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PROJECT IP-5

Tropical grasses and legumes: Optimizing genetic diversity for multipurpose use

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

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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 detech 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 (SB-2). Selected grasses and legumes evaluated in production systems (PE-5) in collaboration with national partners (SN-2).

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

Project Logframe 1998

Narrative Summary	Verifiable Indicators	Means of Verification	Critical Assumptions
Project 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	Number of new cultivars of grasses and legumes released by NARS partners and used by farmers in different production systems by 2001	Statistics on income and natural resource conservation in smallholder livestock farms in LAC	Governments put in place policies to favor sustainable livestock/forage development in marginal areas occupied by small farmers
Project Purpose To identify superior gene pools of tropical grasses and legumes for sustainable agricultural systems in sub-humid and humid tropics	Genetic diversity in selected grasses and legumes species for key quality attributes, pest and diseases, environmental adaptation and utility to farmers defined by 2001	Statistics on adoption of new forage cultivars by farmers and on their impact on production and natural resource conservation	Donors and NARS support strong livestock/forage related research and development programs in tropical areas
Output 1. Grass and legume gene pools with known quality attributes	Selected grass and legume accessions with known quality and antinutrional attributes and animal production potential delivered to partners by 2000	Scientific publications, annual reports and postgraduate thesis	Continued availability of funds from Colombia and other donors and effective collaboration with CIAT's Projects, NARS and advanced research institutes
Suboutput 1.1 Role of antinutrional factors in grasses and legumes in digestion and metabolism of ruminant animals identified	**List of legume species with known quality attributes by 1999** **(1998)** • Papers with results of tannin research submitted for publication • List of selected shrub legumes characterized for types (chemical nature and molecular weights) of CT • Extent of CT degradation by rumen microorganisms • List of enzymes (fungal origin) affected by CT and nature of the effect • List of *Brachiaria* genotypes screened for the presence of saponins with hemolytic effects	• PhD thesis, scientific publications and annual report	Continued collaboration with IGER, UK
Suboutput 1.2 Defined environmental "niches" to grow herbaceous and shrub legumes with tannins as feed resources	**Soil quality and climatic parameters associated with quality attributes of selected legumes by 1999** **(1998)** • List of selected genotypes of *Desmodium* and *Arachis* to grow in different locations • Defined quality parameters of *Calliandra* provenances as affected by location	• Pre-graduate and PhD thesis, scientific publications, annual report • Seed of selected accessions of *Arachis* and *Desmodium*	Continued collaboration with OFI, UK and with the U. of Hohenheim, Germany

Narrative Summary	Verifiable Indicators	Means of Verification	Critical Assumptions
Suboutput 1.3 Identified synergism in quality parameters among contrasting forages	**Regression model to predict milk yield response fed forage based supplements based on MUN as a nutritional indicator by 1999** **(1998)** • Defined nitrogen deficiency indices associated with different forage mixtures • Established relationship between response in milk yield to forage based supplements and milk urea nitrogen (MUN)	• Pre-graduate thesis • Annual report	Continued availability of funds from Colombia and from TROPILECHE (ILRI, IDB)
Suboutput 1.4 Known quality and animal production potential of selected grasses, herbaceous and shrub legumes	**Level of milk yield increment with elite grass and legume species by 1999** **(1998)** • Established pastures (Quilichao) with selected ecotype and commercial cultivars of *Brachiaria* to assess milk yield • Defined level of milk yield increment of cows fed with legume-based supplements and grazing legume-based pastures	• Ms thesis • Annual report	Seed of selected *Brachiaria* ecotype is available
Output 2. Grass and legume gene pools with known diversity in host/parasite/ symbiont interactions	*Selected grasses* and legumes with known reaction to major pest an disease delivered to partners by 2000	Scientific publications, annual reports and postgraduate thesis	Continued availability of funds from Colombia and other donors and effective collaboration with other CIAT's Projects, NARS and advanced research institutes
Suboutput 2.1 Host-plant relationships, ecology and population dynamics of spittlebug understood	**Complete description of the variation in biology and abundance *of spittlebug* species in Colombia by 1999** **(1998)** • List of lowland spittlebug species and description of their biology and natural enemies correctly identified • Papers submitted for publication (population dynamics of spittlebug, *spittlebug rearing unit* and vibrational communication of spittlebug) • Published guide for studying the biology and ecology of grassland cercopids	• Papers submitted for publication • New project proposal on biological control of spittlebug to COLCIENCIAS • Technical guide on grassland cercopids • Annual report	• Additional funds to support Entomology Post Doc are found • CORPOICA delivers to CIAT funds from collaborative project funded by FEDEGAN • Funds form NESTLE are available to support spittlebug bioecology work in Caquetá
Suboutput 2.2 Spittlebug resistance in *Brachiaria* genotypes assessed and characterized	**Improved glasshouse and field screening procedures for assessing spittlebug resistance in *Brachiaria* genotypes by 1999** **(1998)** • Improved and less costly mass rearing technique for spittlebug • Defined spittlebug nymphal damage on susceptible and resistant *Brachiaria* genotypes • List of *Brachiaria* genotypes evaluated for spittlebug resistance under field conditions • Paper submitted for publication on methodologies for screening for spittlebug resistance	• Scientific publication on screening methodology for *spittlebug resistance* submitted • Annual report	• Special funds from PRONATA are available for development of field screening method for spittlebug resistance • Funds from NESTLE support screening *Brachiaria* in the field for spittlebug resistance • Additional funds from FEDEGAN are available to support work on antibiotic effects of *Brachiaria* on spittlebug

Narrative Summary	Verifiable Indicators	Means of Verification	Critical Assumptions
Suboutput 2.3 *Brachiaria* resistant genotypes to spittlebug identified and reconfirmed	**Genotypes of *Brachiaria* resistant to spittlebug under glasshouse and field conditions available for multisite evaluation by 2000** **(1998)** • Reconfirmed and new sources of resistance to spittlebug for the *Brachiaria* improvement program • At least 500 *Brachiaria* genotypes screened for spittlebug resistance in the glasshouse • List of *Brachiaria* genotypes evaluated for spittlebug resistance under field conditions	• List of *Brachiaria* genotypes with resistance to spittlebug • Annual report	Effective flow of *Brachiaria* genetic recombinants for screening for spittlebug resistance
Suboutput 2.4 Genetic control and molecular markers identified for spittlebug resistance and apomixis in *Brachiaria*	**Known potential to use marker assisted selection (MAS) for spittlebug resistance in *Brachiaria* by 2000** **(1998)** • 100 new *Brachiaria* hybrids • 100 marker loci mapped • 50 resistant sexual clones selected as parentals for 1999 cycle • Marker linked (< 2cM) to apomixis locus • Conserved genome sequence identified	• Annual report	• Special funding from FEDEGAN is available to develop molecular map of *Brachiaria* • Special funds from ACIAR are available for comparative mapping with pearl millet • Continued and effective collaboration with SB-2
Suboutput 2.5 Role of endophytes in tropical grasses elucidated	**Protocols for detection of endophytes and list of endophytes properly identified in tropical grasses by 1999** **(1998)** • List of new endophytes in *Brachiaria* • List of *Brachiaria* accessions examined for presence of endophytes using specific antisera • Defined degree of association between endophytes and spittlebug resistance in *Brachiaria* • Manuscripts submitted for publication on endophyte research	• Scientific publications submitted • Annual report	• Continued funding from Japan to support endophyte research • Effective collaboration with Rutgers University on endophyte taxonomy • Effective collaboration with the Entomologist in IP-5
Suboutput 2.6 Interactions between host and pathogens (bacteria, fungus, virus) identified for key forage species	**Number of isolates of *Colletotrichum* characterized and list of *Stylosanthes* and *Arachis* genotypes with durable resistance to anthracnose by 1999** **(1998)** • Number of characterized isolates of *Colletotrichum* from *Arachis pintoi* • Manuscript submitted for publication	• Scientific publications submitted • Annual report	Funds from ACIAR channeled through CSIRO for anthracnose research are made available to CIAT
Suboutput 2.7 Information on genetic diversity of *Brachiaria* and *Arachis* linked with biotic constraints	**Genetic characterization of *Brachiaria* and *Arachis* integrated into GIS by 1999** **(1998)** • Initial assessment of genetic diversity in *Brachiaria* and *Arachis* collections using isozyme data	• Annual report	• Funds are identified for isozyme data analysis in collaboration with SB-2 • Effective input from Biometrician in analysis of isozyme data
Output 3. Grass and legume gene pools with known adaptation to edaphic and	Selected grasses and legumes with known adaptation to acid infertile soils and diverse climatic conditions delivered to partners by 2000	Scientific publications, annual report, postgraduate thesis	Continued availability of funds from Colombia and other donors and effective collaboration with other

Narrative Summary	Verifiable Indicators	Means of Verification	Critical Assumptions
climatic constraints			CIAT's Projects, NARS and advanced research institutes
Suboutput 3.1 Genotypes of *Brachiaria*, *Panicum* and *Arachis* with adaptation to low soil fertility soils identified and characterized	**Methodology to screen forage grass and legumes for adaptation to low soil fertility and list of *Brachiaria* genetic recombinants, and *Arachis* accessions with superior adaptation to low soils fertility by 1999** **(1998)** • List of *Brachiaria* genotypes with adaptation to low fertility soils as measured in the greenhouse • Field trial established in the Llanos (Matazul) to evaluate edaphic adaptation of *Brachiaria* genotypes • Field experiment established in forest margins (Caquetá) to evaluate P accumulation and utilization in genotypes of *Arachis* • Established differences in nutrient acquisition by contrasting legumes in association with grasses under field conditions	• Scientific publications submitted • Annual report	• Continued collaboration with Hokkaido University, Japan • Completion of PhD thesis by P. Wenzl • Funds from NESTLE are available to work in Caquetá • Carry over funds from the JIRCAS Project are available to continue work on legume persistence in Carimagua
Suboutput 3.2 Genotypes of *Brachiaria*, *Arachis* and *Calliandra* with dry season tolerance identified and characterized	**List of characterized accessions of *Brachiaria, Arachis* and *Calliandra* with dry season tolerance by 1999** **(1998)** • Field trial established in the Llanos (Matazul) to evaluate dry season tolerance in *Brachiaria* • Established correlation between nonstructural CHO and ash content with dry season tolerance in *Brachiaria* • List of *Brachiaria*, *Arachis* and *Calliandra* ecotypes selected for superior dry season performance from regional trials	• Seed of selected accessions of Brachiaria, *Calliandra* and *Arachis* • Annual report	• Forage Biologist completes analysis of G x E trials with *Arachis* • Continue to have support from Forage Agronomist in Costa Rica • Seed of accessions of *Brachiaria* and *Arachis* is available to plant in the llanos
Suboutput 3.3 Genotypes of *Brachiaria* and *Paspalum* with adaptation to poorly drained soils identified and characterized	**List of characterized accessions of *Brachiaria* and *Paspalum* selected for poorly drained soils by 1999** **(1998)** • Seed of *Paspalum* accessions multiplied for regional testing	• Annual report	Continued support from Forage Agronomist in Costa Rica
Suboutput 3.4. Genotypes of shrub legume species with tolerance to cool temperatures identified and characterized	**List of characterized accessions of shrub legumes selected for tolerance to cool temperatures by 1999** **(1998)** • Field trials established in hillsides of Costa Rica and Honduras with new *Leucaena* species • List of shrub legumes selected for adaptation to mid altitude hillsides in Cauca	• Annual report	• Continued support from Forage Agronomist in Costa Rica • Effective collaboration with OFI, UK, MAG, Costa Rica
Suboutput 3.5 Defined genotype x environment interactions on performance of *Brachiaria*, *Arachis* and *Desmodium*	**List of characterized accessions of *Brachiaria* and *Arachis* for specific environments by 1999** **(1998)**	• Kilograms of seed of selected accessions of *Desmodium*, *Arachis* and *Brachiaria* harvested	• Funds from SENA requested by CORPOICA are available for on-farm evaluation of new *Brachiaria* ecotypes

Narrative Summary	Verifiable Indicators	Means of Verification	Critical Assumptions
	• Defined performance of *Brachiaria, Arachis* and *Desmodium* genotypes in different environments • Seed of new accessions of *Brachiaria* and *Desmodium* multiplied for on-farm evaluation	• Workshop proceedings on performance of *Desmodium* and *Brachiaria* in Colombia • Annual report	• Continued funding from FEDEGAN to support the *Brachiaria* Network in Colombia • Adequate climatic conditions to harvest seed of *Arachis* in Bolivia and of *Brachiaria* in Costa Rica and Colombia
Suboutput 3.6 Information on genetic diversity of *Brachiaria, Arachis, Desmodium* and selected shrub legumes linked with environmental adaptation	**Degree of correlation of isozyme characterization data with environmental conditions (soils and climate) by 2000** **(1998)** • Initial genetic diversity of *Brachiaria* and *Arachis* quantified through isozyme analysis	• Map showing "niches" to grow *Arachis* • Annual report	• Effective collaboration with SB-2 for isozyme data analysis • Effective input from Biometrician for analysis of isozyme data
Output 4. Superior and diverse grasses and legumes delivered to partners for evaluation	Number of grasses and legumes released by NARS and being adopted by farmers in different environments and production systems by 2000	Statistic on early adoption by farmers of new grasses and legumes	• Continued availability of funds from Colombia and other donors and effective collaboration with other CIAT's Projects, NARS and advanced research institutes
Suboutput 4.1 Release and deployment to farmers in different production systems of successful grass and legume cultivars through partnerships	**Number of grasses and legumes species released in Colombia and Central America by 2000** **(1998)** • Seed of promising accessions of *Brachiaria* and *Desmodium* multiplied for on-farm evaluation inColombia • One grass (*B. bryzantha* 26110), one herbaceous legume (*A. pintoi* 18744) and one shrub legume (*C. argentea*) included in on-farm trials in Costa Rica and Colombia	• Technical bulletins on grasses and legumes for release • Annual report	• Effective collaboration with NARS that form part of TROPILECHE • Continued financial support from NESTLÉ • New NARS partners interested in joining TROPILECHE
Suboutput 4.2 Defined "niches' for selected grass and legume cultivars based on analysis of G x E interactions	**List of grasses and legumes with well defined attributes suited for different agroecologies by 2000** **(1998)** • List of accessions of *Arachis, Desmodium* and *Brachiaria* selected on the basis of performance in multilocational trials	• Kilograms of seed of selected accessions available for distribution • Annual report	Effective collaboration with PE-4 forage data to GIS
Suboutput 4.3 Expert systems on forage biodiveristy by linking geographic information with biological data	**Forage Data base in CD-ROM for use by NARS by 1999** **(1998)** • Working group assembled to link forage data bases with GIS	• Diskettes with Forage Database • Annual report	Input from Database Specialist in CIAT
Suboutput 4.4 Effective communication of research results through newsletters, journals and workshops	**Number of institutions and individuals receiving/contributing to publications by 2000** **(1998)** • Three project Newsletters • Three volumes of Pasturas Tropicales	• Number of recipients of Newsletters • Number of published articles in Pasturas Tropicales • Program for Workshop on spittlebug	• Effective contributions of IP-5 members to the Newsletter • IP-5 members and NARS partners continue to submit papers for publication in Pasturas Tropicales

Narrative Summary	Verifiable Indicators	Means of Verification	Critical Assumptions
	• Workshop on spittlebug • Number of trained NARS partners		• Funds are identified to organize workshop on spittlebug
Inputs **Personnel** 3.5 SS/Y 1 SRF 1 PDF **Funding in 1998:** **US$1,319,041** Core (7%), Highly Restricted Core-Colombian Government (46%) and Special Projects (47%)	**Scientist Disciplines** 0.7 Ruminant Nutritionist and PM (SS) 1.0 Plant Breeder 1.0 Plant Pathologist 1.0 Plant Entomologist (PDF) 1.0 Forage Biologist (SRF) 0.5 Plant Entomologist (SS) 0.3 Plant Nutritionist (SS)	• Annual reports • Scientific publications from team members	• Continued funding from the Colombian Government and from core to pay for salaries of SS in the Project • Additional funding is obtained to support the Entomology PDF • Team is effective in attracting special funding to support operations

Research Highlights in 1998

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

Condensed tannins in tropical legumes

- Found differences in the ability of soluble and bound condensed tannins extracted from tropical legumes to inhibit fermentation by rumen microorganisms. In addition, legume species differed in tannin structure (proanthocyanidin units and molecular weights) and in the ability of CT to inhibit activity of enzymes from fungal origin that degrade protein and fiber.

Synergistic effects of improved forages

- Found a large response in milk yield with supplementation of shrub legumes in combination with sugarcane when level of crude protein in the grass only pasture was deficient and demonstrated that milk urea N (MUN) can be a valuable nutritional indicator for matching improved forages with nutritional requirements of milking cows.

Intake and animal production with improved forages

- Continued to show that in farms of the forest margins cows grazing *Arachis pintoi* based-pastures produce more milk (0.5 to 1 liter/d) than in grass only pastures, provided they have high nutritional genetic potential requirements and to respond to the improved nutrition.

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

Bioecology of spittlebug

- Field studies with spittlebug showed greater population synchrony and abundance fluctuations in seasonally dry areas compared to continuously humid and no role of drought as a preovipositional cue in egg diapause was detected.

- Spittlebug abundance (density) was found to be similar in *Brachiaria* pastures with and without *Arachis pintoi*, but insect load increases by 22% in the grass-legume pasture.

- Six classes of spittlebug natural enemies were found including a parasitic fly that may be the first neotropical report. A total of 19 isolates of entomopathogenic fungi have now been collected from grassland spittlebugs in Colombia.

Screening *Brachiaria* for spittlebug resistance

- The new greenhouse spittlebug screening technique developed in 1997 was fully implemented with excellent results and a new technique for screening for resistance under field conditions was developed.

- For the first time ever, simultaneous studies on the resistance of *Brachiaria* spp. genotypes to four species of spittlebug were conducted.

- Five hundred hybrid-derived sexual genotypes were screened for spittlebug resistance, resulting in eleven final selections . New *Brachiaria* hybrids with very high levels of resistance to the spittlebug *Aeneolamia varia* were also identified.

Molecular markers in *Brachiaria*

- Over 200 additional *Brachiaria* hybrids were produced to augment existing mapping population. A total of 100 maker loci are now mapped and isolation of microsatellites was initiated.

Endophytes in tropical grasses

- Additional endophytes were isolated in pure culture and PCR analysis showed that there were differences among isolates in species of *Brachiaria*

- Artificial inoculation methods to introduce endophytes into *Brachiaria* were implemented and assays with *Brachiaria* clones with or without endophytes showed that aphids preferred to feed on endophyte-free plants to endophyte-infected one and that endophytes were effective against certain fungal pathogens

Anthracnose in *Stylosanthes* and *Arachis*

- Determined pathogenic variability of 183 isolates of *Colletotrichum gloeosporioides* isolated from *Arachis pintoi*. Pathogenic specialization was observed and genetic diversity of 91 isolates was measured by RAPD analysis. There were at least five lineages of *C. gloeosporioides* in the isolates studied.

- Host range studies showed that isolates of *C. gloeosporioides* cross-infect *Stylosanthes* and *Arachis*.

- A chitinase- encoding gene cloned from rice was recloned in an appropriate vector for *Agrobacterium*-mediated transformation and an in vitro assay showed that crude preparations of the chitinase enzyme had antifugal activity against *C. gloeosporioides*

Genetic diversity in *Brachiaria* and *Arachis*

- The analysis of isozyme data in 411 *Brachiaria* spp. and 28 accessions of *Arachis pintoi* separated distinct genotypes.

Output 3: Development of forage gene pools with known adaptation to environmental constraints

Adaptation of grasses and legumes to low fertility acid soils

- A stepwise screening methodology based on physiological and biochemical plant mechanisms was developed to assess adaptation of *Brachiaria* genotypes to acid soil stress

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

- Selected *A. pintoi* CIAT 22259 for superior adaptation to low nutrient soil supply and persistence at two contrasting sites in savannas

Adaptation of grasses and legumes to drought

- The accession *Brachiaria brizantha* CIAT 26110 was found to have high production of fine root and to maintain a high proportion of root length to leaf area, which confers drought tolerance.

- The provenance *Calliandra calothyrsus* was selected CIAT 22310 due to good performance in the wet and dry seasons

- Found that low levels of shoot Ca and ash content combined with greater levels of total nonstructural carbohydrates in shoot tissue may serve as indicators of dry season tolerance in species of *Brachiaria* and *Arachis*.

Adaptation of grasses to poorly drained soils

- Found that *B. dictyoneura* cv. Llanero (CIAT 6133) had no symptoms of foliar or root fungal disease under waterlogged conditions, and that *B. brizantha* CIAT 26110 showed only mild symptoms of root fungal symptoms caused by *Pythium* sp. under similar conditions.

- Confirmed that within a small core collection of *Paspalum*, there are accession that perform well in poorly drained acid soils and seed of selected materials is now been multiplied for regional testing

Adaptation of shrub legumes to cool temperatures in hillsides

- The shrub *Rhynchosia schomburgkii* CIAT 19235 was selected due to outstanding vigor in poor soils in hillsides of Cauca.

- Recorded differences in DM yield, dry season performance and psyllid tolerance among new *Leucaena* species in hillsides of Costa Rica.

G x E interactions with grasses and legumes

- New *Brachiaria* (10) and *Desmodium ovalifolium* (4) ecotypes were selected from multilocational trials for seed multiplication and on-farm grazing trials in 1999.

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

Delivery of elite grasses and legume to partners

- Release by MAG (Ministerio de Agricultura y Ganadería) in Costa Rica of *Arachis pintoi* CIAT 18744 as cv. Porvenir for multipurpose use.

- New ecotypes of *Arachis pintoi* now under evaluation in farms of the forest margins of the amazon, Llanos and humid hillsides in Colombia

Targeting forage germplasm and linking forage databases to GIS

- Formation of a working group with participants form different CIAT Projects to link forage databases with GIS and of a multidisciplinary team for participatory evaluation and targeting of multipurpose forages in hillsides of Central America

Effective communication with partners

- Training workshops on spittlebug bioecology and management were held in Colombia and Ecuador with participation of 49 researchers from 10 institutions. A workshop with participants of the Brachiaria Network in Colombia was also held to present results and plan future work

- Continued to publish a Forage Newsletter and a Journal, both of which are reaching 350 forage researchers.

Progress towards achieving Output Milestones

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

- **List of legume species as feed resources with known quality attributes (1999)**

Results indicate that in tropical legumes the insoluble fraction of condensed tannins (CT) bound to fiber has a greater negative effect on forage digestibility than the soluble fraction, but that this later fraction can negatively affect protein degradation by rumen microorganisms. We have also shown that the biological activity of CT is different among legume species and between genotypes of the same species and that this is related to different proanthocyanidin ratios in the soluble tannin fraction and to different molecular weights.

Our increased understanding on the effects of soluble and bound CT on the nutritive values of a range of tropical legumes has allowed us to develop better screening methods to assess quality of selected legumes. These improved methods are currently being used to characterize quality attributes of a range of herbaceous and shrub legume species to define their potential feed value.

Further definition of the effect in ruminant animals of the different fractions of condensed tannins determined by laboratory procedures, will be required for refinement of improved screening procedures for quality evaluation of tropical legumes.

- **Defined soil quality and climatic parameters associated with quality attributes of selected legume species (1999)**

We have defined how environmental factors such as soil fertility and rainfall, affect quality of *Desmodium ovalifolium*. This information will be valuable for targeting to specific niches other legume species with high tannin contents. The assessment of how soil physical parameters affect yield and quality of *D. ovalifolium* genotypes will also improve our ability to identify environmental niches to grow *Desmodium* and other legume species with tannins as feed resources.

With the results from the work on G x E, we were able to select four new *Desmodium ovalifolium* genotypes with wide adaptation and good forage quality, which will be tested in on-farm grazing trials next year.

A productive and high quality *D. ovalifolium* cultivar would represent a significant advancement for developing grass/legume pasture for the humid tropics where the only persistent herbaceous legume options at present is *A. pintoi*. Advantages of *D. Ovalifolium* over *A. pintoi* are that it is better adapted to conditions of low soil fertility and much easier and cheaper to establish.

- **Nutritional indicators to predict milk yield response to forage-based supplements (1999)**

We have a better guidelines on how to maximize the nutritional benefits of introduced shrub legumes as protein supplements for dairy cows in dual-purpose cattle systems. Our results from last year indicated that level and frequency of legume supplementation affected the utilization of nutrients in sheep fed low quality grasses. Results from this year indicate that there is a response in milk yield with supplementation of shrub legumes in combination with

sugar cane, when the grass available in the pasture is limiting in quantity and quality, particularly protein, and when cows have the genetic potential to respond.

We also have results that indicate that the use of nutritional markers such a Milk Urea Nitrogen (MUN) can be a helpful tool for matching feed resources with nutritional requirements of dairy cows. What is lacking now is the validation of these results in on-farm trials, which is an objective being pursued in collaboration with the Tropileche Consortium housed in PE-5.

Our immediate challenge is to develop a fast and reliable laboratory in vitro method that allows us to evaluate the synergistic effect of different forage combinations in terms of fermentation parameters. Steps on this direction will be made in 1999.

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

- **Complete description of the variation in biology and abundance of spittlebug species in Colombia (1999)**

Given the known variation in spittlebug bioecology, taxonomic diversity and pasture management systems, it is clear that the pest's impact will vary considerably across its range. A 2-year project near completion has been a "rapid bioecological assessment" of Colombia's lowland grassland spittlebugs to describe this variation and open up avenues for advances in management of the pest. Significant amount of new basic information on bioecology of spittlebug has been acquired over this period. The North Coast of Colombia and the Amazonian Piedmont have rapidly emerged as benchmark sites for spittlebug management, representing 2 distinct ecosystems.

First time information on species biology and distribution, population dynamics and ecology of spittlebug serve as the foundation for present and future studies on management strategies and the ultimate establishment of an integrated pest management program. As the most important insect pest in the most extensive agricultural activity in the Americas, grassland spittlebugs merit a widening of research efforts focused on the IMP tools upon which holistic management depends. The next steps should included studies to quantify impact, monitoring programs, diapause studies and additional studies on the basic biology of this diverse group.

- **Improved glasshouse and field screening procedures for assessing spittlebug resistance in Brachiaria genotypes (1999)**

Major advances have been achieved in capacity and reliability of screening for spittlebug resistance. In 1998, a total throughput of over 1,200 genotypes, including germplasm accessions and recombinants from the *Brachiaria* breeding project was achieved. This is nearly six times the capacity of previously available screening methodology and the full potential of the new methodology has yet to be reached.

It is worthy of note that a new standard of host plant resistance is now recognized. *B. brizantha* CIAT 6294 (cv. Marandú) was included as a resistant check in all early screenings. For the past year, a hybrid-derived clone (BR93NO/1371) has been adopted as standard resistant check. This recombinant consistently shows an even greater degree of antibiotic resistance than cv. Marandú.

Important questions remain, that we will have to address in the near future. There is need to continue and expand the studies on the resistance of *Brachiaria* spp. to several other spittlebug

species different from *A. varia*. If we confirm preliminary results which suggested that the mechanism of resistance to *Zulia* spp. is different from that responsible for resistance to *A. varia*, changes in breeding strategies will have to be made in order to incorporate resistance to other major insect species.

As in previous occasions, we would like to insist on the need to allocate funds to study the biochemical basis of resistance to spittlebug as a key element in the understanding of resistance and in the possible development of simple biochemical markers for selection.

- **Genotypes of *Brachiaria* resistant to spittlebug under glasshouse and field conditions available for multilocational evaluation (2000)**

During 1997 and 1998 a population of over 3,000 individual has been evaluated in two field trials (Carimagua and Caquetá) and 500 pre-selections assessed for spittlebug resistance under artificial infestation. Eleven tetraploid sexual clones with spittlebug resistance were selected and these are being recombined to produce a new cycle population. New sources of resistance in germplasm accessions have also been identified, and these are being incorporated in breeding populations

With the new greenhouse and field screening methodologies and very rigorous selection, it ought to be possible to achieve high mean levels of resistance in a random-mating sexual population. Crosses of elite apomictic genotypes to this population will then generate large numbers of superior apomictic segregants to be tested for commercial release.

Protocols for detection of endophytes and list of endophytes properly identified in tropical grasses (1999)

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We now have protocols to detect endophytes in tropical grasses and in collaboration with the US-based Company BioWorld, specific antisera were developed for detection of endophytes in *Brachiaria*. In collaboration with Rutgers University, the taxonomy of the endophytes of *Brachiaria* was determined in relation to other endophytic fungi. RAPD analysis showed that isolates of endophytes from species of *Brachiaria* were variable

We have also made progress in defining the potential benefits of endophytes. An effective artificial inoculation method was implemented for use in *Brachiaria* and endophyte-free *Brachiaria* clones were generated using systemic fungicides. These clones along with clones containing endophytes are being tested for drought, insect and pathogen tolerance. The beneficial effects of endophytes on a fungal pathogen and aphids were determined.

- **Number of isolates of *Colletotrichum* characterized and list of *Stylosanthes* genotypes with variable resistance to anthracnose (1999)**

A comprehensive study on genetic and pathogenic variability among several isolates of *C. gloeosporioides* was conducted and the results are useful for breeding for anthracnose resistance in *Stylosanthes*. Host range studies also showed that isolates of *C. gloeosporioides* cross infect *Stylosanthes* and *Arachis*, which poses potential risk on forage *Arachis*.

Progress has also been made in advancing anthracnose mangement methods through genetic engineering. A chitanase- encoding gene cloned from rice and recloned in a vector for *Agrobacterium*-mediated transformation, showed in vitro fungal activity against anthracnose.

Output 3: Development of forage gene pools with known adaptation to environmental constraints

- **Improved methodology to screen forage grasses and legumes for adaptation to low fertility soils (2000).**

We were successful in developing a screening method to evaluate aluminum tolerance in *Brachiaria*. This method uses relative root elongation as a simple measure to identify aluminum sensitive genotypes. But this method so far has worked with seedlings developed from seed. We need to adapt this method for vegetative stem cuttings so that we can evaluate large numbers of *Brachiaria* hybrids for their aluminum tolerance.

One major problem in adapting the screening method to stem cuttings is the variability among cuttings in terms of nutrient status that could interact with the degree of aluminum tolerance. In order to reduce variability, we need to use a large number (at least 30) of replicates for each treatment. We will also try to test this method to determine genotypic differences in tolerance to aluminum stress in *Arachis pintoi*.

- **List of characterized accessions of grasses and legumes with tolerance to dry season (1999)**

We identified a provenance of *Calliandra calothyrsus* with good agronomic performance and with drought tolerance. In addition, field evaluation in the better soils found in Atenas, Costa Rica allowed us to identify a number of promising accessions of *Brachiaria* and *Arachis* with tolerance to dry season. We have also made advances in establishing fertilizer requirements for the establishment of *Cratylia argentea*, which is well know for its drought tolerance.

Atenas in Costa Rica appears to be a good site for evaluation of dry season tolerance of forages. This is mainly because it is a subhumid tropical hillside located at 2000 masl, 1600 mm annual rainfall with a long dry season (5 to 6 months) and a mean temperature of 23.7 C. Soils at this site are inceptisols with medium level of fertility. The other sites used (Carimagua and Quilichao) for dry season evaluation, , may not be suitable due to the interaction of low soil fertility with dry season and limited intensity of water stress. Thus we intend to continue our efforts at Atenas site to select grasses and legumes with dry season tolerance and to carry out detailed studies to identify plant attributes that confer tolerance to dry season.

- **List of characterized shrub legumes for tolerance to cool temperatures in mid altitude hillsides (1999)**

Work in hillsides of Cauca resulted in the identification of a range of shrub legumes species with adaptation to poor soils and to cool temperatures. The most promising species are *Rhynchosia schomburgkii*, *Calliandra* sp., *Calliandra houstonia, Leucaena diversifolia, Pueraria wallichii* and *Flemingia macrophylla*. However, for most of these species we still need to define their feeding value and effects on the soil.

The multilocational evaluation of *Leucaena* has allowed us to make progress in identifying new *Leucaena* species tolerant to psyllid and with desirable agronomic attributes. Interactions in performance between *Leucaena* species and site are also being documented. As a result we will be able to better target *Leucaena* species to different environments including mid altitude hillsides with variable rainfall.

- **List of characterized accessions of *Brachiaria* and *Paspalum* for poorly drained soils (1999)**

We made progress toward identifying species of *Brachiaria* with adaptation to poorly drained soils and to diseases associated with waterlogging. The mechanisms responsible for plant infection with fungi are not well understood, but the intraspecific variation within *Brachiaria,* has allowed us to advance with new ecotypes tolerant to soil fungal diseases and with desirable agronomic attributes. In addition, we reconfirmed that among the *Paspalum* collection obtained for EMBRAPA, Brazil there are species well adapted to poorly drained soils. Seed of these lines is being multiplied for wider testing.

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

- **List of grass and legume cultivars released by NARS in the region (2000)**

Our ongoing collaboration on forage evaluation with different partners in the region continues to to be an effective way to catalyze releases of key forage species. Through the *Brachiaria* Network in Colombia we were able to identify ecotypes with wide adaptation to different environments and with agronomic attributes superior to commercial cultivars. Availability in 1999 of basic seed of the selected *Brachiaria* ecotypes will allow our Colombian partners to test them under grazing in livestock farms, which is a key step in the process leading to release of new forage cultivars.

We have made significant progress in the on-farm evaluation of new *Arachis pintoi* ecotypes in subhumid hillsides and forest margins. In Costa Rica, *A. pintoi* CIAT 18744 will be released this year by MAG (Ministerio de Agricultura y Ganaderia) as cv. Porvenir. This release will be accompanied by a Technical Bulletin and by basic seed multiplied by SEFO –SAM in Bolivia under a contract with CIAT. In forest margins of Colombia three new ecotypes of *A. pintoi* are under on-farm evaluation in low fertility soils and work is underway to determine P requirements for establishment.

Considerable advances have been made in demonstrating the benefits of *Cratylia argentea* as a dry season supplement for milking cows in dual-purpose cattle farms in Costa Rica. As result, some commercial seed producers are now multiplying seed to satisfy an increasing demand of this legume by farmers. We expect that this demand will also increase in Honduras and Nicaragua given that farmers participating in Tropileche in these countries are evaluating *C. argentea.* Results from on-farm work will be key in the decision by countries in Central America to release *Cratylia* in *the* near future.

- **Integrated forage data bases with GIS in friendly user format (1999)**

A working group was formed to assist in the integration of forage databases with GIS and progress was made in defining a conceptual framework and in transforming the forage databases into formats suitable for GIS analysis Progress also was made in putting a large forage database in a more friendly user format.

A refined GIS model to match genetic diversity and sites of origin will be available for use by scientists by early 1999. The validity of the model could be tested with comparative studies of genetic biodiversity of selected forage species(i.*e. Arachis*) using molecular markers. However, these studies will require additional funds and human resources for the molecular studies.

Initial contacts have been made with possible collaborators in and outside CIAT to investigate possibilities to carry out this important work

- **Institutions and individuals receiving/contributing to forage related publications (2000)**

Trough Workshops, Forages Newsletter and Pasturas Tropicales we are reaching a wide audience of forage researchers in LAC. Both publication 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 we face is to continue publishing high quality original research papers and most important to secure alternative funds to maintain or expand the number of subscribers, which is not an easy task.

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

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

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

Over the past three years we have collaborated with IGER (Institute of Grassland and Environmental Research) in the UK through a project (Amelioration of antinutritional factors in Tropical Legumes) funded by DFID (Department for International Development) and carried out by a Ph.D student (R. Barahona) in the U. of Reading. In addition, during 1997 we initiated collaboration with OFI (Oxford Forestry Institute) in the UK through a project to investigate factors affecting the nutritive value of *Calliandra calothyrsus* as a browse legume for ruminants, which is also funded by DFID. To carry out the research, a range of contrasting legume species (*Desmodium ovalifolium, Flemingia macrophylla, Leucaena leucocephala, L. pallida, L. macrophylla, Calliandra calothyrsus, Clitoria fairchildiana* and *Cratylia argentea*) have been included in vitro experiments to study the effect of CT and cell wall composition on forage quality.

Previous results had show differences among legume species in: a) proportion of soluble and bound tannins, b) extent of digestion and end products of fermentation by rumen microorganisms, and c) condensed tannin (proanthocyanidins) types as indicated by different ratios of cyanidin, delphinidin, pelargadinin and fisetinidin. In addition, in 1997 we reported that condensed tannins bound to the forage had a greater effect on digestion than soluble condensed tannins. However, our results also indicated that differences in quality among tropical legume species were not only related to tannins but also to potential degradability of cell wall constituents.

During 1998 we investigated the effect of bound and soluble tannins on the fermentation of D-glucose and grass cells. In addition, we determined types and of molecular weights of condensed tannins extracted from selected legume species and defined the effect of tannins from different sources on activity of enzymes of fungal origin.

Relative nutritional effects of bound and condensed tannins from tropical legumes on fermentation of glucose and plant cells (R. Barahona, M. Theodorou, P. Morris and C.E. Lascano)

Highlight

- Large differences in the ability of soluble and bound condensed tannins from tropical legumes to inhibit fermentation by rumen microorganisms.

Rationale: Condensed tannins (CT) can be found in two fractions in plant tissue: a) as soluble (easily extractable by aqueous organic solvents) and b) as bound (residue remaining after extraction with organic solvent) to cell protein and/or carbohydrates. Previous results (AR-97) had shown that by far bound CT were more effective in inhibiting fermentation by rumen

microorganisms than soluble CT. As a follow-up to results reported in 1997, additional experiments were carried out in 1998 to compare the relative nutritional effects of soluble and bound condensed tannins from 6 different legumes on fermentation of D-glucose and plant cells.

Methods: Tannins extracted from immature and mature leaves of *Desmodium ovalifolium, Leucaena leucocephala, L. pallida, Calliandra calothyrsus and Clitoria fairchildiana.* were included in two experiments using the pressure transducer technique. The experiments were:

a) *Soluble CT and fermentation of D-glucose*: Different CT were dissolved in a 50% aqueous methanol solution and added to triplicate serum bottles to give a final concentration of 1400g of tannins/Kg of D-glucose. The inoculated bottles were incubated with rumen microorganisms at 39°C for 48-h. Gas accumulation profiles and concentrations of volatile fatty acids (VFA) were used as response variables.

b) *Bound CT and fermentation of grass cells.* Tannin-grass cells (*Festuca arundinacea*) were prepared as described in the AR-97, but the level of tannins bound to the plants was 3% and not 6% as used previously. Triplicate samples of the tannin-plant cell complex were incubated with rumen microorganisms at 39°C and at the of 48- h gas production and VFA were measured.

Results: The addition of soluble CT to a media with the D-glucose resulted in at least a 20% reduction of gas production by rumen microorganisms when compared to the control (Table 1). The maximum reduction of gas accumulation (up to 39% of control) was observed with CT from immature leaves of *C. fairchildiana, L. pallida* and *C .calothyrsus.* On the other hand, the addition of CT from *L. leucocephala* resulted in the lowest reduction (20%) of gas production relative to the control. Results also showed that soluble CT from immature leaves of *C. fairchildiana, F. macrophylla* and *L. pallida* were more effective in inhibiting fermentation of D-glucose by rumen microorganisms than CT from mature leaves (Table 1).

Table 1. Effect of different types of condensed tannins (CT) extracted from immature and mature leaves of tropical legumes on the fermentation of glucose and *Festuca arundinacea* plant cells by rumen microorganisms.

	Gas accumulated at 48 h, ml	
CT source	Extractable CT and glucose[1]	Bound CT and plant cells[2]
Immature *D. ovalifolium*	460.5 ^{defg}	200.9 ^{d}
Mature *D. ovalifolium*	458.3 ^{defg}	192.3 ^{d}
Immature *F. macrophylla*	452.8 ^{efg}	142.7 ^{f}
Mature *F. macrophylla*	516.3 ^{bc}	150.5 ^{e}
Immature *L. leucocephala*	536.7 ^{b}	219.3 ^{bc}
Mature *L. leucocephala*	531.7 ^{bc}	225.8 ^{ab}
Immature *L. pallida*	418.3 ^{g}	164.6 ^{e}
Mature *L. pallida*	504.2 ^{bcd}	165.8 ^{e}
Immature *C. calothyrsus*	413.4 ^{fg}	208.2 ^{cd}
Mature *C. calothyrsus*	468.6 ^{def}	196.9 ^{d}
Immature *C. fairchildiana*	429.3 ^{g}	200.7 ^{d}
Mature *C. fairchildiana*	485.0 ^{cde}	200.0 ^{d}
Control	677.1 ^{a}	240.3 ^{a}

[1] Effect of extractable CT from different sources on the fermentation of D-glucose by rumen microorganisms. Extractable CT were added in concentrations of 1400 g of CT per kg of D-glucose.

[2] Effect of bound CT from different sources on the fermentation of *Festuca arundinacea* plant cells by rumen microorganisms. Bound CT were added in concentrations of 30 g of CT per kg of plant cells.

^{a,b,c,d,e,f,g} Means within a column without common superscript letters differ ($P<0.05$).

The addition of CT to plant cells also resulted in reduction in gas production relative to the control, as shown in Table 1. However, we observed great variability in the magnitude of the reduction in gas production relative to the control, which ranged from 6 to 40%.The highest reduction in gas production was observed with CT from *F. macrophylla*, whereas the lowest was observed with CT from *L. leucocephala*. Contrary to the case of soluble CT, plant maturity did not have a significant influence on the ability of CT to inhibit fermentation of plant cells. The exception was CT from immature leaves *F. macrophylla*, which had a greater inhibitory effect on fermentation of plant cells as compared to CT from mature leaves (Table 1).

Volatile fatty acids produced during fermentation of D-glucose were also affected by the addition of soluble CT, the exception being valerate (Table 2). Reduction of total VFA concentration ranged between 11 and 23% relative to the control with no CT. The highest concentration of total VFA was observed with *L. leucocephala*, although differences among legumes were not statistically significant. With 4 of the 6 legumes included in the experiment, the higher depression on VFA production was observed with CT from immature leaves. Examination of individual VFA fractions showed that iso-butyrate and iso-valerate were reduced the most and that acetate and propionate were reduced the least in the fermentation of D-glucose with addition of soluble CT (Table 2).

Table 2. Volatile fatty acid (VFA) concentration after fermentation of D-glucose by rumen microorganisms as affected by the presence of soluble condensed tannins (CT; 1400 g per kg of D-glucose) extracted from mature and immature leaves of six tropical legumes.

CT source	mMoles per litre[1]						
	Total VFA	Straight chain VFA				Branched chain VFA	
		Acetate	Propionate	Butyrate	Valerate	Iso-butyrate	Iso-valerate
Immature *D. ovalifolium*	466.4 ^{c}	329.6 ^{d}	87.6 ^{b}	37.0 ^{abc}	1.6 ^{ab}	1.6 ^{d}	8.6 ^{bc}
Mature *D. ovalifolium*	528.4 ^{bc}	376.0 ^{abc}	100.8 ^{b}	37.0 ^{abc}	0.0 ^{b}	6.8 ^{ab}	8.0 ^{bc}
Immature *F. macrophylla*	486.0 ^{bc}	341.6 ^{bcd}	93.6 ^{b}	36.0 ^{abc}	2.5 ^{ab}	3.6 ^{bcd}	8.8 ^{bc}
Mature *F. macrophylla*	518.0 ^{bc}	368.8 ^{bcd}	100.8 ^{b}	36.3 ^{abc}	0.0 ^{b}	3.0 ^{cd}	9.4 ^{bc}
Immature *L. leucocephala*	539.6 ^{b}	384.0 ^{ab}	104.0 ^{b}	38.4 ^{ab}	0.0 ^{b}	3.6 ^{bcd}	9.8 ^{bc}
Mature *L. leucocephala*	527.2 ^{bc}	366.0 ^{bcd}	102.4 ^{b}	37.0 ^{abc}	5.3 ^{a}	5.7 ^{abc}	11.2 ^{b}
Immature *L. pallida*	486.8 ^{bc}	346.8 ^{bcd}	96.8 ^{b}	34.4 ^{bc}	1.4 ^{ab}	1.1 ^{d}	6.2 ^{bc}
Mature *L. pallida*	524.8 ^{bc}	376.8 ^{abc}	100.4 ^{b}	35.7 ^{bc}	0.8 ^{ab}	4.0 ^{bcd}	7.4 ^{bc}
Immature *C. calothyrsus*	475.6 ^{bc}	342.0 ^{bcd}	98.0 ^{b}	27.6 ^{c}	0.0 ^{b}	2.6 ^{cd}	4.9 ^{c}
Mature *C. calothyrsus*	472.0 ^{bc}	332.2 ^{cd}	94.0 ^{b}	34.6 ^{bc}	0.0 ^{b}	2.6 ^{cd}	7.7 ^{bc}
Immature *C. fairchildiana*	482.0 ^{bc}	344.0 ^{bcd}	94.4 ^{b}	34.9 ^{bc}	1.0 ^{ab}	0.8 ^{d}	7.1 ^{bc}
Mature *C. fairchildiana*	520.8 ^{bc}	365.6 ^{bcd}	103.2 ^{b}	38.8 ^{ab}	0.0 ^{b}	4.0 ^{bcd}	9.3 ^{bc}
Control	604.0 ^{a}	412.0 ^{a}	119.6 ^{a}	45.9 ^{a}	0.9 ^{ab}	8.4 ^{a}	17.0 ^{a}

[1]After 48 h of incubation, values corrected for blank (containing only fermentation media and rumen fluid).

^{a,b,c,d} Means within a column without common superscript letters differ ($P<0.05$).

In the fermentation of plant cells with addition of CT we also observed a reduction of total VFA production ranging from 5 to 55% relative to the control (Table 3). The highest reduction in concentration of total VFA was with CT from *F. macrophylla*, whereas the lowest reduction was recorded with CT from *L .leucocephala*. Maturity of legume leaves from which CT were extracted to bind to plant cells had no measurable effect on VFA production (Table 3).

Discussion: Results indicate that soluble and bound CT from different legumes can have different consequences on animal nutrition. Analysis of gas accumulation data confirm previous findings (AR-97) that indicated that bound CT are more effective in inhibiting fermentation of substrates than soluble or extractable CT. Much lower bound CT-substrate (plant cells) ratios were required to obtain comparable levels of inhibition (reduction of

accumulated gas) than those required with soluble CT to inhibit fermentation of substrate (D-glucose).

Table 3. Volatile fatty acid (VFA) concentration after fermentation of plant cells from *Festuca arundinacea* by rumen microorganisms as affected by the presence of bound condensed tannins (CT; 30 g per kg of plant cells) extracted from mature and immature leaves of six tropical legumes.

CT source	mMoles per litre[1]						
	Total VFA	Straight chain VFA				Branched chain VFA	
		Acetate	Propionate	Butyrate	Valerate	Iso-butyrate	Iso-valerate
Immature *D. ovalifolium*	80.8 ^{abc}	39.3 ^{ab}	28.3 ^{ab}	10.3 ^{abc}	1.32	0.0	1.6 ^{b}
Mature *D. ovalifolium*	71.4 ^{bc}	36.5 ^{ab}	25.4 ^{ab}	8.9 ^{abc}	0.00	0.0	0.6 ^{b}
Immature *F. macrophylla*	59.5 ^{c}	33.1 ^{b}	21.0 ^{b}	5.3 ^{c}	0.00	0.0	0.1 ^{b}
Mature *F. macrophylla*	83.7 ^{abc}	45.5 ^{ab}	27.8 ^{ab}	9.1 ^{abc}	0.00	0.0	1.3 ^{b}
Immature *L. leucocephala*	128.9 ^{ab}	56.5 ^{ab}	38.9 ^{a}	13.8 ^{ab}	0.41	0.0	1.9 ^{b}
Mature *L. leucocephala*	109.2 ^{abc}	59.4 ^{ab}	35.4 ^{ab}	12.7 ^{ab}	0.35	0.0	1.4 ^{b}
Immature *L. pallida*	83.2 ^{abc}	47.3 ^{ab}	26.2 ^{ab}	8.5 ^{bc}	0.00	0.0	1.2 ^{b}
Mature *L. pallida*	77.5 ^{abc}	43.2 ^{ab}	25.4 ^{ab}	7.8 ^{bc}	0.00	0.0	1.1 ^{b}
Immature *C. calothyrsus*	117.7 ^{abc}	68.1 ^{ab}	34.1 ^{ab}	11.7 ^{ab}	0.46	0.0	1.7 ^{b}
Mature *C. calothyrsus*	105.8 ^{abc}	59.1 ^{ab}	32.9 ^{ab}	11.9 ^{ab}	0.00	0.0	1.5 ^{b}
Immature *C. fairchildiana*	83.3 ^{abc}	46.1 ^{ab}	27.1 ^{ab}	8.9 ^{abc}	0.00	0.0	1.2 ^{b}
Mature *C. fairchildiana*	78.6 ^{abc}	41.8 ^{ab}	26.3 ^{ab}	8.8 ^{abc}	0.39	0.0	1.3 ^{b}
Control	134.6 ^{a}	71.0 ^{a}	42.1 ^{a}	14.6 ^{a}	1.4	0.0	3.9 ^{a}

[1]After 48 h of incubation, values corrected for blank (containing only fermentation media and rumen fluid).

^{a,b,c} Means within a column without common superscript letters differ ($P<0.05$).

Soluble and bound CT also had different effects on VFA concentration at the end of the fermentation period .The fraction most affected by the presence of soluble CT were the branched (iso-butyrate and iso-valerate) VFA's. However, the greatest reduction in VFA's concentration due to presence of bound CT was with straight chain group (acetate, propionate and butyrate).

Given that branched chain VFA's arise mainly from degradation of plant amino acids (i.e. valine and leucine) by rumen microorganisms, our results support the hypothesis that soluble CT have their largest effect on protein degradability by rumen microorganisms due to the formation of indigestible complexes. On the other hand, the significant reduction of VFA concentration after fermentation of plant cells bound to CT support the idea that bound CT mainly restrict fiber digestibility.

Great differences were also observed in the ability of CT from different tropical legumes to inhibit fermentation of D-glucose and plant cells. Without exception, the lowest inhibition of fermentation of D-glucose and plant cells was observed with CT from *L. leucocephala*. In contrast, the CT that produced the highest inhibition of fermentation of D-glucose and plant cells were those extracted from *F. macrophylla, L. pallida* and *C. fairchildiana*. These differences in ability of CT form different legume species could be related to a number of factors including molecular weight as will be discussed in another section of this report.

Quality attributes in provenance of *Calliandra calothyrsus* (A. Issac, N. Narvaez, J. Stewart and C.E. Lascano)

Highlight

- Difference between provenance of *Calliandra calothyrsus* in proanthocyanidin units, which is related to tannin biological activity or ability to bind proteins.

Rationale: *Calliandra calothyrsus* is a tree legume native in Mexico and Central America which is of interest throughout the humid and subhumid tropics owing to its fast growth and its tolerance of soils with low pH. However, reports of its feeding values vary widely apparently due to interactions of post-harvest management of the forage with condensed tannins. Several workers have reported a large reduction in digestibility and intake when the forage is fed dried as compared to fresh. The mechanism of this change is probably related to changes in polymerization of tannins present. On the other hand, very little is known about the environmental effects on *Calliandra* quality. A collaborative OFI/CIAT project is underway since 1996 to study the effect of provenance, environment and management on quality of *Calliandra* forage. In the AR-97 we highlighted differences in tannin level and types between provenance of *C. calothyrsus* grown in sites with contrasting soil fertility. However, we subsequently found some problems with the identification of provenance in the field and decide to repeat the analysis.

Methods: Two provenances from Guatemala (Patulul-CIAT 22316) and Nicaragua (San Ramon-CIAT 22310) of *Calliandra calothyrsus* selected in OFI on the basis of different forage quality (i.e. digestibility and tannins) were sampled in Palmira and Quilichao for forage quality. Samples of immature and mature edible material from each provenance harvested at the two sites were freeze dried and analyzed for IVDMD, CP, fiber (NDF and ADF), and level and types of CT present. In addition, ratios of prodelfinidin: procianidin: propelargonidin were analyzed in purified CT using HPLC. Biological activity (astringency) of purified CT of the two provenance was determined with radial diffusion assay, which is based on the ability of tannins to bind to BSA (protein) placed in a petri dish.

Results: Large differences in quality were observed between provenance grown at the two sites, but no interaction of provenance x site was observed for quality parameters measured (Table 4). The provenance CIAT 22316 had higher IVDMD, less fiber (NFD and AFD), less bound CT, but more soluble CT than CIAT 22310. In addition, results showed that forage harvested in Palmira had more CP and was more digestible than forage harvested in Quilichao, even though fiber content and soluble CT level did not change with site. However, the level of bound CT was higher in forage harvested in Quilichao. As expected, mature forage was less digestible and had less CP than immature forage (results not shown). Reduced IVDMD due to maturity was accompanied by increased fiber content, particularly AFD (cellulose and lignin). Immature forage had more soluble CT than mature forage, which was not the case for bound CT.

Of special interest were the types of CT (proanthocyanidin units) present in the two *C. calothyrsus* provenance under evaluation. Results in Table 5 show that purified CT in CIAT 22310 were higher in delfinidin relative to cyanidin and pelargonidin units. In contrast, the CT purified from CIAT 22316 had more cyanidin relative to delfinidin and pelargonidin units. Contrary to what was reported in 1997, the units forming the CT present in the two *Calliandra* provenance did not change with site. On the other hand, astringency (ability of CT to bind protein) from the two provenance was 18% higher in CIAT 22310 (1.3 g of protein bound /g of CT) than in CIAT 22316 (1.1 g of protein bound/g of CT).

