % protein ($F = 93.71$; $df = 1, 4$; $P = 0.001$), 8.42 and 12.44% for associated and unassociated treatments, respectively.

Discussion: This initial experiment failed to effectively test how *Brachiaria* might benefit from the company of *Arachis* in terms of tolerance to spittlebug damage. Linking results from experimental greenhouse tubs to the field are complicated especially when the opposing processes of competition for resources and exchange of nitrogen are considered. Field experiments would probably be the most easy to interpret and therefore most rewarding in completing this assessment of the response of grassland spittlebugs to, and their effect on, grass/legume associations.

Progress towards achieving output milestone

- Defined variation in the biology and abundance of spittlebug species in Colombia.

New methodologies for biological studies, population ecology studies, analysis of substrate communication and small and large scale rearing colonies have been established, evaluated and implemented. These tools must now be transferred to partners throughout the neotropics to support and foment quality research of high impact in other regions where spittlebugs are pests. Contrasting sites in Colombia have been successfully developed as model systems for linking bioecological studies with improvements in pest management as well as to broaden our understanding of the bioecological variation exhibited by this particular group of pests and by the family Cercopidae in general.

Activity 2.2 Diagnosis of spittlebug for elaborating IPM components

Highlights

- Confirmed 11 species of spittlebugs from 5 genera for graminaceous crops in Colombia; 3 of these are new reports. Four of these species are shared by Ecuador where 6 species were confirmed.
- Found that nymph mortality of *A. varia* was influenced by plant age and water stress, but no effect was found on egg diapause.
- Established new ceparium to isolate and house fungal entomopathogens from which 145 monosporic strains have been obtained, originating from both spittlebug life stages (nymphs, adults), 6 species and 6 departments of Colombia.
- Developed new methodology to screen for virulence of fungal entomopathogens against spittlebug adults.
- Found up to 77% efficiency in the control of *A. varia* adults by selected fungal entomopathogens.
- Characterized growth patterns of fungal entomopathogen colonies as a tool for screening virulence.

The tools required to advance the integrated pest management of spittlebugs are rudimentary of nonexistent. Results from CIAT's research program on the comparative bioecology of Colombia's spittlebugs offer the most detailed information on the pest complex for any country to date. This investigation serves as a template for other regions or countries confronting their own problems with this pest, also to stimulate research by national and regional institutions. Linking these recent results to advances in spittlebug IPM will depend on the transfer and diffusion of new information, technologies and diagnostic tools. Efforts in spittlebug management have been compromised by an inadequate consideration of the bioecological variation in the group, difficult access to the literature, inappropriate research methodologies, and lack of a model system for tailoring IPM to the contrasting regions and livestock systems where spittlebug occur. In 1999 we continued studies on egg diapause to advance a mechanistic understanding of early season population synchrony in seasonal sites and to support monitoring schemes. Information was gathered to develop guides for the identification and distribution of spittlebugs in Colombia and Ecuador. Finally, extensive studies were made on fungal entomopathogens, the most promising natural enemy spittlebug biological control. We established a ceparium to house diversity collected from spittlebugs throughout Colombia, developed an evaluation methodology to screen this diversity for virulence, and initiated studies on biological aspects of the isolates as characters to assist in gauging virulence and formulation qualities.

2.2.1 Identity and distribution of pasture and sugar cane spittlebugs in Colombia and Ecuador (D. Peck)

Rationale: Diagnosis for launching effective IPM programs depends on information on the distribution and identity of species in the pest complex. Correct species determinations and identification keys are of high priority for advancing in management and fomenting new research.

Methods: Distribution information will be collected from museum specimens housed in national institutions such as universities and CIAT. These data will be augmented with collection and site data being obtained in the course of other studies. Species determinations will be made through collaboration with outside specialists. Thus far data have been summarized from partial analysis of CIAT's collection plus new observations made over the last three years and from a recent visit to the British Museum of Natural History (London, UK) where many of the type specimens are housed.

Results: We have been able to confirm 14 spittlebug species from 6 genera associated with graminaceous crops of Colombia. Very superficial assessments in Ecuador have reported 6 species from 4 genera (Table 25). A major change in the nomenclature of one Colombian species was discovered. Comparisons with type specimens determined that *Z. colombiana* is a junior synonym of *Z. pubescens* and should therefore be retired from usage. What CIAT has referred to as *Z. colombiana* in this annual report and over the last two decades is in fact *Z. carbonaria*. Two species from Colombia were determined to be undescribed (*Mahanarva* sp. and *Zulia* sp.). We have also obtained novel diversity and distribution data in the last three years (Table 26).

Table 25. Known diversity of and distribution of Ecuador's grassland spittlebugs.

Species	Geographic region
Isozulia astralis	Amazonia
Mahanarva andigena	Coast
Mahanarva sp. nov. [1]	Amazonia, Coast
Sphenorhina sp.	Amazonia
Zulia pubescens	Amazonia
Zulia sp. nov. [1]	Coast

[1] Corresponds to the same undescribed *Mahanarva* and *Zulia* species from Colombia.

Table 26. Diversity and distribution of Colombia's grassland spittlebugs.

Species	Geographic region
Aeneolamia lepidior	North Coast, Cauca Valley, Magdalena Valley
Aeneolamia reducta	North Coast, Magdalena Valley, Llanos
Aeneolamia varia	Llanos, Amazonia
Mahanarva andigena	Pacific Coast
Mahanarva phantastica	Western and Central Cordilleras
Mahanarva sp. nov.	Amazonia
Notozulia entreriana	Llanos
Prosapia simulans	Cauca Valley
Sphenorhina rubra	Llanos
Sphenorhina sp.	Central and Eastern Cordilleras
Zulia birubromaculata	Pacific Coast
Zulia carbonaria	Cauca Valley, Amazonia, Cordilleras
Zulia pubescens	Cauca Valley, Amazonia, Cordilleras
Zulia sp. nov.	Pacific Coast

These include *Z. carbonaria* in Amazonia, *M. andigena, N. entreriana* and *Zulia* sp. nov. in Colombia, and *Prosapia simulans* in South America where no published accounts have yet been made. In 1999 *P. simulans* was detected for probably the first time in Colombia and is likely the first report of this genus in South America. This species occurs from Costa Rica to Mexico and the possibility that it is a new species invasion of Colombia merits serious attention given its status as the second most important spittlebug pest in sugar cane of Costa Rica and Nicaragua. Distribution range varies from species to species and the coarse data already gathered suggests that different geographic zones support a distinct spittlebug complex.

Discussion: Despite the comparatively high level of spittlebug research in Colombia and the relatively well-studied fauna, we have obtained reports of new species and new ranges. Evidence suggests that invasions to new areas are a risk. Ongoing work will enable us to assemble a range map for Colombia's spittlebug species as a start to interpreting distribution patterns. Keys will be established to enable researchers and technicians to reliably determine spittlebug species and thereby more actively and productively participate in studies on the basic biology, behavior and management of the species complex in particular zones. This diagnostic phase will serve as a template for studying the fauna in other countries and geographic regions where spittlebugs are major forage grass and sugar cane pests.

2.2.2 Studies on the determinants of diapause in spittlebug eggs (U. Castro and D. Peck)

Rationale: Egg diapause allows synchrony between spittlebug populations and the wet season during with growth and development are possible. In some species it has been shown that the incidence of diapause (i.e. the proportion of eggs that are not immediately developing) is lowest among early season females (first generations) and highest among late season females (last generation). An explanation for seasonal changes in the incidence of diapause is hindered by little information on preovipositional environmental cues that serve as token stimuli to the insect. Photoperiod and plant quality, for example, are thought to play a role in diapause regulation of spittlebugs but experimental data are lacking. Our goal is to describe the role of certain preovipositional cues in regulating diapause in eggs of *A. varia.* Plant age and drought stress are both factors perceived by nymphs and coincident with the advent of the dry season; there should be a selective advantage to adult females that lay diapause eggs at that time of the year.

Methods: Experiments in 1998 did not detect an effect of host plant water stress on diapause. Therefore we moved on to assess plant age and the combination of plant age with water stress in two experiments. In the first, a completely randomized block design (7 repetitions) assessed plant age. *Brachiaria ruziziensis* plants were allowed 4, 6 and 8 weeks to establish in 17.5 cm pots and produce surface roots adequate for infestation with 100 eggs of *A. varia.* In the second experiment a 2 x 2 factorial design (6 repetitions) tested water stress (drought, control) with plant age (4 and 8 weeks). Plants in the drought treatment received only 32 ml water every other day . Adults that survived each treatment repetition were transferred to separate oviposition chambers to recover eggs and follow their time to eclosion under incubation (darkness, 27°C, 100% RH). The effect of experimental conditions on diapause were measured as percent of eggs in diapause and time to eclosion. Nymph mortality, plant dry weight and quality were measured to confirm an effect of treatments on the host plant.

Results: There was no effect of plant age on the plant quality attributes % protein or % neutral detergent fiber, but % in vitro digestibility decreased significantly with age (4 weeks 65.4, 6 weeks 63.5, 8 weeks 62.3) (Table 27). Plant age had no effect on incidence of diapause or on days to eclosion for nondiapausing or diapausing eggs. Partial results (4 of 6

repetitions) of the combination of plant age with drought stress indicated an effect of both factors on nymph mortality (ANOVA, P<0.03). Of 2297 eggs followed, only 3 eggs were diapausing (34, 37, 37 days to eclosion) so there were no differences detected in the incidence of diapause (Table 28). No differences were detected in days to eclosion of nondiapause eggs; means for the four treatment combinations only varied from 19.7-20.1.

Table 27. Effect of plant age on nymph survivorship and egg diapause.

Treatment (weeks old)	Nymph survivorship (%)	Nondiapause eggs (%)	Days to eclosion nondiapause	Days to eclosion diapause
4	7.0 a	96.0 a	20.6 a	69.1 a
6	6.5 a	97.6 a	19.7 a	82.3 a
8	3.5 b	97.3 a	19.5 a	94.0 a

For each columns, means followed by different letter are significantly different (P<0.05)

Table 28. Effect of plant age and drought stress on nymph survivorship and egg diapause (partial analysis after 4 of 6 planned repetitions).

Treatment		Nymph survivorship (%)	Days to eclosion nondiapause
Water stress	Plant Age (weeks)		
Drought	4	7.0 a	19.6 a
Drought	8	3.3 ab	19.7 a
Humid	4	10.4 bc	20.1 a
Humid	8	5.8 ab	20.6 a

For each column, means followed by different letters are significantly different (P<0.05)

Discussion: No effect of water stress, plant age or their combination was detected on diapause. Although there were effects of treatments on nymph mortality, treatments may not have induced enough of a plant response for the signal to be perceived by the developing nymphs. We still lack a mechanistic explanation of initial outbreaks and phenology of spittlebugs in Colombia's seasonal environments. Photoperiod and temperature are other obvious seasonal factors that are widely used as token stimuli by diapausing insects. It is time to consider the importance of these variables in regulating the diapause of spittlebugs, but in Colombia seasonal changes in both factors are relatively minor compared to regions at higher latitudes.

2.2.3 Identity and incidence of fungal entomopathogens and other natural enemies of spittlebugs in contrasting sites of Colombia (D. Peck, A. Morales, A. Bolaños, U. Castro, F. López)

Rationale: Natural enemies of spittlebugs in Colombia have not been assessed on a broad level. Detailed population surveys in five regions of the country, however, have permitted the simultaneous collection of data on the seasonal incidence of predators, parasitoids and pathogens. In addition to new information on abundance, seasonality, species and life stages attacked, we hope to encounter new natural enemies as a first step to gauge their potential as biological control agents.

Methods: Natural enemies were collected as part of spittle mass and sweep net surveys in population studies at five lowland sites, and also during the course of different activities or visits to other regions. Entomopathogens were isolated from cadavers of nymphs and adults found in the field or greenhouse. Insect tissue was sterilized, fungi were grown on agar medium under incubation, monosporic isolates were prepared once reproductive growth was obtained, and isolates were stored dry at –20°C for future evaluation. The following results update those reported in 1998.

Results: Five classes of natural enemies have been encountered in Colombia: predaceous flies, parasitic flies, parasitic nematodes, parasitic mites and fungal entomopathogens (Table 29).

Salpinogaster nigra (Diptera: Syrphidae) is the most well-known enemy of grassland spittlebugs; the larvae attack nymphs inside their spittle mass. This enemy is relatively most abundant in field sites at Santander de Quilichao (Cauca) where over the course of two spittlebug generations 146 larvae, 79 pupae and 9 adults were encountered in 19 weekly field surveys for spittlebug population studies.

A new report for Colombia was made of a parasitoid (Hymenoptera: Encyrtidae) of *S. nigra* pupae. As many as 98 wasps emerged from parasitized pupae. This parasitoid could represent an important factor limiting the abundance of this spittlebug natural enemy. After a first report in 1998, no new cases of parasitism of adults by Pipunculidae were discovered. It is believed that this is the first report of this fly family attacking the superfamily Cercopoidea in the New World.

Parasitic nematodes probably belong to the genus *Hexamermis* (Nematoda: Mermithidae); identification is limited because nematodes that emerge from nymphs and adults represent late juvenile stages that are difficult to identify. The parasitic mites are probably *Leptus* spp. (Acari: Erythraeidae) that feed on host hemolymph and are the most abundant and widely distributed natural enemy of spittlebugs in Colombia. Although they can cause host death in other insects, it is doubtful they represent an important mortality factor.

Table 29. Incidence of spittlebug natural enemies in four geographic regions of Colombia (1996-1999).

Region	Natural enemy	J	F	M	A	M	J	J	A	S	O	N	D
North Coast	Parasitic mites					x	x	x	x	x	x	x	x
(Córdoba, Sucre)	Parasitic nematodes					x	x						
Eastern Llanos	Fungal entomopathogens							x					
(Meta)	Parasitic mites			x	x	x	x	x				x	x
	Parasitic nematodes				x	x	x	x	x	x	x		
	Predaceous flies (Syrphidae)					x	x						
Cauca Valley	Fungal entomopathogens		x		x	x	x		x				
(Valle, Cauca)	Parasitic mites	x	x	x	x	x	x						
	Parasitic flies (Pipunculidae)	x											
	Predaceous flies (Asilidae)								x				
	Predaceous flies (Syrphidae)				x				x	x			
Amazonia	Fungal entomopathogens	x	x	x			x	x					
(Caquetá)	Parasitic mites	x		x	x	x	x	x	x	x	x	x	x
	Parasitic nematodes	x	x										
	Predaceous flies (Syrphidae)	x				x	x	x		x		x	x

The newly established ceparium for fungal entomopathogens now includes 45 multisporic isolates from which 145 monosporic isolates have been obtained (Table 30).

These isolates originate from both spittlebug life stages (nymphs, adults), representing 6 species (*A. reducta, A. varia, Mahanarva* sp. 1, *Mahanarva* sp. 2, *Z. colombiana, Z. pubescens*) and were collected in 6 departments of the country.

Table 30. Collection of multisporic isolates of entomopathogenic fungi from grassland spittlebugs.

	Host					Date
Isolate	Genus	Species	Sex	Life Stage	Department	collected
C – 1	*Aeneolamia*	*reducta*	Female	Adult	Sucre	Jun-98
F-11	*Aeneolamia*	*varia*	Male	Adult	Caquetá	Jun-98
36	*Aeneolamia*	*varia*	Female	Adult	Meta	Jul-97
37	*Aeneolamia*	*varia*		Adult	Meta	Jul-97
34A	*Aeneolamia*	*varia*	Male	Adult	Meta	Jul-97
34B	*Aeneolamia*	*varia*	Male	Adult	Meta	Jul-97
P-1	*Aeneolamia*	*varia*		Adult	Valle	Feb-99
P-2	*Aeneolamia*	*varia*		Nymph	Valle	May-99
P-3	*Aeneolamia*	*varia*		Adult	Valle	May-99
P-4	*Aeneolamia*	*varia*		Adult	Valle	May-99
P-5	*Aeneolamia*	*varia*		Adult	Valle	May-99
P-6	*Aeneolamia*	*varia*		Nymph	Valle	May-99
F-18	*Mahanarva*	sp. 1	Male	Adult	Caquetá	Jul-99
F-19	*Mahanarva*	sp. 1	Male	Adult	Caquetá	Jun-98
T-1	*Mahanarva*	sp. 2		Adult	Nariño	Jul-99
T-2	*Mahanarva*	sp. 2		Adult	Nariño	Aug-99
S – 1	*Zulia*	*colombiana*	Female	Adult	Cauca	Jun-98
Q-1	*Zulia*	*colombiana*		Adult	Cauca	Apr-99
Q-2	*Zulia*	*colombiana*	Female	Adult	Cauca	Apr-99
Q-3	*Zulia*	*colombiana*		Adult	Cauca	May-99
Q-4	*Zulia*	*colombiana*		Adult	Cauca	May-99
Q-5	*Zulia*	*colombiana*	Male	Adult	Cauca	May-99
Q-6	*Zulia*	*colombiana*		Adult	Cauca	May-99
Q-7	*Zulia*	*colombiana*		Instar 4	Cauca	May-99
Q-8	*Zulia*	*colombiana*		Instar 5	Cauca	May-99
Q-9	*Zulia*	*colombiana*	Male	Adult	Cauca	May-99
Q-10	*Zulia*	*colombiana*		Adult	Cauca	May-99
Q-11	*Zulia*	*colombiana*		Adult	Cauca	Jul-99
Q-12	*Zulia*	*colombiana*	Male	Adult	Cauca	May-99
Q-13	*Zulia*	*colombiana*		Adult	Cauca	Aug-99
F –7	*Zulia*	*pubescens*		Adult	Caquetá	Feb-98
F-1	*Zulia*	*pubescens*		Adult	Caquetá	Jun-98
F-10	*Zulia*	*pubescens*	Male	Adult	Caquetá	Jun-98
F-20	*Zulia*	*pubescens*	Female	Adult	Caquetá	Jun-98
F-14	*Zulia*	*pubescens*		Adult	Caquetá	Jun-98
24	*Zulia*	*pubescens*		Adult	Caquetá	Jan-97
14	*Zulia*	*pubescens*		Adult	Caquetá	Jul-97
17	*Zulia*	*pubescens*		Adult	Caquetá	May-97
23B	*Zulia*	*pubescens*	Male	Adult	Caquetá	May-97
47	*Zulia*	*pubescens*		Adult	Caquetá	May-97
62-1	undet.	undet.		Nymph	Caquetá	Feb-97
62-2	undet.	undet.		Nymph	Caquetá	Feb-97
48-1	undet.	undet.		Instar 5	Caquetá	Jan-97
48-2	undet.	undet.		Instar 5	Caquetá	Jan-97
B-1	undet.	undet.		Adult	Sao Paulo, Brazil	May-99

Discussion: Few natural enemies of spittlebugs have ever been seriously assessed for their potential as agents of biological control. Of those reported in Colombia, predaceous flies (Syrphidae), parasitic flies, and fungal entomopathogens are the most promising candidates for studies on biological control potential. The discovery of previously unknown enemies suggests that others remain to be identified and considered for biological control.

Collections in Colombia have particularly yielded a diverse array of fungal entomopathogens. This diversity is currently being evaluated in laboratory trials for virulence with the future hope of employing the most promising isolates in the field for evaluation of formulation and field testing methodologies.

2.2.4 Evaluation methodology for measuring virulence of fungal entomopathogens on spittlebugs (A. Morales, R. Tobón, A. Bolaños and D. Peck)

Rationale: Fungal entomopathogens currently demonstrate more potential for spittlebug management than any other class of natural enemy. Despite high levels of virulence in the laboratory, however, effectiveness in pastures has never been demonstrated. Focus on a narrow diversity of isolates and commercial products, lack of consideration for insect-pathogen interactions, and poor formulation and field-testing methodologies have compromised successful deployment. Ongoing population surveys have gathered a diversity of isolates from Colombian spittlebugs. Exploiting and assessing this diversity for biological control must begin with a dependable and rapid methodology for quantifying virulence in the laboratory and screening this collection of isolates.

Methods: Evaluation units were 30-day old plants (7-10 stems) of *B. ruziziensis* in pots (13 cm diam) covered by acetate cylinders (15 cm diam x 40 tall). These plants were infested with 10 adult tenerals (< 24 d old) of *A. varia*. Two or tree hours after infestation plants were sprayed with 5 ml of concentrated conidial suspension (10^8 conidia/ml) with an airbrush and compressor (10 psi). Ten repetitions (plants) were performed for each isolate under evaluation and each block had a control consisting of water plus tween (0.05%). All evaluated isolates were monosporic and previously reactivated on adults of *A. varia* and all but isolate 62-2 were identified as *Metarhizium* spp. The sprayed plants with their insects were maintained in a growth chamber (7°C ± 2°C, 80% ± 10% RH). Five days later insects were scored as alive, dead, and dead with evidence of micosis. Dead insects without micosis were stored in petri dishes with moist filter paper for 3-4 days to ascertain evidence of fungal infection.

Results: To date 18 isolates have been evaluated originating from 2 life stages and 3 species of spittlebugs (Table 31). All isolates were collected from the Department of Caquetá.

All evaluated isolates demonstrated pathogenic activity on *A. varia*. Absolute mortality covered a wide range from 20– 85% (Figure 26). Mortality in the control was 25%.

Table 31. List and origin of fungal entomopathogens isolates evaluated.

	Origin			Origin	
Isolate	Species	Life stage	Isolate	Species	Life stage
F-11	*Aeneolamia varia*	Adult	F-10e	*Zulia pubescens*	Adult
F-18	*Mahanarva* sp.	Adult	F-10f	*Zulia pubescens*	Adult
F-18a	*Mahanarva* sp.	Adult	F-20	*Zulia pubescens*	Adult
F-18e	*Mahanarva* sp.	Adult	F-20a	*Zulia pubescens*	Adult
F-19	*Mahanarva* sp.	Adult	F-20b	*Zulia pubescens*	Adult
F-1	*Zulia pubescens*	Adult	F-20c	*Zulia pubescens*	Adult
F-10	*Zulia pubescens*	Adult	F-20f	*Zulia* pubescens	Adult
F-10c	*Zulia pubescens*	Adult	48-1	undetermined	Nymph
F-10d	*Zulia pubescens*	Adult	62-2	undetermined	Nymph

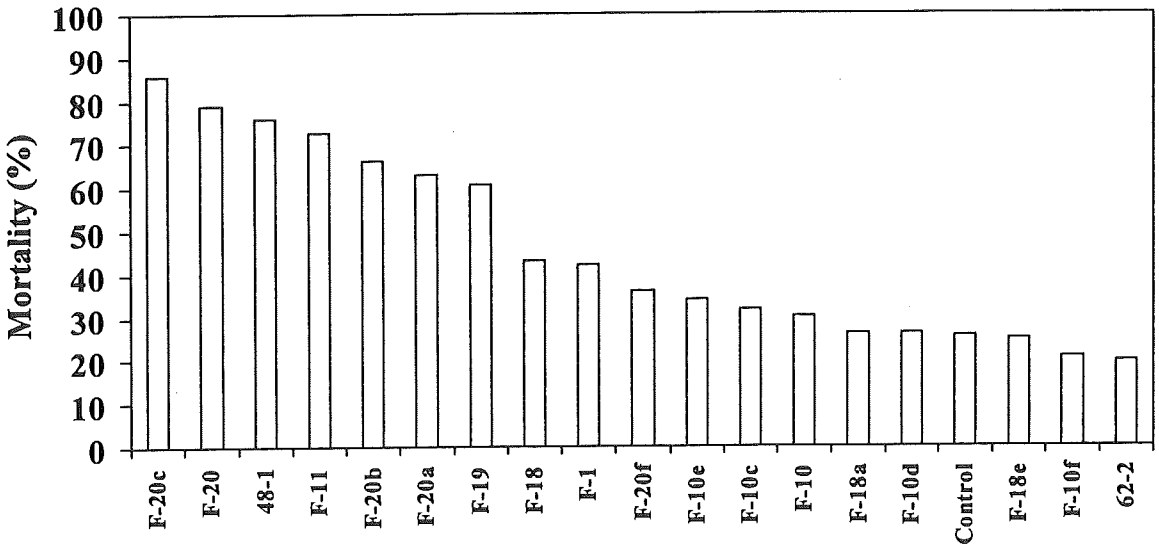


Figure 26. Mean absolute percent mortality of *A. varia* adults applied different isolates of fungal entomopathogens.

High and low virulence groups were distinguished. The low virulence group (11/18 isolates) was considered those with mortality below 40%. The high virulence group contained several monosporic strains of F-20 as well as one strain isolated from the nymphal life stage (Figure 16). Monosporic isolates from the same multisporic differed greatly in virulence. In the case of F-20, monosporic isolates were evaluated at efficiencies (Henderson & Tilton) of 76.8%, 66.5%, 53.8%, 48.7% and 16.9% (Figure 17). The isolate F-10, on the other hand, showed no efficient monosporic strains.

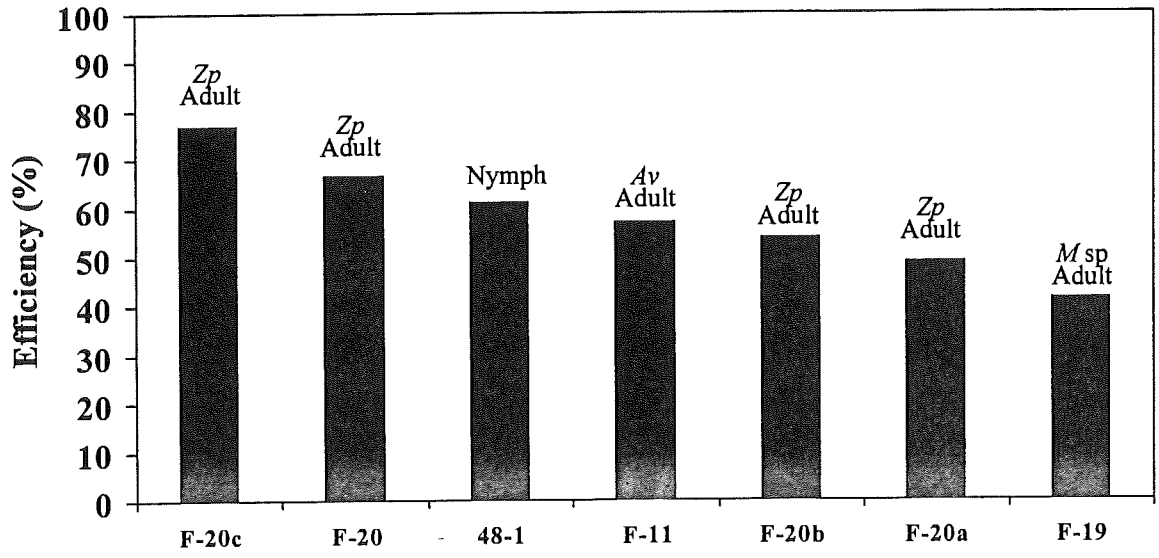


Figure 27. Efficiency (Henderson & Tilton) of the most virulent fungal entomopathogen isolates evaluated on *A. varia*. Initials indicate origin of the strain: Zp = *Zulia pubescens*, Av = *Aeneolamia varia*, Msp = *Mahanarva* sp.

Discussion: Evaluation of virulence against *A. varia* adults in the laboratory requires a high survivorship of control adults and the current methodology proves adequate; control mortality is 25%. These first evaluations indicate a wide range of virulence among a small

group of the ceparium's collection. The methodology is effective for segregating high and low virulence material for screening. No correlation was detected between life stage or species of origin and virulence against adult *A. varia* because in the high virulence group there are strains originally from *A. varia*, *Mahanarva* sp. and *Z. pubescens*, adults as well as nymphs. Once isolates have been screened on *A. varia* adults, the methodology will be adapted for evaluation on *A. varia* eggs and nymphs to identify the most susceptible life stages. Ultimately these most promising isolates will be screened on other spittlebug species.

2.2.5 Characterizing the growth and colony patterns in fungal entomopathogens of spittlebugs. (A. Bolaños, A. Morales and D. Peck)

Rationale: The evaluation of morphological characteristics and colony growth are parameters that permit a better understanding of the biology, mode of action and diversity of fungal entomopathogens. Certain of these characteristics accompany or contribute to variation in virulence and therefore can be used as a tool to more effectively select the most pathogenic isolates for laboratory and field trials and spittlebug control. The objective of this study was to evaluate growth and observe differences among colonies to understand the factors most relevant for future correlation between growth characteristics and application and selection for field trials.

Methods: The 11 isolates used in this study were taken from the newly established spittlebug fungal entomopathogen collection where material is stored on filter paper at -20 °C. Physiological and morphological studies were carried out on modified Sabouraud agar (10 g neopeptone, 40 g dextrose, 5 g yeast extract, 20 g agar, 1000 ml distilled water, chloramphenicol). Petri dishes with agar were inoculated with disks (diam 5 mm) of actively growing mycelium for incubation (28°C). Growth was measured along four radiating lines (from the edge of the disk to edge of colony) originating in the disk center every two days until growth stopped or filled the dish. Observations on other colony characteristics were made when the colony had grown 25% of the distance to the edge of the petri dish Three repetitions were evaluated for each isolate. These isolates included *Metarhizium* spp., *Paecilomyces* spp. and one other unidentified genus.

Results and Discussion: Colonies of all 7 *Metarhizium* isolates were white, green and yellow in color depending on zonation and all but one were subcircular in form (Table 32). More variable aspects of colony growth and appearance included aspect that varied from rhizomorphic to cottony to aerial growth; time to sporulation 2-6 days; exudate color clear or yellow; and time to exudation 6-13 days. Formation of exudate is thought to be positively related to pathogenicity. Aerial (versus flat) growth is also thought to be an important character because it probably interferes with sporulation. The *Paecilomyces* colonies evaluated were very distinct in color from *Metarhizium* featuring white and purple growth (Table 24). The two isolates differed in aspect (rhizomorphic vs. cottony) and sectorization.

The period of major growth for all colonies under these conditions was 3-25 days (Figure 28). The fastest growing isolate F-14 occupied the usable area of the petri dish in 15 days. There was a significant effect of isolate on growth rate as measured by the number of days required to grow 30 linear mm (ANOVA, $P<0.001$). The *Metarhizium* isolate F-14 and the undetermined 48-2 had significantly higher growth rates than the other isolates, requiring 9.9 and 12.8 days, respectively to grow 30 linear mm. High growth rate indicates greater aggressiveness in acquiring nutrients from the growth medium. Slow growth rates indicate that isolates were less tolerable of media contamination and drying out, rendering them less effective as control agents in the adverse conditions of the field. There were no distinguishable differences in growth rate between *Metarhizium* and *Paecilomyces*.

Table 32. Characterization of colony growth in certain fungal entomopathogen isolates.

Isolate	Genus	Aspect [1]	Color [2]	Days to sporulation	Form [3]	Zonation	Defined margin	Sector-ization	Days to exudation (color)
F-1	*Metarhizium*	Rhizomorphic	W, G, Y	4	SC	+.	-	–	6 (clear)
F – 11	*Metarhizium*	Rhizomorphic	W, G, Y	5	SC	+	-	–	7 (yellow)
F – 18	*Metarhizium*	Intermediate	W, G, Y	2	C	+	+	+	11 (clear)
F – 19	*Metarhizium*	Aerial growth	W, G, Y	6	SC	+	+	–	13 (clear)
F – 20	*Metarhizium*	Intermediate	W, G, Y	4	SC	+	-	–	8 (clear)
48-1	*Metarhizium*	Intermediate	W, G, Y	2	SC	+	-	–	(clear)
62 – 1	*Metarhizium*	Aerial growth	W, G, Y	4	SC	+	+	–	(clear)
24	*Paecilomyces*	Cottony	W, V	no det.	C	+	+	+	–
62 – 2	*Paecilomyces*	Rhizomorphic	W, V	no det.	C	+	+	–	–
48- 2	undetermined	Rhizomorphic	W, B	no det.	SC	–	+	–	(clear)
F – 14	undetermined	Cottony	P, O	no det.	SC	–	+	-	2 (clear)

[1] Intermediate means between rhizomorphic and cottony.
[2] B=brown, G=green, O=orange, P=pink, V=violet, W=white, Y=yellow
[3] C = Circular, SC = Subcircular

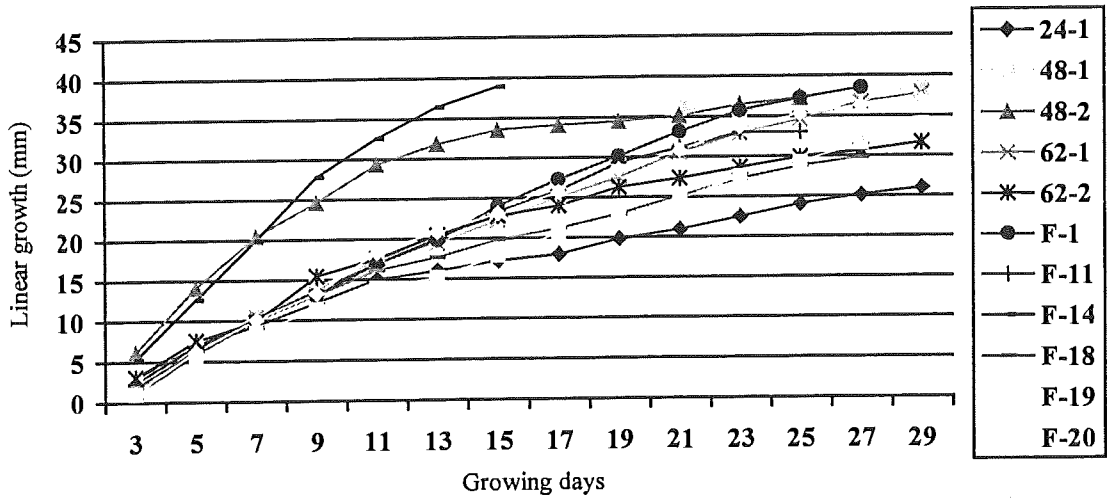


Figure 28. Growth rates of 11 fungal entomopathogen isolates.

Ongoing and future studies will evaluate these parameters for other isolates, evaluate additional parameters such as rate of sporulation and dry mass production, and relate these characteristics to levels of pathogenicity determined in laboratory trials. It is hoped that some of these characters can be used as tools to screen entomopathogens for both virulence and formulation qualities.

Progress towards achieving output milestone

- IPM components relevant to spittlebug species in Colombia better understood.

Advances in the elaboration of IPM components were limited due to lack of new funding sources. The strategic research fund of CIAT, however, supported a highly successful program to study fungal entomopathogens. Through those studies we have acquired the facilities, expertise and methodologies to seriously assess fungal entomopathogens for

biological control potential. Isolates of high virulence were identified and shown to affect adults of *A. varia*. High priority now is the confirmation of virulence of selected strains of entomopathogens against other life stages and species, and employment in the field through new studies that test formulation, application and field evaluation technologies.

Activity 2.3 Develop *Brachiaria* hybrid populations to screen for spittlebug resistance, edaphic adaptation, and quality

Highlights

- Produced, propagated and established in field nurseries over 3,000 open pollinated *Brachiaria* progenies.

2.3.1 Generate open-pollinated sexual population of *Brachiaria* hybrids from seed harvested on 1998 crossing block (F. Feijoo; J. Miles)

Rationale: A recurrent selection scheme is being applied to a heterogeneous tetraploid sexual *Brachiaria* population to upgrade mean spittlebug resistance. The pervious cycle, initiated in 1997, was completed with the identification of 10 parental clones in mid-1998. OP seed of these clones was germinated in early 1999 to give rise to the current selection cycle population.

Methods: Open pollinated seeds, harvested from pot-grown plants at CIAT headquarters, was aged (to overcome dormancy), hand scarified, imbibed on moist filter paper in petrie dishes, and germinated in a quartz sand medium in plastic planting flats. Seedlings were transplanted to 10-cm (4-in) plastic pots in the greenhouse. A total of 3,865 seedlings were established. Of these, 651 were culled on poor performance (lack of vegetative vigor) prior to propagation.

Results and discussion: A total of 3,215 seedlings were obtained from open pollinated (polycross) seed produced from an isolated crossing block established in mid-1998 with 10 selected parental clones.

2.3.2 Propagate and establish space-planted nurseries of *Brachiaria* hybrids at contrasting sites (Matazul and Caquetá) (F. Feijoo; G. Sotelo; C. Plazas; D. Vergara; A. Ortega; J. Miles)

Rationale: The current methodology used to select parental clones each selection cycle is first, to cull on visual observation of unreplicated clones established as single, spaced plants in field nurseries at each of two locations (clones vegetatively replicated over locations). "Pre-selections" (approx. 500-1000 clones) are then evaluated for spittlebug resistance in replicated, artificially inoculated glasshouse screenings in two stages. Seedlings produced in output 2.3.1 were established in field trials. The site in Caquetá where we have been working since 1993, was abandoned this year for reasons of public safety. A second field site was established at CIAT-Quilichao station, a not entirely satisfactory substitute owing to lower spittlebug pressure at Quilichao.

Methods: A single vegetative propagule was taken from each of 3215 seedlings and established in a 5-cm peat pot. The original seedlings were transplanted on 04 May 1999 at the CIAT-Quilichao station. Vegetative replicates were transplanted to a similar field nursery at the Matazul Farm, Puerto López (Meta) in the Colombian Llanos. Clones were independently randomized at the two locations. Clones were transplanted into half-sib

family rows of 15 sibs each at Quilichao (10-sib rows at Matazul). Transplants were set at approx. 1.5 m apart in rows 1.5 m apart.

Results and discussion: Establishment was satisfactory with only 16 plants (0.5%) lost at Quilichao and 47 (1.5%) lost at Matazul.

2.3.3 Visually evaluate and cull down to 500-1,000 pre-selections of *Brachiaria* hybrids (J. Miles)

Rationale: Current capacity to assess spittlebug resistance under controlled, artificially infested conditions has increased dramatically over the past two years, but still is limited. Given the volume of OP progenies generated, an initial culling is required to evaluate spittlebug resistance only on clones with at least some evidence of satisfactory agronomic field performance. Field nurseries provide the opportunity to observe performance and cull on unsatisfactory growth or plant type before propagation for spittlebug trials.

Methods: At the time of preparation of this report (30 August 1999), one visual evaluation (on a very simple, 4-point scale: "Cull"; "Questionable"; "Satisfactory"; "Outstanding") has been conducted at Matazul, on 03 August. Following rating, plants were cut back to approx. 10 cm. Several ratings have been done on the nursery at Quilichao and plants have been cut once, to approx. 10 cm in early July. Preliminary selections, of approx. 500-1000 clones, will be made by 01 November, and pre-selections propagated from the Quilichao nursery for spittlebug trials at CIAT.

Results and discussion: Based on early observations, some clones have already been culled. A "final" culling will be made by 01 November, based on two additional ratings at Matazul and at Quilichao. These 500-1000 "pre-selections" will be propagated vegetatively from the Quilichao field nursery to a greenhouse at CIAT to provide material for entomological evaluation beginning in January, 2000.

2.3.4 Production of sexual x apomictic *Brachiaria* hybrid populations using selected sexual clones and elite apomictic genotypes (A. Ortega; H. Franco; C. Plazas; J. Miles)

Rationale: Candidate *Brachiaria* clones for commercial release must have apomictic reproduction. The sexual tetraploid breeding population produces no apomicts, as sexuality is the homozygous recessive condition (aaaa). In order to generate superior apomicts, elite sexual clones must be crossed (as females) with elite apomictic accessions or hybrids (as pollen parent). This sexual x apomictic hybridization can be done with each new cohort of sexual selections.

Methods: The 10 clones selected from the 1997 sexual progenies, in addition to being recombined among themselves to produce a new, fully sexual population, were exposed to pollination by apomicts under two different scenarios: Individual propagules were set in a small agronomic trial at Matazul in 1998. This trial contained a diversity of elite, apomictic *Brachiaria* accessions (plus a few hybrids). The same elite sexual clones were set out in seed multiplication plots at CIAT-Popayán to be exposed to pollen of our most promising apomictic accessions (and one apomictic hybrids) under more controlled conditions.

Results and discussion: The 10 elite sexual clones cover a considerable range in flowering date, and synchronization with pollen source was not always ideal. Open pollinated seed was harvested from the sexual clones at Matazul during second semester, 1998. Two hundred eighty-three seedlings were generated from this seed. These seedlings are probably hybrids. They were vegetatively propagated and established in the space-planted nurseries at Matazul

and Quilichao. Seed was harvested on the isolated, elite sexual plants at Popayán too late to be included in the 1999 plantings. This seed will be held and germinated for evaluation during the 2000 season.

Progress towards achieving output milestone

- Tetraploid sexual parental clones of *Brachiaria* selected for spittlebug resistance, edaphic adaptation and forage quality

Evaluation nurseries containing >3,000 progenies were successfully established at two contrasting sites (Quilichao and Matazul). Visual scoring and initial culling began and on the order of 20-50 final selections will be identified by mid-2000 to screen for spittlebug resistance, tolerance to high levels of Al in the soil and in vitro digestibility.

Activity 2.4. Identify host mechanisms for spittlebug resistance in *Brachiaria*

Highlights

- Made further progress in understanding mechanisms underlying resistance of *Brachiaria* spp. to four species of spittlebug.

2.4.1 Studies on resistance of *Brachiaria* to spittlebug (C. Cardona y G. Sotelo)

Rationale: Studies on the resistance of *Brachiaria* genotypes to species of spittlebug other than *Aeneolamia varia* continued in 1999. This is important, as it can not be assumed that resistance to *A. varia* (the species that had always been used for resistance evaluation) applies across the board to all spittlebugs affecting *Brachiaria* spp. in the Tropics. Resistance to *Zulia colombiana, Z. pubescens*, and *Mahanarva* sp. was studied again in 1999.

Methods: Two greenhouse tests were conducted. A third one including more genotypes is in progress. The screening methodology was the same that has been adopted for mass screening of genotypes for resistance to *A. varia*. Four genotypes well known for their resistance or susceptibility to *A. varia* were used as test materials.

Results and Discussion: There was not a significant interaction between trials. The combined analysis of variance followed by mean separation indicated that there were significant differences in terms of visual damage scores and percentage nymphal survival (Table 33). As in 1998, lower nymphal survival due to high levels of antibiotic resistance to *Mahanarva* sp. and *A. varia* were detected in the resistant genotypes CIAT 6294 (Marandú) and the hybrid CIAT 36062 (BR93-NO/1371).

Z. pubescens and *Z. colombiana* caused significantly less damage to the resistant genotypes but there were no indications of antibiosis to these two species in the resistant genotypes. Percentage emergence of both *Zulia* species recorded on CIAT 6294 and CIAT 1317 did not differ from that recorded on the susceptible genotypes CIAT 0606 and CIAT 0654. The lower levels of damage caused by *Zulia* spp. on the resistant genotypes could be due to tolerance or another mechanism that remains to be elucidated.

Table 33. Damage scores (D.S.) and percentage nymphal survival in four *Brachiaria* spp. genotypes exposed to attack by nymphs of four different spittlebug species. Means of two trials, 10 replications per trial for each genotype-insect species combination.

Genotype	*Aeneolamia varia*		*Zulia colombiana*		*Zulia pubescens*		*Mahanarva* sp.	
	D.S.	% survival	D.S.	% survival	D.S.	% survival	D.S.	% survival
CIAT 0606	4.a	88.5a	4.1a	56.0a	4.4a	57.8a	4.6a	46.50a
CIAT 0654	4.1b	74.5b	4.3a	60.5a	4.2a	68.4a	4.2a	37.0a
CIAT 6294	1.6c	25.50c	2.6b	47.0a	2.3b	48.7a	1.6b	0.5b
CIAT 36062 (BR93-NO/1371)	1.2c	3.5d	2.4b	43.0a	1.9c	46.0a	1.1b	0.5b

Means within a column followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test). Each species analyzed separately.

Further analysis of the data from four consecutive trials clearly showed that the high levels of antibiotic resistance to *A. varia* and *Mahanarva* sp. found in CIAT 6294 and CIAT 36062 (BR93-NO/1371) do not seem to operate against *Z. colombiana* and *Z. pubescens.* Nymphal survival in the *Zulia* complex was significantly higher (Table 34). These results are important for future breeding strategies and fully justify the need to conduct simultaneous screening under field conditions in areas of the Tropics where several species of spittlebug may coexist.

Table 34. Percentage nymphal survival of four spittlebug species reared on two *Brachiaria* genotypes fully characterized for their high levels of resistance to *A. varia*. Means of four greenhouse trials, 10 replications per trial for each genotype-insect species combination.

Spittlebug species	CIAT 6294	CIAT 36062 (BR93-NO/1371)
Aeneolamia varia	25.5b	3.5b
Zulia colombiana	48.7a	44.0a
Zulia pubescens	37.0a	43.0a
Mahanarva sp.	0.5c	1.0c

Means within a column followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test). Each genotype analyzed separately.

Progress towards achieving output milestones

- Mechanisms of resistance to spittlebug complex better understood.

We have now confirmed that the high levels of antibiotic resistance of some *Brachiaria* hybrids to *Aeneolamia varia* and *Mahanarva* does not operate with *Zulia* species. Biochemical studies are urgently needed to better understand the mechanisms of antibiotic resistance in selected hybrids.

Activity 2.5: Identify *Brachiaria* genotypes resistant to spittlebug

Highlights

- Selected in the glasshouse new *Brachiaria* hybrids with more spittlebug resistance than checks.
- Demonstrated the feasibility of using a field methodology to screen *Brachiaria* hybrids for spittlebug resistance.

2.5.1 Propagation and delivery of selected *Brachiaria* hybrid sexual clones (F. Feijoo; G. Sotelo; W. Mera; J.Miles)

Rationale: New methodology is being developed by the Forage Entomology group to facilitate deployment of spittlebug nymphs on host test-plants in the field. These methodologies require validation using host genotypes of known spittlebug reaction under relevant field conditions such as prevail in the Amazonian piedmont of Caquetá.

Methods: Ten selections from a large progeny population produced in 1997 (1998 IP5 AR) were vegetatively propagated and shipped by air as potted plants to Florencia for propagation and establishment of field trials at the Corpoica Macagual experiment station.

Results and discussion: Plants were successfully delivered from CIAT-Palmira to Caquetá for controlled field evaluation for resistance to spittlebug.

2.5.2 Seed multiplication of a *Brachiaria* hybrid CIAT 36062 (BR93-NO/1371) highly resistant to spittlebug (A. Ortega; I. M. Rao; J. W. Miles)

Rationale: The *Brachiaria* clone CIAT 36062 (BR93-NO/1371) -- an apomictic hybrid originally selected in 1993 -- has many positive attributes. It exhibits essentially complete antibiosis to *Aeneolamia varia* nymphs in controlled glasshouse tests. It has a strongly stoloniferous growth habit, and high production of leaf tissue. It appears to maintain a dark green color under most conditions of growth, suggesting that it may be effective at hosting N-fixing bacteria. Although total forage yield is not among the best of our promising apomictic hybrid clones, it was thought worthwhile to test this particular clone under realistic grazing conditions to assess its real commercial potential. While flowering has been very meagre at CIAT-Palmira and other low-elevation, low-latitud sites in Colombia, preliminary observations of flowering at Popayán (1,800 msnm) suggested that seed production might be feasible there.

Methods: A 1.200 m^2 seed plot was established at CIAT-Popayán with vegetative material, obtained both at CIAT-Popayán and at CIAT.

Results and discussion: Flowering has been erratic and uneven in the seed plot. Approx. 1.2 kg of clean seed has been harvested to date (30 August 1999). We expect to continue harvest of this plot through 1999, so as to have sufficient seed by early 2000 to be able to establish one or more grazed plots.

2.5.3 Seed multiplication of promising new apomictic-*Brachiaria* hybrids (A. Ortega; J. Miles)

Rationale: Only one apomictic hybrid product of the *Brachiaria* breeding project CIAT 36061 (FM9201/1873) is under large-scale seed multiplication at the moment. Several more recent selections are showing promise under limited field evaluation. Definitive assessment requires larger quantities of seed. We established a small plot (approx. 120 m^2) of one additional promising apomictic hybrid (FM9503/S046/024) at CIAT-Popayán in mid-1999, so as to generate larger quantities of seed for more extensive testing during 2000.

Methods: The seed plot was established during July 1999, with vegetative material from a very small (2.5 m) row-plot at Popayán. Seed will be hand harvested and processed.

Results and discussion: Seed plot is in the establishment phase and we expect to begin seed harvest next year (2000).

2.5.4 Greenhouse screening of *Brachiaria* accessions and hybrids for resistance to spittlebug (C. Cardona, G. Sotelo, J. W. Miles)

Rationale: As indicated in the 1998 report, assessment of large numbers of genotypes developed by breeding activities at CIAT has been facilitated and expanded by the development of a new mass screening methodology. In 1999, a large series of genotypes were screened for different purposes.

Methods: Accessions from CIAT's *Brachiaria* collection were infested in the greenhouse using the experimental unit developed in 1997. Each unit was infested with 10 mature eggs of *A. varia* previously selected in the laboratory. Eggs were checked 24 hours after infestation and unhatched eggs were replaced. Infestation was allowed to proceed without interference until all nymphs were fully mature or adult emergence first occurred. At this point, plants (10 replications per genotype) were scored for symptoms using a damage score scale. Percentage nymphal survival was calculated. Genotypes were then classified for resistance.

Results and Discussion: Out of 65 accessions tested, 7 were classified as resistant, 2 were intermediate and 54 were susceptible (Table 35). As in previous occasions, there was a significant correlation ($r = 0.607$, $P<0.001$) between visual damage scores and percentage nymphal survival.

Table 35. Levels of resistance to *Aeneolamia varia* in selected *Brachiaria* spp. genotypes as determined using a greenhouse resistance evaluation technique. Means of 10 replicates per genotype.

Accession	Species	Damage scores[a]	Percentage nymphal survival	Rating (R, resistant; I, intermediate; S, susceptible)
CIAT 16835	*B. brizantha*	1.8c	18.9e	R
CIAT 26126	*B. brizantha*	2.7bc	30.0cd	R
CIAT 16843	*Brachiaria* sp	2.9b	32.0cd	I
CIAT 26172	*B. ruziziensis*	2.9b	3.3g	I
CIAT 16828	*B. brizantha*	3.0b	-	I
CIAT 16872	*B. brizantha*	3.0b	57.5b	S
CIAT 26741	*B. arrecta*	3.0b	39.0c	I
CIAT 36062 (BR93-NO/1371)[b]	Hybrid	1.4d	13.3ef	R
CIAT 6294[b]	*B. brizantha*	1.6c	27.7d	R
CIAT 0606[c]	*B. decumbens*	4.0a	76.0a	S
BRX-44-02[c]	*B. ruziziensis*	4.9a	71.2a	S
C. V. (%)		15.0	26.8	

[a] 1, no damage; 5, very heavy damage, plant killed
[b] Resistant checks
[c] Susceptible checks
Means within a column followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

A set of 92 hybrids used for molecular marking of genes governing apomixis or spittlebug resistance was evaluated in 1999. These were tested in comparison with four well known checks, the resistant hybrid CIAT 36062 (BR93-NO/1371) and the accessions CIAT 6294 (resistant), CIAT 0606 (susceptible) and CIAT 0654 (susceptible). Ten replications per genotype were used. The hybrids selected on the basis of visual damage scores (≤ 3.0 in a 1-5 scale) were then inspected for nymphal survival and rated as resistant (<30% survival), intermediate (31-50%), and susceptible (>50%). Resistant and intermediate genotypes are shown in Table 36.