Table 4. Quality parameters in edible material of two provenance of *Calliandra calothyrsus* grown at to sites (values are average of mature and immature material).

Provenance	Quality Parameters					
	CP	IVDMD	NDF	ADF	SCT[1]	BCT[2]
			Palmira site			
San Ramón (22310)	17.6	32.8	28.4	23.3	14.4	10.3
Patulul (22316)	18.0	45.1	17.8	16.0	22.5	6.2
Mean	17.8 a	38.9 a	23.6	19.6	18.5	8.2 b
			Quilichao Site			
San Ramón (22310)	16.0	24.0	27.6	23.4	14.7	12.7
Patulul (22316)	16.3	36.4	19.7	15.0	21.9	9.3
Mean	16.1 b	30.2 b	23.1	19.2	18.3	11.0

[1]SCT= Soluble condensed tannins [2]BCT= Bound condensed tannins
[a,b] Means within a column without common superscript letters differ (P<0.05).

Discussion: As expected, the quality of the two *Calliandra* provenance measured in terms of digestibility was higher in the site with more fertile soils (Palmira) than in the site with acid low fertility soils (Quilichao). Results also indicate differences in quality attributes between provenance, which can not be attributed to level of soluble CT. In vitro digestibility of *Calliandra* forage was negatively correlated to bound CT (r=-0.86), to acid detergent fiber (r=-0.80) and to a lesser extent to neutral detergent fiber (r=-0.72). These finding support the results from in vitro experiments reported in the previous section that indicate that digestibility of some tropical legumes is more related to the level of bound CT than to soluble CT.
In addition, results support the idea that low digestibility of tropical legumes with CT in some cases is more related to fiber level and composition than to CT.

Table 5. Proanthocyanidin ratios in purified condensed tannins from immature forage of two provenance of *Calliandra calothyrsus* grown at two sites.

Provenance	Proanthocyanidin ratios		
	Delfinidin	Cyanidin	Pelargonidin
		Palmira Site	
San Ramón (22310)	58.0	34.0	8.0
Patulul (22316)	3.3	92.4	4.3
		Quilichao Site	
San Ramón (22310)	69.6	34.0	5.4
Patulul (22316)	8.3	88.6	3.0

It was interesting to note that the two *Calliandra provenance* differed in composition of soluble CT present in leaf tissue. The higher delphinidin: cyanidin ratio in soluble CT recorded in CIAT 22310 compared with CIAT 22316, which had a higher cyanidin: delphinidin ratio, is consistent with the higher astringency observed with CT of CIAT 22310. Results with temperate legumes (*Lotus pedunculatus* and *L. corniculatus*) indicate that reactivity of CT increases with increasing delphinidin: cyanidin ratios. In this context, CT in *L. corniculatus* are mostly cyanidin units, whereas CT in *L. pedunculatus* are mostly delphinidin units. It is known that CT from *L. Pedunculatus* are more effective in reducing in vitro degradation of Rubisco than CT from *L. corniculatus*, which is related to higher proportion of delphinidin units relative to cyanidin. These results indicate that the composition of CT may vary within genotypes of the specie and further support the idea that soluble CT in tropical legumes are more related to protein degradation in the rumen than bound CT, possibly due to their capacity to bind to microbial enzymes.

Molecular weight of purified condensed tannins from tropical legumes (R. Barahona, M. Theodorou, P. Morris and C.E. Lascano)

Highlight

- Difference among legumes in MW of tannins possibly related to biological activity

Rationale: Molecular weights (MW) of CT found in forage species varies widely. Previous work carried in CIAT's Forage Quality laboratory had indicated different degrees of polymerization in the tannins extracted from five shrub legumes, which is an indication of different MW. In temperate legumes, variability of tannin MW is also large. For example, estimates of MW of polymer units ranged between 2000 for *Lotus corniculatus* to 4000 for *L. pedunculatus*.

The degree of polymerization or MW of CT can have a significant effect on the formation of complexes with other molecules such as protein and carbohydrates. Phenolic compounds of low MW are thought to form unstable cross-links with proteins, while those with very high MW are said to be ineffective as protein binding agents. Thus, to better define how CT from different legumes interact with other molecules such as protein, there is a need to determine their MW.

Methods: For the determination of MW, peracetate derivatives of purified CT from immature and mature leaves of 6 legumes (*D. ovalifolium, F. macrophylla, L. leucocephala, L. pallida, C. colothyrsus* and *C. fairchildiana)* were prepared as follows. Ten mg of CT was mixed with 2.5 ml of a 1;1 (v/v) solution of pyridine: acetic anhydride and the resulting mixture was left to stand overnight at room temperature. This was followed by the addition of 5 ml of distilled water and cooling of the mixture before centrifuging at 2500 rpm for 10 minutes. The pellet resulting was washed twice prior to centrifugation. The recovered peracetates were dried and dissolved in 2 ml of tetrahydrofuran and then injected into an HPLC (Shimadzu LC4A system fitted with 2 PLGel 5 μm 500 A column series). Under these conditions the operating pressure was of 88 kg/cm^2. The GPC calibration was performed with polystyrene standards of MW ranging from 162 to 22-00 Dalton. Data was analyzed with a PC equipped with GPC6000 chromatography software.

Results: Under the conditions of the assay, analyses were complete by 25 minutes, but 5 more minute were allowed for the elution process to take place to set greater assurance that results were accurate. Results (not shown) of the calibration runs with the polystyrene standards indicated that the best fit was with cubic equation where the minimum, maximum and standard deviation were 0.51%, 0.56% and 0.0140, respectively. In Table 6 we show the MW of CT of different legumes which were determined using the technique described above. There was great variation in MW among legumes. For example, CT from *D ovalifolium and F. macrophylla* were found to have MW greater than 4700 Dalton. In contrast, CT from *L. leucocephala* had MW of only 1800 Dalton. Differences were also observed in MW between species, such as in *L. leucocephala* and *L. pallida*. MW of CT were higher in mature than in immature leaves (results not shown), the exception being *F. macrophylla* and *D. ovalifolium*.

Table 6. Molecular weights of the peracetate derivatives of a condensed tannins from different tropical legumes.

Legume	Retention time (minutes)	MWPK (Dalton)
D. ovalifolium	15.18	4736
F. macrophylla	15.07	5044
L. leucocephala	16.75	1874
L. pallida	16.15	2685
C. calothyrsus	15.87	3159
C. fairchildiana	15.22	4645

Discussion: The range of MW (1874 to 4736 Dalton) observed in young leaf tissue is similar to what has been observed in temperate species such as Lotus. It is generally accepted that the biological activity of CT measured in terms of ability to precipitate proteins is related to MW usually following a quadratic relationship. The fact that CT in *L. leucocephala have* lower reactivity with protein than CT from *D. ovalifolium* and *F. macrophylla* is consistent with difference in MW of tannins found in this study. In addition, the MW of CT in *L. pallida* was higher than in *L. leucocephala*, which is consistent with their higher biological activity.

Within the range of MW recorded in CT of legumes examined, it would seem that there is a positive relationship between MW and there biological activity. This is consistent from results in *Lotus*, where researchers have found that differences between *L. pedunculatus* and *L. corniculatus* in reducing protein degradation are related to MW. Tannins in L. *pedunculatus* with a MW of 4000 are more effective in reducing protein degradation than CT from *L. corniculatus* with MW of 2000.

More work is needed in order to establish the real biological meaning of different MW of CT in tropical legumes.

Effect of condensed tannins from tropical legumes on the activity of fungal enzymes (S. Sánchez, R. Barahona, M. Theodorou, P. Morris and C.E. Lascano)

Highlight

- Difference among legumes in the ability of CT to inhibit activity of enzymes from fungal origin that degrade protein and fiber

Rationale: It is well known that one major effect of CT is to form complexes with proteins, which are either reversible (formation hydrophobic and/or hydrogen bonds) or irreversible (oxidation of the phenolic compound to reactive quinones). It follows that soluble CT from ingested feed would mainly act by interacting directly with the rumen microorganisms or their enzymes. Thus we were interested in defining how soluble and bound CT from different legume affect the activity of selected enzymes from fungal origin.

Methods: Condensed tannins were purified from freeze dried leaves of *Desmodium ovalifolium*, *Flemingia macrophylla*, *Leucaena leucocephla*, *L. pallida*, *Calliandra calothyrsus* and *Clitoria fairchildiana* . Enzymes used in the study, which were facilitated by the Microbiology group in IGER, UK were: a) Carboxymethylcellulase (CMCase), b) β-D glucosidase, c) Xylanase, and d) β-D -xylosidase. However, we will only report results obtained with CMCase. In addition, we evaluated the effect of CT bound to plant cells on activity of a mixture of fungal enzymes.

Initial experiments were conducted to study the effect of adding different concentrations of CT from *D. ovalifolium* to reaction mixtures with CMCase and β-D –xylosidase . In a second group of experiments CT extracted from all 6 legumes were added at different concentrations to the reaction mixture containing the enzymes.

CMCase activity in the presence of CT was measured by allowing the enzymatic reaction to take place for 30 minutes at 50°C. Because the enzyme and CT solutions could contain some free glucose, two assays were carried out. A first set of samples received 100 µl of tannin solution, 100 µl of enzyme and 800 µl of substrate. Samples in a second set received 800 µl of citrate-phosphate buffer, pH 6.5 instead of the substrate solution. The enzymatic reaction was allowed to take place for 30 minutes at 50°C. The net released glucose was defined as the amount in set 1 minus glucose in set 2.

In a final assay was carried out to compare the effects of bound vs. soluble CT on enzymatic degradation of plant cells as measured by glucose net liberation. CT extracted from all legumes were either added to the solution with plant cells (soluble CT) or bound to plant cells (0.3 mg of plant cell/ml of reaction mixture) isolated from *Festuca arundinace.* Prior to the reaction, plant cells were washed with distilled water to eliminate excess sugars and other soluble constituents.

Results: Data from the first set of experiments showed a clear inhibitory effect of CT from *D ovalifolium* on the activity of CMCase as measured by net glucose liberated (results not shown). It was also observed that the concentrations of CT (range: 0 to 0.05 mg/l) were adequate to measure enzyme activity in subsequent experiments.

Results from other experiments ,which compared the effect of soluble CT extracted from different legumes indicated differences among legumes in the inhibitory effects of CT on the activity of CMCase. For example, the highest degree of enzyme inhibition was with CT of *C. fairchildiana, F. macrophylla, D. ovalifolium* and *L. pallida,* whereas the lowest inhibitory effect was observed with CT from *L. leucocephala* and *C. callothyrsus* (Figure 1).

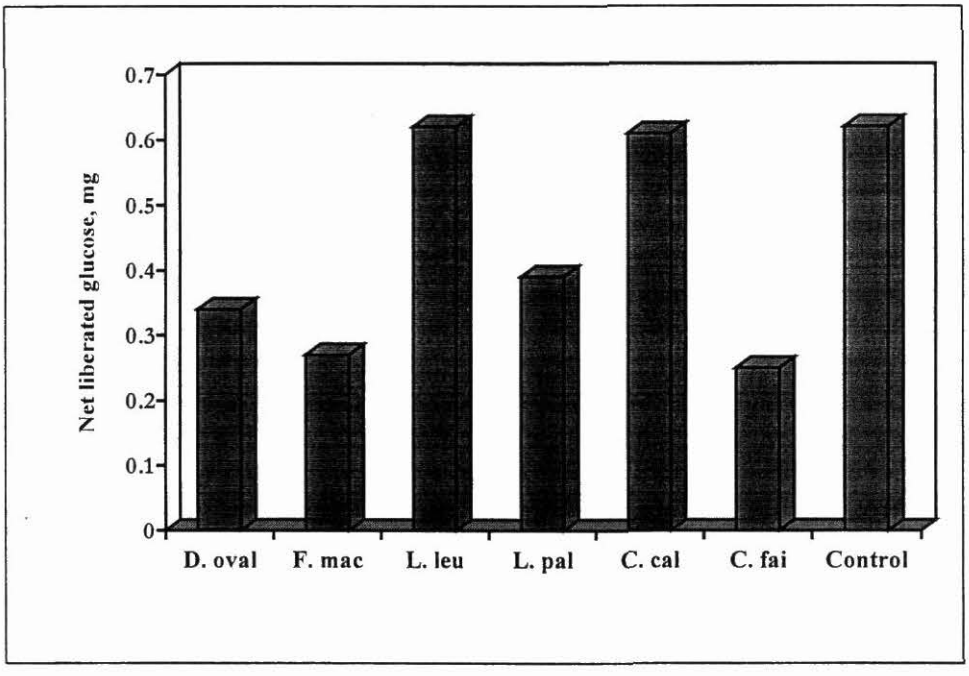

Figure 1. CMCase activity on the presence of soluble CT. Effect of CT from six tropical legumes (0.025 mg/ ml of reaction mixture) on the activity of CMCase from *Neocallimastyx hurleyensis.* D. oval = *Desmodium ovalifolium*; F. mac = *Flemingya macrophylla*; L. leu = *Leucaena leucocephala*; L.pal = *L. pallida*; C. cal = *Calliandra calothyrsus* and C. fai = *Clitoria fairchildiana.*

The inhibitory effect of CT on the activity of β-D –xylosidase (results not shown), indicated that this enzyme was more sensitive to tannins than β-D- glucosidase and that the activity varied with the source of CT. For example, CT of *C. fairchildiana* were the most effective in reducing enzyme activity whereas CT from *L. palliida* were the least effective in inhibiting the enzyme.

Results on the effect of soluble and bound CT on activity of a mixture of fungal enzymes indicate that in most cases bound CT were more effective as inhibitors of microbial enzyme activity than soluble CT (Figure 2). However, it was interesting to note differences among

sources of CT. For example, bound CT from *F. macrophylla* had the highest inhibitory effect relative to the control, whereas bound CT from *L. pallida* and *D. ovalifolium* had the least effect.

Discussion: Results from this work indicate that CT from different legumes have the potential to inhibit enzyme activity and as result affect forage digestibility and protein degradation by cellulases and proteolytic enzymes produced by rumen microorganisms. However, it is evident that the effect of CT on enzyme activity varies with the source of CT and that some enzymes are more sensitive to CT than other enzymes. In addition, it would seem that the protection of substrate by bound CT during fermentation is a more effective way to deter enzymatic activity than precipitation of enzymes by soluble CT.

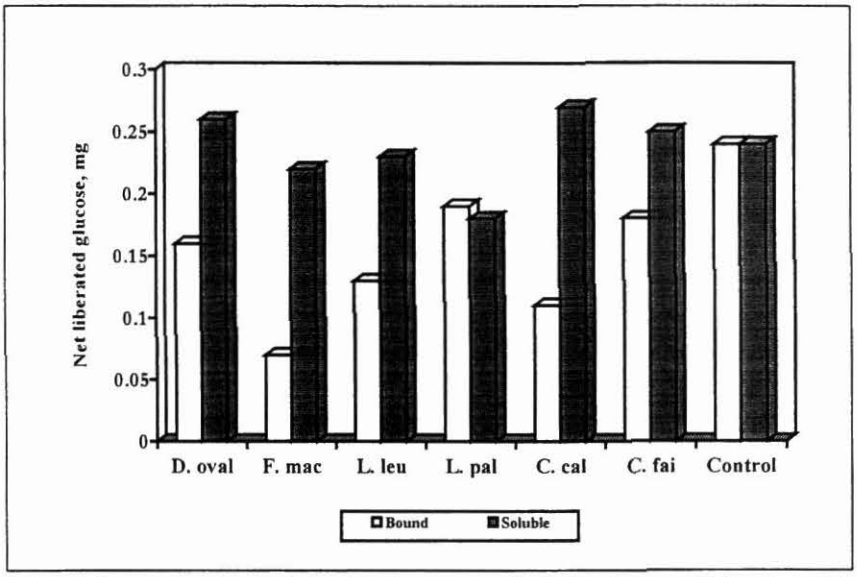

Figure 2. Enzymatic degradation of *Festuca arundinacea* plant cells in the presence of soluble and bound CT. Relative effect of soluble and bound CT from six tropical legumes (0.3 mg of plant cell DM/ml of reaction mixture) on the activity of a mixture of enzymes from *Neocallimastyx hurleyensis*. **D. oval** = *Desmodium ovalifolium*; **F. mac** = *Flemingya macrophylla*; **L. leu** = *Leucaena leucocephala*; **L. pal** = *L. pallida*; **C. cal** = *Calliandra calothyrsus* and **C. fai** = *Clitoria fairchildiana*.

Progress towards achieving output milestone

- **List of legume species as feed resources with known quality attributes (1999)**

Results so far indicate that in tropical legumes the fraction of CT bound to fiber (insoluble) has a greater negative effect on forage digestibility than the soluble fraction, but that this later fraction can negatively affect protein degradation by rumen microorganisms. We have also shown that the biological activity of CT can be different among legume species and even between genotypes of the same species and that this is related to different proanthocyanidin ratios in the soluble tannin fraction and to different molecular weights.

Increased understanding on the effects of soluble and bound CT on the nutritive value of a range of tropical legumes has allowed us to to develop better screening methodologies to assess quality of selected legumes. These improved methods are currently being used to characterize quality attributes of a range of herbaceous and shrub legume species with potential value as feed resources.

Further definition of the true behavior in the ruminant animal of the different fractions of condensed tannins determined by laboratory procedures will be required for the refinement of improved screening procedures for quality evaluation of tropical legumes.

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

It is well documented that tannin production in tropical legumes can be affected by growing conditions. Plants grown under stress (i.e. soil nutrient deficiencies, water deficit) have higher tannin levels than when grown under no stress. Thus to be able to define "niches" for particular legume species, it is important to quantify the effect of soil and climatic stress on tannin production and forage quality in general. To accomplish these objectives, a special project funded by 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. ovalifolium*. A total of 18 genotypes of *D. ovalifolium* were planted in 6 contrasting sites in Colombia, which represent major ecosystems (subhumid and humid hillsides, savannas, and forest margins) and soil texture and fertility. Expected outputs from the Project include (1) quantification of the effects of changes in soil fertility and climate (rainfall and temperature) on quality parameters of different *Desmodium* genotypes and (2) selection of appropriate *Desmodium* genotypes for different ecological "niches". This year we completed the statistical analysis of results on agronomic and quality attributes of *Desmodium* genotypes as affected by soil fertility and rainfall.

Effects of environmental factors (soils, climate) on quality of *D. ovalifolium* (A. Schmidt, C. E. Lascano, N. Narváez, R. Barahona, G. Ramirez and R. Schultze-Kraft)

Highlight

- Quality of *Desmodium ovalifolium* genotypes influenced by fertilizer level and by rainfall

***Rationale*:** Tannin concentration in plant tissue has been shown to vary with plant species, plant part, plant maturity and soil fertility. It is also known the forage quality and acceptability of *D. ovalifolium* by grazing ruminants has been observed to change from one environment to the other. Changes in CT content and structure could be related to this grazing behavior. In order to select superior genotypes for specific regions in the tropics it is indispensable to understand the effects of climate and soil conditions on quality of *Desmodium ovalifolium*.

Table 7. Sites in Colombia where genotypes of *Desmodium ovalifolium* were evaluated

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

H= high M= moderate L= low

Methods: A multilocational trial was carried out from April to June 1995 in order to evaluate the agronomic performance and quality of a 18 genotype core collection of D. ovalifolium at 6 contrasting sites representing typical high rainfall areas in Colombia (Table 7). A total of 108

plots (6x5 m each) were established at each site. The 18 genotypes were arranged in a split-plot design with 2 fertility levels and 3 replicates. As described in AR-97 a large data set was obtained through a series of evaluations of agronomic and quality parameters since 1995 including lab analysis and cafeteria-trials. NIRS was used for quality analysis in order to handle the 2000 plant tissue samples generated throughout the project. A variety of statistical procedures including multiple-stepwise regressions and stability analysis were applied in order to rank environments and genotypes, to define genotype stability across sites and environmental factors determining plant performance.

Results: Data analysis (ANOVA) indicated that in *Desmodium* grown in the Carimagua, Macagual and Cauca sites there was an increase of leaf tissue digestibility (IVDMD) and a decrease of soluble CT and CT astringency with higher rainfall. This effect was reverse at La Rueda and Chinchiná sites, where forage quality decreased with higher rainfall (Table 8). Fertilizer levels had also an effect on digestibility and soluble CT content, thus confirming results reported last year. With the application of a high fertilizer level (A) there were increase in quality parameters, with the exception of the Cauca and the Chinchiná site where this effect could not be observed (Table 9).

Table 8. Differences of quality parameters of *D. ovalifolium* in relation to seasons at each site averaged across fertilizer levels

Sites	Season	IVDMD (%)	CP (%)	SCT* (%)	BCT** (%)	Astringency (gPP/gCT)
Alcancía	Wet	45.94a	16.40a	6.79b	2.61a	6.58b
	Dry	42.33b	14.59b	7.92a	2.26b	7.86a
Maquenque	Wet	44.57a	13.98b	6.90b	2.77a	9.23a
	Dry	39.93b	14.40a	7.85a	2.32b	8.23b
Macagual	Wet	44.66a	15.70b	7.60a	2.42b	5.73b
	Dry	42.52b	17.45a	7.34b	2.69a	6.89a
La Rueda	Wet	35.97b	12.70b	8.12a	2.88a	7.52a
	Dry	42.89a	14.10a	6.75b	2.50b	5.77b
Chinchiná	Wet	44.35b	14.35b	6.82a	2.48b	9.62a
	Dry	46.47a	14.95a	6.84a	2.65a	8.79b
Cauca	Wet	44.12a	12.92b	6.66b	3.28b	10.89a
	Dry	37.29b	14.11a	7.04a	3.71a	10.42b

[a,b] Means within a column without common superscript letters differ (P<0.05).
*Soluble condensed tannins
**Bound condensed tannins

Table 9. Differences of quality parameters of *D. ovalifolium* in relation to fertility levels at each site (A = high; B = low) averaged across seasons

Sites	Fertility	IVDMD (%)	CP (%)	SCT (%)	BCT (%)	Astringency (gPP/gTC)
Alcancía	A	49.29a	17.8	6.54b	2.37b	5.60b
	B	39.01b	13.2	8.16a	2.51a	8.83a
Maquenque	A	49.38a	16.35	6.20b	2.43b	6.42b
	B	34.92b	11.94	8.58a	2.68a	11.15a
Macagual	A	45.06a	16.29	7.12b	2.57a	5.91b
	B	42.11b	16.86	7.81a	2.54a	6.71a
La Rueda	A	40.36a	13.52a	7.26a	2.67a	6.46a
	B	38.51b	13.29a	7.61a	2.71a	6.84a
Chinchiná	A	46.37a	14.75a	6.61a	2.59a	9.03a
	B	44.44a	14.55a	7.05a	2.53a	9.39a
Cauca	A	40.57a	13.51a	6.80a	3.55a	10.76a
	B	40.83a	13.51a	6.89a	3.44a	10.56a

[a,b] Means within a column without common superscript letters differ (P<0.05).
*Soluble condensed tannins
**Bound condensed tannins

Discussion: Results clearly showed the influence of climate and fertilizer levels on quality parameters in *Desmodium ovalifolium*. High fertilizer levels and high precipitation resulted in higher forage quality. However, the lower forage quality of *Desmodium* recorded at La Rueda (Forest Margins) and Chinchiná (Humid Hillsides) may be due to poor soil physical conditions (low air permeability) at these sites in the rainy season, which is indicative of a soil x climate interaction. Characterization of soil physical properties was started in May 1998 in order to assess the influence and the magnitude of the mentioned soil climate interaction.

New study on soil physical properties and their interactions with forage quality of *D. ovalifolium* (A. Salamanca, A. Schmidt, E. Amézquita and M. Peters)

Rationale: The quality of *D. ovalifolium* was shown to be influenced by a environmental factors. High rainfall and high fertilizer levels increase quality, but at some specific sites a negative correlation with precipitation patterns was found. In order to explain these negative correlation and to better define specific interactions between soil and climate factors on quality of *D. ovalifolium*,, studies on physical soil properties and their relation to precipitation started in May 1998. Excess rainfall and its consequences on plant development will be measured in during the study. A pregraduate student from the Universidad Nacional Palmira carries out the experiment and sampling.

Methods: Soil samples from 6 sites (see Table 7) are currently being taken and analysed. Soil physical properties in the field (resistance to penetration), root depth and distribution are being measured on each site. Laboratory analysis includes:

a) parameters bulk density
b) hydraulic conductivity,
c) air permeability,
d) porosity, texture and
e) chemical analysis.

Samples will be taken once in the wet and the dry season. Additional greenhouse experiments with a limited set of soils from three sites and three genotypes will be established under 4 irrigation treatments in late 1998 in order to assess the effect of compaction and soil humidity on development and quality patterns of *D. ovalifolium*. Preliminary results are expected by the end of 1998.

***Desmodium* Workshop**

On the 4th of March, 1998 a second workshop was organized with the theme "G x E interactions in a Core Collection of the Tropical Cover Crop and Forage Legume *Desmodium ovalifolium*" . This event, which was held at CIAT, had 62 participants from 17 institutions of 5 countries who discussed results and future perspectives of *Desmodium* as a cover crop and pasture legume.

Presentations of regional experiences from Venezuela, Perú and Colombia complemented previous information with *Desmodium* in Latin America that was presented at the first workshop in 1996. Results of the multilocational trials were presented and discussed and two working groups provided recommendations on future research and development needs in relation to *D. ovalifolium* . New projects in collaboration with institutions, farmers and the private sector were initiated and broad interest in the legume for multipurpose use was achieved.

Progress towards achieving output milestone

- **Defined soil quality and climatic parameters associated with quality attributes of selected legume species (1999)**

We have defined how environmental factors such as soil fertility and rainfall, affect quality of *Desmodium ovalifolium*. This information will be valuable for targeting to specific niches other legume species with high tannin contents. The assessment of how soil physical parameters affect yield and quality of *D. ovalifolium* genotypes will also improve our ability to identify environmental niches to grow *Desmodium* and other legume species with tannins as feed resources.

Furthermore, with the results from this work we were able to select four new *Desmodium ovalifolium* genotypes with higher forage quality than the commercial cultivar (CIAT 350) , which will be tested in large animal grazing trials early next year. Trials will include other legumes like *A. pinto* and several *Brachiaria* species.

A productive and high quality *D. ovalifolium* cultivar would represent a significant advancement for developing grass/legume pastures in the humid tropics where the only persistent herbaceous legume options at present is *A. pintoi*. Advantages of *D. ovalifolium* over *A. pintoi* are that it is better adapted to conditions of low soil fertility and much easier and cheaper to establish.

Suboutput 1.3 Identified synergism in quality parameters among contrasting forages

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

To derive some principles on how best to match feed resources a number of experiments were carried out during 1998 in the Quilichao research station with grazing milking cows fed forage-based supplements. These studies have been partially supported by COLCIENCIAS through scholarship program for young researchers.

Supplementation of grazing milking cows with *Cratylia argentea* in combination with sugar cane (P. Avila and C.E. Lascano)

Highlight

- Responses in milk yield to legume supplementation in combination with sugarcane shown to be related to quality of forage in the pasture.

***Rationale*:** Results from short-term grazing experiments with milking cows had indicated that milk yield response to legumes in association with grasses is dependent on the season (dry, rainy) of the year. Thus, it was of interest to examine the interaction of forage-based supplements with pasture attributes (quantity and quality) on milk yield of cows during both the dry and rainy seasons.

Methods: Experiments with grazing milking cows in the dry and wet seasons were carried out in the Quilichao Research Stations using a 4x4 Latin Square design with 14 days per period, of which 7 were for adaptation and 7 for measurements. In each experiment there were two breed groups of four cows each (Brahman and Holstein x Brahman crossbreeds) grazing a *Brachiaria decumbens* pasture (2 AU/ha). All cows were supplemented with the following forage based supplements: T1: 100% sugar cane, T2: 75% sugar cane + 25% *C. argentea*, T3: 50% sugar cane + 50% *C. argentea*, and T4: 25% sugar cane + 75% *C. argentea*. The level of supplement offered per cow/day was on a DM basis 1.5% of BW, which insured that cows could select. Milk yields were recorded daily during 7days in each experimental period. In addition, forage availability and quality were measured in each experiment.

Results: Milk yield of Brahma type cows did not increase with legume supplementation in the dry or wet seasons (results not show). However, fat corrected milk yield of Holstein crossbred cows supplemented with different proportions of *C. argentea* in combination with sugar was increased in the dry season relative to only sugar cane, which was not the case in the wet season (Table 10). In the dry season milk yield of crossbred cows grazing *B. decumbens* pastures increased from 12 to 30% with the addition of the shrub legume in the sugar based supplement. An analysis of the forage on offer in the pasture revealed that as expected in the dry season the grass was deficient in crude protein (4%) whereas in the wet season, protein in the grass (7%) was within the range expected in pastures without N fertilization. It is well known that a protein deficiency in the basal diet of ruminants can negatively affect voluntary intake and consequently milk production.

Table 10. Fat-corrected milk yield of crossbred cows supplemented with different protein to energy ratios from forage sources and biomass availability, digestibility, and crude protein content of *Brachiaria decumbens* used as basal grass during the dry and rainy seasons.

	Milk yield during dry season (kg/cow/day)	Milk yield during rainy season (kg/cow/day)
Treatments		
100% Sugarcane	6.0 b	7.9
75% Sugarcane + 25% *Cratylia*	7.0 a	7.5
50% Sugarcane + 50% *Cratylia*	7.2 a	7.7
25% Sugarcane + 75% *Cratylia*	7.8 a	8.3
Grass in pasture		
* Biomass availability (kg/haDM)	2164	2502
* Digestibility (%)	63.4	66.6
* Crude Protein (%)	4.4	7.1

[a,b] Means within a column without common superscript letters differ (P<0.05).

Discussion: Results indicate that in dual- purpose cattle herds, legume supplementation should be used strategically, especially during the dry season when the basal grass is limiting in quantity and quality as compared to the rainy season. However, this supplementation with shrub legumes will only be successful in terms of improving milk yield with crossbred cows that have the genetic potential to respond as we have shown in a number of on-station and on farm experiments. The legume technology being developed for small dairy producers has excellent potential for adoption by farmers given the fact that the capital investment required to establish it is low. However, there is a need to evaluate legume supplementation with cows with even higher genetic potential than used so far and to validate these on- station results under farm conditions within the Tropileche Consortia.

Milk Urea Nitrogen (MUN) as an indicator of the energy: protein status of milking cows
(P. Avila and C.E. Lascano)

Highlight

- Demonstration that milk urea N (MUN) can be an important tool to better match improved forages with nutritional requirements of milking cows

Rationale: In ruminant animals, when there is an excess of protein relative to energy in the rumen, the level of Rumania ammonia increases. Unused ruminal ammonia enters the portal blood through the rumen wall and is transferred to the liver where it is detoxified by conversion to urea. The liver also produced urea from deamination of amino acids rising from postruminal digestion and protein turnover. Urea then circulates in the blood to the kidneys and is excreted with the urine or it can be diffused from the blood into milk.

When there is a deficiency of dietary protein as often occurs with tropical grasses, ruminal ammonia concentrations are relatively low and the proportion of N recycled back to the rumen as urea increases. As a result of these metabolic transactions, blood urea (BUN) is highly correlated with ruminal ammonia and with milk urea nitrogen (MUN). Therefore, in healthy ruminants, MUN concentrations could be a good indicator of the protein to energy ratio in the diet and serve to define the need to supplement protein from legume sources.

Methods: Data on MUN from grazing experiments with milking cows receiving forage based supplements were used to determine the value of MUN as an indicator of energy: protein status. A total of 48 observations on MUN from Holstein (H) and Brahma (B) crosses were included in a non-linear regression analysis, where Y was the % increment in milk production due to legume supplementation and X was the corresponding level of MUN in the control receiving no legume in the supplement.

Results: In Figure 3 we show the relationship between milk yield increases and MUN of cows supplemented with *Cratylia argentea* in different combinations with sugar cane. Large variation was observed in MUN values and consequently the regression curve has to be taken with caution for predictive purposes.

Four distinct groups of cows were identified:

a) cows, which showed an increment in milk, yield with legume supplementation when the level of MUN was <10 mg/dL,
b) cows that did not respond to legume supplementation when the level of MUN was <10 mg/dL,
c) cows, which showed modest increment in milk yield with legume supplementation when MUN was >10 mg/dL and
d) cows, which did not produce more milk due to legumes when MUN was >10mg/dL.

Most of the cows with MUN levels of <10mg/dL that responded to legume supplementation where Holstein crosses (Figure 3). It is interesting to observe that there were a small number of Holstein and Brahma crosses, which responded to legume supplementation even though their MUN values were <10mg/dL.

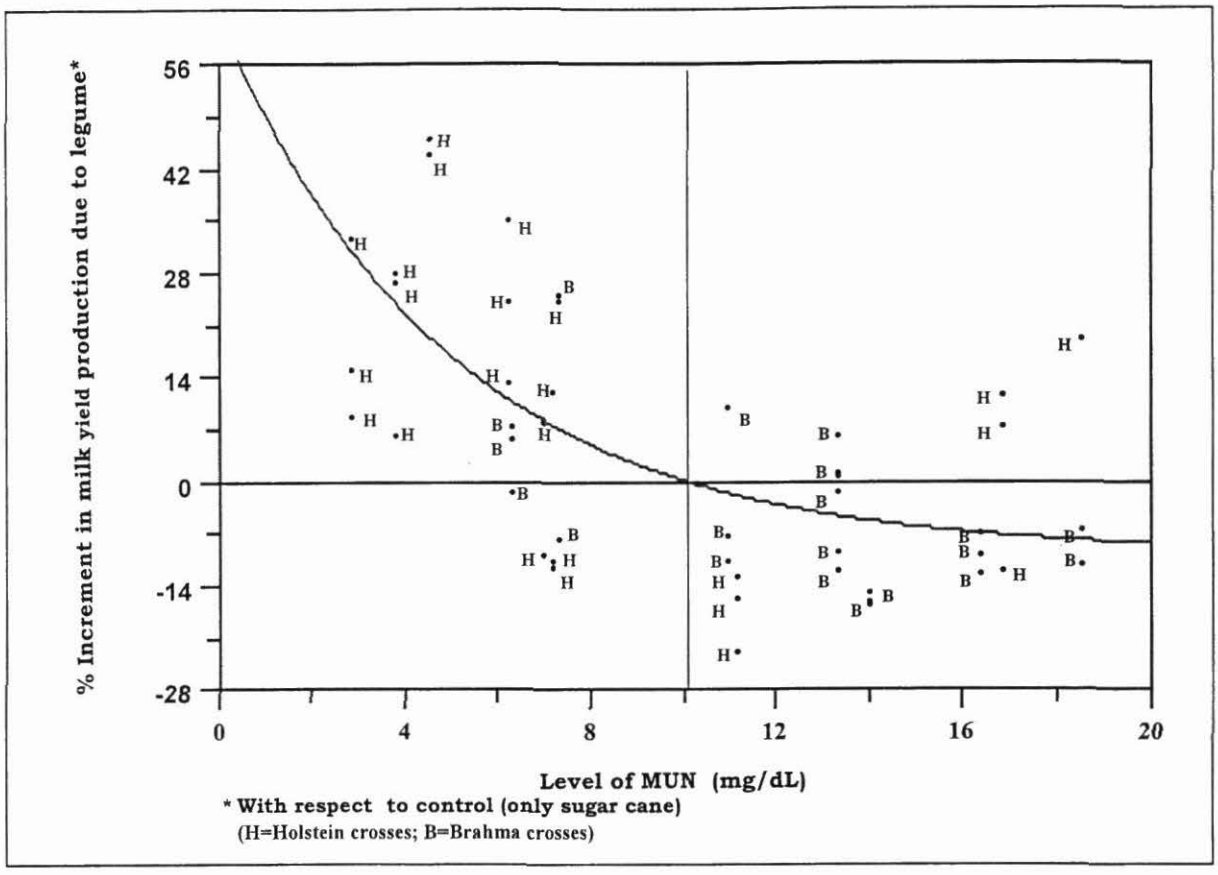

Figure 3. Relationship between milk yield increases and MUN of cows supplemented with *Cratylia argentea* in combination with sugar cane.

Discussion: These results confirm previous findings that indicate that MUN varies with the protein : energy ration in the diet of milking cows. In addition, 10 mg/dL seem to be a critical level for MUN, bellow which there are high probabilities of obtaining a response to legume supplementation provided the cows have the genetic potential to respond to the improved nutrition. In view of these results, the use of MUN as a nutritional indicator seems to be a good tool for attempting to maximize the synergistic effect of introduced legume forages with existing forages and for matching improved forages with the nutritional requirements of dairy cows in dual-purpose cattle systems.

Progress towards achieving output milestone

- **Nutritional indicators to predict milk yield response to forage-based supplements (1999)**

We now have better guidelines to maximize the nutritional benefits of introduced shrub legumes as protein supplements for dairy cows in dual-purpose cattle systems. Our results from last year had indicated that level and frequency of legume supplementation affected the utilization of nutrients in sheep fed low quality grasses. Results from this year indicate that there is a response in milk yield due to supplementation of legumes in combination with sugar cane, when the grass available is limiting in quantity and quality, particularly protein, and when cows have the genetic potential to respond.

We also have results that indicate that the use of nutritional markers such a Milk Urea Nitrogen (MUN) can be a helpful tool for matching feed resources with nutritional requirements

of dairy cows. What is lacking now is the validation of these results in on -farm trials, which is an objective being pursued in collaboration with the Tropileche Consortium housed in PE-5.

Our immediate challenge is to develop a fast and reliable laboratory in vitro method that allows us to evaluate the synergistic effect of different forage combinations in terms of fermentation parameters. Steps on this direction will be taken in 1999.

Suboutput 1.4 Known quality and animal production with selected herbaceous and shrub legumes

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

This year we evaluated quality parameters in two provenance of *Calliandra calothyrsus* grown at two sites and results were presented and discussed in Suboutput 1.1 of this report. We also continued to evaluate different strategies for feeding *Cratylia argentea* to milking cows and results were reported in Suboutput 3.0 of this report and in AR-98 of PE-5, as part of Tropileche. During 1998 we carried out a trial with sheep to determine differences in palatability between provenance of *Calliandra calothyrsus* grown in Quilichao. In addition, we continued to monitor milk production in pastures with and without *Arachis pintoi* in dual-purpose farms in Caqueta, Colombia as part of the Nestle Project and initiated collaboration with CORPOICA in Caquetá, Colombia to evaluate the use of *Stylosanthes guianensis* with pre-weaned calves.

Assessment of palatability of *Calliandra calothyrsus* provenances fed to sheep (P. Avila, J. Stewart and C.E. Lascano)

Highlight

- No difference in palatability between *Calliandra calothyrsus* provenance in spite of having different levels of soluble CT.

***Rationale*:** Level of voluntary intake is an important quality attribute of legumes since it is highly correlated with animal performance. Intake in turn is a function of the palatability of the forage and its chemical composition as it affects fermentation in the rumen and turnover. A simple and reliable method to assess palatability of forages is through feeding small quantities of the test forage to animals and measuring the rate of intake. It has been suggested that intake of *Calliandra calothyrsus* is low due to high levels of CT in the edible forage. However, other results from Australia showed that the feed value of this legume could be enhanced if fed fresh rather than dry. Thus it was of interest to measure short-term intake, as a measure of palatability, of selected provenance of *Calliandra* currently under evaluation in Quilichao.

***Methods*:** Edible forage (leaf and thin stems) of *C. calothyrsus* CIAT 22310 and 22316 grown in Quilichao was fed to 8 sheep receiving a grass basal diet in a 4 x 4 Latin Square design arrangement. Each provenance was offered either fresh or sun-dried in the morning and afternoon and animal were allowed to consume the forage for 30 minutes. Intake recorded was transformed to DM intake/hour per unit of body weight.

Results: Results of short-term intake of the two *Calliandra* provenance fed fresh and sun-dried to sheep indicated that intake of *Calliandra* was low regardless of provenance or post-harvest treatment. The amount consumed ranged from 27 to 33% of that offered.

Discussion: Based on Australian results we had expected higher intake with fresh than with sun-dried *Calliandra*. In addition, based on chemical analysis, we expected that rate of intake of *Calliandra calothyrsus* would be higher with CIAT 22310 given that is has lees soluble CT than CIAT 22316, even though its digestibility and fiber levels are lower (see Table 4). Thus it would seem that differences measured in soluble CT (15 and 22% for CIAT 22310 and CIAT 22316, respectively) are not enough to affect palatability of the forage as measured using the short-term intake methodology. In fact the low level of soluble CT may still be to high and as a result negatively affect palatability of the forage. A study is underway to assess the effect of supplementing dry and fresh *Calliandra* in terms of intake, digestibility and N utilization by sheep fed a low quality grass basal diet.

Milk production in *Arachis*-based pastures in dual-purpose cattle farms in forest margins
(G.A. Ruiz, J. Rozo and C.E. Lascano)

Highlight

- Continue to show that in farms of the forest margins cows grazing *Arachis pintoi* pastures produce more milk than in grass only pastures

Rationale: The NESTLE Project, which aims to establish the basis for recuperation of degraded pastures with legumes in forest margins, entered into its fourth year of activities in dual-purpose cattle farms in the piedmont region of Caquetá, Colombia. Due to logistical difficulties, we have only made detailed measurements of milk yield in one of the farms where *Arachis pintoi* was introduced in 1995. In all other farms we continue to monitor botanical composition of pastures and bulk milk production in the grass and grass legume pastures.

Methods: Milk yield is being measured periodically in grass and grass/legume pastures managed by the farmer in a rotational sequence. Results on daily milk production have been classified according to breed of cow and to stage of lactation. In addition, we have measured milk urea N (MUN) as indicator of legume consumption by grazing cows.

Results: The content of *Arachis pintoi* in the mixed grass/legume pasture where milk yield has been monitored is in the order of 30%. In this pasture, daily milk production of crossbreed Holstein cows has been 8 to 11% higher than in the grass only pasture (Table 11).

Table 11. Milk yield and milk urea N (MUN) in cows grazing pastures with and without *A. pintoi* in farms of the forest margins (Caquetá, Colombia).

Pasture	Stage lactation (days)	Milk yield (kg/cow/d)	MUN (mg/dL)
Brachiaria spp.	1-90	6.3	6.5
	91-180	5.7	67
	>180	5.0	7.5
Mean		5.7	6.9
Brachiaria spp./*A. pintoi*	1-90	7.0	10.2
	91-180	6.2	12.8
	>180	5.4	13.4
Mean		6.2	12.1

As expected, the highest response to the legume was recorded with cows in the first and second third of lactation. The higher MUN levels recorded were accompanied by increased milk yield in the grass/legume pasture, which gives us a certain amount of confidence that the observed response is due to legume intake.

Discussion: The legume content in the pasture being monitored increased from 15 % in 1996 to over 30% in 1998. This increase in *Arachis pintoi* in the pasture has been accompanied by a corresponding increase in milk yield. For example, in 1997 the average effect of legume on milk yield of the total herd (47 cows) was 0.13 liters (3%), whereas this year the increment for the herd (41 cows) was 0.30 liters (6%). It continues to be evident that the greatest response to *Arachis pintoi* is with crossbred Holstein cows and within this group, with cows in early lactation.

An additional benefit of the *Arachis*-based pasture technology has been in terms of soil earthworms. Results (not show) indicate that in pastures with *Arachis*, the population of earthworms ($192/m^2$) is considerably greater than in the grass only pasture ($80/m^2$) and native pasture ($16/m^2$). Similar results were also observed in grass and grass/legume pastures being monitored for earthworms in other farms participating in the project. It is postulated that this increased earthworm population is contributing to improved nutrient cycling and to improved internal drainage of the soils, which is a major constraint in pastures in the region.

Use of *Stylosanthes guianensis* with pre-weaned calves in dual-purpose cattle systems in forest margins of Colombia (J. Velasquez, G.A. Ruiz and C.E. Lascano)

Highlight

- Reconfirmed that by feeding *Stylosanthes guianensis* to pre-weaned calves their is more milk for sale and improved calf performance

Rationale: In dual-purpose cattle farms two main outputs are milk and weaned calves for fattening. Under traditional management, farmers usually favor selling as much milk as possible to increase their cash flow, but as a result calves suffer from under nutrition and mortality rates are high. Therefore, development of feeding systems that allow farmers to obtain more milk for sale and the same time result in good performance of pre-weaned calves is of high priority in dual-purpose cattle systems.

The idea of using *Stylosanthes guianensis* for grazing pre-weaned calves has been tested in Pucallpa, Peru. Results indicate that with this alternative the farmer can sell almost one more liter of milk/cow day and still maintain adequate growth of their calves, which has important economical implications (See AR-98 of PE-5). To further test the use of Stylo for pre-weaned calves, we initiated a collaborative study this year with COPRPOICA partners in Macagual, Caqueta, Colombia.

Methods: A small paddock (2 ha) of Stylo was established in the CORPOICA research station in Macagual, Caquetá to allow 1 to 3 months calves to freely graze after milking. Calves with access to Stylo also received residual milk (milk remaining in the udder after hand milking) after each milking. Calves in the control treatment received milk from one quarter of the udder at milking and had access to a grass pasture after milking. In al cases calves remained with the dam for 3 to 4 hours after milking, before going to the grass or Stylo pastures.

Results: The amount of milk for sale resulting from the use of Stylo by pre-weaned calves was 21% higher than recorded with cows that had calves managed in the traditional systems (Table

12). In addition, liveweight gain of calves with access to Stylo was 30% higher than in the control animal during a 90-day period.

Table 12. Milk for sale and growth of pre-weaned calves with and without access to a *Stylosanthes guianensis* (Stylo) pasture (CORPOICA, Macagual, Caquetá, Colombia).

Item	Control[1]	Stylo pasture[2]
Milk for sale (l/cow/day)	3.3	4.0
Liveweight gain of pre-weaned calves (g/A/d)	297	389

[1]Six cows with calves
[2]Six cows with calves

Discussion: The results obtained by CORPOICA in forest margins of Colombia on the use of Stylo for pre-weaned calves are in agreement with those obtained in small dairy farms of Pucallpa, Perú.

This Stylo technology could be very attractive to small dairy producers given that the cost of establishment of the legume is less than the establishment of legume-based pastures for the milking herd and it results in increased cash flow due to the extra milk for sale, without affecting calf performance. In addition, the Stylo technology could form part of crop-pasture rotation system eliminating the need to fallow land for secondary forest regeneration and subsequent slash and burn for annual crop production. This is because well managed Stylo pastures could persist for 3 or 4 years and during this time produce a beneficial effect on the soil through N fixation and nutrient cycling.

Progress towards achieving output milestone

- **Higher milk yield increments with elite grass and legume species (1999)**

Our results from this year further confirm that legumes can have a significant impact in animal production when used in different feeding systems in dual-cattle production farms. We have consistently demonstrated through on-farm trials that with the use of herbaceous legumes (*Arachis pintoi*) in association with grasses milk yield of cows can increase 10 to 15% over the straight grass, provided cows have the genetic potential to respond to the improved nutrition. On-station results also indicate that by supplementing shrub legumes (*Cratylia argentea*) in combination with cut and carry grasses (sugarcane) milk yield improves, particularly when the biomass in the pasture on offer is limiting in quantity and quality. Finally we have on-farm and on-station results that indicate that with small areas of high quality herbaceous legumes (*Stylosanthes guianensis*) for pre-weaned calves, there is a positive effect not only in calf performance but also in milk for sale, which has important economical implications for smallholders.

The challenge ahead is to enhance adoption of herbaceous and shrub legumes by farmers by strengthening on- farm participatory research and by facilitating seed of selected legumes to farmers. To more effectively accomplish these objectives we need to continue building strong linkages with other CIAT Projects, NARS partners and with the private livestock and seed sectors.

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

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

Grassland spittlebugs (Homoptera: Cercopidae) pose the greatest biotic limit to forage grass production in the neotropics. Outbreaks of these native, xylem-sucking insects dramatically reduce forage quality and productivity, causing declines in persistence and limiting the establishment of improved forage species such as *Brachiaria decumbens*. There is still no effective strategy for their integrated management. Part of the problem stems from the tendency to overgeneralize similarities among the diverse species and genera. This is aggravated by a deficient understanding of their basic biology and behavior, a scarcity of detailed site-specific studies on their ecology, and the fact that spittlebugs attack pastures across a wide range of ecological zones. Despite the similarities in bioecology and pasture habitat, the nature of their impact on forage grasses could vary greatly among regions given the taxonomic diversity of the group superimposed on this range of environments and forms of pasture management.

The present research addresses the components of bioecology most relevant to an interpretation of pest status through a detailed elaboration of taxonomic, seasonal, local and regional determinants of abundance and distribution. The principal objective is to gather the basic information necessary to launch the establishment of an integrated pest management program for grassland spittlebugs. A secondary objective is to support concurrent studies on host plant resistance.

A 2-year research program on the comparative biology and ecology of grassland spittlebugs in the lowland of Colombia was initiated in September of 1996. Post-doctoral fellow Daniel Peck was contracted to lead bioecology studies as part of a special project funded by the Colombian Fondo Nacional de Ganado awarded to CORPOICA Regional 2 (C.I. Turipaná) and coordinated by Nora Jiménez. CIAT and the Organization of American States funded one half of the position and further operational expenses were awarded through Nestlé de Colombia to ensure detailed comparative studies in 4 departments of the country most affected by spittlebugs.

Success of this project has depended on the creation of a national network of researchers, which over the 2 years has included the direct involvement of 5 research assistants, 3 university professors and 18 undergraduate students. The comparative population dynamics studies, which form the foundation for most other activities, take place in Montería (Córdoba), Corozal (Sucre), Villavicencio (Meta) and Florencia (Caquetá) with the collaboration of CORPOICA and the Universities of Amazonía, Sucre and los Llanos.

In 1997 we reported on the initiation of first time studies on (1) a new rearing unit to facilitate biological studies (2) the biology of three species, (3) vibrational communication, and (4) seasonal variation in egg diapause. The first year of population studies identified 6 species in the 4 survey sites. In the 3 seasonally dry sites 1 species dominated and abundance corresponded with the wet seasons; in the continuously wet site 2 species dominated and there was a higher incidence of natural enemies. We also reported initial results that spittlebug abundance does not vary between pastures with and without *Arachis pintoi* associations.

Population studies were continued for a second year in 1998 in combination with biological studies on 4 species previously unstudied. Comparative methodologies have thus far yielded

detailed information on the biology of 2 species and the population dynamics in 4 regions. We have advanced research on substrate communication and egg diapause, evaluated the response of spittlebugs to *Brachiaria*/*Arachis* associations, and carried out 2 new spittlebug training workshops. This report identifies research highlights and summarizes the new information gathered to date. Because most studies are ongoing, we outline our future analyses and perspectives.

Studies on the biology and behavior of major spittlebug species

Highlights

- First-time studies on the biology of 4 lowland spittlebug species were initiated.
- Apparatus to study substrate communication among adult spittlebugs was established and the first recordings produced.

Rearing unit that facilitates biological studies of spittlebugs (U. Castro, D. Peck)

Rationale: Biological studies of spittlebugs benefit from rearing colonies that overcome the seasonality of the insect and provide access to all life stages year round. Current rearing techniques, however, such as CIAT's mass rearing program, are beyond the capabilities of small research groups given the high labor, space and time requirements and the daily effort of collecting emerging adults by hand and transferring them to oviposition chambers. Biological studies, furthermore, should be carried out *in situ*. Anecdotal evidence for the introduction of spittlebug species to regions where they do not normally occur argues strongly against the transfer of species from one geographic zone to the next due to the risk of new pest introductions. Here we describe improvements and initial efficiency measures of a new design reported in 1997.

Methods: We sought to develop a small-scale rearing unit adaptable for different species and genera of spittlebugs that has reduced labor and material requirements. One particular focus was the combination of nymph rearing and egg laying chambers to eliminate a major bottleneck of CIAT's colony, the individual capture of newly emerged adults from nymphal rearing pots for release into oviposition cages.

Two models were evaluated. Model 1 was described in 1997 and features a tray (61.6 x 31.4 cm) with host plant roots for nymphal development. *B. ruziziensis* requires approximately 3 weeks for roots of shallowly planted stems to descend through the network of holes in the bottom of the tray and form a mat between the planted tray and a second tray stacked below. Three weeks after infestation of the roots with eggs, the tray is fitted on top of a 10 cm tall walled wooden frame that allows roots to descend. When adults are ready to emerge the tray is fitted on top of a 32.3 cm tall rectangular aluminum frame elevated by 4 feet (15.5 cm). The frame is wrapped with a sheet of black nylon mesh to form walls that reduce light, raise humidity and prevent escape of the insects. An identical tray that contains oviposition substrate slides in at the bottom of the frame. Stems of potted host plant enter the unit through the side to provide adults with food.

Model 2 has 2 components. The nymphal box is the same 10 cm tall wooden frame that allows the roots to descend. Newly emerged adults exit the box through a slit (1.5 cm tall by 40.0 cm long) in the wall, attracted to the relative light of the oviposition chamber attached alongside. The oviposition chamber is identical to the aluminum frame above but with the nymphal rearing tray replaced by a lid.