Table 36. Levels of resistance to *Aeneolamia varia* in selected *Brachiaria* genotypes (means of 10 replications per genotype)

Genotype	Damage scores[a]	Percentage nymphal survival	Rating (R, resistant; I, intermediate; S, susceptible)
BP 1028/0027	2.3b	39.0c	I
BP 1016/0008	2.3b	40.0c	I
BP 1027/0128	2.4b	25.0cd	I
BP 1017/0005	2.4b	5.6d	R
BP 1027/0118	2.6b	35.0c	I
BP 1016/0018	2.7b	5.0d	R
BP 1028/0022	2.8b	60.0b	S
BP 1016/0037	2.9b	35.0c	I
BP 1016/0009	2.9b	40.0c	I
BP 1028/0017	2.9b	78.0ab	S
BP 1027/0116	3.0b	75.0ab	S
BP 10270136	3.0b	64.0ab	S
CIAT 36062 (BR93-NO/1371)[b]	1.7c	12.0d	R
CIAT 6294[b]	1.3c	23.0cd	R
CIAT 0606[c]	4.4a	84.0a	S
CIAT 0654[c]	4.7a	77.0ab	S
C. V. (%)	15.0	32.1	

[a] 1, no damage; 5, very heavy damage, plant killed; [b] Resistant checks; [c] Susceptible checks
Means within a column followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

2.5.5 Evaluation of field screening methodology for evaluating resistance of *Brachiaria* to spittlebug (C. Cardona, G. Sotelo, J. W. Miles)

Rationale: Assessment of spittlebug resistance under natural levels of infestation in the field is very difficult due to the focal, unpredictable occurrence of the insect. The technique based on the initial infestation of the plants in the greenhouse and the subsequent transfer of the infested material to the field was used extensively in 1999. This work was conducted at CIAT in Palmira and at the Macagual Station of Corpoica in Caquetá.

Methods: Using the experimental unit described in 1998, the genotypes (usually 10 replicates) are infested in the greenhouse with an average of 10 eggs per stem. Once the infestation is well established, with all nymphs feeding on the roots, the units are transferred to the field and transplanted 10-15 days after infestation. The infestation is then allowed to proceed without interference until all the nymphs have developed and adults emerge some 30-35 days thereafter. The plants are then scored for damage by means of the 1-5 visual scale utilized in greenhouse screenings. The number of stems per clump is counted before and after infestation.

Results and Discussion: Using the technique described above, four genotypes well known for their reaction to *A. varia* were evaluated. As shown in Table 37, there were significant differences between resistant and susceptible genotypes. Damage ratings for resistant genotypes ranged from 1.3 to 1.5 while those for susceptible genotypes ranged from 3.7 to 4.9. While the resistant genotypes CIAT 6294 and CIAT 36062 (BR93-NO/1371) more than doubled the number of stems per clump, the susceptible genotypes CIAT 0606 and CIAT 0654 did not show an increase in tillering as a result of insect feeding damage. There was a perfect match between field and greenhouse ratings for resistance or susceptibility. Three large-scale field tests were conducted in Macagual, Caquetá. Equal sets of 40 genotypes (11 hybrids, 29 accessions) of known reaction to *A. varia* in greenhouse conditions were evaluated in the field for resistance to *A. varia* (2 tests) and *Z. pubescens* (1 test).

Table 37. Response of *Brachiaria* spp. genotypes to attack by nymphs of the spittlebug *Aeneolamia varia* under field conditions. Means of 10 replications.

Genotype	Damage scores (1-5)[a]	No. of stems per clump			Resistance ratings	
		Before infestation	40 days after infestation	% Increase	Greenhouse conditions	Field conditions
CIAT 0606	3.7b	20.1a	20.8b	3.5	S	S
CIAT 0654	4.9a	24.2a	23.3b	-3.7	S	S
CIAT 6294	1.3c	22.8a	40.2a	76.3	R	R
CIAT 36062 (BR93-NO/1371)	1.5c	12.6b	36.9a	192.8	R	R
C.V. (%)	12.5	27.7	25.9			

[a] 1, no damage; 5, very heavy damage, plant killed
Means within a column followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

Ten replications of each genotype were used. The materials were rated on the basis of damage scores. Damage scores for *A. varia* ranged from 1.0 to 5.0 (mean: 3.4). Damage by *Z. pubescens* ranged from 1.7 to 4.1 (mean: 3.0). In general, ratings for resistance under field conditions matched previous greenhouse ratings (Table 38). Curiously, the hybrid BR 97NO/0083, susceptible in the greenhouse, showed field resistance to both insects. One of the hybrids (BR97NO/0402) showed resistance to *A. varia* but not to *Z. pubescens*. Most accessions were susceptible to both species of spittlebug. An opportunistic evaluation of 10 hybrids selected in 1998 was made in the "La Rueda" farm in Caquetá. Under very high natural infestation, the hybrids BR 97NO/0047, BR 97NO/0235, BR97NO/0402, and BR97NO/0457 showed levels of resistance comparable to those of the resistant checks CIAT 36062 (BR93-NO/1371) and CIAT 6294 (Figure 29). Resistance ratings matched those previously reported (IP5 1998 AR).

Table 38. Comparative responses of hybrids and accessions of *Brachiaria* tested for resistance to two species of spittlebug under field conditions. Caqueta, 1999.

	Reaction[a] to *Aenelolamia varia*			
	In the	Under field conditions		Reaction to *Zulia pubescens*
Genotype	greenhouse	Test 1	Test 2	
BR 97NO/0047	R	I	R	I
BR 97NO/0082	S	S	S	I
BR 97NO/0155	I	I	I	R
BR 97NO/0235	R	I	I	R
BR 97NO/0083	S	S	R	R
BR 97NO/0402	R	R	R	S
BR 97NO/0405	S	S	S	S
BR 97NO0410	S	R	R	R
BR 97NO/0457	R	R	R	R
BR 97NO/1143	I	I	I	R
BR 97NO/2965	I	I	R	I
CIAT 16113	I	S	S	S
CIAT 16212	S	S	S	S
CIAT 16467	S	S	S	S
CIAT 16871	S	S	S	S
CIAT 26180	S	S	S	S
CIAT 0654[a]	S	S	S	S
CIAT 0606[a]	S	S	S	S
CIAT 6294[b]	R	R	I	R
CIAT 36062 (BR93-NO/1371)[b]	R	R	R	R

[a] Standard susceptible checks
[b] Standard resistant checks

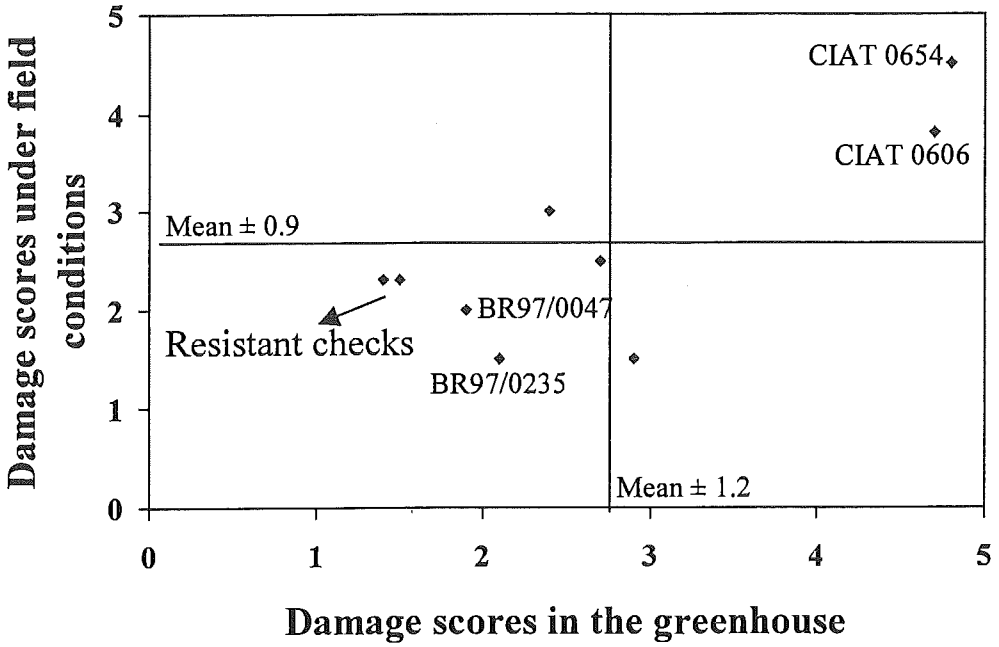


Figure 29. The relationship between greenhouse and field resistance or susceptibility to spittlebug in 9 *Brachiaria* genotypes.

Progress towards achieving output milestone

- Apomictic genotypes of *Brachiaria* resistant to spittlebug under glasshouse and field conditions available for multisite evaluation

We now have results which indicate that with few exceptions, ratings for resistance to spittlebug under field match ratings obtained in the glasshouse. Seed multiplication of a spittlebug-resistant hybrid CIAT 36062 (BR93-NO/1371) was initiated and we should have sufficient seed for grazing trials in 2000.

Activity 2. 6 Identify genetic control and molecular markers for spittlebug resistance and apomixis in *Brachiaria*

Highlights

- Developed mapping population for identification of markers of apomixis and QTL analysis of spittlebug resistance.
- Completed phenotypic evaluation of spittlebug resistance with 226 hybrids used for molecular marker development.

2.6.1 Propagate F_1 genotypes (4X *B. ruziziensis* x *B. brizantha* cv. Marandú) for spittlebug screening and for molecular marker assessment (F. Feijoo; G. Sotelo)

Rationale: We hypothesize that molecular genetic markers linked to QTL conditioning resistance to spittlebug will improve selection efficiency for this trait in *Brachiaria* breeding populations. Marker and phenotypic data need to be obtained from a structured population in order to identify marker-QTL associations.

Methods: A hybrid population was formed by crossing the spittlebug-susceptible tetraploidized sexual *B. ruziziensis* with the spittlebug-resistant, natural tetraploid apomictic *B. brizantha* CIAT 6294 (cv. Marandú), to generate 238 confirmed (with αβ-esterase isozymes) hybrid genotypes. A smaller population (74 hybrid individuals) already existed for this same cross.

Results and discussion: Hybrids were produced by controlled pollination and confirmed by the presence of paternal isozyme bands. These hybrid clones have been propagated vegetatively evaluated for spittlebug reaction under controlled glasshouse conditions with artificial infestation (see next sub-activity).

2.6.2 Variability in spittlebug resistance in a *Brachiaria* marking population (C. Cardona, G. Sotelo and J. W. Miles)

Rationale: There is need to provide both breeders and molecular scientists with reliable phenotypic characterization of the levels of resistance or susceptibility present in hybrid *Brachiaria* populations that are going to be used to develop molecular markers for apomixis and/or spittlebug resistance. This was accomplished in 1999.

Methods: A set of 226 hybrids received from the breeding program and 5 checks were infested in the greenhouse using the experimental unit developed in 1997. Each unit was infested with 10 mature eggs of *A. varia* previously selected in the laboratory. Eggs were checked 24 hours after infestation and unhatched eggs were replaced. Infestation was allowed to proceed without interference until all nymphs were fully mature or adult emergence first occurred. At this point, plants (5 replications per genotype) were scored for symptoms using a damage score scale. The hybrids selected on the basis of visual damage scores (≤ 3.0 in a 1-5 scale) were then inspected or nymphal survival and rated as resistant (<30% survival), intermediate (31-50%), and susceptible (> 50%).

Results and Discussion: The frequency distribution of genotypes according to visual damage scores is shown in Figure 30.

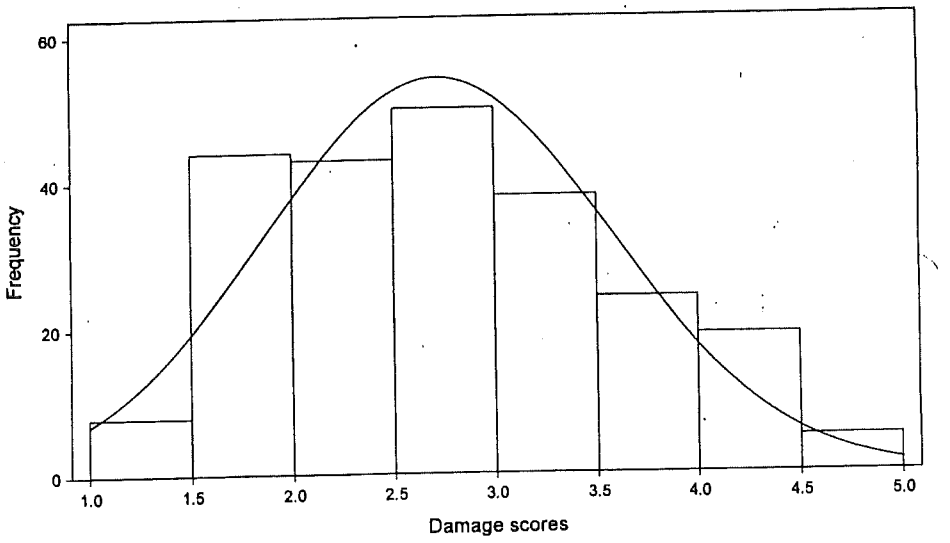


Figure 30. Frequency histogram of damage scores in a population of 226 *Brachiaria* hybrids and 5 checks tested for resistance to *Aeneolamia varia*.

The phenotypic evaluation of these materials and the histogram based on damage scores do not suggest monogenic resistance. The histogram shows that the range between hybrid populations covers the range of damage of the two parents, the resistant *B. brizantha* cultivar CIAT 6294 (2.0) and the susceptible *B. ruziziensis* accession CIAT 0654 (4.2). This could be an indication of a relatively simple inheritance.

Progress towards achieving output milestone

- Known potential to use marker assisted selection (MAS) for apomixis and spittlebug resistance in *Brachiaria.*

The *Brachiaria* mapping population has now been developed and spittlebug resistance phenotyping was completed.

Activity 2.7. Elucidate the role of endophytes in tropical forage grasses

Highlights

- Found that antiserum that differentiated isolates of endophytes was consistent with DNA data.
- Successfully introduced an endophytic fungus isolated from *B. brizantha* CIAT 6780 into *B. decumbens* CIAT 606.
- Found that endophytic isolates from *B. brizantha* 6780, *B. decumbens* 606 and *B. arrecta* 16845 are closely related to each other whereas the isolate from *B. brizantha* 26110 is distinct.
- Showed that growth of fungal and bacterial pathogens are inhibited by isolates of endophytes from *Brachiaria.*
- Generated endophyte-free and –infected clones of *B. brizantha* CIAT 26110 for drought tolerance and spittlebug resistance studies.
- Showed that endophytes isolated from *Brachiaria* have a positive effect on growth of *B. arrecta* subject to severe drought, but no effect on confering resistance to spittlebug was found.

Endophytic fungi have one of the most complex associations with their hosts resulting in physiological responses in terms of growth, water relations, nutrient acquisition and utilization, and secondary metabolite production. Evidence exists that some grass species require their specific endophytes for maximum fitness under environmental stresses. Although considerable information is available on temperate grass-endophyte interactions, it is acutely short on tropical grasses and endophytes. In fact, it is widely believed that the most widely studied *Acremonium* endophytes exist only in temperate grasses as quoted here: "It is noteworthy that the seed-borne *Acremonium* (Link) Fr. Endophytes are known only from the cool-season subfamily Pooideae with the C3 photosynthetic mechanism" (quoted from: Clay, K. The potential role of endophytes in ecosystems. In: Biotechnology of endophytic fungi of grasses. 1994. C.W. Bacon and J. F. White (eds). CRC Press). However, the work we have been conducting showed that this statement no longer holds true as we discovered *Acremonium* endophytes in C4 grasses as well.

The following research activities have been carried out in 1999:
- Testing of antiserum developed against the endophytic fungus isolated from *Brachiaria brizantha* CIAT 6780.
- Artificial inoculations of *B. decumbens* CIAT 606
- Surveys and documentation
- Characterization of isolates of endophytes

- Fungal culture preparations for alkaloid detection
- Experiments for endophyte eradication
- Effect of endophytes on pathogens *in vitro* and *in vivo*

Rationale: Endophytes have been demonstrated to confer resistance to several insects and diseases in a number of grasses. There is need to test their possible role in conferring resistance to spittlebug.

2.7.1 Testing of antiserum developed against the endophytic fungus isolated from *Brachiaria brizantha* CIAT 6780 (G. Segura, A. C. Velasco and S. Kelemu)

Rationale: Quick and specific methods of endophyte detection are important for large scale screening of field materials. Pure endophyte cultures were sent to the company BioWorld Laboratory (USA) for antiserum production.

Materials and Methods: A monoconidial culture of *A. implicatum* isolated from *B. brizantha* 6780 was used for antiserum production by the BioWorld laboratory. Several vials of antisera were received some 6 months later. Two of the samples received (production bleed- Bio 93 and final bleed-Bio 94) were purified and conjugated with the enzyme alkaline phosphatase. Preliminary tests included agar diffusion plates (Ouchterlony) which consist of agar gels where preparations of test sample and antibodies are placed in adjacent wells. Enzyme-linked immunosorbent assays (ELISA) were also tested. ELISA allows enzyme-mediated amplification of detection sensitivity of antigen-antibody reactions.

Results and Discussion: Ouchterlony double diffusion test is one of the least sensitive serological methods as it requires high concentrations of antigen and specific antiserum for precipitin bands to develop. Many serological tests depend on visual antigen-antibody precipitate. However, ELISA reveals the presence of antigen-antibody interactions by enzymatic development of a colored product. Results from double diffusion were unsatisfactory in differentiating the various tests. With purified and conjugated antisera, it was possible to detect significantly the antigen i.e. the pure endophytic fungus isolated from *B. brizantha* CIAT 6780, when applied in pure form and at high concentration (Table 39).

Table 39. Comparison of DAS-ELISA values (absorbance at 405 nm, 60 minutes reactions) with different concentrations of gamma globulin (IgG) and conjugated antiserum.

	IgG dilution 1/2000			IgG dilution 1/10,000		
	Conjugated Dil. 1/1000	Conjugated Dil. 1/2000	Conjugated Dil. 1/6000	Conjugated Dil. 1/1000	Conjugated Dil. 1/2000	Conjugated Dil. 1/6000
Blank control: 1x PBS	0.256	0.211	0.104	0.246	0.143	0.077
Pure endophyte culture on PDA (conc. OD = 0.12)	0.154	0.110	0.096	0.232	0.160	0.050
Pure endophyte culture on PDA (dil. 1/1000)	0.147	0.109	0.112	0.233	0.152	0.009
Pure endophyte culture in nurient broth (conc. OD = 0.52)	1.499	1.279	1.051	1.766	2.388	0.755
Pure endophyte culture in nutrient broth (dil. 1/100)	0.117	0.134	0.098	0.219	0.163	0.017
B. brizantha extract with no endophyte (treated with fungicide) dil. 1/100	0.119	0.127	0.085	0.191	0.100	0.002
"Healthy" rice plant extract (dil. 1/100)	0.096	0.088	0.080	0.091	0.084	0.001

However, endophyte detection in tissues of *B. brizantha* CIAT 6780, it was consistent in differentiating between tissues with endophytes and those without endophytes (treated with the fungicide Folicur to eliminate endophytes) at low concentration. However, the antiserum could differentiate the various isolates of endophytic fungi in a manner consistent with the DNA data we have to date (Table 40). DNA analysis and morphological data showed that the fungal isolate from *B. brizantha* CIAT 26110 is distinct from the other endophyte isolates from other accessions of *Brachiaria*, whereas all others tested were identical.

Table 40. Endophyte isolates and their DAS-ELISA values (absorbance at 405 nm, 60 minutes reactions).

	IgG dilution 1/4000		IgG dilution 1/8000		IgG dilution 1/16000	
	Conjugated Dil. 1/6000	Conjugated Dil. 1/12000	Conjugated Dil. 1/6000	Conjugated Dil. 1/12000	Conjugated Dil. 1/6000	Conjugated Dil. 1/12000
Blank control: 1x PBS	0.197	0.210	0.200	0.298	0.205	0.435
Nutrient broth	0.147	0.113	0.133	0.176	0.199	0.222
Endophyte from accession 6780	1.505	1.036	1.608	1.126	1.748	1.075
Endophyte from accession 26110	0.100	0.116	0.101	0.102	0.098	0.101
Endophyte from accession 16845	1.567	1.070	1.651	1.097	1.643	1.101
Endophyte from accession 606	1.670	1.223	1.815	1.101	1.858	1.150

2.7.2 Artificial inoculations of *B. decumbens* CIAT 606 with an isolate of endophytic fungus from *B. brizantha* CIAT 6780 (X. Bonilla, F. Muñoz and S. Kelemu)

Rationale: *B. decumbens* has traits, such as reactions to insects, distinct from *B. brizantha*. The introduction of an endophyte from *B. brizantha* into *B. decumbens* will allow us to examine new traits (if any) acquired by the recipient host attributed to the endophyte.

Materials and Methods: Seeds of *B. decumbens* 606 were cleaned manually removing all plant debris. The seeds were then surface sterilized with 70% ethanol for 2 minutes, 2.5 % NaOCl for 10 minutes and then washed several times with sterile distilled water. Excess moisture was then removed using sterile filter paper. Eight to ten seeds were then placed in pots containing MS medium. Approximately three weeks later seedlings were artificially inoculated with a needle in the area of the apical meristem.

Using stereo microscope and a very delicate entomological needle, a tiny portion of endophyte mycelium from an actively growing culture was carefully introduced into the apical meristem of each seedling. The inoculation point was then carefully sealed with sterile vaseline to avoid fungal growth onto the MS medium. The inoculated plants were replanted onto MS medium for about 9 days more. The plants were then removed from the medium and placed in pots containing nutrient solution for 24 hours before transplanting them to pots containing sterile soil.

Results and Discussion: A total of 218 *Brachiaria decumbens* 606 were artificially inoculated with an endophytic isolate of *B. brizantha* 6780. Of these, only 55 plants survived. The remaining plants died due to meristem damage during inoculations. These 55 inoculated plants were tested for the presence of endophytes 4-5 months after inoculations on potato dextrose agar and under light microscopy. Four plants tested positive in both tests.

2.7.3 Characterization of isolates of endophytes (Y. Takayama, F. Muñoz, J. White and S. Kelemu)

Rationale: Endophytes are of considerable agronomic interest. Apart from reports of cattle toxicity, endophytes provide various desirable traits to their grass hosts. Detailed cultural, morphological and molecular characterizations of isolates are useful in differentiating the isolates and determining traits.

Materials and Methods: DNA was isolated using standard procedures from fungal mycelia/conidia collected directly from agar medium. DNA amplifications were conducted using 10-base oligonucleotide primers, programmed in a Programmable Thermal Controller with 45 cycles of a 1 min (2 min for the first cycle) denaturation step at 94 °C, annealing for 1 min at 35 °C, and primer extension for 1 min (7 min in the final cycle) at 72 °C. The amplification products were resolved by electrophoresis in a 1.2 % agarose gel, stained with ethidium bromide, and photographed under UV lighting.

The isolates were also characterized using 18S rDNA and ITS rDNA. Antiserum from *A. implicatum* (isolated from *B. brizantha* 6780) was used to test isolates as described earlier. The isolates from *B. decumbens* 606 and *B. brizantha* 26110 were sent to the International Mycological Institute (IMI, UK) for confirmation of identification.

Results and Discussion: PCR data with two of the primers used are shown in Figure P. Work is in progress to develop specific DNA probes. Results from 18S rDNA and ITS rDNA probes are shown in Figures 31 and 32.

The 18 S rDNA tree is very informative placing the *Brachiaria* isolates firmly in the genus *Acremonium* close to *A. strictum* and *A. kiliense*. The ITS rDNA data shows that the isolates are closely related. The IMI report identifies the isolate from *B. decumbens* 606 as: "*Acremonium terricola* (J. H. Mill., Giddens & A.A. Foster) W. Gams. This isolate produced individual conidiogenous cells on otherwise unmodified vegetative hyphae. The conidiogenous cells produced conidia from their apex by a replacement wall-building apex system with percurrent proliferation, and the conidia adhered in weak unbranched chains with the youngest at the base.

These characters enable the fungus to be placed in the genus *Acremonium*, and there, using my keys, it comes out as this species. It is important to note, however, that identification of *Acremonium* isolates to species level is frequently problematic, so that this identification must be regarded as tentative. Many members of the Clavicipitales (which include grass endophytes) produce conidial states of this general type."

The fungus isolated from *B. brizantha* 26110 was identified by IMI as *Hyalodendron* sp. and describes as follows: "Species of this genus produce large numbers of colorless, thin-walled, smooth, aseptate conidia holoblastically from colorless, thin-walled, smooth conidiogenous cells arising at right angles from the vegetative mycelium. The conidia themselves give rise holoblastically to more conidia in branched chains with the youngest conidia at the apices, and these chains are held together more or less strongly, with the conidia seceding schizolytically.

When conidial production is abundant, the colonies and microscope slides can give the impression that the fungus is yeast-like. *Hyalodendron* is a difficult and poorly-understood genus which has tended to be used as a dumping ground for taxonomically rather diverse fungi. An identification to species level is not practical."

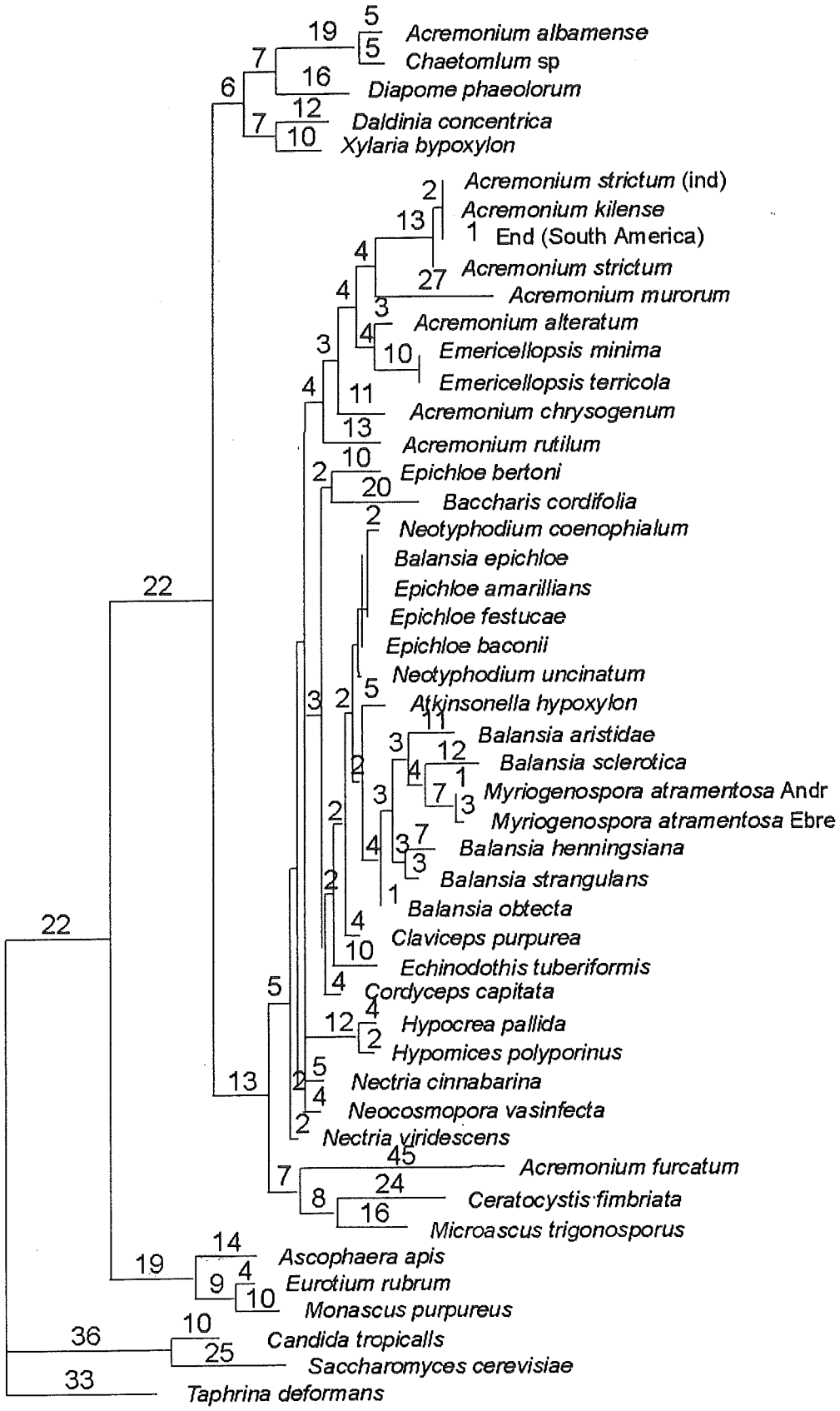


Figure 31. Dendrogram tree using 18 S rDNA.

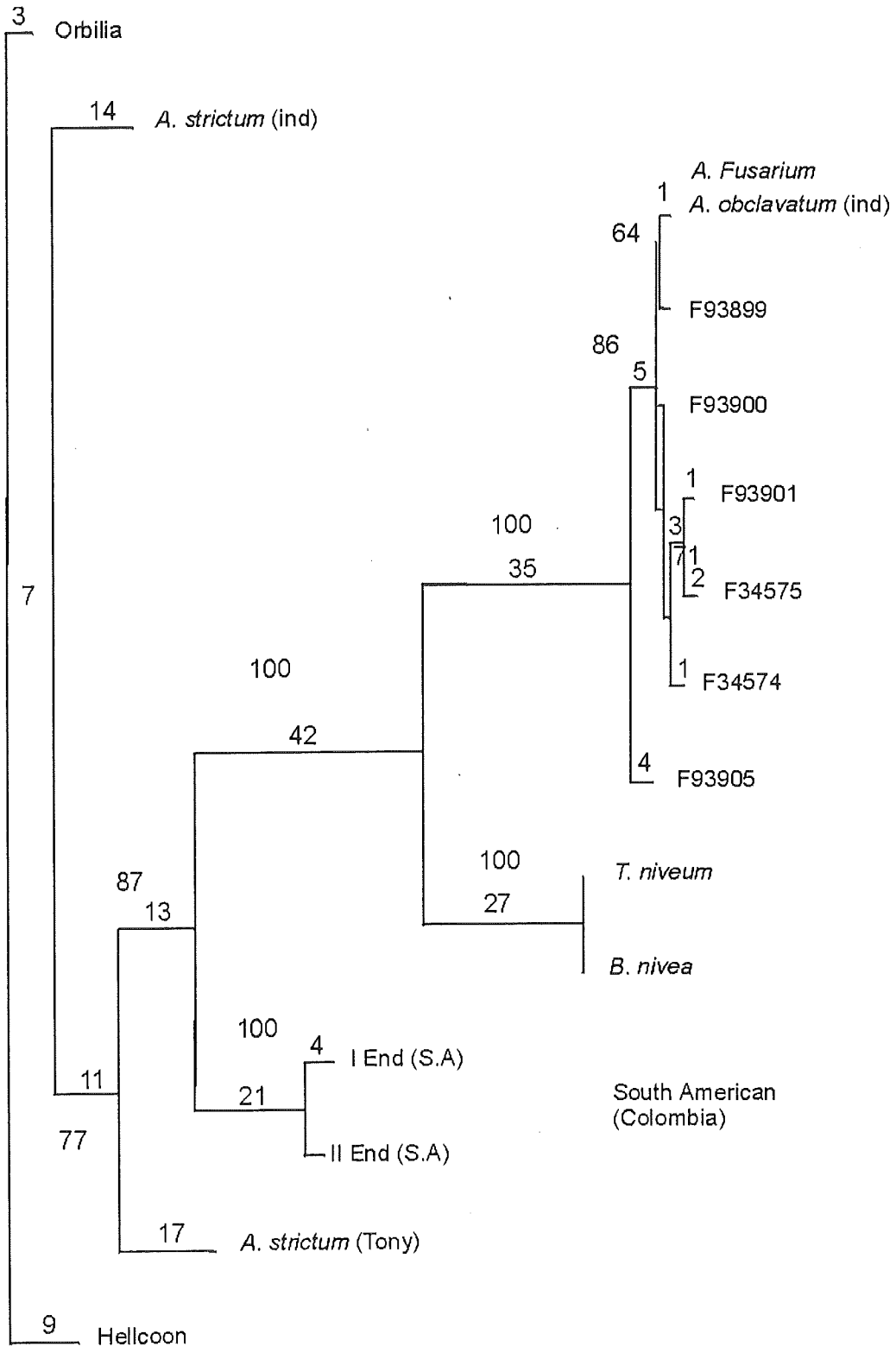


Figure 32. Dendrogram tree using ITS r DNA probe.

Antisera from endophytes may be used to study the relatedness among endophytes. Antiserum produced from the endophyte isolate 6780 indeed shows the relatedness of the isolates from *B. arrecta* 16845, *B. decumbens* 606 and *B. brizantha* 6780 consistently confirming DNA data (Table 40, Figure 33).

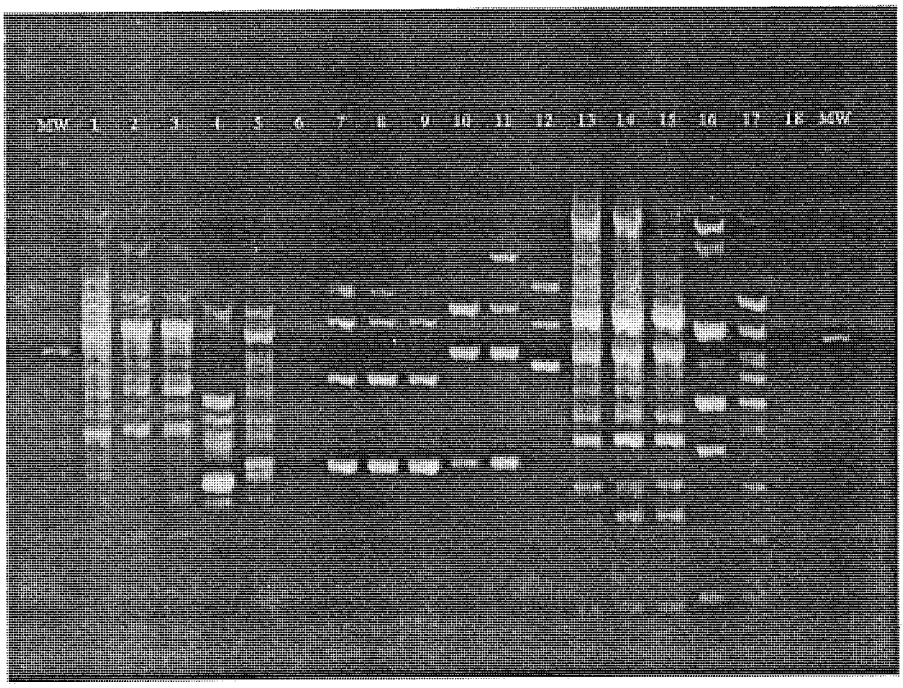


Figure 33. RAPD profile of endophyte isolates with three random primers (lanes 1-6, primer AK9; 7-12, primer AK10; 13-18, primer AK19). Lanes 1,7,13 are DNA of isolate from *Brachiaria decumbens* 606; lanes 2, 8, 14 from *B. brizantha* 6780 parcela 201; lanes 3, 9, 15 from from *B. arrecta* 16845 parcela 904; lanes 4, 10, 16 from *B. brizantha* 6780 parcela 501; lanes 5, 11, 17 from *B. arrecta* 16845 parcela 909; lanes 6, 12, 18 from *B. brizantha* 26110.

2.7.4 Fungal culture preparations for alkaloid detection (X. Bonilla and S. Kelemu)

Rationale: Various cattle health syndromes have been reported in association with grazing *Brachiaria* pastures. Cattle deaths have also been reported in the Brazilian Cerrados and the Venezuelan Llanos. However, the exact cause of the problem is still a subject of speculations. Examining *Brachiaria* endophytes for chemical alkaloid production will be a step towards establishing the cause of the health syndrome.

Materials and Methods: Special alkaloid media were used to grow endophyte isolates. Flasks containing 50 ml M102 medium (30 g sucrose, 20 g malt extract, 2 g bacto peptone, 1 g yeast extract, 0.5 g $MgSO_4$, 1 g KH_2PO_4, 0.5 g KCl and distilled water to 1L, pH 6.0) were inoculated with fungal mycelium (20-40 pieces of 2 x 4 mm mycelial agar discs). The cultures were incubated on a rotary shaker (200 rpm) at 25 °C. Five ml of the two week M102 culture was used to inoculate 500 ml flasks containing 100 ml of SM medium (100 g sorbitol, 40 g glucose, 10 g succinic acid, 1 g KH_2PO_4, 0.3 g $MgSO_4$, 1 g yeast extract, 20 ml 50x Vogel's salts [150 g Na_2 Citrate, 250 g KH_2PO_4, 100 g NH_4NO_3, 10 g $MgSO_4$, 5 g $CaCl_2$, 5 ml trace elements, 5 ml 0.1 mg/ml biotin, 750 ml distilled water; pH 6.8], to 1L distilled water; pH to 5.6 with NH_4OH).

The trace elements solution contained 5 g citric acid, 5 g $ZnSO_4.6H_2O$, 1 g $Fe(NH_4)2\ (SO_4)2.\ 6H_2O$, 250 mg $CuSO_4.5H_2O$, 50 $MnSO_4$, 50 mg boric acid, 50 mg $Na_2MoO_4.2H_2O$, and distilled water to 100 ml. The cultures were incubated for a week at 25 °C on the rotary shaker as earlier. At the end of one week, the cultures were moved to a stationary position where they remained undisturbed for about 4 months. A film of mat of mycelium is expected to form over the surface of liquid medium. At the end of the incubation time, the endophyte mycelium will be collected, freeze-dried and shipped for alkaloid analysis.

Results and Discussion: Results of chemical analysis are not available at the time of this report writing.

2.7.5 Eradication of endophyte from infected plants (F. Muñoz, X. Bonilla and S. Kelemu)

Rationale: Removal of endophytes from plants in order to create endophyte-free and endophyte-infected genetically identical *Brachiaria* tillers are necessary to study the effects of endophytes on physiological and morphological plant responses.

Materials and Methods: Based on the literature on eradication of endophytes in temperate grasses and our own earlier results, the fungicide folicur was used to remove an endophytic fungus from *B. brizantha* CIAT 26110. The method used involves the following steps:

(1) split the plants into separate tillers, wash off the soil completely, and trim the leaves. Five-10 tillers of the same mother plant were selected for treatment; (2) the tillers were then placed in a beaker containing a solution of 0.1 ml/L of folicur (250 g ai/L) for at least 6 hours; (3) the tillers were then transplanted singly in pots (with bottom drainage) containing sterile soils; (4) the plants were grown for 4 weeks and then tissues were examined for the presence or absence of endophytes using staining protocols and under light microscopy as well as isolations on culture media.

Results: Various microscopic and culture tests showed that the great majority (>95%) of treated plants showed no endophyte presence. This indicates that the fungicide had successfully eradicated the endophyte.

2.7.6 Effects of endophytes on pathogens *in vitro* and *in planta* (X. Bonilla and S. Kelemu)

Rationale: Endophyte-infected grasses have been shown to possess various properties of applied value. It has been demonstrated that nematode counts were significantly higher in plots of endophyte-free tall fescue than in plots of infected ones. Endophyte-infected grasses are also more resistant to fungal pathogens. Culture studies have shown that antagonism between endophytic fungi and various grass pathogens exist. We have carried out experiments to examine if this is also the case in endophytes of *Brachiaria* and some pathogens.

Materials and Methods: Antifungal activity of *Brachiaria* endophyte isolates was examined on 3 pathogens (*Rhizoctonia solani*, causal agent of foliar blight of *Brachiaria*; *Pyricularia oryzae*, causal agent of rice blast; *Drechslera* sp., casual agent of leaf spot disease of many grasses including *Brachiaria*). Four endophytic isolates (isolated from *B. brizantha* 6780, *B. decumbens* 606, *B. arrecta* 16845, *B. brizantha* 26110) were used. All isolates were grown on either potato dextrose agar (PDA) or SM media. Each endophyte isolate was grown at opposite corners of petri plates containing SM media and incubated at 28 °C for a week.

Agar discs (6 mm in diameter) of test pathogens were placed in the center of each plate and incubated further until pathogens on control plates containing no endophytes either cover the whole plate or more than 3/4 of the plates. The area covered by fungal mycelia was copied on to transparent overheads, scanned and then analysed using a software. For tests *in planta*, either whole *Brachiaria* plants or detached tissues with and without endophytes were inoculated with pathogens.

Agar discs of endophytic mycelia (6 mm in diameter) were placed on sterile filter papers placed on plates containing nutrient agar and incubated for about 12 days at 28 °C until ¾ of the filter paper area was covered with endophyte mycelia. The filter papers were then carefully removed off the plates, and the same plates were used to culture dilutions of bacterial (*Xanthomonas* sp. pathogenic on *Brachiaria*) suspensions. Dilutions series were made from an original bacterial concentration of $OD_{600} = 0.1$. Bacterial colony counts were done 24 hours later.

Results and Discussion: All endophyte isolates, except the one isolated from *B. brizantha* 26110, inhibited all the test fungal pathogens *in vitro* (eg. Figure 34). The endophyte isolates from *B. brizantha* 6780, *B. decumbens* 606, and *B. arrecta* 16845 consistently and strongly inhibited the growth of all the test pathogens (Table 41). Interestingly, *in vitro* inhibition studies on *Xanthomonas* sp. showed that all endophytic isolates had inhibitory effects. The isolate from 26110 had the highest inhibitory effect on the test bacterium (Table 42).

Endophyte effects *in planta*, however, was not consistent between expriments. This inconsistency may be due to variations in diffusible antifungal substances produced *in vitro* and *in planta*. It may also be due to variations in endophyte concentrations which we can control *in vitro*, but not *in planta*.

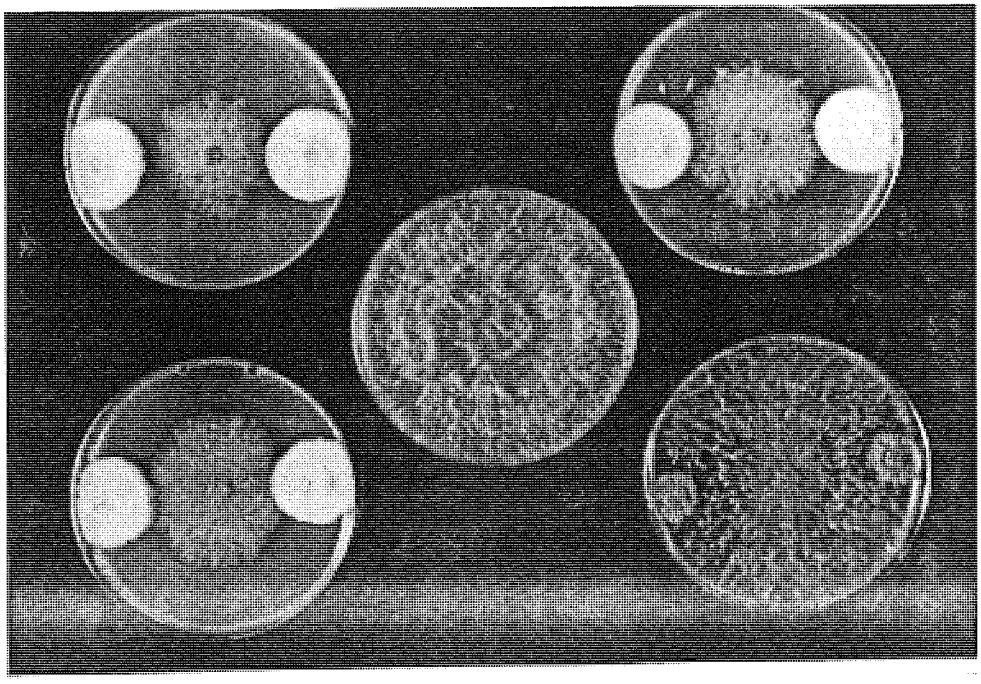


Figure 34. Growth inhibition of *Rhizoctonia solani* (the causal agent of foliar blight of *Brachiaria*) by isolates of endophytic fungi in SM media (see media for alkaloid detection). Clockwise from top left endophytes from: a) *Brachiaria brizantha* 6780, b) *B. decumbens* 606, c) *B. brizantha* 26110, and d) *B. arrecta* 16845. Center plate is control plate with *R. solani* only. The fungus in the center of each plate if *R. solani*.

Table 41. The growth of fungal pathogens in the presence or absence of endophytic isolates.

Pathogen	Endophyte isolate	Growth area (mm^2)[1]
Rhizoctonia solani	Control	1176.26a
	26110	311.26b
	16845	263.79b
	6780	248.71b
	606	209.70b
Drechslera sp.	Control	1238.74a
	26110	758.23b
	6780	574.73c
	16845	557.06c
	606	537.13c
Pyricularia oryzae	Control	656.47a
	26110	567.36a
	16845	218.90b
	6780	192.83b
	606	165.44b

[1]Values are means of 5 repetitions. Data in vertical columns followed by the same letter are not significantly different (p=0.01) according to Duncan's multiple range test.

Table 42. Bacterial growth in the presence and absence of diffusible substances from isolates of endophytes.

Substance-Producing Isolate	Bacterial dilution series					
	10^{-2}		10^{-3}		10^{-4}	
	+[1]	-	+	-	+	-
6780	382[2]	882	73	111	73	197
16845	425	921	57	130	21	72
606	403	931	314	641	35	200
26110	55	785	27	167	6	87

[1] + = presence of diffusible substance from endophyte isolates; - = absence of diffusible substance from endophyte isolates.
[2] Values are bacterial colony counts.

2.7.7 Studies on the effect of endophytes on resistance to spittlebug in *Brachiaria* (C. Cardona, S. Kelemu, G. Sotelo)

Methods: To test the role of endophytes several genotypes were chosen. In a first trial, the accession CIAT 6780 (known to harbor the endophyte reported by CIAT) was treated with a fungicide to remove the endophyte. An untreated check as well as other untreated checks was used for comparison. In two more trials, seeds of an identical set of genotypes were treated with high temperature (40° C) for two weeks. Untreated checks were also used. Methods of infestation and variables measured were identical to those currently used for resistance screenings.

Results and Discussion: In terms of insect damage scores and percentage nymphal survival no significant differences were found between fungicide-treated and untreated plants of CIAT 6780. All other genotypes expressed resistance or susceptibility as in previous numerous tests (Table 43).

Table 43. The effect of fungicide treatment used to kill endophytes in the expression of resistance to *Aeneolamia varia* in accession CIAT 6780. Means of 10 replications.

Genotype	Damage scores (1-5)[a]	Percentage nymphal survival
CIAT 6780 treated with fungicide	1.8b	27.4d
CIAT 6780 untreated	1.7b	37.9cd
BR 93NO/36062 (BR93-NO/1371)[b]	1.0c	1.5e
CIAT 6294[b]	1.6b	38.3c
CIAT 0654[c]	4.5a	88.2a
CIAT 0606[c]	4.8a	78.0b
C.V. (%)	16.9	31.5

[a] 1, no damage; 5, very heavy damage, plant killed
[b] Resistant checks
[c] Susceptible checks
Means within a column followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

The combined analysis of two consecutive trials with high temperature treatment did not show differences between treated and untreated plants. Resistance and susceptibility expressions were consistent with genetic differences among genotypes in numerous previous studies. Although these preliminary trials do not suggest a possible role of endophytes in spitthebug resistance, there is need for further studies (Table 44).

Table 44. The effect of heat treatment used to kill endophytes in the expression of resistance to *Aeneolamia varia* in five *Brachiaria* accessions. Means of two trials, 10 replications per treatment per trial.

Genotype	Damage scores[a]		Percentage nymphal survival	
	Treated	Untreated	Treated	Untreated
CIAT 6294	1.9c	1.8b	41.5b	38.5b
CIAT 6297	1.8c	1.9b	35.0b	39.5b
CIAT 6780	2.4b	1.7b	41.0b	35.0b
CIAT 0606[b]	4.5a	4.8a	71.5a	74.0a

[a] 1, no damage; 5, very heavy damage, plant killed
[b] Susceptible check
Means within a column followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

2.7.8 Role of endophytes in drought tolerance of *Brachiaria* species (A. C. Bolaños, J. Ricaurte, S. Kelemu and I. M. Rao)

Rationale: Within the past two decades several experimental studies have provided insight into grass-endophyte biology and have suggested possible uses of a fungus-grass relationship as biological control and tolerance mechanisms for biotic and abiotic stresses. A mutualistic symbiosis is known to exist between the endophytic fungus and the grass in that the host provides nutrition and a means of dissemination for the fungus, while the fungus provides resistance to herbivory and enhances host growth and survival under stressful environments, particularly drought. Plants endure or survive drought with a variety of escape and tolerance

mechanisms, all of which serve to improve water utilization or water conservation. Drought escape mechanisms (e.g., rapid phenological development during periods of adequate soil moisture) are primarily associated with annual species. The perennial nature of endophyte-infected grasses suggests that drought escape is not an alternative, so tolerance mechanisms (e.g., osmotic adjustment) are utilized. Dry season tolerance and persistence are two major agronomic traits that determine the economic usefulness of tropical grasses in the subhumid regions. A glasshouse study was conducted to determine the role of endophyte (*Acremonium implicatum*) infection on drought tolerance of *Brachiaria arrecta*.

Materials and Methods: An endophytic fungus, *Acremonium implicatum* (J. Gilman and E. V. Abbott) W. Gams, was isolated from *Brachiaria arrecta* CIAT 16845. Plantlets were propagated from the original mother plant containing the endophyte. Half of these plants were treated with the fungicide (Folicur) to eliminate the endophyte while the remaining half was left untreated. These genetically identical plants were subjected to three levels of drought stress (control with no drought stress; 60% field capacity with moderate drought stress; and 30% field capacity with severe drought stress). Drought stress was imposed in pots (4 kg soil) by withholding water supply to soil at desired field capacity. An Andisol (with low available phosphorus) from Darien (Valle del Cauca, Colombia) was amended with 80 kg P/ha to ensure adequate supply of essential nutrients. The trial was conducted as a split-plot design (endophyte treatments as main plots and drought treatments as subplots) with 3 replications (2 plants/pot). A number of plant attributes were monitored during plant growth and the plants were harvested at 50 days after standarization.

Results and Discussion: The presence of endophyte had no significant effect on shoot growth characteristics of the grass at control and moderate drought stress levels (Table 45). However, the endophyte infected plants maintained better leaf expansion and produced significantly greater leaf biomass under severe drought stress conditions.

Table 45. Influence of endophytic fungus (*Acremonium implicatum*) infection on drought tolerance of *Brachiaria arrecta* CIAT 16845 grown in pots (4 kg soil) in the greenhouse at three levels of water stress.

Plant attributes	*Endophytic infection* Status	Water stress level*			LSD ($P = 0.05$)
		Control	Moderate	Severe	
Shoot biomass (g/pot)	+	30.4	21.7	6.89	
	-	31.1	21.3	5.46	2.25
	$LSD_{(p = 0.05)}$	NS	NS	1.21	
Leaf biomass (g/pot)	+	5.77	4.95	2.34	0.58
	-	6.22	4.90	1.80	
	$LSD_{(p = 0.05)}$	NS	NS	0.32	
Shoot biomass (g/pot)	+	24..6	16.8	4.55	
	-	24.9	16.4	3.65	1.85
	$LSD_{(p = 0.05)}$	NS	NS	NS	
Leaf areaa (cm^2/pot)	+	1308	1142	445	
	-	1378	1158	326	172
	$LSD_{(p = 0.05)}$	NS	NS	87	
Specific leaf area (m^2/kg)	+	31.1	28.8	19.6	
	-	30.1	28.6	18.4	3.1
	$LSD_{(p = 0.05)}$	NS	NS	NS	
Leaf stem ratio (g/g)	+	0.23	0.30	0.52	
	-	0.25	0.30	0.50	0.05
	$LSD_{(p = 0.05)}$	NS	NS	NS	

+ = with endophyte infection; - = without endophyte infection.
* Control= 100% field capacity; Moderate= 60% field capacity; Severe= 30% field capacity
NS = not significant.