The efficiencies of both models to produce adults was measured. Approximately 3 weeks after planting, roots were infested with 500 eggs. Adults were collected daily from the oviposition chamber in Model 1. In this study Model 2 only comprised the nymphal box; adults exited into a large emergence cage that surrounded the unit. Only adults that emerged through the slit into the emergence cage were collected. Six paired repetitions were carried out between October 1997 and May 1998.

Results: A major limitation of Model 1 was the problem of overwetting the oviposition substrate while watering the roots above. This will probably lead to a decrease in oviposition that Model 2 would not experience. In Model 1 roots deteriorated more rapidly than Model 2 probably due to increased exposure on the top of the aluminum frame. Model 2 encouraged adults to leave toward the light of the exterior while protecting the roots from exposure. Twice as many adults were recovered from Model 2 than Model 1 (Table 13) The 60% efficiency of adult production from eggs in Model 2 is similar to that of CIAT's mass rearing colony. Observations indicate, however, that nymphal development was extended by 1 - 1.5 weeks and adult emergence was less synchronous compared with the mass rearing colony.

Table 13. Production of adults from 500 eggs in 2 models of a rearing unit.

	N	Mean Adults Recovered	Mean Proportion M:F	Mean Efficiency
Model 1	6	142 a	1.24	28.5% a
Model 2	6	300 b	1.06	60.0% b

Within columns, values with different letters are significantly different at P<0.05.

Discussion: While this unit probably is not appropriate for mass rearing, it does offer an alternative for other study situations. Despite the limitations of the early rearing unit (Model 1), it is currently being used as a tool to support biological studies of *Aeneolamia reducta*, *A. lepidior*, *Mahanarva* sp, and *Zulia pubescens* in different regions of Colombia. The improved unit (Model 2) is twice as efficient in adult production owing to better protection of nymphal feeding sites (roots) and the method of using light to lure adults into nearby oviposition chambers. Continuing studies are underway to measure production of both adults and the second generation of eggs from the complete Model 2 (nymphal box alongside oviposition chamber). The egg collections will confirm that the design eliminates the need to individually capture each newly emerged adult.

Biology and habits of four lowland spittlebug species (D. Peck, W. Medina, B. León, Y. Ballesteros, C. Gallego and A. Pérez)

Rationale: An inadequate understanding of the basic biology and behavior of most spittlebug species probably contributes to their ineffective management. For 4 of 6 lowland pasture species in Colombia, we lack information on all aspects of biology that are relevant to the refinement of management tactics. *A. varia* has been well-studied given its pest status in sugar cane, while studies of *Z. colombiana* have been carried out previously at CIAT.

Methods: Studies to characterize the biology, habits and morphology of spittlebug life stages were initiated for *A. lepidior* in Sucre, *A. reducta* in Córdoba, *Mahanarva* sp. in Caquetá and *Z. pubescens* in Meta. Comparative methodologies were employed to study egg development (see following section), morphological characterization of the life stages, duration of the life stages, oviposition sites and reproductive biology.

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 nymph and presence of the molting exuvia we determined advancement to the next instar. Adult longevity was measured by daily assessing mortality in small groups of teneral adults (~6) confined over host plants under sleeve cages. To determine oviposition sites groups of field-collected females (10-15) were confined under sleeve cages over plants with the presence of litter and surface of soil sieved to facilitate extraction of eggs. Eggs were recovered from the soil, litter, beneath the leaf sheath or stuck to the plant, and inserted into plant tissue. Reproductive biology was assessed by enclosing teneral adult females in large (15.0 x 2.5 cm) petri dishes lined with moist filter paper for oviposition, host plant stems in a vial, and the continual presence of 1 male. These arenas were observed continuously for 3 days or until first oviposition to measure precopulation and preoviposition periods, number and duration of copula, and egg laying rates.

Table 14. Duration of life stages of *A. reducta* and *Mahanarva* sp.

		Duration of Life Stage (Days)							
		Nymphal Instar						Adult Sex	
Species		1	2	3	4	5	1 - 5	M	F
A. reducta	Mean	5.9	5.4	5.3	4.8	4.5	26.1	5.5	6.2
	Min-Max	5-7	5-6	4-7	4-6	4-5	24-28	5-7	5-8
	N	72	65	45	43	43	43	9	14
Mahanarva sp.	Mean	6.9	11.4	10.8	7.3	8.2	44.2	7.5	7.7
	Min-Max	4-11	7-15	8-14	6-10	8-9	40-48	3-11	3-11
	N	30	28	25	25	20	20	19	41

Results: Partial results are presented for *A. reducta* and *Mahanarva*. Certain differences in the biology and habits of these 2 species illustrate the variation among this pest complex. These differences include time to complete nymphal development, relative time in each instar, adult longevity and propensity to lay eggs in litter (Tables 14, 15).

Table 15. Oviposition sites of *A. reducta* and *Mahanarva* sp.

	% Eggs Recovered (No. Eggs)			
Species	Soil	Litter	Plant Surface	Plant Tissue
A. reducta	90.1 (1308)	8.5 (122)	0.9 (13)	0.0 (0)
Mahanarva sp.	77.8 (552)	19.9 (141)	2.4 (17)	0.0 (0)

That *Mahanarva* requires 1.7 times more time to complete nymphal development probably reflects the extreme size differences between the 2 species. The study on reproductive biology in *Mahanarva* yielded detailed data on time to first copulation, time to first oviposition and daily oviposition rates (Table 16). Under these experimental conditions, *Mahanarva* females mate on average only 1.6 days after emergence and laid their first eggs after only 2.75 days; these brief periods match the short duration of the adult life stage.

Table 16. Reproductive biology of *Mahanarva* sp.

Species		Hours to Copulation			Duration of Copulation			Hours to Oviposition			Eggs per Oviposition		
		1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Manaharva sp.	Mean	38.6	48.9	60.7	6.7	1.7	1.0	55.9	65.9	70.1	10.8	5.3	4.4
	Min	47.0	58.3	68.4	9.5	3.3	2.3	68.2	75.0	72.0	15	9	7
	Max	30.5	38.5	50.0	2.0	0.6	43.4	56.5	67.2	6	2		
	N	20	20	17	20	20	17	20	20	6	20	20	5

Discussion: Additional data for 4 species are being collected on descriptive morphology of the life stages including measurements of head capsule width, stylet length, wing pad length (nymphs), body length and body width (adults). New information from these 4 species will be compared with relatively well-known species such as *A. varia* to further our understanding of similarities and differences among members of this pest complex.

Comparative egg morphology and development (D. Peck)

Rationale: Combating spittlebug outbreaks in seasonally dry regions will probably depend on the identification and suppression of the first generation. An understanding of the biology of the egg stage is critical to linking population data with climactic data and for building predictive models of the phenology and synchrony of early season spittlebug populations. This information is also absent for our understanding of the basic biology and behavior of many species and will complement studies on the determinants of egg diapause. Comparative studies will broaden our understanding of variation and similarities among this pest complex.

Methods: Studies were initiated to describe and compare egg development stages among 4 species of spittlebug under controlled conditions of temperature and humidity. The objectives were to measure the duration, size and external morphology of development stages in 4 species, determine the stage that is prolonged in diapause, and compare the variation in development within and between 3 genera of spittlebugs. Eggs of *A. lepidior, A. reducta* and *Mahanarva* sp have been examined thus far. Eggs were obtained from groups of field-collected females enclosed in petri dishes lined with moist filter paper that served as oviposition substrate. After 24 hours of oviposition, eggs were incubated at CIAT under continually moist conditions (27°C, without light). Individual eggs were followed daily to record changes in development; at certain developmental stages measures of width and length were made with an ocular micrometer.

Results: Certain externally visible changes associated with egg development were held in common by *A. lepidior, A. reducta* and *Mahanarva* sp. All progressed through the 4 generalized stages (S1, S2, S3, S4) described for other species. S1 eggs were recently laid, pale yellow, and showed no 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. In S3, the chorion split along the hatching line exposing the dark black surface of the operculum. A pale red pigment spot was visible under the midpoint of the operculum; in the late S3 stage this spot migrated to 3/4 of the distance to the posterior pole. Finally, eggs in S4 revealed 2 pairs of red pigment spots (ocular and abdominal) associated with the nymph about to hatch. The proportion of developmental time spent in each stage was similar between *A. reducta* and *Mahanarva*: 35.5, 10.5, 26.0 and 28.0% in stages S1-S4 respectively (Table 17). Due to low sample size *A. lepidior* was not comparable The largest species, *Mahanarva* sp., required significantly more time for development but only 1.2 days more than *A. reducta*.

Table 17. Duration (days) of egg developmental stages for 3 species (27°C, 100% RH, without light).

	Species					
	Aeneolamia lepidior		*Aeneolamia reducta*		*Mahanarva* sp.	
Stage	Mean (n)	Min-Max	Mean (n)[1]	Min-Max[1]	Mean (n)	Min-Max
S1	10.1 (6)	6.0-18.5	5.7 (92)	5.0-8.0	5.9 (104)	5.0-7.0
S2	2.1 (6)	1.0-4.0	1.6 (91)	0.0-8.0	1.9 (102)	1.0-5.0
S3	2.4 (6)	0.0-5.0	4.1 (88)	0.0-5.0	4.5 (102)	2.0-6.0
S4	4.0 (6)	3.0-5.5	4.4 (86)	3.0-7.5	4.7 (100)	3.0-9.0
Total	18.6 (6)	13.0-23.0	15.8 (86)	15.0-22.0	17.0 (100)	15.0-23.0

[1] Does not include outlier of 18.0 days in S1.

Certain details of development varied among species. In both *Aeneolamia*, a pale red pigment spot was visible late in the S1 stage, appearing from 1/2-3/4 and 1/4-1/2 down from anterior to posterior pole for *A. lepidior* and *A. reducta* respectively. This spot was absent in *Mahanarva* sp. during S1. In S2, the red spot was visible under the midpoint of the operculum in most *Aeneolamia* but was visible in only a minority (~10%) of *Mahanarva* sp. For all 3 species, size measures demonstrated that the eggs swelled between S2 and S3 probably due to the opening of the chorion and the intake of water as development proceeds. Differences in egg sizes among species corresponded with the clear size differences in the nymphal and adult life stages of these species Among the eggs incubated in this study, no evidence for diapause was observed in *A. lepidior*, *A. reducta* or *Mahanarva* sp (n=102, 826, 743 eggs respectively). Previous studies, however, reported diapause in the S2 stage for *A. reducta*, with eclosion occurring 29-206 days after oviposition. Although an extended S1 stage is not reported in spittlebugs (diapause in known to occur in S2), among eggs followed individually in this study 2 *A. lepidior* and 1 *A. reducta* demonstrated an extended S1 stage of 15.0, 18.5 and 18.0 days respectively Table 18).

Table 18. Size (mm) of egg developmental stages for 3 species.

	Species					
	Aeneolamia lepidior		*Aeneolamia reducta*		*Mahanarva* sp.	
Stage	Mean (n)	Min-Max	Mean (n)	Min-Max	Mean (n)	Min-Max
Length						
S1	0.92 (100) a	0.86-1.01	0.84 (100) a	0.74-0.90	1.14 (100) a	1.09-1.23
S2	1.00 (3) b	0.00-1.00			1.16 (90) b	1.09-1.26
S3	0.99 (3) b	0.97-1.00	0.87 (100) b	0.78-0.97	1.17 (100) c	1.09-1.29
S4	0.99 (5) b	0.97-1.01	0.90 (100) c	0.81-0.97	1.20 (80) d	1.12-1.29
Width						
S1	0.29 (100) a	0.25-0.33	0.27 (100) a	0.23-0.30	0.35 (100) a	0.30-0.38
S2	0.30 (3) a	0.29-0.30			0.35 (90) a	0.30-0.38
S3	0.33 (3) b	0.30-0.36	0.29 (100) b	0.26-0.33	0.38 (100) b	0.33-0.42
S4	0.35 (5) b	0.33-0.36	0.00 (100) c	0.29-0.36	0.42 (80) b	0.38-0.45

Within species, length and width measures with different letters are significantly different at $P<0.05$.

Discussion: These methods show that it is feasible to compare certain taxonomically variable characteristics of egg development and morphology among the spittlebug species under study. These measures include size and physical description, externally visible changes that accompany development, the stage extended in diapause, and the duration of stages under controlled conditions. Along with parallel studies on oviposition behavior and reproductive biology (see previous section), this new information will offer the detailed basic understanding currently lacking in many spittlebug species.

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

Rationale: Vibrational communication is one fundamental aspect of adult spittlebug behavior that has not been examined in detail beyond sound recording and personal observations that verify its existence. Among related insects, bioaccoustics is 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. Our research objectives are to establish the recording methodology, characterize the different acoustic signals and determine the role they play in reproductive biology.

Methods: Much of 1998 was dedicated to designing a methodology to record, visualize and interpret the sounds produced by these insects. Apparatus were established to capture both air-borne and substrate-borne signals. Fifty hours of recordings were made and analyzed to verify existence of the signals in *Z. colombiana* and to determine the periods and conditions of highest activity. A small colony of this species was established and maintained throughout this period to provide adults for studies. *Z. colombiana* was chosen as the focus species because it is larger and more docile than *A. varia*.

Results: Two methods were utilized to capture communication signals, a phonographic cartridge and a microphone. In each case the signal was transferred to a MacAdios acquisition card that amplified and digitized the signal for electronic storage on a computer. To record vibrations directly, insects were housed on the surface of a *Brachiaria* leaf held taut between two supports (Figure 4). A phonographic needle of ceramic crystal in direct contact with the plant surface recorded the substrate-borne vibrations initiated by the insects as small electrical signals. To capture the aerial component of the signal, a microphone (Azden ECZ 990) was employed when the insect was confined in a small space sufficiently isolated from environmental noise.

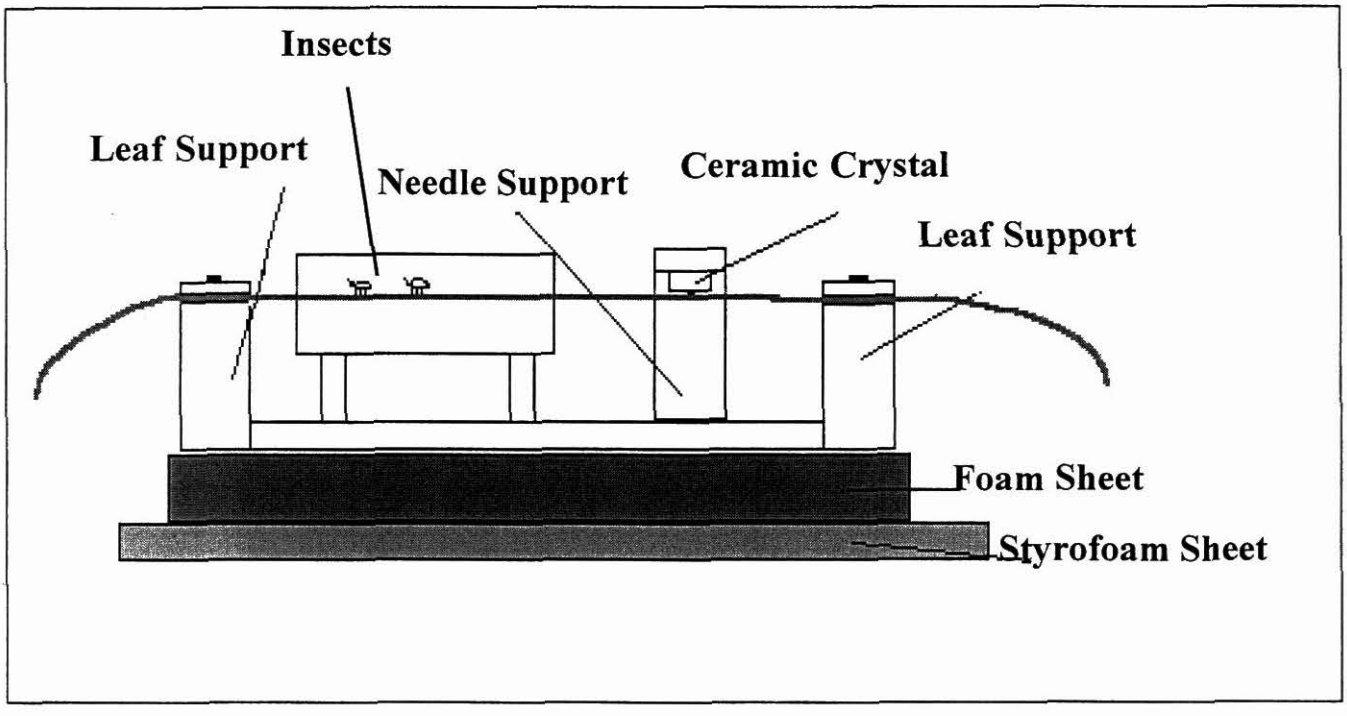

Figure 4. *Brachiaria* leaf support for recording adult vibrations by means of ceramic crystal phonografic needle.

Three substrate recordings were made continuously from 16:00 until 09:00 the next day using 3 groups: 3 males, 3 females and 2 males with 2 females. The highest acoustic activity occurred in the mixed sex group and during the hours of 16:30-18:00. Analyses of the phonographic recordings from the mixed sex group demonstrated patterns that comprised a series of pulses spaced every ~1.5 seconds (Figure 5). The form of the pulse varied in 2 distinct ways but it is unknown if that variation was due to different calls or different individuals.

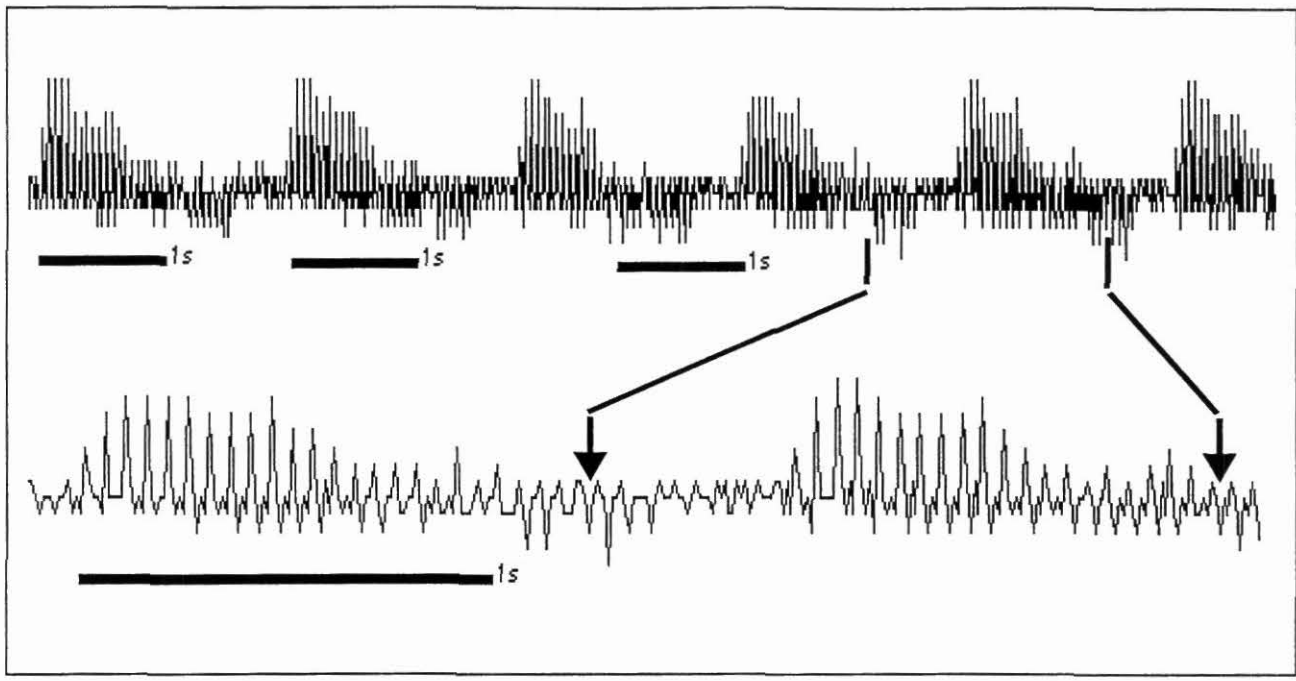

Figure 5. Fifteen second fragment of a recording made from a group of *Z. colombiana* adults (2 male and 2 female). Arrows indicate the repetitive pulse attributed to tymbal variation.

The ceramic crystal of the phonographic cartridge is sensitive to electromagnetic interference, therefore the recordings are less amplifiable. Microphone recordings, however, can be amplified to a greater degree and may be of utility under certain circumstances.

Discussion: Acoustic signals have been verified for *Z. colombiana* and can be recorded directly via substrate or indirectly via air. Substrate borne signals are known to be more biologically relevant than air-borne, yet limitations of the recordings techniques may make use of the microphone more appropriate under certain circumstances. Future studies are planned to refine the techniques and increase the fidelity of the final recordings.

With the technical phase of this project complete we are describing the physical properties and circumstances of the different calls and initiating studies on the relevance of vibrational communication for the reproductive biology of *Z. colombiana*.

Studies on the effects of environmental conditions on spittlebug egg development

Highlights

- Studies on seasonal changes in egg diapause in 4 regions were initiated
- No role of drought as a preovipositional cue in egg diapause was detected

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

Rationale: Egg diapause permits synchrony between spittlebug populations and the wet season during which growth and development are possible. In some species it has been shown that the incidence of diapause (i.e. the percentage of eggs that are not immediately developing) is lowest among early season females (first generations) and highest among late season females (last generation). A description of seasonal changes in diapause incidence - and how it varies

among species and regions - is therefore an essential element in understanding the synchrony and timing of early season nymph populations.

Methods: We continued studies initiated and reported in 1997 that were designed to measure diapause incidence among females occurring at the same farm sites in 4 regions of the country where there are concurrent population studies. To examine differences among species and regions, 3 species were each sought in 2 regions: *A. reducta* in Córdoba and Sucre, *A. varia* in Meta and Caquetá, and *Z. pubescens* in Meta and Caquetá. Depending on availability at each collection date, approximately every month during the active spittlebug season groups of 1-34 females were collected from each half of 3 population study plots (i.e. 6 groups) and allowed 3 days to oviposit in large petri dishes lined with moist filter paper that served as oviposition substrate. After express delivery to CIAT, eggs were maintained under moist conditions in incubation (27°C, without light) and scored for eclosion of nymphs twice weekly.

Results: Limited availability of insects and personnel prohibited collections in certain months and excluded studies of *Z. pubescens* in Meta (Table 19). Incidence of diapause was extremely low in the surveyed sites and species, but the completed surveys only consisted of first or second generation females. Surveys will continue through the end of the year to overlap with late season generations of females where we expect to encounter a higher incidence of diapause eggs.

Discussion: A continuation of this study through the end of 1998 will give us an understanding of changes in the incidence of diapause in correlation with changes in seasonality (precipitation). It will also broaden our understanding of the variation in diapause "strategies" among species and across different geographic ranges for the same species. This study, in combination with concurrent studies on population dynamics and the determinants of diapause (following section), will offer a foundation for the development of integrated pest management tools such as models to predict the magnitude, timing and synchrony of early season outbreaks.

Table 19. Incidence of non-diapause eggs and duration of egg development in 3 spittlebug species based on site and month.

	Mean % Diapause/ Mean Days to Eclosion of all Eggs[1]					
	A. reducta		*A. varia*		*Z. pubescens*	
Date	Córdoba	Sucre	Meta	Caquetá	Meta	Caquetá
Jul 97	-	-	99.1/18.1	-	-	-
Aug 97	-	-	99.7/17.2	-	-	-
Feb 98	-	-	-	99.3/18.2	-	99.3/15.1
Mar 98	-	-	-	98.8/17.9	-	100.0/18.0
Apr 98	-	-	-	75.5/25.4	-	100.0/23.0
May 98	-	92.0/42.0	99.4/18.8	100.0/15.4	-	100.0/15.0
Jun 98	-	97.7/18.5	99.3/18.9	99.9/17.3	-	100.0/19.8
Jul 98	99.4/19.1	-	-	Pending	-	100.0/16.4
Aug 98	Pending	Pending	Pending	Pending	-	100.0/16.0

[1]Mean based on ovipositing female groups (1-6 depending on availability in field and oviposition success). Eggs obtained per group varied from 158-373 (Ar), 15-745 (Av) and 2-122 (Zp)

Preoviposition determinants of diapause in spittlebug eggs (U. Castro and D. Peck)

Rationale: 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*. Since establishment of the methodologies in 1997 we have completed an experiment with drought and initiated studies with plant phenology. 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: We assessed the effect of drought stress in a completely random block design (12 repetitions) that included all 4 treatment combinations of water stress (yes or no) and infestation (yes or no). *B. ruziziensis* plants (were allowed 2 weeks to establish in pots (17.5 cm diameter and height) after which 100 eggs about to hatch were added to infested plants. Drought was imposed on water stressed plants (receiving only 1/4 the water of control plants) after a 4-day window to permit spittle mass establishment. Treatment means in each repetition means were based on 5 pots each.

Adults from each treatment repetition were transferred to separate oviposition chambers. At peak emergence, females were allowed 3 days to lay eggs that were subsequently incubated (27°C, without light, 100% RH) and assessed twice weekly for eclosion. The effect of experimental drought conditions on diapause was measured as % eggs entering diapause and days until eclosion. Nymph mortality (% successful adult emergence) and plant dry weight were measured to confirm an effect of water stress on the host plants.

Results: Nymph mortality was higher on drought stressed plants ($P>|t|=0.001$) than controls; mean mortality was 90.07 and 78.15% respectively. Mean plant dry weight at the end of the experiment was 5.7 and 8.3 g/5 pots for drought-stressed and control plants respectively, but not significantly different ($P>|t|=0.535$). Drought stress had no significant effect on the incidence of diapause in eggs of surviving adults or on mean days to eclosion of all egg types (Table 20). Although not significantly different, mean days to eclosion for diapause eggs from adults subjected to water stress was 21 days longer than those in the control.

Table 20. Effect of drought stress during the nymphal stage on development of eggs from surviving adults.

	Mean (Range) [1]	Mean Days to Eclosion (range) [1]		
Treatment	% Diapause	Non Diapause	Diapause	All Eggs
Drought	2.89 (0.0-14.0)	18.48 (13.5-22.2)	67.33 (38.5-134.2)	19.34
Control	2.62 (0.0-12.9)	19.23 (16.8-21.6)	46.87 (34-76.7)	19.85

[1] Within columns, means are not statistically different at $P<0.05$.

Discussion: Water stress as applied to nymphs in this experiment did not influence diapause in eggs produced by the adults. Results suggest that duration of diapause - in addition to incidence - is a component that must be examined in future studies. In combination with other environmental factors, however, drought could still be implicated in the regulation of diapause. Additional studies using this successful experimental methodology will assess the effect of photoperiod and phenology, or age of the nymphal host plant, on diapause.

Studies on comparative population ecology of spittlebugs in four lowland regions

Highlights

- Field studies show greater population synchrony and abundance fluctuations in seasonally dry areas compared to continuously humid.
- North coast populations of *A. reducta* in *Bothriocloa pertusa* pastures completed 3-4 generations in 1997.

- Suppression of the initial generation or outbreak of spittlebugs may a be a valuable management tactic in seasonally dry areas.
- Six classes of spittlebug natural enemies were encountered including a parasitic fly that may be the first neotropical report.

Comparative population dynamics of spittlebugs: local and regional variation in species composition, abundance, phenology and synchrony (D. Peck, Y. Ballesteros, M. Barrios, C. Gallego, W. Medina, J. Rojas, L. Rojas, J. Rubio)

Rationale: There are very few detailed site-specific studies of 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 the determination of all life stages. Identical survey methodologies employed at different sites allow the comparison of population performance measures such as abundance, phenology, synchrony, voltinism and species composition. An understanding of the patterns and variation in these measures is critical to assess and predict pest status.

Methods: In 1998 we continued population surveys initiated at the start of 1997 in 4 lowland sites in departments of Colombia most affected by the insect (Table 21). As reported in 1997, 3 0.5 ha plots were in different pastures on each of 4 farms representative of the regions. Nymph surveys comprised counts in 2 0.25 m^2 cuadrats in each quarter plot. Adults surveys comprised 50 sweeps of an insect net in each quarter plot. Surveys were performed approximately twice weekly during the spittlebug season but only every 2 weeks during the dry season. All nymphs were determined to instar and adults to sex and species.

Results: Data were analyzed from January 1997 through June 1998; a total of 6384 nymphs and 41,656 adults were collected and analyzed. There are marked difference between Caquetá, the continuously humid site, and the 3 seasonally dry sites. As reported in the 1997, 1 species dominated in Córdoba, Sucre and Meta, while pastures in Caquetá were shared by 3 species, 2 in high abundance. Córdoba and Sucre showed the greatest population fluctuation over the period of study, based on the disappearance of the insect during the dry season months and extreme abundance peaks (Figure 6). Meta did not present such extreme population fluctuations probably due to a less severe and prolonged dry season. Caquetá demonstrated the presence of spittlebug nymphs and adults throughout the entire study period corresponding to the lack of a pronounced dry season.

Table 21. Population survey sites and species composition.

Site	Dominant forage	Adult Species Composition (%)					
		Aeneolamia		*Mahanarva*		*Zulia*	
		lepidior	*reducta*	*varia*	sp.	*colombiana*	*pubescens*
		97/98	97/98	97/98	97/98	97/98	97/98
Córdoba: Montería	*Bothriocloa pertusa*	<1/*	100/100	-	-	-	-
Sucre : Corozal	*Bothriocloa pertusa*	*/*	100/100	-	-	-	-
Meta :V/cencio	*Brachiaria decumbens*	-	1/4	98/96	-	-	1/<1
Caquetá: Florencia	*Brachiaria decumbens*	-	-	65/66	1/2	*/*	34/33

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

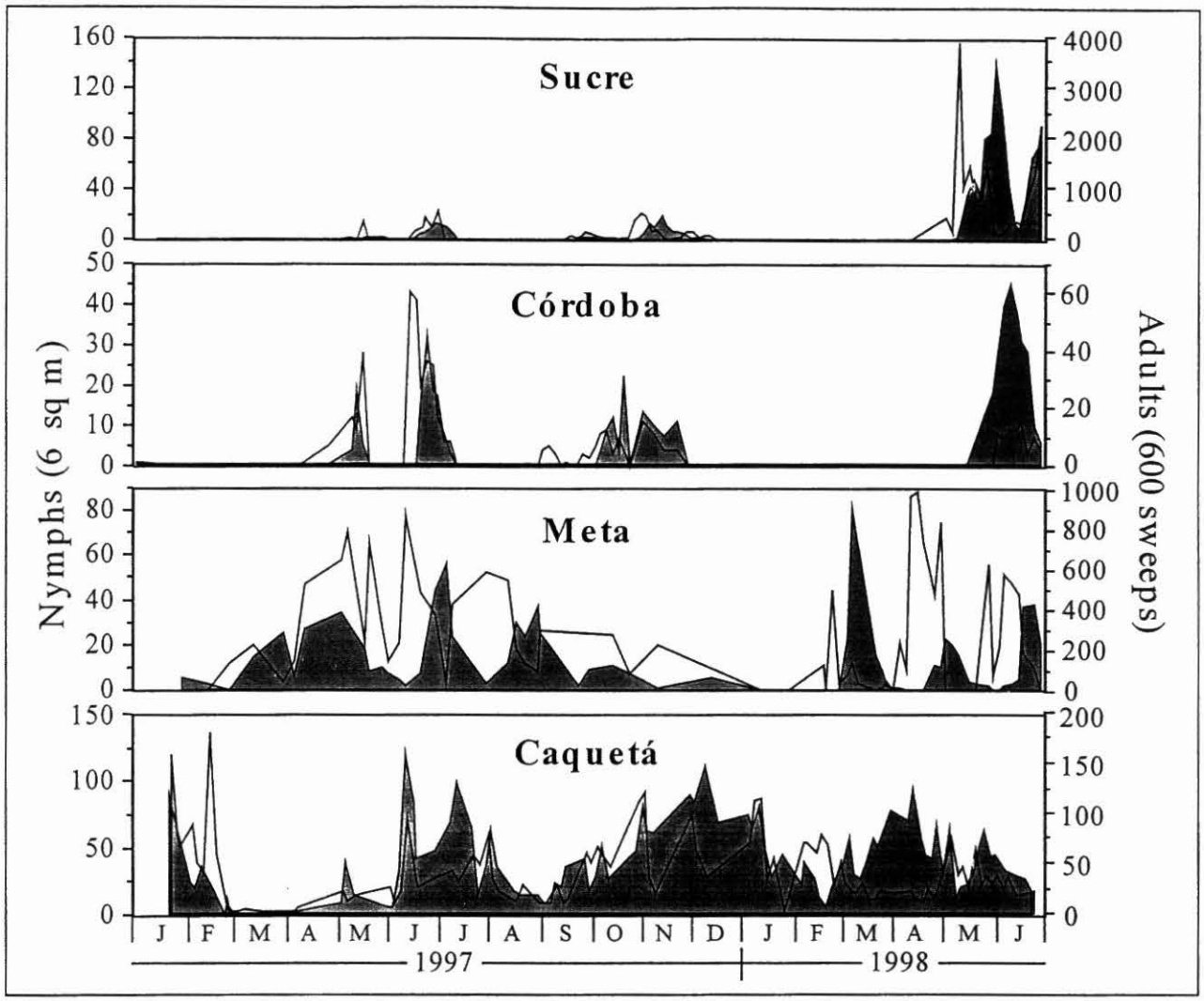

Figure 6. Seasonal fluctuations of total nymph and adult spittlebug abundance in 4 survey sites. Totals are the sum of 3 plots at each site. Córdoba and Sucre are *Bothriocloa pertusa* pastures; Meta and Caquetá *Brachiaria decumbens*.

Spittlebugs survive the dry season months as drought-resistant diapause eggs. Concurrent experiments have noted extremely low rates of diapause in eggs from field-collected *Z. pubescens* females in Caquetá (see Activities 2.1.1 and 2.1.2). Although we know some species may be incapable of diapause, continually humid conditions in this region may not stimulate their production. Although *A. varia* and *Z. pubescens* were present all year, there were fluctuations that may relate to subtle climate or precipitation changes. *Z. pubescens*, in particular, demonstrated population synchrony in the form of broad abundance peaks (Figure 7).

In addition, the period of peak *Z. pubescens* abundance occurred during a period of *A. varia* decline (October-January). Therefore climate changes might provoke diverging species population responses that manifests as a temporal pattern of resource partitioning. Other studies suggest a spatial partitioning owing to diverging habitat preferences.

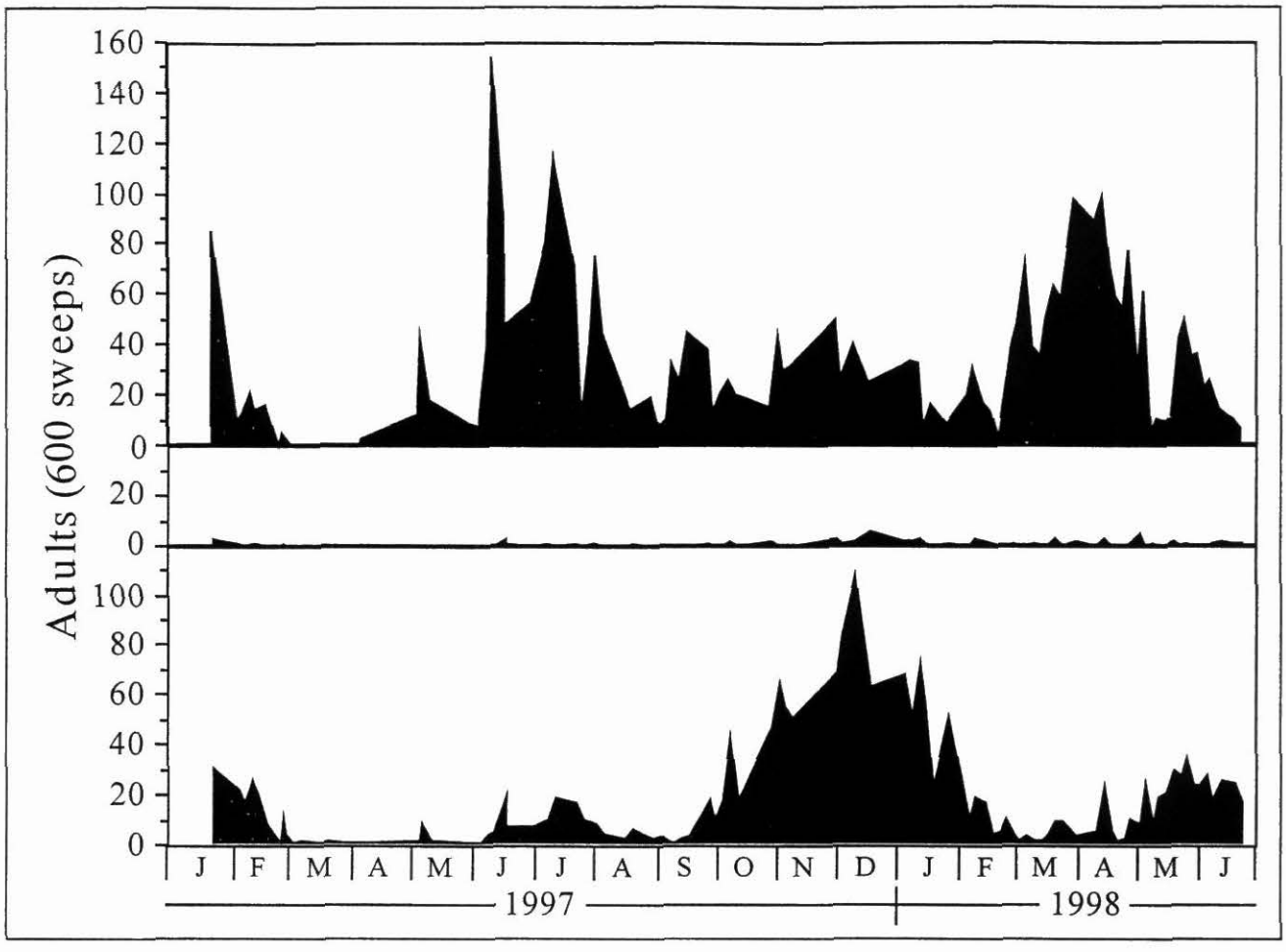

Figure 7. Seasonal fluctuations of spittlebug adults in the Caquetá survey site. Totals for each species are the sum of 3 plots.

Population synchrony is much more pronounced in the seasonal sites. Eggs eclose en masse at the start of the rainy season initiating a synchronous first population that can give rise to very large subsequent generations. In such regions, control of the first generation foci might be a key strategy for management. In the Sucre site, for instance, dry season fires in 2 plots completely eliminated local spittlebug populations. Adults began reappearing in early June (despite the absence of local nymphs), coincident with the maturation of local nymph populations in the 3rd site. This suggests that focal outbreaks can rapidly invade surrounding areas through the movement of adults.

Spittlebug phenology and synchrony can be interpreted in detail at the seasonally dry sites with a single dominant species. Determination of instars has allowed us to lend a high level of resolution to local population dynamics and accurately assess the number of generations a year. Córdoba and Sucre, for instance, each showed 2 discrete generations in May - July, followed by overlapping third and fourth generations in September-November (Figure 8). There was surprisingly little variation in phenology between the 2 sites, but the first 3 generations in Córdoba peaked approximately 1 week earlier.

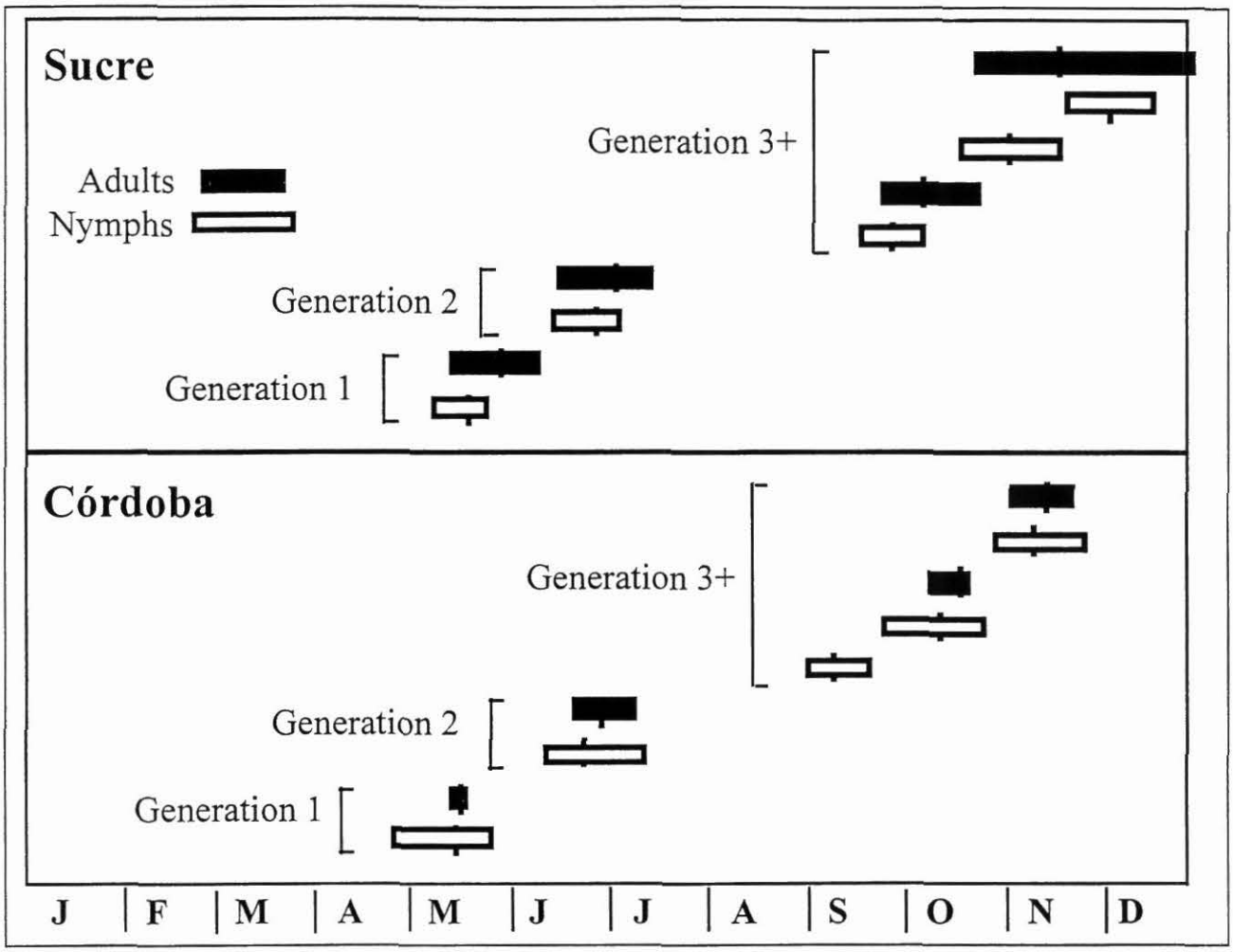

Figure 8. Phenogram of *A. reducta* populations *Bothriocloa pertusa* pastures of 2 survey sites. Horizontal bars indicate the period of occurrence of the life stage; vertical lines indicate the accumulation of 50% of the individuals of that group (accumulated insect days).

Discussion: Complete analyses will be performed after the accumulation of 2 sequential years of data. All sites will be analyzed by each life stage to assess variation in population performance measures at the farm, regional and species levels. A critical analysis still pending is the correlation of these measures with precipitation patterns to determine if climactic data can assist in predicting the timing and magnitude of early season spittlebug outbreaks.

Identification, abundance and phenology of natural enemies associated with spittlebugs (D. Peck)

Rationale: Natural enemies of spittlebugs in Colombia have not been assessed on a broad level. Detailed population surveys in 4 regions of the country, however, have permitted the simultaneous collection of data on the incidence of predators, parasitoids and pathogens. In addition to new information on abundance, seasonality and life stages attacked, we hope to encounter new natural enemies and gauge their future potential as biological control agents.

Materials: Natural enemies were collected as part of spittle mass and sweep net surveys in population dynamics studies at 4 lowland sites. Other observations of natural enemies were recorded over the course of other activities.

Results: Six classes of natural enemies were encountered in Colombia (Table 22). Predaceous larvae of *Salpingogaster nigra* (Diptera: Syrphidae) were found in spittle masses where they attacked nymphal and teneral adult occupants. Adult flies were recorded from sweep net surveys and pupae were occasionally encountered on grass stems. Adult and nymph cadavers that were encountered in population surveys were sent to CIAT for isolation and identification of any associated entomopathogenic fungi. The genera *Metarhizium* and *Paecilomyces* have been identified thus far.

Parasitic nematodes (Nematoda: Mermithidae) emerged from nymphs and adults upon immersion in alcohol for preservation of samples. Among the nymphs, only instars 4 and 5 were recorded with nematodes. The presence of parasitic mites was recorded when samples from adult surveys were being assessed under the microscope. As many as 4 were found on the same individual.

Two new enemies were encountered in 1998, but each of them on only a single occasion. An adult robber fly (Diptera: Asilidae) was found feeding an adult *Z. colombiana*. Robber flies are generalist aerial predators known to include adult spittlebugs in their diet. Two minute larvae of a parasitic fly (Diptera: Pipunculidae) were dissected from adult *Z. colombiana* after reproductive capacity of CIAT's small colony fell. This may be the first report of this parasite in neotropical spittlebugs. Parasitism only occurs in adults and is known to cause atrophy in the reproductive system of females. The adult spittlebug hosts had originally been wild caught on CIAT campus. Unfortunately, numerous dissections and searching have not turned up additional cases of parasitism.

Table 22. Incidence of spittlebug natural enemies in Colombia.

Site	Enemy	Spittlebug species attacked	Life stage attacked	J	F	M	A	M	J	J	A	S	O	N	D
Caquetá	Nematodes	Mah.	Nymph	■	■						■	■	■		
	Mites	Av, Mah, Zc, Zp	Adult	■		■	■	■	■	■	■		■	■	■
	S. nigra		Nymph	■					■	■			■	■	
	Metarhizium	Av, Mah, Zp	Ad, Nym	■	■				■						
	Paecilomyces	Zp	Ad, Nym	■	■										
Cauca	*S. nigra*	Zc	Nymph				■				■	■			
	Asilidae	Zc	Adult									■			
	Pipunculidae	Zc	Adult	■											
Córdoba	Nematodes	Ar	Adult					■							
Meta	Nematodes	Av	Ad, Nym				■	■	■	■	■				
	Mites	Av				■	■	■	■	■				■	
	S. nigra							■	■						
Sucre	Nematodes	Ar	Adult						■						
	Mites	Ar	Adult					■	■	■					

Discussion: The highest abundance and diversity of natural enemies resides in Caquetá and could be correlated with any of the factors that distinguishes this region: high precipitation, lack of a dry season, high spittlebug diversity, and presence of spittlebugs all year round. The same argument matches the relative scarcity of enemies in Córdoba and Sucre: severe dry season and 1 dominant spittlebug species. The identifications of all natural enemies is being investigated. Among the different classes, fungal entomopathogens are currently being assessed for their biological control potential. *S. nigra* is a well-known pan-neotropical predator of grassland spittlebugs that might be augmented through certain cultural practices that reduce limits to the abundance of other life stages (e.g. pupation safe sites, adult food sources).

Parasitic nematodes are known to be abundant in certain sugar cane systems yet they have not been evaluated for biological control potential. Finally, the discovery of a parasitic Pipunculidae - possible the first report for neotropical spittlebugs - offers a new agent for future consideration and suggests that other natural enemies have not yet been registered.

Studies on response of spittlebugs to grass/legume associations (D. Peck, J. Correa, W. Puentes, C. Ramirez, G. Ruíz)

Highlights

- Spittlebug abundance (density) is similar between *Brachiaria* pastures with and without *Arachis pintoi,* but insect load in the associated pasture increased by 22%.
- Diverging habitat preferences may contribute to resource partitioning in two sympatric species.

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. 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. In 1997 we reported initial results of a study on the response of spittlebugs to grass/legume associations in terms of abundance. We now report a complete analysis of that study plus results from an additional field study on insect load in terms of abundance per availability of resource.

Methods: Differences in spittlebug abundance were examined at 6 on-farm trials in Caquetá that each featured paired plots of *Brachiaria* and *Brachiaria/Arachis pintoi* established earlier in other IP-5 activities (Table 23). Plots of 0.5 ha were marked in each treatment and sampled monthly in 1997 for nymph and adult populations. Spittle mass and sweep net surveys consisted of 20 counts in 25 x 25 cm cuadrats and 10 series of 20 sweeps, respectively, per treatment. All nymphs were scored to instar and adults to species and sex. Five additional surveys on 4 farms were carried out in 1998 to record adult abundance together with availability of food resources. Number of *Brachiaria* stems were counted in 20 25 x 25 cm cuadrats per treatment, a measure of resource availability that substituted for and was highly correlated with wet weight ($r^2 = 0.794$).

Table 23. Description of experimental sites and species composition

Site	Date Estab	Forage-Grass	Adult Species Composition (%)			
			Aeneolamia varia	*Mahanarva* sp.	*Zulia colombiana*	*Zulia pubescens*
Southern Zone:						
Diamante	1995	*B. decumbens*	49.9	2.6	0.3	47.2
Norglandia	1995	*B. decumbens, humidicola*	35.4	3.2	0.0	61.4
Villa Clarita	1995	*B. decumbens, brizantha*	35.6	0.1	0.0	64.3
Northern Zone:						
Caña Brava	1995	*B. humidicola*	100.0	0.0	0.0	0.0
Higuerón	1989	*B. decumbens*	77.6	0.6	17.3	4.5
Primavera	1995	*B. decumbens*	43.4	0.0	0.0	56.6
Overall:			48.1	2.0	3.2	46.7

Results: Abundance did not differ between treatments for any spittlebug life stage or species when farm was used as the unit of repetition (Table 24). The responses of spittlebugs at different farms was highly variable. In Norglandia, for instance, nymphs were almost always

more abundant in the associated pasture while in Villa Clarita they were more abundant in the monoculture (Figure 9). In many cases these differences were consistent over the entire year or half year. In the 3 farms with highest spittlebug populations (Diamante, Norglandia, Villa Clarita), the treatment preferred by adults switched mid year; Norglandia had the opposite response of the other two farms (Figure 9). These results demonstrate that there are other characteristics of the habitat more important than *Arachis* association in driving spittlebug abundance.

Table 24. Probability values (paired t-test) for differences between monoculture and associated pastures (n=6).

Life Stage	Prob>|t|
Total Nymphs	0.562
Instar 1	0.563
Instar 2	0.530
Instar 3	0.773
Instar 4	0.675
Instar 5a	0.462
Instar 5b	0.792
Total Adults	0.256
A. varia	0.439
Mahanarva sp.	0.545
Z. colombiana	0.268
Z. pubescens	0.870

Separate analysis of the 2 most abundant species showed that *A. varia* and *Z. pubescens* appeared to partition themselves spatially, largely preferring opposite treatments (Figure 10). Like overall adult and nymph abundance, preferences of these two species were not related to association and varied greatly from 1 farm to the next. At 1 one farm, however, differences were largely consistent across the entire duration of the study (13 months). Spittlebug abundance was much greater in the region south of Florencia. Over the months of the experiment, mean number of nymphs (per 2.5 m^2) and adults (per 400 sweeps) was 41.1 and 15.1 in the south and 87.1 and 22.4 in the north. Species composition varied considerably among farms but with no clear differences between regions or treatments. *A. varia* and *Z. pubescens* were overwhelmingly the most abundant species; *Mahanarva* sp. occurred at 4 farms, *Z. colombiana* at 2. The report of 6 *Z. colombiana* on 1 date in Diamante is probably an error of handling specimens because the species has been consistently limited to Higuerón. The supplemental surveys showed a clear difference in the number of *Brachiaria* stems between monoculture and association (Figure 11). Mean number of stems per 0.0625 m^2 was 29.5 and 22.9 for the monoculture and association respectively. This difference translates into a 22.4% greater insect load for associated pastures.

Discussion: This study demonstrates that spittlebug abundance does not differ between *Brachiaria* monocultures and *Brachiaria/Arachis* associations in Caquetá. But due to a reduced grass component in associations, insect load, or the number of insects per unit grass, increases 22.4% under the association. Greater herbivore pressure, however, does not necessarily translate into greater impact in associations. Grass grown in the company of nitrogen fixing plants may profit from enhanced vigor and increased tolerance to spittlebug damage. A complete answer to producers in Caquetá and other parts of Latin America where grass/legume associations are being promoted will depend on the response of the pasture to the spittlebugs. We have therefore initiated a third phase of this study, namely a greenhouse

experiment to assess spittlebug damage on grass grown with and without the company of *A. pintoi*.