These results indicate that the presence of endophyte in tropical grasses such as *Brachiaria* can significantly improve dry season tolerance and contribute to greater production of meat and milk in the tropics. Further research work is in progress to test the role of endophytes on root and shoot attributes that are linked to drought tolerance in *Brachiaria*.

Progress towards achieving output milestone

- Alkaloid profiles of endophytes isolated from *Brachiaria* are determined.
- Effects of endophytes in *Brachiaria* on spittlebug and rhizoctonia resistance, and a drought tolerance defined

The identification of alkaloids induced by endophytes isolated from tropicas forage is in progress in collaboration with a US based coli. Antisera developed interacted with isolates of endophytes consistent with DNA data and work is in progress to develop specific DNA probes for quick endophyte detection. Our first results indicate that endophytes had no effect on confering resistance to spittlebug in *Brachiaria*, but do have an effect on confering resistance to severe drought, at least in *B. arrecta*. Isolates of endophytes from *Brachiaria* inhibited in vitro growth of bacterial pathogens, but the effect was variable in planta possibly due to differences in concentration of endophytes in the tissue.

Activity 2. 8. Define interactions between host and pathogen in *Brachiaria*, *Arachis*, and *Stylosanthes*

Highlights

- Successfully introduced a rice chitinase gene into *Stylosanthes guianensis* CIAT 184 and transgenic plants generated showed high level of anthracnose resistance in both laboratory assays and greenhouse tests.
- Found that some isolates of *C. gloeosporioides* isolated from infected *A. pintoi* are also pathogenic to *S. guianensis*, and some which originated from accessions of *S. guianensis* can infect *A. pintoi*.
- Determined pathogenic and genetic diversity of 53 isolates of *Colletotrichum gloeosporioides*, and established a field plot of *Stylosanthes guianensis* for epidemiological studies of anthracnose disease.
- Identified a *Xanthomonas* sp. as the casual agent of a wilt disease of *Brachiaria*, and a developed an inoculation method.

The fungal disease anthracnose is an important disease of key forage legumes and other major tropical crops. The pathogen, *Colletotrichum gloeosporioides*, consists of various host specific populations. Work on pathogen population structure and disease management continues to be the major focus.

The following activities were conducted during 1999:

- Completion of a graduate thesis on anthracnose disease management through genetic engineering
- Pathogen population studies and data analysis
- Studies on a bacterial wilt disease of *Brachiaria*
- Establishment of field plots for epidemiology studies
- New studies initiated

2.8.1 Developing transgenic *Stylosanthes* plants for a rice chitinase gene for resistance to anthracnose (Jiang Changshun and Segenet Kelemu)

Rationale: Although sources of anthracnose resistance are available in accessions of *Stylosanthes*, the high level of genetic and pathogenic variability in isolates of *C. gloeosporioides* made it difficult for long-term stable resistance. Additional cost-effective disease management strategies are, therefore, needed for this host-pathogen system.

Chitinases are proteins that are expressed by plants in response to infections. They have been reported from both mono- and dicotyledons and occur in widely different tissues, including embryos, seeds, cotyledons, leaves, stems, roots, flowers, etc. Chitinases are also secreted by many different microorganisms, including actinomycetes, soil bacteria, and various fungi. These proteins catalyze the hydrolysis of the β-1,4 linkages of the N-acetyl-D-glucosamine polymer chitin. Chitin is a structural component in organisms such as fungi. Chitinases are usually expressed constitutively at low levels in plants, but biotic and abiotic stresses can induce higher levels of expression.

These enzymes probably play a defensive role against pathogenic fungi. For example, a chitinase from *S. guianensis* leaves was reported to kill *C. gloeosporioides* hyphae, and increased activity can enhance this plant's resistance. Some chitinase-encoding genes, such as the rice chitinase gene, have been isolated from plants. *Stylosanthes* transformation and regeneration from calli has been demonstrated. But no transgenic *Stylosanthes* plants, carrying a chitinase gene to control anthracnose, have so far been reported.

This and other research results in the scientific literature plus the availability of a cloned rice chitinase gene made us believe that the use of a chitinase gene may provide an additional disease control measure. The research describes: (1) the use of a 1.1-kb, chitinase-encoding gene, isolated from rice, to generate transgenic *S. guianensis* CIAT 184 plants; (2) the effects of chitinase enzyme preparations on the anthracnose fungus *in vitro*; (3) the reactions of transgenic *S. guianensis* plants to anthracnose, caused by *C. gloeosporioides*.

Materials and methods: A rice chitinase gene, cloned in the plasmid pBSKS-G11(R), was kindly provided by Dr. S. Muthukrishnan of Kansas State University. The binary vector pCAMBIA2301 (Figure35) was kindly provided by Dr. R. Jefferson of CAMBIA, Australia. The *A. tumefaciens* strain LBA4404 was provided by Biotechnology Research Unit, CIAT. The enzymes *Hind*III, *Bam*HI, calf intestinal alkaline phosphate (CIP), and T_4 DNA ligase were purchased from GIBCO/BRL, Life Technology, Grand Island, NY. Reagents for recovering DNA from gels and for SDS-PAGE were from BIO-RAD Laboratories, Hercules, CA. Plasmid kits used were those of the QIAGEN Company, Hilden, Germany. All other chemicals were purchased from the SIGMA Company, St. Louis, MO.

Bacterial transformation: Competent cells of *E. coli* DH5α were prepared and subsequent transformations with plasmids carried out according to the protocol described by Inoue, 1990 (Gene 96: 23-28). Transformed cells were cultured on LB-agar medium with appropriate antibiotics (100 μg/mL ampicillin for pBSKS-G11(R), and 50 μg/mL kanamycin for pCAMBIA2301 and its derivatives) and incubated overnight at 37°C. For α-complementation screening, 40 μL/plate X-gal and 4 μL/plate ITPG were used.

Agrobacterium tumefaciens transformation was carried out, using the freeze-thaw method (Holsters et al., 1978. Mol. Gen. Genet. 163:181-187). Competent cells of *A. tumefaciens* were prepared, using YEP liquid medium (5 g yeast extract, 10 g peptone, 5 g NaCl per L), and treated with 10 mM $CaCl_2$. After adding plasmid DNA, cells were frozen in liquid nitrogen, thawed at 37°C in a water bath for 5 min, and incubated in 1 mL YEP liquid

medium with vigorous shaking at 28°C for 3 h. Transformed cells were selected by plating them on LB-agar medium with 25 μg/mL streptomycin and 50 μg/mL kanamycin. Transformants were maintained at −80°C in 20% glycerol.

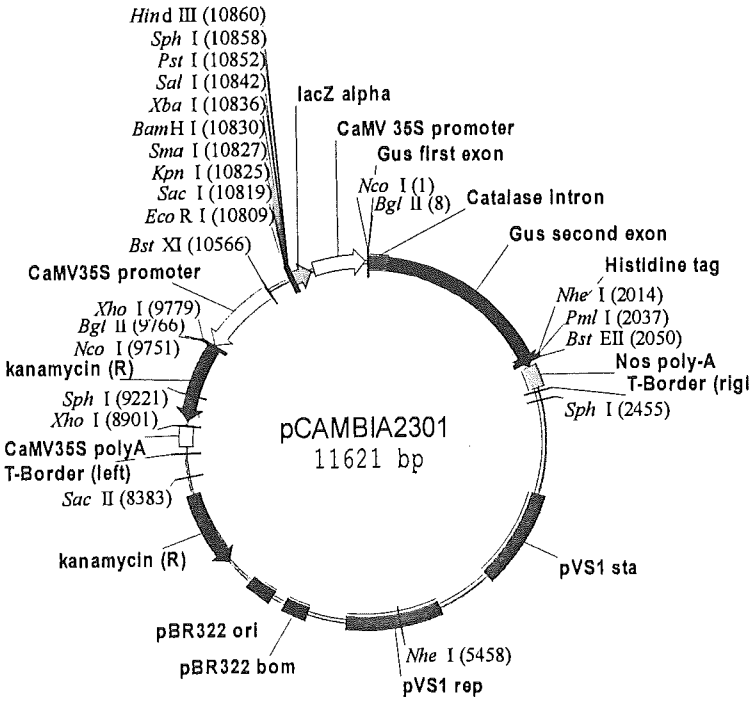


Figure 35. Map of pCAMBIA2301 vector. The chitinase gene was inserted at *Hind* III site.

Plasmid isolation: Highly purified plasmids were extracted with the QIAGEN plasmid kit and protocols provided by the manufacturer. Plasmid isolations from *Agrobacterium* were carried out according to some modifications of existing protocols.

Constructing a vector carrying a chitinase gene: Samples of 30 μg of DNA from each of pBSKS-G11(R) and pCAMBIA2301 were digested with *Hind*III to completion. A subsample of 1 μg of digested DNA was run on 0.8% agarose gel at 45 V for 2 h, to check that digestion was complete. After digestion, plasmid pCAMBIA2301 was extracted with phenol (pH = 8.0) and/or phenol:chloroform:isoamyl alcohol (25:24:1), precipitated with isopropanol, washed with 70% ethanol, and redissolved in 1× TE.

Dephosphorylation of plasmid pCAMBIA2301 was carried out according to the method described by Sambrook et al. (1989). A 1.5-kb DNA fragment, containing a 1.1-kb rice chitinase gene and the CaMV 35S promoter, was recovered from the digested plasmid pBSKS-G11(R), using the BIO-RAD DNA recovery kit according to its manufacturer's instructions. For ligation, 1 μg of the recovered DNA fragment, 0.25 μg of the dephosphorylated plasmid pCAMBIA2301, 1 μL of T_4 DNA ligase (1U/μL), 1 μL of ten-fold ligation buffer, and sterilized distilled water were thoroughly mixed, making a total volume of 10 μL. The ligation mix was then incubated at 15°C for 16 h, after which a sample was run on 0.8% agarose gel to check ligation efficiency.

The ligated DNA product was used to transform *E. coli* DH5α according to the method described above. The success of the cloning was verified by isolating the recombinant DNA from the transformants and digesting it with restriction enzymes *Hind*III and *BamH*I. Two vectors with the chitinase gene, designated pCAMBIACH1 and pCAMBIACH2, were

constructed. Purified pCAMBIACH2 DNA was used to transform the *A. tumefaciens* strain LBA4404. Recombinant DNA was isolated from transformant *A. tumefaciens* cells and digested with *Hind*III for verification. All recombinant DNA techniques were carried out with standard procedures (Sambrook et al., 1989).

In vitro assays of chitinase activity against the anthracnose fungus: Protein extracts, and cell-free filtrates were prepared from *E. coli* DH5α transformants containing the chitinase gene according to documented methods. Preparations of chitinase were obtained from the lysed cells by centrifuging at 5,000 rpm for 30 min, filtering supernatant through a 0.22-μm membrane filter, and concentrating with centricon-10 (Amicon Company, Beverly, MA). Culture supernatant was filtered through a 0.22-μm membrane filter to obtain cell-free filtrates.

These preparations containing the chitinase enzyme were tested for their anti-*C. gloeosporioides* activity, using documented methods (Mauch et al.1988. Plant Physiol. 88:936-942; Kelemu et al. 1995. Australas. Plant Pathol 24:168-172). Fungal spores were harvested from adequately sporulating colonies and suspended in sterilized distilled water. Spore concentrations were adjusted to optical density of 0.02 at 600 nm (OD600 = 0.02). A 50-μL sample of spore suspension was evenly spread on plates containing nutrient agar. Sterilized filter-paper discs were soaked with crude preparations of chitinase, transferred to plates containing fungal spores, and incubated at 28°C for 48-72 h to test for anti-fungal activity. To test the effect of chitinase from cell-free culture filtrates of *E. coli* with chitinase clones on *C. gloeosporioides,* 100 mL of filtrates were placed in a 250-mL Nephelo sidearm flask and inoculated with 1 mL of spore suspension (OD_{600} = 0.05). These were incubated at 28°C with shaking at 200 rpm. Anti-fungal activity was evaluated at intervals from 0 to 8 days. Control treatments had no chitinase preparations.

In vitro transformation and regeneration of Stylosanthes plants: Seeds of *S. guianensis* CIAT 184 were surface-sterilized with 3.0% NaOCl solution for 15 min and rinsed with sterilized distilled water, then treated for 5 min with 70% ethanol and rinsed three times with sterilized distilled water. Seeds were germinated on a basal MS medium (Murashige and Skoog, 1962. Physiol Plant 15:473-495). Cultures were maintained under fluorescent light at 55 μE/m2 per second at 24°C, with a 12-h photoperiod. Segments were excised from leaves for transformation.

Agrobacterium tumefaciens LBA4404, containing plasmid pCAMBIACH2, was left to grow overnight at 28°C, with shaking at 200 rpm, in 10 mL of LB liquid medium, containing 100 μM acetosyringone, 25 μg/mL streptomycin, and 50 μg/mL kanamycin. For co-cultivation, cells were collected from the overnight cultures by centrifuging and resuspended in fresh LB liquid medium. Leaf-segment explants were inoculated by swirling for 2-5 min in the bacterial suspension, blotted dry on sterilized filter paper, plated onto regeneration medium (basal medium with 1.0 μg/mL α-NAA and 4.0 μg/mL BAP), and incubated at 28°C in the dark for 2 days. The explants were then washed in sterilized distilled water, blotted dry on sterilized filter paper, and cultured on the regeneration medium, containing 15 μg/mL kanamycin and 250 μg/mL carbenicillin. After 2 weeks, all growing calli were transferred to a fresh regeneration medium for further selection. The selected green calli were then transferred to basal medium, containing 0.01 μg/mL α-NAA and 4.0 μg/mL BAP for shoot induction. After shoots appeared, the regenerated plantlets were transferred to basal medium, containing 0.1 μg/mL α-NAA and 0.4 μg/mL BAP for elongation. Shoots were excised and cultured on the basal medium with quarter-strength salt and 0.1 μg/mL α-NAA for rooting. Kanamycin and carbenicillin were used in all regeneration steps. Regenerated plantlets were transferred to pots containing autoclaved soil and placed in a greenhouse.

Histochemical test for GUS activity: Tests for GUS activity were carried out according to the histochemical assay procedures described by Jefferson (1987, Plant Mol Biol Rep 5:386-405) and Kosugi (1990, Plant Sci 70:133-140). Tissues from putative transformants were soaked in X-gal buffer at 37°C for 3 h to detect GUS activity.

Inoculating transgenic plants with isolates of C. gloeosporioides, and assessing disease reaction: Resistance of transformed and control plants to C. gloeosporioides was assessed, using two methods: laboratory testing of detached leaves and whole plant inoculation. For laboratory testing, leaves from the transformed and control plants maintained in the greenhouse were placed on wet sterilized filter paper in petri dishes. A 30-μL sample of freshly prepared C. gloeosporioides suspension (OD600 = 0.12) was then added to leaf surfaces. The petri dishes were sealed with parafilm and incubated at room temperature for 3-7 days to evaluate the developing lesions on the leaves. Inoculation on plants was carried out using the methods described by Kelemu et al. (1996, Plant Dis. 80:1355-1358). Inoculum was prepared by washing conida from 1-week-old OMA cultures with sterile deionized water and adjusting the conidial suspension to OD_{600} = 0.12. Each plant was spray-inoculated with conidial suspension until leaves were dripping. The inoculated plants were then transferred to a dark room with high relative humidity (>90%) and temperatures between 21 and 29°C for 2 days. The plants were then transferred to a greenhouse with temperatures between 19 and 30°C until disease symptoms were expressed and evaluations were made.

Results and Discussion

***Anti-fungal activity of the chitinase in vitro*:** The chitinase gene expressed a 35-kD protein in two constructs. The enzyme showed *in vitro* anti-fungal activity against *C. gloeosporioides* (Figure 36). Further studies showed that crude preparations of chitinase enzyme from *E. coli* DH5α, containing plasmid pCAMBIACH2, had a 2.2 times higher anti-fungal activity than those from *E. coli* DH5α, containing plasmid pCAMBIACH1. Evaluation was done by estimating areas of growth inhibition of *C. gloeosporioides* in culture plates.

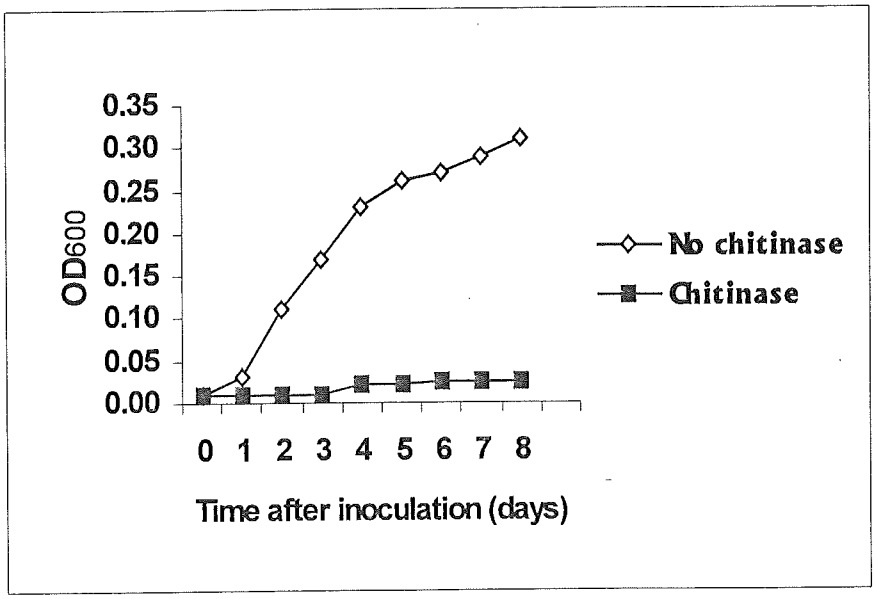


Figure 36. Effect of chitinase on the growth of *Colletotrichum gloeosporioides*. Optical density (OD_{600}) of *C. gloeosporioides* cultures in the presence of cell-free culture filtrates of chitinase and no-chitinase clones.

In vitro plant transformation and regeneration: Calli were induced from leaf segments infected by *Agrobacterium* on selective medium, containing 15 mg/L kanamycin. Shoots were induced from kanamycin-resistant calli cultured on regeneration medium with half-strength salt. These shoots grown from kanamycin-resistant calli could be elongated on regeneration medium, containing 20 mg/L kanamycin (Figure 37). Rooting occurred from shoots excised from calli (Figure 38). Leaves of the primary transformed plants were capable of callus induction. Some kanamycin-resistant plants showed GUS activity.

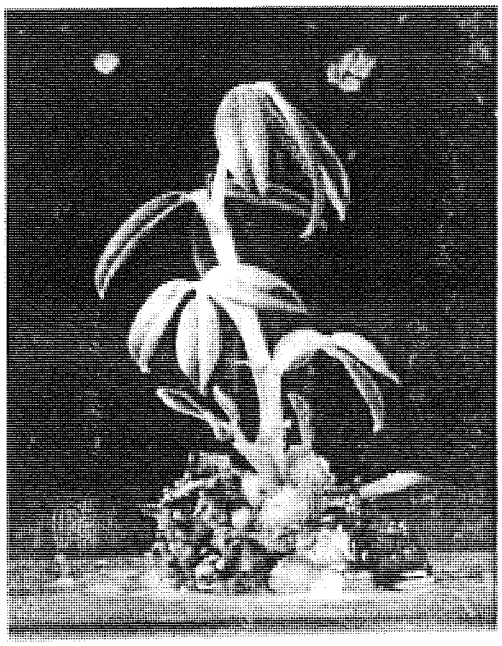


Figure 37. Shoots of *Stylosanthes* plantlets growing from putatively tranformed calli on a regeneration medium .

Figure 38. Rooting of *Stylosanthes* plantlets from putatively transformed calli in a selective medium.

Reaction of transgenic plants to infections by the anthracnose fungus: Results from both laboratory and greenhouse tests showed that transgenic *Stylosanthes* plants had higher levels resistance to *C. gloeosporioides* than control untransformed plants (Figure 39). In lab tests, developing lesions on detached leaves of transgenic plants were smaller than those of untransformed plants (data not shown), and the first disease symptoms of transgenic plants were expressed later than those on control (1 day after inoculation for control, 6 days after inoculation for transgenic plants).

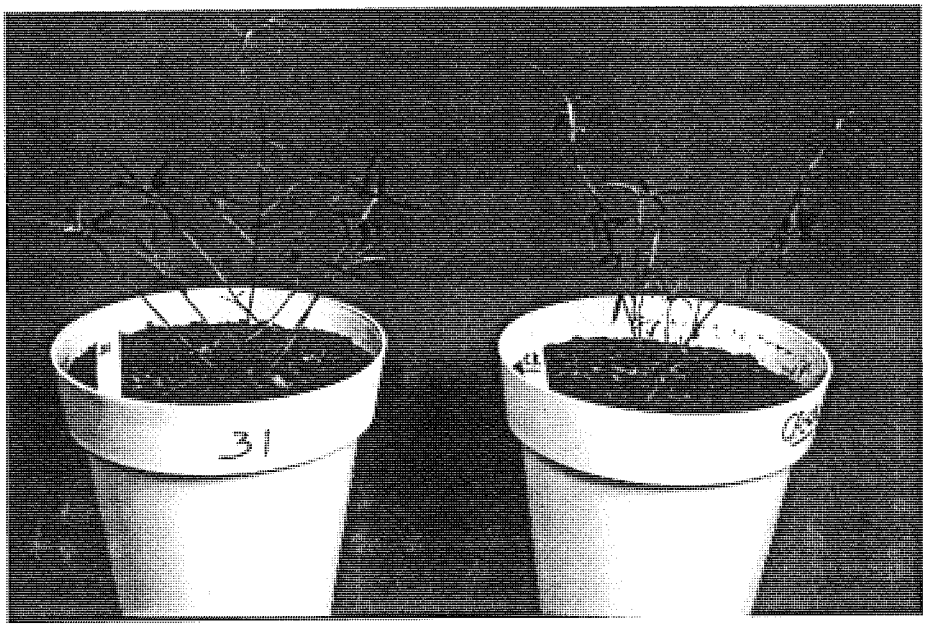


Figure 39. Reactions of transgenic *Stylosanthes* plants (left) and control plants (right) to anthracnose 6 days after inoculations with *C. gloeosporioides* CIAT16100.

2.8.2 Pathogen Population Studies in *Arachis* and *Stylosanthes*

Pathogen population studies in *Arachis pintoi* (F. Muñoz, M. X. Rodriguez and S. Kelemu)

Rationale: *Arachis pintoi* which is native to Brazil, has shown high potential as a forage and as a cover crop in various plantations. Anthracnose disease caused by *Colletotrichum gloeosporioides* is of increasing importance on *A. pintoi* in Colombia. The pathogen causes severe stolon dieback, leaf spots and may kill entire plants. The practice of using vegetative material to propagate *A. pintoi* has probably contributed to the widespread occurrence of the disease.

In this study, we examined the genetic diversity among isolates of *C. gloeosporioides* on *A. pintoi* collected in four regions of Colombia, Caquetá (Amazon), Carimagua (savannas), Palmira and Popayan (seasonally dry mid-altitude hillsides), using randomly amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP). Five accessions of A. pintoi were used to determine pathogenic variability of these isolates.

Materials and Methods

Fungal isolates. Isolates were obtained from diseased tissues of various accessions of *A. pintoi* using established protocols (Kelemu et al., 1996, Plant Disease 80:1355).

Monoconidial cultures of each isolate were grown on sterile filter papers and stored in sealed envelopes at 4 (C.

Pathogenicity test. Mature, healthy trifoliates were detached from each of 5 accessions of *A. pintoi* (CIAT 17434, CIAT 18744, CIAT 18748, CIAT 22160 and the original accession corresponding to each isolate). For cross-infectivity tests, a highly susceptible *Stylosanthes guianensis* accession CIAT 2312 was included. The trifoliates were surface sterilized and placed in sterile sand containing tubes for inoculations as described by Subrahmanyan et al (1983, Plant Disease 67:209). Inoculum preparations and inoculations were conducted as described by Kelemu et al (1996, Plant Disease 80:1355).

DNA isolations, amplification conditions and RFLP analysis. For DNA isolations, fungal cultures were grown in fresh V-8 juice liquid medium for 3 days at 28 °C on a shaker at 200 rpm. DNA was isolated using the method of Dellaporta et al (1983, Plant Molecular Biology Reporter 1:19). Amplification conditions and RFLP analysis were conducted as described by Kelemu et al (1999, European Journal of Plant Pathology 105: 262). Nine, arbitrary 10-base, oligonucleotide primers from Operon Technologies were used for PCR amplifications. An ECL direct nucleic acid detection systems kit (Amersham) was used as directed by the manufacturer to detect hybridized bands and label the probe containing a repetitive element.

Statistical data analysis . Comparisons of each banding profile for each primer were conducted on the basis of presence or absence (1/0) of RAPD products of the same size. Bands of the same size were scored as identical. The pooled data set was analyzed using the Numerical Taxonomy System for personal computer, NTSYS-pc (Exeter software, 1994). A three-dimensional graph of the first three dimensions of a multiple correspondence analysis was constructed, using the "spin" platform of JMP software (SAS, 1995) to provide a visual representation of the association.

Results and Discussion. Anthracnose, caused by *Colletotrichum gloeosporioides*, is a disease of increasing importance on *Arachis pintoi.* Disease symptoms are shown in Figure 40. The pathogenicity of 91 isolates of *C. gloeosporioides* isolated from *A. pintoi* in four regions of Colombia was studied on five accessions (CIAT 17434, CIAT 18744, CIAT 18748, CIAT 22160, and the original host accession corresponding to each isolate).

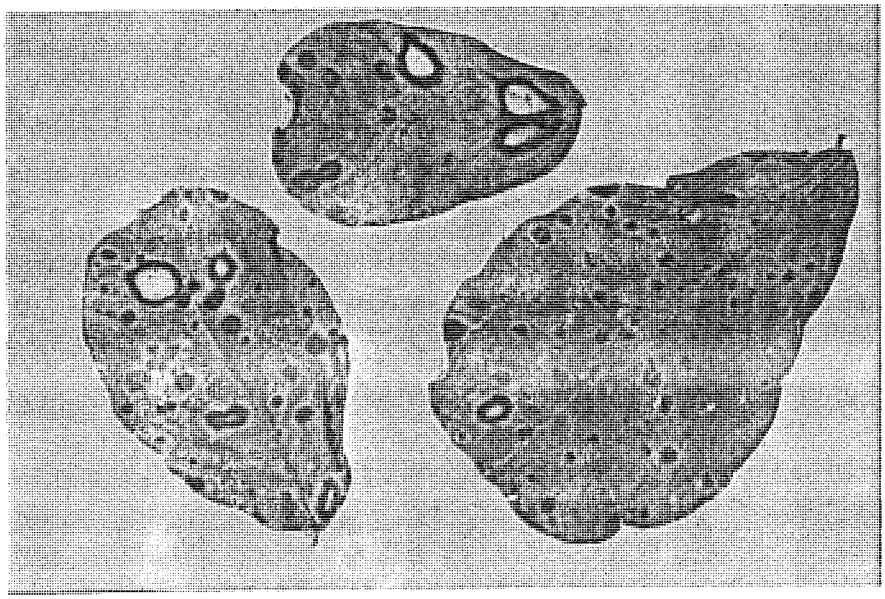


Figure 40. Anthracnose symptoms on *Arachis pintoi.*

Eighty-four of the isolates were pathogenic on at least one host accession, whereas the remaining seven were non-pathogenic. Differential reactions were observed indicating the existence of pathogenic specialization in *C. gloeosporioides* on *A. pintoi*. The genetic variability among these isolates was measured at molecular level by random amplified polymorphic DNA (RAPD), and Southern blot analysis using a repetitive DNA probe (termed CgT1: *C. gloeosporioides* Transposon 1) generated from an isolate of the pathogen infecting the forage legume *Stylosanthes guianensis*. A total of 81 band positions were scored and analysis of the RAPD data revealed at least five groups, with the isolates from one region distributed in all the groups.

A dendrogram figure separated the 91 isolates into five groups (data not shown). Isolates from Caquetá were distributed within four groups, whereas the fifth group consisted of isolates from Palmira. The cluster analysis generated seven groups as shown in Figure 41.

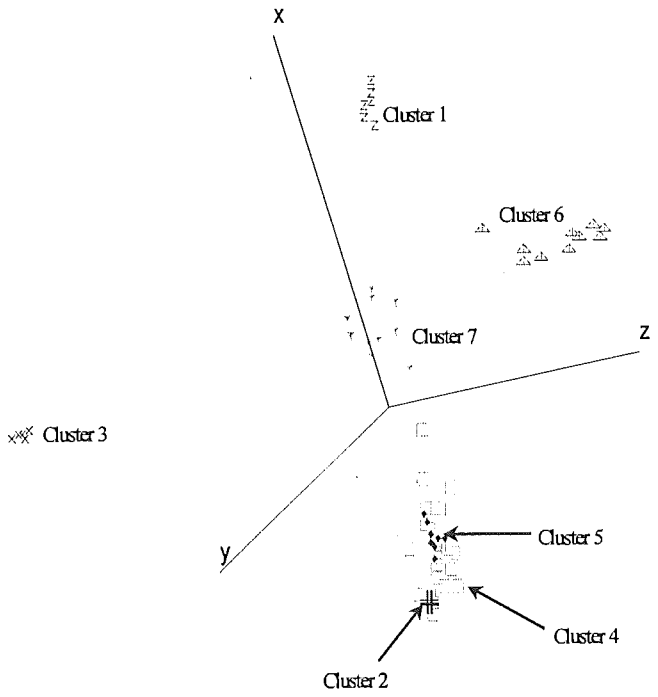


Figure 41. Ninety-one isolates of *Colletotrichum gloeosporioides* clustered based on RAPD data using the spin platform of JMP software (SAS, 1995).

A high concordance was observed between the dendrogram and cluster analysis (Table 46). Clusters 1 (all isolates from Palmira), 3 (Caquetá and Palmira) and 6 (Caquetá and Carimagua) had high Cluster Consistency Index (CCI) [Table 0]. Cluster 1 consisted of 14 isolates from Palmira, cluster 3 had 7 isolates from Caquetá and 3 from Palmira, and cluster 6 had isolates from Caquetá (8) and from Carimagua (3).

Cluster 7 consisted of 6 isolates from Caquetá, 3 isolates from Palmira and one from Popayan. The ten isolates in cluster 3 (with the highest CCI) originated from Palmira and Caquetá. Records of the *Arachis* hosts from which the isolates from Palmira were isolated indicated that the vegetative planting materials originally came from Caquetá. This shows that the isolates from Palmira probably were introduced from Caquetá. It is also likely that almost all locations had some introductions from Caquetá through vegetative materials.

RFLP data revealed that DNA from only 5 of the 91 isolates (CIAT 16453, CIAT 16485, CIAT 16486, CIAT 16576, CIAT 16499) hybridized with the repetitive DNA probe pCHB1 (cloned from an isolate specific to *S. guianensis*). Interestingly, these isolates also caused anthracnose symptoms on a highly susceptible accession of *S. guianensis*.

Table 46. Concordance of dendrogram groups and cluster assignments of isolates of *Colletotrichum gloeosporioides*, and Cluster Consistency Index (CCI).

Dendrogram Groups	Cluster identification in Cluster Analysis	Cluster Consistency Index (CCI)*
A	3	1.00
B	2	0.29
	4	0.00
	5	0.00
C	7	0.28
D	6	0.72
E	1	0.93

*High CCI values indicate high cluster stability.

Pathogen population studies in anthracnose disease of *Stylosanthes* (C. I. Giraldo and S. Kelemu)

Rationale: Anthracnose disease continues to be the most important limitation to *Stylosanthes* production. The pathogen population is diverse and complex. Knowledge on the pathogen population structure is crucial to effective disease management.

Materials and Methods: Fungal cultures, DNA isolations, PCR analysis, plant inoculations, and pathogenicity evaluations, statistical data analysis were all as described before in various publications (Kelemu, et al., 1996, 1997 and 1999). In addition to 13 random primers of 10 bases, two18-base primers containing fungal microsatellite sequences were used.

Results and Discussion: Isolates of C. gloeosporioides collected from S. guianensis fields in Palmira, Carimagua, Caquetá and Brazil were characterized. The isolates were collected in 1993 and 1994. The PCR amplifications revealed a total of 102 scorable DNA bands. The dendrogram of the RAPD data (Figure 42) is primarily organized into two groups which diverge with a genetic similarity value of 0.4. The larger of the two group, which contains 69% of the isolates, consists mostly of isolates from Caquetá with a similarity value of 0.77.

This group also contains three of the four isolates from Palmira. The second group, however, is diverse with isolates from all four locations. This second group had a subgroup containing 6 of the 7 Brazilian isolates with a similarity value between 0.76 and 0.96, and another subgroup with isolates from Carimagua and Caquetá. Pathogenicity evaluations on S. guianensis differentials resulted with distinct 5 races and others in between (Figure 43).

Most of the Brazilian isolates were in group 2. All of the isolates in groups 3 and 4 were from Caquetá. Group 5, which was the most pathogenic, contained isolates from Carimagua, Caquetá and Palmira. Isolates 16097 (Caquetá), 16096 (Caquetá), 16066 (Carimagua), 16020 (Palmira), 16051 (Palmira), 16072 (Brazil) and 16058 (Palmira) were the most pathogenic. Of the differential hosts used, S. gianenisis 2312 was the most susceptible followed by cv. Endeavour. cv. Mineiraó, CIAT 1283 and CIAT 2340 were highly resistant.

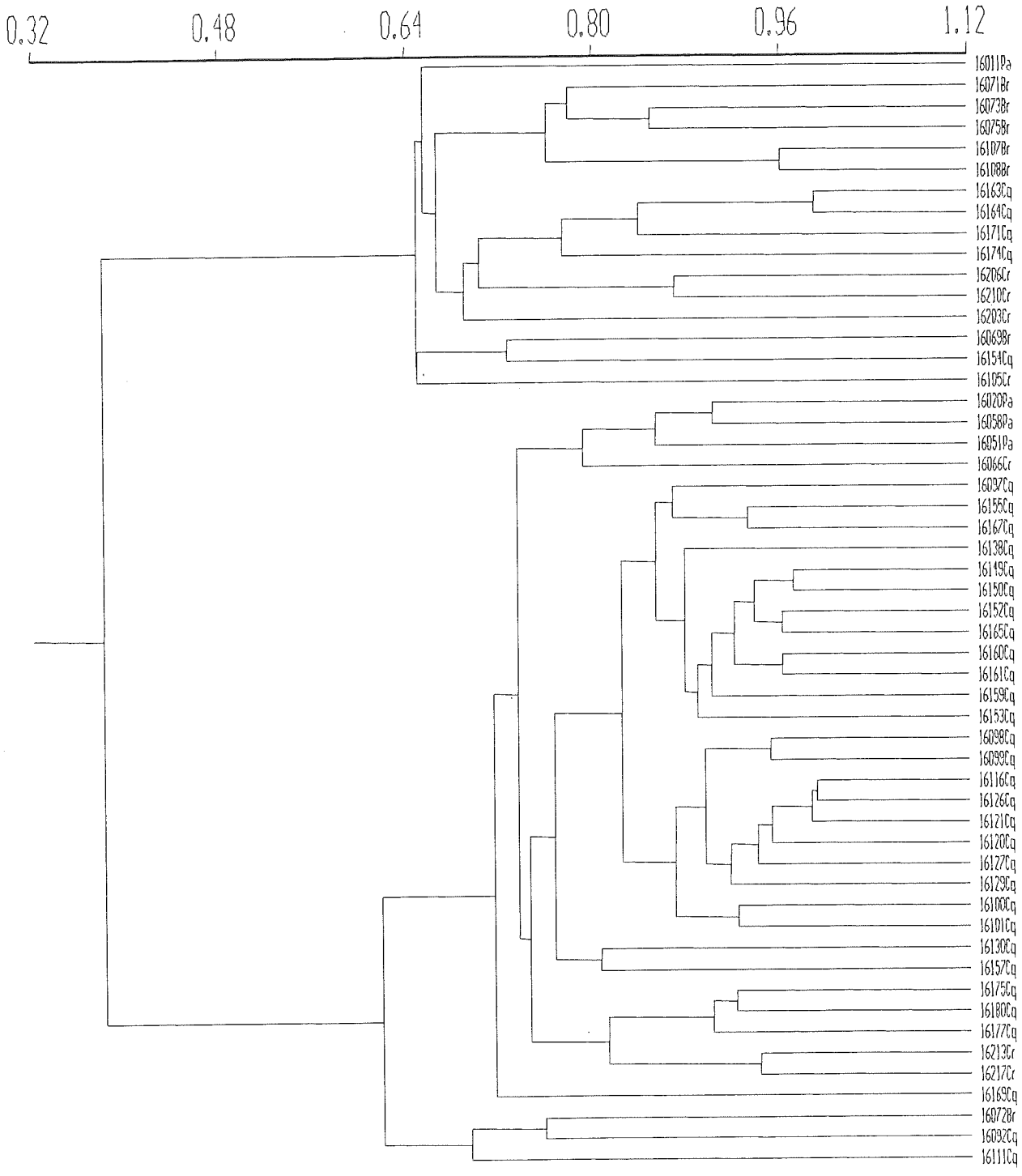


Figure 42. Similarity dendrogram of isolates of *C. gloeosporioides* constructed based on RAPD loci using NTYS.

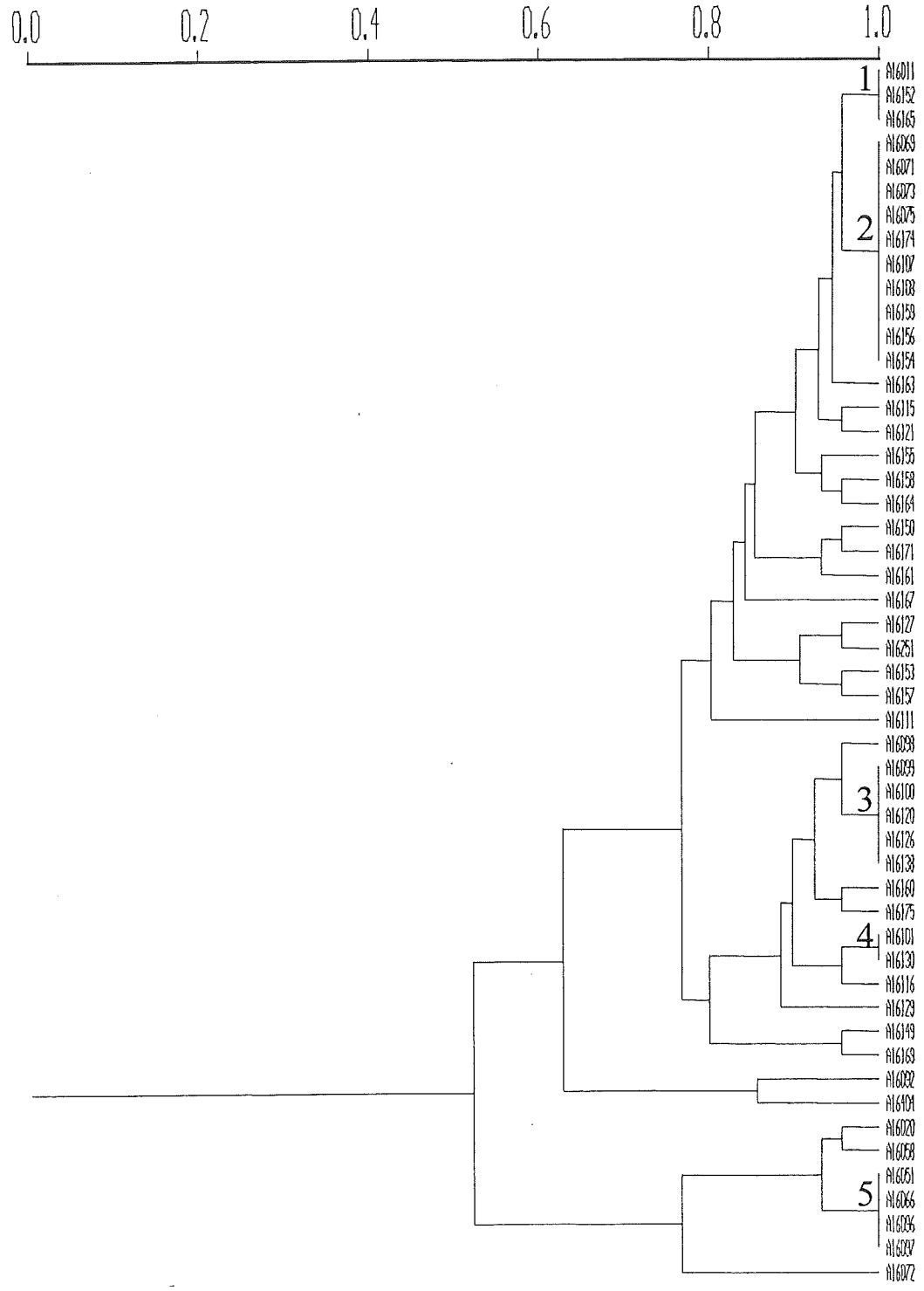


Figure 43. Similarity dendrogram of isolates of *C. gloeosporioides* constructed based on pathogenicity on differential hosts.

2.8.3 Establishment of field plots for anthracnose epidemiology studies (F. Muñoz, G. Segura and S. Kelemu)

A field plot of various accessions of *S. guianensis* with various levels of anthracnose resistance were established at the CIAT station in Quilichao. The objectives of this work are to: 1) study anthracnose development on various accessions of *S. guianensis* in relation to weather parameters, 2) study pathogen population dynamics in the field plot over a period of three years, and 3) pathogen race distribution on resistant accessions over a period of three years.

Rationale: Disease epidemiology is basically the study of pathogen populations in host populations and the development of disease resulting under environmental interactions. In short it is the study of disease dynamics under natural conditions. Epidemiology, thus, provides information on disease development in the field. This information in turn contributes to disease management strategies.

Materials and Methods: Thirteen selected *S. guianensis* genotypes (CIAT 184, CIAT 136, cv. Mineiraó, CIAT 11833, CIAT 11844, bulk breeding populations FM9405 parcelas 1-5, GC 1578, CIAT 2312, CIAT 2340, cv. Endeavour) and 2 cultivars of *S. scabra* (cv. Fitzroy, cv. Seca) were planted in three replications at a field site in Quilichao. In addition, differential host genotypes were planted in one block for isolate collections.

Results and Discussion: Due to unusually heavy rains, a number of the planted materials were destroyed. It took several attempts and transplanting to finally establish the field. The weather monitor was transported from Caquetá station. One of the important parts of the monitor, the humidity sensor required replacement. A new replacement humidity sensor shipped from Australia, unfortunately, was damaged on the way to Colombia due to poor packaging. In any case, we expect to receive another sensor in good conditions and the system will be running before the end of the year. The pathogen population dynamics in the field and the disease progress in relation to weather parameters and pathogen diversity will be studied.

2.8.4 Studies on a bacterial wilt disease of *Brachiaria* (C. Zuleta and S. Kelemu)

Rationale: A number of *Brachiaria* genotypes maintained in the glass house at CIAT showed a wilt disease which eventually killed affected plants. Some of these materials have been incorporated in the breeding program, which gives the possibility of having susceptible genetic backgrounds in promising breeding products. Work has been initiated to establish the cause of the problem and to possibly look for control measures.

Materials and Methods: The bacterial wilt disease appeared in CIAT 1015, a cross between *B. ruziziensis* (44-03) and *B. decumbens* CIAT 606. Isolations were conducted on general bacterial (nutrient agar) and fungal (potato dextrose agar, PDA) media. Small leaf tissues were surface sterilized in 1% NaOCl solution for 3 min, in 70% ethanol for 1 min, and rinsed three times with sterile distilled water. A portion of the samples was plated on PDA and incubated at 28 °C. The remaining tissue samples were macerated in sterile distilled water, spread on nutrient agar plates and incubated at 28 °C.

Healthy tillers of *Brachiaria* plants were separated and planted pots containing sterile soil. Experiments involved to determine inoculation method(s) and disease agent were: needle inoculation method, root soaking in bacterial suspension for 12 h, cutting of leaves with scissors immersed in bacterial suspension, spray inoculation with bacterial suspension containing 5% twin 20, and use of forceps with needles glued on one side and piece of

sponge in another for bacterial suspension introduction into tissues. Control plants were inoculated treated similarly with sterile distilled water. Plants were then placed in humidity chambers for 48 hours and then moved to a growth chamber with RH of 70% and temperature 30 °C. Bacterial identification was done using standard protocols.

Results and Discussion: The disease is devastating under optimum conditions eventually killing the entire plant (Figure 44). It can also be transmitted during plant handling and transplanting. Three types of bacterial colonies were isolated on nutrient agar (colors white, cream and yellow). No fungal colonies were isolated. Of the three bacterial colonies, the yellow colony consistently produced disease symptoms after artificial inoculations. The most effective inoculation methods were the use of scissors to introduce bacteria and forceps with needles and sponges. The bacterium was identified as *Xanthomonas* sp.

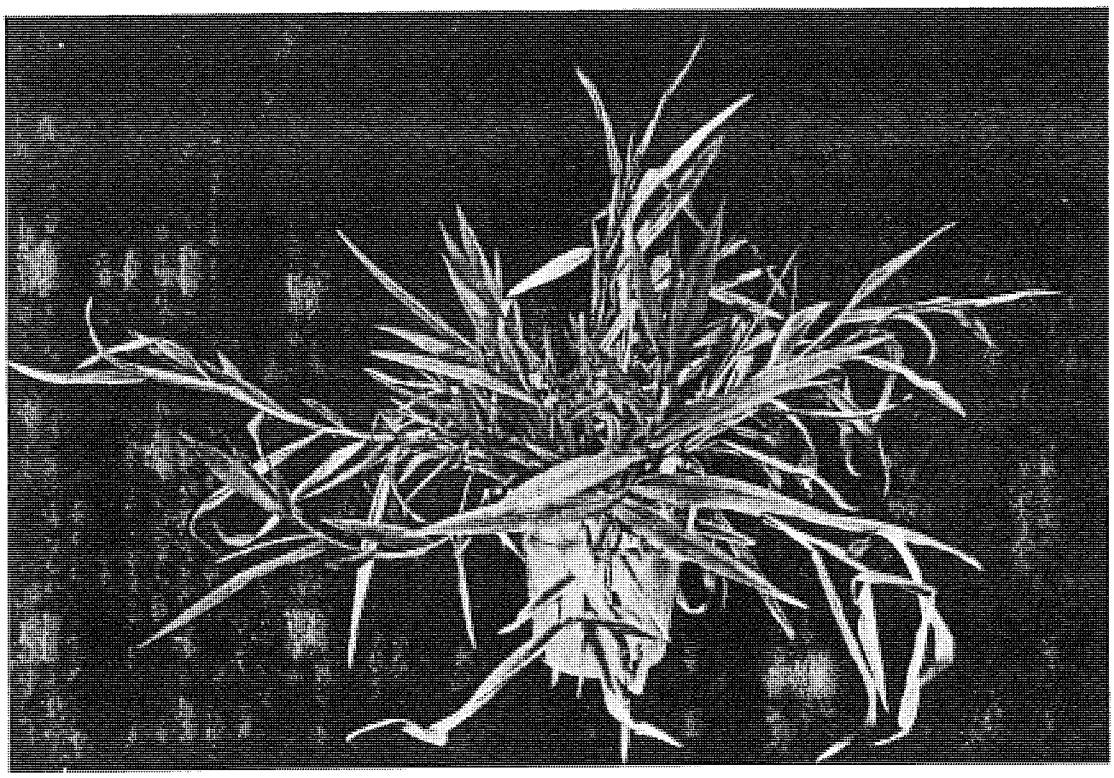


Figure 44. Bacterial disease symptoms in *Brachiaria.*

Identifications of the wilt disease causing bacterium were based on characteristics shown in Table 47. A number of chemical tests were conducted to confirm the identification (data not shown). The devastating nature of the disease and the fact that it appeared among the breeding background materials required attention. The symptom can easily be confused with that of spittlebug damage. We hope to look for sources of resistance in *Brachiaria.*

Table 47. Differential characters for bacterial identification.

Tests	Results
Gram stain	Gram negative
Yellow colonies on yeast extract-dextrose-$CaCO_2$ (colonies mucoid)	+
Growth on MS agar	-
Pectate degradation	-

New work initiated:

1) **Bacterial endophytes** (Viviana Pizo and S. Kelemu): An undergraduate thesis has been initiated on bacterial endophytes in *Brachiaria*.

2) **Antifungal compounds** (Gustavo Segura and S. Kelemu): A large collection of wild forage legumes exist in CIAT. These materials could harbor unique anti-fungal compounds for potential use in disease control.

Progress towards achieving output milestone

- Isolates (100 of *C. gloeosporioides* are isolated from *S. guianensis* using PCR and differential host.
- Benefit of chitinase gene on confering resistance to anthracnose in transformed *Stylosanthes* is demonstrated.

A paper describing a comprehensive anthracnose pathogen population study was published in the European Journal of Plant Pathology. This paper analyses the pathogen population in various *Stylosanthes* growing locations.

Transgenic *Stylosanthes guianensis* plants containing a rice chitinase encoding gene were generated and these plants showed high level of anthracnose resistance in the laboratory and glasshouse tests performed. Our results from this year also indicate that anthracnose disease may be a serious threat to *Arachis pintoi*.

Output 3: Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed

Activity 3.1: Identify genotypes of *Brachiaria, Panicum* and *Arachis* with adaptation to low fertility soils

Highlights

- Showed that internal detoxification of Al in root apices by malic and citric acid is a mechanism of Al tolerance in *B. decumbens.*
- Developed a rapid and reliable screening procedure to evaluate aluminum tolerance of *Brachiaria* genotypes using vegetative cuttings.
- Nominated a new *Brachiaria* accessions for grazing trials based on regional agronomic trials in Colombia and Costa Rica.
- Selected *A. pintoi* CIAT 18747 for rapid establishment in acid soils.
- Showed that three accessions of *A. pintoi* (CIAT 18744, 18747 and 18751) are more dependent on VAM association when rock P is applied as P source as compared with other accessions (CIAT 22160 and 22172).
- Demonstrated that P supply to soil is more important for *A. pintoi* establishment than tillage.
- Selected *Panicum maximum* CIAT 36000 as an alternative for acid infertile soils given its low N and P requirements as compared to commercial cultivars.

A major limitation for forage production on low fertility acid soils is the low supply of nutrients. Tropical grasses and legumes that are adapted to infertile acid soils have root and shoot attributes that are linked to strategies to acquire nutrients in a low pH and high Al (aluminum) environment. Identification of those plant attributes is fundamental to develop more efficient screening procedures for germplasm evaluation and/or improvement.

3.1.1 Studies on mechanisms of acid soil adaptation in *Brachiaria* cultivars and development of a screening method (P. Wenzl, G. M. Patiño, A. L. Chaves and I. M. Rao)

Last year, we reported significant progress in defining specific physiological and biochemical mechanisms that contribute to superior adaptation of *Brachiaria decumbens* to low supply of phosphorus and nitrogen (1998 IP5 AR). We also elucidated the chemical structure of two secondary metabolites that are induced in roots of *Brachiaria* species under phosphorus and nitrogen deficiency.

Furthermore, we demonstrated that the level of Al tolerance of *B. decumbens* is markedly superior to that of other *Brachiaria* species and other crop species such as corn and wheat. During 1999, we achieved significant progress in (*i*) the elucidation of physiological mechanisms underlying Al tolerance of *B. decumbens* and (*ii*) the modification of a simple Al tolerance screening procedure for application to vegetative cuttings instead of seedlings.