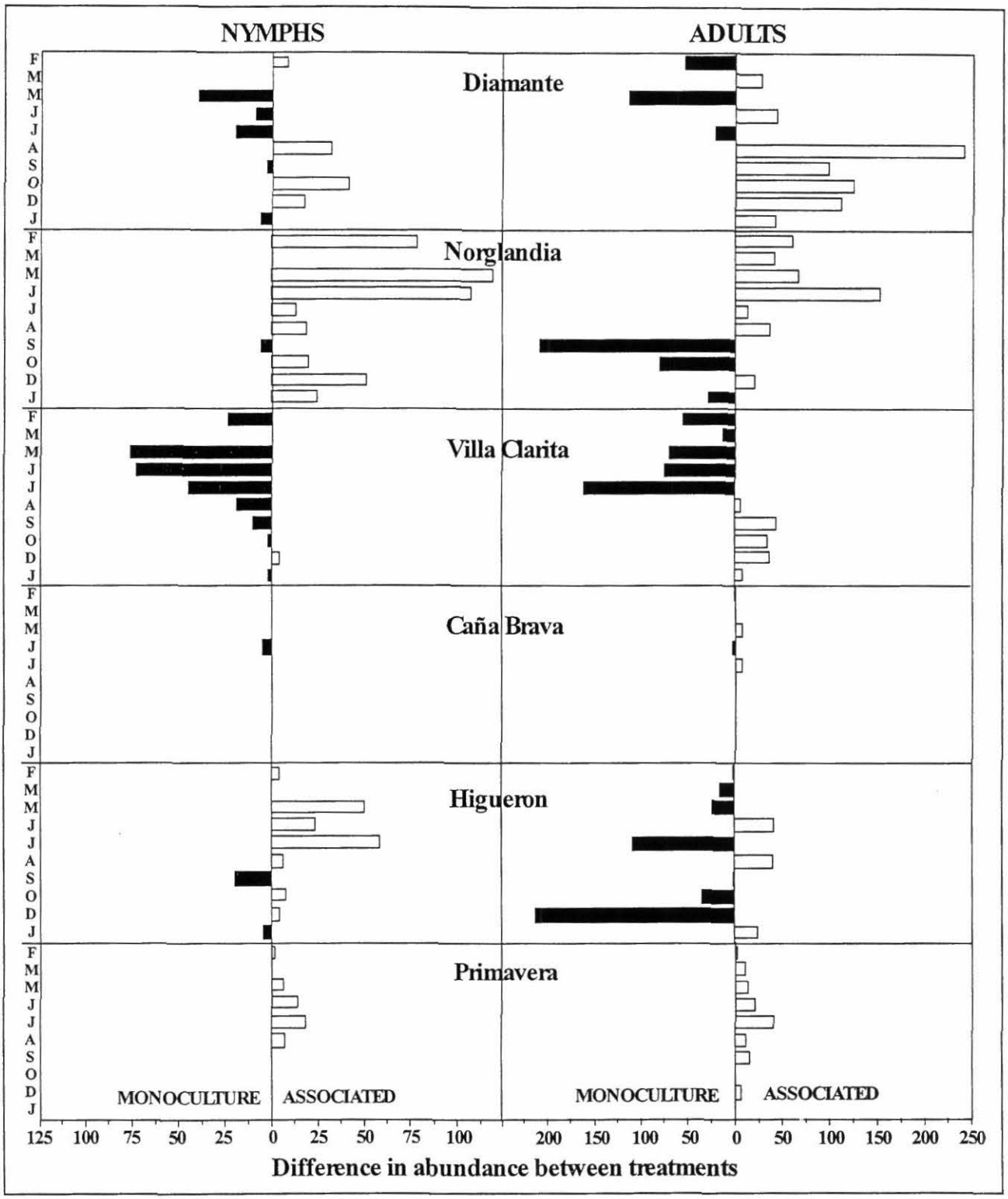

Figure 9. Abundance of total nymph and adult populations in paired treatments on 6 farms. Data are the difference between treatments for each month. Nymph surveys were not performed in March.

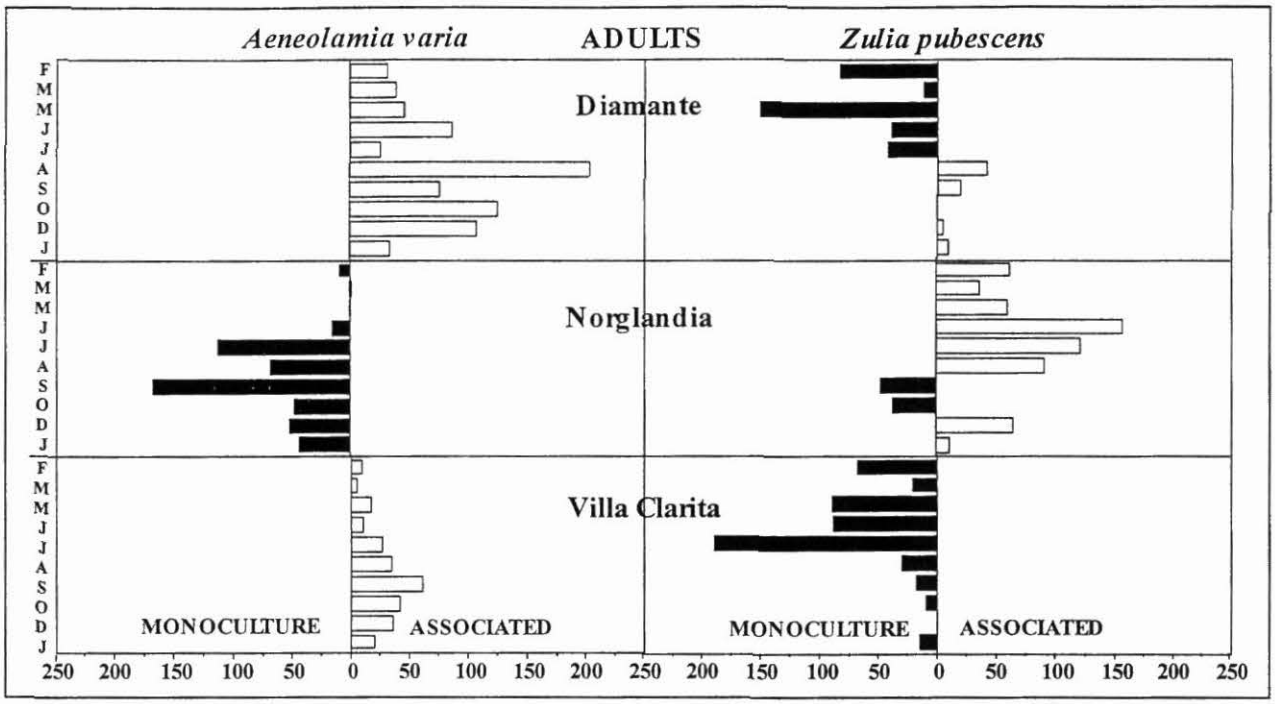

Figure 10. Abundance of adult *Aeneolami varia* and *Zulia pubescens* populations in paired treatments on three farms where they were most abundant. Data are the difference between treatments for each month.

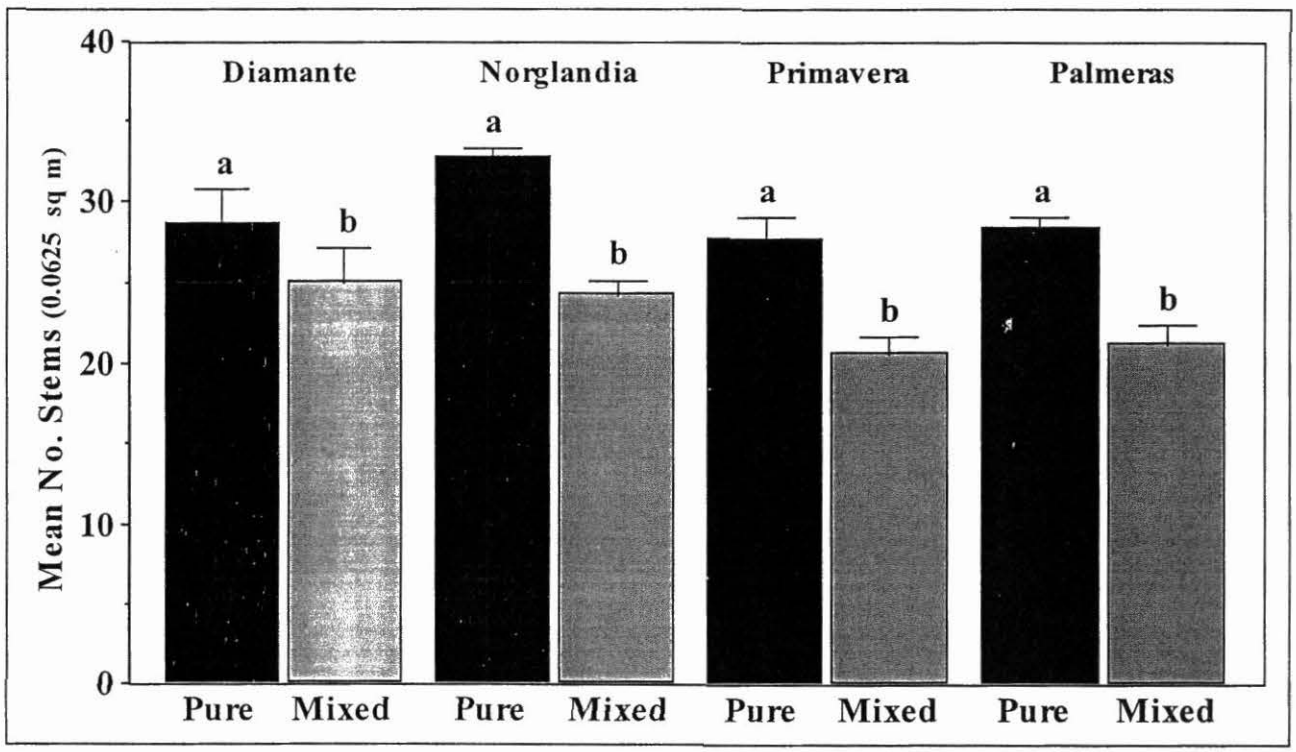

Figure 11 Difference in the availability of *Brachiaria* grass in monoculture and associated pastures. Bars are standard errors. Within farms, means with different letters are significantly different at P<0.05.

Studies on evaluation of fungi entomopathogenic to spittlebugs (A. Morales, A. C. Bolaños, D. Peck)

Highlight

- 19 isolates of entomopathogenic fungi have been collected from grassland spittlebugs in Colombia
- CIAT's new "box" design offers a resource-efficient alternative for mass rearing of spittlebugs

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 not been demonstrated. Focus on a narrow diversity of isolates, 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 2 genera of entomopathogenic fungi and 3 genera of spittlebugs. Exploiting and assessing this diversity for biological control must begin with a dependable and rapid methodology for quantifying virulence in the laboratory.

Methods: This CIAT strategic research initiative began June 1998. Thus far work has progressed in 3 activities, establishment of the ceparium, a new spittlebug colony and an evaluation methodology. Entomopathogens were isolated from cadavers of adults and nymphs found in the field in the departments of Sucre, Meta, Caquetá and Cauca. Insect tissue was sterilized, fungi were propagated 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.

Establishment of a new mass rearing colony of *A. varia* was necessary to provide sufficient insects for experimentation and to avoid risk of contamination of a single source of insects. A new "box" design that previously demonstrated savings in time, materials and labor was employed. This design features host plants (*Brachiaria ruziziensis*) of 6-8 stems transplanted onto a thin layer of soil in an array of 16 mounds per wooden box (1.6 x 0.6 x 0.1m). Adequate conditions for root and nymph development were achieved by covering the rooting space with a layer of cloth through which the foliage emerges. Each of the 16 plants was infested with 100-150 eggs. Once adults appeared an emergence cage was placed over the unit to facilitate collection. Two generations (2 boxes each) infested with 150 eggs per plant have been completed to date.

Trials were initiated to improve adult survival under experimental conditions of fungus evaluation, a limitation of previous efforts to evaluate entomopathogens against *A. varia*. The experimental units comprised acetate cylinders over 12 cm diameter pots with 6-8 *B. ruziziensis* stems. Ten adults <1 d old were added to each unit and the mortality followed daily. Because manipulation of recently emerged adult (tenerals) probably increases mortality, 2 collection techniques were evaluated: aspirator and manual collection with soft forceps.

Results: To date, fungi have been obtained from 19 insects, of which 11 were advanced to monosporic isolates and placed under long-term storage (Table 25). Including the 5 isolates that have not yet sporulated (non-reproductive mycelia only) the collection comprises 2 genera of entomopathogens (*Metarhizium*, *Paecilomyces*) obtained from 2 life stages (nymphs, adults of both sexes) of 5 species (*Aeneolamia reducta*, *A. varia*, *Mahanarva* sp., *Z. colombiana*, *Z. pubescens*), from 4 departments (Caquetá, Cauca, Meta, Sucre).

Results from the first large-scale trial of the new mass rearing design were positive. The 2 completed trials yielded an efficiency of 50.8 and 55.8% production of adults from eggs, with a peak of 26.2 and 27.4% emergence of individuals over a 3-d period. This efficiency matches the traditional CIAT mass rearing methodology (60%) but falls short of previous small-scale experiments of a similar box design (70%). Rapid deterioration of host plants during the final days of nymphal tenure suggest that a reduction in number of eggs infested could increase efficiency. Studies are underway to gauge the efficiency of the design with an infestation of 100 eggs and implementation of minor management adjustments. In the trial of the experimental evaluation units, adult mortality after 6 days was 38.5 and 32.0% for the aspirator and manual collection methods, respectively. There was no significant difference in 6-day mortality between collection styles (Prob>F=0.314).

Table 25. Entomopathogenic fungi isolated from grassland spittlebugs in Colombia.

Host Access. No.	Host Species	Host Sex	Host Life Stage	Department of Origin	Fungus Genus	Status [1]
C-1	*Aeneolamia reducta*	Female	Adult	Sucre	Undet.	UnSP
C-5	*Aeneolamia reducta*		Adult	Sucre	Undet.	UnSP
F – 11	*Aeneolamia varia*	Male	Adult	Caquetá	*Metarhizium*	MS
V-34 A	*Aeneolamia varia*	Male	Adult	Meta	Undet.	SP
V-34 B	*Aeneolamia varia*	Male	Adult	Meta	Undet.	SP
V-36	*Aeneolamia varia*	Female	Adult	Meta	Undet.	SP
V-37	*Aeneolamia varia*		Adult	Meta	Undet.	SP
F - 18	*Mahanarva* sp.	Male	Adult	Caquetá	*Metarhizium*	MS
F - 19	*Mahanarva* sp.	Male	Adult	Caquetá	*Metarhizium*	MS
S-1	*Zulia colombiana*	Female	Adult	Cauca	Undet.	UnSP
S-5	*Zulia colombiana*		Adult	Cauca	Undet.	UnSP
F - 1	*Zulia pubescens*		Adult	Caquetá	*Metarhizium*	MS
F - 10	*Zulia pubescens*	Male	Adult	Caquetá	*Metarhizium*	MS
F - 20	*Zulia pubescens*	Female	Adult	Caquetá	*Metarhizium*	MS
24	*Zulia pubescens*		Adult	Caquetá	*Paecilomyces*	MS
14	*Zulia pubescens*		Adult	Caquetá	Undet.	SP
17	*Zulia pubescens*		Adult	Caquetá	Undet.	SP
23 B	*Zulia pubescens*	Male	Adult	Caquetá	Undet.	SP
47	*Zulia pubescens*		Adult	Caquetá	Undet.	SP
F-7	*Zulia pubescens*		Adult	Caquetá	Undet.	UnSP
48-1	undetermined		Instar 5	Caquetá	*Metarhizium*	MS
62-2	undetermined		Nymph	Caquetá	*Metarhizium*	MS
48-2	undetermined		Instar 5	Caquetá	*Paecilomyces*	MS
62-1	undetermined		Nymph	Caquetá	*Paecilomyces*	MS

[1] UnSP is unsporulated mycelia; SP is sporulated; MS is monosporic isolate

Discussion: Collections in Colombia have yielded a diverse array of fungal entomopathogen isolates. Additional collections from different host species and regions will consolidate future opportunities to evaluate a broad diversity of isolates for virulence against *A. varia* and other species with the goals of (1) describing variation in virulence that depends on life stage and host species and (2) identifying candidates for field evaluations.

Evaluation of virulence against *A. varia* adults in the laboratory depends on high survivorship of control adults. The results obtained so far show an acceptable range of < 50% mortality after 6 days and indicate that improvements in other aspects of virulence evaluation against this life stage can be obtained. Early results from the first large scale deployment of a new rearing design for *A. varia* are very positive. After the integration of a few changes in the methodology and a proper analysis of efficiency, this design should prove to be equally efficient and dependable and much less costly in labor, time and material inputs than the traditional method.

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

Highlights

- Training workshops on spittlebug bioecology and management have involved 49 participants from 10 institutions

Rationale: Despite the impact of grassland spittlebugs in Colombia and the rest of tropical America, there exists little regional expertise on their biology and management outside of CIAT. Access to information is also extremely limited because there is no text that summarizes our knowledge of the family Cercopidae and existing guides to pasture spittlebugs are outdated, inaccurate and ignore family level bioecology. This gap, and the establishment of a new spittlebug bioecology research group in 1997, prompted the first "Workshop on the Bioecolgy and Management of Grassland Spittlebugs" in April 1997. Success of the first event stimulated 2 additional workshops in 1998.

Methods: The second workshop took place from 19-25 April, 1998 in CIAT. Like the first event, the objective was to unite all participants directly involved in the spittlebug bioecology project for 5 days of intensive lectures, labs and discussions with the goal of reinforcing comparative methodologies, team building and giving a solid information foundation on the insect, its habitat and issues critical to its management. It was also the opportunity to advance thesis projects and acquire literature relevant to particular projects. The Servicio Ecuatoriano de Sanidad Agropecuaria (SESA) also invited CIAT to lead a similar event in Puyo, Ecuador from 1-4 September.

Results: Thirty-one participants representing 6 universities and 4 other national institutions attended the training workshops in 1998 (Table 26). In the CIAT workshop, 8 participants were students directly involved in bioecological studies; the others were invited due to interest in related research. Detailed notes, diagrams and reference lists accompanied the 6 core lectures and the 6 core labs. This material was improved over 1997 and is now being prepared for future publication as a guide to the study of grassland spittlebug bioecology. Participants were also provided a package of 20 core readings on basic and applied themes of the family Cercopidae.

Table 26. List of institutions attending training workshops in 1998.

Event	Institutions	No. Participants
CIAT	Laverlam, S.A.	2
Workshop	Universidad de la Amazonia	2
	Universidad de los Llanos	3
	Universidad Javeriana	1
	Universidad Nacional	3
	Universidad de Sucre	2
	Universidad del Valle	1
SESA	Ministry of Agriculture and Ranching	2
Workshop	Puyo Sugar Cane Growers Association	1
	SESA	14

Discussion: This workshop had great success as a tool for team building, training and the transfer of information and methodologies. It will be repeated in subsequent years to continue promoting regional and national experience in this widely distributed pest complex, stimulating research accurately grounded in the insect's natural history, and advancing management through an awareness of the variation in the nature of spittlebug impact.

Progress towards achieving output milestone

- **Complete description of the variation in biology and abundance of spittlebug species in Colombia (1999)**

Given the known variation in spittlebug bioecology, taxonomic diversity and pasture management systems, it is clear that the pest's impact will vary considerably across its range. This 2-year project has been a "rapid bioecological assessment" of Colombia's lowland grassland spittlebugs to describe this variation and open up avenues for advances in management of the pest. Significant amount of new basic information on the biology of spittlebug has been acquired over this period. The North Coast of Colombia and the Amazonian Piedmont have rapidly emerged as benchmark sites for spittlebug management, representing 2 distinct systems.

First time information on species biology and distribution, population dynamics and ecology serve as the foundation for present and future studies on management strategies and the ultimate establishment of an integrated pest management program. As the most important insect pest in the most extensive agricultural activity in the Americas, grassland spittlebugs merit a widening of research efforts focused on the IMP tools upon which holistic management depends. The next steps should included studies to quantify impact, monitoring programs, diapause studies and additional studies on the basic biology of this diverse group.

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

Large-scale evaluation of *Brachiaria* spp. genotypes for resistance to spittlebug
(C. Cardona, G. Sotelo and J. Miles)

Highlights

- New greenhouse spittlebug resistance evaluation technique developed in 1997 fully implemented with excellent results.

- *Brachiaria* hybrids with very high levels of resistance to the spittlebug *Aeneolamia varia* were identified.

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

A new, more efficient methodology for mass screening of resistance under greenhouse conditions was developed and tested (see 1997 Annual Report). In 1998, the new methodology was fully implemented for large-scale screening of genotypes.

Methods: Based on previous results, a series of *Brachiaria* genotypes and 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 were scored for symptoms using a visual damage score scale. Genotypes were then classified for resistance.

Results and Discussion. In a first set of experiments, 61 germplasm accessions taken from CIAT collection and 16 hybrids developed at CIAT were screened. Of the 16 hybrids tested, seven were classified as resistant for having damage scores significantly lower than the susceptible checks, CIAT 0606 and CIAT 0654. Three hybrids were classified as highly resistant based on a combination of low damage scores and significant antibiotic effects on spittlebug nymphs. Of the 61 germplasm accessions studied, 13 were resistant (Table 27).

Full implementation of the new greenhouse methodology for mass screening of *Brachiaria* spp. genotypes for resistance to spittlebug was initiated in 1998 when 501 hybrids were evaluated. A preliminary screening was conducted using two replications per hybrid in comparison with 10 replications of each of the checks, the resistant hybrid BR93NO/1371 and the accessions CIAT 0606 (S), CIAT 0654 (S), and CIAT 6294 (R). Those 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%).

Table 27. Levels of resistance to *Aeneolamia varia* in selected *Brachiaria* spp. genotypes as determined using a new greenhouse resistance evaluation technique. Means of 10 replicates

Genotype	Species	Visual damage scores[a]	Percentage nymph survival	Rating[b]
CIAT 0606[c]	*B. decumbens*	4.9	73.0	S
CIAT 0654[c]	*B. ruziziensis*	5.0	67.0	S
CIAT 06294[d]	*B. brizantha*	2.3	20.0	R
CIAT 06297	*B. brizantha*	1.8	8.0	R
CIAT 06780	*B. brizantha*	1.9	21.0	R
CIAT 16077	*B. brizantha*	2.2	26.0	R
CIAT 16099	*B. brizantha*	2.1	19.0	R
CIAT 16307	*B. brizantha*	1.3	4.0	R
CIAT 16310	*B. brizantha*	2.2	28.0	R
CIAT 16827	*B. brizantha*	3.0	24.0	I
CIAT 16829	*B. brizantha*	2.3	13.0	R
CIAT 16830	*B. brizantha*	2.0	12.0	R
CIAT 16961	*B. subulifolia*	2.0	12.0	R
CIAT 16964	*B. subulifolia*	2.9	8.0	R
CIAT 26298	*B. decumbens*	2.6	0.0	R
CIAT 26300	*B. decumbens*	2.8	10.0	R
FM9503/057/014	Hybrid	1.6	3.0	R
FM9503/015/010	Hybrid	1.7	8.0	R
FM9503/070/016	Hybrid	2.4	18.0	R
BR93NO/1371[d]	Hybrid	1.5	0.0	R
LSD 5%		0.7	4.6	

[a] In a 1-5 damage score scale (1, no damage; 5, dead plant)
[b] R, resistant; I, intermediate; S, susceptible
[c] Susceptible check
[d] Resistant check.

Results of the preliminary screening indicated that 61 of the hybrids exhibited damage levels lower than 3.0. When these were checked for antibiosis (percentage nymph survival), most were classified as susceptible with survival rates above that of the resistant parent, CIAT 6294. However, hybrids BR97NO/0235 and BR97NO/0047 were classified as highly resistant in comparison with the best resistant check, the hybrid BR93NO/1371. The levels of tolerance and/or antibiosis present on selected genotypes are shown in Figure 12. The high levels of resistance in the new hybrids BR97NO/0235 and BR97NO/0047 were reconfirmed when the

61 hybrids selected in the preliminary test were reconfirmed using 10 replications per genotype (Table 28). Three other hybrids were classified as intermediate with survival rates statistically the same as that of the resistant parent, CIAT 6294 ('Marandú'). These results confirmed the reliability and capacity of the screen. The high levels of resistance in some of the hybrids are indication of the excellent progress made in incorporating resistance to spittlebug

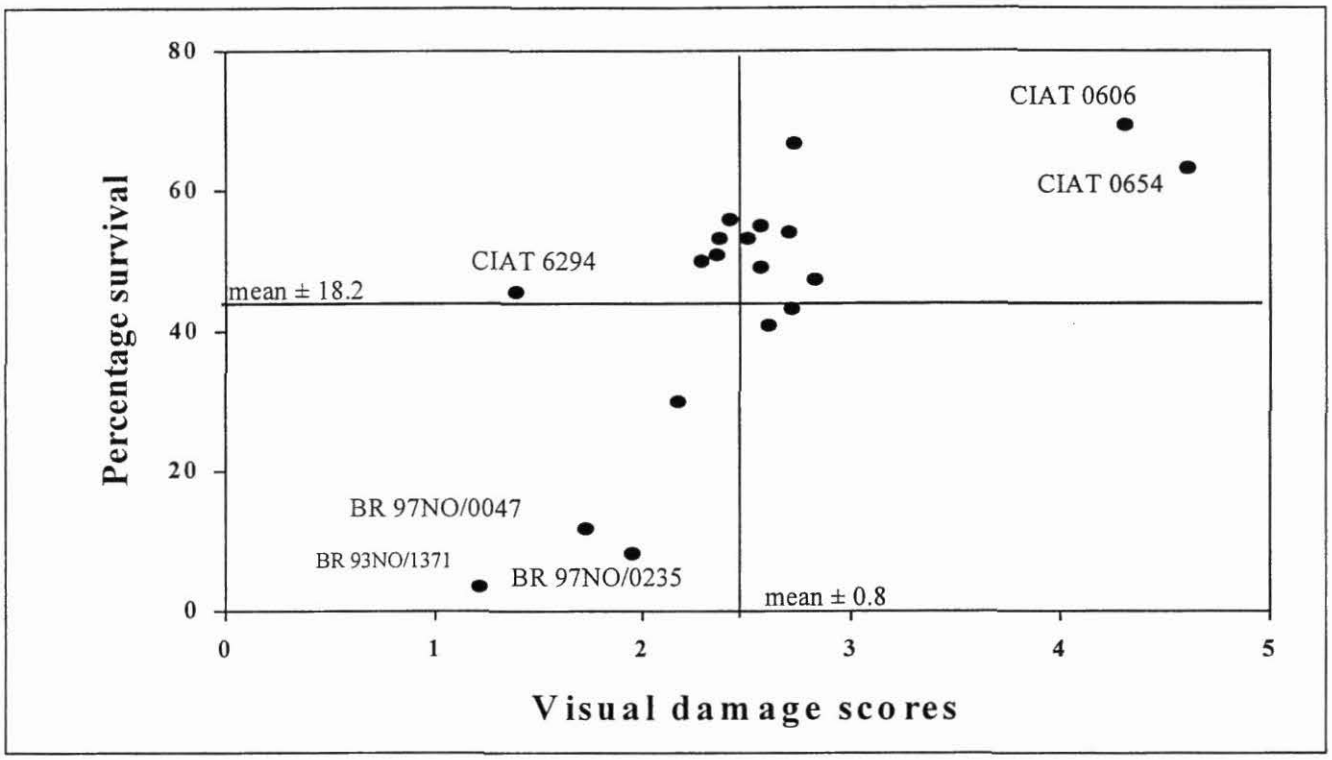

Figure 12. The relationship between visual damage scores and percentage nymph survival in 15 *Brachiaria* hybrids selected for resistance to spittlebug in preliminary tests using the new evaluation technique (two reps per genotype). CIAT 0606 and CIAT 0654 are susceptible checks; CIAT 6294 ('Marandú') and the hybrid BR93NO/1371 are resistant checks.

Table 28. Levels of resistance to *Aeneolamia varia* in the five best *Brachiaria* hybrids selected in 1998. Means of 10 replications per genotype.

Genotype	Damage scores[a]	% nymph survival[b]	Rating[c]
BR97NO/0047	1.9de	13.3c	R
BR97NO/0235	2.1cd	10.0c	R
BR97NO/0155	2.4bcd	34.0b	I
BR97NO/0402	2.7bc	40.0b	I
BR97NO/0457	2.9b	40.0b	I
BR93NO/1371[d]	1.4e	0.0d	R
CIAT 6294[d]	1.4e	40.0b	R
CIAT 0606[e]	4.7a	60.0a	S
CIAT 0654[e]	4.8a	65.7a	S
C. V. (%)	24.5	39.4	

[a] In a 1-5 visual damage score scale (1, no damage; 5, dead plant)
[b] Analyzed as arcsine square root of proportion . Untransformed data are presented.
[c] R, resistant; I, intermediate; S, susceptible
[d] Resistant checks. CIAT 6294 ('Marandú') is the resistant parent
[e] Susceptible checks.
Means within a column followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

Development of a new methodology for resistance screening under field conditions (C. Cardona, G. Sotelo and W. Mera)

Highlight

- A new technique for evaluation of resistance of *Brachiaria* to spittlebug under field conditions was developed.

Rationale: As stated, assessment of resistance to spittlebug under natural levels of infestation in the field has been impossible due to the focal, unpredictable occurrence of the insect. As indicated in the Annual Report 1997, different alternatives to develop a reliable artificial infestation technique were tested. Among these, the initial infestation of plants in the greenhouse and the subsequent transfer of the infested plants to the field was considered promising. Intensive work in 1998 allowed us to further improve upon the methodology in such a manner that we are now in the position of testing in the field in Caquetá all those hybrids rated resistant to spittlebug in the greenhouse at CIAT Palmira.

Methods: In trying to develop a methodology for field infestation with spittlebug, two main aspects were considered: 1) The source of infestation; 2) The host plant and the creation of a microenvironment in its base that is suitable for adequate development of nymphs (root proliferation, shade, high humidity). The source of infestation has not been a problem. Mass rearing techniques developed at CIAT and at Macagual Station in Caquetá allow us to produce as many insects as needed for extensive screening of *Brachiaria* genotypes. Through trial and error excellent results were obtained with the technique that we are calling "inverted pot". This can be described as follows (Figure 13): a 20-stem division of a mother plant is taken from the field and transferred to the greenhouse (step A).

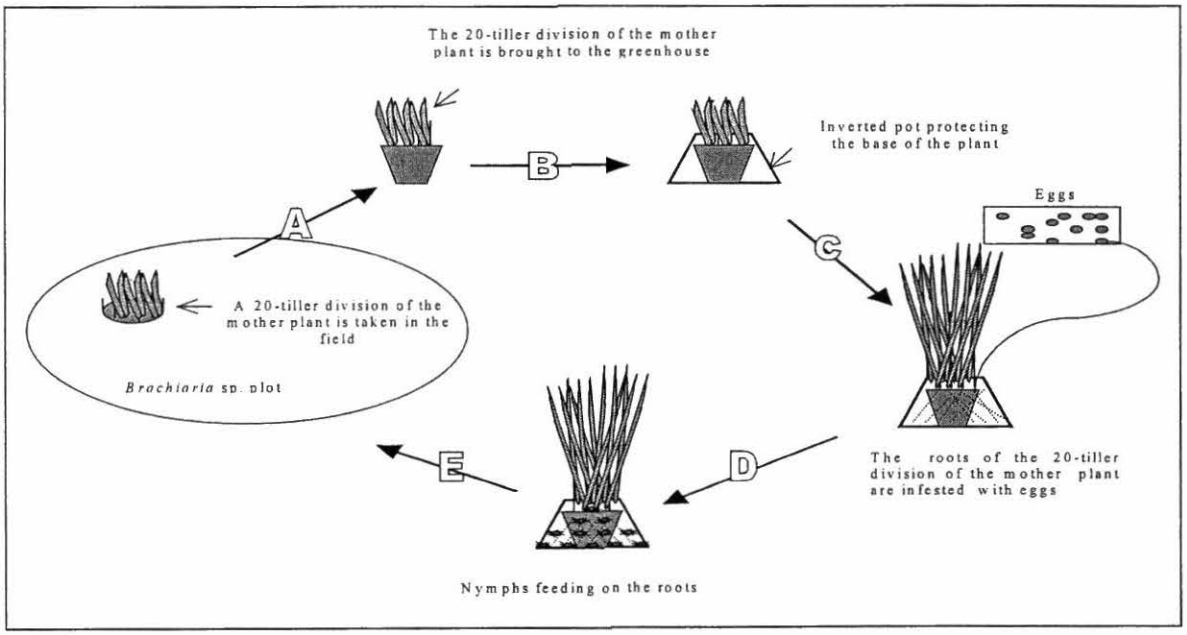

Figure 13. Methodology to evaluate *Brachiaria* spp. genotypes for resistance to spittlebug under field conditions. A. A 20-tiller division of the mother plant is taken from the field, transferred to the greenhouse and conditioned to promote root proliferation; B. The root area of the plant is protected (covered) with an inverted greenhouse pot; C. The roots are infested with eggs; D. Nymphs feeding on roots are fully established; E. Infested plants, still protected with the inverted pot, are transferred to the field.

After disinfestation with a mild insecticide, the 20-stem division is placed on a bench and its base covered with an inverted pot open at both ends (step B). The purpose of the inverted pot is to provide the root area of the plant with a dark, humid microenvironment that will promote the production of new secondary roots. These in turn will serve as feeding sites for nymphs. After rooting for 10-12 days, the 20-stem sections or clumps are infested in the greenhouse with an average of 10 eggs per stem (step C). Once the infestation is well established, with all nymphs feeding on the roots (step D), the units are transferred to the field and transplanted 10-15 days after infestation (step E). The infestation is then allowed to proceed without interference until all nymphs have developed and adults emerge some 30-35 days thereafter. The plants are then scored for damage by means of the same visual scale utilized in greenhouse screenings.

Results and Discussion: Using the technique described above, 10 *Brachiaria* genotypes were evaluated for spittlebug resistance in Caquetá. The materials were tested in a grass-covered field as well as in a field without any cover. As shown in Table 29, the presence of a grass cover did not have any effect on resistance ratings. With or without grass cover, susceptible and resistant genotypes were clearly identified. In spite of the fact that damage scores were taken prematurely (when nymphs had barely reached the fourth instar stage), significant differences between the resistant checks and the susceptible genotypes were detected with such precision that resistance ratings matched those obtained in previous greenhouse evaluations.

Table 29. Response of *Brachiaria* spp. genotypes to attack by nymphs of the spittlebug *Aeneolamia varia* under field conditions. Caquetá, Colombia. Means of five replications.

Genotype	Rating[a] under greenhouse conditions	Damage[b] in pasture-covered field	Damage in field without pasture coverage	Rating under field conditions
CIAT 0606[c]	S	3.4a	3.2a	S
CIAT 06387	?	3.4a	2.8ab	S
CIAT 01737	?	2.8b	2.1c	S
CIAT 06133	S	2.7b	2.5bc	S
CIAT 16327	S	2.6bc	2.2c	S
CIAT 01873	?	2.5bc	2.3c	S
CIAT 16871	S	2.5bc	2.5bc	S
CIAT 16867	S	2.1c	2.1c	S
CIAT 6294[d]	R	1.0d	1.0d	R
BR93NO/1371[d]	R	1.0d	1.0d	R

[a] R, resistant; I, intermediate; S, susceptible
[b] In a 1-5 visual damage score scale (1, no damage symptoms; 5, dead plant)
[c] Susceptible check
[d] Resistant check
Means within a column followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test).

Identical results were obtained at CIAT Palmira where the infestation was allowed to proceed without interference until full emergence of adults occurred. There were significant differences between resistant and susceptible genotypes . Damage ratings for resistant genotypes ranged from 1.3 to 1.7 while those for susceptible genotypes ranged from 4.9 to 5.0 (Table 30).

The impact of the insect on plant growth and development was measured with precision. While the resistant genotypes CIAT 6294 and BR93NO/1371 doubled the number of stems per clump, the susceptible genotypes CIAT 0654 and CIAT 0606 did not produce any new stem. All plants in the susceptible genotypes died as a result of insect damage while all plants in the resistant checks survived with very little foliar damage. There was a perfect match between field and greenhouse ratings.

Table 30. Response of *Brachiaria* spp. genotypes to attack by nymphs of the spittlebug *Aeneolamia varia* under field conditions. CIAT, Palmira. Means of 10 replications.

Genotype	Rating under greenhouse conditions[a]	Stems/clump upon infestation	Stems/clump 40 days after infestation	Damage[b]	Rating under field conditions
CIAT 0654	S	25.1ab	25.6c	5.0a	S
CIAT 0606	S	26.9a	25.2c	4.9a	S
CIAT 6294	R	18.2c	32.6b	1.7b	R
BR93NO/1371	R	20.3bc	41.7a	1.3c	R

[a] R, resistant; S, susceptible

[b] In a 1-5 visual damage score scale (1, no symptoms; 5, dead plant)

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

Studies on the nature of antibiosis to spittlebug (G. Díaz, G. Sotelo and C. Cardona)

Highlight

- New knowledge on the nature of antibiosis in *Brachiaria* to spittlebug was obtained.

Rationale: A series of detailed studies on the nature of antibiosis as a mechanism of resistance to *A. varia* in *Brachiaria* spp. was initiated in order to: a) Understand the effects of resistance on the biology of the insect; b) Compare the nature of damage caused by nymphs of spittlebug on resistant and susceptible genotypes; c) Determine the impact of antibiosis on the population dynamics of the insect as a result of the interaction between insect and plant in resistant genotypes. In addition, these studies are necessary to develop better breeding strategies, to shed light on the most suitable methodology to screen for resistance and to assist in the interpretation of results in current efforts to develop molecular markers for spittlebug resistance. They will also constitute basic knowledge for future studies on the biochemical basis of resistance to the insect.

Methods: To study the antibiosis mechanism associated with spittlebug resistance under greenhouse conditions, two contrasting *Brachiaria* genotypes were chosen: the highly susceptible accession CIAT 0654 and the highly resistant hybrid BR93NO/1371. These materials were infested in the greenhouse and large cohorts of nymphs were obtained. Daily samples of nymphs of a known age were taken in order to measure different biological parameters, to make observations and to describe the damage caused by the insects at different stages of their life cycle. The following is a preliminary summary of the main results obtained to this date.

Results and Discussion: The first effect of antibiosis on the biology of the spittlebug is a significant prolongation of the time required by all nymphal instars to complete their development (Figure 14). In two consecutive experiments, the impact of the antibiosis mechanism present in the resistant *Brachiaria* hybrid was so high that the duration of the fifth instar could not be calculated with precision because very few insects reached this stage of development. The second significant effect on the biology of the spittlebug is a very high level of nymphal mortality (Figure 15). Percentage survival to the adult stage on the susceptible genotype averaged 87% while survival on the resistant hybrid averaged 26.5%. Data on percentage survival through time, fitted a linear regression model. Analysis of the regression of percentage survival on days after infestation for the two experiments gave no evidence either of differences neither in intercepts nor of nonhomogeneity of the two regression coefficient estimates for either the susceptible accession CIAT 0654 or the resistant hybrid BR93NO/1371. Hence, data from the two experiments were pooled and a single regression was calculated for

each genotype. The slope of the regression for the susceptible genotype (y = 100.6 – 0.33x) was significantly different (P<0.05) from that calculated for the resistant genotype (y =114.4–2.10x).

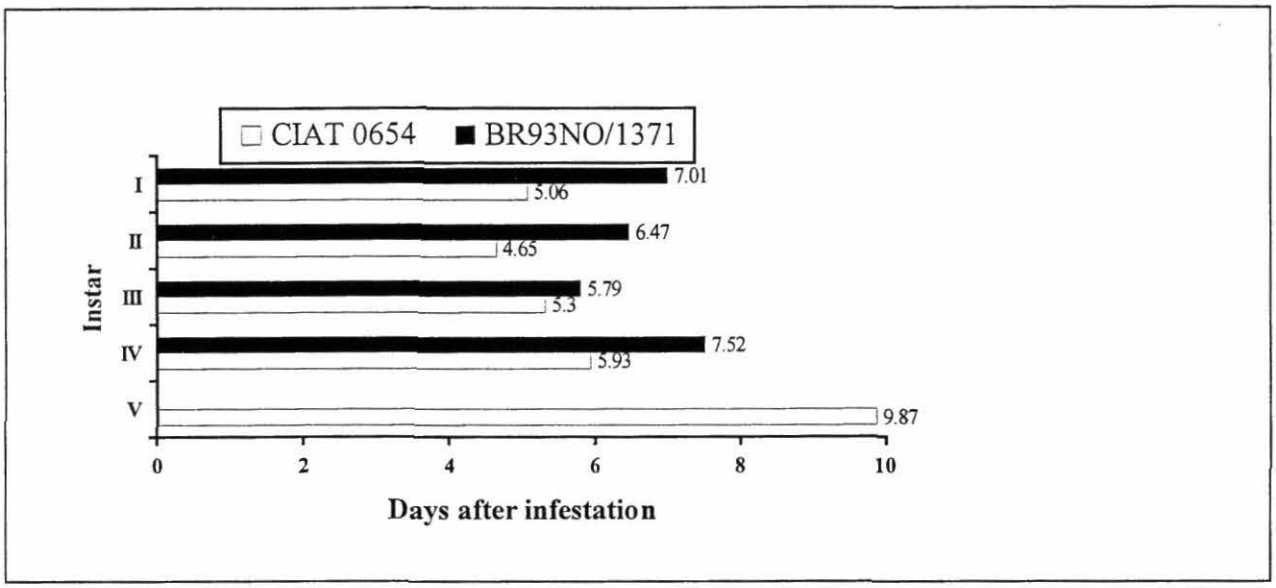

Figure 14. Effect of the resistant hybrid BR93NO/1371 on the duration of nymphal instars of *Aeneolamia varia*. CIAT 0654 is a highly susceptible accession.

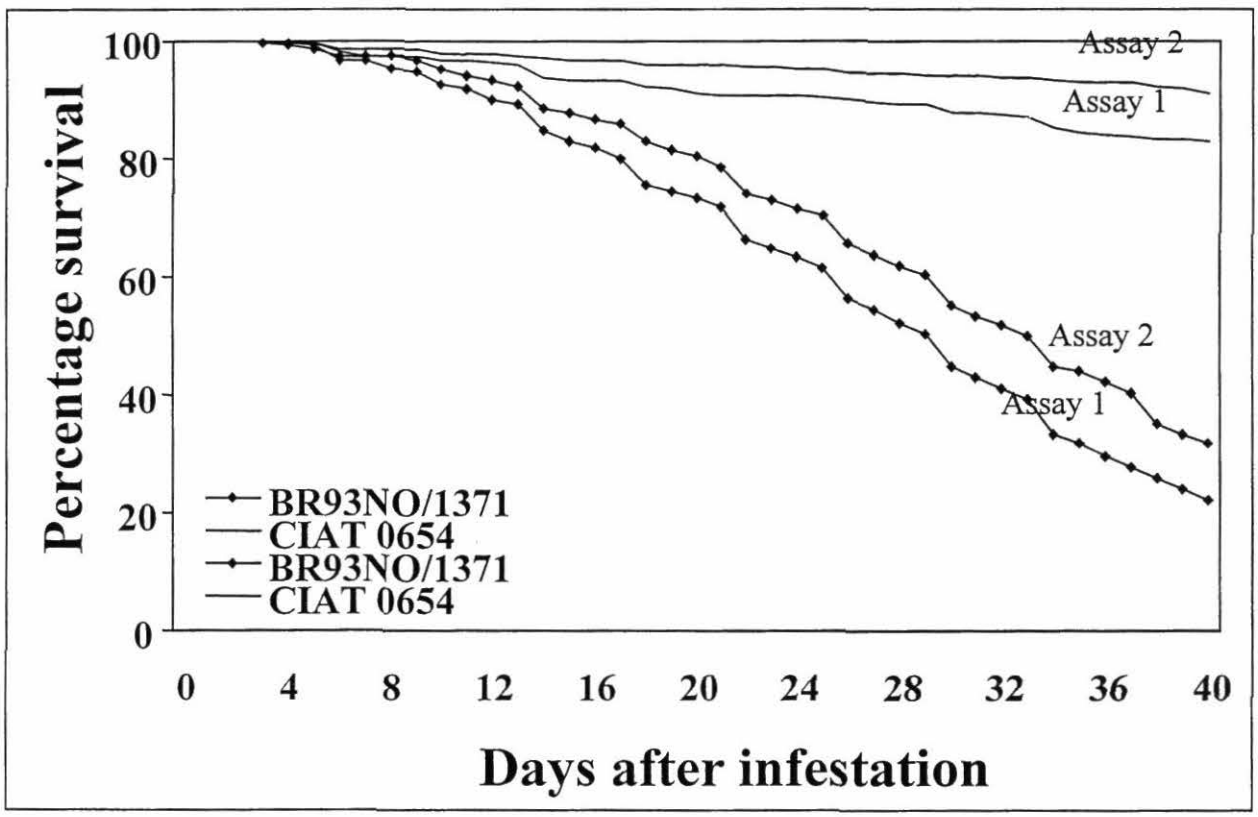

Figure 15. Survival curves for nymphs of *Aeneolamia varia* reared on two *Brachiaria* sp. Genotypes. CIAT 0654 is a highly susceptible accession; BR93NO/1371 is a highly resistant hybrid

Antibiosis also affects the size of surviving nymphs. The mean dry weight of nymphs reared on the susceptible genotype was significantly different ($P<0.05$) from the mean dry weight of nymphs reared on the resistant genotype. The reduction in nymphal weight was consistent through instars (Figure 16).

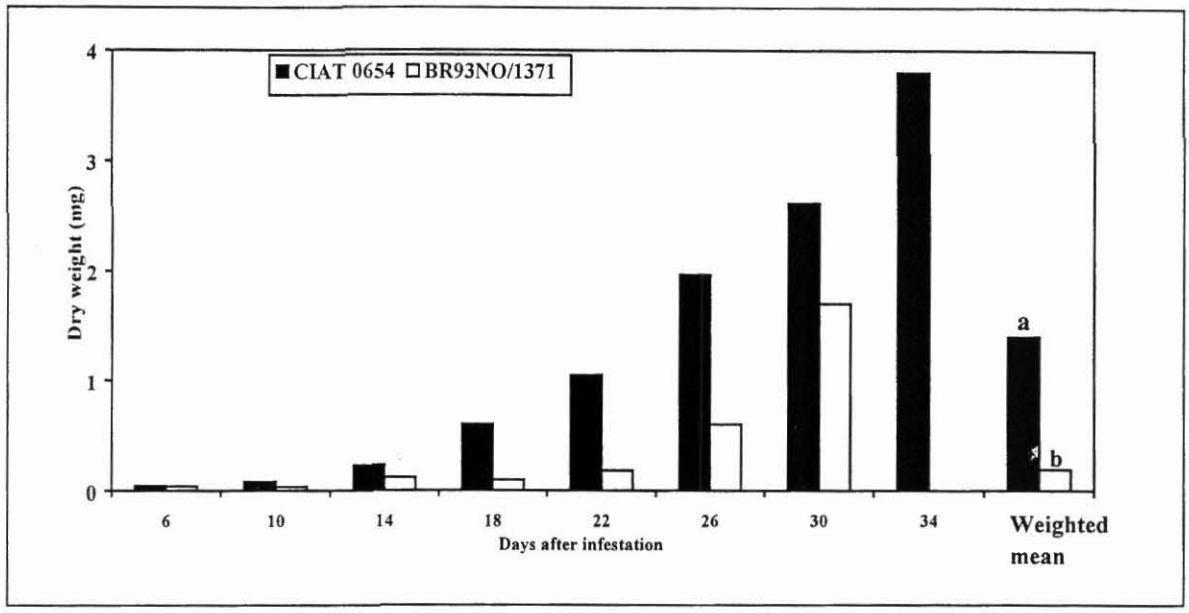

Figure 16. Dry weight of nymphs of *Aeneolamia varia* reared on two *Brachiaria* spp. genotypes. CIAT 0654 is a highly susceptible accession; BR93N0/1371 is a highly resistant hybrid.

Antibiosis effects can also be detected by the differential amount of spittle produced by nymphs feeding on susceptible or resistant genotypes. Consistently, nymphs feeding on the resistant hybrid produced less spittle than those feeding on the susceptible accession (Figure 17).

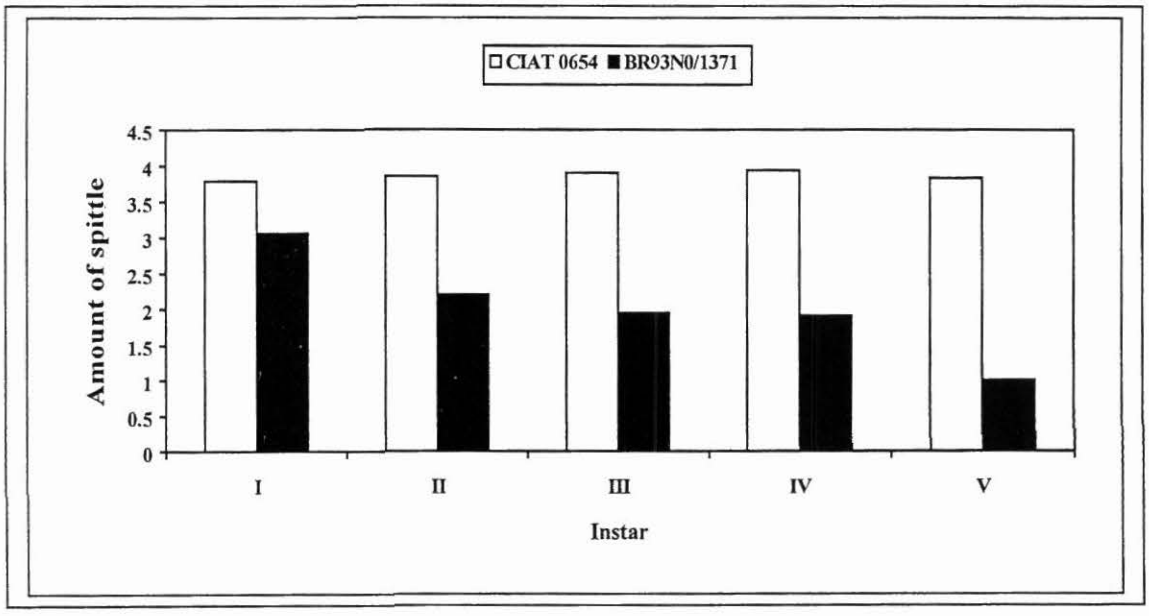

Figure 17. Amount of spittle produced by nymphs of *Aeneolamia varia* reared on two *Brachiaria* spp. genotypes. CIAT 0654 is a highly susceptible accession. BR93N0/1371 is a highly resistant hybrid. The amount of spittle was estimated by means of a 1-4 visual scale (1, no spittle; 4, abundant).

A detailed analysis of damage caused to the foliage as a result of nymphal feeding on the roots of the susceptible genotype CIAT 0654 was also performed. Symptoms appear 14-20 days after infestation (Figure 18), that is to say when nymphs reach the late third instar or the early fourth instar. Symptoms first appear on the lower leaves of the plant and advance upward to the apex. On any given leaf, symptoms first appear on the tip of the leaf and advance to the lower base. There is an initial loss of color of the tissues, followed by yellowing of the border areas, total yellowing of the leaf blade, and necrosis of the tissues. Damage intensifies 38 days after infestation (Figure 18) and when the genotype is very susceptible, all leaves show symptoms and all leaves die. Most severe damage is caused by fifth instar nymphs.

These are very important results as they clearly indicate that visual damage scores must be taken at least 38 days after infestation and not before. This will allow full expression of damage, a condition that is necessary in order to clearly differentiate between resistant and susceptible genotypes of *Brachiaria*

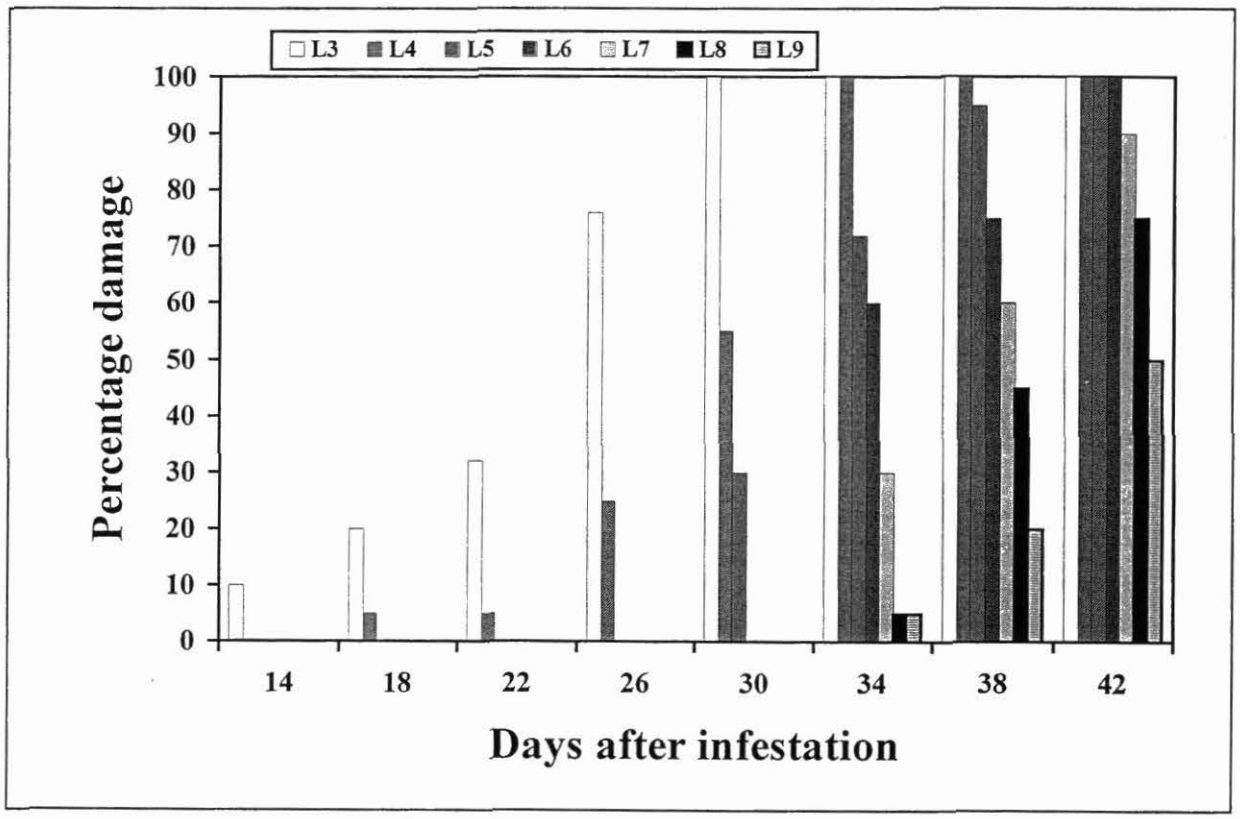

Figure 18. Leaf (L) damage resulting from feeding by nymphs of the spittlebug *Aeneolamia varia* on the roots of a highly susceptible *Brachiaria* sp. Genotype (CIAT 0654).

Resistance of *Brachiaria* spp. to other spittlebug species (C. Cardona and G. Sotelo)

Highlight

- For the first time ever, simultaneous studies on the resistance of *Brachiaria* spp. genotypes to four species of spitllebug were conducted.

Rationale: Studies on the resistance of *Brachiaria* genotypes to species of spittlebug other than *A. varia* were initiated in 1998. This is important as it can not be assumed that resistance to *A. varia* (the species that has always been used for resistance evaluation) applies across the board to all spittlebug species affecting *Brachiaria* in the Tropics. The species *Zulia colombiana, Z.*

pubescens, and *Mahanarva fimbriolata*, important in other areas of Colombia, were included in the studies.

Methods: Two greenhouse tests were conducted during 1998. 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 susceptibility or resistance to *A. varia* were used as test materials.

Results and Discussion: Results of two consecutive trials were consistent and are summarized in Table 31. As with *A. varia*, high levels of antibiosis resistance to *M. fimbriolata* were detected in the resistant *Brachiaria* genotypes CIAT 6294 and BR93NO/1371. *Z. pubescens* and *Z. colombiana* caused significantly less damage to the resistant genotypes, possibly as a result of tolerance.

Table 31. Damage scores (DS) and percentage nymph survival (%S)[1] in four *Brachiaria* spp. genotypes exposed to attack by nymphs of four different spittlebug species. Means of 10 replications for each genotype-insect species combination.

Genotype	*Aeneolamia varia*		*Zulia colombiana*		*Zulia pubescens*		*Mahanarva fimbriolata*	
	DS	%S	DS	%S	DS	%S	DS	%S
CIAT 0654[2]	4.2b	85.0a	4.7a	51.0a	4.9a	62.9a	4.9a	29.0a
CIAT 0606[2]	4.9a	65.0b	4.6a	46.0a	4.9a	55.7a	4.5b	33.0a
CIAT 6294[3]	1.4c	24.0c	2.7b	56.0a	2.9b	61.3a	1.6c	1.0b
BR93NO/1371[3]	1.3c	5.0d	2.8b	44.0a	2.0c	46.2a	1.1d	1.0b

[1]Traditional susceptible check for *A. varia*
[2]Traditional resistant check for *A. varia*
[3]Analyzed as arcsine square root of proportion. Untransformed means are presented
Means within a column followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test). Each insect species analyzed separately.

It seems that antibiosis is not the mechanism of resistance to the *Zulia* spp. complex because there were no significant differences in terms of nymphal survival when the genotypes were infested with nymphs of these species. Comparison of percentage survival of three species on the two resistant genotypes (Figure 19) clearly showed that the mechanism of resistance to *Zulia* spp. could be different from that known to occur for *A. varia*.

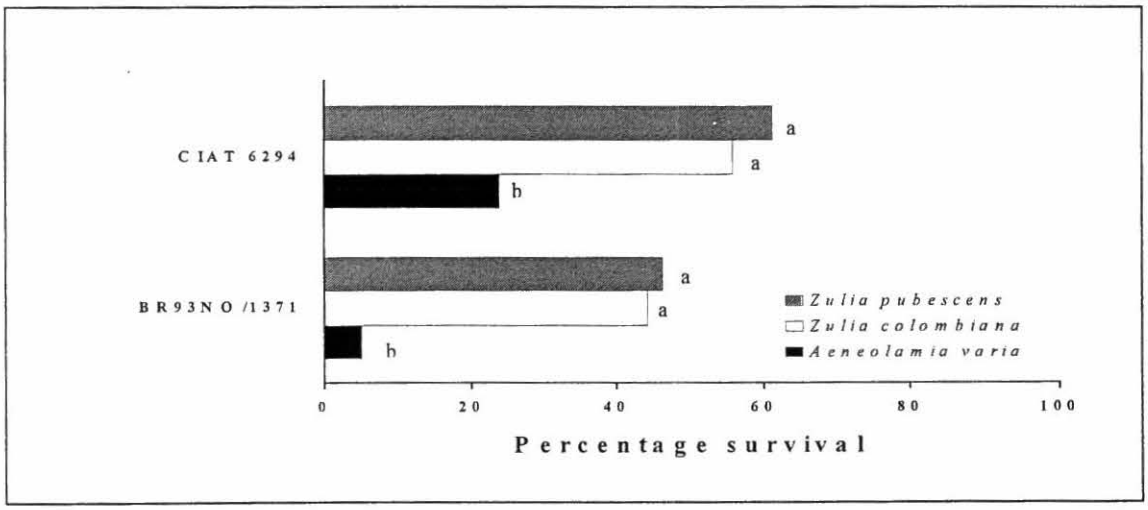

Figure 19. Fate of nymphs of *Zulia colombiana* and *Z. pubescens* reared on two *Brachiaria* genotypes resistant to *Aeneolamia varia*. Letters indicate statistical differences at the 5% level of confidence. Each genotype analyzed separately.

This has serious implications in developing breeding strategies for those areas of the Tropics where a complex of spittlebug species may occur. These studies will continue, but based on our preliminary results, the decision has been made to screen for resistance to *Z. pubescens* (more abundant in Caquetá than *Z. colombiana*) all those genotypes rated resistant to *A. varia*.

Progress towards achieving output milestone

- **Improved glasshouse and field screening procedures for assessing spittlebug resistance in *Brachiaria* genotypes (1999)**

Major advances have been achieved in capacity and reliability of screening for spittlebug resistance. In 1998, a total throughput of over 1,200 genotypes, including germplasm accessions and materials from the *Brachiaria* breeding project was achieved. This is nearly six times the capacity of previously available screening methodology and the full potential of the new methodology has yet to be reached.

During 1997 and 1998 a population of over 3,000 sexual genotypes has been evaluated in two field trials (Carimagua and Caquetá) and 500 pre-selections assessed for spittlebug resistance under artificial infestation. Eleven clones have been selected and these are being recombined to produce a new cycle population. With the new greenhouse and field screening methodologies and very rigorous selection, it ought to be possible to achieve high mean levels of resistance in a random-mating sexual population. Crosses of elite apomictic *Brachiaria* genotypes to this population will then generate large numbers of superior apomictic segregants to be tested for commercial release.

New sources of resistance in germplasm accessions have been identified, and these are being incorporated in breeding populations. It is worthy of note that a new standard of host plant resistance is now recognized. *B. brizantha* CIAT 6294 (cv. Marandú) was included as a resistant check in all early screenings. However, for the past year, a hybrid-derived clone (BR93NO/1371) has been adopted as standard resistant check. This recombinant consistently shows an even greater degree of antibiotic resistance than cv. Marandú.

Important questions remain, that we will have to address in the near future. There is need to continue and expand the studies on the resistance of *Brachiaria spp.* to several other spittlebug species different from *A. varia*. If we confirm preliminary results suggesting that the mechanism of resistance to *Zulia* spp. is different from that responsible for resistance to *A. varia*, changes in breeding strategies will have to be made in order to incorporate resistance to other major insect species. As in previous occasions, we would like to insist on the need to allocate funds to study the biochemical basis of resistance to spittlebug as a key element in the understanding of resistance and in the possible development of simple biochemical markers for selection.

Suboutput 2.3 *Brachiaria* resistant genotypes to spittlebug identified and reconfirmed

Screening of *Brachiaria* populations for spittlebug resistance (J. W. Miles)

Highlight

- Three sets of *Brachiaria* germplasm accessions have been (re)evaluated for spittlebug resistance.

Rationale: By 1997, only approximately one-half of the total *Brachiaria* germplasm collection had been evaluated for spittlebug resistance using older screening methods. Evaluation of the entire collection, particularly accessions of *B. brizantha* and *B. decumbens* accessible to the plant breeding project, was urgent. Appropriate plant material was necessary for these screenings with newer methods being developed.

Methods: Vegetative material collected from introduction plots at Popayán, was transferred to CIAT, maintained, propagated, and established for resistance testing.

Results and Discussion: Two screenings, totaling approx. 130 accessions, will be concluded in 1998. The first screening - of 61 accessions - resulted in the reconfirmation or new identification of 13 resistant genotypes. A second screening is in progress (late September) and the initiation of a third is anticipated before year's end. Sources of resistance newly identified in accessions belonging to *B. brizantha* or *B. decumbens* are being and will continue to be introgressed into sexual breeding populations to broaden the genetic base of resistance.

Propagation of appropriate hybrid-derived plant material for glasshouse screenings
(J. W. Miles)

Highlight

- Five hundred hybrid-derived sexual genotypes were screened for spittlebug resistance, resulting in eleven final selections.

Rationale: The *Brachiaria* breeding project focuses on a heterogeneous tetraploid sexual population containing genes from three *Brachiaria* species. Large recombinant populations are generated each breeding cycle. In 1997 over 3,000 individuals were produced for initial field evaluation at Carimagua and Caquetá. Field evaluation of large populations can roughly assess gross agronomic attributes, and allows initial culling, but provides essentially no information regarding reaction to spittlebug. Pre-selections, based on field performance, need to be rigorously screened for spittlebug reaction before final selections are made for recombination. New screening methodology now available allows a tremendous expansion in capacity.

Methods: At the beginning of the year, 500 pre-selections were identified on field performance from 1997 plantings at Carimagua and Caquetá. Two vegetative replications of each of these clones, plus 10 replications each of four standard check genotypes, were prepared for an initial screening with artificial infestation with spittlebug in the glasshouse. Based on this first screening with infestation, 50 clones were selected for more detailed evaluation with 10 vegetative replications each.

Results and Discussion: Eleven tetraploid sexual clones, fully characterized for spittlebug reaction, were identified by early June 1998, from the initial 3,000 individuals produced for 1997 plantings.

For the first time since *Brachiaria* breeding began at CIAT 10 years ago, we have achieved rigorous selection , from a large initial population, of a small number of clones with desirable agronomic attributes and spittlebug resistance to serve as parentals of the subsequent breeding cycle. Owing to several previous cycles advanced with essentially no information on spittlebug reaction, the breeding population has low average resistance. A very small proportion of the population (ca. 0.3%) shows a useful level of resistance to spittlebug. Following this rigorous selection, the next cycle population should have greatly enhanced average resistance.

Progress towards achieving output milestone:

- **Genotypes of *Brachiaria* resistant to spittlebug under glasshouse and filed conditions available for multilocational evaluation (2000)**

After screening at least 500 *Brachiaria* genotypes for spittlebug resistance in the glasshouse, we have identified a few recombinants resistant to spittlebug, which will now be screened under field condition and tested for acid soil adaptation. We have also reconfirmed and new sources of resistance to spittlebug for the *Brachiaria* improvement program. Thirteen germplasm accessions identified in the first set evaluated are already being introgressed into the sexual breeding population. Accessions identified in subsequent screenings will be incorporated into the population as soon as possible.

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

Production of *Brachiaria* hybrids for mapping population (J. W. Miles and Joe Tohme)

Highlight

- Over 200 additional hybrids produced to augment existing mapping population.

Rationale: Molecular markers of apomixis and of other attributes such as spittlebug resistance should improve efficiency of selection. Identification of marker(s) of the apomixis locus and QTL's for spittlebug resistance requires establishing associations between markers and the desired phenotype(s) in appropriate, large, segregating populations. A small mapping population consisting of hybrids between a very susceptible (tetraploid *B. ruziziensis*) and very resistant (*B. brizantha* cv. Marandú) genotypes had previously been generated. Initial spittlebug data were available on 78 individuals in this population. Additional hybrids were needed to enhance population size to a number adequate for reliable identification of QTL's.

Methods: Additional hybrids were produced by controlled pollination in the glasshouse. Two hundred seventy-six seedlings have been produced. These will be screened (with isozymes) to eliminate any accidental selfs. A total mapping population of 150-200 is sought.

Results and Discussion: An additional 276 putative hybrid seedlings are presently growing in the greenhouse. Isozyme analyses will soon commence. By year's end, we ought to have the additional 100 or more confirmed hybrids to bring total mapping population size to the desired range. Generating a new controlled hybrid mapping population -- a straightforward task in nearly any crop species -- is a far from trivial exercise in *Brachiaria*. Synchronization of flowering is often difficult. Controlled pollination always produces a proportion of unintentional selfs, which need to be identified and culled. The advantage with *Brachiaria* is that, once produced, a hybrid mapping population is essentially immortal (by vegetative propagation).

Propagation of individual clones from mapping population

Highlight

- Original mapping population clones have been maintained and new clones produced.

Rationale: QTL mapping in *Brachiaria* requires clonal replicates of large numbers of individual segregants from well defined genetic population(s). A small initial mapping population (94 individuals) was produced several years ago for studies aimed at mapping the apomixis locus in *Brachiaria*. This population has been maintained by vegetative propagation. New hybrids from the same full-sib family are being produced, confirmed (with isozymes) and propagated for QTL studies of spittlebug resistance.

Methods: Plants are maintained and propagated as part of the *Brachiaria* breeding activities and small population is being augmented with new hybrids.

Results and Discussion: An original population of 94 individuals has been maintained for several years. They have recently been propagated for detailed phenotyping for spittlebug reaction. We are seeking to produce an additional 100 sibs to allow more precise estimation of QTL's. In addition, appropriate plant materials have been increased and made available for marker analysis and for characterization of spittlebug reaction.

Progress towards achieving output milestone

- **Known potential to use marker assisted selection (MAS) for spittlebug resistance in *Brachiaria* (2000)**

Additional hybrids were produced to augment existing mapping population and currently the identity of 100 hybrids is being confirmed with isozymes. In addition, we have 100 maker loci mapped and isolation of microsatellites has been initiated . Mapping of theses markers will follow.

Suboutput 2.5 Role of endophytes in tropical grasses elucidated

Endophytes of forage and turfgrasses are among the most extensively used biological control agents in plant protection. These fungi grow intercellularly in tissues, ovules and seeds of infected plants. These endophytes also protect plants from drought. However, these same endophytes also cause toxicosis and other undesirable effects on livestock grazing the infected grass. All these positive and negative attributes are reported in temperate grasses and their respective endophytes.

Although toxicity and some subclinical symptoms of livestock grazing on certain grasses have been observed in tropical grasses in a number of South American countries, the cause(s) has not been determined. These beneficial and hazardous effects of these mysterious fungi reported in temperate grasses warrant research on tropical grasses.

One of the bottlenecks in endophyte research in *Brachiaria* had been detection of endophytic fungi. Detection protocols in plant tissues and seeds were developed and improved further in 1997. Using these protocols, new endophytes, which resemble those reported in temperate grasses, were identified in species of *Brachiaria*.

During 1998 the following research activities were carried out as part of the endophyte project:

- Development of artificial inoculation methods
- Endophyte detection: examination under light microscopy and isolations culture media
- Surveys and documentation
- Characterization of endophyte isolates

- Taxonomy of endophytes from *Brachiaria*
- Experiments with systemic fungicides to eliminate endophytes in *Brachiaria*
- Effect of endophytes on pathogens *in planta*
- Effect of endophytes on pathogens *in vitro*
- Effect of endophytes on aphid populations

Development of artificial inoculation methods with endophytes (S. Kelemu and M. X. Rodriguez)

Highlights

- Artificial inoculation methods to introduce endophytes into *Brachiaria* were identified.

Rationale: Artificial introduction of endophytes is useful to study specific host-fungus interactions and resistance to pest and pathogens, alkaloid synthesis, host persistence, drought tolerance, toxicity to cattle, etc.

Methods: The successful method we used in infecting *Brachiaria* was the seedling shoot apical meristem inoculation method described by Latch and Christensen in 1985 (Latch, G. C. M. and Christensen, M. J. Artificial infections of grasses with endophytes. Ann. Appl. Biol. 107: 17-24). Seeds were surface sterilized with 2.62% sodium hypochlorite for 15-30 minutes and 50% sulfuric acid for 15-20 minutes and then washed three times with sterile distilled water. The time to sterilize depends on the species and the seed health. Seeds were germinated in the dark on water agar at room temperature.

A small amount of endophyte mycelia was placed into a vertically slit apical meristem using a binocular stereomicroscope in a laminar flow hood. Seedlings were incubated for an additional 7 days in the dark. They were then placed under fluorescent light for about 7 days at room temperature. They were removed from the agar and planted in potted soil under plastic covers to maintain high humidity, incubated for 2 weeks at room temperature and then placed in the greenhouse. The seedlings are grown without plastic cover until tillers were established. The presence or absence of the endophyte was determined by culturing and/or staining leaf sheaths (methods reported in Annual Report 1997).

Results and Discussion: There was a high (as high as 70%) loss of inoculated plants due to damage done to the meristem during inoculations. About 10% of the surviving plants showed the presence of endophytes in subsequent tests involving culturing on media (Figure 20. Pure culture of *Acremonium* isolated from inoculated *Brachiaria* plants) and/or staining tissues and examining under light microscope.

Attempts to infect mature plants through wounds were not successful so far. A final successful introduction of endophytes into plants must show asymptomatic presence, have high seed transmission rate, and the association should show the new desired trait. To avoid less persistent infections, multiple tillers of each plant should be tested for a period of up to 6 months. Once persistent symbiosis is demonstrated, these plants can be used for various studies both in the greenhouse and the field.

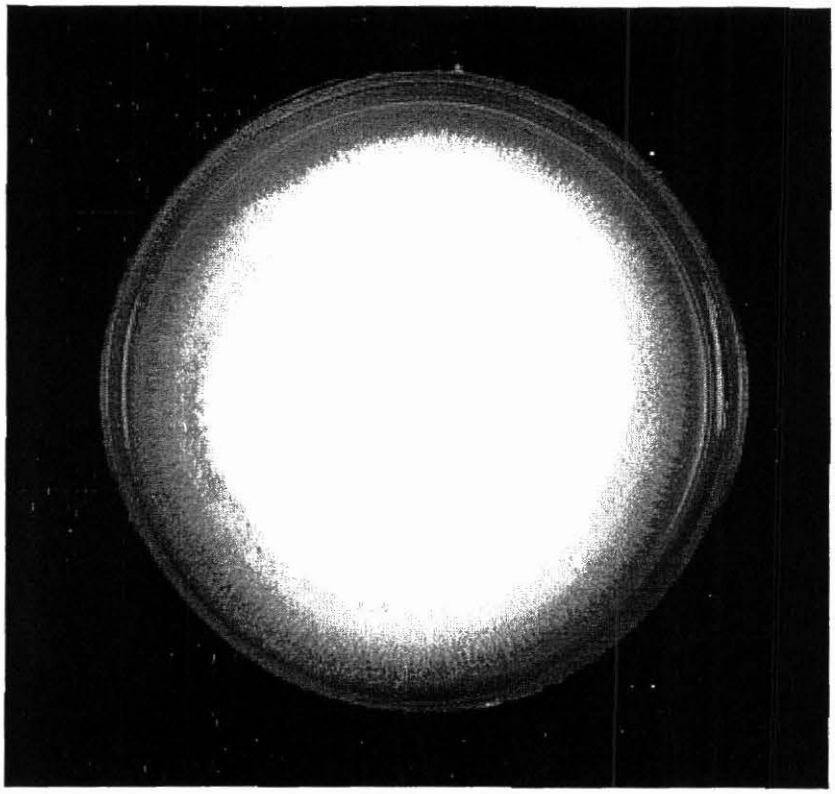

Figure 20. Pure culture of Acremonium isolated from an artificially inoculated *Brachiaria* plant.

Surveys and documentation of endophytes in tropical grasses (Y. Ando, S. Kelemu and Ximena Bonilla)

Highlight

- Additional endophytes were isolated in pure culture.

Rationale: Surveys and documentations of the presence of endophytes in native grasses in the llanos of Colombia and in *Brachiaria* sp. are needed to establish endophyte distribution in tropical America. It is through these surveys that endophytes are collected for further studies.

Methods: Plant tissues collected from the field and greenhouse were prepared as described in Annual Report 1997. Tissues were stored in a 6:3:1 ethyl alcohol: chloroform: 85% glacial acetic acid solution for up to 12 weeks if not examined immediately for detection of endophytes. The samples, which showed presence of endophyte-like mycelia, were cultured on media for isolations.

Results and Discussion: **Field** surveys of native grasses and *Brachiaria* sp. continued in order to determine the extent of endophyte occurrence in species of *Brachiaria* and native grasses. Our surveys confirmed earlier observations that *Balansia* spp. are present in association with native grasses (e.g. Figure 21). In addition, an endophyte-like fungi were isolated in pure culture from *Brachiaria decumbens* CIAT 606 and *B. brizantha* CIAT 26110. Further examinations are needed in order to establish the identity of these fungi.

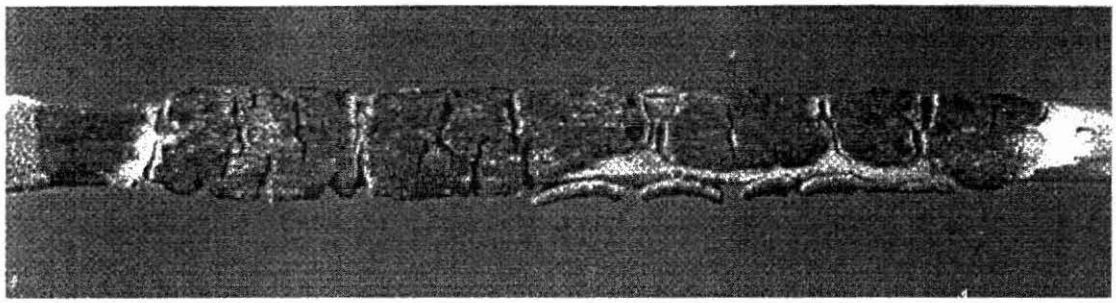

Figure 21. Signs of *Balansia* sp. on a native grass.

Characterization of isolates of endophytes (M. C. Zuleta, G. A. Iriarte and S.Kelemu)

Highlight

- PCR analysis showed that there were differences among isolates of endophytes in species of *Brachiaria*.

***Rationale*:** The purpose of isolate characterization studies is to learn and understand various traits of the microbes in question. One of the big problems in endophyte studies is the lack of quick and reliable detection methods. The methods currently being used are time consuming and labor intensive. One of our objectives in this study is to develop a PCR based detection method.

***Methods*:** DNA was extracted from isolates of endophytes using standard methods. Various primers were tested to randomly amplify portions of the DNA using polymerase chain reactions. A total of 7 isolates from *B. brizantha* 6780 and *B. arrecta* 16485 were tested.

***Results and Discussion*:** Nine primers were identified which gave various polymorphic bands. These preliminary data show that two distinct endophytic fungi were isolated from *B. arrecta* accession CIAT 16485. Eight of the nine primers used failed to amplify the DNA of one of the isolates from accession CIAT 16485, isolate 16485 910 V (e.g. Figure 22). However, a more detailed work is needed to characterize more isolates and to determine the distinction among them.

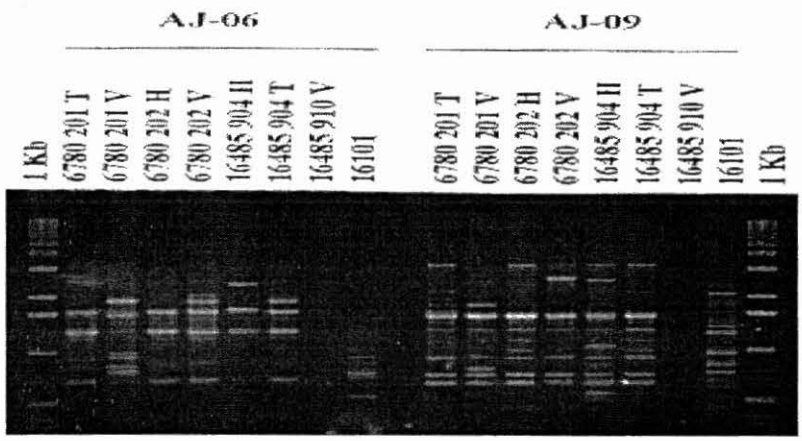

Figure 22. RAPD profiles of isolates of *Acremonium* isolated from accessions of *Brachiaria*.

Taxonomy of endophytes isolated from *Brachiaria* (J. White and S. Kelemu)

Highlight

- Using partial sequences of the nuclear small subunit ribosomal DNA (18 S rDNA), many of the endophyte isolates from species of *Brachiaria* were put in clad with *A. strictum* and *A. kiliense.*

***Rationale*:** Detailed studies of endophyte taxonomy are essential in order to determine the evolutionary relationship among various endophytes and establish their correct identity. Classification systems based on culture characteristics and DNA probes provide reliable information.

***Methods*:** Endophytes were isolated on standard laboratory media such as potato dextrose agar supplemented with tetracycline to deter bacterial contamination. Once pure fungal cultures were obtained, they were examined for culture characteristics under light and scanning electron microscopy. Those, which resembled Acremonium endophytes, were sent to the International Mycological Institute in UK for further confirmation. In addition, these isolates were sent to Rutgers University for further detailed DNA analysis.

***Results and Discussion*:** The International Mycological Institute confirmed the fungi identified from species and accessions of *Brachiaria* to be either *Acremonium implicatum* or *Acremonium* sp. Using partial sequences of the nuclear small subunit ribosomal DNA (18 S rDNA), these isolates were put in clad with *A. strictum* and *A. kiliense.* This indicates that the identification of the isolates as *A. implicatum* was probably a correct one.

Use of systemic fungicides to eliminate endophytes in species of *Brachiaria* (C. E. Posso, M. X. Rodriguez, X. Bonilla, Y. Takayama, and S. Kelemu)

Highlight

- *Brachiaria* clones with or without endophytes were produced.

***Rationale*:** Endophyte-free and endophyte-infected plants are useful to study a number of responses of plants and the role endophytes play in these responses. In addition to artificial inoculations to introduce endophytes into plants with no endophytes, fungicides can be used to eliminate from endophytic fungi from plants and thus creating endophyte-free plants.

***Methods*:** Two fungicides were used to generate endophyte-free clones of species of *Brachiaria.* Propiconazole, a systemic fungicide with a broad range of activity, was used to remove endophytes from *B. brizantha* CIAT 6780 and *B. arrecta* CIAT 16845. Several young tillers were removed from each genotype and each was planted in a cup without draining holes, which contain washed and sterilized sand. Propiconazole solution at 6 µg/ml concentration was added to the pot to completely immerse the tillers. Every two days the cups were weighed and water was added to the original weight for the purpose of maintaining the original fungicide concentration. After growing for 5 weeks, each plant was removed, washed and replanted in pots containing normal greenhouse soil. Three months later, these plants were checked for endophyte presence.

Folicur, another fungicide, was used to eliminate endophytes in *Brachiaria.* Three to five washed tillers of each genotype were prepared. These tillers were placed in a beaker containing a solution of 0.1 ml/l of Folicur, which has 250 g ai/l for about 6 hours. The treated tillers were

then planted individually in pots containing soil. The plants were allowed to grow until new tillers are produced. The leaf sheath of the tillers were examined for absence of endophytes.

Results and Discussion: The method involving Folicur was preferable to use as the procedure allowed to generate endophyte-free tillers in a much shorter time than with the propiconazole method. Subsequent tests revealed that the success rate of eliminating the endophyte was around 30%.

Effect of endophytes on a leaf spot pathogen in *Brachiaria* (Y. Takayama, F. Muñoz, and S. Kelemu)

Highlight

- *In vitro* and *in vivo* assays showed that endophytes are effective against certain fungal pathogens

Rationale: Many endophyte-infected grasses exhibit enhanced resistance to insects and pathogens. Endophyte-infected grasses possess other properties of applied value as well. The adverse effect of endophyte/grass association on pathogens has been reported in only temperate grass/endophyte symbiosis so far. It is important to study the role of endohytes in disease control in tropical grasses like species of *Brachiaria*.

Methods: An endophytic fungus, *Acremonium implicatum* (J. Gilman and E.V.Abbott) W. Gams, was isolated from *Brachiaria brizantha* 6780. Plantlets were propagated from the original mother plant containing the endophyte. Half of these plantlets were treated with the fungicide Folicur to eliminate the endophyte while the remaining half was left untreated. These genetically identical plants were then challenged with *Drechslera* sp., a casual agent for a leaf spot disease.

Results and Discussion: Results indicate that *Acremonium implicatum* protects *Brachiaria brizantha* CIAT 6780 from infections by *Drechslera* sp. Endophyte-infected plants had fewer and smaller lesions than endophyte-free plants (Figure 23).

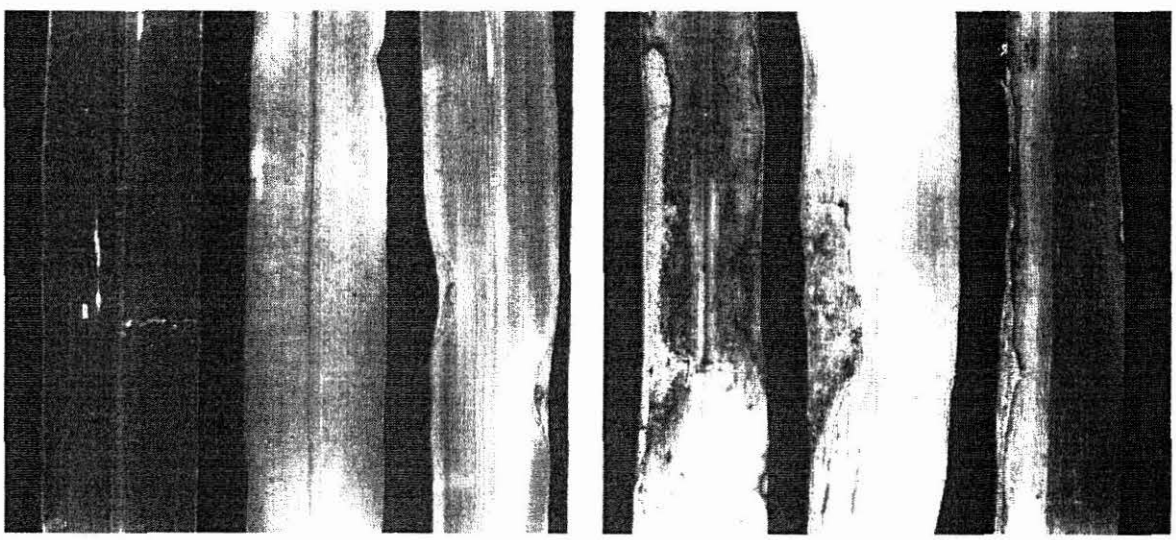

Figure 23. Endophyte-containing and endophyte-free tissues of *Brachiaria brizantha* challenged with the pathogen *Drechslera* sp. Tissues on the left contain an endophyte.

Sporulation of *Drechslera* sp. on artificially inoculated, detached leaf sheaths was less on tissue containing endophyte than on endophyte-free tissue (Figure 24).

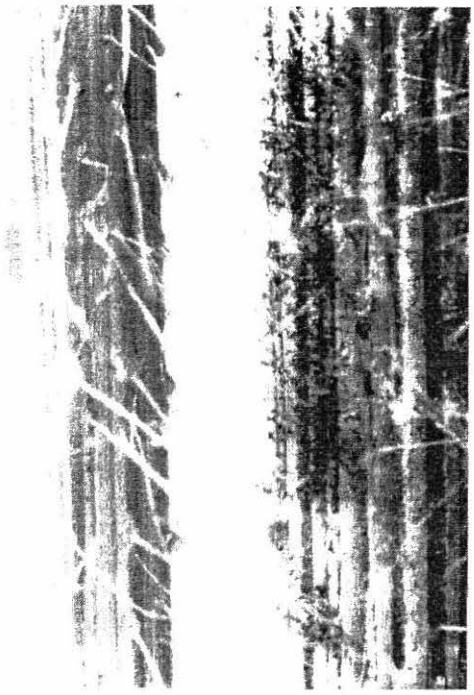

Figure 24. Endophyte-containing and endophyte-free leaf sheaths inoculated with the pathogen *Drechslera* sp. Tissue on the left contains an endophyte. Note the extent of pathogen sporulation on the tissue, which is free of endophytes.

***In vitro* effects of *A. implicatum* on the pathogen *Drechslera* sp.** (S. Kelemu, X. Bonilla, and F. Muñoz)

Highlight

- Plate assays showed that a diffusible compound (s) of endophytes inhibit the growth of the fungal pathogen *Drechslera* sp.

***Rationale*:** Several *Acremonium* endophytes of temperate grasses have been reported to inhibit the growth of pathogenic fungi *in vitro*. No such information, however, is available on *Acremonium* endophytes of tropical grasses. Knowledge on *in vitro* effects of endophytes on plant pathogenic fungi may help us understand and design disease management strategies in grasses.

***Methods*:** An agar plate assay was used to determine *in vitro* antifungal activities of *A. implicatum*. The fungus *Drechslera* sp. which causes a leaf spot disease of *Brachiaria* was used in the test. An agar disk of *Acremonium* was placed in the center of a potato dextrose agar plate containing tetracycline. A sterile filter paper the same size as the bottom of the petri plate was placed on top of the agar completely in contact with the agar. The plates were incubated at 28 C until a fourth of the area was covered with fungal mycelia and conidia. A plug of *Drechslera* was then placed on the center of the filter paper and incubated further. Control plates had the same treatment except they had no *Acremonium*. When *Drechslera* mycelial growth covered the control plates, measurements were taken. Plates were scanned and areas of *Drechslera* growth recorded using image analyzer, WinRHIZO, Version 3.6 D (Regent Instruments Inc, Canada). In

another experiment, *Acremonium* plug was placed at the center of a sterile filter paper, which was placed on top of a PDA plate and incubated until a fourth of the filter paper was covered with endophyte growth. The paper was then carefully removed from the plate. A *Dechslera* plug was placed at the center of the agar plate and incubated at 28 C.

Results and Discussion: This simple assay indicated a strong antibiosis by *Acremonium* occurred with *Drechslera* sp, an important pathogen of a wide range of grasses. Figure 25 shows *Drechslera* mycelial growth area in the presence or absence of *Acremonium*.
It is important to remember that this assay does not imply that the endophyte demonstrates antifungal activity *in planta*. Figure 26 shows pathogen growth inhibition by some compound(s) of the endophyte.

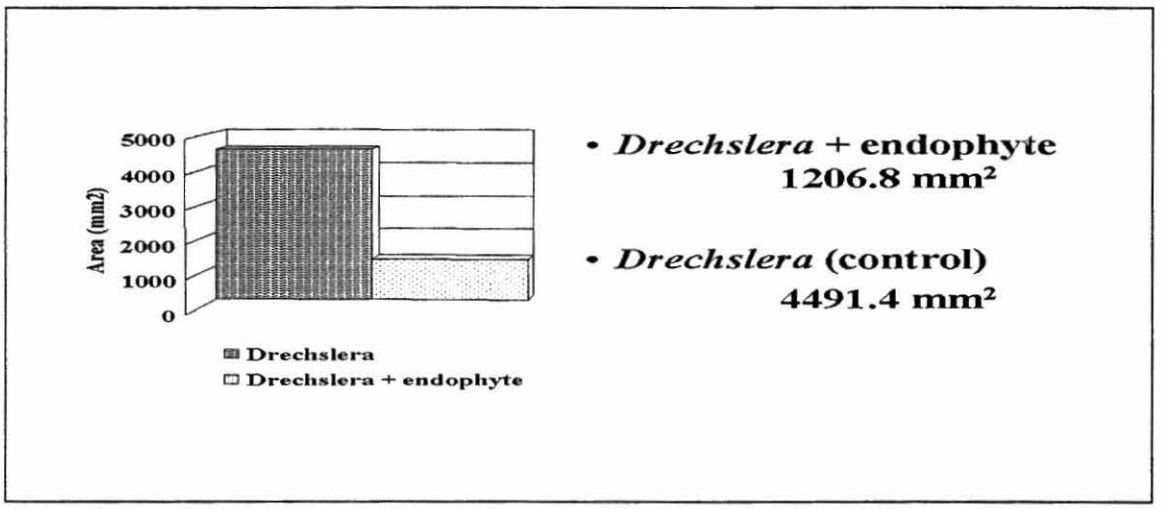

Figure 25. Mycelial growth of *Drechslera* sp. in the presence or absence of *Acremonium*.

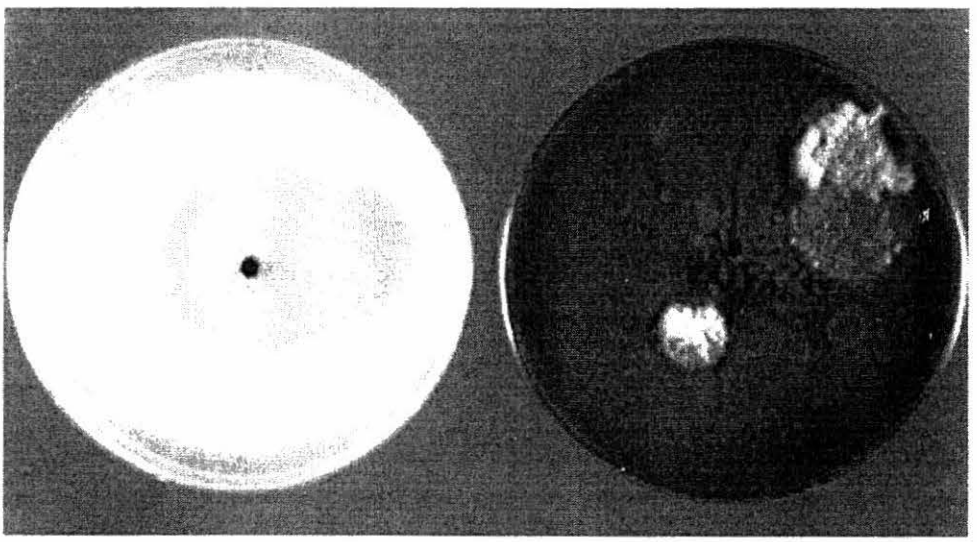

Figure 26. Growth inhibition of *Drechslera* by a diffusible compound(s) of *Acremonium*. The plate on the right is control plate.

Effect of endophytes on aphids in *Brachiaria* (F. Muñoz, C.E. Posso, and S. Kelemu)

Highlights

- Assays conducted on *Brachiaria* clones showed that aphids preferred to feed on endophyte-free plants to endophyte-infected ones.
- Some level of toxicity to aphids may exist in endophyte-containing *Brachiaria* clones.

Rationale: Endophyte-induced resistance of temperate grasses to insects has been well documented. The most intensively studied one is the interaction of perennial ryegrass with the Argentine stem weevil. Many other insect species including aphids have been reported to be adversely affected by endophytes in a number of temperate grasses. This kind of information is, however, not available in tropical grasses

Methods: Endophyte-free and endophyte-infected *B. arrecta* clones were created using the fungicide Folicur as described above. Two types of tests were conducted: 1) Ten aphids (*Rhopalosiphum maidis*) were placed on each plant. Aphid-infested endophye-free or endohyte-infected plants were placed separately and isolated from each other under transparent plastic boxes in order to confine the aphids to either endophyte-free or endophyte infected plants. The treatments were arranged in a randomized complete block design with 4 blocks in the greenhouse. Aphid population counts were conducted for a period of 15 days. 2) Ten aphids were placed on each plant. Endophyte-infected and endophyte-free plants infested with aphids were then placed under a transparent plastic box in order to give the aphids a choice between endophyte-infected or endophyte-free plants. Aphid counts were conducted for a period of 20 days.

Results and Discussion: Results of experiment 1 and experiment 2 are presented in Figures 27 and 28, respectively. Deterrence and some level of toxicity appear to be involved.

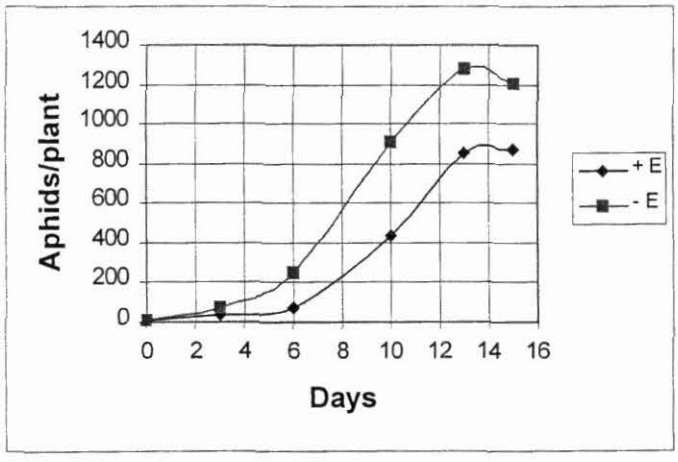

Figure 27. Populations of aphids on endophyte-containing or endophyte-free plants of *Brachiaria arrecta* (*no choice* assay).

As Figure 28 shows, there is an apparent preference for endophyte-free *Brachiaria* plants by the aphids when they had a choice between endophyte-infected or endophyte-free plants as demonstrated by higher number of aphid population on endophyte-free plants. When no choice was given and the aphids were confined strictly to either endophyte-free or endophyte-infected

plants, still a higher number of aphids were counted on endophyte-free plants than on endophyte-infected ones although to a lesser degree (Figure 27) than when a choice was provided, indicating perhaps that some toxicity by endophytes may exist. These observations are in agreement with those reported in endophyte/temperate grass symbiosis.

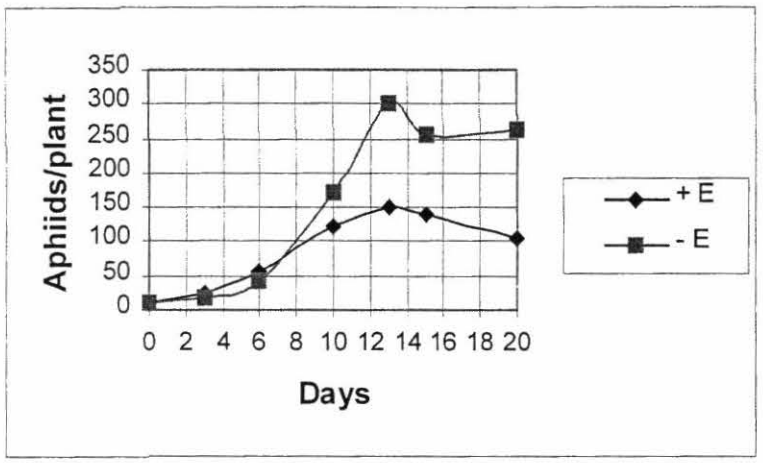

Figure 28. Populations of aphids on endophyte-containing or endophyte-free plants of *Brachiaria arrecta* (preference assay).

Progress towards achieving output milestone

- **Protocols for detection of endophytes and list of endophytes properly identified in tropical grasses (1999)**

We have adapted protocols to detect endophytes in tropical grasses and in collaboration with the US-based company BioWorld, specific antisera were developed for detection of endophytes in *Brachiaria* . In collaboration with Rutgers University, the taxonomy of the endophytes of *Brachiaria* has been determined in relation to other endophytic fungi. RAPD analysis showed that isolates of endophytes from species of *Brachiaria* were variable.

We have also made progress in defining the potential benefits of endophytes. An effective artificial inoculation method has been adopted for use in *Brachiaria* and endophyte-free *Brachiaria* clones were generated using systemic fungicides. These clones along with clones containing endophytes are being tested for drought, insect and pathogen tolerance. Effects of endophytes on a fungal pathogen and aphids was determined.

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

Rationale: Anthracnose, caused by *Colletotrichum gloeosporioides*, is an important disease of *Stylosanthes guianensis*, and of world-wide distribution. It is also becoming increasingly important on *Arachis pintoi*. Total dry matter losses and reduced nutritional value have been reported in Colombia and other countries.

The pathogen is a heterogeneous and complex species, consisting of various host specific populations. It exhibits extreme variability in both morphology and pathogenicity. The cheapest

and environmentally clean method of disease control is the use of resistant plant genotypes. Understanding the pathogenic and genetic variability among isolates of the pathogen is key to creating effective breeding programs for anthracnose resistance and deployment of resistance. Work on a new disease of *Stylosanthes* caused by the fungus *Lasiodiplodia theobromae* was completed in 1997.

This work included the identification of the causal agent, development of inoculation and resistance assessment methods, and identification of resistant genotypes of *S. guianensis*. A paper was published in the Canadian Journal of Plant Pathology on this work (see List of Publications). Effective artificial inoculation methods were developed for some foliar diseases of *Arachis pintoi* and sources of anthracnose resistance were identified in *Stylosanthes* genotypes. Genetic and pathogenic diversity among isolates of *C. gloeosporioides* collected from various geographic locations were determined, and several publications describe this work (see List of Publications).

During 1998 the following activities were carried out:
- Biodiversity studies on the anthracnose pathogen of *Arachis*
- Molecular data analysis
- Graduate thesis development and supervision on anthracnose disease management
- *C. gloeosporioides* cross-infectivity studies in isolates from *Stylosanthes* and *Arachis*
- Manuscript preparation

Biodiversity studies on the anthracnose pathogen of forage *Arachis* (F. Muñoz, M. X. Rodriguez and S. Kelemu)

Highlights

- Pathogenic variability of 183 isolates of *Colletotrichum gloeosporioides* isolated from *Arachis pintoi* was determined on 5 accessions of *A. pintoi* (CIAT 17434, 18744, 18748, 22160) and the original accessions from which the isolates were obtained. These isolates were collected from Carimagua, Caquetá, Palmira and Popayan.

- Pathogenic specialization of isolates was observed and the genetic diversity of 91 of these isolates was measured by RAPD analysis.

- Results show there were at least five lineages of *C. gloeosporioides* in the isolates studied. Caquetá area had the highest diversity of *C. gloeosporioides* isolates of the forage *Arachis*.

Rationale: *Arachis pintoi* is an important legume with potential as forage, hay, and cover crop. Accession CIAT 17434 is currently the most widely distributed. This narrow genetic base has perhaps the associated potential risks of severe disease epidemics. Density of legumes in pastures is generally higher than those found in native habitats, thus providing a favorable microclimate for disease development including *C. gloeosporioides*.

Although anthracnose is not currently a significant constraint for *A. pintoi*, it is a disease often observed in experimental plots in various locations. The common practice of using vegetative propagation materials probably has contributed to its distribution. It is, thus, important to study the pathogenic and genetic diversity of the pathogen in preparation for effective disease management strategies.

Methods: Isolates were collected and purified using standard methods described earlier. Five accessions of *A. pintoi* (CIAT 17434, 18744, 18748, 22160) were inoculated with each of the

183 isolates. A corresponding accession from which each isolate was isolated was included as a positive check for pathogenicity. An inoculation method developed for foliar pathogens of peanuts was adopted where detached leaves instead of whole plants were used.

DNA was isolated from all isolates, which showed pathogenicity on at least the original host accession, and some non-pathogenic isolates, using standard methods. Nine arbitrary primers were used to randomly amplify DNA using protocols described in earlier reports (Annual Report, 1997).

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. A statistical analysis of the genetic variation over the entire data set was undertaken to determine the possible number of genetic lineages present using NTSYS-pc.

The similarity index for qualitative data was calculated using Simple Matching Coefficient. A clustering program with UPGMA was used to construct a dendrogram .

Results and Discussion: Of the 183 isolates evaluated, only 78 isolates consistently produced gray spots with a brown to black sharp edge or brown to black spots. These symptoms were scored as anthracnose symptoms. Differential reactions were observed in isolate/host genotype interactions, indicating pathogenic specialization among isolates of *C. gloeosporioides* in *A. pintoi.*

Figure 29 shows a dendrogram, which classified the 91 isolates tested into five groups (A, B, C, D, and E). The 91 isolates analyzed were separated first in two groups at 0.47 similarity level : one group with 81 isolates and another group with 10 isolates.

This second group had a similarity index of 0.97 to 1.00. The group with 81 isolates was divided into two groups at similarity index of 0.60. One of these was group B, with 47 isolates from Caquetá (32 isolates) and Popayán (15 isolates), had a similarity index of 0.85 to 1.00 (Figure 29).

The other group with 34 isolates was further divided at similarity index of 0.70 in 2 groups. Group E consisted of 14 isolates with similarity index of 0.84 to 1.00 and another group with 20 isolates was separated at 0.78 level to form group C with similarity index of 0.82 to 0.96, and group D with similarity index of 0.86 to 0.97. Groups C and D consisted of 9 and 11 isolates, respectively.

Isolates from Caquetá appeared in all of the groups indicating that Caquetá had isolates with high genetic diversity. Isolates from Popayan and Caquetá showed a high degree of similarity indicating that one originated from the other. Group E consisted of isolates from Palmira.

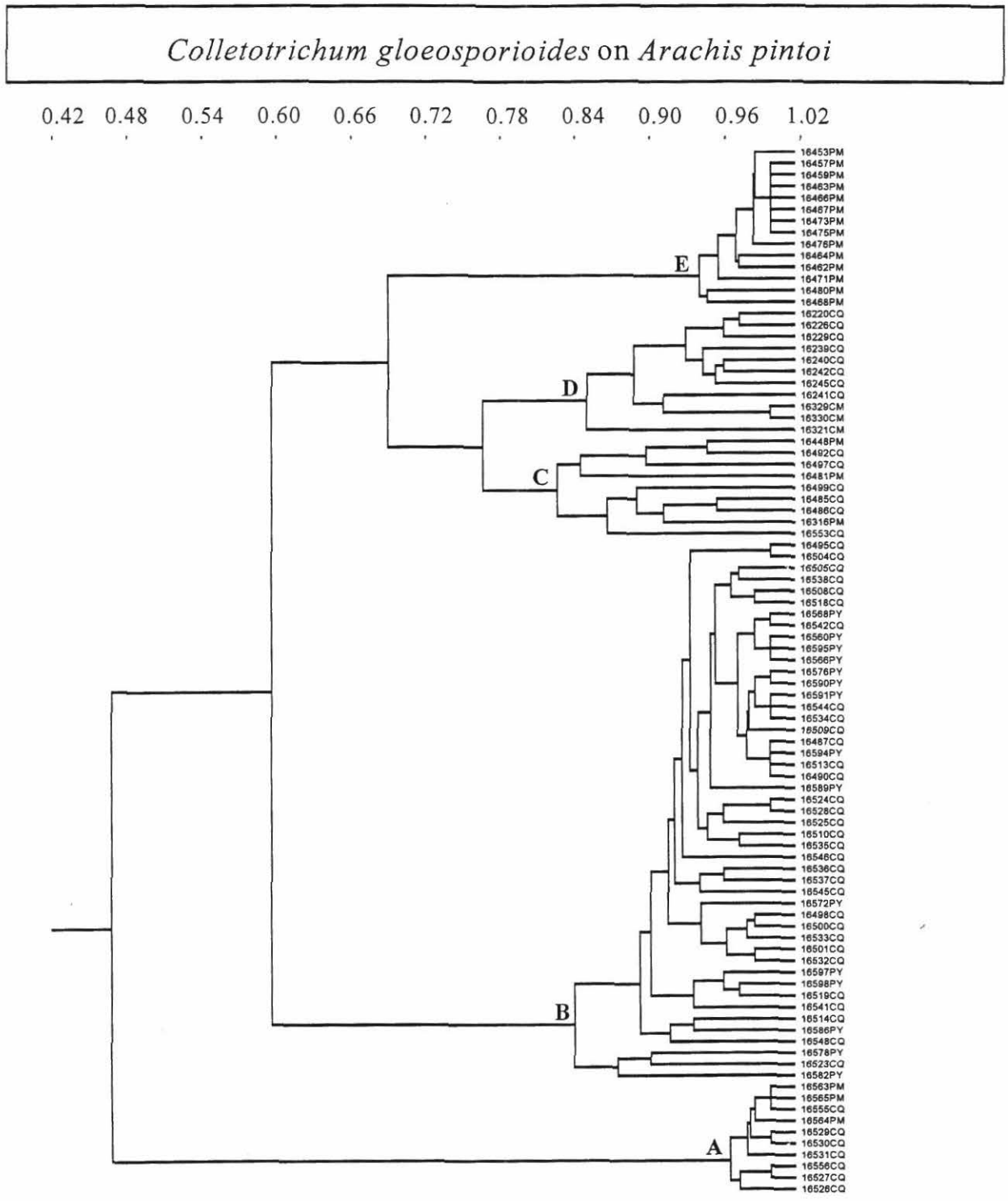

Figure 29. Dendrogram shows the 91 isolates tested into five groups (A, B, C, D, and E)

Anthracnose disease management methods: Genetic engineering of *Stylosanthes guianensis* using chitinase encoding gene (J. Changshun and S. Kelemu)

Highlights

- A chitinase- encoding gene cloned from rice was recloned in an appropriate vector for *Agrobacterium*-mediated transformation.
- *In vitro* effects of crude preparations of the chitinase enzyme on *C. gloeosporioides,* isolated from *Stylosanthes* sp., were determined.