Species of *Brachiaria* species are the most widely planted tropical forage grasses in the world. For example, in Brazil alone, more than 70 Million hectares are planted with *Brachiaria* pastures. The center of diversity of *Brachiaria* lies in eastern Africa, from where they have been exported to Australia, South America and Asia. Introduced pastures of *Brachiaria* contributed to a marked increase in livestock production, particularly in the tropical savanna regions of South America. Most of the currently used cultivars have been directly derived from natural apomictic germplasm. All existing commercial cultivars have recognized defects, mainly susceptibility to spittlebugs or poor edaphic adaptation.

This was the main reason for the initiation of the *Brachiaria* breeding program at CIAT. It aims at combining superior acid soil adaptation, found in *B. decumbens*, with resistance to spittlebugs, found in *B. brizantha*. Both species are apomictic, but produce fertile pollen, which can be used to pollinate the sexual species, *B. ruziziensis*. In this way, it is possible to combine genes of the two apomictic species. The challenge is to develop rapid and reliable screening procedures to evaluate thousands of genetically recombined individual plants for their level of adaptation to acid soils, since the traditional field-based methods are expensive and time-consuming.

Previous research has demonstrated that *Brachiaria* species, when cultivated under nutrient-limited conditions similar to those found in soil solutions of infertile acid soils, exhibit significant interspecific variation in Al tolerance, with *B. decumbens* cv. Basilisk being the most tolerant (1998 IP5 AR). This year we investigated putative physiological mechanisms contributing to the high level of Al tolerance of *B. decumbens* and evaluated the suitability of the root elongation method for evaluating Al tolerance of *Brachiaria* genotypes using vegetative cuttings instead of seedlings.

A) Investigation of Al tolerance mechanisms of *B. decumbens*

Methods: Groups of two hundred seedlings each of *B. ruziziensis* cv. Common and *B. decumbens* cv. Basilisk were cultivated for 13 days in a nutrient solution that simulated the nutrient-deficient conditions found in soil solutions of typical acid soils (1997 IP5 AR). Al was added to these solutions to achieve an Al^{3+} activity of 44 or 114 µM, along with a control treatment lacking Al. Al, organic acids and P were analyzed in root apices, the entire root system and shoots using atomic absorption spectroscopy with a graphite furnace atomizer, HPLC and the malachite green method, respectively.

Organic acids were also analyzed in exudates collected from intact root systems. Callose in root apices was quantified fluorimetrically. Efflux of organic acids and phosphate from root apices was measured using excised root tips exposed to solutions containing increasing Al levels. Excised root apices were also employed to quantify proton influx, the site of which was located using bromocresol green as a pH-sensitive dye.

Furthermore, Al fractions in roots and shoots were determined by extraction of frozen tissues at pH 7.5 and subsequent ultrafiltration using a membrane with a size exclusion limit of 5,000 Dalton. The surface area of root apices and the entire root system of plants was determined using a root image analysis software (WinRHIZO) after scanning stained roots with a flatbed scanner.

Results and Discussion: Comparison of growth of two *Brachiaria* species with contrasting acid soil adaptation demonstrated that the acid soil-adapted species, *B. decumbens*, was much less affected by Al toxicity than *B. ruziziensis*, whose persistence in acid soils is poor. The interspecific difference in growth was also confirmed by differential staining of root apices with hematoxylin (Figure 45).

It was clear that the level of Al tolerance of *B. decumbens* is remarkable. The total concentration of Al in the more concentrated treatment was 222 µM, which is considerably higher than the sum of all other cations, and the phosphate concentration was just 1 µM. Quantitative measurements of root growth, root apical diameter and callose content in root apices confirmed the marked interspecific difference in Al tolerance between the two species (Figure 46).

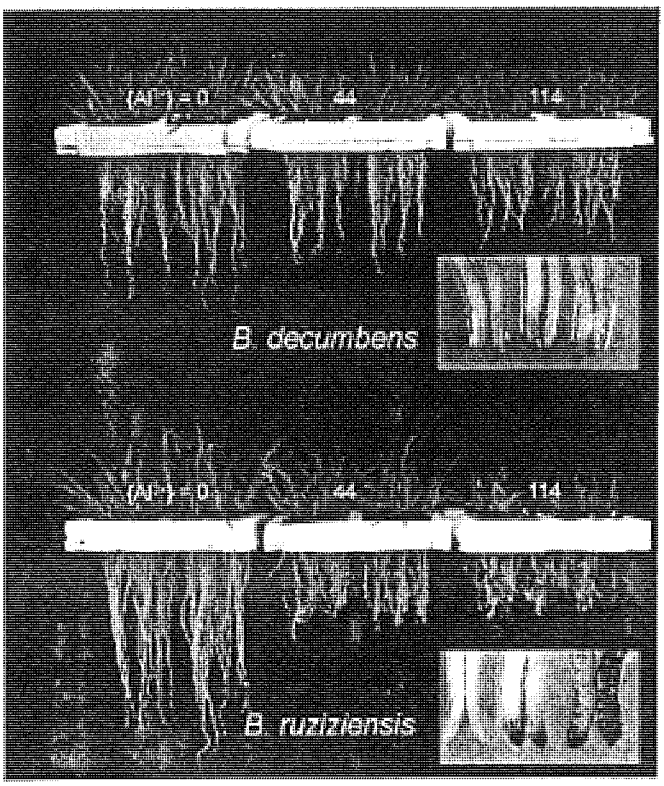


Figure 45. Growth of *B. decumbens* and *B. ruziziensis* in nutrient solutions containing increasing concentrations of aluminum (Al^{3+} activity of 0, 44 and 114 μM) and sub-optimal levels of nutrients similar to those found in acid soils (see IP-5 Annual Report, 1997). The inserts display hematoxylin-stained root apices of plants taken from the three treatments.

Since the level of Al tolerance of *B. decumbens* appeared to be so high, we were interested to compare it to other species, for which Al tolerance mechanisms have been investigated. A root elongation bioassay with seedlings demonstrated that Al tolerance of *B. decumbens* was markedly superior to all crop species for which similar assays have been reported in the literature (1998 IP5 AR).

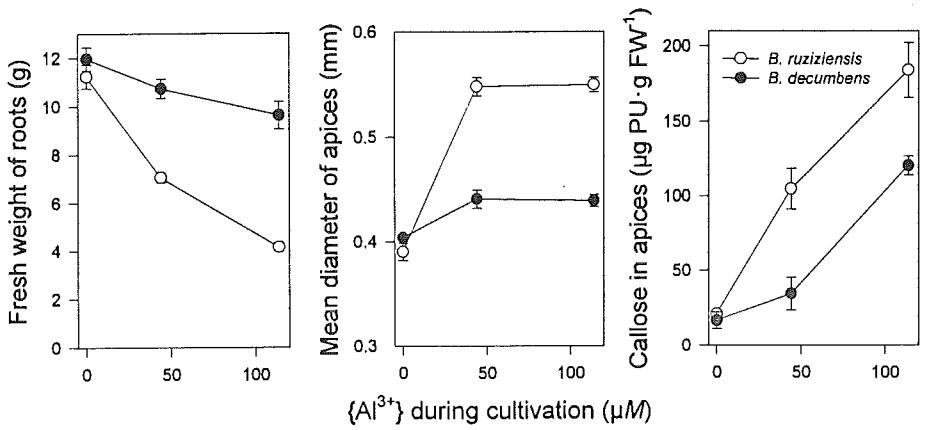


Figure 46. Interspecific difference in Al tolerance between *B. decumbens* and *B. ruziziensis*. Overall root growth (*left half*), diameter of apices of adventitious roots (*middle*) and callose content of apices of adventitious roots (*right half*) indicate that *B. decumbens* is significantly more Al tolerant than *B. ruziziensis*.

With the exception of high-Al treatment, which severely damaged *B. ruziziensis* plants, *B.decumbens* did not take up much less Al than *B.ruziziensis* (Figure 47, left half). This observation indicates the importance of internal Al tolerance mechanisms as opposed to Al resistant mechanisms based on exclusion of Al by external detoxification. Only in root apices of *B.decumbens*, the Al concentration seemed to be somewhat lower in the low-Al treatment, suggesting that Al exclusion mechanisms may also contribute to the interspecific difference in Al tolerance. The Al content of shoots was much lower than that of roots, even in the case of *B. ruziziensis* that was cultivated in the high-Al treatment. It thus seems that both species can retain Al taken up by roots within the root system and prevent transport to shoots.

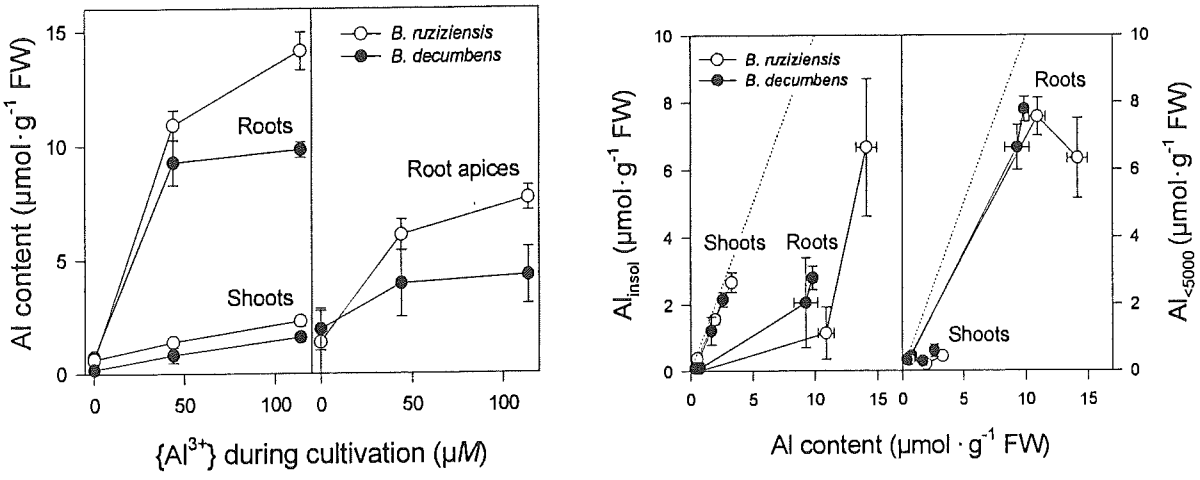


Figure 47. Uptake of Al by *B. decumbens* and *B. ruziziensis*. *Left half:* comparison of Al concentrations in roots, root apices and shoots. *Right half:* Al fractions in roots and shoots. Dotted lines display the total Al content.

The fact that most of the Al extracted with a high-pH buffer from roots passed through a filter with a size exclusion limit of 5,000 Dalton (Figure 47, right half, right graph), suggests that Al in roots is largely bound to low-molecular-weight compounds. In shoots however, most of the Al was found in the insoluble fraction, i.e. bound to cell walls or membranes (Figure 47, right half, left graph). A notable exception to this pattern is the marked elevation of the level of insoluble Al at the expense of Al bound to low-molecular-weight compounds found in roots of *B. ruziziensis* from the high-Al treatment. This probably reflects a severe intoxication of roots with Al.

In several species, including wheat, corn, buckwheat and snapbeans, exudation of organic acids by roots and particularly root apices, has been inferred to confer Al resistance through chelation of Al^{3+} in the rhizosphere. Given its remarkable level of Al tolerance in *B. decumbens*, we hypothesized that there are probably multiple Al tolerance mechanisms, one of which might be organic acid exudation. Indeed, experiments designed to measure organic acids exuded by the entire root system of hydroponically cultivated plants, confirmed exudation of oxalic, citric and malic acid by roots of *Brachiaria* species. Exudation of any of these three organic acids has been found to increase Al tolerance in other species.

However, when plants are grown in the presence of Al, the less Al-tolerant species, *B. ruziziensis*, was found to exude larger quantities of oxalic and citric acid (Figure 48). Furthermore, in *B. ruziziensis* the exudation of these two acids was stimulated by Al, while in *B. decumbens* this was only the case for citric acid.

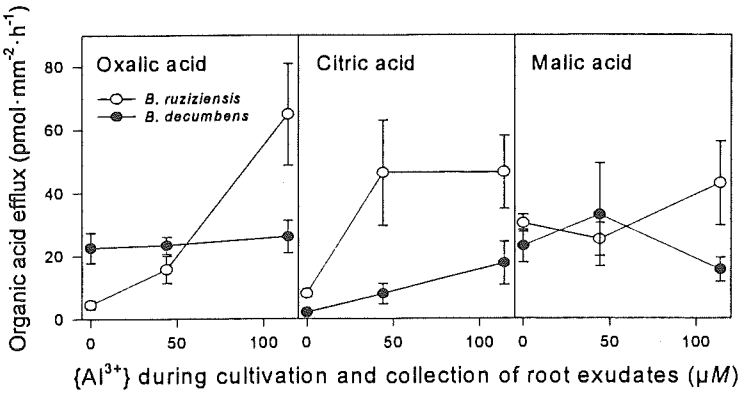


Figure 48. Exudation of organic acids (per root surface area) by intact root systems of *B. decumbens* and *B. ruziziensis*.

Next, we focused our attention to quantify differences in exudation of organic acids by root apices. Using excised root apices from adventitious roots, we found that exposure to Al during cultivation increased exudation of oxalic and citric acid by root apices. *B .decumbens* exuded more oxalic acid while *B.ruziziensis* exuded more citric acid. Root apices of *B. decumbens* also exuded malic acid (Figure 49, left half). However, it should be noted that the level of exudation (as measured per root surface area) was only about 20 - 50 % of that measured for the entire root system (compare Figures 48 and 49). Also, root apices of other species, which are less Al tolerant, exuded considerably higher levels of organic acids (Figure 49, right half). Taken together, these data cast considerable doubt on the idea that exudation of organic acids by root apices is the major Al tolerance mechanism of *B. decumbens*. On the contrary, they suggest that other strategies for Al detoxification are necessary to explain the markedly superior level of Al tolerance of *B. decumbens*.

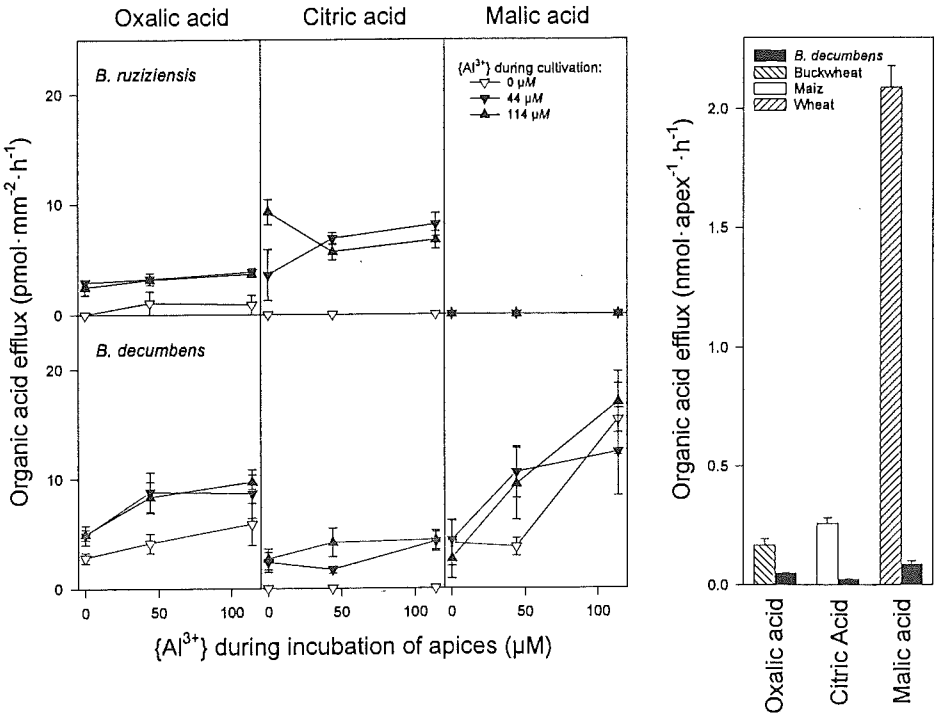


Figure 49. *Left half:* exudation (per root surface area) of organic acids by excised root apices. *Right half:* comparison with Al-induced organic acid exudation by root apices of other species as reported in the literature.

In contrast to the low level of organic acid exudation, high concentrations of organic acids accumulated in root apices. Organic acid levels in root apices of *Brachiaria* species were typically greater than that of the other species. In both species, Al uptake by root apices stimulated the accumulation of citric acid. In the case of malic acid, a similar Al-stimulated accumulation was only found in *B.decumbens.* In *B. ruziziensis*, Al had the reverse effect, i.e. it caused a significant decrease of the malate concentration within root apices (Figure 50, lower row). It follows that a more efficient detoxification of Al by chelation with large amounts of malic in addition to citric acid in root apices may be a reason for the high Al tolerance of *B. decumbens* compared to *B. ruziziensis*. However, it should be noted that out of the three organic acids found in root apices of *Brachiaria* species, malate is the least efficient Al chelator, while oxalate, which was only found at very low concentrations, is the most efficient. We further investigated whether Al stimulated organic acid accumulation in other tissues as well. At the level of the root system as a whole, i.e. including root apices and mature parts of roots, we found that Al uptake exclusively stimulated accumulation of citric acid, suggesting that accumulation of malate in root apices of *B. decumbens* was confined to root apices (Figure 50, middle).

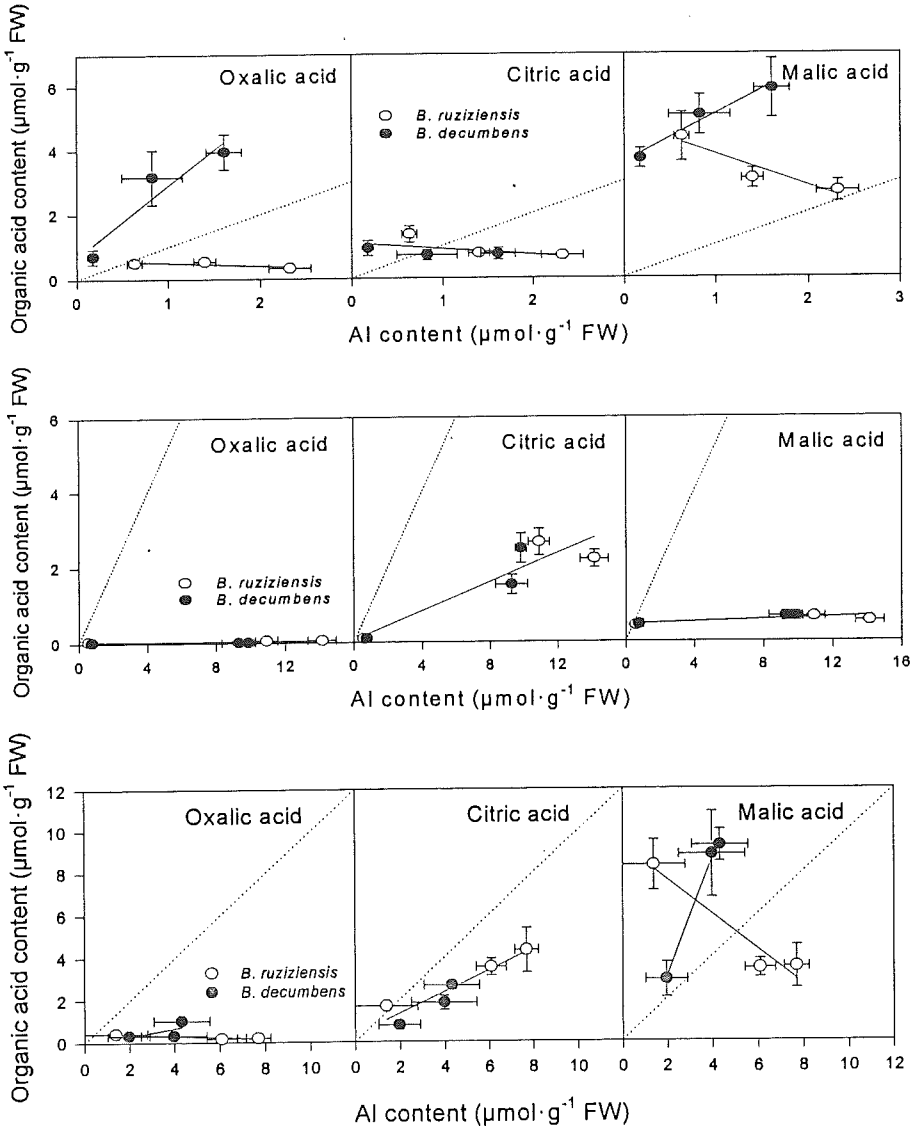


Figure 50. Accumulation of organic acids in shoots (*upper row*), roots (*middle*) and root apices (*lower row*) as stimulated by Al within the tissue. Dotted lines represent a 1:1 molar ratio between Al and the respective organic acid.

Al transported from the root system to shoots stimulated accumulation of oxalic and malic acid in shoots of *B. decumbens*. No comparable effect was detected in shoots of *B. ruziziensis* (Figure 50, top row). Hence, a more efficient detoxification of Al within shoots might also contribute to the superior level of Al tolerance of *B. decumbens*. However, the fact that most of the Al in shoots was found in the insoluble fraction (Figure 47, right side) suggests that Al detoxification by low-molecular-weight compounds in shoots may not be important.

We continued our survey of possible Al tolerance mechanisms by investigating pH changes around root apices. A stimulation of the H^+ influx at root apices was found to increase Al tolerance of *Arabidopsis*. Using a simple staining method, we showed that both species increase the apoplastic pH of root apices (not shown). The region of root apices with the strongest pH increase appeared to be the transition zone, where cells prepare for rapid elongation. This region has been shown to be the primary target site of rhizotoxic Al ions.

Since only small amounts of organic acids were exuded and possible pH changes caused by their efflux are expected to be small, we quantified the pH increase in terms of an influx of protons. The results indicated that: (i) both species exhibit a substantial proton influx at root apices, and (ii) proton influx of *B. decumbens* seems to be less affected by Al toxicity than that of *B. ruziziensis* (Figure 51).

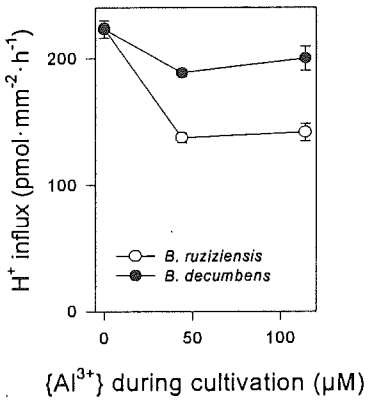


Figure 51. Alkalinization of the incubation medium caused by excised root apices. Influx of H^+ into root apices was calculated from the pH-increase measured during incubation of excised root apices in 200 µM $CaCl_2$ pH 4.20.

Apart from the experiments outlined so far, we also investigated the possible contribution of an efflux of phosphate from root apices to Al tolerance of *Brachiaria* species. We found that phosphate efflux was significantly lower than that of a wheat variety classified as Al tolerant. Also, root apices of *B. ruziziensis* exuded more phosphate than that of *B. decumbens* (not shown). These data imply that phosphate exudation does not contribute to the superior level of Al tolerance of *B. decumbens*.

When measuring total P content in root apices, however, we found that Al toxicity caused a dramatic decrease in the P content of root apices of *B. ruziziensis*, while that of *B. decumbens* was virtually unaffected by Al exposure (Figure 52, left half). Apparently, the decrease of the P content in root apices was caused by an inhibition of P transport from mature portions of the root system into apices (Figure 52, right half).

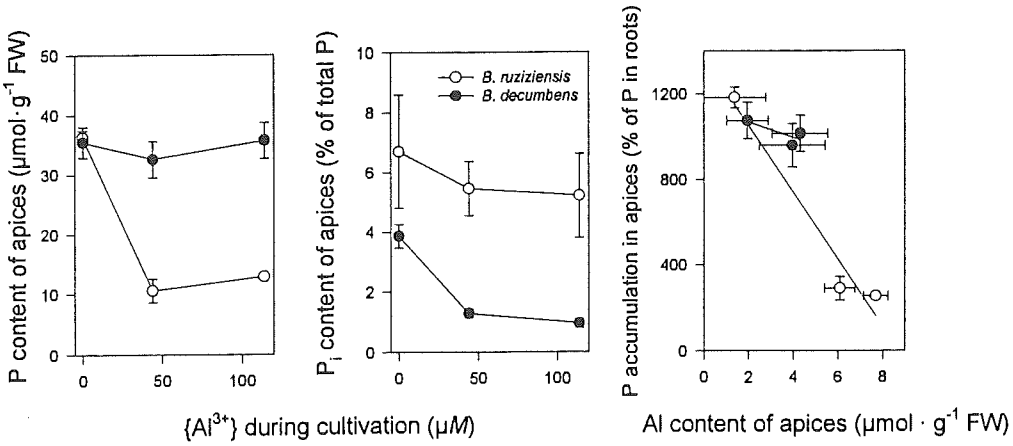


Figure 52. Al-P interactions in root apices. *Left half:* Total P content of root apices. *Middle:* phosphate content of root apices as % of total P. *Right half:* Inhibition of P accumulation in root apices by Al.

We thus hypothesized that the higher P content of root apices of *B. decumbens* could account for the superior Al tolerance of *B. decumbens*, provided that Al was detoxified within root apical cells by binding to phosphate ions. However, measurement of the phosphate concentration of root apices ruled out this possibility. The phosphate concentration of root apices of *B. decumbens* was found to be significantly lower than that of apices of *B. ruziziensis*, and exposure to Al decreased it further (Figure 52, middle). Still, the possibility remains that other phosphate-containing organic compounds detoxify Al ions within cells of root apices.

In summary, data obtained so far suggest that:

(i) *B. decumbens* exhibits an outstanding level of Al tolerance when compared to *B. ruziziensis* and crop species such as wheat or corn,
(ii) exudation of organic acids and phosphate by root apices contributes probably little to Al tolerance of either *Brachiaria* species,
(iii) the increase in pH around root apices contributes to Al tolerance in both *Brachiaria* species,
(iv) both *Brachiaria* species detoxify Al in all parts of the root system by chelation with citrate,
(v) detoxification of Al in root apices by malate in addition to citrate may enhance Al tolerance of *B. decumbens*,
(vi) both *Brachiaria* species retain Al within the root system, and
(vii) the relatively small amount of Al that is translocated into shoots is detoxified by oxalic and malic acids in *B. decumbens*, while *B. ruziziensis* seems to lack an Al-responsive detoxification mechanism based on chelation by organic acids in shoots.

Still, the very high level of Al tolerance of *B. decumbens* gives reason to suspect that, in addition to internal chelation of Al by organic acids, other so far uncharacterized Al tolerance mechanisms may be at work.

B) Development of a screening procedure for acid soil adaptation of *Brachiaria*

Rationale: A stepwise screening procedure was proposed to evaluate seedlings of genetic recombinants of *Brachiaria* for their degree of adaptation to acid soils (IP5, 1998 AR). However, availability of adequate amounts of seed for evaluation of newly generated recombinants is a major limitation to apply the seedling method. Therefore, we tried to adapt the root elongation method to stem cuttings.

Methods: Stem cuttings were rooted in a $CaCl_2$ (200µM) solution, selected for uniformity and transferred to a solution containing 200µ*M* $CaCl_2$ (pH 4.2) and increasing levels of $AlCl_3$ (0-200µM). The solution was replaced every third day, and the length of the longest root was monitored for each stem cutting during 21 days.

Results and Discussion: Although stem cuttings of all three *Brachiaria* species could be rooted in a $CaCl_2$ solution, roots of *B. ruziziensis* did not elongate significantly in 200µ*M* $CaCl_2$ (pH4.2), even in the absence of Al (Figure 53a, left half). This may be a consequence of its lack of adaptation to nutrient-deficient conditions, especially N deficiency (IP5, 1998 AR).

In contrast, roots of *B. decumbens* and *B. brizantha* continued to elongate during three weeks in the absence of externally supplied nutrients (Figure 53a, left half). When 200µ*M* $AlCl_3$ was added to this solution, root elongation of *B. brizantha* but not of *B. decumbens* was severely inhibited (Figure 53a, right half). Evaluation of various Al levels suggested that 200µ*M* $AlCl_3$ was the most appropriate level to distinguish Al tolerance levels (Figure 53b).

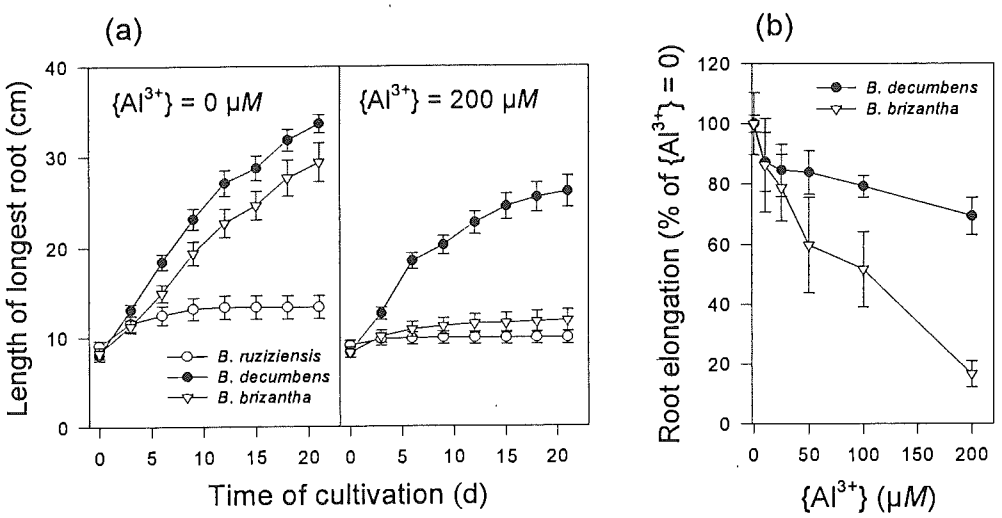


Figure 53. Screening for Al tolerance using stem cuttings and the root elongation method. *(a)* time course of root elongation in 200µ*M* $CaCl_2$ ± 200µ*M* $AlCl_3$ (pH 4.2). *(b)* relative root elongation as a function of the Al^{3+} activity in the solution.

Hence, only roots of stem cuttings of *B. decumbens* can elongate significantly in a solution containing 200µ*M* $CaCl_2$ and 200µ*M* $AlCl_3$ (pH 4.2) (Figure 54).

Root growth of *B. brizantha* is inhibited because of its greater sensitivity to Al, while root growth of *B. ruziziensis* is most likely inhibited because of a combination of its poor adaptation to nutrient deficiency and Al toxicity It follows that this method may be a quick and efficient approach to screen genetic recombinants of *Brachiaria* for their level of Al tolerance and their adaptation to nutrient deficiency in a single step. Further biochemical criteria such as malate and P content of root apices, may be used in the future to complement the screening procedure.

Although it is unlikely that this approach takes into account all factors contributing to acid soil adaptation, we believe that it may be a very useful strategy to significantly reduce the number of individual plants that are finally evaluated in the field.

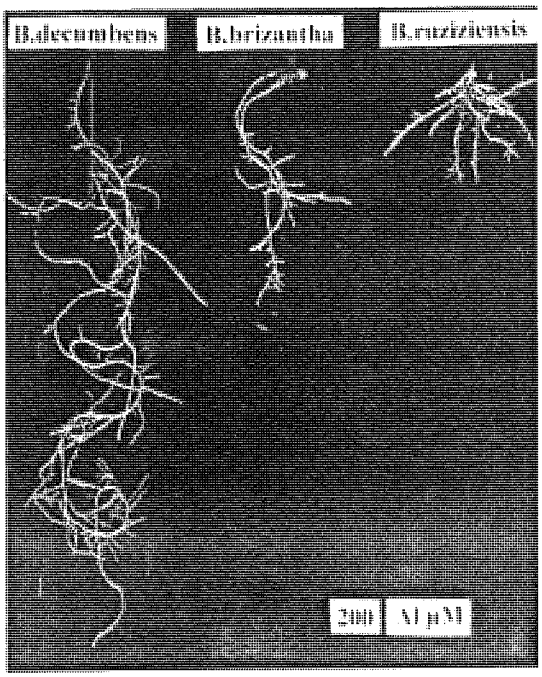


Figure 54. Vegetative cuttings cultivated during 21 days in 200µM $CaCl_2$ and 200µM $AlCl_3$ (pH 4.2). Only roots of *B. decumbens* can elongate at this Al level in the absence of externally supplied nutrients.

C) Conclusions and future research implications (P. Wenzl and I.M. Rao)

Virtually all experiments carried out thus far have demonstrated that *B. decumbens* is better adapted to the various components of the "acid soil syndrome" than the other two *Brachiaria* species. This is true for Al toxicity as well as P and N deficiency. We therefore believe that *B. decumbens* may be an extremely useful model species to understand how different mechanisms contributing to the acquisition and the efficient use of nutrients under Al-toxic conditions could work together to adapt a plant to the typically infertile and acid soils of the tropics.

There is a need to identify and isolate the genes that contribute to the outstanding adaptation of *B. decumbens* to infertile acid soils. It is expected that this will open up the possibility to improve acid soil adaptation of other important crops that are less adapted to acid soils. This can be achieved either by direct transfer of genes from *B. decumbens* or by manipulation of endogenous gene expression based on insights gained from genetic studies of *B. decumbens*.

3.1.2 Identification of genotypes of *Brachiaria* for advanced evaluation in grazing trials

(Members of the *Brachiaria* Network; L.H. Franco; C. Plazas; G. Sotelo; J. Miles)

Rationale: A series of regional agronomic trials of *Brachiaria* accessions was conducted throughout Colombia (Brachiaria Network) in order to assess adaptation and to select promising accessions (or hybrids) for grazing trials.

Methods: Small-plot trials were established during 1996/97 at 12 locations from the north coast, to the Amazonian piedmont. Progress of these trials is reported in periodic reports submitted by the individual collaborators. A final meeting of collaborators of the Brachiaria Network was held in late February, 1999 in Yopal, Casanare.

Results and discussion: The reports of this meeting, including the nomination of accessions for advance to grazing trials, are found in a separate, unpublished document (Miles, J.W. (ed.) 1999. IV Reunión de la Red de *Brachiaria*, Memorias). Selected accessions (and hybrids) are shown in Table 48 for the various test sites, which varied in rainfall (amount and distribution) and soil fertility. A further analysis of results obtained in the Brachiaria Network is made in Activity 4.1 of this Report.

This table also includes a final column indicating the resources required to establish the appropriate grazing trial to test the selections from the agronomic trial phase. Owing mainly to lack of financial support, grazing trial(s) were successfully establish during 1999 at only a limited number of sites. Five different *Brachiaria* entries were established in separate 3-ha plots on a private farm in the Magdalena Valley (Barrancabermeja, Santander, Colombia) by Corpoica collaborator Ing. Henry Mateus. Additional on-farm grazing trials were established by CIAT personal in the Llanos and Amazonian piedmont.

Table 48. Listing of resources required and those available at each site.

Site	Accession (or hybrid)	Duration of trial	Resources available	Area (ha/accession)	Resources required
Chiriguana	6387; 26110	3	Land; Machinery; Fencing; Student help	4	Fertilizers; Seed; Travel expenses
B/bermeja	26318; 36061 (FM9201/1873); 6387; 26159; 26427	3	Land; Machinery; Fencing; Student help	3	Fertilizers; Seed; Travel expenses
Porvenir	6133; 26110	3	Land; Machinery; Fencing; Student help	5	Seeds; Inputs
Puerto López	26110; 36061 (FM9201/1873); (26318)	3	Land	20	Seeds
La Florida Puerto López	26110; 36061 (FM9201/1873); 36060 (BR94NO/1737); 26318	3	Land; Machinery; Fencing; Scales	10	Technical assistance; Seeds
Caqueta	36062 (BR93NO/1371); 36061 (FM9201/1873); 26110; 16113; 16886; 16888; 16180; 26318	3	Land; Machinery	3	Fertilizers; Labor; Transportation expenses; Fences; Cost of student help
El Nus	26110; 36061 (FM9201/1873)	3	Land; Machinery; Fencing	3	Seeds; Fertilizer; Travel expenses; Labor costs for planting
Casanare	16113; 26556	3	Land; Machinery; Fencing	3	Fertilizers; Labor costs for planting (50 %); Transportation costs (50%)
La Dorada	6387; 26110	3	Land	3	Inputs; Seed; Machinery; Travel expenses; Cost of planting
Turipana	26110; 16322	3	Land; Machinery; Fencing;	3	Seeds; Inputs; Travel expenses

3.1.3 Studies on genotypic variation in *Arachis pintoi* for tolerance to low phosphorus supply

(N. Castañeda, R. Garcia, N. Claassen and I. M. Rao)

Rationale: Grass alone pastures are the predominant land use system of the tropical grasslands. This is because of their fast establishment, high biomass production (C_4 pathway of photosynthesis) and lack of a persistent legume to grow in association with grasses. Presently there are different tropical legume species that can be grown in association with aggressive grasses. Arachis pintoi (Ap) is unique among them in its ability to form stable grass-legume associations with vigorous grasses such as Brachiarias.

One feature that makes Ap persistent in these associations is its stoloniferous growth habit that allows it to readily invade any bare ground. The stolons root freely and contribute to greater acquisition of nutrients and water. Another plant attribute that improves persistence is its ability to propagate both vegetatively and from the seed it puts underground (geocarpic plant). The most widely used Ap genotypes in the tropical and subtropical regions originate from Brazil. Introduced legume-based pastures with Ap genotypes (CIAT 17434 or 18744) are highly nutritive in quality and not only contribute to increased milk and beef production but also are known to improve physical, chemical and biological conditions of degraded soils. However, establishment of Ap is slow due to variability in mortality of plants after establishment, particularly on acid soils with poor internal drainage.

The major causes for the low pasture productivity can be directly or indirectly attributed to biotic and abiotic factors. Ap genotypes have shown low susceptibility to biotic factors but the abiotic factors, which include edaphic and climatic constraints, account for their lack of productivity during and after establishment. Among the edaphic factors, low availability of P is a major constraint to pasture production in the tropics. Amelioration of P deficiency with fertilizers is not a viable option for many resource-poor farmers. Crop and forage genotypes that can acquire and utilize scarce P resources more efficiently from low-P tropical soils could both improve and stabilize agricultural production. The availability of insoluble P fertilizers such as phosphate rocks (PR) to plants could depend largely on the solubility product of the mineral, which is controlled by its mineralogical characteristics. Since the products of PR dissolution are Ca and phosphate ions, and since dissolution involves an acid reaction, Ca and Pi concentration in soil solution and soil pH are important external factors governing PR availability to plants. Of these three factors, Ca sink size seems to be the strongest factor driving dissolution.

This suggests that a plant with superior ability to acquire Ca may encourage PR dissolution and could be efficient in use of P from PR sources. Genotypes, which can better exploit the residual fertilizer P, would substantially improve the returns on strategic P inputs as well as "capital investments" in large basal or corrective P fertilizer applications. The sustained pasture productivity of low P tropical soils, therefore, requires that adapted forage genotypes make the most efficient use of available soil P in order to reduce the demand for P applications. The genetic characteristics of tropical forage cultivars and the environment in which they are grown influence their growth and productivity. Improved adaptation of forage plants to low P-supplying soils could be due to plant mechanisms which contribute to their high P uptake ability at low P concentrations and/or more efficient internal use of P for increased forage yields. Genetic variability in plant ability to absorb, translocate, distribute, accumulate, and use P are important in adapting plants to low P-supplying tropical soils.

However, only recently has this variability been conscientiously considered for the purpose of adapting plants to low P soil conditions. The mechanisms, which improve P acquisition are: (1) root morphological characteristics (root growth and distribution, root diameter, root-hair development, mycorrhizal association); and (2) root physiological characteristics (P uptake system and P mobilization in the rhizosphere). The mechanisms that improve P utilization are: (1) partitioning of P within the plant (remobilization of P within the plant, P status of the harvested organ); and (2) efficient utilization of P at the cellular level (intracellular compartmentation of P and metabolic requirements of P). Earlier agronomic evaluation work of *Ap* genotypes in different environmental conditions (Australia, Brazil, Colombia, Costa Rica, Philippines and Malaysia) indicated that CIAT 18747, 18748, 18750 and 22160 are better adapted to low fertility soils than CIAT 17434 and 18744. Greenhouse studies at CIAT indicated that *Ap* (CIAT 17434) grown in soil either as in monoculture or in association with *B. dictyoneura* (CIAT 6133) could acquire greater amounts of P from Ca and Al-P sources than that of the grass alone. Acquisition of P was greater from Ca-P than from Al-P source.

The relationship between shoot P uptake and root length showed that the legume roots acquired markedly greater amount of P per unit root length than the grass roots.
Although inter- and intra-species differences in P uptake, accumulation and use are well known for several species, the mechanisms responsible for the differential abilities of tropical forage species to grow at low or high P supply are not completely understood and few have been described to any extent. Understanding these mechanisms is a prerequisite to the identification, selection and improvement of forage germplasm for low-P soils. Field and greenhouse studies are in progress to determine genotypic differences among ten accessions of *Ap* in P-acquisition and utilization from low P soil. Research progress made thus far is reported below.

General Methods: Three field trials were established in humid tropical lowland sites (Caquetá, Colombia) and two greenhouse trials were conducted at CIAT-Palmira. Soil (0-20 cm soil depth) from Montañita, one of the field sites (a clay loam Ultisol), was used for the greenhouse trials. The field was under native pasture. A phosphate rock from Huila, Colombia, (commercial fertilizer "Calfomag") was used in all trials. Its chemical composition (%) is 5.2 P, 25 Ca, 6 Mg and 1 S. Basal fertilizer was applied (kg ha^{-1}) at 20 K (KCl), 50 Ca (agricultural lime plus dolomitic lime), 14Mg (dolomitic lime) and 10S (elemental sulfur).

Leaf laminas were separated and scanned for area (cm^2) (LI 3100 Area Meter (LI-Cor Inc., Lincoln-NE, USA), dried at 70°C for two days and then weighed. The leaf area index (LAI, m^2 of leaf area per m^2 of ground area) and the specific leaf area (SLA, m^2 of leaf area per kg of dried leaves) were determined. Total P acquisition (shoot + root), P acquisition efficiency (mg of P uptake in shoot biomass per unit root biomass or root length), P use efficiency (g of forage production per g of total P acquisition), and P transport index {percentage net transport of P to shoot – [(shoot P content/total P content) x 100]} were thereafter calculated. Inorganic P in leaf and root, acid phosphatase activity in leaf and root were determined.

Contents of P and Ca of the plant parts, soil pH_{H2O} (soil 1: water 1), P (Bray II), Ca, acidity (Al + H), Al and soil organic matter content were also measured. Data from the trials were subjected to an analysis of variance using the SAS computer program. Least-significant differences were calculated by an F-test. A probability level of 0.05 was considered statistically significant.

A) Genotypic differences in *Arachis pintoi* on mycorrhizal dependence and P acquisition from an infertile acid soil

Methods: To determine genotypic differences among *Ap* accessions in terms of mycorrhizal dependence and P acquisition from soil, a greenhouse study was conducted. A 10 x 3 x 2 factorial arranged in a RCBD [ten genotypes [CIAT 17434, 18744, 18745, 18747, 18748, 18751, 22159, 22155, 22160, 22172] x three P sources (NP, PR, TSP) x two levels of VA-mycorrhizae (without or with *Glomus fasciculatum*)] was used and replicated three times. To generate without and with mycorrhizae treatments, soil was sterilized with application of steam and reinoculated with soil microflora for without VAM treatment and with microflora and VAM for with VAM treatment. To determine the rates of P-influx, plants were grown in two batches, one harvested at 45 days and the other at 90 days after planting.

Results and Discussion: As expected, at 45 days after planting (results not shown), the effects of VAM were less marked than at 90 days. This may be due to the lag time in establishment of VAM association. At 90 days after planting, significant genotypic variation in mycorrhizal dependence was observed in terms of leaf area production (Figure 55), shoot biomass production (Figure 56) and shoot P uptake (Figure 57). Although genotypic differences in leaf area production with VAM infection were not marked, three genotypes (CIAT 18744, 18748 and 22160) showed better leaf area development with different P treatments (Figure 55).

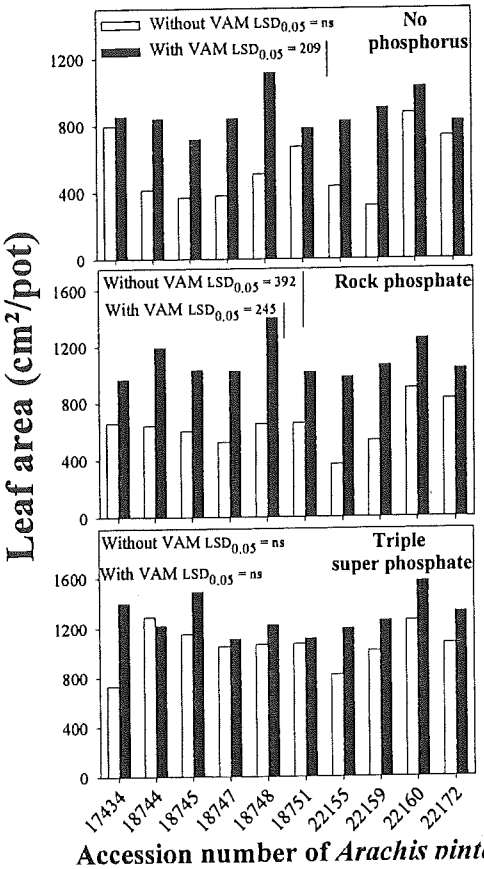


Figure 55. Influence of VA-mycorrhizae on leaf area production of ten accessions of *A. pintoi*. Measurements were made after 90 days of growth.

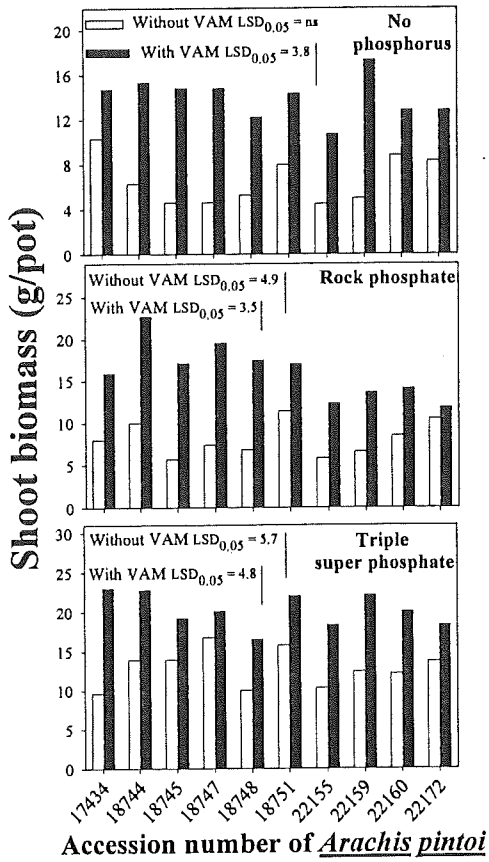


Figure 56. Influence of VA-mycorrhizae on shoot biomass production of ten accessions of *A. pintoi*. Measurements were made after 90 days of growth

In terms of leaf area production, CIAT 18748 was very dependent on VAM association with almost a 2-fold response to inoculation. VAM association significantly improved shoot biomass production with NP (no phosphorus) and RP (rock phosphate) treatments while it was less marked with TSP (triple super phosphate) treatment (Figure 56). Two genotypes, CIAT 18744 and 18747 were outstanding in their response to VAM with RP treatment while three genotypes (CIAT 17434, 22160 and 22172) were less dependent to produce shoot biomass. Association with VAM significantly increased P uptake by three genotypes (CIAT 18747, 18751 and 18744) with NP and RP treatments but not with TSP treatment (Figure 57).

As expected, mycorrhizal dependence was greater with low supply of P to soil (no fertilizer application). It is important to note that mycorrhizal dependence was also greater with rock P application than with TSP application. Colonization of VAM increased the ability to acquire P by 2-fold in some of the genotypes including CIAT 18741, 18748, 18751, 22159 and the commercial cultivar, 17434. Thus the presence of good inoculum of VAM in soil is important for these accessions to establish rapidly in soils when no P is applied. Three genotypes (CIAT 18744, 18745 and 18747) responded better to rock P application than the other genotypes. Among the three genotypes, CIAT 18747 was particularly superior in acquiring P from rock P in association with VAM. It is possible that this accession may have developed extensive hyphal system to acquire P from a less soluble P source. Among the 10 accessions tested, CIAT 22172 and 22160 were less dependent on VAM association.

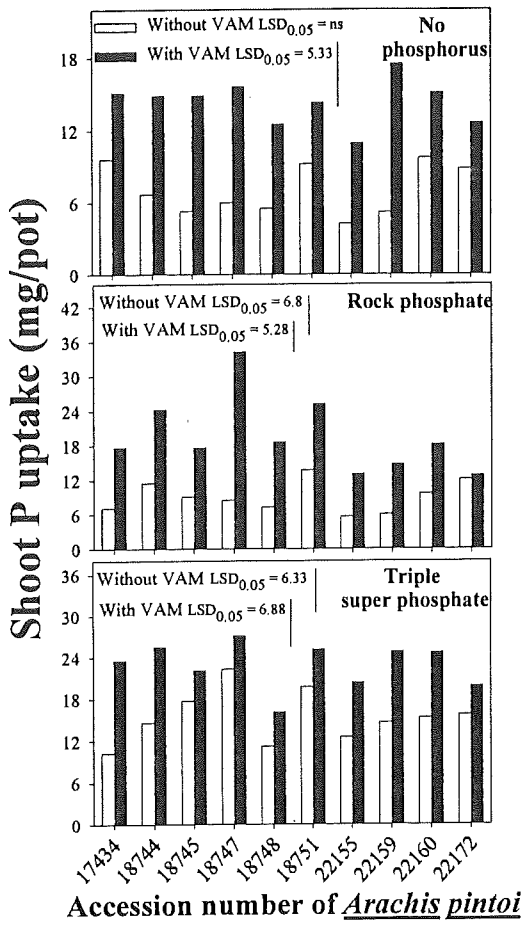


Figure 57. Influence of VA-mycorrhizae on shoot P uptake by ten accessions of *A. pintoi*. Measurements were made after 90 days of growth.

These two genotypes may be better suited to soils where native inoculum of VAM may be very low. These results indicate that three accessions of *A. pintoi* (CIAT 18744, 18747 and 18751) are more dependent on VAM association particularly when rock P is applied as P source. Further research work is needed to identify specific mechanisms responsible for this greater ability of these three genotypes.

B) Identification of *Arachis pintoi* genotypes with adaptation to low P supply in the field

Methods: A field study was conducted at "La Rueda" ranch, Montañita, Caquetá (latitude 1° 25' N, longitude 75° 27' W and 180 m.a.s.l). Plant growth was monitored during June to September 1998. The mean rainfall, temperature and relative humidity were 3500 mm/year, 25°C and 75% respectively. The experiment was laid down in a split plot RCBD with three P levels [native P (NP), phosphate rock (PR), triple super phosphate (TSP)] as main plots and ten genotypes [CIAT 17434 (commercial), 18744, 18748, 22159, 18745, 18751, 22160, 18747, 22155, 22172] as subplots. The experiment was replicated three times. Application (kg P ha^{-1}) of PR and TSP was at 50 and 20, respectively. Plants were harvested at 45 and 90 days after planting.

Results and Discussion: At 45 days after establishment, CIAT 18747 was outstanding in terms of rapid establishment as determined by leaf area index, shoot biomass and shoot P uptake (results not shown). Another accession, CIAT 18751 was also rapid in establishment, particularly with rock P treatment. Among the ten accessions tested, two accessions (CIAT 22159 and 22172) were found to be slow in establishment. At 90 days after establishment, the same two accessions (CIAT 18747 and 18751) maintained their vigor and ability to acquire P from different P sources (Figure 58).

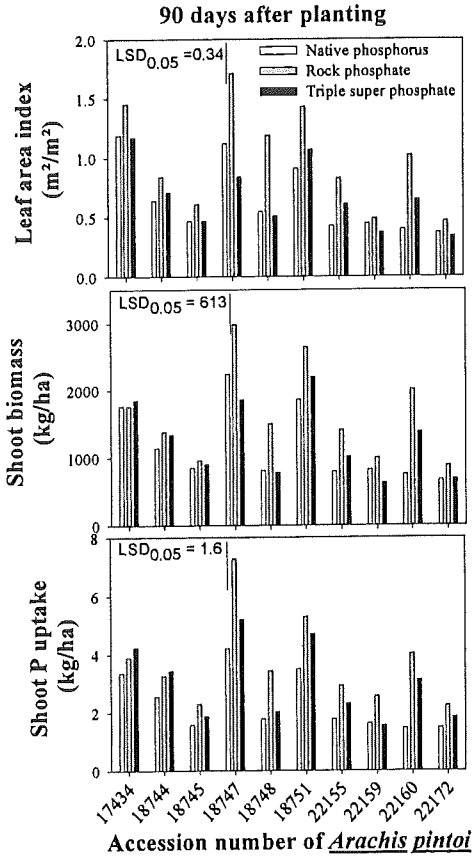


Figure 58. Influence of P fertilizer source on genotypic differences in shoot growth and nutrient acquisition by *Arachis pintoi* from a low P soil at Montañita, Caquetá. Measurements were made at 90 days after planting.

The commercial accession CIAT 17434 also performed well while three accessions (CIAT 18745, 22155 and 22172) were less productive with different treatments of P application. The superior performance of CIAT 18747 was associated with its greater ability to acquire Ca, particularly from rock P source (Table 49). Two other accessions (CIAT 17434 and 18751) also acquired greater amounts of Ca while two accessions (CIAT 18745 and 22172) were particularly inferior to the other accessions.

The superior performance of CIAT 18747 to acquire P from sparingly available P sources may be related to its ability to associate better with VAM combined with exudation of organic acids in addition to better root architecture. But root development in compacted soils may be significantly affected by soil physical characteristics. Further research work is needed to identify specific mechanisms that contribute to superior performance of CIAT 18747.

Table 49. Influence of P fertilizer source on genotypic differences in shoot Ca uptake by *Arachis pintoi* from a low P soil at Montañita, Caquetá. Measurements were made at 90 days after planting.

Plant attribute	Source	Accession of *A. pintoi*										$LSD_{0.05}$
		17434	18744	18745	18747	18748	18751	22155	22159	22160	22172	
Shoot Ca uptake (kg/ha)	NP	15.6	8.67	5.16	17	5.22	12.3	6.45	6.32	5.2	5	
	RP	18.02	10.1	5.28	22.8	9.57	11.1	9.77	7.78	11.86	7.27	
	TSP	19.11	9.8	6.25	19.6	6.57	15.5	6.94	4.24	10.56	5.16	
												5.8

NP= Native phosphorus (0 kg P/ha); RP= Rock phosphate (50 kg P/ha); TSP= Triple super phosphate (20 kg P/ha)

C) Response of *Arachis pintoi* to rock phosphate application

Methods: A glasshouse trial was conducted at CIAT-Palmira, Valle del Cauca at latitude 3° 30' N, longitude 76° 21' W and 965 m.a.s.l. The maximum and minimum day/night temperatures and relative humidities in the glasshouse were 36°/18°C and 94/45%, respectively. The maximum photon flux density during the day was 1100 µmol m^{-2} s^{-1}. Plants were harvested at 90 days after planting. The trial was conducted as a 3 x 8 x 2 factorial in a RCBD [three genotypes (CIAT 17434, 18744, 22160) x 8 levels of PR (kg P/ha) (P_0, P_2, P_5, P_{10}, P_{15}, P_{20}, P_{50}, P_{100}) x two levels of basal fertilizer (kg/ha) (without or with K_{20}, Ca_{50}, Mg_{14}, S_{10})] was used and replicated three times.

Results and Discussion: Response to rock P application was marked up to 50 kg P/ha for all three accessions tested with or without basal application of other nutrients (Figure 59).

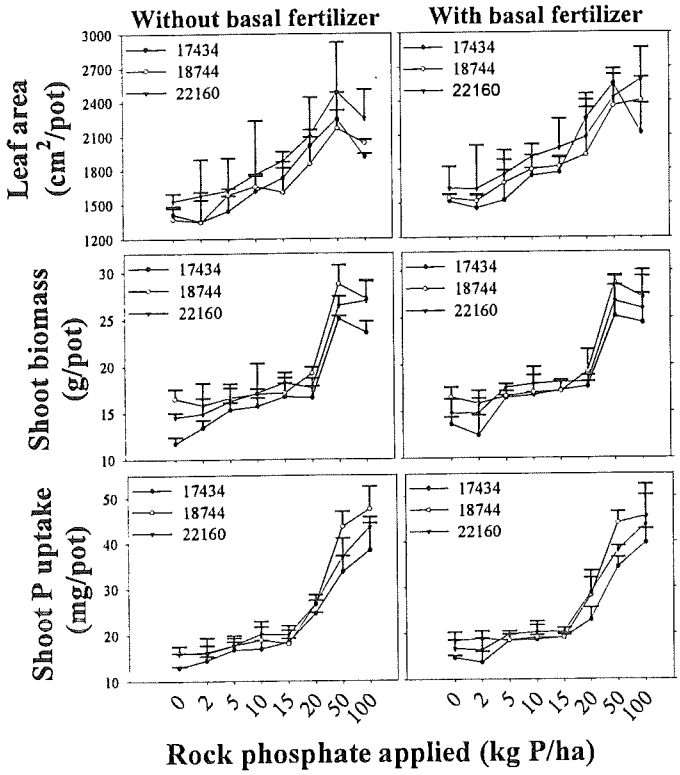


Figure 59. Response of three accessions of *A. pintoi* to rock phosphate application in terms of leaf area production, shoot biomass production and shoot P uptake. Rock phosphate was applied either with (right panel) or without (left panel) application of basal nutrients. Measurements were made after 90 days of growth.

Response to basal application of other nutrients (K, Ca, Mg and S) was not significant for any of the three accessions tested. Compared to commercial accession, CIAT 17434, response to P application was greater for the other two accessions, CIAT 18744 and 22160.

The high level of P application (100 kg P/ha) had only slightly improved shoot P content but not shoot biomass production compared with 50 kg P/ha rate. The lack of response to basal application of nutrients may be due to the presence of Ca (26%) and Mg (6%) in the rock P source. These concentrations will provide for an application of 50 kg P/ha in the form of Calfomag an amount of 211 kg/ha of Ca and 57 kg/ha of Mg.

Since the soil used in this study showed 80% saturation of Al, the levels of essential cations in this soil were low and therefore the supply of Ca and Mg could markedly improve the performance of *A. pintoi* accessions. These results indicate that P application significantly improves the performance of *A. pintoi* accessions

D) Effect of tillage, fertilization, planting system and their interaction on establishment of *A. pintoi* genotypes

Methods: Field study was conducted at "La Esperanza" ranch, Morelia, Caquetá (latitude 1° 23' N, longitude 75° 42' W and 200 masl). Plants were grown during September 1998 to February 1999. The mean rainfall, temperature and relative humidity were 3500 mm/year, 25°C and 85% respectively. The experiment was laid down in a split-split plot RCBD with a factorial (2 x 2) arrangement.

Two planting systems (monoculture and grass-legume association) as main plots, two genotypes (CIAT 17434, 18744) as subplots and two P levels (native phosphorus (NP), and rock phosphate (RP)) plus two tillage methods (without or with tillage) as sub-sub-plots in a factorial arrangement. Application of PR was at 50 kg ha^{-1}. The experiment was replicated three times.

Results and Discussion: The two accessions when grown in monoculture did not respond to tillage in the absence of RP application (Table 50). However, when RP was applied, the response to tillage was marked. Shoot P uptake was markedly improved by RP application than tillage. Among the two accessions tested, CIAT 18748 responded better to the combination of RP application and tillage.

When grown in association with the grass (Bd), the commercial accession CIAT 17434 did not respond to tillage but responded to RP application in terms of shoot biomass. However, the other accession, CIAT 18748 responded to both tillage (58% increase) and RP application (171%). The lack of response of CIAT 17434 to tillage may related to its superior ability to develop root system in the absence of tillage.

Field observations indicated that the root system of CIAT 18748 may be thicker than that of CIAT 17434 at 0 to 20 cm soil depth. But tillage treatment improved root elongation of both genotypes. Application of RP improved not only shoot P uptake but also shoot N uptake and efficiency of P uptake per unit root length. This field study indicates that P supply to soil is more important for *A. pintoi* establishment than tillage.

Table 50. Influence of tillage and P application on the performance of two accessions of *A. pintoi* grown either in monoculture or in association with *B. dictyoneura* (Bd). The values for the legume grown in association are shown in parenthesis.

Plant attribute	Source	Accession of *A. pintoi*									
		Monoculture				$LSD_{0.05}$	Association with *B. dictyoneura*				$LSD_{0.05}$
		17434		18748			17434		18748		
		-T	+T	-T	+T		-T	+T	-T	+T	
Leaf area index (m^2/m^2)	NP	1.13	1.79	1.02	1.48		1.16 (0.38)	2.14 (0.54)	0.84 (0.14)	1.24 (0.28)	
	RP	1.66	1.92	1.19	1.53	0.61	1.1 (0.5)	1.15 (0.55)	1.2 (0.3)	1.69 (0.34)	0.7 (0.33)
Shoot biomass (kg/ha)	NP	1498	1811	1147	1393		2064 (625)	3812 (631)	1523 (170)	2331 (270)	
	RP	1677	1917	1010	1704	587	1949 (580)	2659 (614)	2307 (461)	3141 (329)	1299 (429)
Root biomass (kg/ha)	NP	704	1756	2252	2897		(907)	(1140)	(1119)	(1777)	
	RP	1092	1014	2484	2689	601	(1044)	(912)	(1660)	(1696)	(374)
Root length (km/m^2)	NP	0.24	0.35	0.17	0.25		(0.19)	(0.27)	(0.13)	(0.19)	
	RP	0.27	0.31	0.27	0.41	0.09	(0.24)	(0.26)	(0.16)	(0.2)	(0.06)
Shoot P uptake (kg/ha)	NP	1.7	2.2	1.2	1.6		1.3 (0.5)	2.9 (0.8)	1.0 (0.2)	1.4 (0.3)	
	RP	2.6	3.5	1.8	3.7	0.8	1.9 (0.8)	2.9 (1)	2.5 (0.7)	3.5 (0.5)	1.3 (0.5)
Shoot N uptake (kg/ha)	NP	28.6	38.3	25.6	29.5		18.7 (10.2)	38.2 (12.6)	11.9 (3.3)	19.4 (6.2)	
	RP	35.4	39.1	26.5	39.4	12.2	18.1 (10)	23.5 (11.3)	18.6 (9.6)	21.7 (6.9)	12 (8.4)
P uptake efficiency (µg/m)	NP	984	1038	1417	1439		(471)	(568)	(686)	(607)	
	RP	1470	1797	1587	1786	436	(855)	(751)	(1659)	(1193)	(437)

NP = Native phosphorus
RP = Rock phosphate
(*) data of *A. pintoi*
- T = without tillage
+ T = with tillage

3.1.4 Determine N and P requirements of *Panicum maximum* genotypes (I. M. Rao, P. Argel, J. Ricaurte and R. Garcia)

Rationale: Accesions of *Panicum maximum* evaluated under the numbers, CIAT 6799 and 6944 were outstanding in the agronomic and grazing trials carried out at Carimagua in the Llanos of Colombia. Biochemical (isozyme), cytological (embryo-sac) and morphological analysis conducted by the Genetic Resources Unit at CIAT indicated that the so called accessions CIAT 6799 and 6944 are probably only one accession which was given rise to CIAT 36000. Agronomic evaluation of 17 accessions of *Panicum maximum* in an infertile acid soil at San Isidro, Costa Rica indicated that two accessions, CIAT16061 and 16051 were outstanding in both wet and dry

season performance compared to a number of commercial cultivars (Tobiatá, Centenario, Tanzania, Vencedor and Common).

Two separate glasshouse studies were conducted with the objective of determining genotypic differences in N and P requirements of the above selected accessions compared to commercial cultivars. We tested the following three hypotheses: (i) internal N and P requirements of selected CIAT accessions (36000; 16061; 16051) are lower than those of commercial cultivars; (ii) selected CIAT accessions are capable of acquiring greater amounts of N and P from lower level of N and P supply; and (iii) partitioning of N and P to leaves, particularly at low N and P supply, is grater with the selected CIAT accessions compared to commercial cultivars.

Methods: A sandy loam oxisol from Carimagua was used to grow the plants (4 kg of soil/pot). Basal nutrient supply (kg/ha) included 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo. For the experiment on N requirements a basal supply (kg/ha) of 50 P and seven levels of N (0, 10, 20, 40, 80, 160 and 320) were used. For the experiment on P requirements, a basal supply (kg/ha) of 80 N and six levels of P (0, 10, 20, 40, 80 and 160) were used. At the time of harvest (50 days of growth), plants were separated into the youngest fully expanded leaf, the remainder of the tip (Ytip) and the remainder of the whole leaves (WL) and whole stems (WS). The following measurements were made: leaf area, leaf biomass, stem biomass, total N and P in YEL and Ytip, total N and P in leaf and stem biomass, leaf to stem ratio, shoot N and P uptake and leaf N and P partitioning index.

Genotypic differences in N and P requirements were determined by estimating the amount of N or P required to produce 90% of maximum yield. Genotypic differences in responsiveness to the added N or P over the linear section of the response curve and the shoot yield and N or P uptake without the addition of N or P was determined. The critical values for N or P were determined from the shoot yield data versus N or P concentration response curve at 90% maximum yield or, where a yield plateau was not reached for N or P concentration, by reading the nutrient concentration on the "X"axis at 90% of the maximum yield as determined above.

Results and Discussion: Increasing N supply improved shoot biomass production of all genotypes tested but the extent of increase per unit N supply was markedly superior for two CIAT accessions (16051 and 6177) compared to the commonly used cultivars (Figure 60). The external N requirements of the same two accessions are also markedly lower than those of the other genotypes tested (Table 51). However, when applying these values to field situations, it should be noted that these critical values were derived from pot grown plants at younger stage of plant growth. It is possible that these critical values could be lower for older plants. Among the two cultivars, Vencedor had very high external N requirement. The critical N content of the youngest expanded leaf of accession CIAT 16028 was markedly lower than that of the other genotypes (Figure 61).

Table 51. External and internal critical requirements of N and P among *Panicum maximum* cultivars and accessions.

Cultivar/ Accession	External N requirement (kg/ha)	External P requirement (kg/ha)	Internal N requirement (% of dry weight)	Internal P requirement (% of dry weight)
Vencedor	250	142	3.6	0.11
Tanzania	110	107	2.0	0.07
CIAT 16051	35	122	1.7	0.07
CIAT 16028	63	119	1.2	0.08
CIAT 16061	100	78	1.8	0.07
CIAT 36000	82	120	1.8	0.08
CIAT 6177	35	121	1.8	0.09

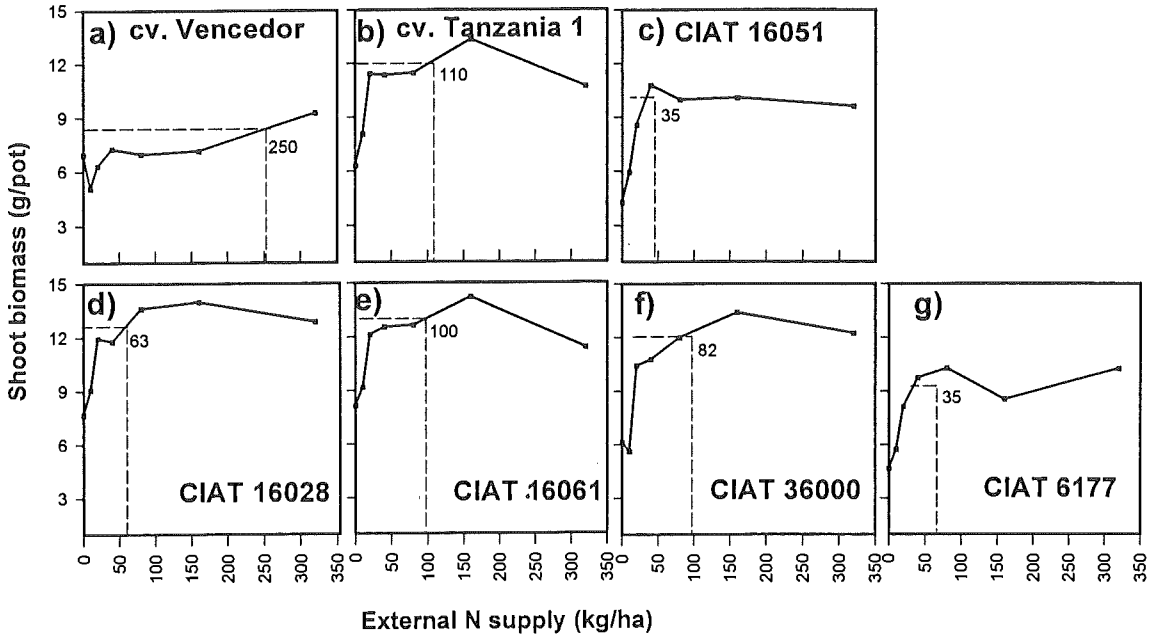


Figure 60. Shoot biomass production response to external N supply to soil of seven genotypes of *Panicum maximum* grown in pots. Critical values for external N requirement are indicated in numbers.

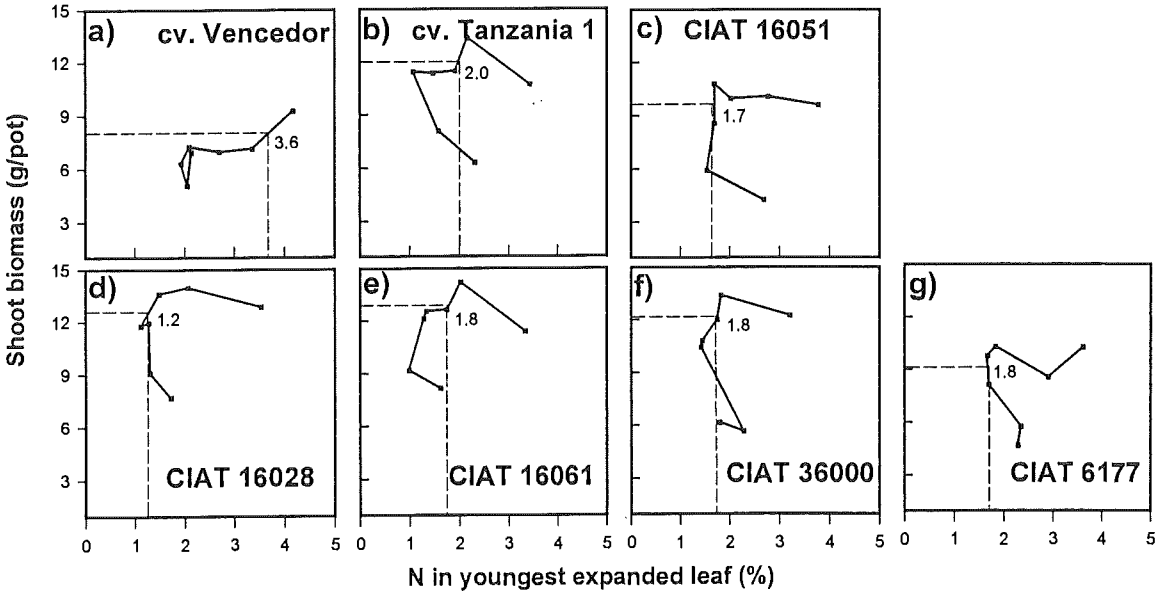


Figure 61. Shoot biomass production response to internal N content of the youngest expanded leaf of seven genotypes of *Panicum maximum* grown in pots. Critical values for internal N requirement are indicated in numbers.

Similar to external N requirement, the critical internal N requirement of cv. Vencedor was markedly greater than that of the other genotypes (Table 51). Increase in P supply markedly improved shoot biomass production of all the genotypes tested indicating that P supply in soil is a major limitation to forage yield (Figure 62).

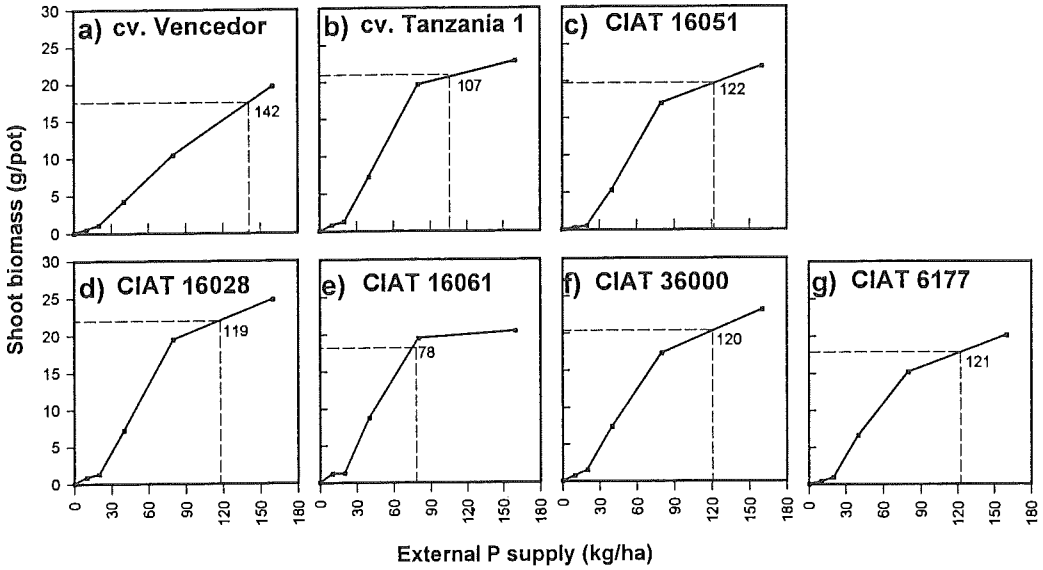


Figure 62. Shoot biomass production response to external P supply to soil of seven genotypes of *Panicum maximum* grown in pots. Critical values for external P requirement are indicated in numbers.

The external P requirement of the accession CIAT 16061 was markedly lower than that of the other genotypes (Figure 62; Table 51), particularly cv. Vencedor which did not reach the asymptote even at 150 kg/ha of P supply. Internal P requirement of cv. Vencedor was also markedly greater than that of the other genotypes (Figure 62; Table 51).

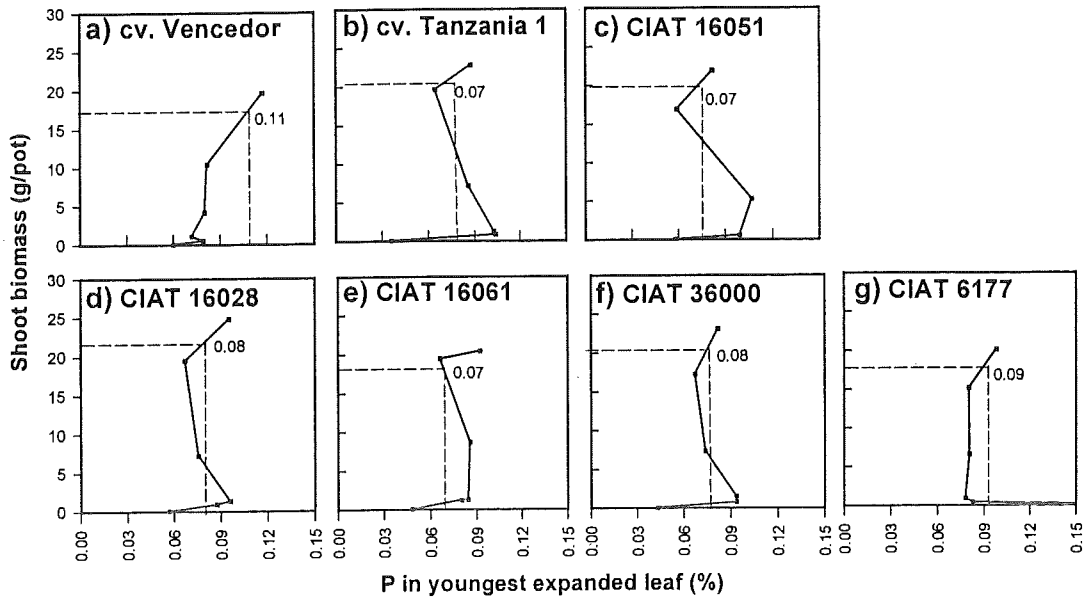


Figure 63. Shoot biomass production response to internal P content of the youngest expanded leaf of seven genotypes of *Panicum maximum* grown in pots. Critical values for internal P requirement are indicated in numbers.

Among the seven genotypes tested, CIAT 36000 produced the more number of tillers at lower supply of either N or P to soil. This particular plant attribute combined with lower external and internal requirements of N and P could make this accession better suited for permanent pastures under grazing in areas with soils of low natural fertility. Another accession, CIAT 16061 which performed well in Central American hillsides agroecosystem could be better suited for cut and carry systems of forage production because of its lower N and P requirements combined with greater production potential at lower levels of N or P supply. The cultivar Vencedor is very demanding for nutrients, but maintains high nutritional value (high values of N and P content in forage tissue) and therefore may be better suited for crop-pasture rotational systems where the input levels are usually much greater than those of the extensive pasture systems.

Progress towards achieving output milestone:

- Selected *Brachiaria*, *Arachis* and *Panicum* genotypes with superior adaptation to low soils fertility available to partners for evaluation.

We were successful in developing a rapid and reliable screening method to evaluate aluminum tolerance of *Brachiaria* genetic recombinants. This method uses relative root elongation as a simple measure to identify aluminum sensitive genotypes. We adapted this method for vegetative stem cuttings so that we can evaluate large numbers of *Brachiaria* hybrids for their aluminum tolerance. We plan to use this method to evaluate a hybrid population of *B. decumbens* x *B. ruziziensis*.

Promising *Brachiaria* accessions have been identified from agronomic trials and seed has been multiplied for evaluation under grazing. However, only a limited number of grazing trials have been established owing to lack of financial support.

We identified accessions of *Arachis pintoi* with variable dependence on VAM association when rock P is applied. Genotypes of *A. pintoi* (CIAT 22160 and 22172) which are less dependent on VAM association when rock phosphate is applied maybe a good option to introduce in degraded pastures where native VAM inoculum could be low.

Activity 3.2 Identify genotypes of grasses and legumes with dry season tolerance

Highlights

- Selected four *Brachiaria brizantha* accessions (CIAT 26110, CIAT 6387, and CIAT 16467) for superior performance during the dry season.
- Showed that low levels of Ca and high levels of total nonstructural carbohydrates in green leaves may serve as indicators of dry season tolerance in *Brachiaria*.

3.2.1 Identification of genotypes of *Brachiaria* with tolerance to drought (L.H. Franco; Members of the *Brachiaria* Network; I. M.Rao; J. Miles)

Rationale: Drought tolerance is a desirable attribute in *Brachiaria* cultivars. Data generated by The Colombian *Brachiaria* network should allow identification of genotypes adapted to drought.

Methods: The Colombian *Brachiaria* network tested 24 *Brachiaria* genotypes over a wide diversity of environments throughout the lowland tropics of Colombia. Dry matter yield data were recorded. A multilocational analysis was performed on the dry matter yield data from these trials.

Results and discussion: The analysis of variance over locations for dry matter production during the period of minimum precipitation gave highly significant ($p<0.001$) F-values for the effects of location; accession, and LxA interaction. The outstanding accessions in terms of dry matter production during the dry season were *B. brizantha* CIAT 26110, CIAT 6387, and CIAT 16467 (Table 52).

Table 52 Comparison of mean dry matter yield of small-plot agronomic evaluation of 20 *Brachiaria* accessions (and hybrids) during period of minimum precipitation.

Duncan					Promedio	N	Accession*
			A		3847.8	21	26110
			A				
	B		A		3441.7	21	6387
	B		A				
	B		A	C	3346.4	21	16467
	B			C			
	B		D	C	3020.8	21	16488
	B		D	C			
	B		D	C	3015.0	21	26318
	B		D	C			
	B	E	D	C	2932.0	21	26556
	B	E	D	C			
	B	E	D	C	2880.5	21	26562
	B	E	D	C			
	B	E	D	C	2846.5	21	16212
	B	E	D	C			
	B	E	D	C	2808.2	21	16113
F	B	E	D	C			
F	B	E	D	C	2800.3	21	16315
F		E	D	C			
F	G	E	D	C	2736.1	21	16121
F	G	E	D	C			
F	G	E	D	C	2708.3	21	1873
F	G	E	D	C			
F	G	E	D	C	2673.4	21	606
F	G	E	D				
F	G	E	D		2539.7	21	16322
F	G	E	D				
F	G	E	D		2526.4	21	16497
F	G	E	D				
F	G	E	D		2476.8	21	26124
F	G	E	D				
F	G	E	D		2377.6	21	6133
F	G	E					
F	G	E			2244.5	21	1737
F	G						
F	G				2144.2	21	26180
	G						
	G				2071.0	21	16327

*Entries 1873 and 1737 are hybrids. All other entries are CIAT accessions.

3.2.2 Determination of the genotypic variation in dry season tolerance in *Brachiaria,* and *Arachis* (I. M. Rao, P. Argel, J. Ricaurte and R. García)

Rationale: Quantity and quality of dry season feed is a major limitation to livestock productivity in subhumid regions of tropical America. Field studies were continued at Atenas, Costa Rica. The main objective was to evaluate genotypic differences in dry season (6 months) tolerance among 17 accessions of *Brachiaria* species and 19 accessions of *Arachis* species. We continued our

work to test the hypothesis that the tolerance to dry season is greater in genotypes that accumulate greater amounts of total nonstructural carbohydrates (TNC) combined with less amounts of minerals (ash content) per unit dry weight of leaves and stems.

Methods: Trial 1 included 17 accessions of *Brachiaria* species and trial 2 included 19 accessions of *Arachis* species selected from agronomic evaluation of the germplasm. Atenas site provided excellent field conditions to evaluate dry season tolerance while keeping nutrient supply in soil adequate for growth. Forage yield, shoot nutrient composition, and nonstructural carbohydrates and ash content in the shoot tissue were measured. The use of shoot attributes such as ash content, Ca content and nonstructural carbohydrate levels as selection criteria for dry season tolerance in *Brachiaria* is being tested further using green leaves developed during dry season compared to the remaining shoot tissue.

Results and Discussion: Forage yield among *Brachiaria* species during dry season ranged from 2150 to 6337 kg/ha and the greatest forage yield was observed with *B. brizantha* CIAT 16488 (Table 53). The superior performance of this accession and of *B. brizantha* CIAT 26110, which maintained greater proportion of green leaves during dry season (visual observation) was associated with lower levels Ca content in green leaves (Table 53).

Table 53. Genotypic variation in forage yield, green leaf nutrient composition, ash content and total nonstructural carbohydrates (TNC) of 17 accessions of *Brachiaria* species grown during dry season at Atenas, Costa Rica.

Genotype (CIAT No.)	Forage yield (kg/ha)	Green leaf composition							TNC (mg/kg)
		C	N	P	K	Ca	Mg	Ash	
		(%)							
B. decumbens (16497)	4322	40.7	0.97	0.13	1.01	0.56	0.42	8.4	164
B. humidicola (16886)	3163	36.6	0.5	0.06	0.77	0.39	0.24	7.2	284
B. brizantha (667)	3955	39.1	0.72	0.11	1.5	0.34	0.22	7.8	162
B. brizantha (6387)	3088	36.4	0.84	0.15	1.13	0.72	0.51	10	151
B. brizantha (16135)	4128	38.7	0.74	0.12	0.88	0.67	0.53	9.2	160
B. brizantha (16168)	5262	38.1	0.68	0.08	0.95	0.57	0.42	9.2	180
B. brizantha (16289)	3661	38.3	0.82	0.1	1.06	0.57	0.48	8	238
B. brizantha (16300)	3334	38.2	0.7	0.1	0.98	0.52	0.35	7	178
B. brizantha (16305)	5678	38.1	0.66	0.09	1.03	0.35	0.39	7	161
B. brizantha (16319)	4879	38.5	0.1	0.09	1.03	0.38	0.37	6.2	165
B. brizantha (16322)	3578	39.0	0.61	0.09	0.98	0.47	0.41	11.2	224
B. brizantha (16467)	4361	37.8	0.97	0.12	1.73	0.44	0.31	11	174
B. brizantha (16488)	6337	38.7	0.61	0.09	1	0.46	0.34	9.4	188
B. brizantha (16549)	2150	36.3	0.76	0.12	1.03	0.73	0.42	9.2	211
B. brizantha (26110)	3843	40.3	0.87	0.08	1.41	0.36	0.23	8	197
B. brizantha (26646)	4534	39.2	0.63	0.09	1.2	0.64	0.5	10	155
Brachiaria hybrid (1873)	5227	38.5	0.73	0.04	1.35	0.36	0.33	6	220
Mean		38.4	0.7	0.1	1.12	0.5	0.38	8.5	189

It appears that green leaf Ca status may serve as a selection guide to evaluate dry season tolerance when combined with green leaf proportion. Determination of TNC in green leaves, dry leaves and dry stems showed that certain accessions with greater forage yield also had greater amounts of TNC in addition to lower levels of Ca and Mg which reflected in decreased levels of ash content (Tables 53 to 55).

Table 54. Genotypic variation in dry leaf nutrient composition, ash content and total nonstructural carbohydrates (TNC) of 17 accessions of *Brachiaria* species grown during dry season at Atenas, Costa Rica.

Genotype	Dry leaf composition							
(CIAT No.)	C	N	P	K	Ca	Mg	Ash	TNC
				(%)				(mg/kg)
B. decumbens (16497)	40.9	0.40	0.06	0.39	0.50	0.36	9.0	195
B. humidicola (16886)	37.1	0.32	0.03	0.48	0.49	0.26	8.8	170
B. brizantha (667)	38.2	0.33	0.06	0.77	0.38	0.26	8.2	163
B. brizantha (6387)	37.5	0.31	0.07	0.51	0.48	0.37	9.4	119
B. brizantha (16135)	37.9	0.34	0.06	0.41	0.51	0.42	10.4	119
B. brizantha (16168)	39.1	0.23	0.03	0.59	0.51	0.35	10.2	149
B. brizantha (16289)	38.8	0.48	0.06	0.65	0.49	0.38	8.8	132
B. brizantha (16300)	38.7	0.24	0.04	0.65	0.37	0.38	7.8	128
B. brizantha (16305)	39.8	0.32	0.05	1.03	0.35	0.39	7.0	161
B. brizantha (16319)	40.3	0.23	0.03	0.46	0.30	0.35	6.2	135
B. brizantha (16322)	37.6	0.23	0.04	0.73	0.45	0.33	11.8	120
B. brizantha (16467)	39.2	0.37	0.06	0.97	0.43	0.31	12.2	156
B. brizantha (16488)	37.9	0.25	0.03	0.49	0.42	0.28	9.4	144
B. brizantha (16549)	37.0	0.30	0.04	0.33	0.48	0.29	8.4	132
B. brizantha (26110)	39.4	0.37	0.04	0.86	0.51	0.29	9.0	144
B. brizantha (26646)	39.9	0.26	0.04	1.55	0.48	0.40	8.6	152
Brachiaria hybrid (1873)	38.1	0.73	0.02	0.53	0.60	0.58	6.6	152
Mean	38.7	0.34	0.04	0.67	0.46	0.35	8.9	145

Table 55. Genotypic variation in stem nutrient composition, ash content and total nonstructural carbohydrates (TNC) of 18 accessions of *Brachiaria* species grown during dry season at Atenas, Costa Rica.

Genotype	Stem composition							
(CIAT No.)	C	N	P	K	Ca	Mg	Ash	TNC
				(%)				(mg/kg)
B. decumbens (16497)	40.6	0.29	0.06	0.55	0.12	0.14	3.8	334
B. humidicola (16886)								
B. brizantha (667)	41.8	0.25	0.07	1.50	0	0.22	7.8	162
B. brizantha (6387)	36.4	0.84	0.15	0.77	0.09	0.08	3.8	302
B. brizantha (16135)	40.3	0.26	0.05	0.54	0.12	0.15	4.0	206
B. brizantha (16168)	41.4	0.20	0.04	0.53	0.16	0.10	5.2	176
B. brizantha (16289)	40.5	0.29	0.04	0.65	0.14	0.12	4.2	248
B. brizantha (16300)	41.3	0.30	0.05	0.45	0.19	0.15	2.6	166
B. brizantha (16305)	40.7	0.18	0.03	0.45	0.07	0.13	3.8	113
B. brizantha (16319)	39.9	0.17	0.03	0.37	0.11	0.16	3.0	119
B. brizantha (16322)	39.4	0.19	0.03	0.49	0.18	0.11	7.4	131
B. brizantha (16467)	39.6	0.21	0.04	0.99	0.12	0.13	4.6	112
B. brizantha (16488)	40.9	0.16	0.02	0.39	0.16	0.12	3.6	106
B. brizantha (16549)	40.4	0.23	0.04	0.44	0.13	0.14	3.6	211
B. brizantha (26110)	-	-	-	-	-	-	-	-
B. brizantha (26646)	40.6	0.18	0.03	0.39	0.16	0.18	3.2	116
Brachiaria hybrid (1873)	39.9	1.05	0.02	0.21	0.19	0.14	2.2	161
Mean	40.2	0.32	0.05	0.58	0.13	0.14	4.2	178

Although forage yield data were not available, among the 19 accessions of *Arachis* species tested, *A. repens* CIAT 22164 contained greater concentration of N in shoots (Table 56). This accession also showed greater levels of Ca and ash content in shoot tissue. Among the 14 accessions of *A. pintoi*, CIAT 22148 showed lower levels of Ca and ash content. The level of TNC in this

accession was also lower than the commercial cultivar (CIAT 17434). It is important to note that *A. pintoi* CIAT 22160 which was selected as dry season tolerant accession from field evaluation in cerrados of Brazil showed lower contents of K, Ca and total nonstructural carbohydrates (TNC) in the shoot tissue. Shoot Ca, Mg and ash contents of *A. pintoi* CIAT 22148 were the lowest among the 19 accessions tested. Similar results were obtained on this accession in the previous dry season. There is a need to further evaluate this accession compared with CIAT 22160 for their tolerance to dry season.

Table 56. Genotypic variation in shoot composition of nutrients, ash content and total nonstructural carbohydrates (TNC) of 19 accessions of *Arachis* species grown during dry season at Atenas, Costa Rica.

Genotype (CIAT No.)	Shoot composition							
	C	N	P	K	Ca	Mg	Ash	TNC
				(%)				(mg/kg)
A. pintoi (17434)	37.4	1.38	0.04	0.31	2.64	0.67	9.8	161
A. pintoi (18744)	39.9	1.81	0.06	0.38	2.89	0.74	10.2	133
A. pintoi (22148)	39.7	1.92	0.06	0.68	1.78	0.32	7.0	149
A. pintoi (22149)	38.7	1.91	0.07	0.62	2.26	0.37	7.8	154
A. pintoi (22150)	42.1	1.25	0.07	0.84	1.97	0.46	7.6	146
A. pintoi (22151)	38.1	2.01	0.08	0.56	2.43	0.45	8.4	125
A. pintoi (22152)	36.9	2.21	0.08	0.40	2.25	0.61	8.8	147
A. pintoi (22154)	36.6	1.93	0.06	0.44	2.41	0.69	8.4	146
A. pintoi (22155)	36.3	1.85	0.07	0.91	2.19	0.49	7.8	139
A. pintoi (22156)	37.6	1.84	0.06	0.89	2.17	0.41	7.8	149
A. pintoi (22157)	37.1	2.00	0.08	0.66	2.34	0.41	8.8	138
A. pintoi (22158)	35.8	2.01	0.07	0.59	2.56	0.47	9.4	136
A. pintoi (22159)	38.2	1.87	0.06	0.39	2.34	0.66	10.0	130
A. pintoi (22160)	38.3	1.74	0.05	0.27	2.22	0.86	8.2	91
A. repens (22161)	37.4	2.01	0.08	0.30	2.4	0.75	9.2	95
A. repens (22162)	35.5	1.90	0.10	0.34	2.94	0.59	9.6	179
A. repens (22163)	34.5	2.01	0.10	0.36	3.1	0.82	10.4	106
A. repens (22164)	36.1	2.61	0.14	0.41	2.38	0.63	8.2	101
A. repens (22165)	37.3	1.94	0.08	0.41	2.31	0.77	8.0	85
Mean	37.6	1.91	0.07	0.51	2.40	0.59	8.7	132

Progress towards achieving output milestones

- Plant attributes of *Brachiaria, Arachis* and *Calliandra* for adaptation to dry season stress are defined
- New genotypes of *Brachiaria, Arachis, Cratylia* and *Calliandra* with dry season tolerance are available for evaluation with NARS partners.

We continued our efforts at Atenas site in Costa Rica to identify plant attributes that confer tolerance to dry season. Results obtained so far indicate that lower levels of Ca and higher levels of total nonstructural carbohydrates in green leaves and a greater proportion of green leaves compared to dry leaves during dry season may serve as indicators of dry season tolerance in *Brachiaria* and possibly *Arachis*.

Two accessions of *B. brizantha* (CIAT 16488 and 26110) were identified as promising materials for areas with prolonged drought and seed has been multiplied for distribution to partners for regional evaluation. Among the *Arachis* accessions tested, two accessions of *A. pintoi* (CIAT 22148 and 22160) were identified as having some drought tolerance. Further testing is needed to identify plant indicators for dry season tolerance.

Activity 3.3. Identify accessions of *Brachiaria* and *Paspalum* with adaptation to poorly drained soils

Highlight

- Selected accessions of *Paspalum* spp. and *Brachiaria* spp. were established in a field trial in well and poorly drained soils in Costa Rica.

Large areas of poorly drained savannas occur in the Colombian and 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 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. In addition, CIAT acquired a collection of *Paspalum* species most of which are thought to be tolerant to waterlogged conditions. This collection is being evaluated in Quilichao in a poorly drained site and results are summarized in the report.

3.3.1 Genotypic variation in *Paspalum* for adaptation to poorly drained soils–Colombia (M. Peters, L.H. Franco, P. Avila, B. Hincapie)

Rationale: 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, which are currently under evaluation in Quilichao and Costa Rica.

Methods: Eight accessions of *Paspalum* spp. were established in 1996 in a poorly drained site in Quilichao in replicated randomized plots. Measurements include plant survival , DM yield, forage quality and observations on flowering and seed production.

Results and Discussion: Among the original eight accessions of *Paspalum* planted at Quilichao seven persisted and of these, *Paspalum plicatulum* CIAT 26989, *Paspalum arundinellum* CIAT 26987, and *Paspalum atratum* CIAT 26986 were identified as superior accessions (Figure 64).

It was interesting to observe that forage yield of CIAT 26986 and 26989 was relatively high in the dry season, indicating potential of these accessions to ensure good forage supply through out the year under poor drainage conditions.

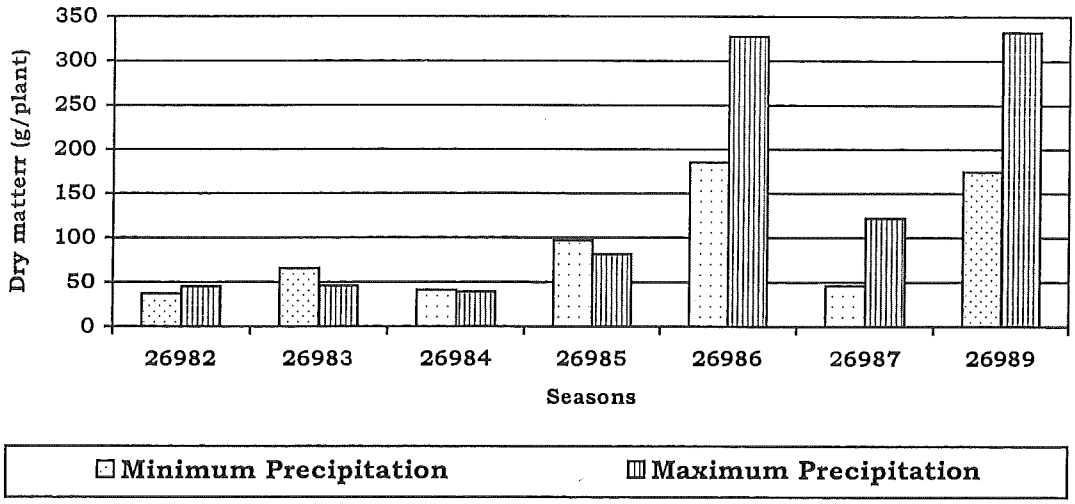


Figure 64. Dry matter production in a collection of *Paspalum* spp. in different seasons, growing under conditions including acid and poorly drained soils, Santander de Quilichao, Cauca, Colombia.

3.3.2 Adaptation of *Brachiaria* and *Paspalum* ecotypes to both well drained and poorly drained soil conditions (P. J. Argel, M. Villarreal (ITCR) and G. Pérez)

Rationale: The landscape of cattle production areas in subhumid and humid tropical environments is not uniform, thus a combination of well drained and poorly drained regions are frequently found. Farmers generally use different pasture species according to the draining condition of the soils, but the options are limitted to few species of *Brachiaria,* to *Echinochloa polystachya* (pasto Alemán), and to native poor quality species of *Hymenachne* and *Paspalum fasciculatum.* On the other hand, many sites with very heavy clay-loam soils (cracking soils) experience waterlogged conditions during the wet period but lose water and crack during the dry season; thus, at these sites persistent forages need to be adapted to both wet and dry periodical soil conditions.

Presently there are promising new genotypes of *Brachiaria* and *Paspalum,* potentially well adapted and productive under waterlogged conditions.

Methods: The evaluation of a number of grasses (*B. brizantha* CIAT 26159, CIAT 26427, CIAT 26318, CIAT 26124 and CIAT 26110; *B. humidicola* CIAT 16113; *Brachiaria* hybrids CIAT 36062 (BR93-NO/1371), CIAT 36061 (FM9201/1873), and *P. guenoarum* CIAT 26985, *P. atratum* CIAT 26986, *P. plicatulum* CIAT 26989, and *B. brizantha* cv. Marandú as check) was initiated this year in Costa Rica. Two contrasting sites were selected for the experiment. A well drained site is located at Atenas, and the other is a poorly drained site located in San Carlos. Direct planting was made at Atenas, while in San Carlos because high humidity of the soil, seeds were pregerminated and seedlings transplanted to the field.

Atenas represents a subhumid tropical forest located at 460 masl, 1600 mm of rainfall distributed from may to november, mean temperature of 23.7 °C and well drained inceptisol soils (sandy loams) of medium fertility, pH 5.9, good levels of OM (7.6 %), medium levels of P (3.6 ppm), high levels of calcium (9.5 meq/100 g), low levels of aluminum and acceptable levels of micronutrients. The site in San Carlos is a humid tropical forest located at 225 masl, 3430 mm of rainfall with a very short dry season, mean temperature of 26.0 °C and poorly drained inceptisol isohipertermic soils of medium fertility, pH 5.3, 5-16 ppm of P, 18.5 meq/100 of calcium and low levels of aluminum.

A total of 28 plants, spaced 0.5 m X 0.5 m, were planted in 2.5 m X 3.0 m plots, and replicated 3 times. Plant survival, height and plant cover will be measured at 4, 8 and 12 weeks after planting. A uniformity cut will be made at the latter date and plant survival and DM yields measured every 5-6 weeks of regrowth. The leaf/stem ratio will be measured in 2 of the evaluation cuts. The experiement is expected to last two growing seasons.

Progress towards achieving output milestones

- New accessions of *Brachiaria* and *Paspalum* selected for poorly drained soils are available to partners for evaluation.

A list of accessions of *Paspalum* spp. well adapted to poorly drained soils is available. Seed production of these accessions has been poor in Quilichao and Popayán but not in Costa Rica. A small plot trial with different genotypes of *Brachiaria* and *Paspalum* was established this year in well drained and poorly drained soils in Costa Rica in collaboration with partners.

Activity 3.4: Identify accessions of shrub legumes with adaptation to different environments

Highlights

- Identified new species of *Leucaena* with broad adaptation to biotic and abiotic constraints in humid and subhumid environments.
- Selected two ecotypes of *Calliandra calothyrsus* for mid-altitude hillsides with acid soils.
- Selected four accessions of *Rhynchosia schomburgkii* for higher-altitude hillsides.
- Increased the number of accessions of *Cratylia argentea* in the Genetic Resources Unit.

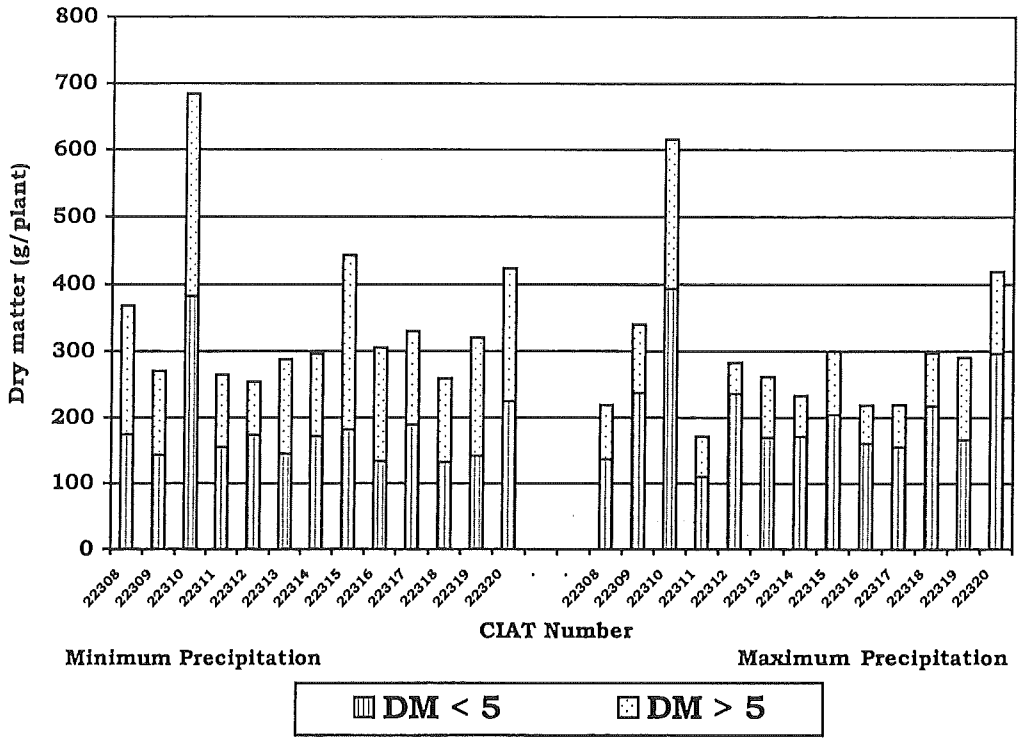
3.4.1 Evaluation of *Calliandra* provenances for agronomic performance in the wet and dry season (M. Peters, A. Pottinger and P. Avila)

Rationale: *Calliandra calothyrsus* is a shrub legume with potential use as dry season supplement for livestock. Thus a small collection is currently being evaluated for quality and agronomic performance at the Quilichao Research station in collaboration with the Oxford Forestry Institute (OFI).

Methods: A total of 13 accessions from OFI, UK were planted in Quilichao during 1996 in single rows of 9 plants each. The design was a complete block with three replications. Measurements include dry matter production, ratio of edible to total dry matter, nutritive value of edible dry matter and regrowth after cutting.