Rationale: Chitinases are a group of proteins expressed in plants in response to infections by plant pathogens. Chitinases are also secreted by number of microbes, including soil bacteria. Chitin is a major cell wall component of many fungi. Chitinases catalyze the hydrolysis of chitin. Some chitinases also have lysozymal activity and can hydrolyze the peptidoglycans in bacterial cell walls.

It has been proposed that chitinase activity induction in plants is a defense mechanism by plants. Induction of chitinase activity has been reported in *Stylosanthes* following infections by plant pathogens. Anthracnose is an important disease of *Stylosanthes*. Although sources of anthracnose resistance are available in *Stylosanthes*, the high level of genetic and pathogenic variability in the pathogen made it difficult for long-term resistance. It is necessary, therefore, to study other disease management methods in addition to host resistance.

Methods: DR. S. Muthukrishnan of Kansas State University kindly provided the chitinase gene cloned from rice. The chitinase gene was recloned in our laboratory in a different vector kindly provided by Dr. R. Jefferson of CAMBIA, Australia. *In vitro* activity of the newly cloned gene was studied on isolates of *C. gloeosporoides* pathogenic on *Stylosanthes*. Crude enzyme preparations from cultures of *E.coli* containing the clone were concentrated and used to test their effectiveness against *C. gloeosporioides*. This active chitinase expressing clone was mobilized to strains of *Agrobacterium tumefaciens* using triparental mating with helper plasmid PRK2013.

Results and Discussion: The chitinase enzyme showed *in vitro* antifungal activity against *C. gloeosporioides* (Figures 30, 31).

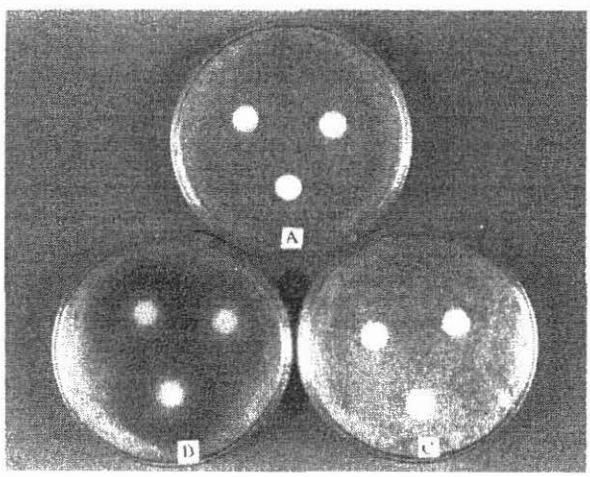

Figure 30. Inhibition of *Colletotrichum gloeosporioides* growth by crude preparations of chitinase. Note the cleared zone caused by chitinase enzyme on plate B as opposed to control plates A and C.

Because the gene encoding the enzyme was isolated from rice and because it naturally exists in a number of plants, the strategy of introducing it to another plant through genetic engineering should pose no biosafety concerns. Chitinases have been shown to have a wide antifungal and even antibacterial activities at least *in vitro*. If these activities are also possible *in planta*, then we may end up with transgenic plants with resistance to a number of pathogens. This work is a graduate thesis work by a student from the University of Southern China, the People's Republic of China.

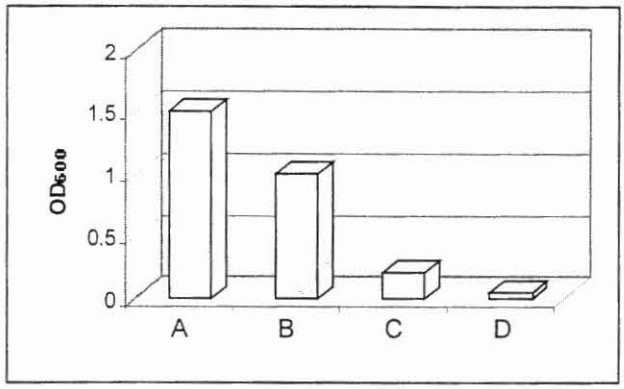

Figure 31. Optical density of *Colletotrichum gloeosporioides* cultures in the presence of cell-free culture filtrates of chitinase (C,D) or no chitinase clones (A,B).

Anthracnose host range studies in forage legumes (C. I. Giraldo, F. Muñoz, and S. Kelemu)

Highlights

- Host range studies showed that isolates of *C. gloeosporioides* cross-infect *Stylosanthes* and *Arachis*.

Rationale: *C. gloeosporioides* is a pathogen, which causes anthracnose in a number of plants. This species is the most ubiquitous of all *Colletotrichum* species with very wide geographic distribution. We studied the cross-infectivity of isolates of *A. pintoi* and *S. guianensis* in order to understand the risk posed by the pathogen particularly on forage *Arachis*.

Methods: Highly virulent isolates of *C. gloeosporioides* isolated from *S. guianensis* and *A. pintoi* were used to determine their cross-infectivity. Isolations, culture maintenance, inoculum preparations, inoculations and disease evaluations were conducted using established protocols described in earlier reports and articles. Isolates of *S. guianensis* used in this study were CIAT 16117, 16134, 16135, 16162 and 16555. Those of *A. pintoi* were CIAT 16481, 16541, 16544, 16590 and 16499. The *A. pintoi* accessions used in the host range studies were CIAT 17434, 18744, 18748 and 22160. The *S. guianensis* accession used is the nearly universal suscept 2312.

Results and Discussion: Results show that isolates cross-infect *Arachis* and *Stylosanthes* (Table 32). This is the first report of cross-infectivity of a pathogen between the two genera. The results also showed differential reactions by isolates of *C. gloeosporioides* on accessions of *A. pintoi*. It is, thus, possible to establish differential hosts in order to characterize races of the pathogen on *Arachis*. If and when the pathogen becomes a major obstacle to the use of forage

Arachis, breeding strategies similar to those of *Stylosanthes* may be used to manage the disease. Information is available on the genetic diversity and distribution of isolates of the pathogen infecting *Stylosanthes*. This information may then be useful and applicable to *Arachis* as well.

Table 32. Reaction of accessions of *Arachis pintoi* and *Stylosanthes guianensis* to isolates of *Colletotrichum gloeosporioides* (% infection)[1].

Isolates[2]	Accessions of *A. pintoi*				*S. guianensis*
	17434	18744	18748	22160	CIAT 2312
16134 sg	80	40	40	20	100
16162 sg	20	40	80	60	100
16135 sg	100	100	40	60	100
16117 sg	40	40	60	40	40
16499 sg	80	60	100	40	40
16555 sg	80	60	60	60	40
16544 ap	60[3]				60
16541 ap	40[3]				80
16590 ap	20[3]				100
16481 ap	100[3]				80

[1] Results are the higher of two inoculations.
[2] s.g indicates isolates originating from *S. guianensis*; a.p indicates isolates originating from *A. pintoi*.
[3] Results are on their respective original host accession.

Progress towards achieving output milestone

- **Number of isolates of *Colletotrichum* characterized and list of *Stylosanthes* genotypes with variable resistance to anthracnose (1999)**

A comprehensive study on genetic and pathogenic variability among several isolates of *C. gloeosporioides* was conducted. The results are useful for breeding for anthracnose resistance in *Stylosanthes*.

The cause of another important disease of *Stylosanthes*, which contributed to poor persistence in Stylosanthes stands, was determined, and sources of resistance were identified.

More detailed epidemiological studies on anthracnose and additional pathogen population studies could not begin in 1998, because funds from ACIAR were not released on time to support the work.

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

Analysis of isozyme data of *Brachiaria* (M. C. Duque, J. W. Miles and G. Ramírez)

Highlight

- Isozyme data on a collection of 411 *Brachiaria* spp. accessions were analyzed and certain species separated clearly from the rest.

Rationale: Characterization of genetic diversity within a species or genus will become more precise and meaningful as classification criteria more directly related to variations in DNA are used. Isozymes might be considered intermediate between morphological markers and direct molecular DNA markers (e.g. RFLP, RAPD, or AFLP markers). Large isozyme data sets existed, but had never been submitted to proper analysis.

Methods: Four hundred eleven germplasm accessions, representing five major species (*B. decumbens*, *B. brizantha*, *B. ruziziensis*, *B. dictyoneura*, and *B. humidicola*) were used. Presence/absence of 32 recognizable isozyme bands was recorded. These data were submitted to correspondence analysis to detect groupings on similarity of banding pattern. Results were graphed in three dimensions by principal coordinates. (Figure 32).

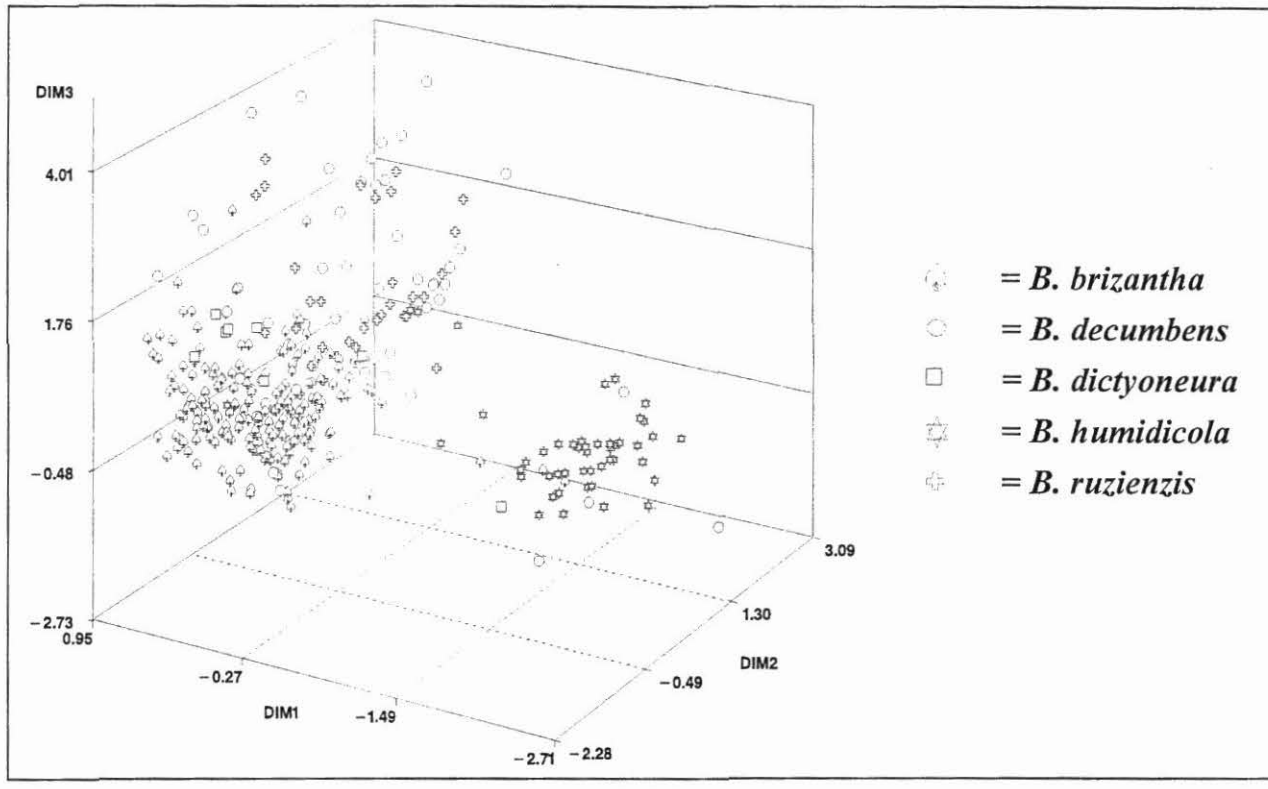

Figure 32. Distribution on three principal component axes of 411 *Brachiaria* spp. accesions, based on diversity of isozyme banding patterns.

Results and Discussion: *B. humidicola* accessions separated clearly from the remainder of accessions on the first component axis. *B. dictyoneura* accessions grouped more closely with *B. brizantha* accessions than with *B. humidicola*, a result contradicting conventional taxonomic classifications which place *B. dictyoneura* and *B. humidicola* in the same sub-specific group (Renvoize, et al. 1996). The only "*B. dictyoneura*" accession falling in the *B. humidicola* group is CIAT 6133, cv. Llanero, which has been reclassified as *B. humidicola* The *B. brizantha*, *B. decumbens*, and *B. ruziziensis* accessions formed much less coherent groups, with no apparent separation at all between *B. decumbens* and *B. ruziziensis*. This is perhaps to be expected, based on the tremendous variability traditionally lumped into *B. brizantha*, the taxonomic confusions among the three species, and the undeniable evidence for proximity of phylogenetic relationship as given by ease of hybridization.

These results for analysis of isozyme variation are the first large-scale attempt at characterizing diversity among the agronomically important *Brachiaria* spp. based on other than morphological or agronomic attributes. It suggests considerable promise for understanding intrageneric diversity when direct DNA markers can be brought to bear. This sort of information should have important implications in applied areas such as plant breeding. Comparison of similarity patterns based on morphological attributes should offer even greater understanding of genetic diversity in *Brachiaria* and its practical consequences.

Analysis of isozymes data of *Arachis* (M. C. Duque, M. Peters and G. Ramírez)

Highlight

- Analysis of isoenzymes in a small collection of *Arachis pintoi* was completed and seven distinct groups based which included genotypes different from the common cultivars.

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

Methods: Isozyme data collected from 28 accessions of *Arachis pintoi* were submitted to the appropriate analysis. A total of 93 bands corresponding to 6 isoenzymes (α esterase, β esterase, Acid phosphatase (acp), Peroxidase, Glutamate Oxalacetate Transaminase (got), Diaforase (dia)) were investigated, of which 3 bands of got, one of acp and one of dia were utilized.

The similarity of accessions (individuals) was calculated by the DICE coefficient followed by the UPGMA method to obtain a dendrogram, utilizing the following equation:

$$Sij = \frac{2a}{2a+b+c}$$

Where:
a=number of shared bands for individuals i,j
b=number of exclusive bands for individual i
c=number of exclusive bands for element j
For analysis, the NTSYS (Numerical taxonomy and multivariate analysis system) package, version 1.8 was utilized.

Results and Discussion: It was possible to differentiate the 28 accessions of *Arachis pintoi* under study into 8 different groups (Figure 33). An earlier isoenzyme study of 37 accessions of *Arachis pintoi* identified 10 distinct groups. It will now be important to relate the groups formed with their origin and agronomic performance. From a preliminary observation of the data, some groups identified by the isoenzyme analysis (mainly the 94' accessions) as closely linked, are also part of the group with superior agronomic performance (CIAT 18747, 18748, 18751 and 22159) in different environments. It is also interesting to note that the commercial cv of *Arachis pintoi (*CIAT 17434) was separated from the rest of accessions, which suggest that it genetically different.

Progress towards achieving output milestone

- **Degree of correlation of isozyme characterization data with germplasm performace in different environmental (2000)**

A important initial step at understanding the organization of genetic diversity in the genus *Brachiaria* and *Arachis* was made with the proper analysis of existing isozyme data on a large germplasm collection. The challenge ahead is to relate isozyme data with passport data and with agronomic performance in different environments.

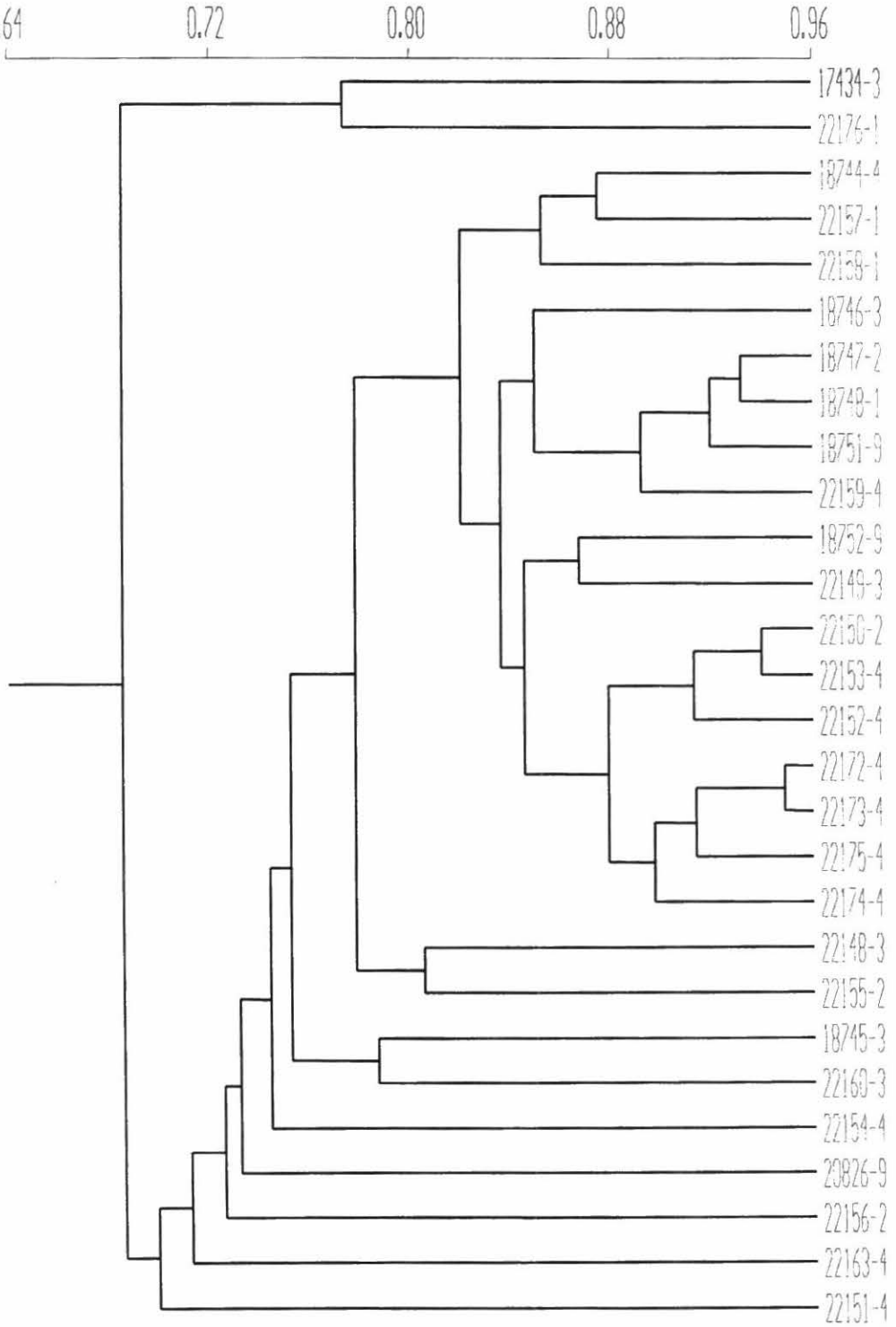

Figure 33. Dendrogram of *Arachis pintoi* based on isozymes analysis using Dice coefficient similarity and UPGMA for classification

Output 3: Development of forage gene pools with known adaptation to environmental constraints

Sub-output 3.1: Genotypes of *Brachiaria*, and *Arachis* with adaptation to low fertility soils identified and characterized

Low nutrient supply, particularly phosphorus (P), is a major limitation of forage adaptation and production in low fertility acid soils. The use of tropical forage grasses and legumes adapted to nutrient stress is one of the most effective means of managing these infertile acid soils. Adapted grass and legume plants have root and shoot attributes that are linked to strategies to acquire nutrients in a low pH and high aluminum (Al) environment. Thus identification of these plant attributes is fundamental to develop more efficient screening procedures for germplasm evaluation and/or improvement.

Significant progress in identification of adaptive attributes of grass (*Brachiaria*) and legume (*Arachis*) species to acid soils has been achieved through collaborative research efforts with the University of Hohenheim (Germany), University of Vienna (Austria) and Hokkaido University (Japan). Both grass and legume species seem to adapt to low nutrient supply in acid soils by increasing the amount of dry matter partitioned to roots at the expense of leaf expansion and shoot growth. But they differ in: (i) shoot and root growth response to phosphorus (P) application - the grass responded better than the legume; (ii) efficiency in acquisition and utilization of P from inorganic and organic P sources - legume was more efficient in acquiring P from less available P sources.

Using a simulated acid soil stress solution and Proton-induced X-ray Emission (PIXE) technique, P accumulation in root apices of *Brachiaria decumbens* (CIAT 606) was identified as a specific mechanism for tolerance to Al. In addition to Al tolerance, this well adapted grass secreted greater amounts of the enzyme phytase that helps to acquire P from organic P sources. These two specific mechanisms could contribute to the outstanding performance of this grass in acid soils.

Significant genotypic variation in a number of shoot and root attributes was observed in species of *Brachiaria* and *Arachis pintoi* when grown with low supply of nutrients (CIAT-IP-5 - Annual. Report 1996; 1997). Field evaluation resulted in identification of one spittlebug-resistant genetic recombinant (BRN093/1371) of *Brachiaria*. This hybrid combined a number of desirable plant attributes such as superior leaf area and leaf biomass, greater nitrogen (N) content in leaves, and greater partitioning of N and P to leaves that could contribute to superior adaptation to infertile acid soils. One accession of *Arachis pintoi* (CIAT 18748) was identified as outstanding in its adaptation to low fertility acid soil based on its ability to partition greater amounts of N to leaves (CIAT-IP-5 - Annual. Rep. 1997).

During 1998, we made significant progress in developing screening methodology to evaluate aluminum tolerance in *Brachiaria* species. We have also made substantial progress in defining specific physiological and biochemical mechanisms that contribute to superior adaptation of *Brachiaria decumbens* to low supply of phosphorus and nitrogen. Furthermore, we have elucidated the chemical structure of two secondary metabolites that are induced in roots of *Brachiaria* species under phosphorus and nitrogen stress conditions.

Studies on mechanisms of acid soil adaptation in *Brachiaria* cultivars

Highlight

- Significant progress was made toward identifying specific physiological and biochemical mechanisms of acid soil adaptation and developing screening methodology to assess edaphic adaptation of *Brachiaria* genotypes to acid soil stress.

Rationale: *Brachiaria* cultivars are the most widely sown forage grasses used for livestock production in acid soil regions of Latin America, particularly in tropical savannas. They can increase livestock productivity by a factor of 5 to 10 with respect to native savanna vegetation, thus representing a significant contribution to the income of farmers. However, all *Brachiaria* species that have attained importance as commercial cultivars have one or more recognized agricultural deficiencies that limit their usefulness, productivity or persistence. Consequently, pasture degradation has caused a widespread negative environmental impact in Latin America, a situation that could be alleviated by the use of an improved set of cultivars that are genetically adapted to both biotic and abiotic stresses.

An ongoing breeding program at CIAT seeks to combine favorable traits, such as adaptation to acid soils, resistance to spittlebug and forage quality, within new apomictic cultivars. Easy and quick methods are thus required to screen large progenies for these traits. The current lack of understanding of the factors contributing to acid soil adaptation of *Brachiaria* species is the main reason why the development of screening methods for edaphic adaptation lags behind the design of screening procedures for other traits, including apomixis and spittlebug resistance. Several studies were conducted to define acid soil adaptation mechanisms in the three *Brachiaria* cultivars that are used in the breeding program: *B. decumbens* cv. Basilisk, *B. ruziziensis* cv. Común, and *B. brizantha* cv. Marandú.

Methods: We focused our activities on three major factors that might contribute to the poor persistence of less adapted *Brachiaria* cultivars when grown on low fertility acid soils: Al-toxicity, P-deficiency and N-deficiency. Using simulated acid soil stress conditions, we first screened for interspecific differences in adaptation to these edaphic constraints. Then we investigated physiological/biochemical responses, which are triggered by these stress factors and might thus contribute to the plants' overall adaptation to acid soils. What follows is a summary of results from different activities completed this year.

Differential aluminum tolerance of *Brachiaria* species (P. Wenzl, G. M. Patiño, A. L. Chávez, I. M. Rao and J.E. Mayer)

Initially, it was believed that all *Brachiaria* species are Al-tolerant. However, we used low ionic strength nutrient solutions, with nutrient levels similar to those found in soil solutions of two Oxisols of contrasting texture (sandy loam and clay loam) from the Colombian Llanos, to identify interspecific differences. Using this system, we showed that *B. ruziziensis* is more sensitive to Al than *B. decumbens* and *B. brizantha* (CIAT- IP-5 Annual Report, 1997). With the aim of developing an easy-to-use screening procedure, we further simplified the solution culture technique. Short-term growth (3 d) of the primary root of 4 to 5 d old seedlings in solutions containing only Al^{3+}, Ca^{2+}, and Cl^- ions was measured with a ruler to evaluate whether genotypic differences in Al-tolerance can be resolved in this simple manner. Solutions were designed with GEOCHEM software (Table 33). Relative root length (RRL) was calculated from the difference between the root length after 3 days and the root length at the beginning of the Al-treatment. The results demonstrated that (i) Al-tolerance of all *Brachiaria* species is superior to that reported even for the most tolerant crop varieties, and that (ii) *B. ruziziensis* was clearly less Al-tolerant than *B. decumbens* and *B. brizantha* (Figure 34).

During the past few years, evidence has been accumulating that exudation of organic acids by root tips and accumulation of organic acids in specific tissues are both important strategies for Al-detoxification in plants. Preliminary experiments had indeed indicated that accumulation of citric acid (and eventually phosphate) in roots might act as an Al-tolerance mechanisms in *Brachiaria* species (CIAT- IP-5 annual report, 1997). Initial technical limitations during the analysis of exuded organic acids and phosphate have been overcome through improvements of several analytical techniques. At present, HPLC analyses of organic acids are underway. Preliminary results suggest that not only citric acid but also other organic acids might contribute to the high level of Al-tolerance in *Brachiaria* species.

Table 33. Concentrations and activities of Al^{3+} and Ca^{2+} in solutions used for Al-toxicity assays. The amount of HCl required to adjust the pH to 4.2 was calculated with GEOCHEM.

$[Al^{3+}]$ conc.	$\{Al^{3+}\}$ active.	$[Ca^{2+}]$ conc.	$\{Ca^{2+}\}$ active.	[HCl] conc.
		µM		
0	0	200.0	177.8	64.9
5	3.3	200.4	177.7	64.2
10	6.6	200.9	177.7	63.5
25	16.3	202.4	177.8	61.5
50	31.8	204.5	177.8	58.1
100	61.1	208.4	177.7	51.6
200	114.2	215.1	177.7	39.2

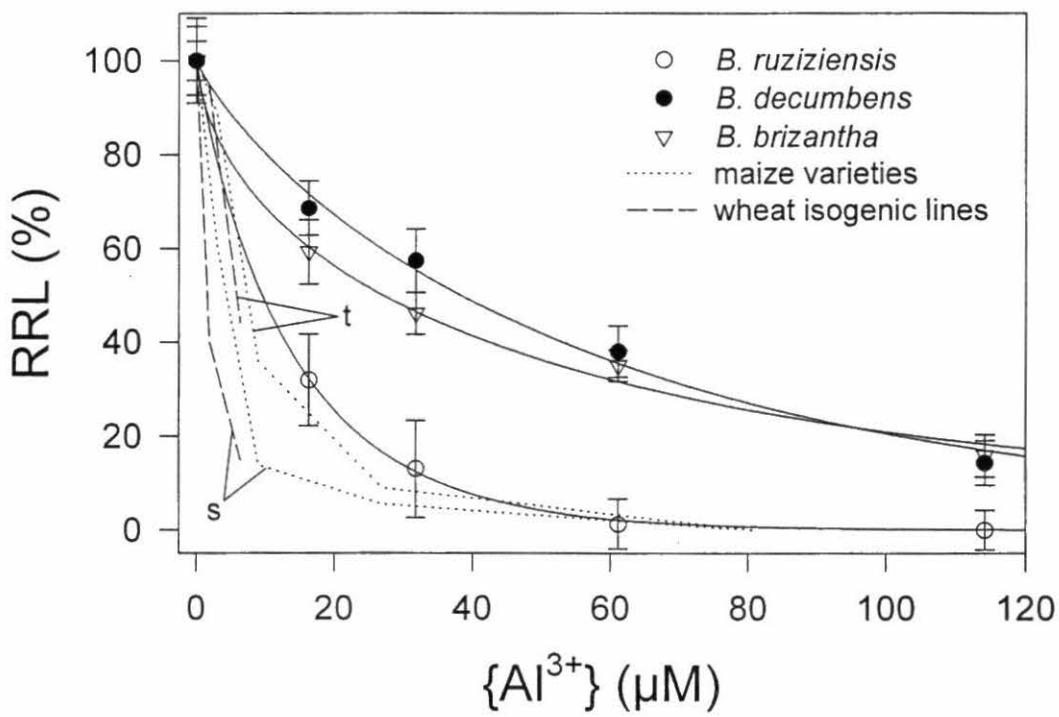

Figure 34. Relative root length (RRL) of three *Brachiaria* species as compared to maize and wheat varieties of contrasting Al-tolerance. Error bars denote SE (n = 28-36). Al-sensitive (s) and tolerant (t) maize varieties were Tuxpeño and South American 3. Al-sensitive (s) and tolerant (t) wheat isogenic lines were ES3 and ET3.

Electrostatic screening effect during nutrient uptake under Al-stress (A. Hernández, P. Wenzl, I. M. Rao and J. E. Mayer)

Al-toxicity does not only inhibit root growth but also modifies and/or inhibits the uptake of other essential nutrients, thereby causing nutritional disorders. For example, Al^{3+} ions seem to compete with Mg^{2+} and Ca^{2+} ions at the uptake sites in roots. We thus evaluated uptake of cationic nutrients by plants under Al-stress as compared to non-stressed plants. We evaluated the effects of Al at two levels of external nutrient supply in solution. Plants were grown in hydroponic culture in the greenhouse under high and low nutrient supply, with and without Al ("control", "control+Al", "stress" and "stress+Al" solutions; see CIAT-IP-5-Report, 1997). Upon harvest, concentrations of cationic nutrients were determined in root tissue using atomic absorption spectroscopy. Isolated and purified root cell walls were either equilibrated with 10 mM NaCl (pH 6.5) for five days to determine their cation exchange capacity (CEC) or equilibrated with an Al-containing nutrient solution ("stress+Al") to detect whether certain cations are preferentially adsorbed by cell walls.

In all three species, exposure to Al^{3+} ions in nutrient solution caused a significant shift in the uptake of cations from divalent to monovalent (K^+) ions by roots - expressed as the equivalent ratio of monovalent to divalent cations (Figure 35 *left half*). This suggests that an electrostatic screening effect on a negatively charged surface close to the sites of nutrient uptake might determine the preferential uptake of cations of different charges. According to the Gouy-Chapman theory of charged surfaces, a decreased surface charge density –e.g. caused by the binding of Al^3 ions– would increase the surface concentration ratio of monovalent to divalent cations. The latter is expected to increase the uptake rate of monovalent cations and decrease the uptake rate of divalent cations, which was experimentally confirmed.

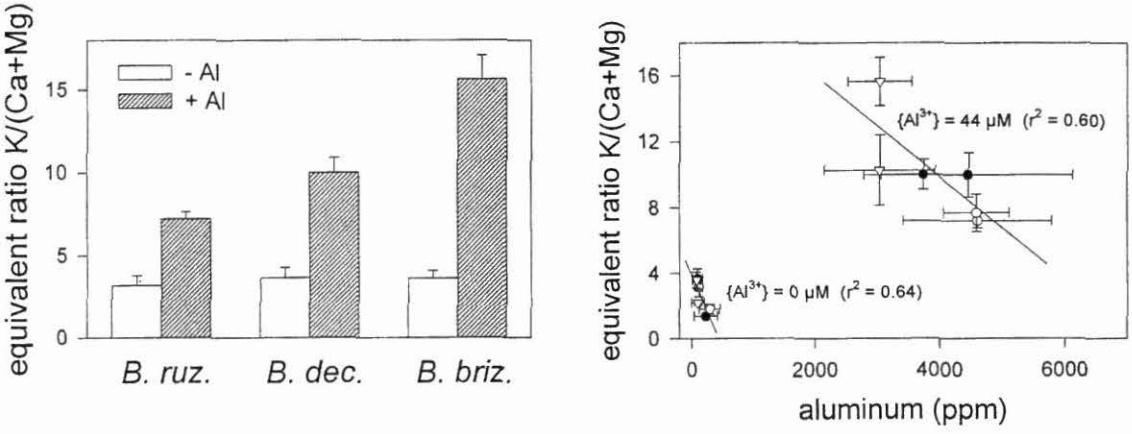

Figure 35. Effect of Al supplied under limiting nutrient supply on nutrient and Al uptake. *Left half:* equivalent ratio of monovalent cations (K) to divalent cations (Ca plus Mg) in root tissue. *Right half:* relation between equivalent ratio and Al-uptake by roots (white circles *B. ruziziensis*; black circles *B. decumbens*; white triangles *B. brizantha*). The group of data points at the upper right represents plants grown in Al-containing solutions ($\{Al^{3+}\}$ = 44 µM); the lower left represents plants grown without Al.

The fact that the increase of the equivalent ratio under Al-stress was negatively correlated with Al-uptake by roots (r^2 = 0.60; Figure 35 right half) further points to the decisive role of electrostatic screening during nutrient uptake. It seems that the larger the surface charge density is, the higher the surface concentration of Al^{3+} ions will be, thus increasing the uptake of Al. A high surface charge density also results in a lower surface concentration ratio of monovalent to divalent cations, thus resulting in a low equivalent ratio upon nutrient uptake. The increase of the equivalent ratio under Al-exposure was most pronounced in *B. brizantha* and less pronounced in *B. ruziziensis* (Figure 35; left half). According to the electrostatic

screening hypothesis, surface charge densities of plant roots grown in Al-containing solutions are thus predicted to be in the order *B. ruziziensis* >*B. decumbens* >*B. brizantha* (Figure 35). When these *Brachiaria* grasses are grown in association with tropical forage legumes, an Al-induced stimulation of K-uptake in *B. brizantha* might sequester large amounts of soil K into the grass thus reducing the availability of K in soil to the associated legumes. These results have important implications to reduce competition for K and to improve the persistence of legumes on K-deficient, sandy loam Oxisols.

The negative charges causing these interspecific differences could be located either in the cell wall or at the plasma membrane. Results indicated that root cell wall properties relevant for cation uptake were rather insensitive to Al-stress and did not vary among species. This was the case for total CEC (Figure 36; left half), the relative ratios of different cations adsorbed by cell walls from a nutrient solution, and for the amount of excess Al in the cell wall, which probably reflects polymerized Al (Figure 36; right half).

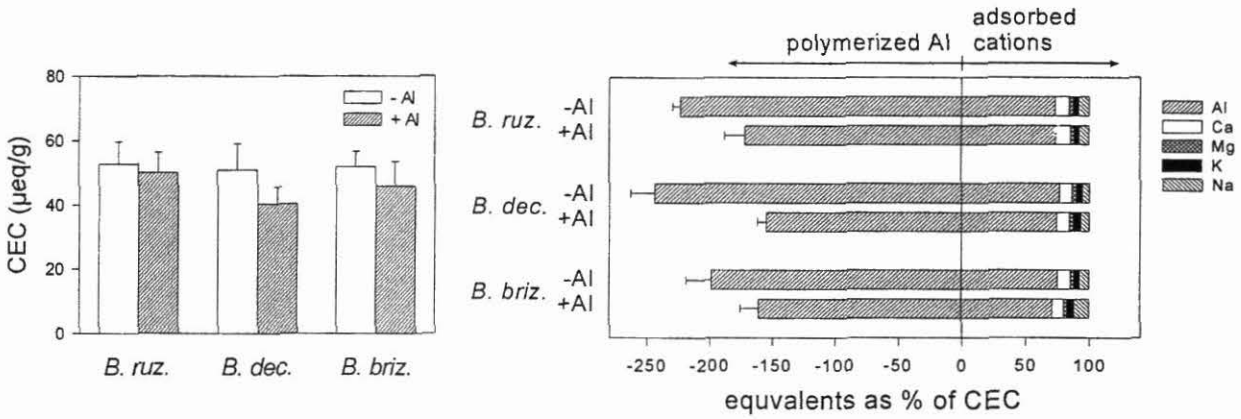

Figure 36. Root cell wall properties relevant for the uptake of cationic nutrients. *Left half:* root cell wall CEC of plants grown under low nutrient supply with and without Al. *Right half:* amount of different cations adsorbed from an Al-containing nutrient solution given in % of the respective CEC of root cell walls. Error bars indicate SE (n = 4).

It thus follows that root cell walls do not seem to cause the interspecific differences in the electrostatic screening effect during nutrient uptake under Al-exposure. This points to a putative involvement of surface charges of the outer layer of the plasma membrane, a possibility that is currently being addressed in additional experiments.

Fine root system contribute to efficient P-uptake in *B. decumbens* (N. L. Lasso, P. Wenzl, I. M. Rao and J. E. Mayer)

P-uptake in low fertility acid soils is limited by the chemical fixation of phosphate by Al^{3+} and Fe^{3+} ions. The result is an extremely low concentration of phosphate in the soil solution (typically < 1 µM) and a low mobility of phosphate in the soil. Consequently, plants adapted to such soils not only have to possess an efficient P-uptake system but also their root systems have to explore large soil volumes for active exploitation of immobile P-reserves. This can be accomplished by promoting root elongation process. However, construction costs of extensive root systems under P-deficient conditions is certainly a limiting factor under P-deficient conditions.

Since longer roots can be constructed with a given amount of biomass when roots are thin, a root system predominantly made of fine roots is expected to be superior with respect to P-acquisition under acid soil conditions. Therefore, an experiment was set up to investigate root

system morphology in relation to P-deficiency. Plants were grown in hydroponic culture with declining P-supply in the greenhouse. Upon harvest, root systems were stained and scanned with a flatbed scanner. The resulting images were then analyzed with WinRHIZO (software for root system image analysis). Dry weight and P-content were determined for different plant organs as well. It was shown that *B. decumbens* possesses the finest root system, independent of the level of P in the nutrient solution, and that *B. ruziziensis* produced the thickest roots (Figure 37; left half). Based on geometric considerations one can estimate that *B. ruziziensis* needs 50-55% more biomass than *B. decumbens* to construct a root system of a given length. Under P-deficient conditions this can be expected to be a significant advantage given the importance of active foraging for soil P reserves.

Leaf expansion and photosynthetic activity per unit leaf area are highly dependent on P supply to the plant. Therefore an attribute which relates root elongation to leaf expansion could be a good measure of the plant's performance under P-stress. Indeed, the higher ratio of root length to leaf area indicates that *B. decumbens* might supply greater amounts of P for leaf expansion and photosynthesis more reliably than the other two species, especially under P-deficiency (Figure 37; right half). Consequently, root geometrical traits and carbon allocation may both play an important role in adaptation of *B. decumbens* to P-deficiency.

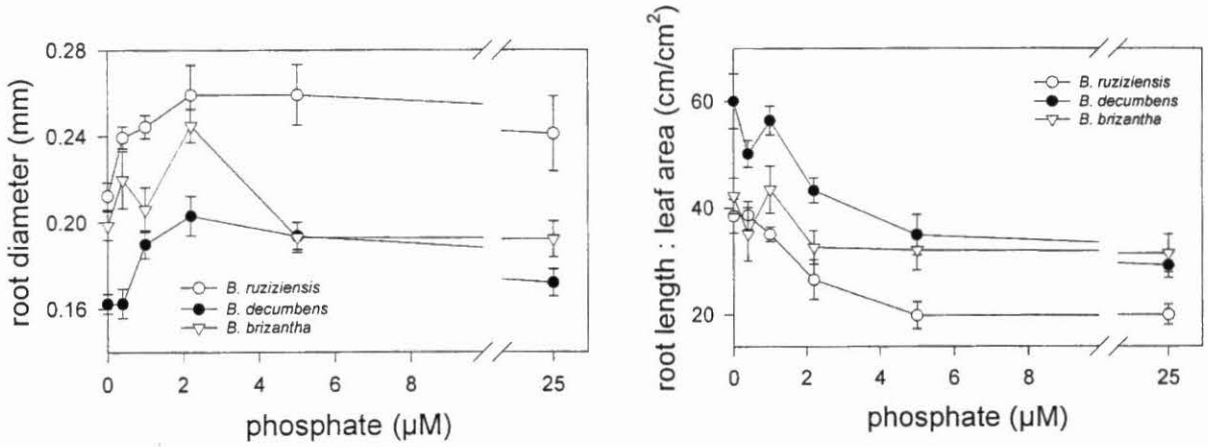

Figure 37. Root diameter under different levels of P-supply in relation to leaf area production. *Left half:* root diameter. *Right half:* ratio of root length to leaf area. Error bars indicate SE (n = 8).

Differential adaptation to N-deficiency could be due to differences in NO_3^- uptake efficiency (N. L. Lasso, P. Wenzl, I. M. Rao and J. E. Mayer)

Another important edaphic constraint in acid soils is the low availability of mineral nitrogen. Pasture degradation is often discussed in the context of a decline of soil N availability. An experiment was conducted to evaluate whether there are interspecific differences in tolerance to N-deficiency. Plants were grown hydroponically in the greenhouse under declining N-supply. Upon harvest, root systems were stained, scanned and analyzed in a similar manner as above. Dry weights and tissues N-content were measured as well.

Total dry matter production of plants was evaluated as percentage of the respective maximum yield (Figure 38). Significant variability among the cultivars was found. Dry matter production of *B. decumbens* was half-saturated at 50 µM of mineral nitrogen (90 % nitrate, 10 % ammonium), while *B. brizantha* required about 2 times and *B. ruziziensis* about 4 times more

nitrogen to attain 50 % growth. Most plants respond to low N supply by stimulation of root growth at the expense of shoot growth and this response seems to reflect the plant's internal N-status. It can be expressed as the root weight ratio (RWR), which is the fraction of the plant's total biomass allocated to the roots.

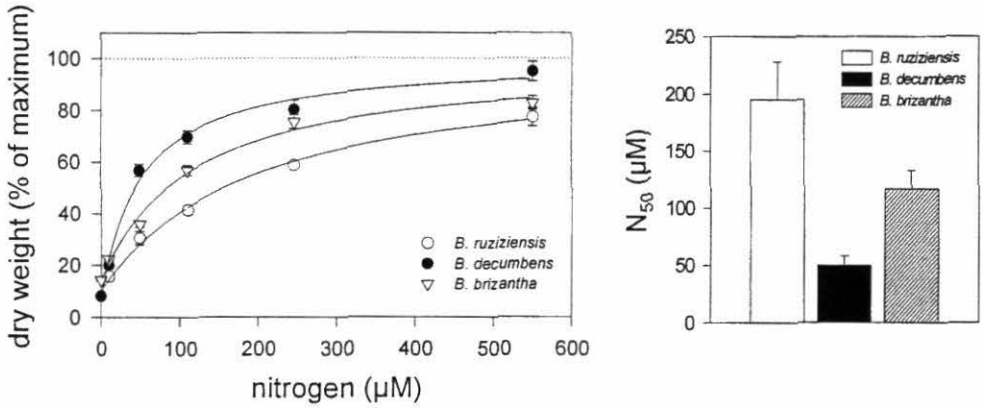

Figure 38. Differential adaptation to N-deficiency as visualized by dry matter production. *Left half:* total dry weight as a function of the level of mineral nitrogen supplied in the nutrient solution (90 % nitrate, 10 % ammonium). Error bars indicate SE (n = 8). *Right half:* comparison of the level of nitrogen at which 50 % of maximum growth was achieved (N_{50}). The latter was calculated from the data in the left half of the figure by non-linear curve fitting with the Marquardt-Levenberg algorithm using a Michaelis-Menten-like function. Error bars indicate SD.

Figure 39 shows that *B. ruziziensis* started to increase its RWR at higher N-concentrations than the other two species, which required a more severe N-deficiency to increase their RWR. This confirms the above finding that *B. ruziziensis* seems to be less adapted to N-depleted soils.

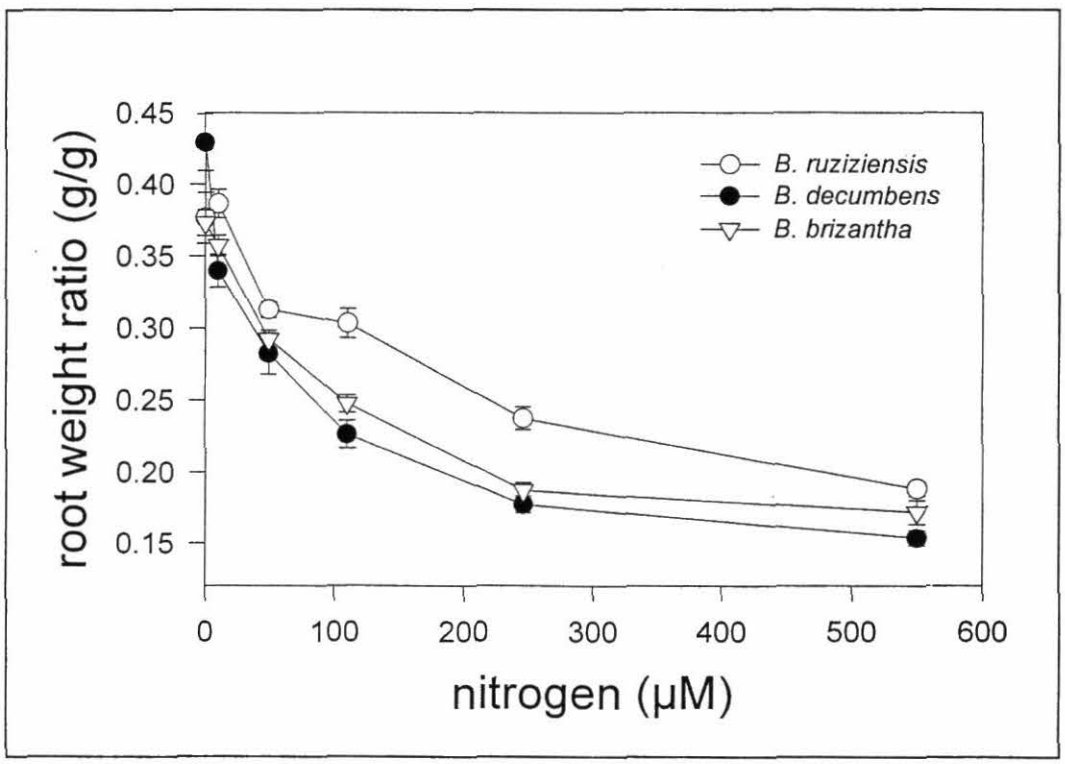

Figure 39. Biomass allocation in response to varying N-supply. Root weight ratio is the fraction of the total biomass allocated to the root system. Error bars indicate SE (n = 8).

In principle, poor adaptation to N-depleted soils could be attributed to low N-uptake efficiency and/or low N-use efficiency. Figure 40 demonstrates that N-uptake efficiency seems to be the determining factor. *B. ruziziensis* acquires less mineral N per unit root surface area than *B. decumbens*. These results are very useful to identify the gene(s) coding for nitrate uptake in *Brachiaria*.

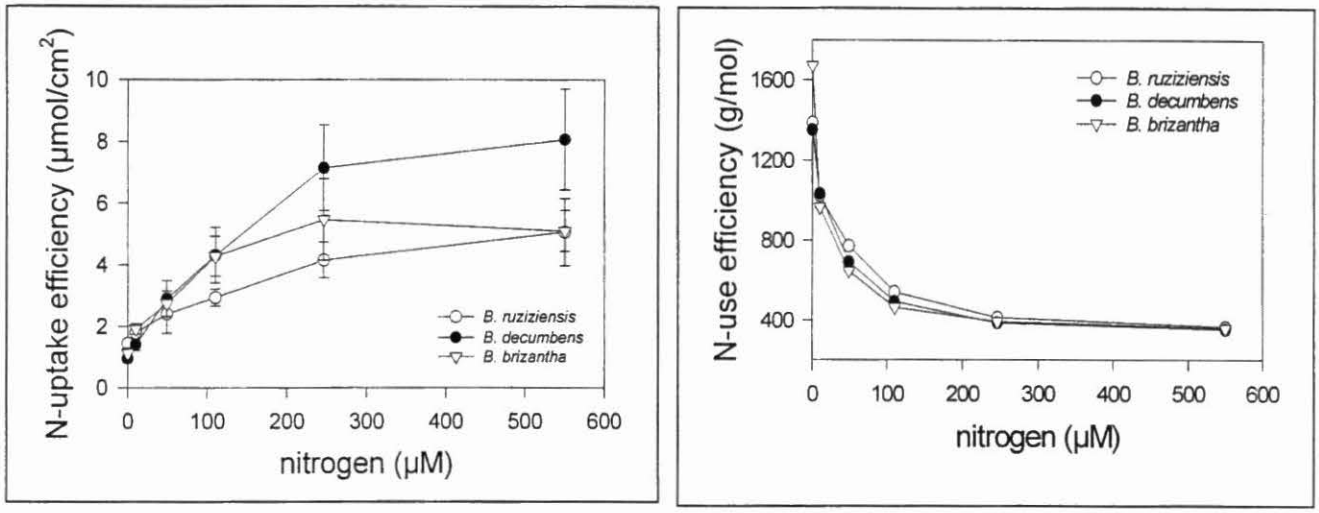

Figure 40. Growth and N supply *Left half*: N-uptake efficiency calculated as the amount of mineral nitrogen taken up per root surface area. Right half: N-use efficiency calculated as the amount of biomass produced per mol of N taken up. Error bars indicate SE (n = 8).

Structural elucidation of two secondary metabolites induced in roots under P- and N-deficiency (P. Wenzl, A. L. Chávez, M. Nair, I. M. Rao and J. E. Mayer)

It is well known that *Brachiaria* species benefit from symbioses with soil microorganisms. They are excellent hosts for arbuscular-mycorrhizae (AM) when grown on low-fertility acid soil. Also, associative nitrogen fixation by soil bacteria in the rhizosphere can contribute significantly to nitrogen uptake in certain *Brachiaria* species.

There is substantial published evidence demonstrating that secondary aromatic compounds are involved in signal exchange between plants and soil microorganisms. Given the importance of the mentioned symbiotic associations for the acquisition of phosphorus and nitrogen by *Brachiaria* species, we thus focused research on aromatic metabolites produced under acid soil-stress in roots. Using reverse-phase HPLC, two dominant aromatic metabolites were detected in roots of the three *Brachiaria* species tested, when grown either in soil or nutrient solution. Both P- and N-deficiency strongly stimulated the accumulation of these two compounds. Although these compounds were detected in all three *Brachiaria* species (*B. decumbens, B. ruziziensis, B. brizantha*) investigated, their level was highest in *B. ruziziensis*.

In order to get insights into their putative role in adaptation to low P or low N supply, a purification protocol was developed and their molecular structure was elucidated. Washed and lyophylized roots of soil-grown plants were extracted with methanol. The extract was evaporated to dryness and the resulting residue was separated by MPLC. Fractions containing the two most dominant UV-absorbing compounds (comp. A and B) were pooled and dissolved in methanol.

The two compounds were further purified by preparative HPLC and dissolved separately in DMSO-d_6. To elucidate their structure ^{1}H-NMR, ^{13}C-NMR, COSY, positive-ion FAB-mass, and CD (circular dichroism) spectra were recorded.

The ^{1}H-NMR spectrum of compound A revealed the presence of two feruloyl moieties and also showed six shielded protons and one partially deshielded proton (Figure 41). The FAB (fast atom bombardment) mass spectral data confirmed the feruloyl moieties and indicated a molecular weight of 544. Assuming ester bonds, the ^{1}H-NMR spectra and FAB-MS data together suggested that compound A contains a structural subunit with a molecular weight of 192 in addition to two feruloyl moieties. This is the molecular weight of quinic acid, which is frequently found to be esterified to hydroxycinnamic acids. ^{13}C-NMR data further confirmed the quinic acid moiety (Figure 41).

The substitution pattern of the two feruloyl moieties on the quinic acid was then resolved with COSY (Figure 42).

All expected couplings could be detected, with exception of the coupling between the axial proton at C6 and the equatorial proton at C5. This may be due to the extremely broad multiplet observed for the axial proton at C6. CD spectra revealed that Compound A is the L (-) enantiomer (Figure 43).

This is in agreement with the CD of cynarin, an analogous compound that contains two caffeoyl moieties. In compound B one of the two feruloyl moieties was shown to be replaced by a p-coumaroyl moiety. It was thus concluded that compound A is L-1,3-di-*O-trans*-feruloylquinic acid (DFQA) and compound B is L-1-*O-trans-p*-coumaroyl-3-*O-trans*-feruloylquinic acid or L-1-*O-trans*- feruloyl-3-*O-trans-p*-coumaroylquinic acid (FCQA). Both assume the chair conformation in which both hydroxycinnamoyl moities are placed in equatorial positions (Figure 44).

DFQA and FCQA are new hydroxycinnamic acid conjugates of quinic acid. Both the hydroxycinnamoyl and the quinic acid portions are synthesized via the shikimate pathway. Shikimate kinase, an enzyme in the middle of the shikimate pathway, is subject to control by energy charge. Under energetically unfavorable conditions, a larger portion of shikimate molecules is diverted from the main trunk into secondary products such as quinic acid and its derivatives. This could explain their accumulation in roots of *Brachiaria* species under P- and N-deficiency.

Transgenic tobacco plants with suppressed levels of phenylpropanoid products showed increased disease susceptibility. This indicates that 5-*O*-caffeoylquinic acid (chlorogenic acid) might be a chemical barrier against microbial attack, since it is the major soluble phenylpropanoid in tobacco.

In a similar way, DFQA and FCQA might accumulate in roots of *Brachiaria* species as preformed protectants against attacks of soil-born fungi and by this increase root lifespan. A longer root lifespan is expected to increase the amount of nutrients taken up per unit of biomass invested in roots. Under nutrient deficient conditions where new biomass is costly to construct, this might improve the plant's overall performance.

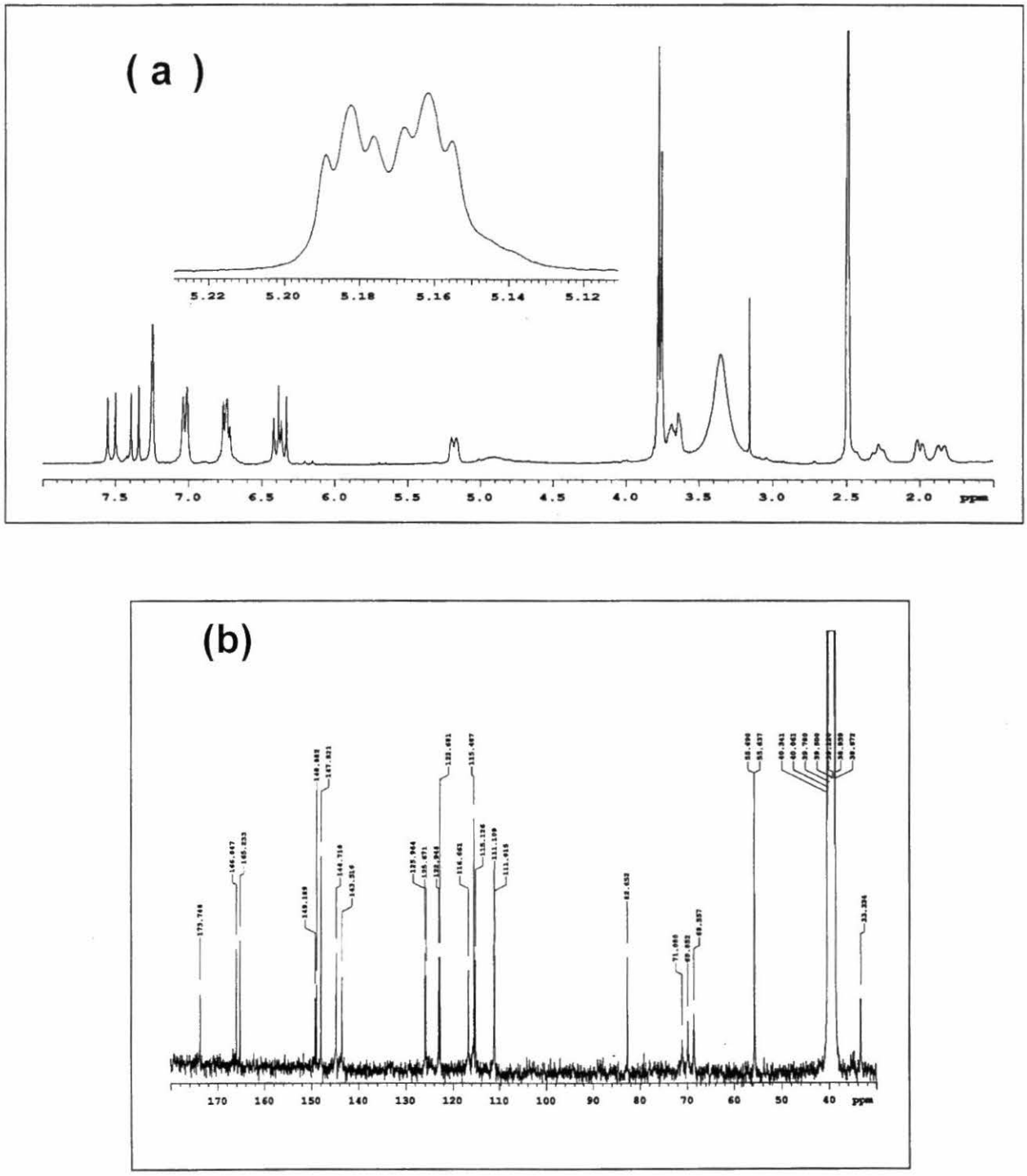

Figure 41 (a) ^{1}H-NMR spectrum of compound A in DMSO-d_6. The broad peak at 3.35 ppm is residual H_2O, the peak at 3.15 ppm is residual methanol and the peak at 2.49 ppm is DMSO. The insert depicts the C3-H_{ax} of quinic acid, which indicates the substitution pattern. Signals assigned to the feruloyl moieties: C2'-H and C2"-H 7.24 ppm, C5'-H and C5"-H 6.73 and 6.75 ppm, C6'-H and C6"-H 7.03 ppm, C7'-H and C7"-H 7.37 and 7.52 ppm, C8'-H and C8"-H 6.36 and 6.39 ppm. Signal assigned to the quinic acid moiety: C2-H_{ax} 2.45-2.50 ppm, C2-H_{eq} 2.00 ppm, C3-H_{ax} 5.18 ppm, C4-H_{eq} 3.64 ppm, C5-H_{eq} 3.69 ppm, C6-H_{ax} 2.28 ppm, C6-H_{eq} 1.85 ppm. (b) ^{13}C-NMR spectrum of compound A. The broad peak at about 40 ppm is caused by DMSO-d_6. Quinic acid signals: C1 82.6 ppm, C2 33.3 ppm, C3 69.8 ppm, C4 71.1 ppm, C5 68.5 ppm, C6 obscured by the DMSO-d_6 peak, C7 173.7 ppm.

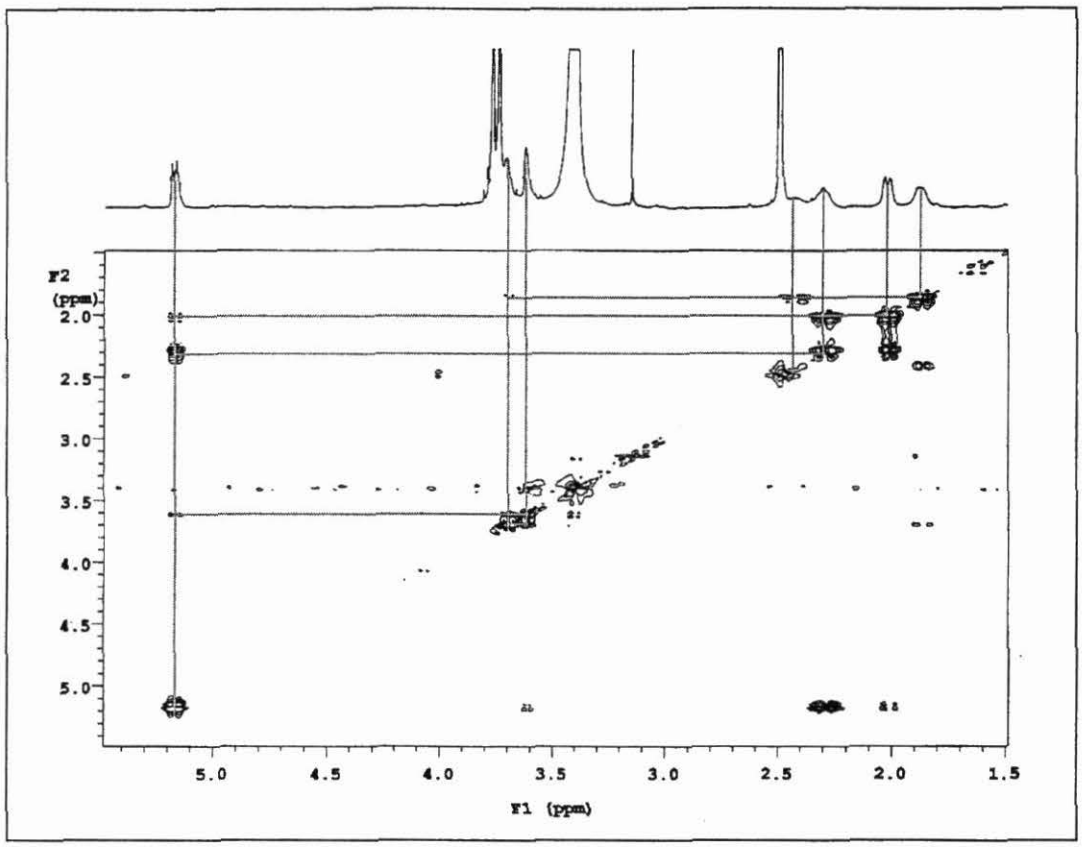

Figure 42 . 2-D homonuclear correlated proton spectra (COSY) of compound A in DMSO-d_6 + D_2O. The portion containing the quinic acid protons is depicted. Proton signals are assigned to: C3-H_{ax} (5.18 ppm), C5-H_{eq} (3.70 ppm), C4-H_{eq} (3.62 ppm), C6-H_{ax} (2.42 ppm), C2-H_{ax} (2.30 ppm), C2-H_{eq} (2.01 ppm) and C6-H_{eq} (1.88 ppm).

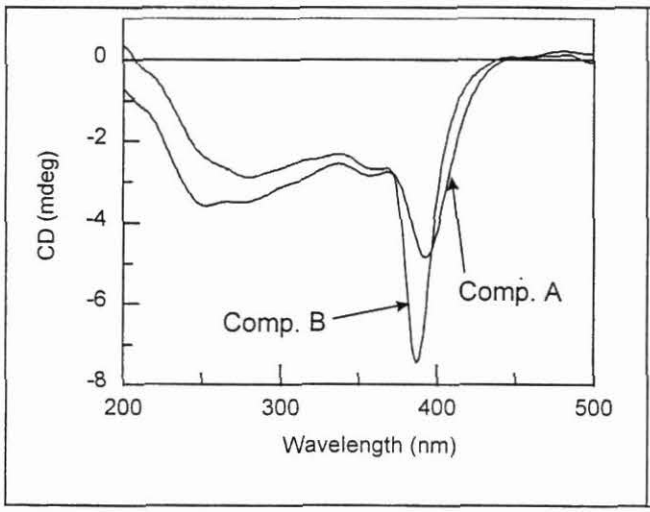

Figure 43. CD spectra of compound A and B. The negative absorptions indicate that both are L (-) enantiomers.

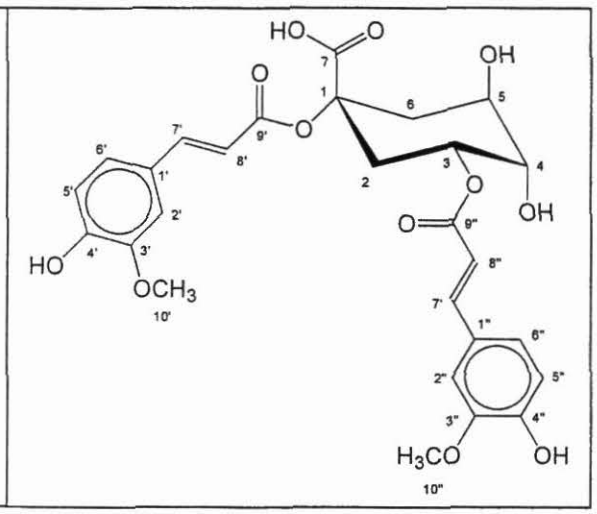

Figure 44. Structure of compound A. Compound B lacks an -OCH_3 group on one of the two feruloyl moieties.

Preliminary results also indicate that roots not only accumulate but also exude DFQA and FCQA. Both compounds seem to accumulate in soil. Consequently, they could also influence the soil microbial community in the rhizosphere. Additional experiments are required to determine whether they are involved in signal exchange between the plant and symbiontic microorganisms such as AM and N_2-fixing bacteria.

Proposed screening procedure for assessing acid soil adaptation of *Brachiaria* (P. Wenzl, I. M. Rao and J. E. Mayer)

Given the complex nature of the acid soil syndrome, it is intuitively obvious that acid soil adaptation must be a polygenic aggregate trait. Consequently, no single screening procedure can suffice to cover even the most significant traits contributing to acid soil adaptation. For this reason we propose a stepwise procedure as outlined in Figure 45

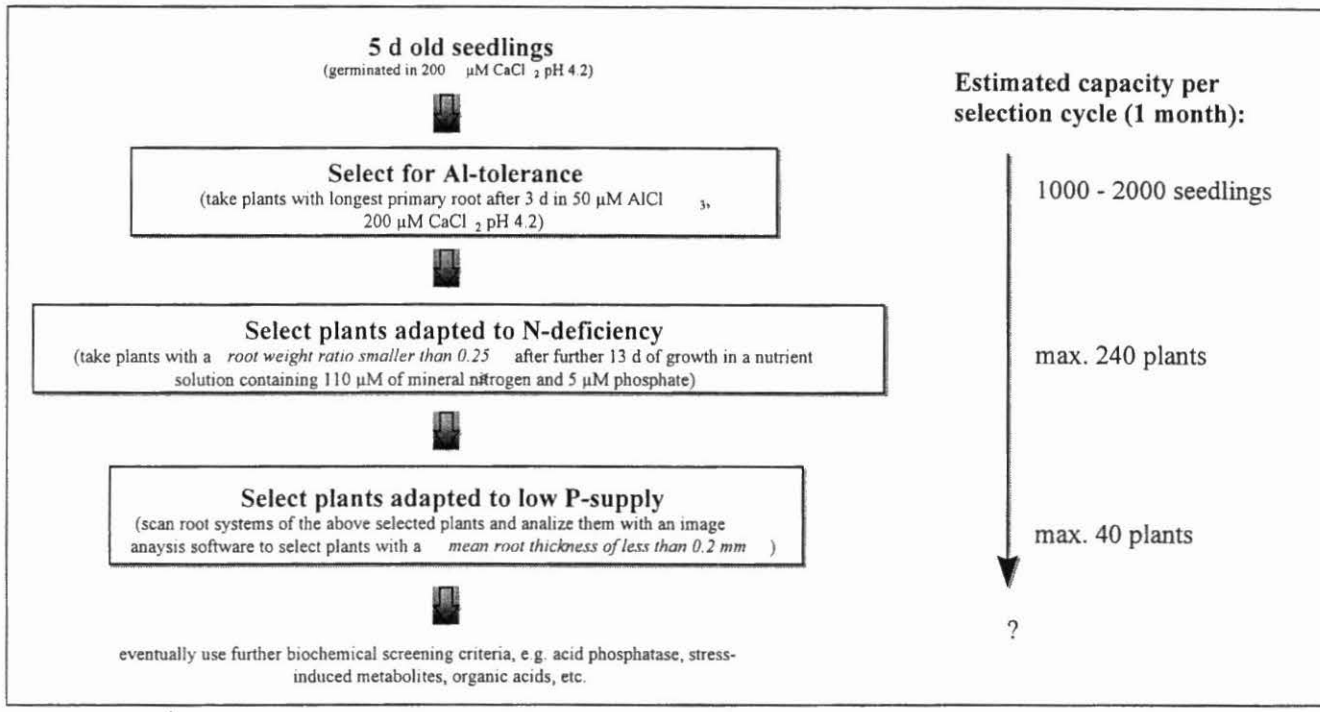

Figure 45. Proposed scheme for a large-scale screening procedure to select acid soil-adapted *Brachiaria* genotypes during the ongoing breeding program, based on analysis of growth in nutrient solutions. For further explanation see text.

It is based on differential growth of seedlings in nutrient solutions and is designed to eliminate non-adapted germplasm with a genetic background similar to that of *B. ruziziensis*. The scheme starts with technically and/or logistically simple selection methods and then shifts toward more sophisticated methods as the number of selected plants decreases. It includes screening steps for the three major edaphic stress factors of infertile acid soils: Al-toxicity, P-deficiency, and N-deficiency. In the proposed procedure seeds are germinated in a $CaCl_2$ solution. The first selection step is designed to discard Al-sensitive seedlings through measurement of short-term elongation of the primary root (with a ruler) in a solution containing 50 µM $AlCl_3$ and 200 µM $CaCl_2$ at pH 4.2. At this Al-level, interspecific differences can be easily resolved (see Figure 34). Al-tolerant seedlings are then transferred to a nutrient solution containing sufficient nutrients ("control" solution; see CIAT-IP-5-Annual Report, 1997) with the exception of nitrate and ammonium, which are adjusted to 100 µM and 10 µM, respectively. This is the level at which interspecific differences in N-response are most pronounced (see Figuress 38 and 39). After 13 d of growth, shoots and roots are separated –leaving only the shortest

adventitious root attached to the stem– and weighted separately to determine the root weight ratio (RWR). Genotypes adapted to N-deficiency should have a RWR of ≤ 0.25 when grown at this N-level (see Figure 39). The older leaves of the shoots of N-adapted genotypes are removed to reduce transpiration, thus leaving only the stem plus one adventitious root plus the youngest leaf. In this form they could be transferred to sand culture. Their root systems are stained and scanned with a flatbed scanner to measure the mean root thickness with WinRHIZO software. Genotypes adapted to P-deficiency should have a mean root thickness similar to *B. decumbens*, i.e. ≤ 0.2 mm at the level of P supplied in the nutrient solution (5 µM; see Figure 37). P-adapted plants could then be transferred from sand culture to soil either as whole plants or as stem cuttings. Ongoing biochemical experiments might come up with additional screening criteria, which could be used on plants that have gone through the above outlined selection procedure.

Throughout this research activity, all experiments have been conducted with seedlings, because logistic as well as physiological constraints prohibited the use of stem cuttings for experiments designed to gain knowledge about adaptation mechanisms. An implementation of the described screening procedure for stem cuttings would require additional experiments for the validation and/or adaptation of the proposed methods. We suggest that it may be better to use seedlings for screening of acid soil tolerance for two reasons. First, *Brachiaria* seeds are very small and have only little nutrient reserves while stem cuttings contain considerably greater nutrient reserves. This implies that (i) the error associated with any screening method for acid soil adaptation will be large, and that (ii) longer growth periods would be required to deplete internal nutrient reserves. Second, screening for Al-tolerance in hydroponics based on elongation of the primary root is a well-established and easy method, which allows handling of an extremely large number of seedlings. It could be a powerful method to initially discard non-adapted germplasm and thus facilitate more sophisticated downstream screening steps.

Studies on plant attributes in *Brachiaria* genotypes related to tolerance to low nutrient supply (I. M. Rao, J. W. Miles, C. Plazas, J. Ricaurte and R. Garcia)

Highlights

- Found that two genetic recombinants (BRN093/3204, FM9201/1873) of *Brachiaria* were outstanding in their adaptation to low fertility acid soil conditions.
- Showed that *Brachiaria brizantha* CIAT 26110 is outstanding in fine root production and maintaining greater proportion of root length to leaf area.

A. Field study

Rationale: Evaluation of plant performance under field conditions will help to determine genetic variation in adaptation to low fertility, acid soil stress and to identify specific plant attributes that may be utilized as selection criteria in a genetic enhancement program. A field study is in progress for the past 3 years at Carimagua to evaluate differences in edaphic adaptation and persistence of *Brachiaria* genotypes and to identify key attributes of edaphic adaptation.

Methods: The trial comprises 17 entries, including nine natural accessions (four parents) and eight genetic recombinants. The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots. Plots with high initial fertilizer application received maintenace fertilizer application in the third year (kg/ha of 10P, 10K, 17Ca, 7 Mg and 5 S). The trial is submitted to periodic mob grazing according to forage on offer.

Results and Discussion: Measurements of forage yield and leaf area index during wet season during 1995 to 1997 indicated marked genotypic variation (Table 34). With low initial fertilizer application two genetic recombinants, BRN093/3204 and FM9201/1873, were found outstanding in forage yield and leaf area production when compared to other hybrids. One of the genetic recombinants (BRN093/1371) which is very resistant to spittlebug infestation (C. Cardona, unpublished data) was found to be responsive to initial fertilizer application in terms of leaf area production and forage yield.

Table 34. Genotypic variation as influenced by fertilizer application in forage yield and leaf area index of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Carimagua, Colombia. Data are means of 3 measurements made over 3 years (1995-1997)

Genotypes	Forage yield		Leaf area index	
	Low fertilizer	High fertilizer	Low fertilizer	High fertilizer
	(t/ha)		(m2/ m2)	
Recombinants:				
FM9201/1873	4.79	8.69	3.26	5.01
FM9201/209	3.51	6.69	2.11	3.49
BRN093//1371	3.52	8.52	2.49	4.06
BRN093/2602	2.72	4.96	1.79	2.69
BRN093/3204	5.89	8.91	3.10	3.62
BRN093/3788	4.51	7.78	3.01	3.51
BRN093/766	3.83	7.46	2.53	4.43
Parents:				
CIAT 606	5.15	9.07	3.04	5.24
CIAT 6780	6.23	9.95	2.84	4.00
BRUZ/44-02	1.16	3.04	0.53	0.89
CIAT 26646	5.92	7.06	2.41	2.98
Accessions:				
CIAT 16467	7.56	10.2	3.22	3.72
CIAT 16473	6.25	11.5	2.38	3.52
CIAT 26556	4.93	9.02	1.98	3.70
CIAT 6133	1.96	2.52	1.41	1.63
CIAT 679	2.55	3.32	1.31	1.55
Mean	**4.40**	**7.43**	**2.35**	**3.36**
LSD (P =0.05)	1.63	2.73	1.01	1.25

B. Glasshouse study

Rationale: There is a need to explore the extent of genotypic variation between and within species in order to develop nutrient efficient genotypes, which could meet the mineral nutrient requirements of ruminants. A glasshouse experiment examined genotypic differences in tolerance to low nutrient supply among 11 genotypes of *Brachiaria* (2 genetic recombinants, 4 parents and 5 most promising accessions).

Methods: A sandy loam oxisol from Carimagua was used to grow the plants (4 kg of soil/pot). Nutrients were supplied before planting at three levels (nil, low and high). Low nutrient supply (kg/ha) included 20 P, 20 K, 33 Ca, 14 Mg and 10 S while the high nutrient supply included 80N, 50 P, 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo). At the time of harvest (58 and 142 days of growth), several shoot and root attributes such as forage yield, leaf area, root length, and root length/leaf area were determined. Total forage yield and leaf area production (two harvests) were determined.

Table 35. Genotypic variation as influenced by nutrient supply (nil, low and high) in forage yield and leaf area production of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown for 142 days in pots (4 kg soil/pot) in a sandy loam oxisol from Carimagua, Colombia. Data are total values of 2 harvests made at 58 and 142 days after establishment in pots.

Genotypes	Forage yield			Leaf area		
	nil	low	high	nil	low	high
	(g/pot)			(cm^2/pot)		
Recombinants:						
FM9201/1873	3.64	8.32	17.0	240	562	1493
BRN093/1371	1.02	7.66	15.5	122	563	1495
Parents:						
CIAT 606	2.62	11.9	30.2	181	855	1965
CIAT 6780	4.72	8.54	25.0	402	861	1922
BRUZ/44-02	3.04	7.71	16.5	245	564	1210
CIAT 26646	2.43	12.3	30.0	176	623	1604
Accessions:						
CIAT 16488	3.59	11.2	28.3	274	630	1652
CIAT 26032	4.39	11.2	28.6	431	943	2011
CIAT 26110	2.55	9.96	25.9	291	797	1931
CIAT 26124	3.82	12.8	31.6	278	763	1914
CIAT 26318	5.06	11.4	33.0	286	585	1494
Mean	**3.35**	**10.3**	**25.6**	**266**	**704**	**1684**
LSD (P =0.05)	1.51	2.7	5.6	127	117	243

Results and Discussion: Shoot and root attributes were influenced by genotype and also the level of nutrient supply to soil (Tables 35 and 36). As expected, increase in nutrient supply improved forage yield as a result of stimulation of leaf area production. *B. brizantha* CIAT 26110 was outstanding in root length and root length/leaf area relationship.

Table 36. Genotypic variation as influenced by nutrient supply (nil, low and high) in root length and root length to leaf area ratio of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown for 142 days in pots (4 kg soil/pot) in a sandy loam oxisol from Carimagua, Colombia. Data are total values of 2 harvests made at 58 and 142 days after establishment in pots.

Genotypes	Root length			Root length/leaf area		
	nil	low	high	nil	low	high
	(m/pot)			((km/m^2)		
Recombinants						
FM9201/1873	63.8	154	320	10.6	9.75	6.96
BRN093/1371	34.0	156	245	9.18	7.81	4.83
Parents:						
CIAT 606	34.5	121	371	4.32	4.81	11.8
CIAT 6780	124	231	387	8.62	7.03	8.23
BRUZ44-02	67.6	138	353	10.7	6.84	8.82
CIAT 26646	52.3	140	316	7.80	6.31	8.85
Accessions:						
CIAT 16488	95.4	147	305	7.36	7.22	8.77
CIAT 26032	142	246	372	7.85	7.41	7.65
CIAT 26110	140	317	516	11.1	11.6	10.8
CIAT 26124	119	199	442	9.25	7.52	11.2
CIAT 26318	118	183	416	8.89	9.49	14.8
Mean	**91**	**185**	**368**	**8.70**	**7.80**	**12.3**
LSD (P=0.05)	72	64	107	NS	3.06	4.08

Studies on genotypic variation in *Arachis pintoi* for tolerance to low nutrient supply
(I. M. Rao, B. Maass, J. W. Miles, M. Peters and C. Plazas)

Highlight

- Showed that *A. pintoi*, CIAT 22259, was outstanding in its adaptation (to low nutrient supply in soil) and persistence as revealed by leaf area index and legume biomass proportion when associated with an aggressive grass at two sites in Carimagua.

Rationale: Intergeneric and interspecific variation in tolerance to low nutrient supply has been identified among a number of tropical forage legumes when grown in soils of contrasting texture. But knowledge on intraspecific variation in tolerance to low nutrient supply is limited.

Methods: A field experiment examined genotypic differences in tolerance to low nutrient supply among 39 accessions of *A. pintoi* grown for 2 years in association with *B. dictyoneura* cv. Llanero at two sites of contrasting soil texture (Alcancia – clay loam oxisol; Maquenque – sandy loam oxisol) over two years under grazing. Nutrients were supplied 15 days after planting (20 P, 20 K, 12 Mg and 12 S). Maintenance fertilizer at half the rates of initial fertilizer levels was applied 2 years after establishment. Several shoot attributes such as forage yield, legume DM yield, % legume biomass, legume stolon length and legume leaf area index were determined at 2 months after maintenance fertilizer application..

Table 37. Genotypic differences in adaptation and persistence of 39 accessions of *Arachis pintoi* after 2 years of grazing at two sites (Alcancia and Maquenque) in Carimagua, Colombia. The legume accessions were associated with *Brachiaria dictyoneura* cv. Llanero.

Genotype (CIAT No.)	Site	Total DM yield (g/m^2)	Legume DM yield (g/m^2)	Legume biomass (%)	Legume stolon length (cm)*	Legume leaf area index (m^2/m^2)
18744	Alcancia	300	137	45	20	1.08
	Maquenque	302	98	35	22	0.81
18748	Alcancia	365	161	44	25	1.32
18750	Maquenque	343	141	41	50	0.64
22152	Alcancia	378	176	47	18	1.53
22160	Alcancia	274	127	46	23	0.77
	Maquenque	406	134	33	34	0.87
22175	Alcancia	322	128	40	28	1.04
22231	Alcancia	325	12.8	33	12	0.76
	Maquenque	281	73	26	15	0.46
22233	Alcancia	260	116	44	36	0.94
	Maquenque	239	43	18	12	0.20
22234	Alcancia	381	174	46	33	1.42
	Maquenque	322	134	41	21	0.83
22236	Alcancia	359	167	47	21	1.29
	Maquenque	363	154	42	19	0.88
22238	Alcancia	312	141	45	23	1.32
	Maquenque	309	88	30	18	0.42
22241	Alcancia	295	137	47	12	1.15
22259	Alcancia	406	189	47	26	1.59
	Maquenque	455	225	49	15	1.28
22260	Alcancia	188	81	43	31	0.62
	Maquenque	426	105	25	32	0.65
22263	Maquenque	328	87	26	11	0.31
22264	Alcancia	341	134	39	16	0.92
22265	Alcancia	131	46	35	10	0.37
22269	Alcancia	326	157	48	17	1.09
	Maquenque	433	149	34	25	0.88
22270	Alcancia	470	205	44	20	1.83
22271	Alcancia	422	189	45	39	1.40
Mean	**Alcancia**	**324**	**145**	**44**	**21**	**1.13**
	Maquenque	**352**	**122**	**36**	**21**	**0.75**
LSD(P=0.05)	Alcancia	NS	NS	10	15	0.53
	Maquenque	NS	NS	NS	NS	0.33

Alcancia: clay loam oxisol; Maquenque: sandy loam oxisol *Average of 5 stolons

Results and Discussion: The extent of leaf area production and legume DM yield exhibited greater genotypic variation (Table 37). Among 39 accessions of *A. pintoi*, CIAT 22259 was outstanding in maintaining greater proportion of legume biomass in an association due to its ability to maintain greater expansion of leaves, as revealed by the values of leaf area index.

Progress towards achieving output milestone

- **Improved methodology to screen forage grasses and legumes for adaptation to low fertility soils (2000).**

We have been successful in developing a screening method to evaluate aluminum tolerance in *Brachiaria*. This method uses relative root elongation as a simple measure to identify aluminum sensitive genotypes. But this method so far has worked with seedlings developed from seed. We need to adapt this method for vegetative stem cuttings so that we can evaluate large numbers of *Brachiaria* hybrids for their aluminum tolerance.

One major problem in adapting the screening method to stem cuttings is the variability among cuttings in terms of nutrient status that could interact with the degree of aluminum tolerance. In order to reduce variability, we need to use a large number (at least 30) of replicates for each treatment. We will also try to test this method to determine genotypic differences in tolerance to aluminum stress in *Arachis pintoi*.

Suboutput 3.2: Genotypes of grasses and legumes with dry season tolerance identified and characterized

The emphasis in the past was to select grasses and legume for acid soils and extensive livestock systems. However, there is a great demand for forages suitable for intensive smallholder livestock systems most of which have seasonal shortages of forages and consequently contribute to environmental degradation due to overgrazing. Thus, an important goal of the Forage Project is to identify grasses and legumes adapted to subhumid environments (4 to 6 month dry season) found in many tropical regions.

Evaluation of *Calliandra* provenances for agronomic performance (M. Peters, A. Pottinger and P. Avila)

Highlight

- Selected on agronomic merits an outstanding *Calliandra calothyrsus* provenance (CIAT 22310)

Rationale: *Calliandra calothyrsus* is a shrub legume with potential use as dry seasons cut and carry supplement for livestock. Thus a collection of 13 accessions 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. In terms of edible dry matter, accession CIAT 22310 is outstanding, followed by CIAT 22320 (Tables 38a, b). With time the performance of CIAT 22312 and CIAT 22314 seems to have improved, including a favorable relation of edible to total dry matter.

Table 38a. Edible dry matter, percentage of edible dry matter (DM) and regrowth of 13 Calliandra calothyrsus accessions in the wet season of the first year of production (3 harvests)

Accession CIAT No.	Edible DM (g/plant)	Ratio Edible DM/Total DM (%)	Average number of new shoots
22310	1522	55.8	21
22320	1055	48.3	24
22309	951	56.1	18
22315	764	41.3	14
22312	744	65.6	31
22314	726	55.3	15
22317	712	52.9	14
22318	709	57.4	20
22313	687	50.8	17
22319	683	43.5	14
22308	675	47.5	10
22316	564	45.2	13
22311	526	56.9	11

Increased dry matter yields in CIAT 22312 were associated with the ability to form a great number of new shoots, (50 new shoots per cut) during the rainy season. A result from this work is the selection CIAT 22310 as an outstanding accession of *Calliandra calothyrsus*, given its excellent performance in the wet and dry seasons. It will be necessary to multiply seeds and test this – and other – accessions in other hillsides sites to investigate the potential fodder value of *C. calothysrus* as an alternative to *L. leucocephala.*

Table 38b. Edible dry matter, percentage of edible dry matter (DM) and regrowth of 13 *Calliandra calothyrsus* in the dry season of the first production year (two harvests)

Accession CIAT No.	Edible DM (g/plant)	Ratio Edib.le DM/Total DM (%)	Average number of new shoots
22310	809	87.1	33
22320	572	95.3	34
22315	385	80.5	25
22318	359	91.6	32
22312	348	93.8	41
22309	320	97.9	30
22313	319	92.5	29
22319	307	83.4	23
22314	307	94.5	20
22317	267	91.1	23
22316	256	92.4	20
22311	220	81.8	16
22308	206	73.8	17

Plant attributes associated with genotypic variation in dry season tolerance among *Calliandra* provenances at two sites (I. M. Rao, J. W. Miles, P. Argel and M. Peters)

Highlight

- Selected *Calliandra calothyrsus* CIAT 22310 due to outstanding performance in the dry season.

Rationale: *Calliandra calothyrsus* is a fast growing tree legume adapted to the humid and subhumid tropics. Its growth performance, biomass production, nutrient recycling potential and contribution to soil improvement are comparable or better than that of *Leucaena leucocephala*. Other multiple uses of *Calliandra* include fodder value, pulp and paper production and land rehabilitation. It has great potential as dry season feed in the subhumid regions of Latin America. However, there is very little information on provenance evaluation of *Calliandra* for dry season tolerance. We have been interested in defining plant attributes associated with dry season performance of genotypes in core collection of *Calliandra* from OFI to complement the agronomic evaluation work that is in progress (see previous section).

Methods: At the Quilichao Experimental Station, a core collections of 13 accessions from OFI were planted in November 1996. Forage samples were collected twice during 1997 to determine differences among provenances in tolerance to short dry season. Parameters measured included: shoot biomass, leaf nutrient composition, leaf ash content and leaf TNC (total nonstructural carbohydrates). At Atenas, Costa Rica, a set of two provenances were sampled at the end of a long dry season for the same measurements.

Table 39. Variation among provenances in shoot biomass production, leaf nutrient composition, leaf ash content and leaf TNC (total nonstructural carbohydrates) of 13 accessions of *Calliandra* grown in an Oxisol at Quilichao, Colombia. Measurements were made twice during the year of 1997.

Provenance	Shoot biomass	Leaf Ca	Leaf K	Leaf Mg	Leaf ash	Leaf TNC
(CIAT No.)	(t/ha)	(%)				(mg/g)
5 August 1997:						
22308	5.02	0.34	0.61	0.17	3.22	171
22309	3.35	0.34	0.56	0.18	3.89	185
22310	8.00	0.41	0.56	0.21	4.07	138
22311	3.75	0.49	0.55	0.21	4.68	220
22312	2.38	0.38	0.64	0.12	3.36	180
22313	2.67	0.47	0.52	0.25	5.72	171
22314	3.79	0.37	0.61	0.19	4.01	150
22315	5.38	0.29	0.63	0.17	3.76	116
22316	4.15	0.63	0.47	0.20	4.45	169
22317	4.33	0.47	0.57	0.14	3.54	184
22318	2.43	0.58	0.48	0.23	3.75	136
22319	3.47	0.53	0.60	0.19	3.75	192
22320	4.56	0.35	0.56	0.17	4.44	183
Mean	**4.10**	**0.44**	**0.57**	**0.19**	**4.05**	**169**
LSD(P=0.05)	2.72	0.15	0.11	0.04	1.64	39
3 December 1997:						
22308	2.58	0.43	0.76	0.20	5.09	74.3
22309	6.31	0.69	0.64	0.26	5.93	60.9
22310	6.12	0.65	0.62	0.30	5.57	51.5
22311	1.31	0.60	0.74	0.27	5.22	86.8
22312	3.18	0.59	0.83	0.19	6.74	67.5
22313	3.50	0.63	0.19	0.30	5.99	65.4
22314	3.39	0.49	0.69	0.20	4.19	74.0
22315	4.24	0.49	0.68	0.21	3.74	85.5
22316	2.61	0.58	0.65	0.20	4.57	76.8
22317	3.29	0.77	0.63	0.27	4.47	57.6
22318	3.66	0.69	0.71	0.28	4.87	69.9
22319	4.82	0.64	0.75	0.24	3.84	67.0
22320	7.15	0.79	0.67	0.28	4.58	69.8
Mean	**4.01**	**0.62**	**0.70**	**0.24**	**4.98**	**69.8**
LSD(P=0.05)	2.60	0.23	0.10	0.09	1.97	22.5

Results and Discussion: Results from the **Quilichao site** indicate that there is significant variation in performance among provenances (Table 39). Among 13 accessions, CIAT 22310 was outstanding in shoot biomass production. This superior performance seems to be not closely related to any of the other parameters measured. It is possible that soil nutrient status (infertile Oxisol) may have interacted with dry season tolerance.

At the **Atenas site,** comparison of two provenances for their shoot nutrient status, ash content and TNC indicated differences in those parameters (Table 40). Based on these preliminary results, there is a need to evaluate a large number of provenances at Atenas site for identification of plant attributes for dry season tolerance. This site may be better suited for dry season tolerance because of moderately fertile soil conditions and long dry season.

Table 40. Differences in shoot nutrient composition, ash content and total nonstructural carbohydrates (TNC) between two accessions of *Calliandra* growing during dry season at Atenas, Costa Rica.

Provenances (CIAT number)	Shoot composition							
	C	N	P	K	Ca	Mg	Ash	TNC
	----------(%)----------							(mg/g)
Calliandra spp. (20399)	47.1	2.43	0.09	0.54	1.62	0.32	7.4	76.2
C. grandiflora (20400)	46.7	2.23	0.10	0.44	2.00	0.31	8.4	82.8

Response of *Cratylia argentea* during establishment to applications of phosphorus and calcium (M. Jiménez, P. J. Argel and G. Pérez).

Highlight

- Found that *C. argentea* responded during establishment up to 60 kg/ha of P in an inceptisol of medium fertility, but not to Ca.

Rationale: *C. argentea* is a shrub legume that adapts to a wide range of soils, tolerates drought and has excellent regrowth after frequent cutting. A limitation of *C. argentea is* that it establishes slowly, but results from Brazil indicated that growth can be improved with the application of fertilizers, particularly phosphorus. For this reason it was important to study the response of *C. argentea* to P and Ca during the establishment phase in more fertile soils found in subhumid areas of Costa Rica.

Methods: A field trial was conducted in Atenas, Costa Rica that has the following soil and climatic characteristics: subhumid tropical forest ecosystem located at 200 m.a.s.l, 1600 mm of annual rainfall, mean temperature of 23.7 °C and inceptisol soils (sandy loams) of medium fertility with pH close to neutrality (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 aceptable levels of micronutrients.

Inoculated seeds of *C. argentea* CIAT18668 were planted in single rows at 0.5 m between plants, and fertilized (fertilizer not incorporated) one month later with the following phosphorus (P) and calcium levels (Ca) as treatments: P: 0, 20, 40 and 60 kg/ha; Ca: 0, 200, 400 and 600 kg/ha. The sources of P and Ca were Triple Super (45% P_2O_5) and Calcium Carbonate (34 % of Ca), respectively. A factorial arrange of treatments was made in a complete randomized block design. Plots consisted of 10 plants and were replicated 4 times. DM yields of edible (leaves and thin stems) and not edible (thick stems) foliage was measured in four central plants from each plot 5 months after establishment.

Results: DM yields of *C. argentea* increased significantly (P<0.0001) with P supply, but did not respond to Ca (P>0.22) fertilization; similarly the interaction P x Ca was not significant (P>0.49). Results in Table 41 show significant increases in both total and edible DM yields in response to P supply. The magnitude of the increase was higher with 60 kg/ha of P, and statistically different (P < 0.05) between 20 and 40 kg/ha of P. The relationship between the P levels studied and DM yields was linear and highly correlated (r^2=0.97; P<0.01); this relationship was represented by the equation: Y (DM yields) = 49.37 + 0.99 P.

Table 41. Response during establishment of *C. argentea* CIAT 18668 to phosphorus and calcium fertilization in and inceptisol of Atenas, Costa Rica.

P	DM yields (g/plant)		DM yields (g/plant)	
(kg/ha)	Total	Edible	Ca (kg/ha)	Total
0	54.3c	50.9c	0	81.1a
20	73.8bc	69.4bc	200	71.0a
40	90.9b	84.3b	400	95.0a
60	124.9a*	112.3a	600	97.3a

* Means followed by the same letter within a column are not significant (P<0.05)

Discussion: Low phosphorus (P) supply limits forage production, particularly in low fertility acid soils of the tropics. *C. argentea* is a shrub legume that establish slowly, but growth can be improved with the applications of P and calcium (Ca) as reported in poor acid soils (oxisols) of Brazil. In the Brazilian work *C. argentea* responded to application of up to 420 kg of P/ha which was associated with the application of 4 t/ha of Ca.

In our experiment the soil is not acid and the levels of P and Ca are not low as those in the Cerrados of Brazil. But in spite of this, *C. argentea* responded to P applications, indicating the high requirement that this legume has for this element. Plants responded linearly up to 60 kg of P/ha (136 kg of P_2O_5/ha), but we were not able to measure a response above this level. In an acid oxisol of Brazil (low P, pH 4,6 and high Al), the external P requirements of *C. argentea* to obtain 80 % of DM growth in glasshouse conditions, was estimated to be 126 kg/ha of P_2O_5, which corresponded to 7.85 ppm of P in the soil. These values may change under field conditions since efficiency of use and application methods and environmental conditions may affect P uptake. In our case P and Ca were not incorporated into the soil.

The calcium carbonate utilized in our experiment was applied as a source of Ca and not to amend the soil. However, there was a tendency to increased yields of *C. argentea* with the highest levels of Ca, but results were not statistically significant. Thus our results indicate that the Ca levels tested had little influence on growth of *C. argentea* in this particular soil, perhaps because of the high level of this element in the soil used to grow the plants.

Studies on genotypic variation in dry season tolerance in *Brachiaria* and *Arachis*
(I. M. Rao, P. Argel, J. W. Miles, J. Ricaurte and R. García)

Highlight

- Showed that low levels of shoot Ca and ash content combined with greater levels of total nonstructural carbohydrates in shoot tissue may serve as indicators of dry season tolerance in species of *Brachiaria* and *Arachis*.

Rationale: Quantity and quality of dry season feed is a major limitation to livestock productivity in tropical America. Field studies were conducted at Atenas, Costa Rica. The main objective was to evaluate genotypic differences in dry season (6 months) tolerance among 18 accessions of *Brachiaria* species and 19 accessions of *Arachis* species. We tested 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: The trial included 18 accessions of *Brachiaria* species and 19 accessions of *Arachis* species selected from agronomic evaluation of the germplasm. This 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.

Results and Discussion: Forage yield among *Brachiaria* species during dry season ranged from 135 to 375 g/m^2 and the greatest forage yield was observed with *B. brizantha* CIAT 16305. This accession also had greater concentration of N in forage tissue (Table 42).

Table 42. Genotypic variation in forage yield, shoot nutrient composition, ash content and total nonstructural carbohydrates (TNC) of 18 accessions of *Brachiaria* species grown during dry season at Atenas, Costa Rica.

Genotypes CIAT No.	Forage yield (g/m^2)	Shoot composition							
		C	N	P	K	Ca	Mg	Ash	TNC
		(%)							(mg/g)
B. decumbens (16497)	200	40.9	0.86	0.10	0.86	0.66	0.49	10.8	149
B. humidicola (16886)	249	40.2	0.75	0.09	0.81	0.42	0.27	9.4	184
B. humidicola (26149)	215	39.4	0.67	0.05	0.54	0.40	0.48	11.4	104
B. brizantha (26646)	180	41.8	0.79	0.08	1.37	0.58	0.40	9.4	172
B. brizantha (16549)	135	40.6	0.69	0.06	0.65	0.74	0.35	10.2	129
B. brizantha (16467)	180	42.1	0.87	0.08	1.21	0.50	0.31	8.6	142
B. brizantha (6387)	204	38.9	0.71	0.09	0.84	0.74	0.52	12.8	128
B. brizantha (16305)	375	41.9	0.96	0.08	1.14	0.35	0.41	8.0	171
B. brizantha (16322)	210	42.0	0.80	0.08	1.44	0.5	0.38	8.4	151
B. brizantha (667)	180	42.5	0.83	0.09	1.22	0.43	0.28	7.4	165
B. brizantha (16168)	270	41.0	0.84	0.08	1.18	0.60	0.46	10.2	127
B. brizantha (26110)	155	42.4	0.84	0.07	1.16	0.42	0.28	6.8	141
B. brizantha (16135)	150	40.6	0.80	0.08	0.83	0.69	0.42	10.1	118
B. brizanhta (16488)	174	40.5	0.70	0.08	1.18	0.49	0.31	11.0	120
B. brizantha (16289)	125	41.5	0.76	0.07	0.78	0.61	0.36	8.0	113
B. brizantha (16300)	140	41.5	0.80	0.09	1.07	0.57	0.31	8.4	125
B. brizantha (16319)	225	41.3	0.64	0.06	1.11	0.43	0.40	8.6	153
B. brizantha (mixed)	315	42.3	0.95	0.06	0.95	0.67	0.27	7.0	114
Mean	**205**	**41.2**	**0.79**	**0.08**	**1.02**	**0.54**	**0.37**	**9.2**	**139**

The superior performance of this accession and *B. brizantha* CIAT 26110, which maintained greater proportion of green leaves during dry season was associated with lower levels of shoot ash content and particularly the Ca content. It appears that shoot Ca status may serve as a selection guide to evaluate dry season tolerance. Determination of TNC in shoot tissue 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.

Among the 19 accessions of *Arachis* species tested, *A. pintoi* CIAT 22160 contained greater concentration of N in shoots (Table 43). This accession also showed lower levels of Ca and greater concentrations of TNC in shoots. This accession was found to be outstanding in its

adaptation to dry season in the Cerrados of Brazil. Among the 5 accessions of *A. repens*, CIAT 22165 showed lower levels of Ca and ash content. But the level of TNC in this accession was also lower than the other accessions.

The levels of Ca, ash and TNC were greater in *A. glabrata* cv. Florigraze compared to the other *Arachis* species. 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.

Table 43. 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 number	Shoot composition							
	C	N	P	K	Ca	Mg	Ash	TNC
				(%)				(mg/g)
A. glabrata cv. Florigraze	42.4	2.13	0.13	0.62	3.36	0.35	11.2	88.8
A. repens (22163)	42.2	2.23	0.11	0.34	2.83	0.56	9.6	62.9
A. repens (22162)	41.9	2.10	0.10	0.52	3.42	0.42	10.4	64.1
A. repens (22164)	42.6	2.47	0.11	0.39	2.77	0.48	9.0	60.3
A. repens (22165)	43.0	2.36	0.09	0.27	2.41	0.60	7.8	49.2
A. repens (22161)	42.5	2.56	0.12	0.33	2.45	0.51	8.8	77.2
A. pintoi (22152)	42.8	2.91	0.14	0.60	2.49	0.48	8.8	77.3
A. pintoi (22153)	42.2	2.66	0.13	0.57	2.79	0.47	10.0	76.0
A. pintoi (22156)	41.9	2.36	0.11	0.68	2.71	0.37	9.6	94.1
A. pintoi (22160)	43.3	3.09	0.13	0.84	2.12	0.60	7.6	86.2
A. pintoi (22149)	42.5	2.64	0.12	0.73	2.39	0.36	8.6	80.8
A. pintoi (22155)	40.7	1.61	0.05	0.36	3.39	0.44	10.6	59.8
A. pintoi (22159)	41.2	1.83	0.06	0.40	2.82	0.64	8.4	49.3
A. pintoi (22151)	42.5	2.36	0.11	0.59	2.91	0.45	9.4	86.0
A. pintoi (22158)	42.8	2.84	0.13	0.77	2.53	0.53	9.0	82.6
A. pintoi (22157)	42.1	1.99	0.08	0.26	3.28	0.39	10.8	82.9
A. pintoi (22150)	43.0	2.39	0.11	0.84	2.23	0.43	8.2	99.9
A. pintoi (22148)	43.4	2.89	0.15	1.20	1.89	0.38	7.2	76.1
A. pintoi (22154)	41.6	2.88	0.15	0.98	2.54	0.50	9.2	68.0
Mean	42.3	2.44	0.11	0.59	2.70	0.47	9.2	74.8

Progress towards achieving output milestone

- **List of characterized accessions of grasses and legumes with tolerance to dry season (1999)**

We have identified a provenance of *Calliandra calothyrsus* with good agronomic performance and with drought tolerance. In addition, field evaluation in the better soils found in Atenas, Costa Rica has allowed us to identify a number of promising accessions of *Brachiaria* and *Arachis* for tolerance to dry season. We have also made advances in establishing fertilizer requirements for the establishment of *Cratylia argentea*, which is well known for its drought tolerance.

Atenas in Costa Rica appears to be a good site for evaluation of dry season tolerance of forages. This is mainly because it is a subhumid tropical hillside located at 2000 masl, 1600 mm annual rainfall with a long dry season (5 to 6 months) and a mean temperature of 23.7 C. Soils at this site are inceptisols with medium level of fertility. The other sites tested for dry season evaluation, Carimagua and Quilichao may not be suitable due to the interaction of low soil fertility with dry season and the intensity of water stress is limited. Thus we intend to continue

our efforts at Atenas site to select grasses and legumes with dry season tolerance and to carry out detailed studies to identify plant attributes that confer tolerance to dry season.

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

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. Thus we are interested in defining variation among *Brachiaria* and *Paspalum* species to adaptation to poor soil drainage.

Tolerance of *Brachiaria* species to foliage and root fungi under two soil moisture conditions (C. Zúñiga, R. González, E. Bustamente and P. J. Argel).

Highlights

- Showed that *B. brizantha* cv. Marandú and *B. brizantha* CIAT 16322 are very susceptible to root fungal disease caused by *Fusarium* sp. And to a foliage fungal disease caused by *Rhizoctonia* sp. under waterlogged (over saturated) soil conditions.

- Showed that *B. dictyoneura* cv. Llanero had no symptoms of foliar or root fungal disease under waterlogged conditions, and that *B. brizantha* CIAT 26110 showed only mild symptoms of root fungal symptoms caused by *Pythium* sp. under similar conditions.

Rationale: During the last decade new *Brachiaria* cultivars have been widely planted in tropical lowlands of Latin America, and the problems associated with pest and disease have increased. Farmers have the tendency to establish grasses with little technical assistance and failures of pasture persistence due to biotic factors are very common. For instance, *B. brizantha* cv. Marandú shows high plant mortality when planted in poorly drained soils due to root fungal diseases. On the other hand it is known that within the same *Brachiaria* species there are significant differences in plant tolerance to fungal infections. These differences need to be identified and evaluated in order to make progress in defining niches to grow *Brachiaria species*.

Methods: Root and foliage samples of *B. brizantha* cv. Marandú showing symptoms of fungal foliar diseases were collected in farms located in humid tropic conditions of Río Frío, Costa Rica. The fungi *Fusarium* sp. and *Pythium* sp. were found in both roots and foliage of infected *Brachiaria*, while *Rhizoctonia* sp. Was only found in leaves and *Trichoderma* sp. in roots. Both *Fusarium* sp. and *Rhizoctonia* sp. presented the highest percentage of appearance compared to other fungi.

Seedlings of *B. brizantha* cv. Marandú, *B. brizantha* CIAT 26110, CIAT 16322 and *B. dictyoneura* cv. Llanero, were transplanted and thinned to 3 plants per pot with 5 kg sterile soil collected from fungi infected fields. A total of 10 pots were used for each line of *Brachiaria*. After 58 days of growth, treatments consisting on inoculation of plants with different fungi were imposed on each line of *Brachiaria* under two soil water conditions: field capacity (FC) and over saturated soil (OS). These soil conditions were maintained for 28 days while the observations on foliage and fungi appearance and severity were recorded.

Results: Symptoms of foliage damage caused by *Rhizoctonia* sp. and root damage caused by *Fusarium* sp. appeared 4 days after inoculation in either soil condition in both *B. brizantha* cv. Marandú and *B. brizantha* CIAT 16322. The degree of damage caused the fungi remained low at FC, but increased on OS, causing plant mortality between 15 and 26 days after inoculation of the fungi. *B. brizantha* CIAT 26110 was affected only by *Pythium* sp. the roots at OS, but without causing plant mortality, while *B. dictyoneura* cv. Llanero showed no symptoms in any of the growth conditions during the 28 days of the experiment.