Results and Discussion: The evaluation of the 13 accessions of *Calliandra calothyrsus* in Quilichao as part of an OFI study will be terminated by early 2000. Preliminary data analysis of agronomic performance indicates that the accessions CIAT 22310 and 22320 are the most productive in terms of edible forage (Figure 65).

Seed multiplication is underway to evaluate these –and other–accessions at other sites as alternatives to *Leucaena* spp. and *Cratylia argentea* in subhumid mid-altitude hillsides with low fertility soils.



*DM < 5 = Dry matter with a stem less than 5 mm
*DM < 5 = Dry matter with a stem greater than 5 mm

Figure 65. Dry matter production in a collection of *Calliandra calothyrsus* during minimum and maximum rainfall, Santander de Quilichao, Cauca, Colombia.

3.4.2 Evaluation of a collection of *Rhynchosia schomburgkii* (M. Peters, B. Hincapie, P. Avila)

Rationale: From previous evaluation of a range shrub legumes in hillsides of Cauca, *Rhynchosia schomburgkii* emerged as one of the most promising species due to good cool tolerance. Thus we were interested in evaluating a number of accessions of this legume to select those with superior yield.

Methods: A total of 13 accessions of *Rhynchosia schomburgkii*, mostly originating from Colombia, were planted at Quilichao. Plants were transplanted into single-row plots, with 4 replications. Dry matter yield, plant persistence, drought tolerance and forage quality are the main parameters to be evaluated.

Results and discussion: Among the most promising accessions in terms of dry matter production during the wet season are CIAT 918, 17918, 19235 and 22134 (Figure 66. Variation in growth habit in accessions of *Rhynchosia schomburgkii* is low. Accessions being evaluated are intermediate between a herbaceous and woody and have the ability to twine.

While this legume has potential as a soil cover plant, its value as a source of feed will depend on how acceptable the forage is to livestock. Cafetería type trials will be carried out next year to assess acceptability to cattle of the different accessions.

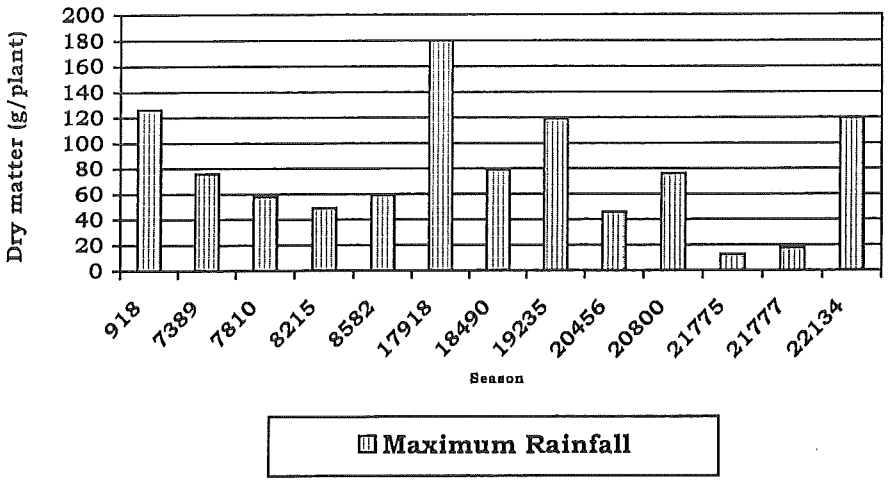


Figure 66. Dry matter production of a collection of *Rhynchosia schomburgkii* during maximum precipitation, Santander de Quilichao, Cauca, Colombia.

3.4.3 Characterization of a collection of *Cratylia argentea* (M. Peters, L. H. Franco, B. Hincapie, P. Avila)

Rationale: It is now well documented through the work of Tropileche that *Cratylia argentea* is an excellent alternative as a cut and carry legume for dry season feeding. Some accessions are currently being evaluated and adopted by farmers, in Central America and Colombia. However, the genetic resource base of this species has been limited up to now and hence activities to acquire new germplasm of *Cratylia argentea* was of high priority.

Methods: New germplasm of *Cratylia argentea* was received this year from EMBRAPA/ CENARGEN in Brasil (see Table 57 for passport data) and were combined with accessions available in the CIAT collection for field evaluation. In total 41 accessions of *Cratylia argentea* were sown in pots in a greenhouse in Palmira and subsequently planted in the field (Quilichao) as a Randomized Complete Block design with three replications.

Table 57. Passport data of *Cratylia argentea.* Received from CENARGEN-EMBRAPA,Brazil during 1999.

Number	Country	State	km	City	Latitude	Longitude	Altitude	Synonyms
22404	Brazil	Goias	24	Campos Belos	13 S 1	56 W 37	650	CO LC-87701; DO BRA-000191
22405	Brazil	Goias	52	Campos Belos	13 S 15	46 W 28	700	CO LC-8703; DO BRA-000213
22406	Brazil	Goias	21	Posse	14 S 15	46 W 30	780	CO LC-8704; DO BRA-000221
22407	Brazil	Goias	180	Brazilia	14 S 54	46 W 56	500	CO GPS-000515; DO BRA-000515
22408	Brazil	Goias	6	Posse	14 S 6	46 W 25	810	CO PZ-0003; DO BRA-000540
22409	Brazil	Goias		Divino Polis Goias	13 S 27	46 W 22	540	CO PZ-006; DO BRA-000566
22410	Brazil	Goias	3	Divino Polis Goias	13 S 17	46 W 25	660	CO PZ-007; DO BRA-000574
22411	Brazil	Mato Grosso	9	Campos Belos	15 S 12	46 W 47	550	CO GPS-3019; DO BRA-000647
22412	Brazil	Goias	56	Barra do Garcas	15 S 42	52 W 43	400	CO GPS-3040; DO BRA-000779

Each replication consists of 7 plants per accession. Measurements will include: seasonal forage yield and quality and seed production. An additional replication with 33 accessions was sown for seed production and morphological observations.

3.4.4. Evaluation of adaptation of new Leucaena species in different sites in tropical America (P.J. Argel, G. Pérez and Alan Pottinger).

Rationale: During 1999 the evaluation of new *Leucaena* species, in collaboration with OFI (England), ended in Atenas, Costa Rica, and continued in Mexico, Honduras, Panamá, Colombia and Brazil. Despite differences in evaluation time, variations within the genus *Leucaena* were confirmed in terms of DM yields, climate and soil adaptation, as well as susceptibility to psyllid insect (see IP5 Annual Reports 1997, 1998). A group of promising new species was selected that showed good adaptation across sites, these lines form the base to further evaluate the genus.

Methods: Nineteen lines of different species of *Leucaena* were planted for evaluation in Costa Rica at the Escuela Centroamericana de Ganadería (ECAG), and in Mexico, Honduras, Panamá, Colombia and Brazil. Considerable variation in soil, altitude and climate characteristics were recorded in sites where the trials established successfully. Altitude over sea level varied from 33 m up to 460 m, and main ecosystems were classified as tropical dry forest, tropical isohypertermic savannas and very humid and subhumid tropical forest. Annual total rainfall varied from 875 mm in Comayagua (Honduras) up to 4500 mm in Gualaca (Panamá), and also varied the distribution of the rains.

For instance, Atenas in Costa Rica, Comayagua and Isla in Mexico have a dry period of approximately 6 months, while this period in Gualaca (Panamá) lasts from 3 to 4 months. On the other hand, mean daily temperature varied from 20.0 °C to 26.0 °C across sites. Variations also existed in soil type, soil pH, and percentage of soil aluminum saturation and P content. Gualaca in Panamá had the more acidic soil recorded, this site had high aluminum content and very low levels of P as well. Meanwhile, Comayagua, Atenas and San Carlos in Costa Rica presented soils with low acidity, no problems with aluminum toxicity and medium levels of P content. Direct planting was made in rows spaced 0.5 m apart. The experimental plot consisted of 10 plants per plot spaced 0.5 m between plant and replicated 4 times. At each experimental site plant height, plant diameter, plant mortality, total and edible DM yields and the incidence of diseases and psyllid insect were measured every eight to twelve weeks of regrowth.

Results: Considerable variation in growth, plant mortality, regrowth capacity following cutting, psyllid tolerance and dry matter yields, were observed between *Leucaena* species across sites. At Atenas, Costa Rica, plant height 10 months after planting varied from 0.5 m to more than 1.5 m; the best initial growth was recorded in *L. collinsii* subsp. *collinsii* 52/88, and the poorest growth was observed in *L. multicapitula* 81/87. Also in Atenas, plant mortality at the end of the second dry season was high for *L. multicapitula* 81/87 (22 plants), *L. trichodes* 61/88 (11 plants) and *L. lempirana* 6/91(8 plants).

No mortality was recorded for cv. Tarramba, *L. macrophylla* subsp. *nelsonii* 47/85, *Leucaena* hybrids 52/87 and 1/95, *L. salvadorensis* 17/86, *L. pallida* 14/96, *L. diversifolia* subsp. *diversifolia* 83/92, *L. collinsii* subsp. *zacapana* 56/88 and *L. esculenta* subsp. *esculenta* 47/87. Other *Leucaena* species had plant mortality that ranged from 1 to 4 plants; meanwhile the different species included in this experiment, *C. callothyrsus* DPI 115690 and *C. argentea* CIAT 18668, had plant mortality of 22 plants and none respectively. Table 58 shows that in Atenas DM yields of edible tissues were close to 100 g/plant/cut during the wet season for the following species: L. *trichandra* 53/88, *L. collinsii* 52/88, cv. Tarramba, *L. pallida* 14/96 and *Leucaena* hybrids 1/95 and 52/87.

Table 58. Edible dry matter (DM) yields of *Leucaena* species and one line each of *Cratylia argentea* and *Calliandra collothyrsus* established in Atenas, Costa Rica (Means of 4 cuts every 8 weeks during the wet season and 4 cuts every 8 – 12 weeks during the dry season).

Species	ID No. (OFI)	Edible DM yields (g/plant) Wet	Dry	Total (g/plant)
L. trichandra	53/88	118.8	96.2	215.0 a*
L. collinsii	52/88	110.5	100.4	210.9 a
L. leucocephala subsp. *glabrata*	34/92	104.2	85.1	189.3 ab
L. pallida	14/96	104.1	65.7	169.8 bc
L. hybrid (unknokwn parents)	1/95	98.7	70.1	168.7 bc
L. macrophylla subsp. *nelsonii*	47/85	79.0	81.8	160.8 bcd
L. leucocephala CIAT	17263	82.0	73.4	155.4 bcd
L. lucocephala x *L. pallida* (F_5)	52/87	102.9	49.9	152.8 bcd
L. salvadorensis	17/86	81.7	70.4	152.1 bcd
L. lanceolata	43/85	75.1	60.1	135.2 cde
L. diversifolia subsp. *diversifolia*	83/92	74.0	59.7	133.6 def
L. pallida	79/92	81.7	44.5	126.2 defg
L. esculenta subsp. *esculenta*	47/87	87.2	38.2	125.4 efg
L. pulverulenta	83/87	64.7	42.0	106.7 efg
L. collinsii subsp. *zacapana*	56/88	57.9	38.4	96.3 fg
L. lempirana	6/91	45.4	47.9	93.4 fg
L. shannonii subsp. *magnifica*	19/84	40.5	47.1	87.7 g
L. trichodes	61/88	11.2	4.1	15.3 h
L. multicapitula	81/87	3.6	1.7	5.3 h
C. argentea CIAT	18668	110.4	102.6	212.9 a
C. callothyrsus DPI	115690	21.5	15.5	40.0 h

*Within a column means followed by the same letter are not significantly different (P>0.05)

These lines also produced high DM yields during the dry period with the exception of *Leucaena* hybrid 52/87 that had a reduction of nearly 50% yield during this period. It was also interesting to note that *L. macrophylla* subsp. *nelsonii* 47/85 (also a promising line) had a tendency to produce more DM yield during the dry period, and indication of tolerance to prolonged dry conditions. Meanwhile, very low DM yields during the same period (less than 50 g/plant/cut) were recorded for *L. lempirana* 6/91, *L. shannonii* subsp. *magnifica* 19/84, *L. trichodes* 61/88 and *L. multicapitula* 81/87. Other *Leucaena* species had intermediate DM yields.

Besides Atenas, Costa Rica, *L. trichandra* 53/88 adapted well to Isla (Mexico), Morretes (Brazil), and to Comayagua (Honduras). Similarly *L. pallida* 14/96, cv. Tarramba and *L. macrophylla* subsp. *nelsonii* 47/85 were among the species with better adaptation across sites; however *L. collinsii* 52/88 did well in Atenas, but poor in other sites with the exception of Comayagua. Meanwhile, *L. lempirana* 6/91, *L. trichodes* 61/88 and *L. multicapitula* 81/87 adapted poorly to most of the sites. Other *Leucaena* species had intermediate DM yields including *L. leucocephala* CIAT 17263, a local check that had been selected before in Atenas out of a collection of 94 accessions.

Damage by the psyllid insect was reported only from the trial at Atenas, but was also present in Comayagua and Gualaca, Panamá. However, the insect did not cause plant mortality, although differences existed between *Leucaena* species related to psyllid tolerance. The highest damage index rated was 4.0 (tips and young leaves severely curled, yellow and covered with sap), and observed in *L. salvadorensis* 17/86, *L. collinsii* subsp. *zacapana* 56/88 and *L. multicapitula* 81/87. *L. trichandra* 53/88, showed good tolerance, while other promising materials like cv. Tarramba and *L. collinsii* 52/88 had ratings of 3.4 and 2.0 respectively (tips and young leaves moderately curled and yellow). Mild attacks of the fungus *Camptomeris leucaenae* were recorded, particularly in old leaves of *Leucaena* species.

Discussion: The *Leucaena* trials should have beenevaluated under an uniform methodology proposed by OFI as it relates to plot size, number of plant per plot, cutting height, psyllid rating and frequency of evaluation. However, local circumstances obliged collaborators to adapt the evaluation of the trials to their own circumstances, particularly in relation with initiation and frequency of the evaluation cuts. Most of the trials had to be replanted because of failures in germination of some of the lines, and this produced some changes in the management of the trials. However, in spite of this problem useful conclusions can be made related to the adaptation of new *Leucaena* species to different environments in the Latin American tropics.

The species *L. trichandra* 53/88, *L. leucocephala* subsp. *glabrata* 34/92 (cv. Tarramba in Australia), *L. pallida* 14/96 and *L. macrophylla* subsp. *nelsonii* 47/85, showed good adaptation across sites. *L. collinsii* 52/88 also performed well in Honduras and Costa Rica (subhumid tropics with medium fertility soils), but poorly in Brazil, Panamá and Mexico; this corresponds with information that indicates that the latter species grows naturally in dry and semi-arid environments of Mexico and Guatemala. The resistance to the psyllid insect of this species and the good protein content reported from Honduras (23.7 %), suggests good forage potential of the species for dry environments.

L. trichandra has considerable variability within the species, and the resistance to the psyllid insect, to poor acid soils and the forage quality, are directly related to plant origin. The present line evaluated *L. trichandra* 53/88, originates from Los Guates (Guatemala), and is regarded as more tolerant to the psyllid insect and of superior growth than other plants from different origins in Mexico and Central America.

L. pallida is highly resistant to the psyllid insect and has been an important source of resistance in plant breeding programs of *Leucaena.* The present line evaluated, *L. pallida* 14/96, is a composite coming from CSIRO (Australia); it showed acceptable DM yields across sites and good level of crude protein (20.6 %); for this reasons it should be considered a species that has also good forage potential.

L. macrophylla is a less known species, and one the characteristics of this plant is the larger size of the leaflets (3 –7 cm long). These trials showed that the species has a wide range of adaptation and grows well in subhumid as well as in humid environments with very acid soils like in Gualaca, Panamá. It had 22.6 % of crude protein as reported from Comayagua, Honduras, indicating that it is a species worth to be considered for further evaluation.

Cultivar Tarramba is presently used widely in Australia in cattle farms. It is susceptible to psyllid and has much of the forage quality characteristics of other *L. leucocephala* species. It showed good agronomic performance across sites and it is a germplasm readily available for commercial planting, since seed is available in Australia. These trials also showed the poor adaptation to all the sites of *L. trichodes* 61/88 (a species originated in Jipijapa, Ecuador), and also poor were *L. lempirana* 6/91 and *L. multicapiula* 81/87. The psyllid insect did not caused serious problems to the new *Leucaena* species at any site, perhaps because the insect has natural enemies in this part of the world. Close observation of the insect was followed in Atenas, Costa Rica, but the foliar damage recorded did no represent significant lost of DM yields.

In general, we can conclude that new species of *Leucaena* were identified as being more productive than the traditional *L. leucocephala* species. However, it is necessary to follow up the best lines and concentrate in agronomic aspects such as seed production potential, forage and wood quality and seedling vigor, to fully understand the opportunities that these new species may have in different production systems.

Progress towards achieving output milestone

- New accessions of *Leucaena, Calliandra, Cratylia* and *Rhynchosia* selected for tolerance to a wide range of environments are available to partners for evaluation.

We have now selected accessions of *Calliandra calothyrsus* and *Rhynchosia schomburgkii* with potential adaptation to mid-altitude hillsides and cool termperatures, respectively. In addition, we have identified species of *Leucaena* (*trichandra, pallida, leucocephala* subs *glabrata, macrophylla* subs *nelsonii*) with good adaptation in subhumid and humid environments. Seed of selected accessions within species is being multiplied for multilocational evaluation with partners.

Activity 3.5 Identify accessions of grasses and legumes adapted to environmental constraints in systems

Highlights

- Planted a collection of cowpea from in Quilichao to determine its utility as green manure.
- Initiated trials to evaluate different legumes as cover in plantations in the Llanos of Colombia.

3.5.1 Evaluation of an IITA collection of multipurpose (forage, soil fertility maintenance, human consumption) accessions of *Vigna unguiculata* for multipurpose use (M. Peters, L. H. Franco, B. Hincapie, P. Avila)

Rationale: Cowpea (*Vigna unguiculata*) is utilized in the subhumid/semi-arid tropics of West Africa and India as a source of food and feed for livestock. Work at CIAT with a limited number of accessions indicated high potential of cowpea for soil improvement in degraded soils in hillsides. However, distribution and evaluation of cowpea in Latin America so far has been limited.

Methods: A core collection of 15 cowpea accessions (Table 59) were obtained from B.B. Singh, cowpea breeder at IITA. These accessions were planted at CIAT's Quilichao site to evaluate grain and forage yield and value as green manure for a succeeding maize crop, to assess equivalent input to the soil from cowpea as grown as a green manure. All lines had high rates of germination (95%), and the establishment in the field was excellent.

Table 59. Lines of *Vigna unguiculata* received from IITA for planting in Quilichao.

IT86D-716	IT89KD-288	IT90K-284-2	IT93K-573-5	IT96D-759*
IT86D-719	IT89KD-391	IT93K-503-1	IT96D-733	IT95K-10882
IT86D-715	IT90K-277-2	IT93K-637-1	IT96D-740	IT95K-1088-4

*IT96D-759 only for multiplication of seed because have few seed for the experiment.

3.5.2 Evaluation of herbaceous legumes as cover crops in rubber and oil palm plantations in the Llanos of Colombia (C. Plazas and M. Peters)

Rationale: Plantations are being promoted in the Llanos of Colombia and thus there is a demand for appropriate covers to reduce weed infestation, to maintain and improve soil fertility, to control erosion and increase the microfauna biomass. In the rubber plantation the target group for this promotion are small to medium size farmers who want to diversify their farming operations.

In the oil palm plantations plots of up to 5 ha are rented out to landless farmers to manage the oil palms for the oil palm industry. Thus the selection of legume cover crop for rubber and oil palm plantation will have beneficial effect on the welfare of resource poor farmers.

Methods: In 1999 a range of legume species were sown under shade and no-shade conditions at two sites representing a well drained savannas and piedmont in the Meta department of Colombia. The following legume accessions were sown: *Arachis pintoi* (CIAT 17434, 18744, 18748, 22159), 22160 (seed rate 10 kg/ha); *Desmodium heterocarpon* ssp. *ovalifolium* CIAT 350, 13105, 13110, 13651, 23762 (0.5 kg/ha), a mixture of *A. pintoi* (CIAT 18744), *D. heterocarpon* ssp. *ovalifolium* (CIAT 13651) and *Pueraria phaseoloides* (CIAT 8042, 9900); a control without any cover crop was included as well. In the well drained savanna site, cover crops were planted in young and old rubber plantation to measure the effect of shade on the legumes. An additional trial with the same legume species was planted in an oil palm plantation in site in the piedmont.

The following parameters will be measured in the trials: (a) *Establishment phase*: germination, vigour and cover, incidence of weeds, flowering; (b) *Production phase:* yield during wet and dry seasons, weed population, tolerance of legumes to shade, decomposition in the soil of legume litter; plantation crop – height, diameter, vigour, productivity of old trees.

Progress towards achieving output milestones

- New accessions of *Pueraria, Centrosema,* cowpea, *Arachis, Desmodium,* and *Calliandra,* are available for evaluation with NARS partners as cover crops and green manures in savannas and hillsides.

The acquisition this year and planting of a collection of cowpea from IITA will allow us to select lines that are suitable as green manures in different production systems. In addition, we initiated the evaluation of different legume species as cover crops in rubber and oil palm plantations in the Llanos of Colombia.

Activity 3.6: Identify genotypes of *Brachiaria*, *Desmodium*, and *Arachis*, with broad edaphic and climatic adaptation

Highlight

- Identified two accessions of *Arachis pintoi* (CIAT 18744 and 22268) as being more productive than the commercial cultivar in sites with good soil fertility.
- Multiplied seed of selected genotypes of *Brachiaria* and *Desmodium* to facilitate on-farm evaluation with partners.

3.6.1 Identification of genotypes of *Brachiaria* with adaptation to biotic and abiotic constraints in Central America (P. J. Argel and G. Pérez)

Rationale: *Brachiaria* grasses are the more widely grown pastures in subhumid and humid tropics, and a number of commercial cultivars are presently available such as cv. Basilisk of *B. decumbens,* cvs. Marandú and La Libertad of *B. brizantha,* cv. Humidicola of *B. humidicola* and cv. Llanero of *B. dictyoneura.* All these cultivars have important roles in different cattle production systems, but also have limitations. For instance, cv. Basilisk adapts well to a wide range of soils and is easy to manage and establish from seed, but it is highly susceptible to spittlebugs and is associated with photosensitization in cattle. Cultivar Marandú is resistant to spittlebug, but requires soils of medium to good fertility, does not tolerate waterlogged soils and it is susceptible to foliar blight caused by *Rhizoctonia solani.* Cultivars Llanero and Humidicola, are also susceptible to spittlebugs and are of medium to low nutritional quality. Thus, it is essential

to have access to promising new *Brachiaria* germplasm in order to select potentially well adapted and more productive cultivars.

Methods: A field trial is currently underway at ECAG facilities in Atenas. This site represents a subhumid tropical forest ecosystem, located at 460 masl. Annual rainfall is1600 mm distributed from may to November and mean temperature is 23.7 °. Soils are classified as inceptisol (sandy loams) of medium fertility, with a pH 5.9, good levels of OM (7.6 %), medium levels of P (3.6 ppm), high levels of calcium (9.5 meq/100 g), low levels of aluminum and acceptable levels of micronutrients.

Scarified seeds of *B. brizantha* CIAT 26110, *Brachiaria* hybrid CIAT 1873 and *B. brizantha* cv. Marandú as check, were planted in rows 0.50 m apart and 0.50 m between plants in 2 x 3 m plot size. Five seeds were planted by site and later thinned to one plant per site. Treatments (lines of *Brachiaria*) were distributed in a randomized block design replicated 4 times. Plots were fertilized with 50 kg of N/ha one month after planting; three months later uniformity cut at 15 cm height was made to all plots. Evaluations on DM yields have been made every 5 weeks of regrowth during the wet season and every 8 weeks during the dry period. The leaf/stem ratio was also measured in one of the cuts during the wet period; a total of 5 evaluation cuts have been made up to date.

Results and Discussion: DM yields have been similar for cv. Marandú and a *Brachiaria* hybrid and significantly lowers ($P< 0.05$) than those recorded for *B. brizantha* CIAT 26110 (Table 60). This has been associated with a better and more uniform and vigorous regrowth after cutting of the latter accession, both during the dry (2 cuts) and the wet period (3 cuts). Cultivar Marandú and the *Brachiaria* hybrid have also showed high susceptibility to foliar blight caused by *Rhizoctonia solani,* particularly during cycles of high temperatures and high relative humidity in the wet season. The fungus show necrotic lesions of cream to greyish-white color on leaves and stems that reduces the foliar area and obviously affects DM yields. The symptoms come on cycles and tend to disappear as the dry season enters. The leaf/stem ratio Was similar in cv. Marandú and CIAT 26110, but slightly lower in the *Brachiaria* hybrid. This latter plant produces very hairy and soft leaves, that look similar to *B. ruziziensis* (one of the parents of this hybrid).

Table 60. Dry matter yields and leaf/stem ratios of promising *Brachiaria* ecotypes established in Atenas, Costa Rica (Means of 5 evaluation cuts – 2 cuts during the dry season and 3 cuts during the wet period).

Ecotypes	DM yields (g/m^2)	Leaf/Stem Ratio
B. brizantha CIAT 26110	291.2 a*	1.4
B. brizantha cv. Marandú	204. 3 b	1.5
Brachiaria hybrid CIAT 1873	202.8 b	1.3

* $P<0.05$

A number of *Brachiaria* species grow well in fertile soils, but also adapt to a wide range of low-fertility soils because they tolerate high Al soil concentrations and low P and Ca contents. The soils at Atenas are inceptisols of medium fertility without Al toxicity and high levels of Ca, and this accounts for the relatively high DM yields recorded in all the *Brachiaria* ecotypes up to date. However, *B. brizantha* CIAT 26110 produced around 30 % more DM yield than the other lines of *Brachiaria,* which indicates the good adaptation that it has to subhumid environments similar to Atenas.

Cultivar Marandú is resistant to spittlebug, and it is one of the parents of the *Brachiaria* hybrid, but this insect has been reported only as present in Atenas and as a consequence tolerance to the insect has not been evaluated up to date. On the other hand, the susceptibility to foliar blight of cv. Marandú and the *Brachiaria* hybrid is of some concern because the reduction in DM yields. However *B. brizantha* CIAT 26110 has not shown up to date any of the foliar symptoms produced by the fungi. This line has also shown to be resistant to *Fusarium* sp. fungi and mild susceptibility to *Pythium* sp. under waterlogged conditions (IP5, 1998 AR). None of these *Brachiaria* lines are stoloniferous, although CIAT 26110 may root decumbent stems in close contact with the soil and thus improve ground cover. On the other hand, the growth habit of these lines facilitates the establishment of the grasses with associated legumes such as *Arachis pintoi,* because low initial competition. In general, our results indicate that *B. brizantha* CIAT 26110 is a highly promising line that deserves further evaluation to better document its forage quality and animal production potential so that it may be considered for release shortly as a new *Brachiaria* cultivar.

3.6.2 Multilocational evaluation of a core collection of *D. heterocarpon* ssp. *ovalifolium:* Influence of physical soil characteristics and its interactions with climatic conditions on productivity and quality (A. Salamanca, A. Schmidt, E. Amézquita, M. Peters)

Rationale: Current research on tropical forages at CIAT aims to identify and develop legume and grass germplasm for marginal conditions such as acid, low-fertility soils in the humid and subhumid tropics in order to contribute to increased livestock production and soil enhancement. To select legume germplasm for different environments and production systems, there is a need to define the influence of soil factors on biomass production, quality attributes and possible interactions with genotypes. Yet the knowledge on how physical and chemical soil factors affect different species and genotypes of tropical legumes is limited.

An important example is *Desmodium heterocarpon* ssp. *ovalifolium* which has high biomass production in acid soils and adapts to wide range of climatic conditions. However, forage quality is negatively affected by high condensed tannin (CT) content, which is influenced by environmental factor as well as by genotype. Identification of *D. heterocarpon* ssp. *ovalifolium* genotypes with high forage production and nutritive value is considered key for developing persistent grass/legume combination for different production systems in the humid tropics.

In the IP5 1998 AR we presented results on the effect of sites on quality of different genotypes of *D. heterocarpon* ssp. *ovalifolium* and indicated that it would appear that soil physical parameters had an influence on forage quality attributes. Thus this year we measured different soil physical characteristics in the sites where genotypes of *D. heterocarpon* spp. *ovalifolium* were evaluated.

Methods*:* Soil samples were collected from the six sites of the multi-locational trial established in 1995 (Table 61). For Alcancía, Maquenque, Macagual and Rueda samples were taken in the periods of low and high rainfall, but only in the wet season for the sites La Romelia and Melcho. In each site 9 plots of accessions 33058, 350 and 13089 (high fertilization level) were compared with three plots without *Desmodium* (control). Samples were taken at six soil depths (0-5, 5-10, 10-15, 15,20, 20-30 y 30-40 cm) and analysed for: apparent density, hydraulic conductivity, air permeabilty, total porosity, size of pores, texture, water retention, and chemical analyses. In addition, measurements on resistance to rupture and penetrabilty was determined together with studies on root distribution and effect of moisture on compaction.

Results and Discussion: In this report we only present the results of soil physical parameters in the differents sites were *D. heterocarpon* ssp. *ovalifolium* genotypes were evaluated. At each sites, the variabilty between accessions and control was low, therefore the results of the three accessions and control were averaged.

Table 61. Sites used in the multilocational trial of *Desmodium heterocarpon* ssp. *ovalifolium.*

Environment	Site	Latitude	Longitude	Altitude msnm	Precipitation mm/year	Temperature Annual average °C	Characterization
Dry hillsides	1. El Melcho	2°44'23"N	76°33'34"O	1555	1800	17.5	Mod. fertility Inceptisol
Humid hillsides	2. La Romelia	4°58'20"N	75°39'58"O	1360	2600	20	Fertile Andisol
Savanna	3. La Alcancía	4°34'37"N	71°21'09"O	150	2300	26	Low fertility High clay Oxisol
	4. Maquenque	4°31'17"N	71°15'41"O	150	2300	26	Low fertility Sandy Oxisol
Tropical Forest	5. La Rueda	1°26'10"N	75°25'47"O	180	3500	25	Low fertility high clay Ultisol
	6. Macagual	1°29'59"N	75°39'33"O	190	3500	25	low fertility High clay Ultisol

Apparent soil density: In Figure 67 we show results on apparent soil density (mg/m^3) as affected by site and season. In the hillsides sites, low soil density values were recorded given that soils are derived from volcanic ashes. In savannas the apparent soil density tended to increase during the wet season and with soil depth, probably due to accumulation of leached sediments in the lower levels during times of high humidity. In the sites in the humid forest, the highest soil density values were recorded in the dry season, probably caused by a contraction of the drying soil.

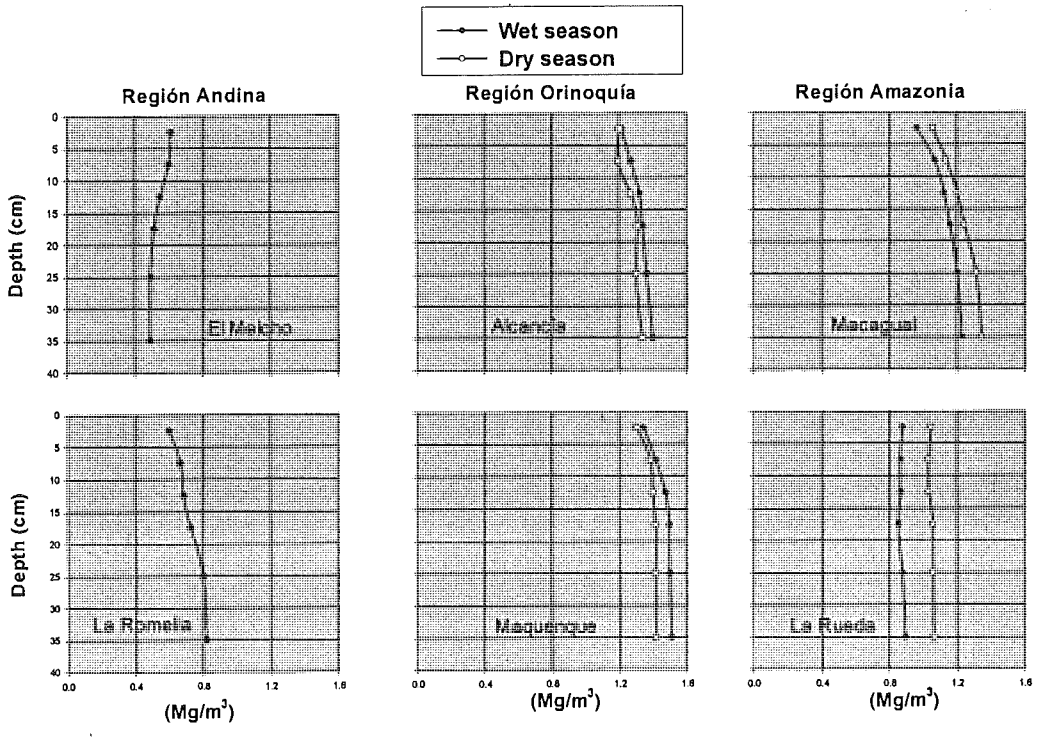


Figure 67. Multilocational trial of *Desmodium heterocarpon* ssp. *ovalifolium* : Apparent density of experimental sites.

While in La Rueda the variation due to soil depth was low, in Macagual an increase in apparent density with soil depth was observed for the two seasons. Fluctuation in soil density due to season was greatest in La Rueda as compared to Macagual, probably due to high clay content of soils. In general, the lowest values for apparent soil density were found up to depth of 15 cm, indicating the potential importance of *D. heterocarpon* ssp. *ovalifolium* as a soil stabilizer given its deep root system.

Hydraulic conductivity: In Figure 68 we show the hydraulic conductivity characteristics of the soil at each site. The two sites in the hillsides showed only minor variation due to soil depth, with a tendency of lower values with increasing depth. These soils – because of their structural characteristics and their saturated conditions – do not allow a high hydric flow, indicating the importance of a legume cover to counteract soil erosion.

The site Maquenque in the savannas had the highest values of soil hydraulic conductivity, due to its sandy texture. However, in the Alcancia site, hydraulic conductivity decreased below 15 cm of depth, indicating that these soils only drain in the upper layers, and thus are easily saturated with excess water. In the humid forest the values for hydraulic conductivity were lower than in other sites.

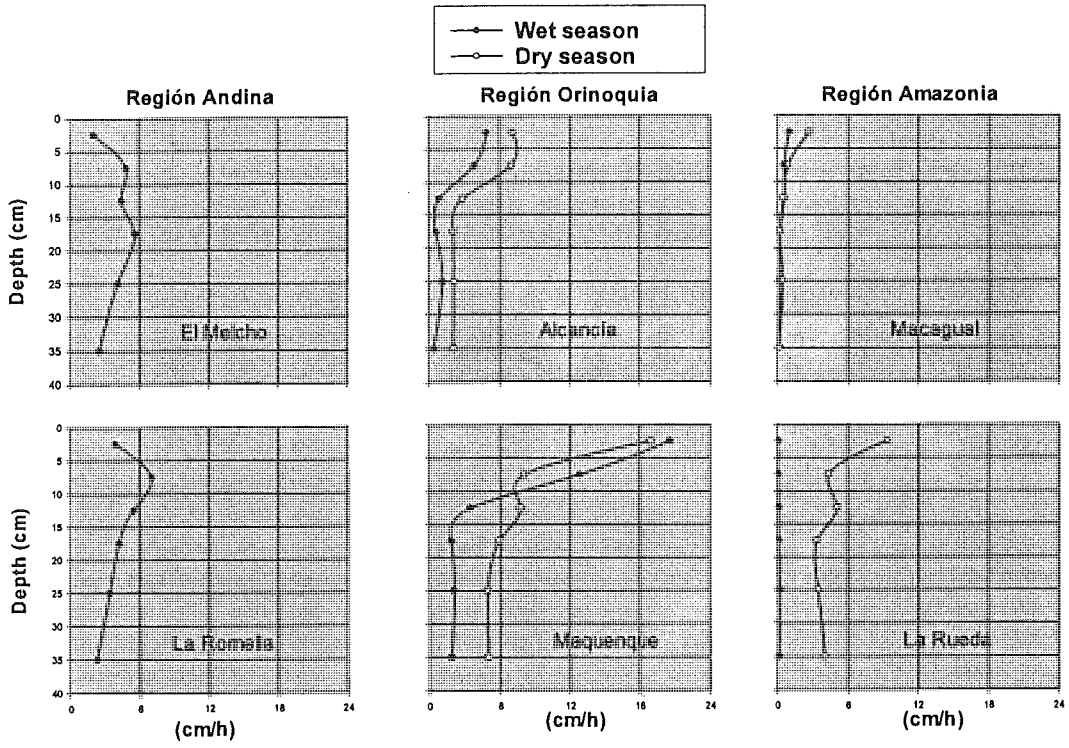


Figure 68. Multilocational trial of *Desmodium heterocarpon* ssp. *ovalifolium* : Saturated hydraulic conductivity of experimental sites.

These soils have poor drainage and oxigen for plant roots is often limited and because of these characteristics behave as dry soils in a humid environment. *Air permeability:* Results on air permeability (Figure 69) indicate constant behaviour in the soils at the two hillside sites, possibly due to high OM content. In the savannas air permeability was high up to 15 cm depth, and then decreases rapidly, showing the importance of a deep rooting legume species, to improve air penetration in the soil. Like the soils in savannas, the soils of the humid forest had low air flow in the dry season.

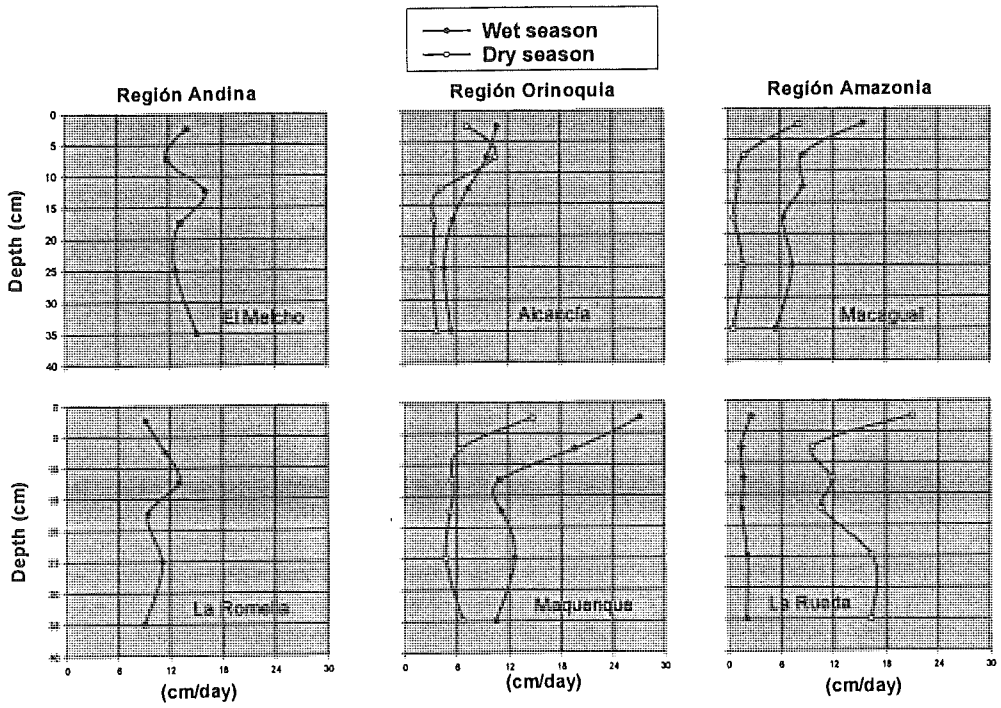


Figure 69. Multilocational trial of *Desmodium heterocarpon* ssp. *ovalifolium* : Permeability of air at 75 cm of experimental sites.

Penetrability: Values for resistance to rupture (kpa) and penetrability (Mpa) in the different sites are shown in Figures 70 and 71. The results reflects the physical status of soils as affected by seasonal variation.

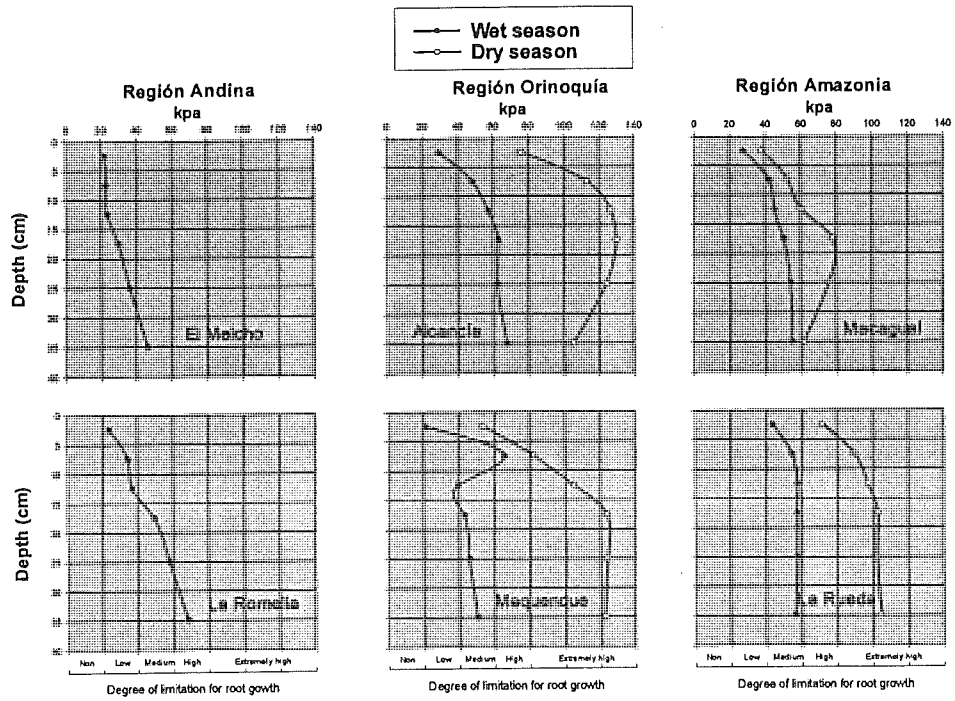


Figure 70. Multilocational trial of *Desmodium heterocarpon* ssp. *ovalifolium* : Resistance to rupture of experimental sites.

There were strong limitations for root development below 10 cm of depth, regardless of site, which has negative effects on plant development.

The soil data presented in this section will be correlated with forage yield and quality of *D. heterocarpon* ssp. *ovalifolium* to define effects of physical soil parameters and interaction with climatic conditions. Based on this we will be able to better define niches where genotypes of *D. heterocarpon* ssp. *ovalifolium* can contribute to soil enhancement.

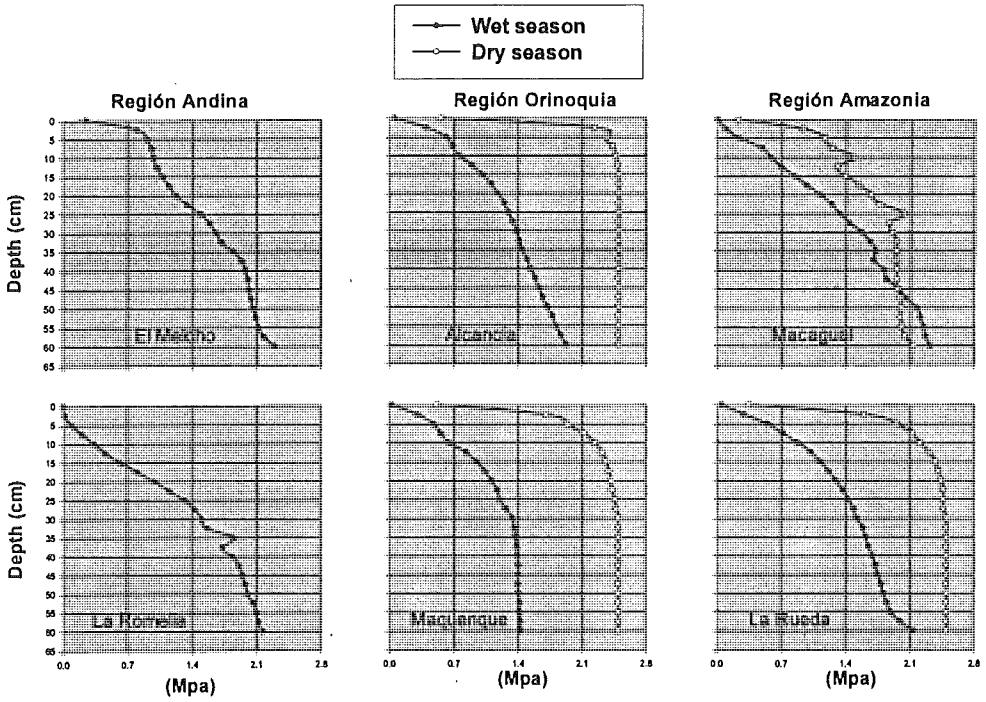


Figure 71. Multilocational trial of *Desmodium heterocarpon* ssp. *ovalifolium* : Penetrability of experimental sites.

3.6.3 Genotype x environment interactions on performance of *Arachis pintoi* (M. Peters, B. L. Maass)

Rationale: Targeting forage germplasm to deferent ecosystems and production systems is a major objective of CIAT's Forage Team. Consequently we assembled core collections of key grass (*Brachiaria*) and legumes (*Arachis, Desmodium)* species to include in multilocational trials, which are now finalized. With the information available, we expect to be able to define genotype x environment interaction information, which will then be linked to GIS to allow extrapolation of results. In this report we present results from a multilocational trial with *Arachis pintoi* carried out in Colombia.

Methods: Two sets of multilocational trials were established, one in 1994 (35 *Arachis* spp. accessions) and one in 1995 (39 *A. pintoi* accessions) (see previous annual reports). Seven accessions were common to both sets of trials, with the commercial variety CIAT 17434 serving as a control. Each set of trials was planted at six sites, ranging from subhumid hillside ecosystem in the Cauca Department of Colombia to the lowland Amazon forest margins in the 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. Data was then subjected to a stability analysis to define performance of germplam across

different agro-ecosystems. In this report we only present the results of the analyses performed in two sites characterized by having high fertility soils, but different rainfall.

Results and discussion: In Tables 62 and 63 we show results of the most promising accessions of *Arachis pintoi* selected in a relatively dry site –Palmira- and in a relatively humid site – Chinchina (coffee zone) with good soil fertility. Results indicate that the accessions CIAT 18744 and 22268 performed well across the two sites, and that based on different agronomic attributes they could have different uses in farmer fields. On the other hand, accessions CIAT 22236, 22238 and 22241 had outstanding agronomic characteristics across sites but have the limitation of producing no or only small amounts of seed. These accessions have a high potential where vegetative propagation is feasible but promotion over large areas will be hindered by lack of seed. In general, the two accessions selected outperformed CIAT 17434, the commercial variety 'Mani Forrajero', in the two environments which are characterized by having contrasting climate but good soil fertility.

Table 62. Selected accessions of *Arachis pintoi* and their different potential uses in a dry environment of Colombia – Palmira

Use and management	Accession CIAT No.	Agronomic characteristics
As cover crop	18744, 22235, 22268	More than 85% of soil covered 12 months after establishment
As green manure	18744, 18748, 22235, 22270	More than 60 g/m² dry matter in the first year of production
As pasture	18744, 22263, 22268	More than 60 g/m² dry matter in the second year of production
As cover crop, green manure and pasture: need for vegetative propagation	22233, 22236, 22238, 22241	High dry matter production (> 60 g/m²) but low seed production (< 7 g/m²).

All selected accessions have high protein contents (> 19%) and high *in-vitro* dry matter digestibilities (>70%)

Table 63. Selected accessions of *Arachis pintoi* and their different potential uses in humid hillsides of the coffee zone of Colombia – Chinchina.

Use and management	Accession CIAT No.	Agronomic characteristics
As cover crop, green manure and pasture	18744, 18746, 18747, 18748, 18751, 22160, 22257, 22260, 22268, 22269	Good soil cover at 4 months after establishment, high forage production, high seed production and high forage quality
As cover crop, green manure and pasture: need for vegetative propagation	22236, 22238, 22241	Excellent soil cover 4 months after establishment, high forage production and high forage quality. But low seed production (<6.0 g/m²).

All selected accessions have high protein contents (> 19%) and high *in-vitro* dry matter digestibilities (>67%)

3.6.4 Delivery of seed of promising accession of *Brachiaria* for on-farm grazing trials

(Members of the *Brachiaria* Network; L.H. Franco; C. Plazas; J. Miles)

Rationale: New forage plants cannot be delivered to commercial users until after they have been tested under realistic production conditions -- i.e. in grazing trials conducted over several years to test persistence under grazing and animal productivity parameters. The Colombian National *Brachiaria* Network always fixed its sights on eventual commercial release of new *Brachiaria* cultivars in Colombia. The first stage of this process was the agronomic, small-plot testing of a

collection of promising accessions over a range of diverse environmental conditions throughout the lowland tropical regions of Colombia. Preliminary results, available by early 1998, indicated approximately a dozen of promising accessions. Actions were taken immediately to multiply seed for grazing trials to be established during 1999.

Methods: Seed production plots (4000-5000 m^2) were established at the CIAT experimental station near Popayán and on rented land on a neighboring private farm. Seventeen lines were established (Table 64).

Table 64. Inventories *Brachiaria* seed produced at CIAT-Popayán during 1998/1999.

Genus	Species	CIAT (No)	Established area	Harvested quantity, classified
			(ha)	(kg)
Brachiaria	*brizantha*	6387	0.33	2.8
Brachiaria	*brizantha*	16113	0.36	82.0
Brachiaria	*brizantha*	16121	0.47	98.0
Brachiaria	*brizantha*	16316	0.14	4.5
Brachiaria	*brizantha*	16322	0.57	192.5
Brachiaria	*brizantha*	16467	0.36	105.5
Brachiaria	*brizantha*	26110	0.40	20.0
Brachiaria	*brizantha*	26124	0.30	68.0
Brachiaria	*brizantha*	26318	0.47	202.0
Brachiaria	*brizantha*	26556	0.47	90.0
Brachiaria	*decumbens*	606	0.30	42.0
Brachiaria	*dictyoneura*	6133	0.10	70.0
Brachiaria	*humidicola*	26159	0.41	116.0
Brachiaria	*humidicola*	26427	0.40	5.0
Brachiaria	Hybrid	36062 (BR93NO/1371)	0.12	1.2
Brachiaria	Hybrid	36060 (BR94NO/1737)	0.32	40.0
Brachiaria	Hybrid	36061 (FM9201/1873)	0.40	36.0
Total			5.90	1175.5

Results and discussion: Seed inventories as of early 1999 are indicated in Table 3. Unfortunately, owing to financial restrictions of our main funding institution for this work (The Colombian National Livestock Producers' Federation: Federación Nacional de Ganaderos de Colombia) it has been possible to establish grazing trials only at a couple of sites where they were funded directly with CIAT resources, and at one site where it was possible to plant a 15-ha grazing trial by tapping the resources of a private rancher, and using seed donated by CIAT. Unfortunately, there appears no reason to expect an improvement in this bleak funding situation for Colombia during 2000.

3.6.5 Delivery of seed of selected genotypes of *Desmodium heterocarpon* ssp. *ovalifolium* for on-farm trials (A. Ortega; H. Franco; J. Miles)

Rationale: A PhD project conducted by a German student (Axel Schmidt) evaluated agronomic performance of a collection of 18 *Desmodium heterocarpon* ssp. *ovalifolium* accessions over a range of environments at six locations in Colombia. A sub-set of accessions was selected on a variety of attributes, including forage production and nutritional quality (digestibility and tannin content). The next logical step is to evaluate this small set of selections under grazing to assess persistence and animal performance relative to standard check accessions. Seed is required for the establishment of these grazing trials.

Methods: Seed multiplication plots were established at CIAT-Quilichao station during 1998 and early 1999. Nine accessions were included (Table 65). Plots are hand harvested and threshed.

Table 65. Seed production parameters of accessions of *Desmodium heterocarpon* ssp. *ovalifolium*: CIAT-Quilichao, 1999.

Genus	Species	CIAT (No)	Established area	Harvested quantity, classified
Desmodium	*heterocarpon* ssp. *ovalifolium*	350	0.16	28.0
Desmodium	*heterocarpon* ssp. *ovalifolium*	13086	0.10	11.9
Desmodium	*heterocarpon* ssp. *ovalifolium*	13089	0.11	18.0
Desmodium	*heterocarpon* ssp. *ovalifolium*	13105	0.18	30.0
Desmodium	*heterocarpon* ssp. *ovalifolium*	13110	0.05	23.0
Desmodium	*heterocarpon* ssp. *ovalifolium*	13651	0.18	30.0
Desmodium	*heterocarpon* ssp. *ovalifolium*	23665	0.05	4.4
Desmodium	*heterocarpon* ssp. *ovalifolium*	23762	0.15	16.3
Desmodium	*heterocarpon* ssp. *ovalifolium*	33058	0.08	0.1
Total			1.06	161.7

Results and discussion: Seed inventories as of first semester, 1999 are listed in Table 4. One very promising accession (33058) has very late and shy flowering, and seed yields to date have been very disappointing. Seed is now available in significant quantities for grazing trials to assess legume persistence and animal performance on new *Desmodium heterocarpon ssp. ovalifolium* accessions.

Progress towards achieving output milestone

- New accessions of *Brachiaria, Desmodium,* and *Arachis* with adaptation to soil and climatic conditions are evaluated with NARS partners and farmer groups.

Agronomic evaluation of accessions of *Brachiaria* and *Desmodium* in different sites was completed and seed of promising accessions multiplied to facilitate the establishment of grazing trials with partners. Two accessions of *Arachis pintoi* (CIAT 18744 and CIAT 22268) were found to outperform the commercial cultivar in sites with good soil fertility in Colombia.

Activity 3.7 Link information on genetic diversity and environmental adaptation for conservation and planning of future collections

Highlight

- Passport data of 10 legume species is now ready to be linked with the GIS-tool FLORAMAP.

3.7.1 Use of FLORAMAP to link forage germplasm passport data to GIS (P. Jones, G. Hyman, M. Peters, L.H. Franco, M.A. Franco, B. Hincapie, G. Ramírez)

Rationale: For the conservation of bio-diversity and for planning of future collections of forage germplasm it will be necessary to determine sites of high genetic diversity. As an additional output, we would like to define collection sites from which accessions of high agronomic interest have originated.

Methods: Two researchers of CIAT, Peter Jones and Alexander Gladkov, developed a GIS-based tool, FLORAMAP for the prediction of potential centres of biodiversity for specific species, based on passport data of collection sites. Ten forage genuses were selected as test species: *Aeschynomene histrix* (81 accessions); *Arachis pintoi* (54); *Centrosema brasilianum* (249); *Centrosema macrocarpum* (389); *Centrosema pubescens* (694); *Stylosanthes capitata* (309);

Stylosanthes guianensis (1185); *Stylosanthes hamata* (142); *Stylosanthes scabra* (714); *Stylosanthes viscosa* (251).

Results and discussion: Available passport data was checked for mismatches and prepared for incorporation into the GIS tool. The model will allow us to predict distribution and areas of possible adaptation of legume species through climate, altitude and geographical data.

We expect to have a preliminary recommendation on sites for in situ conservation of forage germplasm in disturbed environments by 2000. Linkage of sites of origin with isozyme data is still in progress, but will be available for *Arachis pintoi* only in 2000. Analysis of *Brachiaria* data will be done once the a new version of FLORAMAP includes data from Africa (currently the model is restricted to Latin America).

Progress towards output milestone

- Passport, agronomic performance and isozyme marker data for *Brachiaria* and *Arachis* is linked to the FLORAMAP. The classification of agro-ecosystems is being revised for better targeting of forage germplasm to climatic and edaphic conditions in a given site.

Passport data from accessions representing 10 legume species was checked for mismatches and is now ready to link to FLORAMAP.

Activity 3.8: Study the genetics of selected grass and legume species to facilitate conservation and improvement

Highlights

- Identified tentative genetic markers --one co-dominant isozyme marker and one morphological (flower color) marker– for *Arachis pintoi*.
- Produced F_1 hybrids of *Arachis pintoi* and established field plots to generate F_2 seed for complete genetic characterization.

3.8.1 Identify and characterize one or more single-locus genetic markers in *Arachis pintoi*

(M.C. Gutiérrez; F. Feijoo; C. Ocampo; J.W. Miles)

Rationale: No rigorous, quantitative data are known relating to the reproductive biology (in particular, rates of natural outcrossing) in *Arachis pintoi*. This information is important from various points of view, including germplasm maintenance, seed production, and plant breeding procedures. Insect activity (especially honey bees (*Apis mellifera*)) is commonly observed on *A. pintoi* flowers, with apparent manipulation of the flowers and collection of pollen, suggesting the possibility of insect-mediated cross pollination. One or more simple, single-locus genetic markers will facilitate quantitative estimates of rates of natural outcrossing.

Methods: A small collection of *A. pintoi* accessions was assembled and assigned to pairs by possession of contrasting morphological attributes as indicated in previous studies. The initial expectation was that a reliable, single-gene, morphological marker would be found. Methodology for artificial hybridization was worked out, and a program of hybridization initiated, with reciprocal crosses attempted within assigned pairs of accessions. It soon became clear that the putative morphological contrasts were not likely to prove reliable (except, perhaps, flower color). We then sought isozyme polymorphisms between members of pairs where crosses had already been achieved.

Results and discussion: Of a number of enzyme systems tested on paired accessions, one system (PGM) gave a reliable allele difference for one accession pair of *Arachis pintoi* (CIAT 22240 vs. 22266). The maker contrast is co-dominant: inter-accession F_1 hybrids show both parental bands. A number of confirmed hybrids of this accession pair are available.

A flower-color contrast also appears to be monogenic: the typical "lemon-yellow" (e.g. CIAT 22264) vs. a darker, orange-yellow (e.g. CIAT 22175). The marker shows typical recessive/dominance relations: F_1 hybrids have the normal, yellow flowers, regardless of which accession is used as female.

To confirm unequivocally that inheritance of these putative marker loci is monogenic, observation of segregation in the respective F_2 populations is necessary. A small (approx. 4 x 5 m) field plot was established in August 1998 using vegetative propagules of the F_1 hybrid 22240 x 22266. We will produce an F_2 population of seedlings from seed harvested from this plot. We plan to produce approx. 100 F_2 seedlings and analize them for the presence of the two diagnostic PGM bands. We anticipate initiating seed harvest from this field plot during the second-semester rainy season (September/October, 1999).

Two 1 x 1 x 0.5-m-deep square containers were sown with vegetative propagules of the F_1 hybrid 22175 x 22264. Seed harvested from these pots will be used to determine segregation of flower color in the F_2 generation.

3.8.2 Production of "open-pollinated" *Arachis pintoi* populations of parents with contrasting genotypes (M.C. Gutiérrez; F. Feijoo; A. Ortega; J. Miles)

Rationale: Analysis of the open-pollinated progeny of adjacent plants of contrasting marker phenotypes to detect the presence and proportion of hybrid phenotypes allows estimation of outcrossing rate under given conditions. Our isozyme marker is ideal for this type of study as the two parental phenotypes are distinct and distinct from the F_1.

Methods: Single rows of each of two parental accessions of *A. pintoi* (22240 and 22266) were established in the field at CIAT in August 1998. Rows approx. 10-m-long were planted parallel, 2.5 m apart. At the moment of the preparation of this report (30 August 1999), the two rows are closing the intervening space, though growth is very inequal. Both accessions have been flowering almost continuously since shortly after transplanting to the field. These plots will be sampled on several transects perpendicular to the direction of the rows to provide seed from sites at different distances from the contrasting accession. Proportion of hybrid phenotypes will be determined for the seed from different sampling sites. We anticipate initiating seed harvest during the second-semester rainy season (September/October, 1999).

Results and discussion: Rows of parental accessions were established, though growth has been very unequal between the two accessions. Results of this series of studies will provide full characterization of two potential genetic markers in *A. pintoi* and the first reported quantitative estimates of rates of natural outcrossing for this species.

3.8.3 Assessment of outcrossing in *Arachis pintoi* using appropriate marker(s) (F. Feijoo; C. Ocampo; J. Miles)

Rationale: Rate of natural outcrossing is important to design safe seed multiplication strategies as well as for the choice of efficient plant breeding schemes. No known estimate of outcrossing rate in *A. pintoi* exists.

Methods: Open pollinated of *A. pintoi* seed will be germinated and individual seedlings (approx. 200) will be assessed for diagnostic PGM isozyme markers. Proportion of outcrossing can be calculated directly as all three genotypes (both parentals and the F_1) are clearly distinguishable.

Results and discussion: Completion of this activity awaits harvest of open pollinated seed now being produced in the field at CIAT.

Progress towards achieving output milestone

- Genetic markers characterized and level of outcrossing known in *Arachis*

Two, probably single-gene markers have been identified in *Arachis pintoi*. F_2 seed is being produced for complete genetic characterization. Open pollinated seed of two parental genotypes of *A. pintoi* are being produced for estimation of natural outcrossing and quantitative estimates of outcrossing should be available by mid-2000.

OUTPUT 4: Superior and diverse grasses and legumes delivered to NARS partners are evaluated and released to farmers

Activity 4.1 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

Highlights:

- Selected and multiplied seed of new *Brachiaria* accessions and hybrids for the establishment of grazing trials.
- Initiated on–farms trials with new accessions and hybrids of *Brachiaria* in the Llanos, Caquetá piedmont and Magdalena Valley in Colombia.
- Demonstrated the feasibility of introducing *Arachis pintoi* in degraded pastures in dual purpose cattle farms of the amazon piedmont (Colombia) and facilitated the adoption of this legume by over 100 farmers.
- Demonstrated on-farm benefits of *Cratylia argentea* as a dry season supplement for milking cows and facilitated spontaneous adoption of the legume in Costa Rica.

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 through the TROPILECHE Systemwide Livestock Program led by ILRI, both of which are housed in the CIAT's Systems Project (PE-5). Thus an important objective of CIAT's Tropical Grasses and Legumes Project 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) establishment of linkages with CIAT's projects and consortia that form part of Systemwide initiatives (i.e.TROPILECHE) and d) seeking opportunities for special projects and collaboration with NARS partners

During 1999 we continued collaboration with other Projects in CIAT (PE-5), IARC's (ILRI), and NARS partners in Colombia, Peru, and Central America. We also initiated contacts with different institutions in Ecuador in order to establish collaborative work on spittlebug in the coast and amazon rainforest through WB funds.

The Brachiaria Evaluation Network in Colombia (J. Miles and L. H. Franco)

The Brachiaria Network financed until this year by a Colombian Cattlemen Association (FEDEGAN-Fondo Nacional del Ganado), comprised 13 sites and involved as partners forage researchers from CORPOICA (Corporación Colombiana de Investigación Agropecuaria), a Colombian University, SENA and private producers.

A final Workshop was held during February 1999 in Yopal, Casanare and what follows is a summary of the major conclusions. The objective of the Workshop was to report and discuss the final results obtained in agronomic trials and to define concrete actions to advance selected *Brachiaria* genotypes to on-farm grazing trials. A total of 20 accessions of *Brachiaria* grown in 11 sites in Colombia were included in a combined analysis to define the effect of climate and soils on yield of the accessions evaluated. Average yield of all accessions during the rainy season

(3664 kg DM/ha) was 32% higher that in the dry season (2771 kg DM/ha). The locations where the highest yields were recorded during the wet season were in the North Coast (Chiriguana, Cesar and El Porvenir, Cordoba) followed by amazon piedmont (Macagual, Caquetá). During the wet season yields lower than the average across locations were recorded in the llanos (Carimagua) and Magdalena valley (Barrancabermeja).

To analyze the adaptation of *Brachiaria* genotypes to different environments we used the Eberhart and Russell (1966) Methodology modified by Amezquita (1982), in which adaptation is defined as the relative response of each genotype evaluated in different locations. Each location represents an environment with different climatic and edaphic conditions expressed through an Environmental Index (AI = Mean DM yield of all accessions in a given location – Mean DM yield of all accessions across locations). The AI assumes that the best indicator of quality of the environment is the overall productivity or yield of the accessions tested. A linear regression analysis was then performed where: y = Mean production of each accession in each location and x = AI. Mean yield of accessions across locations is represented by the intercept (a) of the regression and the degree of adaptation of accessions is represented by the slope (b) of the regression.

Results indicate that accessions of *B. brizantha* CIAT 26110, 6387 and 16467 had the highest yields and that had good response to improvements in the environment in the wet (Figure 72) and dry (Figure 73) seasons. However it should be noted that accession *B. brizantha* CIAT 26562 responded to improvements in the environment in the wet season but not in the dry season. In contrast, the accession *B. brizantha* CIAT 16488 responded to improvements in the environment in the dry season but not in the wet season.

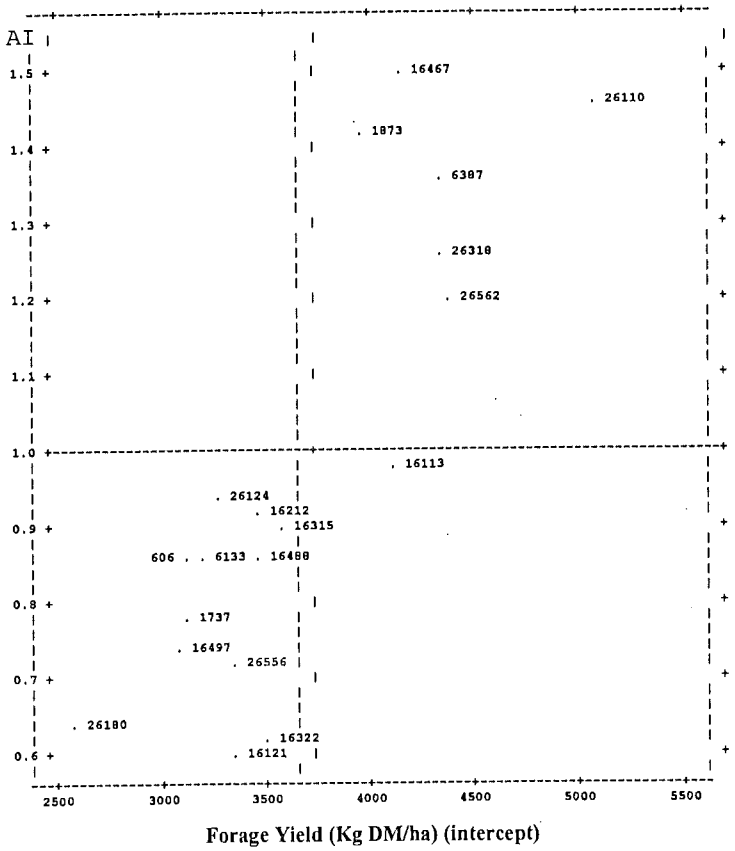


Figure 72. Classification of accessions of *Brachiaria* evaluated during the wet season in different sites of Colombia based on an Adaptation Index (AI = slope of linear regression.

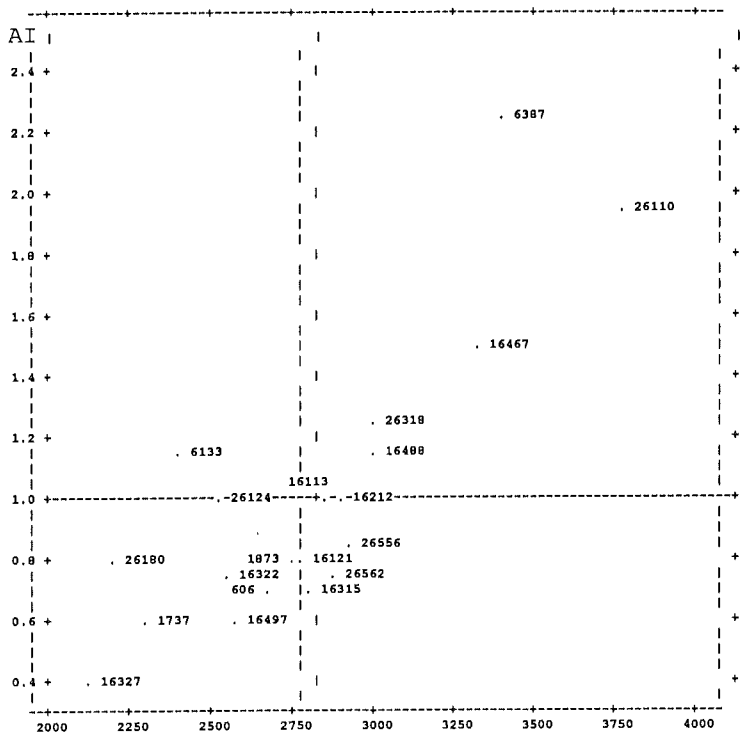


Figure 73. Classification of accessions of *Brachiaria* evaluated during the dry season in different sites of Colombia based on an Adaptation Index (AI = slope of a linear regression)

The *Brachiaria* hybrid CIAT 36061 (FM9201/1873) exhibited high yield in the wet season, but yields were reduced by 50% during the dry season. On the other hand, the hybrid CIAT 36060 (BR94-NO/1737) showed low yields in all locations, both in the wet and dry seasons. It was interesting to note the commercial cultivar *B. decumbens* cv Basilisk (CIAT 606) had a limited response in terms of forage yield to improvements in the environment , which was defined by the average yield of all accessions in each location included in the analysis.

From this results it is clear that *B. brizantha* CIAT 26110 is well adapted to a broad range of environments differing in rainfall (quantity and distribution) and in soil fertility. Thus this accession was selected by all participants in the Brachiaria Network for inclusion in on-farm grazing trials. Other accessions like *B. brizantha* CIAT 6387 and 26318 were also selected by some of the participants for on-farm trials. It was interesting to note that the *Brachiaria* hybrid CIAT 36061 (FM9201/1873) was selected by 5 of the participants in the Network for grazing a local cattlemen association trials due to outstanding performance in the wet season.

The original plan was that each participant in the Brachiaria Network would establish grazing trials in collaboration with farmers in their locations. To support this action, CIAT would multiply seed of the most promising accessions. With the financial assistance of (Agroganadera del Valle) we were able this year to multiply seed in Popayan (see Section 3.6.4 of this Report) of most of the *Brachiaria* accessions selected in the Network. One exception was *B. brizantha* CIAT 26110, which produced very limited quantities of seed. However, in 1998 we delivered 47 kg of seed of this accession produced in Costa Rica to CORPOICA to support there on-farm work in Colombia.

Only one member of the Network obtained funds to establish an on-farm grazing trial in the Magdalena Valley (Barrancabermeja) with the following accessions: *Brachiaria* hybrids CIAT 36061 (FM9201/1873) and CIAT 36060 (BR94-NO/1737)), *B. brizantha* CIAT 26318 and *B. humidicola* (CIAT 26159 and 26427). Each accession was established in 3 ha plots and will be individually grazed to measure liveweight gain. New *Brachiaria* accessions and hybrids were also established by CIAT in farms of the llanos and forest margins of Caquetá. The establishment of grazing trials in other locations in Colombia has been postponed until funding is secured.

TROPILECHE Consortia in Tropical America (F. Holmann, P. Kerridge, P.J. Argel, C.E. Lascano)

We continue active collaboration with PE-5 through the project "Improved Legume-based Feeding Systems for Smallholder Dual-Purpose Cattle Production in Tropical Latin America", which is a CIAT-led consortium know as TROPILECHE that operates under the Systemwide Livestock Program (SLP) convened by ILRI. The consortium consists of scientists from CIAT, ILRI and national agricultural research organizations in Peru (IVITA, CODESU, INIA, FUNDAAM), Costa Rica (MAG, ECAG, CATIE, UCR), Nicaragua (IDR), and Honduras (DICTA). The strategy being used by TROPILECHE improve feeding systems is through: a) on station evaluation of new feed resources to match nutritional requirements of animals, b) on-farm evaluation of new legume-based forage components, and c) economic analysis and acceptability/adoption studies.

The results generated in the third year of the project further confirm that improved grasses and legumes can have a significant impact in animal production.

Major Results: Applied research has demonstrated the value of the shrub legume *Cratylia argentea* fed as silage in addition to its value when fed fresh. Milk yields are maintained at similar levels as with protein sources from commercial concentrates but at lower cost. There is a need for more information on the best manner to manage *Cratylia* for silage production. New information has also been obtained on management of *Cratylia* as a cut and carry forage. A cutting interval of 60 days at a height of 90 cm produces feed with higher crude protein, 19%, than cutting at 90 days, with only a small reduction in dry matter yield. Applied research also provided information on frequency of feeding on N utilization. Results indicated that feeding twice a day with legumes only results in higher N uptake when a high level of supplement is fed (e.g., 1% BW).

On-farm research with *Cratylia argentea* only began last year. It has now been confirmed through on-farm trials that it can be effectively used as a supplement to replace protein concentrates fed either fresh or as silage. The most economical option for a producer in the dry season is to supplement cows with freshly-cut *Cratylia* though feeding as silage is also more economical than feeding purchased commercial concentrates or chicken manure. Producing silage from *Cratylia* during the rainy season when it is not needed as a supplement will reduce the size of the protein bank that farmers need to plant and maintain.

Grass-legume associations are a viable option for improving feed quality and increasing milk yields. *Arachis pintoi-Brachiaria* associations increase the milk yield by 8% over that of straight grass when legume content in the pasture is near 30%, even where cows are supplemented with commercial concentrates. This is provided that cows have a moderate to high genetic potential for milk production.

The results obtained on the use of *Stylosanthes guianensis* (Stylo) in the forest margins of Colombia for pre-weaned calves are similar with those obtained last year in small dairy farms in Pucallpa, Peru. When Stylo was fed to calves, the amount of milk for sale was 21% higher than

recorded with cows whose calves were managed in the traditional system. Liveweight gain of calves with access to Stylo was 30% higher than in the control group. In addition, Stylo was shown to increase the yield of a subsequent rice crop and thus could be included in a rotational system with agricultural crops.

The database available in the Tropileche web page containing research results on dual-purpose cattle systems in LAC since 1960 continues to be expanded and consulted. During the last year an average of 3.1 entries were recorded per day. This web page also contains information about other research centers in LAC working with dual-purpose cattle. We also use it to share research results generated by the project.

Steps to disseminate these technologies are in progress. This year we produced an 11-min video with our national partner Ministry of Agriculture (MAG) in Costa Rica, on the adoption of *Cratylia* and *Arachis*-based technologies by a farmer in Costa Rica. This farmer is currently producing more milk on less area, has doubled his family income, and has released areas from the livestock enterprise to serve as protected areas for timber production and protection of water sources. This videotape will be used for disseminating information to other farmers in Latin America. In addition to this video, farmers collaborating with the project are sharing their experiences with other farmers through field days organized by MAG. Next year we plan to produce and distribute to producers and extension services pamphlets on management of new feeding systems.

The impact generated by the project so far is shown by:

a) Other countries evaluating the new technologies. In Nicaragua and Honduras, our partners IDR and DICTA have established more than 65 ha of improved forages in 20 farms located in 5 sites;
b) Ecuador and Bolivia have expressed their interest in joining the project. Proposals are being prepared with our partners INIAP and CIAT-Santa Cruz to commence research activities in small dual-purpose farms.

Tropileche Workshop: Annual meetings to discuss workplans, current research and future challenges as well as constraints are important activity in TROPILECHE in order to increase the efficiency of research and interchange ideas. This year separate workshops have been organized for South America (July 1999 in Peru) and Central America and the Caribbean (Costa Rica in October 1999).

Participants in TROPILECHE held a workshop to plan and discuss present and future activities in South America during June 27 to July 2, 1999, in Moyobamba, Peru. The objectives of the workshop were to: (a) present the research achievements obtained by the Consortium and pose future challenges; (b) present research results achieved in Peru and to discuss new activities for 1999 and 2000; (c) participate in a field visit to understand and identify opportunities in current animal production systems in the Moyobamba region of the Peruvian Amazon; (d) review strategic and participatory research based on needs and constraints; and (e) analyze and discuss new forms of collaboration with other institutions and in other countries of South America, especially Ecuador, Bolivia and Brazil. Invited participants in the workshop included 22 researchers from Peru, Colombia, Ecuador, Bolivia, and Brazil. The workshop proceedings will be distributed during the month of October. Mayor outcomes of the workshop were:

1) TROPILECHE will continue to reduce research activities in Pucallpa in 2000 and increase activities in Moyobamba. Potential for adoption of new forages in Pucallpa is low due to an oversupply of forage from a reduced herd inventory. In addition, the dual-purpose herd has a low genetic potential for milk yield which reduces economic benefits from investing in new

forage technologies and the market potential for milk in Pucallpa is severely limited as there is no milk plant. The situation in Moyobamba is the reverse. A cooperative milk processing plant has been opened, farmers are improving the genetic potential of their herds for milk production and there is a demand by farmers for improved feeding systems.

2) The Consejo Transitorio Agricola Regional (CTAR), through the Fundacion para el Desarrollo Agricola del Alto Mayo (FUNDAAM) will be our partner in Moyobamba and the institution responsible to carry out all of the research activities agreed during the workshop. The CTAR also agreed to invest as matching funds 35% of the resources that TROPILECHE delivers to Moyobamba.

3) There are good possibilities to expand research activities in Ecuador with our partner INIAP by accessing Wold Bank funds bilaterally and in Bolivia with CIAT-Santa Cruz through a collaborative project with DFID. TROPILECHE agreed with both INIAP and CIAT-Santa Cruz to follow up and develop project proposals for submission to both WB and DFID to access bilateral funds.

The challenge ahead is to facilitate wider evaluation of herbaceous and shrub legumes by small farmers and the production of seed of selected legumes. We are identifying new research needs from problems that are being experienced by farmers evaluating the new technologies. To effectively accomplish these objectives there is a need to continue building strong linkages between the project and other ILRI and CIAT Projects, NARS partners and the private livestock and seed sectors.

NESTLE Project in the Amazon Piedmont in Colombia (G. A. Ruiz, M. Jervis, J. Rozo, J. Velásquez and C. E. Lascano)

An inter-institutional on-farm project financed by NESTLE to recuperate degraded pastures in selected farms of the Colombia Amazon piedmont terminated this year. The specific objectives of the 4-year project were to document the on-farm benefits of Arachis-based pastures, train personnel of different institutions on establishment and utilization of Arachis pastures using participatory methods and initiate and catalyze an active transfer mechanism of the Arachis technology in the region.

Major Results: Pastures of grass alone (76 ha) and grass associated (114 ha) with commercial Arachis (cv. Mani Forrajero) were established in 16 selected farms and grazed by milking cows. Milk yields of individual cows increased from 0.3 to 0.5 liters/day in the grass legume-pastures depending on the genetic potential of the cows and on lactation stage. This response to the legume was associated with increased biomass and quality of the forage on offer and with improved biological activity in the soils. Ex-ante economical analysis indicated that Internal Rates of Return where higher with *Arachis*-based pastures (19. 3%) than with grass alone pastures (12.0%).

The NESTLE project contributed to identify ways of facilitating the diffusion of *Arachis*-based technology in the region, by identifying alternative methods for establishing grass/legume pastures, by in situ demonstrations of proper grazing management and by identifying "bottlenecks" for the adoption of *Arachis*. At the end of the 4 years a total of 3,000 ha had been planted with *Arachis* by over 100 farmers, of which 87% were in association with grasses and the remainder as pure legume stands.

Lessons learnt during the course of the project were the need to have flexible research methods for on-farm pasture evaluation and the need to avoid absentee owners since they do not provide the necessary feedback to researcher and do not act as promoters of the technology. On-farm

research using participatory methods does not by itself accomplish the ultimate goal of diffusion/ adoption of improved pasture technology. Thus alternative strategies for diffusion of new legume-based technology should be part of the overall objective of a pasture/livestock project.

Collaborative work CORPOICA to reclaim degraded pastures in the Llanos of Colombia
(C. Plazas, A. Rincon, J. Miles and C. E. Lascano)

One major limitation for beef and milk production in Neotropical savannas is degradation of introduced grasses, as a result of nitrogen deficiencies and overgrazing. Thus CIAT's Forage Project (IP-5) has been developing improved grasses and legumes that can contribute to reclaim large areas of degraded pastures in tropical regions where livestock is a major land use system.

In collaboration with PE-5, and CORPOICA we initiated in 1998 on-farm evaluation of new grasses and legumes in representative farms of the llanos of Colombia. A total of four farms (two in the well-drained savannas and two in the piedmont) were initially selected to evaluate new ecotypes of the pasture legume *Arachis pintoi.* Selected farms were representative of the two sub-ecosystems and have large areas of degraded pastures. In addition, farmers participating in the Project indicated their willingness to cover some of the cost of the work done in their farms.

In each farm at least 8 ha of degraded *Brachiaria* spp. pastures were used to establish the following treatments:

a) Four ecotypes of *Arachis pintoi* (CIAT 17434-control and 18744, 18748 and 22160) and,
b) Two legume planting densities (3 and 6 kg/ha)

Land was prepared using a chisel plow and a disk harrow following overgrazing of the *Brachiaria* pastures. The seed *Arachis* seed was planted using a conventional grain row planter. The fertilizer used (kg/ha: 250 rock phosphate, 250 dolomite Ca, 150 Potassium chloride, and 25 sulfur) was broadcasted in all the area. Soil physical and chemical characteristics were measured in all farms before planting the legume. Measurements were also made on the above ground biomass (cover, botanical composition, forage on offer and presence of pest and diseases). In order to allow an estimate on changes in the soil over time, additional measurements were done in the soil (physical and chemical) after planting *Arachis*. Post-establishment measurements include: rate of germination of *Arachis* seed (30 days after planting), cover of the legume (at 45 day intervals), botanical composition and legume and grass yield (in the rainy and dry seasons).

Major Results: The establishment of *Arachis* sown in 1998 in association with *Brachiaria* spp was only successful in 1 of the 4 farms selected. In all farms the accession CIAT 22160 had very low germination and there were no measurable difference in the establishment rate of the other accessions (CIAT 18744 and 18748) relative to the commercial cultivar (CIAT 17434).

Pastures reclaimed in 1998 - Piedmont: The farm where their was good legume establishment in association with *B. humidicola* (slow to establish) is located in the piedmont with relatively good soils (2.5% OM) and a native-grass vegetation covering the degraded pasture chosen for rehabilitation. After sowing the legume/grass and applying the fertilizer a large proportion of weeds germinated together with some *B. decumbens* (the original grass introduced in the pasture) but weeds were successfully controlled with a mechanical cutter. Initial grazing began 7 months after establishment with an average stocking rate of 2.5 A/ha. Legume proportion of the pastures was not significantly affected by sowing rate and after 1 year, the botanical composition of the pasture was 34% grass, 40% *Arachis* and 26% weeds. Animal LWG have been very high (1kg/head/day) most likely due to compensatory growth.

The second farm located in the piedmont where establishment of *Arachis* was poor had relatively good soils (2.8% OM), but the area chosen to plant the legume was dominated by *B. decumbens* in poor condition. Initial establishment of the legume following land preparation and fertilization was good, but at the end of the rainy season (December 1998) the proportion was low regardless of ecotype used or sowing rate. This reduction of legume in the pastures was most likely the result of competition from the vigorous grass that germinated after land preparation. It was not possible to minimize this competition given that the farmer did not have animals to graze the pasture when needed. It was decided to re-establish *Arachis* (CIAT 18744 and 18748) in the rainy season of 1999. The amount of *Arachis* is now ranging from 7 to 12% and intermittent grazing is being applied to favor the legume.

Pastures reclaimed in 1998-Well-drained savannas: Establishment of *Arachis* was not successful in the two farms located in the well-drained savannas in spite of having good seed germination and having applied fertilizer. The lower soil fertility found in well-drained savannas as compared with the piedmont together with competition from sown grasses and weeds would explain the poor establishment of *Arachis* in degraded pastures. To obtain good Arachis-based pastures more fertilizer would have to be applied, but it is unlikely that farmers would be willing to pay the extra cost.

In the on-farm work carried out so far in the Llanos of Colombia to rehabilitate degraded pastures we have learned several things, which are summarized as follows:

1. Farmers recognize pasture degradation as a major constraint for increasing milk or beef production, but have serious limitations of machinery for land preparation and sowing of new grass and legume species. In addition, farmers have limitations to invest on fertilizers and on seed of legumes, usually more expensive than that of grasses.
2. Security aspects have determined to a great extent that owners seldom visit their farms and as a consequence we have not been able to involve the owners in the on-farm work as much as we would have liked.
3. Successful establishment of *Arachis* is dependent not only on soil fertility, but also on the original vegetation in the degraded pasture, on the companion grass used and on grazing management.
4. Failures on the establishment of *Arachis pintoi* in degraded pastures in the low fertility soils of the well-drained savannas have determined that we look for other legume options.
5. Demand for new grasses with drought tolerance and for shrub legumes to supplement milking cows in the dry season.

New plantings: High priority was given in 1999 to the selection of new farms to plant in degraded areas new grasses and legumes using as a criteria the willingness of resident farmers to actively participate in the evaluation. In Table 66 we summarize the new plantings in farms of the piedmont and well-drained savannas. As can be seen in Table 66 new plantings include ecotypes of *Brachiaria brizantha* selected in the Brachiaria Network for high forage yield and drought tolerance.

In addition, ecotypes of *D. heterocarpon* ssp. *ovalifolium* selected in a multilocational trial in Colombia were sown as the legume component to recuperate degraded pastures in both ecoregions. It is felt that *D. heterocarpon* ssp. *ovalifolium* is a better option to recuperate pastures in farms in well drained savannas as compared with *Arachis*, given its good adaptation to low fertility soils and low seed rates (400 to 500 g/ha) required for establishment. The shrub legume *Cratylia* was established in two farms as a fodder bank to supplement milking cows in the dry season.

Table 66. Plantings during 1999 in farms of the Llanos of Colombia

Sub-Region	Species planted	Area (ha)
Piedmont		
Farm 1	*B. brizantha* CIAT 26110	2
	B. brizantha CIAT 26318	3
Farm 2	*B. brizantha* CIAT 26318	5
Farm 3	*Desmodiun heterocarpon* ssp *ovalifolium* (mixture of 5 accessions) + *P .phaseoloides (Kudzu)*	20
Farms 4 and 5	*Cratylia argentea*	¼ to 1 ha
Well-drained savannas		
Farms 1 and 2	*D. heterocapon* ssp. *ovalifolium* (mixture of 5 accessions)	7.5 to 48.5
Farm 3	*B. brizantha* CIAT 26318	5

It is to premature to talk about impact of the collaborative on-farm work in the Llanos of Colombia aimed at testing with farmers new grass and legume options to rehabilitate degraded pastures. However, it should be kept in mind that the current economic-social conditions in the regions do not contribute to the desire of farmers to make large investments on pasture renovation on their properties. Thus the work being executed will lay the groundwork for future interventions.

Collaboration with CONDESAN and CIAT's PE-3 –Hillsides of Colombia (R. D. Estrada, A. Ortega and C. E. Lascano)

During 1998 we initiated collaboration with CIAT's PE-3 (Community Management of Watershed Resources in Hillsides Agro-ecosystems) and with CONDESAN (Consorcio para el Desarrollo Comunitario de Cuencas –CIP) for planting *Arachis pintoi* in hillsides of the Municipio de Pensilvania, Caldas, Colombia. In this location their large areas of degraded pastures associated with high rainfall (3000 to 5000 mm/year) and steep topography.

The objective of the Pilot Project is to demonstrate that by improving milk production through pasture reclamation with *Arachis pintoi*, income of poor smallholders in hillsides can be increased. In addition, the project wants to test the hypothesis that partnerships between the private investors and smallholders can lead to increase income of resource poor farmers, while sustaining the natural resource base. Each farmer was to initially establish 2500 m^2 of *Arachis pintoi* (18744) to produce seed. Farmers provide the land, and labor to plant and harvest the seed. We expect that with the income derived form selling *Arachis* seed, the farmers participating in the project will have enough resources to recuperate pastures for milking cows and still have a surplus to purchase an additional cow.

Major Results: A total of 50 plots (2,500 m2/plot) were planted this year with *Arachis pintoi* of which 40 are well established. The performance of Arches was as expected. In sites located in lower altitudes it took 4 months for the legume to completely cover the plots, but it took 6 months at higher site (2,200 masl). Initial estimate of seed production indicates variable yields (336 to 3136 kg/ha) depending on altitude and presence or absence of tress in the plots. The highest yields have been recorded at lower altitudes whereas the lowest yields were in the higher elevations and when *Arachis* was associated with pine trees. The initial idea was to sell the seed produced by farmers to NESTLE, but it turned out that they now prefer to use the seed in the location to harvest the forage and make hay or as a cut and carry to supplement to dairy cattle. A major constraint on the use of *Arachis* has been high cost of establishment and competition with weeds.

Progress towards achieving output milestone

- Selected *Brachiaria* and *Cratylia* cultivars are released in Central America and Colombia

In collaboration with partners we have identified new accessions of *Brachiaria* with broad adaptation and superior agronomic attributes relative to commercial cultivars. Seed of these accessions was multiplied and on station and on –farm grazing trials initiated in Colombia and Costa Rica. Commercial Seed Companies in Costa Rica are already multiplying seed of *B. brizantha* CIAT 26110 and this together with results from on-station and on-farm grazing trials should help NARS decide on future releases.

The *Brachiaria* hybrid CIAT 36062 (BR93-NO/1371) selected for high resistance to spittlebug was included for the first time in a grazing trial in the amazon piedmont, Caqueta.

Activity 4.2. Evaluate and select with farmer participation multipurpose forages for crop/livestock systems

Highlights

- Multiplied seed of a wide range of forage germplasm and distributed seed for evaluation to several countries.
- Demonstrated that germination of seed of *Cratylia argentea* dropped from to 87 to 46 % after two years of storage in uncontrolled ambient conditions (mean temperature -25.6° and relative humidity of -79.5 %).
- Superior grasses were selected by innovative farmers in hillsides of Honduras using participatory methods.

4.2.1 Seed multiplication of selected grass and legume accessions in Palmira for on-farm testing (A. Ortega; H. Franco; J. Miles).

Rationale: Novel forage germplasm cannot be adopted into commercial production systems without some propagation "vehicle". Normally, this is seed. Unlike grain crops, forage plants are not developed primarily for seed production and some are notoriously poor seed producers. In addition, the scale of testing required for forage plants requires rapid expansion of trial areas, from small-plot agronomic trials, through large grazing trials, to commercial adoption. A very common and sometimes severe bottleneck in this process is seed availability. With the objective of facilitating this process, the Forage Project maintains two small seed multiplication units in CIAT-Palmira , Colombia and ECAG- Atenas Costa Rica, to service needs within CIAT for forage plant seeds, and to supply (at cost) extramural needs of national research partners and, if supplies permit, private producers.

Methods: During 1999, the main seed multiplication focus was on continued seed harvest from established plots of *Brachiaria* accessions at Popayán and *Desmodium heterocarpon* ssp. *ovalifolium* plots at CIAT-Quilichao (See sub activities 3.6.4 and 3.6.5). Small plots of a diversity of forage legume germplasm were also established, mainly at CIAT-Quilichao and CIAT-Popayan stations. Most seed of this diversity of species was hand harvested and processed.

Results and discussion: Seed harvests of a wide range of germplasm including a total of 25 accessions representing at least six different species are documented in Table 67.

Table 67. Seed multiplication (genera other than *Brachiaria* and *Desmodium*) at the CIAT-Quilichao, CIAT-Popayan, and CIAT-Palmira experimental stations during the first semester of 1999.

CIAT No.	Genus	Species	Production (kg)
913	*Cajanus*	*Cajan*	5.200
21507	*Cajanus*	*Cajan*	0.950
20400	*Calliandra*	*Calothyrsus*	2.040
21252	*Calliandra*	*Calothyrsus*	0.160
22308	*Calliandra*	*Sp.*	0.165
22309	*Calliandra*	*Sp.*	0.150
22310	*Calliandra*	*Sp.*	1.100
22311	*Calliandra*	*Sp.*	0.050
22312	*Calliandra*	*Sp.*	0.205
22313	*Calliandra*	*Sp.*	0.065
22315	*Calliandra*	*Sp.*	0.110
22316	*Calliandra*	*Sp.*	1.545
22317	*Calliandra*	*Sp.*	0.166
22319	*Calliandra*	*Sp.*	0.375
17009	*Canavalia*	*Brasiliensis*	2.650
18516	*Cratylia*	*Argentea*	8.040
18668	*Cratylia*	*Argentea*	4.330
18672	*Cratylia*	*Argentea*	1.920
18674	*Cratylia*	*Argentea*	2.940
18675	*Cratylia*	*Argentea*	1.430
18676	*Cratylia*	*Argentea*	3.280
18957	*Cratylia*	*Argentea*	2.010
17503	*Leucaena*	*Leucocephala*	0.960
21245	*Leucaena*	*Leucocephala*	3.790
21888	*Leucaena*	*Leucocephala*	14.000

Five genera; 25 distinct genetic materials (accessions).

Seeds were dispatched to a total of six countries (with Colombia representing the major client country) and five institutional types (including to two private individuals) (Table 68).

Table 68. Seed distribution (number of samples and weight (kg)) during the first semester of 1999*

Genus	Number of Sample	Volume (kg)
Aeschynomene	1	0.010
Andropogon	1	10.000
Arachis	20	308.450
Brachiaria	20	6.426
Calopogonium	2	0.070
Canavalia	1	0.150
Centrosema	7	8.260
Cratylia	15	18.155
Desmodium	30	28.403
Flemingia	1	0.040
Leucaena	4	2.250
Mucuna	1	1.000
Panicum	1	0.120
Pueraria	24	3.105
Stylosanthes	5	0.195
Zornia	1	0.010

*These samples were dispatched to 6 countries [China (2); Costa Rica (5); Honduras (4); Nicaragua (4); Peru (3); Sri Lanka (8) and Colombia (108)] and five types of institutions (including two to private individuals): [CIAT (51), CORPOICA (4), Particular (2), University (20), Others-GNO (31)].

4.2.2 Seed multiplication of selected grass and legume accessions in Atenas for on-farm testing (P. Argel)

Experimental and basic seed multiplication is an on- going activity of the Seed Unit in collaboration with the Eschewal Centro Americana de Ganadería (ECAG) at Atenas. This site combines both soils of medium fertility (pH 5.9; 3.6 ppm of P; 7.6 % OM and high Ca contents), and a well distributed rainfall (1600 mm) with a defined dry period that facilitates seed maturation and harvesting. The seed produced is destined to support advanced evaluations of promising pasture germplasm both by CIAT's projects and national programs.

Seed multiplied or acquired: During 1998 and part of 1999, a total of 811.6 kg of experimental and basic seed of promising forages species was either produced or procured by the Seed Unit. The bulk of the seed was formed by *C. argentea* (47.8 kg), *A. pintoi* (649.6 kg), *B. brizantha* CIAT 26110 (79.5 kg), and 24.7 kg of several lines of *C. argentea, P. maximum, D. velutinum, D. heterocarpon* ssp. *ovalifolium, C. ternatea* and *L. leucocephala*

Seed distributed: During the period September 1998 – August 1999 a total of 61 seed requests were processed and 1748.1 kg of experimental and basic seed distributed to Costa Rica, Nicaragua, Guatemala, Honduras, Panamá, Puerto Rico, Colombia, Belize, Jamaica and Nepal. *A. pintoi* lines, *B. brizantha* CIAT 26110 and *C. argentea* were the species more solicited.

4.2.3 Viability of seed of *Cratylia argentea* under uncontrolled ambient conditions (P. J. Argel and G. Pérez)

Rationale: The shrub legume *C. argentea* is well adapted to poor acid soils of the lowland tropics that flowers and sets good quality seed, but little information has been generated up to date on the effects of storage conditions on seed viability of this species. Thus it is important to define storage conditions for seed of *Cratylia* and to make this information available to researchers and farmers interested in multiplying seed.

Methods: Seed pods of *C. argentea* CIAT 18668, were harvested and trashed by hand between February and April (dry period) of 1997. Clean seeds were packed in close plastic jars and stored for two years in uncontrolled ambient conditions at Atenas. At this site, mean temperature during the storage period was 25.6 °C (a minimum mean of 20.1 °C and a maximum mean of 31.1 °C), and the relative humidity had a mean of 79.5 % (minimum of 65 % in January and maximum of 90 % in October). Seed germination tests were carried out two months after the final harvest in April, and then every two to three months for a period of two years. Towel paper on plastic trays watered every second day with a solution of 0.1 % of benomyl, were used for the test. The test lasted 21 days and germinated, hard, dormant (imbibed not germinated) and dead (rotten) seeds, were counted at 3, 7, 10, 14 and 21 days.

Results: Under the storage conditions described, two months after harvest and for a period of one year, seed germination of *C. argentea* seeds was high (more than 60 %), but after that it consistently declined with time and was less than 50 % two years later (Table 69).

Seed dormancy either imposed by the seed coat or by the embryo was low, and thus two months after harvest no hard seeds were recorded, meanwhile embryo dormancy was only 7.5 %, and significantly less ($P < 0.05$) the following months of storage. Similarly, low percentages of hardseedeness developed under the described storage conditions.

Table 69. Percentages of germination, hard, dormant and dead seeds of *C. argentea* CIAT 18668 stored under uncontrolled environmental conditions of Atenas, Costa Rica.

Age (months)	Germination (%)	Hard (%)	Dormant (%)	Dead (%)
2	78.5 a*	0.0 a	7.5 a	14.0 a
4	87.0 a	3.5 b	0.5 b	9.0 a
6	84.8 a	4.0 b	2.4 b	8.8 a
8	83.7 a	4.3 b	2.5 b	9.5 a
13	62.7 b	1.5 b	1.0 b	34.8 b
16	62.7 b	3.8 b	1.5 b	32.0 b
19	59.3 b	4.0 b	3.0 b	33.7 b
22	56.5 b	1.5 b	2.3 b	39.7 b
25	45.8 c	2.3 b	2.5 b	49.4 c

* P< 0.05

Seeds rapidly imbibed water and began to germinate 3 days after planted, but the major proportion of germinated seeds was recorded at day 7 of the test (Figure 74). Germination continued at lower proportions for 21 days. Seeds rapidly imbibed water and began to germinate 3 days after planted, but the major proportion of germinated seeds was recorded at day 7 of the test (Figure 74). Germination continued at lower proportions for 21 days.

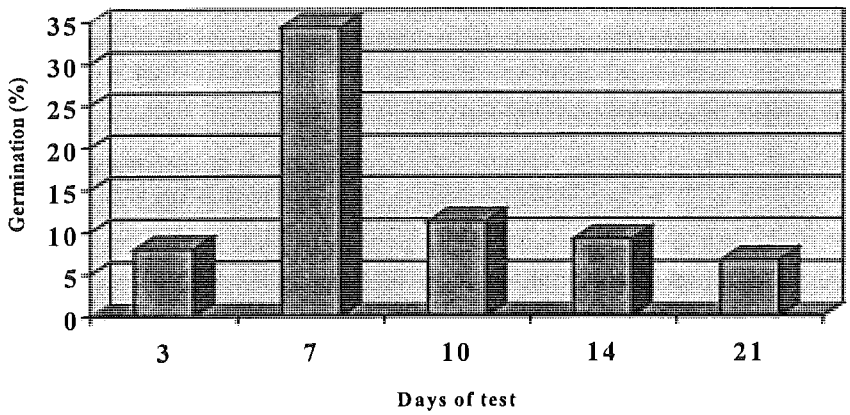


Figure 74. Rate of seed germination in a 21 days germination test of *C. argentea* (CIAT 18668) stored in uncontrolled environmental conditions of Atenas, Costa Rica.

Discussion: Seed deterioration (increasing loss of the ability to germinate) is associated with quantitative and qualitative change of compounds under storage, such as: a) mobilization of food reserves, b) degradation of proteins, c) loss of seed membranes (comprised of lipid layers and proteins), d) enzyme alterations and, e) loss of the ability of the cell to duplicate and divide and thus grow. None of these compounds or processes has been analyzed in *C. argentea* seeds. A decline *in seed germination and an increase in the percentages of dead seeds after 25 months of storage of Cratylia* seed , indicates that some kind of seed degradation is affecting viability of the seed.

Both hardseedeness and embryo dormancy are low in *C. argentea* seeds, therefore we can not expect a prolonged longevity of this seed weathering in the field. However, life span of most kinds of seeds can be extended by storage at low temperatures and low seed moisture content. The latter was not measured in the *C. argentea* seeds used, but the development of hard seeds after 4 months of storage, is an indication of reduction in seed moisture content, since it is well established that legume seeds develop hardseedeness as they lose water to the surrounding atmosphere.

Previous studies have shown that *C. argentea* seeds germinate more readily when planted superficially (no more than 2 cm depth), and the present results indicate that a larger proportion of germinated seeds should be observed after a week of planting. This has practical implications since under appropriate soil humidity conditions, we can expect a high proportion of seedling emergence in a short period, otherwise it may be an indication of either poor seed quality, too deep planting or unfavorable weather conditions.

4.2.4 Participatory evaluation, selection and targeting of multipurpose forage germplasm in the hillsides of Central America (M. Peters, P. Argel, C. Burgos, H. Cruz, M.I. Posas, T. Reyes, N. Espinoza)

Rationale: Forage germplasm in its multiple uses(e.g. as feed, for the suppression of weeds, for maintenance and improvement of soil fertility, for erosion control) could play an important role in improving the well-being of the small and medium size farmers in Central American hillsides. However, adoption - particularly of forage legumes - has been limited, possibly due to lack of direct interaction with the farmers. Therefore it is necessary to develop new forage technologies with farmers, using a participatory approach.

To address this issue, CIAT in collaboration with NARS, NGO's and farmer groups is attempting to select and promote germplasm preferred by farmers. GIS-tools are being developed for strategic targeting of forage germplasm first to environmental and later to socioeconomic niches in hillsides of Central America. The work is also anticipated to contribute to the development of an overall strategy to guide future research on tropical forages and to aid in the diffusion and final adoption of forage- based technology by small farmers. The interaction with strong national partners – alongside the farmers – will be of paramount importance to the success of the approach. As a spin-off, an on-farm experiment to test the ability of improved forages to reclaim land in areas affected by hurricane "Mitch "was established. It is anticipated that appropriate forages can upgrade land without incurring the high costs of other land reclamation practices.

Methods: A combination of agronomic evaluation techniques, participatory technologies, soil indicators, socio-economic studies and GIS tools will be employed. The work links closely with the TROPILECHE project, using some of the same germplasm. On the other hand, Forage germplasm selected from this work will be useful to TROPILECHE and to other projects working in developing new forage alternatives for crop-livestock systems in hillsides.

Results from Honduras: A range of grasses was subject to the assessment and selection (absolute evaluation) by farmers at two sites around Yorito. Honduras (Tables 70 and 71). In both sites *Brachiaria brizantha* CIAT 26110, *Panicum maximum* CIAT 16031(cv.Tanzania) and *Brachiaria* hybrid CIAT 36061 (FM9201/1873) were the overall most preferred grass accessions. Main selection criteria for the farmers were productivity, rooting intensity, color of leaves, and ability to cover the soil. The *Brachiaria* hybrid CIAT 1872 was consistently perceived as an accession with soft leave (seen as an indicator for high palatability) and of high quality and therefore liked by a number of farmers.

Several farmers, however, rated the accession lower for its low forage productivity in comparison to the two other selected accessions. Seed of the three preferred grasses were requested by farmers involved in the test in order to plant larger plots on their farms. In the case of herbaceous legumes farmers were exposed to a high number of accessions.

Therefore a number of open-ended evaluations were executed to narrow down the number of accessions of interest to farmers. A range of 8 accessions, based on perceptions of farmers and agronomic performance was selected for absolute evaluation at one site.

Table 70. Summary of participatory selection of grasses by farmers. Grasses were scored on a scale from 1 (least preferred) to 5 (most preferred species. Site 1: San Jeronimo.

Accessions	Evaluation																Total	Ranking
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
B. brizantha CIAT 26110	18	20	18	18	18	20	18	18	18	20	20	15	6	6	3	5	238	1
B. hybrid 36061 (FM9201/1873)	18	20	16	18	20	20	20	16	14	20	18	13	6	8	3	3	233	3
B. dictyoneura cv. Llanero	12	14	16	14	16	16	14	10	10	8	14	7	6	6	5	5	173	5
P. maximum CIAT 16028	16	14	16	20	16	16	20	16	16	20	16	13	10	6	5	5	225	4
Panicum maximum CIAT 16051	16	12	12	12	8	10	12	12	16	14	14	9	4	4	5	3	163	6
P. maximum cv. Tanzania	16	18	18	20	18	20	18	16	14	18	18	13	10	10	5	3	235	2

Table 71. Summary of participatory selection of grasses by farmers. Grasses were scored on a scale of 1 (least preferred) to 5 (most preferred species. Site 2: San Antonio.

Accessions	Evaluation																Total	Ranking
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
B. brizantha CIAT 26110	18	20	18	18	16	20	18	18	20	18	18	13	13	8	5	5	246	1
B. hybrid 36061 (FM9201/1873)	18	18	14	16	20	14	20	20	18	18	20	13	11	8	3	5	236	4
B. dictyoneura cv. Llanero	12	10	12	14	10	16	12	16	14	14	16	11	13	10	3	5	186	6
P. maximum CIAT 16028	16	16	14	18	18	20	20	18	18	18	16	13	`3	8	5	5	238	3
P. maximum CIAT 16051	14	14	14	18	14	16	14	12	18	18	14	11	9	8	5	5	209	5
P. maximum cv. Tanzania	18	18	20	18	16	16	20	20	16	20	16	13	13	8	5	3	240	2

Preliminary results are shown in Table 72. Farmers were especially interested in the two *Arachis pintoi* accessions CIAT 17434 (cv. Pico Bonito in Honduras) and CIAT 22160 for their ability to cover the soil and high leaf proportion. *Stylosanthes guianensis* CIAT 184 (cv. Pucallpa) was found to be interesting to farmers mainly for its ability to retain green leaves during the dry season. At another site only a small number of legume accessions persisted due to poor adaptation to soil and problems with pest and diseases. However, at that site farmers selected a different range of accessions, with *Centrosema* spp. – in particular *Centrosema plumieri* DICTA – and *Clitoria ternatea* cv. Tejuana being the preferred accessions.

Table 72. Initial selection through participatory evaluation of herbaceous legumes by farmers. Legumes were scored on a scale of 1 (least preferred) to 5 (most preferred species). San Jeronimo.

Accession	Evaluation											Total	Ranking
	1	2	3	4	5	6	7	8	9	10	11		
cv. Pico Bonito in Honduras	5	5	5	5	5	5	5	5	5	5	5	55	1
Arachis pintoi CIAT 22160	5	5	1	1	1	5	5	5	5	5	5	43	3
Centrosema brasilianum CIAT 15387	5	5	1	1	1	1	1	1	1	3	3	23	8
Centrosema macrocarpum CIAT 25222	3	3	1	1	1	3	3	3	3	3	1	25	7
Centrosema plumieri DICTA	5	5	1	1	1	5	5	5	5	5	3	41	4
Centrosema pubescens DICTA (CIAT 434)	5	5	3	3	3	1	1	3	3	1	1	29	5
Desmodium heterocarpon ssp. *ovalifolium* 23762	3	3	1	1	1	5	3	3	3	3	3	29	5
cv. Pucallpa	5	5	5	1	3	3	3	5	5	5	5	45	2

A short-term on-farm experiment for reclamation of land in valley areas damaged by "Mitch" was planted in July 1999. Forages were selected on the basis of earlier agronomic evaluations in the area. The following legume accessions were sown:

a) *Vigna unguiculata* cv. Cowpea Verde Brazil,
b) *Lablab purpureus* DICTA,
c) *Centrosema plumieri* DICTA,
d) *Mucuna pruriens* IITA-Benin,
e) *Canavalia ensiformis* DICTA, and
f) *Brachiaria dictyonuera* cv. Llanero, control.

These legumes will be deferred from utilization for at least for 4-6 months so as to ensure good establishment and incorporate N in the soil. For earlier utilization through grazing at the end of the wet season, the following legumes were planted:

a) *Centrosema plumieri* DICTA,
b) *Centrosema macrocarpum* CIAT 25522,
c) *Centrosema pubescens* DICTA and
d) Natural regrowth included as control.

These plots will be used for participatory selection by farmers of soil recuperation/soil conservation legumes.

Activities in Nicaragua. In July 1999 a new nursery for the participatory selection of forages was established as part of SOL in San Dionisio, near Matagalpa. The following accessions were planted.

A) Grasses:

1. *Brachiaria* hybrid CIAT 36061 (FM9201/1873),
2. *Brachiaria brizantha* CIAT 6387,
3. *Brachiaria brizantha* CIAT 26110,
4. *Brachiaria dictyoneura* CIAT 6133, and
5. *Panicum maximum* CIAT 16028, CIAT 16031, CIAT 16051.

B) Herbaceous legumes:

1. *Arachis pintoi* CIAT 17434 *(cv. Pico Bonito),* CIAT 18744 *(cv. Porvenir),* CIAT 18748, CIAT 22160,
2. *Centrosema acutifolium* CIAT 5568,
3. *Centrosema acutifolium* CIAT 5277,
4. *Centrosema macrocarpum* CIAT 25222,
5. *Centrosema pubescens* CIAT 438, CIAT 5126, CIAT 15160
6. *Clitoria ternatea cv. Tejuana,*
7. *Chamaecrista rotundifolia* CIAT 18252,
8. *Desmodium velutinum* CIAT 13953, and
9. *Desmodium heterocarpon* ssp. *ovalifolium* CIAT 13105, CIAT 13651, CIAT 23762

Once the plots are well established farmers will be invited to select preferred species and accessions within species.

Tropileche Trial in Nicaragua: A trial with grass-legume mixtures (*Brachiaria* spp. and *Arachis pintoi*) was planted as port of SOL in San Dionisio. The pastures will be grazed by dual-

purpose cows. The aim is to offer alternatives to farmer for improving beef and milk production currently based on native pasture of low quantity and quality. The following treatments were applied:

T0 = Native pasture,
T1 = *Brachiaria brizantha* CIAT 26110 in association with *Arachis pintoi* CIAT 22160 and CIAT 18744,
T2 = *Brachiaria brizantha* cv. Marandú in association with *A. pintoi* CIAT 22160 and CIAT 18744,
T3 = *Brachiaria brizantha* cv. La libertad in association with *A. pintoi* CIAT 22160 and CIAT 18744, and
T4 = *Brachiaria dictyoneura* in association with *A. pintoi* CIAT 22160 and CIAT 18744.

Discussion: A number of experiments have been established in hillsides of Honduras and Nicaragua to identify multiple-use forage germplasm according to demand by farmers. The first materials to be selected were grasses for the improvement of pastures. For herbaceous legumes the selection process has entered its 2nd phase. The process is slower than for grasses as legumes are not as frequently used for pasture establishment, as grasses given that they are not well know to farmers. Moreover, the large number of options of legumes offered made it necessary to employ a two-phase process of open-ended evaluation followed by absolute evaluation. A great support to the work on selection by farmers of forage germplasm is the close link to Tropileche, as farmers will observe some accessions on large size plots being grazed by cattle.

4.2.5 Spontaneous adoption of *Arachis pintoi* cultivars and *Cratylia argentea* in Costa Rica, Honduras and Nicaragua (P. J. Argel)

The herbaceous legumes *A. pintoi* CIAT 17434 was released as cv. Pico Bonito and Maní Mejorador respectively in Honduras and Costa Rica in 1993 and 1994; the ecotype CIAT 18744 of this legume was released in Costa Rica as cv. Porvnir in 1998. Meanwhile the shrub *C. argentea* has not been released in any country up to date, although in Costa Rica significant advances on the evaluation of the shrub have been made through participatory on-farm research during the last years, and already spontaneous adoption of the legume is taking place.

Arachis pintoi: It is difficult to quantify the level of adoption of *A. pintoi* in any country and estimate the present area planted either as forage or cover crop; statistics are scarce on new areas planted with pastures and on the volume of seeds traded, besides that this legume propagates easily from cuttings, and many farmers prefer this method of propagation rather than planting by seed because the high cost of the seed. However, in Costa Rica, large volume of seeds of cv. Maní Mejorador began to be commercialized in 1996 (2500 kg of seed imported), this increased to 3000 kg in 1997 and reduced to 1000 kg in 1998, for a total of 6500 kg of seed, which will be sufficient for 812 ha of Maní planted to a rate of 8 kg/ha, either as cover crop or in mixture with grasses. Of the *A. pintoi* cv. Porvenir a total of 450 kg of seeds were imported and commercialized in 1998.

Cratylia argentea: This legume is in status of pre-release in Costa Rica, where it is being evaluated as protein supplement for high energy forages during the dry season, offered either fresh or in silage to milking cows. Pilot Tropileche farms in subhumid areas of Costa Rica are pioneers in the use of *Cratylia* and these farms are utilized by the national extention system to promote its use. The present level of adoption of the shrub can only be estimated from seed sold. A total of 100 kg of experimental seed was sold to 28 farmers by the Seed Unit of CIAT in Atenas and 7 kg of seed sold by one farmer in Esparza. Given that the planting rate is 4 kg/ha of seed, we estimate that approximately 27 ha of *Cratylia* were planted during 1999. The larger demand has come from small and medium size dual purpose farmers of the Nicoya Peninsula and

the counties of Acosta and Puriscal in Costa Rica. These areas hold a high percentage of dual purpose cattle and are subject to dry periods of 5 to 6 months every year.

Statistics on forage adoption in Nicaragua and Honduras are also scarse, but both *A. pintoi* and *C. argentea* are being promoted and evaluated by national research institutes, NGOS, and farmers; the level of adoption however, is less than in Costa Rica.

Progress towards achieving output milestone

- Multipurpose grasses and legumes are tested by farmers in hillsides of Honduras and Nicaragua.
- *Cratylia argentea* is used as a dry season feed by farmers outside the Tropileche benchmark sites by farmers in Costa Rica.

Substantial progress was made this year in the participatory selection by farmers of grasses in Honduras; there is rising interest among participating farmers in some of the herbaceous legumes, in particular *Arachis pintoi*. Efforts are being made to enhance and support artisanal seed production. During the following year it is anticipated that some farmers in Nicaragua and Honduras will establish selected grasses on their farm –seed requests were made – and have selected some legumes. Farmers and NGO's from neighboring communities have shown interest in the work baing carried out and an extension of the approach is planned for the next planting season.

Spontaneous adoption of *Cratylia argentea* is occurring in Costa Rica. During the last 12 months 100 kg of experimental seed was sold to 28 farmers located in four different sites. The continuous adoption of *Cratylia* has stimulated private seed growers in Costa Rica to multiply seed, which will in turn facilitate the official release of this legume by MAG (Ministry of Agriculture and Livestock) in Costa Rica. The shrub legume *C. argentea* has not been included up to now in the farmer participatory evaluation in hillsides of Honduras and Nicaragua. However, it is being evaluated by farmers in the llanos piedmont in Colombia.

Activity 4.3. Develop expert systems for legume biodiversity by linking geographic information with biological data

Highlight

- Developed a trial version of CIAT's forage database in a graphical platform for use in INTRANET by selected CIAT scientists.

4.3.1 Converting the forage database to a graphical platform (Martha Herrera, Arturo Franco, Carlos Lascano, Michael Peters)

Rationale: The Tropical Forage group in CIAT has generated a database on evaluation of germplasm, right from collection or exchange to the release of cultivars by national institutions. A great part of this information has been entered into an ORACLE database, which at present is available for CIAT scientists. For the actualization of the database, an information system based on the fourth generation language ORACLE FORMS 3 was developed; this system is available via in CIAT the Calima Server. However, in view of the technological advances, the requirements of users in CIAT, and the importance of sharing all the research results with other partners institutions through the Internet or via CD-ROM, it is important to convert this information system to a graphical, user-friendly and attractive platform.

Methods: The programs in ORACLE FORMS 3 are converted to ORACLE DEVELOPER 2000, with emphasis on user-friendliness and on an attractive platform. In the first phase, the old programs with information available in character mode are transferred to graphic mode. In the second phase new modules are added. Once completed, the database will be initially tested via CIAT's Intranet and other placed in an Internet CD-ROM.

Results and discussion: A trial version was developed this year. In figures 75 and 76 screenshots of the current versions are shown. The actual version was tested for year 2000 compatibility.

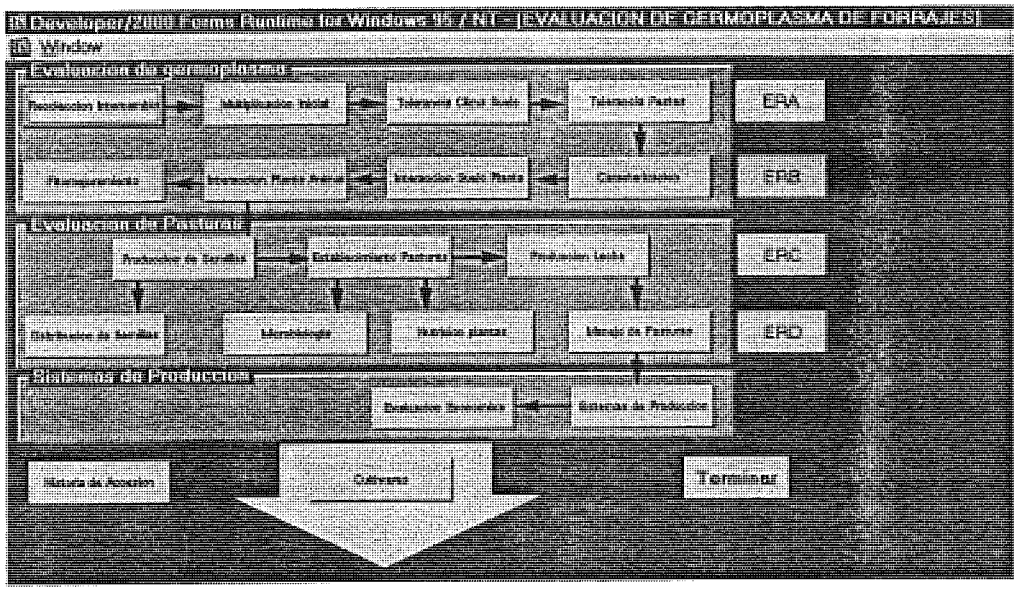


Figure 75. Main screen of the forage database in an attractive graphic platform developed in ORACLE DEVELOPER 2000

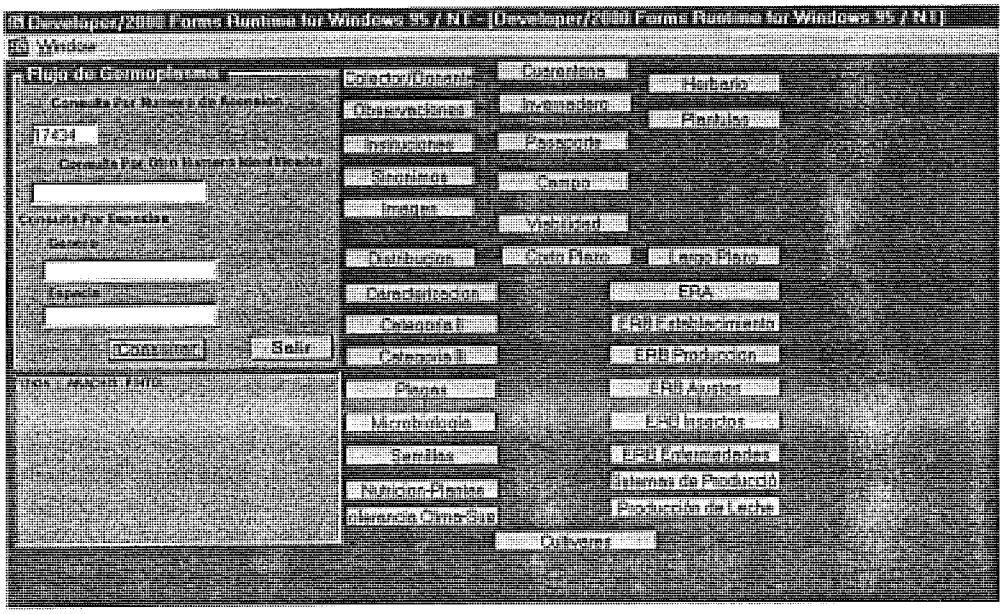


Figure 76. Second screen showing the type of information available for a specific accession

Next steps include the further refinement and testing of the database for Intra- and Internet use and the development of a CD-ROM version. Target group for this CD-ROM are mainly NARS and NGO's in Latin America. In the future an English version of the database is planned. At present, the database contains a total of 1537 accessions, evaluated in 315 sites. In many of these sites, available information is incomplete and therefore a great effort is being made to update the database through capturing information from existing publications and through obtaining data directly from scientists in the Forage (IP5) and Production Systems (PE5) Projects.

4.3.2 Use of GIS models for better targeting forage germplasm (M. Peters, Glen Hyman, Luis Horacio Franco, Arturo Franco, Belisario Hincapie, Gerardo Ramirez, Alexander Gladkov, and P. Jones)

Rationale: The overall approach which intends to integrate agro-ecological, economic and social information, is based on the following two main assumptions.

1. A wealth of information on the agro-ecological adaptation of forage germplasm is available in CIAT's-held forage databases. However, the access and hence utilization of this information needs to be improved.
2. In previous evaluations of forage germplasm adaptation, to environmental conditions, the agro-ecological information is separated from socio-economic factors influencing forage germplasm adoption by farmers.

Based on these assumptions, the targeting of forage germplasm intends to enhance the utility of existing information and, in future, to integrate environmental and socio-economic adaptation of forage germplasm for multiple uses. It is anticipated, that this approach will allow a more accurate and client-oriented prediction of possible entry points for forage germplasm.

Methods: A working group, formed to carry out the work, agreed to follow a step-wise procedure for the development of an expert system, as follows:

1. Inclusion of the existing RIEPT (Red International de Evaluación de Pastos Tropicales) database – to start with the regional trials A and B – into GIS –system to describe agro-ecological adaptation of forage germplasm in Latin America
2. Inclusion of supplementary information on agro-ecological adaptation as it exists in CIAT's-held forage databases e.g. the RABAOC database.
3. Inclusion of experiences of (former) CIAT Scientists and collaborators
4. Incorporation of socio-economic information based on existing results, from characterization studies and from on-going work, first on a regional level (i.e. Central America).

Results: Parameters for the description of ecological adaptation based on agronomic performance of accessions across environments were identified. Currently the group is in the process of revising the classification of agro-ecosystems to be utilized for the database as the classification of agro-ecological zones developed by Thomas T. Cochran may not be suitable for use in the GIS tool to be developed.

Discussion: It is believed that the system to be developed will greatly enhance the availability of integrated information on the agro-ecological and socio-economic adaptation of forage germplasm for multiple uses. The integration of information from different sources will allow the improved targeting of forages to farmer's conditions and demands. As a result it is likely that efficiency and client-orientation of future research will be enhanced, and the dissemination of existing and future research results will be improved.

Progress towards achieving output milestone

- Forage database in CD-ROM is available to NARS and CIAT Projects.
- Decision support system is available for targeting forage germplasm for agronomic performance in agroecosystems.

A preliminary version of the graphically and user-friendly forage database is available for selected scientists through the CIAT's Intranet. During 2000 this database will be refined for utilization via the Internet and a CD-ROM. This database will also be utilized in developing the Decision Support Tool for the targeting of forage germplasm.

Activity 4.4 Facilitate communication through newsletters, journals, workshops and internet

Highlights

- Continued to publish a Forage Newsletter and the Journal Pasturas Tropicales.
- Initiated the design of a Web page for the Forage Project.

Forage 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.

Due to budgetary constraints, in 1999 we only produced only one Newsletter in which we reported advances made in the work on endophytes. If the Project is not successful in attracting additional funds the Newsletter initiative will be suspended.

Journal Pasturas Tropicales (C. Lascano and A. Ramirez)

During 1999, we published volume 20 (3) and Volume 21 (1). The two volumes include 16 contributions covering wide range topics as can be seen in Table 73. From the information presented in Table 73 it is evident that major contributors to Pasturas Tropicales continue to be researchers from Brazil. However, it is interesting to note a growing contribution of papers from Colombia, through CORPOICA researchers.

The demand for publication in Pasturas Tropicales continues at a fast rate given that it is the only Journal in LAC dealing with forages that it has a wide international distribution. The large number of papers received and waiting for technical and editorial review illustrates this increased demand. Given the budgetary constraints we phase in the Forage Project we continue to phase the challenge of publishing Pasturas Tropicales in 2000 and years to come. To meet this challenge we have initiated a consultation with subscribers to explore different alternatives such as:

1. increased cost of subscription,
2. commercial advertisement or
3. electronic journal

Based on the response from subscribers we will make a final decision on the future of Pasturas Tropicales.

Table 73. Major topics covered in the two last issues of the Journal Pasturas Tropicales

Themes	V 20 (3) No of Papers	V 21 (1) No of Papers	Institution	Countries
Germplasm evaluation (*Leucaena*, , *Arachis* and *Centrosema*)	1	2	OFI, CIAT, EMBRAPA	United Kingdom, Colombia, Brazil
Nitrogen fixation in Stylo		1	EMBRAPA	Brazil
Response to fertilizer establishment/production (*Arachis, B. brizantha* cv Marandu, *Panicum maximum* cv Tobiata, *Panicum laxum*)	1	3	EPAMIG, EMBRAPA	Brazil
Use of woody legumes to supplement ruminants	2		CATAS, CIAT, CORPOICA	China, Colombia
Nutritional indicators (Rumen ammonia and Milk Urea Nitrogen)		2	CORPOICACIAT	Colombia
Pasture evaluation with milking cows	1		CORPOICA CIAT	Colombia
Legume seed quality	1		CORPOICA	Colombia
Adoption of *Arachis*		1	CIAT	Colombia

Progress towards achieving output milestone

- Institutions and individuals receive and contribute to publications.
- Web Page of the Forage Project is developed.

Through the Forage Newsletter and the Journal Pasturas Tropicales we continue to reach a wide audience of researchers in LAC. Both publications are being distributed to 350 international subscribers including libraries. As a consequence, we are able to communicate not only our research results but also that of our partners. The challenge that we still have is to identify funds to continue publishing in the Forage Newsletter and Pasturas Tropicales. The communication group of CIAT trained this year a member of the IP5 Team on design of Web pages for Projects in CIAT. We are now working on the design of the Web page and hope to have a first version by mid 2000.

PUBLICATIONS

Journal Papers

Argel, P.J. 1999. Maní Forrajero: Una Leguminosa de Uso Múltiple para el Sector Agropecuario de Costa Rica. Montecillos (Costa Rica). Año XV, No. 102. p. 12-13.

Cárdenas, E.A., B.L. Maass, and Peters, M. 1999. Evaluación de germoplasma nuevo de *Arachis pintoi* en Colombia. 2. Bosque muy húmedo (zona cafetera), caldas. Pasturas Tropicales, (submitted).

Cardona, C., J.W. Miles, and G. Sotelo. 1999. An improved methodology for massive screening of *Brachiaria* spp. genotypes for resistance to *Aeneolamia varia* (Homoptera: Cercopidae). J. Econ. Entomol. 92(2):490-496.

Hess H. D., H. Florez, C.E. Lascano, L.A. Baquero, A. Becerra and Ramos J. 1999. Fuentes de variación en composición de la leche y niveles de urea en sangre y leche de vacas en sistemas doble proposito en el tropico bajo de Colombia. Pastura Tropicales 21 (1): 33-42

Kelemu, S. and Y. Takayama. 1998. An endophytic fungus in the tropical grass *Brachiaria*: Effect on a leaf spot disease (Abstract). Phytopathology 88:S46.

Kelemu, S., F. Muñoz, and M.X. Rodriguez. 1999. Genetic and pathogenic diversity of *Colletotrichum gloeosporioides* isolates infecting *Arachis pintoi* (Abstract). Phytopathology 89: S38.

Kelemu, S., D.Z. Skinner, J.L. Badel, C.X. Moreno, M.X. Rodriguez, C.D. Fernandes, M.J. D'A. Charchar, and S. Chakraborty, 1999. Genetic diversity I South American *Colletotrichum gloeosporioides* isolates from *Stylosanthes guianensis*, a tropical forage legume. European Journal of Plant Pathology 105:261-272.

Merkel, U., M. Peters, S.A. Tarawali, R. Schultze-Kraft, and D.K. Berner. Characterization of a collection of *Aeschynomene histrix* in subhumid nigeria. Journal of Agricultural Science (Cambridge), in press.

Moreno, I.R., B.L. Maass, M. Peters, and E.A. Cárdenas. 1999. Evaluación de germoplasma nuevo de *Arachis pintoi* en Colombia. 1. Trópico seco, Valle del Cauca. Pasturas Tropicales, Vol. 21(1):18-32.

Muhr, L., S.A. Tarawali, M. Peters, and R. Schultze-Kraft. 1999. Forage legumes for improved fallows in agropastoral systems of subhumid west Africa. I. Establishment, herbage and nutritive value of legumes as dry season forage. Tropical Grasslands, accepted.

Muhr, L., S.A.Tarawali, M. Peters, and R. Schultze-Kraft. 1999. Forage legumes for improved fallows in agropastoral systems of subhumid west Africa. II. Green manure production and decomposition after incorporation into the soil. Tropical Grasslands, accepted.

Muhr, L., S.A. Tarawali, M. Peters, and R. Schultze-Kraft. 1999. Forage legumes for improved fallows in agropastoral systems of subhumid west Africa. III. Rotational effects on maize after dry season harvest of forage legumes and their subsequent regrowth as green manure. Tropical Grasslands, accepted.

Muhr, L., S.A. Tarawali, M. Peters, and R. Schultze-Kraft. 1999. Forage legumes for improved fallows in agropastoral systems of subhumid west Africa. Acceptability of forage legumes for improved fallows - first experiences of agropastoralists in subhumid south-west nigeria. Experimental Agriculture, submitted.

Muhr, L., S.A. Tarawali, M. Peters, and R. Schultze-Kraft. 1999. Soil mineral n dynamics and maize grain yields following *Centrosema macrocarpum* and *Stylosanthes guianensis*: Effects of different improved fallow notations and varying levels of fertiliser to maize. Field Crops Research, submitted.

Peck, D. C. 1998. Use of alternative food plants exclusively by adult male froghoppers (Homoptera: Cercopidae). Biotropica 30(4):639-644.

Peck, D. C. 1999. Seasonal fluctuations and phenology of *Prosapia* spittlebugs (Homoptera: Cercopidae) in upland dairy pastures of Costa Rica. Environmental Entomology 28(3):372-386.

Peters, M., S.A. Tarawali, and R. Schultze-Kraft. 1999. Relative palatability and seasonal agronomic performance of selected pasture legumes for species mixtures in subhumid west Africa. Experimental agriculture, in press.

Peters, M., S.A. Tarawali, R. Schultze-Kraft, J.W. Smith, and A. Musa. 1999. Performance of legume mixtures under small-plot periodic grazing. Journal of Agronomy and Crop Science 182, 25-35.

Rao, I. M., D. K. Friesen and W. J. Horst 1999. Opportunities for germplasm selection to influence phosphorus acquisition from low-phosphorus soils. Agroforestry Forum (in press).

Rao, I.M., V. Borrero, J. Ricaurte, and R. Garcia. 1999. Adaptive attributes of tropical forage species to acid soils. V. Differences in phosphorus acquisition from inorganic and organic phosphorus sources. J. Plant Nutr. 22 (7):1175-1196.

Rao, I.M., V. Borrero, J. Ricaurte, and R. Garcia. 1999. Adaptive attributes of tropical forage species to acid soils. IV. Differences in shoot and root growth responses to inorganic and organic phosphorus sources. J. Plant Nutr. 22 (7):1153-1174.

Ricaurte, J., Q. Zhiping, D. Filipe, I. M. Rao and E. Amezquita. 1999. Distribución radicular, absorción de nutrientes y erosión edáfica en sistemas de cultivos y forrajeros en laderas del Cauca, Colombia. Suelos Ecuatoriales (in press).

Sotelo, G., C. Cardona, y J. W. Miles. 1998. Metodología mejorada para evaluación de resistencia de *Brachiaria* spp. al salivazo de los pastos (Homoptera: Cercopidae) en invernadero. Revista Colombiana de Entomología 24(1 y 2):17-22.

Wenzl, P., L. I. Mancilla, J. E. Mayer, I. M. Rao and E. Heberle-Bors. 1999. A novel approach to enrich less abundant cDNAs using paramagnetic beads: a theoretical simulation. Nucl. Acid Res. (in review).

Wenzl, P., A. L. Chávez, J. E. Mayer, I. M. Rao and M. G. Nair. 1999. Two di-hydroxy-cinnamoylquinic acid esters from roots of nutrient-deprived *Brachiaria* species. Phytochemistry (in review).

Yi Kexian, C.E. Lascano, P.C. Kerridge, and P. Avila. 1998. The effect of three tropical shrub legumes on intake rate and acceptability by small ruminants. Pasturas Tropicales 20(3):31-35.

Zhiping, Q., I. M. Rao, J. Ricaurte, E. Amézquita, J. Sanz and P. Kerridge. 1999. Root distribution effects on nutrient uptake and soil erosion in crop-forage systems on Andean hillsides. Trop. Agric. (in review).

Workshop and Conference Papers

Argel, P.J. 1999. Tecnologías Forrajeras para el Desarrollo de una Ganadería más Productiva en el Trópico bajo de Centroamérica. Contribución del CIAT. In: Pomareda, C. (ed). Intensificación de la Ganadería en Centroamérica: Beneficios Económicos y Ambientales (Memorias). FAO/CATIE, mayo, 1999. (In press).

Argel, P.J. and G. Pérez. 1998. Adaptation of new species of Leucaena in Costa Rica, Central America- Preliminary results. In: Shelton, H. M., Gutteridge, R. C., Mullen, B. F. and Bray, R. A. (eds). Leucaena-Adaptation, Quality and Farming Systems. ACIAR Proceedings No. 86, Camberra, Australia. p. 146-149.

Argel, P.J., C.E. Lascano, and L. Ramírez. 1998. Leucaena in Latin American Farming Systems: Challenges for Development. In: Shelton, H. M., Gutteridge, R. C., Mullen, B. F. and Bray, R. A. (eds). Leucaena-Adaptation, Quality and Farming Systems. ACIAR Proceedings No. 86, Camberra, Australia. p. 319-323.

Ayarza, M. A., I. M. Rao and E. Barrios. 1999. Potencial de los mecanismos suelos-planta y de los sistemas integrados en el uso eficiente de los nutrimentos disponibles en suelos tropicales. Invited paper presented at XLV Annual Meeting of PCCMCA, Guatemala City, Guatemala.

Castro, U., A. Morales and D. Peck. 1999. Fenología del mión de los pastos (Homoptera: Cercopidae) en el valle del Río Cauca. XXVI Congreso de la Sociedad Colombiana de Entomología [Bogotá, Colombia].

Chakraborty, S., C.D. Fernández, J.J. Charchar, and S. Kelemu. 1998. Complex extotic races of the anthracnose pathogen pose potential threat to Australian *Stylosanthes* cultivars. In: D.L. Michalk and J. E. Pratley. Agronomy-Growing Greener Future: Proceedings of the 9th Australian Society of Agronomy Conference, Wagga Wagga, NSW, 20-23 July, 1998, pp. 549-550.

Chakraborty, S., C.J. Liu, D.F. Cameron, A. Chandra, C.R. Ramesh, C.D. Fernandes, M.J. Charchar, L. Guodao, and S. Kelemu. 1998. Application of molecular markers to breed and select anthracnose resistant *Stylosanthes* and to characterize endemic and exotic populations of *Colletotrichum gloeosporioides*. Fourth Asia-Pacific Congress on Agricultural Biotechnology, 13-16 July, 1998, Darwin.

Jiang, C. and S. Kelemu. 1999. Agrobacterium-mediated transformation of tropical forage legume *Stylosanthes guianensis* with a rice chitinase gene for resistance to anthracnose disease. Ninth International Congres on Molecular Plant-Microbe Interactions. 25-30 July, 1999, Amsterdam.

Lascano, C. E. 1999. Desarrollo de especies forrajeras para sistemas de producción animal en America tropical. In: Proceedings Simposio Internacional de forrajeras subtropicales. Tucumán, Argentina- September 1-3. p 65-70.

Lascano, C. E. 1999. Selective grazing on grass-legume mixtures in tropical pastures. In: Proceedings of the International Symposium on Grassland Ecophysiology and Grazing Ecology. Curitiba, Parana, Brazil- 24-26 August, 1999. p 151-164

López, M. F. and D. Peck. 1999. Importancia de la comunicación a través del sustrato en el comportamiento reproductivo del salivazo de los pastos (Homoptera: Cercopidae). XXVI Congreso de la Sociedad Colombiana de Entomología [Bogotá, Colombia].

Maass, B.L., M. Peters, and E.A. Cárdenas. 1999. Adaptation of *Arachis pintoi* to tropical environments in Colombia. Poster to be presented at the 43. Jahrestagung der Gesellschaft für Pflanzenbauwissenschaften Göttingen.

Morales, A., A. C. Bolaños and D. Peck. 1999. Evaluación de diferentes cepas de hongos entomopatógenos al salivazo de los pastos *Aeneolamia varia* en condiciones de invernadero. XXVI Congreso de la Sociedad Colombiana de Entomología [Bogotá, Colombia].

Morales, A., U. Castro and D. Peck. 1999. Establecimiento de diferentes metodologias de cría del salivazo de los pastos. XXVI Congreso de la Sociedad Colombiana de Entomología [Bogotá, Colombia].

Muhr, L., M. Peters, S.A. Tarawali, and R. Schultze-Kraft, R. 1998. Fallow improvement with forage legumes: potentials and constraints of an integrative technology for crop-livestock systems in subhumid West Africa. in: Renard, G., Neef, A., Becker, K. and Von Oppen, M. (eds) Soil fertility management in West African land use systems; Niamey, Niger, 4-8 March 1997. Markgraf Verlag, Weikersheim, Germany, 393-398.

Peck, D. C. 1999. Diversidad y distribución. Biología y comportamiento. Ecología e impacto. Avances en el Caquetá y avances en el manejo integrado. Memorias, I Seminario Taller Sobre Bioecología y Manejo del Mión de los Pastos en el Piedemonte Caqueteño [Universidad de la Amazonia, Colombia]

Peck, D. C. 1999. La diversidad y biología del mión de los pastos. La ecología e impacto del mión de los pastos. Avances del proyecto "Bioecología comparativa del salivazo de los pastos." Memorias, XXXVIII Foro Entomológico, Plagas de pastos tropicales y su manejo: caso del mión de los pastos [Universidad Nacional, Palmira, Colombia]

Peck, D. C. 1999. The comparative biology of neotropical grassland cercopids. 10th International Auchenorrhyncha Congress [Cardiff, Gales].

Peters, M, P. Horne, A. Schmidt, F. Holmann, P.C. Kerridge, P.C., S.A. Tarawali, R. Schultze-Kraft, C.E. Lascano, P.J. Argel, W. Stür, S. Fujisaka, K. Müller-Sämann, and C. Wortmann. 1999. The role of forages in reducing poverty and degradation of natural resources in tropical production systems. Paper for the International Workshop: Assessing the impact of agricultural research on poverty alleviation. San José, Costa Rica, 14-16 september 1999.

Peters, M., P.J. Argel, C. Burgos, H. Cruz, M.I. Posas, and A. Braun, A. 1999. Participatory selection of forages in Central America - Concept and preliminary results. Poster for the International Workshop: Working with farmers: the key to adoption of forage technologies. Cagayan de Oro City, Philipinnes, 12-15 october 1999.

Schultze-Kraft, R. and M. Peters. 1998. Leguminosas tropicales y diversidad de sus usos: Una sinopsis. In: Pietrosémoli, S. and J. Hernández (eds.), Seminario Internacional: cobertura de leguminosas en cultivos permanentes: compendio. Santa Bárbara, 1-2 octubre 1998. La Universidad del Zulia, Facultad de Agronomía, Maracaibo, Venezuela. p. 4-28.

Wenzl, P., I. M. Rao, J. E. Mayer and W. Roca. 1999. Aluminum tolerance and other traits contributing to acid soil adaptation in a tropical forage grass, Brachiaria decumbens. Invited paper presented at an international workshop on "Genetic Analysis and Engineering of Aluminum Tolerance in Plants", Campinas, Brazil.

Invited Book Chapters

Argel, P.J. and C.J. Paton. 1999. Overcoming Legume Hardseededness. In: Loch, D.S. and Ferguson, J.E. (eds). Forage Seed Production, Volume 2: Tropical and Subtropical Species. CAB INTERNATIONAL. p. 247-265.

Griffith, K., D. C. Peck and J. Stuckey. 1999. Monteverde agriculture: moving towards sustainability. In: N. M. Nadkarni & N. T. Wheelwright [eds] The Natural History, Ecology and Conservation of Monteverde, Costa Rica. Oxford University Press.

Guimaráes, E. P., J. I. Sanz, I. M. Rao and E. Amézquita. 1999. Investigaciones en sistemas Agropastoriles: Qué hemos aprendido y qué debemos hacer en el futuro. In: E.P. Guimaráes, J. I. Sanz, I. M. Rao, M. C. Amézquita and E. Amézquita (eds.). Sistemas Agropastoriles en Sabanas Tropicales de America Latina. CIAT, Cali, Colombia and EMBRAPA, Brazil.

Lascano, C., G. Ruiz, J. Velásquez, J. Rozo, and M. Jervis. 1999. Developing improved pastures systems for forest margins. In: Fujisaka Sam with collaboration of Jones, Annie (ed.). Systems and Farmer Participatory Research. CIAT publication No. 311, p. 50-60.

Rao, I. M. and N. Terry. 1999. Photosynthetic adaptation to nutrient stress. In: M. Yunus, U. Pathre and P. Mohanty (eds.), Probing Photosynthesis: Mechanism, Regulation and Adaptation. Taylor & Francis, U.K.

Rao, I. M., M. A. Ayarza, P. Herrera y J. Ricaurte. 1999. El papel de las raíces de especies forrajeras en la adquisición, reciclje y almacenamiento de nutrientes en el suelo. Memorias de Curso Internacional "Investigación y Desarrollo de Systemas de Producción Forrajera en el Tropico" (del 1 al 31 de Octubre de 1996). CIAT, Cali, Colombia.(in press).

Rao, I. M., D. K. Friesen and M. Osaki. 1999. Plant adaptation to phosphorus-limited tropical soils. In: M. Pessarakli (ed.) Handbook of Plant and Crop Stress. Marcel Dekker, Inc., New York, USA, pp. 61-95.

Tarawali, S.A., M. Peters, and R. Schultze-Kraft. 1999. Selecting and testing forage legumes for sustainable agriculture and livestock production in subhumid West Africa. International Livestock Research Institute (ILRI), Ibadan, Nigeria. University of Hohenheim (380), Stuttgart, Germany, in press.

Undergraduate Theses

Ballesteros, Y. and C. Gallego. 1999. Biología y comportamiento de *Mahanarva* sp. (Homoptera: Cercopidae) bajo condiciones de invernadero [Universidad de la Amazonia, Florencia].

Puentes, W and C. Ramirez. 1999. Respuesta del mión de los pastos (Homoptera: Cercopidae) en praderas asociadas y no asociadas en dos zonas del Departamento del Caquetá [Universidad de la Amazonia, Florencia].

Blanco, M. and B. Giraldo. 1999. Alternativas para el manejo del mión de los pastos en el piedemonte caqueteño [Universidad de la Amazonia, Florencia].

López, F. 1999. Importancia de la comunicación vibracional en el comportamiento reproductivo del salivazo de los pastos *Zulia colmbiana* (Lallemand) (Homoptera: Cercopidae) [Universidad del Valle, Cali].

List of Donors

Donor/Project	Project duration
Australia – ACIAR	
Anthracnose disease in *Stylosanthes*	1998-2000
Austria – Academy of Sciences	
Mechanisms of acid soil tolerance in *Brachiaria*	1994-1999
Colombia – Colombian Government	1999-2003
Grasses and legumes with high nutritive quality	
Grasses and legumes adapted to low fertility acid soils	
Grasses and legumes with adaptation drought, poor soil drainage and resistant to pest and disease	
Integration of improved grasses and legumes in production systems in savannas	
Colombia – PRONATTA	
Development of field screening methodology for spittlebug	1997-1999
Colombia – Agroganadera del Valle	
Seed multiplication of selected forage species	1998-1999
Colombia- FEDEGAN-Fondo Nacional del Ganado	
Brachiaria Network	1996-1999
Spittlebug bioecology	1996-1999
Development of molecular markers for spittlebug in *Brachiaria*	1998-1999
Colombia – NESTLE DE COLOMBIA	
Pilot Development Program – Caquetá	1995-1999
Germany – BMZ	
Desmodium genotype x environment	1995-1999
Great Britain – DFID	
Anti-quality factors in legumes (with IGER)	1996-1999
Leucaena research (Philippines and Central America) (with OFI)	1996-1999
Evaluation of *Calliandra* provenances (with OFI)	1997-1999
Japan – Ministry of Foreign Affairs-JIRCAS	
The role of endophytes in tropical grasses	1995-2000

LIST OF COLLABORATORS

Austria	R. Albert, University of Vienna E. Heberle-Bors, University of Vienna
Australia	J. E. Mayer, CAMBIA, Canberra Sukumar Chakraborty, CSIRO
Brazil	Cacilda B. Do Valle,, CNPGC/EMBRAPA Claudio Karia, EMBRAPA/CNPGC Ronaldo Andrade, EMBRAPA/CPAC Moacyr J. Sales, EMBRAPA/Floresta Joao A. Pereira, EMBRAPA/Floresta
China	Jiang Changshun, CATAS
Colombia	Fernando Suso, Agroganadera del Valle, Cali Raúl Pérez, CORPOICA-Villavicencio Alvaro Rincón, CORPOICA-Villavicencio Tito Díaz, CORPOICA-Tibaitatá Carlos Escobar, CORPOICA-Macagual Jaime Velásquez, CORPOICA-Macagual María Cristina Cárdona, CENICAFE, Chinchiná Senén Suárez, CENICAFE, Chinchiná Juan Carulla, Universidad Nacional de Colombia, Bogotá Edgar A. Cárdenas, Universidad Nacional de Colombia, Bogotá José Restrepo, FIDAR Antonio Pérez, Universidad de Sucre Gustavo Ruiz, Universidad de la Amazonía, Florencia Silvio Guzmán, Fundación Universitaria San Martín, Barranquilla Justo Barros, CORPOICA-Valledupar
Costa Rica	Carlos Jiménez, UCR Milton Villareal, ITCR Mohammed Ibrahim, CATIE Francisco Romero, ECAG Jesús González, ECAG Guillermo Pérez, CIAT Carlos Hidalgo, MAG Vidal Acuña, MAG Marco Lobo, MAG Francisco Morales MAG Jorge Mario Bolaños, MAG Argerie Cruz, MAG Horacio Chi Chan, MAG
Germany	Norbert Claassen, University of Goettingen, Goettingen Rainer Schultze-Kraft, University of Hohenheim Axel Schmidt, University of Hohenheim
Honduras	Conrado Burgos, DICTA Marlen Iveth Posas, SERTEDESO

Japan	M. Osaki, Hokkaido University, Sapporo T. Tadano, Hokkaido University, Sapporo Y. Saito, National Grassland Research Institute, Kitasaku, Nagano
México	Armando Peralta M., Agroproductos de Iguala, S.A. Francisco Javier Henríquez, INIFAP
Nicaragua	Tito Fariñas, Proyecto de Desarrollo Lechero-IDR José Angel Oporta, CNIA-INTA
Panamá	Bolívar Pinzón, IDIAP
Perú	Miguel Ara, IVITA, Pucallpa Jorge Vela, IIAP, Pucallpa Keneth Reátegui, DEPAAM, Pucallpa Geiner Romero, CIAT, Pucallpa Alfredo Riesco, CODESU, Pucallpa Deisy Lara, FUNDAAM, Moyobamba
South Africa	C. A. Pineda, National Accelerator Center, Faure
United Kingdom	Janet Stewart, OFI Alan Pottinger, OFI Mike Theodorou, IGER Philip Morris, IGER Emyr Owen, University of Reading
United States	M. Nair, Michigan State University, East Lansing, Michigan Jim White, Rutger University J. Porter, USDA/University of Georgia

NETWORKS

Brachiaria Evaluation in Colombia*

Barros Justo A., CORPOICA-CI Motilonia, Córdoba, Colombia
Camargo Maris Elena
Canchila Emiro, Universidad de la Paz
De La Torre Manuel, IVITA, Perú
Delgadillo Liliana, CORPOICA-CI-Carimagua, Meta, Colombia
García Heberth, Semillas 'La Florida', Ltda., Meta, Colombia
Guzmán Silvio, CORPOICA-Turipaná, Córdoba, Colombia (until July, 1998)
Hernández Blanca Hilda, CRECED-CORPOICA, Casanare, Colombia
Macías Luis Fernando, CORPOICA, Colombia
Mateus Herny, CRECED-Magdalena Medio, Santander, Colombiano
Montemiranda del V., Alberto, CRECED Norte de Bolivar-CORPOICA, Colombia
Morán Reynaldo, CORPOICA, Colombia
Pérez Raúl, C.I. La Libertad , Meta, Colombia
Ramírez Carlos Alberto, CRECED Magdalena Medio, Caldas, Colombia
Riesco Alfredo, CODESU, Perú
Rodríguez Roberto, CRECED-CORPOICA, Guaviare, Colombia
Soto Isaac, Semillas 'La Florida', Ltda., Meta, Colombia
Tobón Carlos Jaime, CORPOICA-C.I. Tulio Ospina, Colombia
Vela Jorge, IIAP, Perú
Velásquez, Jaime, CORPOICA-Macagual, Colombia

*It finished on October 1999.

Project Staff List

Senior Staff
Carlos E. Lascano, Ph. D., Forage Quality/Animal Scientist , Project Manager
César Cardona, Ph.D., Entomology
Segenet Kelemu, Ph.D., Plant Pathology
John W. Miles, Ph.D., Plant Breeding/Genetics
Francisco Morales, Ph.D., Virology
Idupulapati M. Rao, Ph.D., Plant Nutrition/Physiology

Research Fellow
Michael Peters, Ph.D., Forage Biology
Daniel Peck, Ph.D., Entomology/Ecology

Consultants IP5 and PE5
Pedro J. Argel M., Ph.D., Forage Agronomy (Stationed in San José, Costa Rica)

Biometrics Specialist
Gerardo Ramírez

Research Associates and Asistants
Patricia Avila, Animal Science
Nelmy Narváez, Animal Science
Gustavo Adolfo Ruiz, MV-Animal Science (until February, 1999)
Martha Lucía Escandón, Agronomy (until June 1999)
Camilo Plazas, MV-Animal Science (shared with PE5)
Guillermo Sotelo, Entomology
Clara Inés Giraldo, Phytopathology
Ana Cristina Bolaños, Entomology (until April 1999)
Ximena Patricia Bonilla, Phytopathology
Fernando Muñoz, Phytopathology
Anuar Morales, Entomology (until October 1999)
Heraldo Cruz Florez, Forage Germplasm and Participatory Methods (stationed in Yoro, Honduras)
Ulises Castro, Entomology (until December 1999)

Specialists
Aristipo Betancourt, Genetics
Ramiro García, Plant Nutrition
Alcibiades Ortega, Seed Multiplication
Belisario Hincapié, Programmer, Germplasm

Technicians
Rosalba Tobón, Entomology
Gustavo Ospinal, Forage Quality
José Reinaldo Pareja, Entomology
Gilberto Córdoba, Entomology
Alvaro Baena, Plant Pathology
Fernando Feijoo, Genetics
Elías Burgos, Agronomy
Humberto Franco, Seed Multiplication
William Mera, Entomology (Caquetá)
Daniel Vergara, Plant Nutrition/Genetics
Gustavo Segura, Plant Pathology
Darío H. Viveros, Phytopathology

Workers
Benilda García, Forage Quality
Orlando Trujillo Filigrana, Forage Quality
Angel Betancourt, Genetics
José Nelson Amaya, Genetics
Hernando Viveros Roso, Forage Evaluation
José Ever Aragón, Forage Evaluation
Harold Orlando Zúñiga, Forage Evaluation
Eduardo Quintero Figueroa, Forage Evaluation
Luis Alberto López, Plant Nutrition

Ph.D. Students
Rolando Barahona, IGER University of Reading, United Kingdom
Nelson Castañeda-Ortiz, Georg August of Goettingen, Germany

MSc. Students
Patricia Avila, Forage Quality, Universidad Nacional de Colombia, Palmira (finished in July 1999)
Nelmy Narváez, Forage Quality, Universidad Nacional de Colombia, Palmira
Lida I. Mancilla, Plant Nutrition, Universidad del Valle, Cali, Colombia
Changshun Jiang, Southern China University of Tropical Agriculture, CATAS, The Peoples Republic of China (until June 1999)

Visiting Research Fellows
Yuka Takayama, Tamagawa University, Tokyo, Japan

Pregraduate Thesis Students
Carolina Zuleta, Universidad del Tolima, Ibague, Colombia
Viviana Piso, Universidad del Cauca, Popayán, Colombia
Francisco López, Universidad del Valle, Cali, Colombia
Jairo Rodríguez, Universidad del Tolima, Ibagué, Colombia
Albeiro Salamanca, Universidad Nacional de Colombia (in collaboration with PE2 CIAT Project and Hohenheim University)
Elías Claros Trujillo, Universidad Nacional de Colombia, Palmira (in collaboration with SN3, PE2 and PE5 CIAT Projects)
Liliana Alvarez, Universidad de la Amazonía, Florencia, Caquéta, Colombia
Vanesa Marinez, Universidad de la Amazonía, Florencia, Caquetá, Colombia
Diana Alvarez, Universidad Nacional de Colombia, Palmira

Trainees
Luz Miryam Serrato, Universidad del Tolima, Ibagué, Colombia, Spittlebug fungal entomopathogens, (4 weeks)
Otoniel Pérez, CORPOICA-La Libertad, Villavicencio, Colombia, Spittlebug field evaluation methodologies (2 weeks)
Magnolia Cano, Agrosemilla, Medellín, Colombia,Spittlebug bioecology methodologies (4 weeks)
Jairo Rodríguez, Universidad del Tolima, Ibagué, Colombia, Spittlebug rearing methodologies (6 weeks)
Paula Andrea Toro Velásquez, Universidad de Antioquia, Medellín, Colombia, Forage evaluation with milking cows (5 months)
Eva Margarita Romero, Universidad Central de Venezuela, Maracay, Venezuela, Forage evaluation with milking cows (4 weeks)
Deisy Lara, FUNDAAM, Moyobamba, Perú, On-farm pasture evaluation in Costa Rica (2 weeks)
Hugo Cuadrado, CORPOICA, Colombia, On-farm pasture evaluation in Costa Rica (4 weeks)

Secretaries
Beatriz Arenas
Carmen Tchira
Eugenia Jiménez, Costa Rica
Julia Gómez Quintero