Discussion: Plant diseases are part of tropical pasture ecosystems and the occurrence and severity is associated with climatic conditions, soil factors and species susceptibility to a particular pathogen. For instance, soil moisture may interact with the balance between the host plant and strain pathogenicity.

In our experiment both *B. brizantha* cv. Marandú and *B. brizantha* CIAT 16322 were highly susceptible to *Fusarium* sp. infection in the roots and to *Rhizoctonia* sp. in the foliage under waterlogged conditions (OS). Cultivar Marandú survived waterlogged conditions by creating aerenchyma tissue and root stratification along the surface of the water table (see IP-5 Annual Report 1997), but it seemed that under these conditions the root tissue either goes through internal changes or secretes root exudates that promote root infection by *Fusarium* sp. This condition seems to have not occurred in *B. brizantha* CIAT 26110 or in *B. dyctioneura* cv. Llanero that showed no symptoms of the root fungal disease.

It is likely that the *Rhizoctonia* sp. isolated in this experiment was *R. solani* Kühn, which causes foliar blight and that is an endemic disease widely spread in the tropics of Latin America, particularly in humid and warm areas of Brazil, Colombia and in Central America. Infection by Rhizoctonia has been reported in all species commercially available of *Brachiaria,* including *B. dyctioneura* cv. Llanero, particularly in light grazed pastures and under prolonged high humidity. However, in our experiments neither cv. Llanero nor *B. brizantha* CIAT 26110, showed symptoms of foliar blight, indicating that perhaps the environmental conditions (humidity and temperature) or the duration of the experiment did not favor the development of the disease in these particular lines

During the last decade, pastures established with species of *Brachiaria* have increased in area in the Latin American tropics. For instances, *B. brizantha* cv Marandú has been widely planted in poorly drained soils and reports indicate that plant mortality is high. The results from our experiment show that *Brachiaria brizantha* cv Marandu should not be planted in poorly drained soils that favor foliar and root fungal diseases.

Genotypic variation in *Paspalum* for adaptation to poorly drained soils (F. R. Casola, N. Vásquez, R. A. González, C. Henríquez, P. J. Argel, I. M. Rao, J. W. Miles, M. Peters)

Highlight

- Confirmed that within a small core collection of *Paspalum* there are accessions that perform well in poorly drained acid soils.

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.

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 and observations on flowering and seed production.

Results: Among the original eight accessions of *Paspalum* planted at Quilichao seven have persisted. Early results show *Paspalum plicatulum* CIAT 26989, *Paspalum arundinellum* CIAT 26987, and *Paspalum atratum* CIAT 26986 having the highest dry matter yields (Figure 46). The *Paspalum* accessions seem to be well adapted to the poorly drained soils. However, at the cut in June, 1998, after commencement of the rains, dry matter yields were low, with 100 g or less per plant. Some accessions failed to flower in Quilichao, but first observations of transplants at Popayán are promising in terms of seed yield.

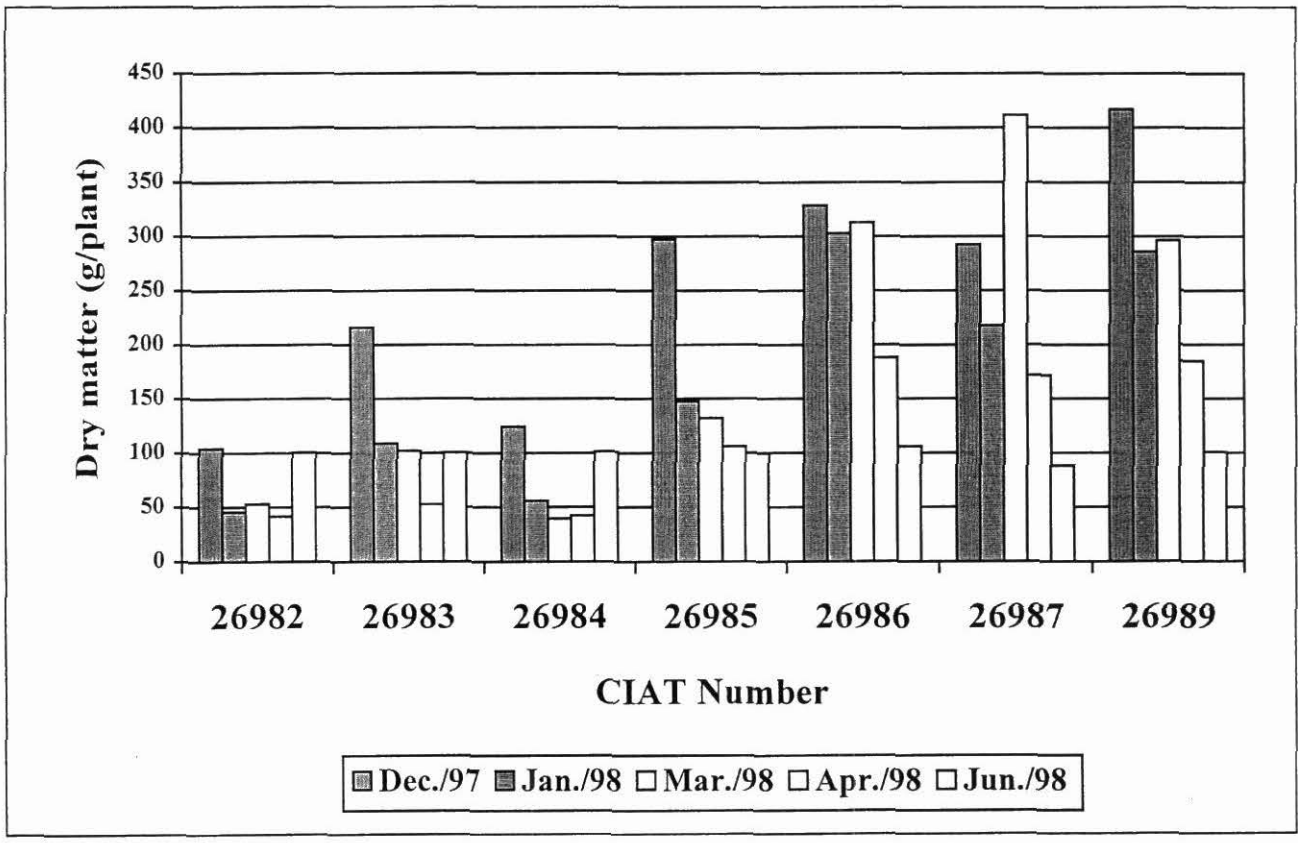

Figure 46. Dry matter production in a collection of *Paspalum* spp. in different seasons, growing under conditions including acid and poorly drained soils, Santander de Quilichao, Cauca, Colombia

Progress towards achieving output milestone

- **List of characterized accessions of *Brachiaria* and *Paspalum* selected for poorly drained soils (1999)**

The genus *Brachiaria* has considerable variation within and between species related to tolerance to biotic and abiotic factors. We have made progress toward identifying species of *Brachiaria* with adaptation to poorly drained soils and diseases associated with waterlogging. The mechanisms responsible for plant infection with fungi are not well understood, but the intraspecific variation within the genus, allows us to advance with new lines tolerant to soil fungal diseases and with desirable agronomic attributes.

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

In the past forage evaluation work in CIAT was concentrated in low lands with poor fertility acid soils and variable rainfall. With the increasing importance given by CIAT to hillsides ecosystems, the Forage Project is dedicating resources to evaluate and select shrub legumes for multipurpose use in this environment. This implies selecting species that adapt to low fertility soils and that tolerate cool temperatures.

A range of shrub legumes genera and species from CIAT's GRU are currently under evaluation in hillsides of Cauca. Work is also in progress in collaboration with OFI, UK to evaluate *Leucaena* species in contrasting environments.

Evaluation of selected shrub legumes in mid altitude hillsides (M. Peters and P. Avila)

Highlights

- Range of shrub legume species selected for further evaluation in hillsides ecosystems

Rationale: A wide range of shrub legumes have been tested for adaptation to tropical lowland conditions, but availability of shrub legumes adapted to mid-altitude hillsides is limited. The identified accessions could be important components in agrosilvopastoral systems to provide forage and mulch, to help in the control of erosion, and to be utilized as live enclosures.

Methods: Forty-five accessions covering a wide range of species and genera are being tested in field trials established during 1996 at two sites in the Cauca Department, at 1,200 (San Vicente) and at 1,600 masl (El Melcho). Plants were transplanted into single-row plots. These plots have been managed by periodic defoliation (with recording of dry matter yield). In addition to dry matter yield, plant persistence is an important attribute. Measurements include height and diameter of plants, the number of regrowing shoots, incidence of pest and diseases, dry matter production and ratio edible/total dry matter. Of the most promising accessions samples are collected for the analysis of nutritive value.

Results: Of the materials planted only 17 accessions and 6 accessions persisted in San Vicente and Melcho, respectively. Preliminary data indicate that *Pueraria wallichii* CIAT 21076 , *Calliandra houstoniana* CIAT 20399, *Calliandra* sp. CIAT 21420, *Flemingia macrophylla* CIAT 19457 and CIAT 17405, *Leucaena diversifolia* CIAT 17251, and *Rhynchosia shomburgkii* CIAT 19235 and CIAT 20800 are promising species in terms of dry matter production, persistence and ability to regrow. (Tables 44, 45, 46).

Discussion: It is known that *Flemingia macrophylla* performs well in mid altitude hillsides, but it has palatability problems. Similar palatability problems were found in *Rhynchosia* germplasm evaluated by CIAT in the early 1980's. The feed value of *Rhynchosia schomburgkii*, *Calliandra houstoniana, Calliandra sp., Leucaena diversifolia* and *Pueraria wallichii* needs to be studied.

Thus selected accessions will be multiplied for wider environmental testing in selected hillside sites and for quality analysis. The appropriateness of these species as feed for livestock and for other uses remains to be determined. The original two *Rhynchosia* lines (CIAT 19235 and CIAT 20800) are currently under evaluation by PE-5 as a soil improvement plant.

Table 44. Average Dry matter yields (g DM/plant) of 6 tree and shrub accessions in El Melcho, Piendamó, and Cauca, Colombia.

	Species	Accession CIAT No.	Edible DM			
			17 jul. 97	25 nov. 97	02 feb.98	06 abr.98
			(g/plant)			
Trees	*Calliandra houstoniana*	20399	346	343	507	135
	Flemingia macrophylla	19457	183	301	224	86
	Senna velutina	18704	106	95	191	13
	Flemingia stricta	21089	76	87	38	0
	Calliandra sp.	21420	0	247	346	129
Shrubs	*Rhynchosia schomburgkii*	19235	496	238	182	50

Table 45. Average Dry matter yields (g/plant) of 17 tree and shrub accessions in San Vicente, Santander de Quilichao, Cauca, Colombia.

	Genera Specie	Accession CIAT No.	Fine Fraction of the Plant			
			17 jul.97	25 nov. 97	02 feb. 98	06 abr.98
			(g/plant)			
Trees	*Flemingia macrophylla*	17405	533	369	0	389
	Calliandra houstoniana	20399	521	576	580	1426
	Pueraria wallichii	21076	323	563	23	341
	Calliandra sp.	21420	300	630	447	780
	Senna velutina	18704	287	334	399	536
	Leucaena diversifolia	17271	270	770	178	786
	Flemingia macrophylla	19457	266	159	7	347
	Senna siamea	20698	216	460	190	598
	Acacia farnesiana	21509	210	165	73	429
	Clitoria fairchildiana	18721	154	227	20	382
	Senna silvestris	7975	145	422	334	462
	Clitoria fairchildiana	18724	144	179	0	314
	Flemingia stricta	21089	73	131	70	170
	Ateleia ovata	7362	31	9	26	94
Shrubs	*Rhynchosia schomburgkii*	19235	481	490	98	581
	Rhynchosia schomburgkii	20800	362	524	210	615
	Flemingia macrophylla	18048	40	38	0	64

Table 46. Number of regrowing shoots (No) and daily growth rate (C./d) in cm, of 14 tree accessions in San Vicente.

Species	Accession	17 jul.97		25 nov. 97		02 feb.98		06-abr.-98	
	CIAT No.	No.	C/d	No.	C/d	No.	C/d	No.	C/d
			cm		cm		cm		cm
Flemingia macrophylla	17405	37	0.69	40	1.13	31	1.61	65	2.24
Calliandra houstoniana	20399	21	0.97	31	1.45	36	2.54	85	3.36
Pueraria wallichii	21076	16	1.17	30	1.78	21	2.15	50	3.10
Calliandra sp.	21420	14	1.02	32	1.47	27	2.72	66	3.41
Senna velutina	18704	6	0.71	26	1.26	16	2.18	32	2.65
Leucaena diversifolia	17271	8	1.53	18	2.38	18	2.79	31	3.68
Flemingia macrophylla	19457	15	0.90	27	1.31	14	1.88	31	2.78
Senna siamea	20698	2	0.80	16	1.50	21	2.19	42	3.03
Acacia farnesiana	21509	5	1.15	7	1.20	10	2.20	25	3.06
Clitoria fairchildiana	18721	9	0.63	18	1.10	20	1.88	28	2.27
Senna silvestris	7975	10	0.69	21	1.28	19	2.02	27	2.68
Clitoria fairchildiana	18724	8	0.67	17	1.15	17	2.02	29	2.54
Flemingia stricta	21089	5	0.62	11	1.11	19	2.02	19	2.49
Ateleia ovata	7362	1	0.59	6	0.87	10	1.80	13	1.99

Evaluation of a core collection of *Rhynchosia schomburgkii* (M. Peters and P. Avila)

Highlight

- Selection of an accession *Rhynchosia schomburgkii* CIAT 19235 with outstanding vigor in poor soils in hillsides of Cauca

Rationale: From the evaluation of a range shrub legumes for tolerance to cool temperatures and work done in Project PE-5, *Rhynchosia schomburgkii* has emerged as one of the most promising accessions for the higher altitude hillsides particularly as a soil cover plant. Therefore, a wider collection of 13 accessions was established at Quilichao in June 1998.

Methods: A total of 13 accessions of *Rhynchosia schomburgkii*, mostly originating from Colombia, 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: Most accessions established satisfactorily, and a large variation was observed the collection in terms of vigor (Table 47). Of the two accessions evaluated in the Cauca, CIAT 19235 is among the most vigorous, while CIAT 20800 exhibited relatively low vigor and survival rates.

Table 47 Survival and vigour of *Rhynchosia schomburgkii* accessions three months after planting at Quilichao

Accession CIAT No.)	Survival (%)	Vigour
8582	100	5
918	100	5
19235	96	5
18490	96	4
7389	93	4
7810	93	3
22134	86	5
21777	82	4
17918	82	4
20456	82	2
20800	68	3
8215	36	2
21775	29	1

Discussion: Phenotypic variation among *Rhynchosia schomburgkii* accessions seems to be low, the habit being intermediate between a herbaceous and shrub legume, with the ability to twine. While the species has potential as a soil cover plant, the value as a forage plant need to be investigated.

Evaluation of new species of *Leucaena* in different environments (P. J. Argel, G. Pérez and A. Pottinger).

Highlights

- Recorded strong interactions between site and *Leucaena* genotypes and site.

- Recorded differences in dry season tolerance, DM yields and psyllid tolerance of new *Leucaena* species

Rationale: *Leucaena leucocephala* is well known for wood and forage quality. However this species adapts poorly to acid soils and is very susceptible to psyllid (*Heteropsylla cubana*). There is considerable variation within the genus *Leucaena* and a wide range of new species are available that need to be studied and characterized to define their range of climatic and soil adaptation, pest resistance, and forage potential.

During 1998 we continued the evaluation of *Leucaena* germplasm in collaboration with OFI (Oxford Forestry Institute of England) (Annual Report IP-5, 1997). A set of regional trails were established jointly with collaborators in Mexico, Honduras, Nicaragua, Costa Rica, Panamá, Colombia, Venezuela and Brazil. In Atenas Costa Rica the trial has entered into the second year of evaluation, while in other countries the evaluations were initiated this year.

Methods: Nineteen lines of different species of *Leucaena* and the addition of checks were planted for evaluation in contrasting sites in different countries. Altitude ranges from 6 to 800 m.a.s.l (mild cool temperatures), rainfall from 886 to 4500 mm per year, soil pH from 4.7 to 8.3 and mean annual temperature from 23 to 26°C.

The Atenas site in Costa Rica is a subhumid tropical hillside located at 200 m.a.s.l, 1600 mm annual rainfall, mean temperature of 23.7 °C and inceptisol soils of medium fertility. This sites has 5 to 6 months of dry season that extends from November to may each year. Plots consists of rows of ten plants spaced 0.5 m apart. *Leucaena* species are replicated four times and measurements taken on plant height and diameter, dry matter yields, psyllid and disease susceptibility, and tolerance to drought.

Results: In the Atenas site we have observed considerable variation within and between species of *Leucaena* with relation to plant growth, psyllid infestation, DM yields and dry season tolerance. Table 48 shows DM yields during the wet (3 cuts) and dry (2 cuts) seasons.

The new specie *L. trichandra* and *L. collinsii* 52/88, *L. pallida* 14/96, cv. Tarramba and the *Leucaena* hybrids, produced relatively high DM yields in both the wet as well as in the dry period; however, at the end of the dry season plant mortality was recorded in *L. trichandra* and *L. collinsii* 52/88. High plant mortality was also recorded in *L. trichodes, L. multicapitula, L. lempirana* 6/91 and in *Calliandra callothyrsus* (control).

These species as well as *L. shannonii magnifica* produced the lowest DM yields. The local check, *L. leucocephala* (CIAT 17263) had intermediate DM yields, that were statistically similar to cv. Tarramba ($P>0.05$). On the other hand, *Cratylia argentea* planted in this trial also as a control, had high DM yields particularly during the dry period, and suffered no plant mortality.

Preliminary results from other locations indicate a strong effect of site on adaptation of *Leucaena* species. For example, *L. lempirana* 6/91 (poor in Atenas, Costa Rica) and *L. diversifolia diversifolia* 83/92 exhibited the best growth in a savanna ecosystem of Isla (Mexico), but poor development in the humid tropics of Los Sanjones (Nicaragua). However, *L. leucocephala* subsp. *Glabrata*.. The cultivar Tarramba, is doing well in subhumid ecosystems such as Atenas and Comayagua in Honduras.

The occurrence of psyllid has been recorded only in Atenas. Mild attacks (less than 25 % foliar damage) has been observed at the end of the wet season of each year of evaluation. Consistently cv. Tarramba, *L. lanceolata* 43/85, *L. salvadorensis* 17/86, *L. sahannonii*

magnifica 19/84, *L. lempirana* 6/91, *L. collinsii zacapana* 56/88, *L. trichodes* 61/88 and *L. multicapitula* 81/87, have had the highest populations of the insect.

Results: For most pasture agronomists the term *Leucaena* means *Leucaena leucocephala*. Most of them, perhaps apart of those that live in Mexico and Central America, are not aware of the great species diversity that exists within the genus *Leucaena*. Now days we recognize 22 different species are recognized as growing in different types of soils, climate, and altitudes (from 0 to 2500 m.a.s.l) and spreading in a band of 40 degrees latitude.

It is known that within and between Leucaena species there are marked differences in tree shape, soil and climate adaptation, wood and foliage quality and tolerance to pests and diseases, particularly to the psyllid attacks (*Heteropsylla cubana*). Our experiment confirm strong interactions between *Leucaena* genotypes and site. For instance, *L. collinsii zacapana* is doing well in Colombia, Mexico and Nicaragua, but not well in Costa Rica. In contrast, cv. Tarramba is one of the best in Costa Rica and Honduras, but not in other sites.

Table 48 Dry matter yields of *Leucaena* and other species (controls) established in subhumid conditions of Atenas, Costa Rica (Means of 3 evaluation cuts every 8 weeks during the wet season and 2 evaluation cuts every 8 to 12 weeks during the dry period).

Species	ID No.	Edible dry matter yields (g/plant)		Plant mortality
	(OFI)	Wet	Dry	(Dry season)
L. trichandra	53/88	106 a*	53 b*	4
L. collinsii	52/88	95 ab	46 bc	1
L. pallida	14/96	90 abc	45 bcd	0
L. l. glabrata (cv. Tarramba)	34/92	89 abcd	56 b	0
L. hybrid	52/87	87 abcd	40 bcde	0
L. hybrid	1/95	87 abcd	47 cb	0
L. pulverulenta	47/87	77 bcde	29 cdef	0
L. pallida	79/92	75 bcde	38 cdef	2
L. diversifolia diversifolia	83/92	69 cdef	42 bcde	0
L. macrophylla nelsonii	47/85	67 def	49 bc	0
L. leucocephala CIAT	17263	66 def	48 bc	1
L. salvadorensis	17/86	63 ef	32 cdef	0
L. lanceolata	43/85	61 ef	32 cdef	2
L. pulverulenta	83/87	59 ef	32 cdef	3
L. collinsii zacapana	56/88	48 fg	14 fgh	0
L. lempirana	6/91	38 g	24 cefg	8
L. shannonii magnifica	19/84	33 gh	25 defg	3
L. trichodes	61/88	13 hi	8 gh	11
L multicapitula	81/87	5 i	3 h	22
Cratylia argentea CIAT	18668	107 a	86 a	0
Calliandra collothyrsus DPI	115690	29 gh	31 cdef	22

* Means within a column followed by the same letter are not significant (P<0.05).

Progress towards achieving output milestone

- **List of shrub legumes characterized and selected for tolerance to cool temperatures in mid altitude hillsides (1999)**

The work in hillsides of Cauca, has allowed us to identify a range of shrub legumes species with adaptation to poor soils and to cool temperatures. The most promising species are *Rhynchosia schomburgkii*, *Calliandra* sp., *Calliandra houstonia*, *Leucaena diversifolia*, *Pueraria wallichii* and

Flemingia macrophylla. However, for most of these species we still need to define their feeding value and effects on the soil.

The multilocational evaluation of *Leucaena* species has allowed us to make progress in selecting *Leucaena* genotypes tolerant to psyllid and with desirable agronomic attributes. Interactions in performance between *Leucaena* species and site are also being documented. As a result will be able to better target *Leucaena* species to different environments including mid altitude hillsides with variable rainfall.

Suboutput 3.5 Defined genotype x environment interactions on performance of selected grasses and legumes

Targeting forage germplasm to deferent ecosystems and production systems is a major objective of CIAT's Forage Team. Consequently we have assembled core collections ok key grass (*Brachiaria*) and legumes (*Arachis, Desmodium)* species to include in multilocational trials. By doing this we expect to be able to define genotype x environment interaction information, which will then be linked to GIS to allow extrapolation of results.

Workshop : Multilocational evaluation of *Brachiaria:* (J.Miles)

Highlight

- New *Brachiaria* ecotypes were selected by partners in the *Brachiaria* Network that operates in Colombia

Rationale: In lieu of periodic site visits, for which time simply is unavailable, periodic contact with regional trial collaborators has been at annual meetings/workshops. During the workshop held in early 1998, visits to three of the trial sites were organized. Formal reports and informal discussions allow assessment of the progress and productivity of the different trials.

Methods: This year's meeting was ably organized by collaborators in the region, namely Ing. Henry Mateus (Corpoica, Barrancabermeja); Prof. Emiro Canchila (U. de la Paz, Barrancabermeja); and Ing. Carlos Alberto Ramírez (Corpoica, La Dorada). It was hosted by local officials of the Corpoica office in Puerto Berrío.

Results: The major result of the meeting was the formulation of a list of 10 best lines based on first-year performance. These lines are the most promising candidates for advance to grazing trials. Final decisions on entries of grazing trials will be made only at the termination of the agronomic trials, at the end of 1998.

In order to be prepared to establish large grazing trials early in 1999, the 10 recommended accessions were advanced to large-scale seed multiplication at Popayán immediately following the March meeting of the regional trials network.

Discussion: More than half of the entries in the regional trials have been effectively culled. Attention -- mainly in the form of large-scale seed multiplication -- is now focused on the remaining lines. When final data are available late in 1998 or early 1999, we will be prepared to advance directly to the following phase of grazing trials as seed will already be available. While several collaborators have dropped out of the network, the original objectives of this exercise are expected to be met.

Multilocational evaluation of a core collection of *D. ovalifolium* (A. Schmidt, C. Lascano, Brigitte Maass, N. Narváez, R. Barahona, G. Ramirez and R. Schultze-Kraft)

Highlights:

- Four new productive *D. ovalifolium* accessions were selected for seed multipllication and further evaluation in on-farm grazing trials in 1999.

Rationale: Current tropical forage research 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 environmental factors on quality and anti-quality components like tannins and possible interactions with genotypes. Yet the knowledge about genotype x environment interactions on quality components and other agronomic attributes of tropical legumes is limited.

An important example for a tropical legume, whose quality seems to be influenced by the environment, is the Southeast Asian *Desmodium ovalifolium*. The species shows high biomass production in acid soils and adapts to wide range of conditions. However, forage quality is negatively affected by high condensed tannin (CT) content, which seem to be influenced by environmental factor as well as by genotypic variation. Identification of *D. ovalifolium* genotypes with high forage production and nutritive value is considered key for developing persistent grass/legume production systems in the humid tropics.

Methods: A core collection of consisting of 18 genotype of *D. ovalifolium* was established in a multilocational trials in six contrasting environments in Colombia. These site are representative of : (1) well-drained savanna, hot (Carimagua, two sites with contrasting soil textures); (2) humid forest margins, hot (Caquetá, two sites with contrasting drainage conditions); (3) dry hillsides, cool (Cauca); and (4) humid hillsides, cool (Caldas). Given that in the selected locations temperature is confounded with soil acidity/fertility; a fertilization treatment with two levels (low/high), adjusted to the specific conditions of the location, was applied. Along with climatic data recording, in order to select high performing new genotypes the following measurements were carried out at each location:

(1) Morphological and production measurements every six and eight weeks in the periods of maximum and minimum rainfall, respectively
(2) Quality analyses in leaves of a six and eight weeks-old regrowth for crude protein (CP), cell wall components (NDF, ADF), in vitro dry matter digestibility (IVDMD), condensed tannins (type and astringency), phosphorus, and sulfur
(3) Consumption of genotypes by grazing cattle through cafeteria-type trials in order to determine relative palatability of genotypes.

Data were analyzed through ANOVA for each site and across fertilizer levels, seasons and all sites. Stability analysis was performed by calculating genotype means against total mean of respective sites (adaptability index) followed by regression analysis across sites. Analysis of regression intercepts and slopes allowed the selection of genotypes for each parameter. Selection focused on high nutritive value accessions with medium to high biomass production.

Results and Discussion: In Figures 47 and 48 we show results of the stability analysis for in vitro digestibility (IVDMD) and astringency of condensed tannin of the core collection of *D. ovalifolium* evaluated in contrasting sites of Colombia, respectively. Intercepts indicate means of accessions and slopes explain the extent of response of a given parameter to environmental

changes. Genotypes with a slope=1 have changes in response parameters that are equal to environmental changes. Slopes<1 indicate that genotypic response is less affected by environmental changes and slopes>1 indicate a larger degree of change in the response parameters than the actual changes in environments. Line Y indicates the overall mean across sites for the respective parameter.

Digestibility and astringency of tannins as key quality criteria for the selection of legumes. With the analysis performed we selected four ecotypes (CIAT 33058, 13105 13651 and 23762) due to their higher digestibility in relation to other ecotypes (Figure 47). The digestibility stability indices of these ecotypes ranged from 0.95 to 1.1, which indicate that changes in digestibility were proportional to changes in the environment. Tannin astringency (i.e. hability of tannins to bind protein) is another key parameter for selecting legumes. Results of the analysis (Figure 48) indicate that most of *D. ovalifolium* selected for digestibility (CIAT 350, 13651, 23762, 33058) also had tannins with lower astringency as compared to other ecotypes. The stability indices for tannin astringency in selected ecotypes was closed to 1, indicating again that changes in this parameter were proportional to change in the environment.

With the help of two cafeteria trials at Chinchiná and La Rueda, the palatability a range as the genotypes could be confirmed. However, since relative acceptability indices may be influenced by a series of factors and replications over time were not possible larger grazing trials with the selected genotypes are inevitable to assess the pasture potential of selected accessions. Multilocational grazing trials will start at the beginning of the wet season 1999. Milk production and liveweight gain will be measured throughout the trials.

A matrix using all parameters measured in the five *D. ovalifolium* genotypes selected was constructed as shown in Table 49.

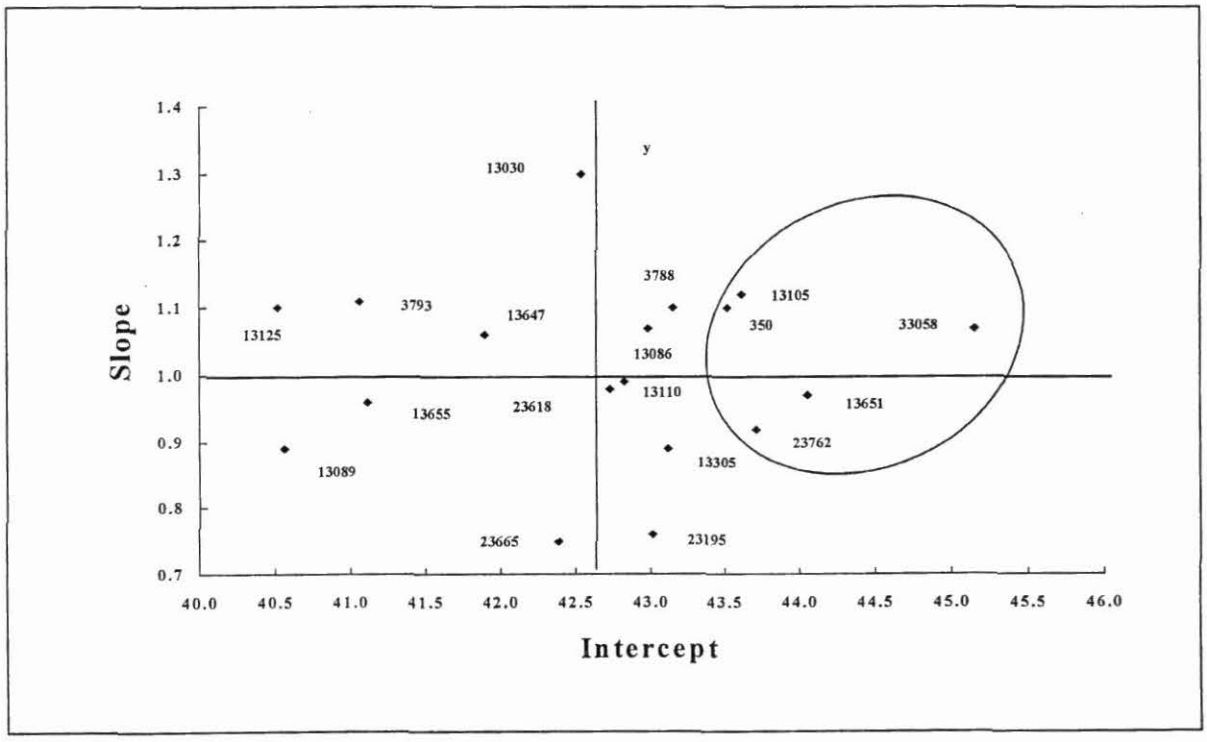

Figure 47. Results on stability analysis of in vitro digestibility in a core collection of *Desmodium ovalifolium* evaluated in contrasting locations.

The selection focused on high forage quality and less on biomass production given that nearly for all genotypes biomass production was more than acceptable. The genotypes CIAT 33058, 13105, 13651, and 23762 were selected for seed multiplication. The accession CIAT 350 , which is the only commercial cultivar of the species at the moment showed, outstanding performance, especially in the Llanos.

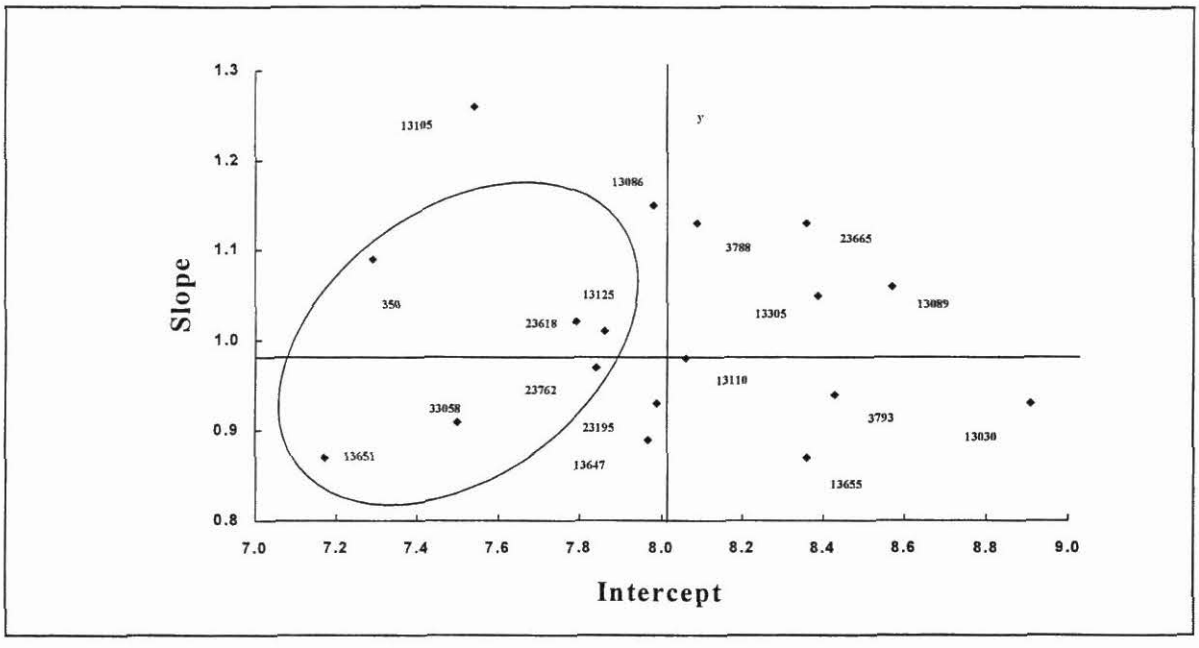

Figure 48. Results on stability analysis of tannin astringency in a core collection of *Desmoidum ovalifolium* evaluated in contrasting environments.

Table 49. Selection matrix for *Desmodium ovalifolium.*

IVDMD	SCT	Astringency	CP	IAC Chinchiná	IAC La Rueda	Leave Biomass	Litter	Cover
350	350	350		350	350	350	350	350
	13086					13086		
13105	13105			13105		13105	13105	13105
			13110					
				13125		13125	13125	13125
					13305			
			13647	13647		13647		
13651	13651	13651	13651	13651		13651		13651
			13655			13655	13655	
					23195			23195
		23618	23618					
23762	23762	23762		23762		23762		
33058		33058	33058	33058	33058		33058	33058

Progress towards achieving output milestone

List of accessions of selected grasses and legumes for specific environments (1999)

Results from the multilocational evaluation of core collections of *Brachiaria* and *Desmodium* in Colombia have allowed us to identify new genotypes with wide soil and climatic adaptation and with equal or superior agronomic and feed value than commercial cultivars. Steps have now been taken to multiply seed of the selected grass and legume genotypes for wider testing and for on-farm evaluation.

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

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

In the past the task of multilocational evaluation of superior gene pools of grasses and legumes was effectively accomplished through forage networks in LAC (RIEPT) and in West Africa (RABAOC), but due to lack of financial support these networks became non-operational. However, elite forage germplasm from CIAT is currently being evaluated with farmer participation in South East Asia through the Forages for Smallholder Project funded by ACIAR and 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 the Tropical Grasses and Legumes Project of CIAT is to identify research partners in LAC that have interest in evaluating selected grasses and legumes for multipurpose use in production systems. The identification of partners for forage evaluation is being accomplished through:

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

Establishment of partnerships and linkages with existing consortia and networks to deliver new grasses and legumes for on-farm testing

Highlights

- Seed multiplication of selected *Brachiaria* accessions and hybrids is in progress for subsequent on-farm grazing evaluation in Colombia.
- New ecotypes of *Arachis pintoi* now under evaluation in farms of the forest margins of the amazon, Llanos and humid hillsides in Colombia.
- Release of *Arachis pintoi* (CIAT 18744 in Costa Rica.

***Brachiaria* Network** (J. Miles and J. Velasquez)

During 1998 the evaluation of selected accessions and hybrids of *Brachiaria* in a range of environments in Colombia was continued through a Network co-financed by FEDEGAN (Federación de Ganaderos de Colombia). The *Brachiaria* Network comprises 13 sites and involves as partners forage researchers from CORPOICA (Corporación Colombiana de Investigación Agropecuaria), a Colombian University, SENA and private producers. A Workshop was held during March 1998 in Puerto Berrio as part of the *Brachiaria* Network and a progress report follows.

The objective of the Workshop was to evaluate progress made in the evaluation and selection of *Brachiaria* genotypes (accessions and hybrids) to edaphic and climatic conditions in different Colombian locations. The event was attended by 20 professionals and by 4 producers participating in the Network and active participants. Reports were presented by 11 of the 13 participants for the following sites:

a) North Coast : 4 sites
b) Magdalena Medio : 2 sites
c) Antioquia: 1 site
d) Llanos : 3 sites and
e) Forest margins of the Amazon: 1 site

A Workshop Proceedings was prepared and sent to participants and to the donor (FEDEGAN). Progress reports were also presented by Drs. J. Miles and C. Cardona on the current status of the work on development of new *Brachiaria* genotypes through breeding and on screening *Brachiaria* for spittlebug. In addition, L. H. Franco from CIAT presented information on methodology for the analysis of multilocational results of *Brachiaria* . The Program also included a Field visit to three sites in the Magdalena Medio.

Results indicate that in all sites there are genotypes of *Brachiaria* with better agronomic attributes than the commercial cultivars (*B. decumbens* CIAT 606, and *B. humidicola* CIAT 6133). Worth mentioning was the effect of a prolonged drought due to "El Niño" and attack of an insect (*Blissus leucopterus*) during the evaluation carried out in 1997. There were also continuous attacks of spittlebug in *Brachiaria* accessions being evaluated particularly in the Llanos (Carimagua) and Forest Margins (Macagual), but in all cases plants recovered.

During the Workshop, a survey was carried out to determine which genotypes of *Brachiaria* ranked highest in preference of agronomist and producers participating in the Network. From this survey 10 accessions of *Brachiaria* were selected as being promising for most locations using vigor and DM yield as selection criteria. Based on preference ranking (numbers in parenthesis indicate number of votes) the following accessions were selected:

a) *B. brizantha* CIAT 26110 (11)
b) *B. humidicola* CIAT 6133 (7)
c) *B. brizantha* CIAT 26124 (6)
d) *Brachiaria* hybrid CIAT 11873 (6)
e) *B. brizantha* CIAT 16467 (4)
f) *B. brizantha* CIAT 6387 (4)
g) *B. humidicola* CIAT 2647 (3)
h) *B. brizantha* CIAT 26318 (3)
i) *B. brizantha* CIAT 26556 (3)
j) *B. humidicola* CIAT 26159 (3)

Worth mentioning is the high preference shown by collaborators for *B. brizantha* CIAT 26110, which has also been selected in subhumid hillsides of Costa Rica as a promising grass due to high DM yield and drought tolerance.

The Workshop was also a good forum to review methodological aspects related to agronomic evaluation of *Brachiaria* accessions. It was decided that in order to facilitate the analysis of data from second year results, it was necessary to standardize cutting frequencies at each site to every 6 weeks and to record DM yield at lest twice in the in the wet season and twice in the dry season. Adjustments were also made to ways of determining leaf : stem ratios in forage sub-sample. To determine spittlebug species , it was recommended that sampling (10 sweeps with a net) be done three times a year at each site and that insects collected be sent to CIAT for identification. In addition, it was recommended that to assess palatability of *Brachiaria* entries, it was necessary to introduce 4 or 5 steers in the plots for 3or 4 days and to record frequency of consumption of individual ecotypes.

As a follow-up to the initial agronomic evaluation, it was proposed that selected *Brachiaria* accessions be multiplied and included in on-farm grazing trials in different locations of Colombia. Under the leadership of Dr. Jaime Velásquez from CORPOICA, a proposal including the on-farm evaluation in 3 to 5 sites of 4 to 5 accessions of *Brachiaria* with and without legumes is to be written and submitted to a donor in Colombia (FEDEGAN and/or PRONATTA).

TROPILECHE (F. Holmann , P. Argel, K.. Reategui, J. Vela, M. Lobo, C. Hidalgo and C. E. Lascano)

The Tropileche Consortia continues to be an important vehicle to test new grass and legume options in farmers fields. A complete report on the progress made during 1998 in Tropileche is found in the Annual Report of PE-5. However, in this Report we would like to highlight some significant accomplishments in Tropileche related to the evaluation of new grasses and legumes and opportunities for adoption and impact.

Pucallpa -benchmark site in Peru (representative of the forest margin): We continue to evaluate the use of *Stylosanthes guianensis* CIAT 184 (Stylo) as an alternative to feed pre-weaned calves in dual-purpose cattle systems. Results continue to show that by planting small areas of Stylo farmers can get at least one more liter of milk for sale and obtain adequate growth rates of calves. In addition, we continue to evaluate new ecotypes of *Arachis pintoi* (18744 and 18748) in pasture systems for milking cows. Improved grazing management of the pastures (heavier grazing) has resulted in more legume in the pasture and in modest increments of milk yield with cows that have low genetic potential.

Results form ex-ante economical analysis carried out in Pucallpa (See AR-98 of PE-5) indicate that with exception of the Stylo technology, the potential for adoption of new pasture options by farmers in Pucallpa are very low. Part of the reasons is the low cattle inventory in the region (low carrying capacity of pastures) and the lack of market for milk.

Consequently, we initiated contacts in Moyobamaba, another region of the forest margins of Perú, with good infrastructure and roads to major markets and where demand for new grasses and legume is high. In this region (Selva Alta, San Martin) livestock farmers are well organized in a cooperative, which sells milk processed in plant owned by them. In addition, there is an ongoing program to improve the genetic potential of milking cows through AI and farmers have been adopting at a fast rate cut and carry grasses to supplement milking cows. Farmers receive technical support from an NGO (FUDAAM) and limited financial support from a state agency (PEAM).

Opportunities for Tropileche in the Moyobamba area are mainly in the introduction of herbaceous and shrub legumes. Herbaceous legumes such as *Arachis pintoi* will be key for the recuperation of degraded pastures, which is major problem phased by producers. On the other hand, shrub legumes such as *Cratylia argentea* will be important protein sources to complement cut and carry grasses being used by farmers and thus replace expensive concentrate.

We are currently in conversations with the Peruvian Representative in the CGIAR to obtain funds to support forage work in Moyobamba through Tropileche and CIAT's IP-5. Work in Pucallpa will continue, particularly as it relates to the development of new forage options for multipurpose use (i.e. cover crops in plantations) in forest margins.

Atenas/Esparza- benchmark sites in Costa Rica (representative of subhumid hillsides): We continue with the on-station and on-farm evaluation of *Cratylia argentea* for cut and carry systems and *Arachis pintoi* (18744) to recuperate degraded pastures. Results indicate that with cows that have high genetic potential (12 liters of milk) supplemented with *C. argentea* farmers can replace at least 35% of expensive concentrate during the dry season. Levels of 70% *Cratylia* and 30% concentrate in the supplement result in 14% less milk than that recorded with cows supplemented with 100% concentrate, but the economics favor the option of using more legume. Other results show that with *Arachis pintoi*-based pastures milk yields are 9 to 11% higher than in the grass alone pasture, regardless of level of supplementation of concentrate.

Through Tropileche, the Forage Project is also testing new grass options with farmers in Costa Rica and as a result a new *Brachiaria brizantha* (CIAT 26110) was selected for high yield and drought tolerance. Seed of this accession has been multiplied for further on-farm testing in Central America and Colombia. Work is also in progress in Atenas, Costa Rica in collaboration with OFI, UK to select new *Leucaena* species with resistance to pest (psyllid) and with drought tolerance.

NESTLE Project (G. A. Ruiz, M. Jervis, J. Rozo , J. Velasquez and C. E. Lascano)

The Forage Project continued to collaborate with the Nestlé Project in forest margins of Coquet, Colombia. New accessions of *Arachis pintoi* (CIAT 18744, 18748 and 22160) have now been planted in poor acid soils found in the topography kwon as "mesones" and where the commercial cultivar (CIAT 17434) does not persist. Early results indicate that the new *Arachis* accessions will not establish in these poor soils without the application of P. Consequently, work is in progress to determine how selected *Arachis pintoi* genotypes respond to different levels of P from commercial sources of fertilizer.

Other forage options being tested in the Caquetá region with help of the Nestlé Project, include the shrub legume *Codaryocalix gyroides* selected on the basis of good adaptation to acid- low fertility soils and high rainfall. This legume has been tested as a cut and carry supplement to milking cows in dual- purpose cattle farm, but results have no been convincing. Cows receiving *C. gyroides* in combination with sugar cane produce the same amount of milk as cows only supplemented with sugarcane. Thus, we consider now that the potential adoption of *C. gyroides* in the region may be very low. In contrast, results with *Stylosanthes guianensis* fed to pre-weaned calves indicate that milk for sale increases while maintaining adequate growth of calves. This legume option for dual-purpose cattle systems may be very attractive to farmers and consequently we foresee that it could be widely adopted if more on- farm demonstrations are done and seed becomes available.

CORPOICA-Plan de Modernización de la Ganaderia –Llanos of Colombia (C. Plazas, R. Perez, A. Rincon and C. E. Lascano)

During 1998 we initiated collaborative work with CORPOICA in the Llanos of Colombia to support to the Plan de Modernizacion de la Ganaderia of Colombia funded by FEDEGAN.

The main objective of the work is to evaluate new ecotypes of *Arachis pintoi* (CIAT 18744, 18748 and 22160 as compared with the commercial cultivar 17434) in livestock farms representing well-drained savannas and piedmont. In addition, we want to test two contrasting planting rates (3 and 6 kg/ha) of *Arachis* in association with *Brachiaria*, given that seed cost is one important limitation for adoption of *Arachis* in the Colombian Llanos.

A total of four farms (two in the well-drained savannas and two in the piedmont) were selected to evaluate the new ecotypes of *Arachis* and the two sowing rates. Selected farms are 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* pastures were used to establish the following replicated treatments in a factorial arrangement:

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 (at 45 days intervals) and legume and grass yield (in the rainy and dry seasons). Legume-based pastures will managed by farmers in a rotational grazing scheme that will include a pure grass pasture. The animal response variable will be liveweight gain (three farms) and milk production (one farm) in *Arachis*-based pastures vs. grass alone pastures.

CONDESAN and CIAT's PE-3 –Hillsides of Colombia (R. D. Estrada 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 for resource-poor farmers, while sustaining the natural resource base.

To accomplish the objectives the following activities have been or are being carried out:

a) Survey among 200 families to measure quality of live and to determine the possibilities of putting in place an improved milk production system to generate additional income
b) Putting in place a mechanism to generate the necessary cash flow to plant *Arachis pintoi* and to purchase tow milking cows by farmers
a) Establish contacts with local investors to finance the purchase of animals, which will be handed over to farmers once the new legume-based pastures are established.

b) Establish contacts with NESTLE to assure that *Arachis* seed produced by farmers will be purchased for pasture reclamation activities in forest margins of Caquetá, as a follow up to the ongoing Nestle Project.

Through a simulation model we evaluated the ex-ante profitability of the intervention with *Arachis* and concluded that it was a viable option. Consequently, it was decided to create a rotary fund to finance the purchase of Arachis seed and fertilizer by participating farmers. Each farmer will initially establish 2000 m² 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.

Half of the harvested seed (estimate of 1000 kg/ha) will be sold to NESTLE ($ US 13.00/Kg) and the remaining seed will be used by the farmer to plant in pastures. In addition, farmers will have access to vegetative material of *Arachis* for planting in the pastures and to use as a cover crop in 3000 ha of timber trees planted in the area.

We envisage that if everything goes as expected, there will be 2000 ha of *Arachis* based pastures and 1000 milking cows in the hands of 200 families by the end of 5 years. A total of 30 plots (6ha) of *Arachis pintoi* have been established and 25 more plots are in the process of establishment.

Seed multiplication of selected forage species for on-farm testing

Seed Unit of CIAT in Palmira (J. Miles and A. Ortega)

Highlight

- Six hectares of *Brachiaria* seed multiplication plots established at CIAT-Popayán and a neighboring private farm.

Seed multiplication

In order for grazing trials to be planted opportunely as soon as the first phase, agronomic trials are completed, seed needed to be multiplied during 1998. Based mainly on preliminary recommendations coming out of the March meeting of the Brachiaria network, 12 accessions and 2 hybrids were chosen for multiplication with financial support of AGROGANADERA del VALLE:

1. CIAT 26556G
2. CIAT 26159
3. CIAT 16322
4. CIAT 26124
5. CIAT 26318
6. CIAT 16467
7. CIAT 26427
8. CIAT 26110
9. CIAT 16121
10. CIAT 16113
11. CIAT 16316
12. CIAT 6387
13. FM9201/1873 (hybrid)
14. BR93NO/1737 (hybrid)

Plots of 3,000 to 5,000 m² per accession were established by direct seeding (where sufficient seed was available) or with vegetative material. Seed multiplication plots were successfully established by late April. First seed harvests are underway at the preparation of this report

(late September). Production goals are to have 50 kg. of classified seed of each of the 13 accessions by planting time (May/June) of the 1999 season.

A short list of promising accessions of *D. ovalifolium* (CIAT 33058; CIAT 23762; CIAT 13110; CIAT 13086; CIAT 13105; CIAT 13651; CIAT 23665) was published at a meeting of collaborators held at CIAT during March 1998. The major unknown of these new accessions is in regards their nutritional quality, as reflected in the performance of grazing animals (liveweight gain or milk production) compared with standard accessions such as CIAT 350 or CIAT 13089. Thus seed multiplication of these accessions is in progress thank to the financial support of AGROGANADERA del VALLE.

Seed distribution

Between 01 January 1998 and 24 September, the Seed Multiplication Unit in Palmira responded to 63 requests for seed. A total of more than 700 kg. of seed, representing 302 different accessions was dispatched to 6 institutions or individuals in Colombia and to 11 foreign countries.

Seed Unit of CIAT in Atenas, Costa Rica: (P. Argel)

Highlight

- Delivered seed of elite grasses and legumes to CORPOICA for on-farm evaluation

Experimental and basic seed multiplication is a continuos activity of the Seed Unit in Atenas, in collaboration with the Escuela Centro Americana de Ganadería (ECAG). This site combines both soils of medium fertility and a well distributed rainfall with a defined dry period that facilitates seed maturation and harvesting. The seed produced is used to support regional forage programs, which are in the process of evaluating new alternatives or of release of new pasture cultivars.

Seed multiplication

During the period October 1997 - August 1998, a total of 392.9 kg of experimental and basic seed was produced. The bulk of the seed was formed by *C. argentea* CIAT 18516/18668 (120.3 kg), *A. pintoi* CIAT 18744 (163.1 kg), *B. brizantha* CIAT 26110 (87.0 kg), and small amounts of *P. maximum, B. brizantha* CIAT 16322, *L. leucocephala, D. velutinum, S. guianensis,* and several lines of *A. pintoi.*

Seed distribution

A total of 319.9 kg of experimental and basic seed was delivered in response to 69 seed requests from 10 countries. CORPOICA from Colombia contracted the production of basic seed and received during 1998 100 kg of *A. pintoi* CIAT 18744, 60 kg of *B. brizantha* CIAT 26110 and 90 kg of *C. argentea* CIAT 18516/18668. The seed is to be used for seed multiplication, and on-farm evaluation under grazing in different regions of Colombia.

An important activity in Costa Rica has been to ensure that basic seed of *A. pintoi* CIAT 18744 is available this year in order to facilitate the release of this ecotype by MAG as cv. Porvenir.

Progress towards achieving output milestone

- **List of grass and legume cultivars released by NARS in the region (2000)**

Ongoing collaboration on evaluation of grasses and legumes with different partners in the region is an effective way to catalyze releases of key forage species. Through the *Brachiaria* Network in Colombia we have been able to identify ecotypes with wide adaptation to different environments and with agronomic attributes superior to commercial cultivars. Availability in 1999 of basic seed of the selected *Brachiaria* ecotypes will allow our Colombian partners to test them under grazing in livestock farms, which is a key step in the process leading to release of new forage cultivars.

We have made significant progress in the on-farm evaluation of new *Arachis pintoi* ecotypes in subhumid hillsides and forest margins. In Costa Rica, *A. pintoi* CIAT 18744 will be released this year a cv. Porvenir by MAG (Ministerio de Agricultura y Ganaderia). This release will be accompanied by a Technical Bulletin and by basic seed multiplied by CIAT in Costa Rica and by SEFO-SAM in Bolivia under a contract with CIAT. In forest margins of Colombia three new ecotypes of *A. pintoi* are under on-farm evaluation in low fertility soils and work is underway to determine P requirements for establishment.

Considerable advances have been made in demonstrating the benefits of *Cratylia argentea* as a dry season supplement for milking cows in dual-purpose cattle farms in Costa Rica. As result, some commercial seed producers are now multiplying seed to satisfy an increasing demand of this legume by farmers. We expect that this demand will also increase in Honduras and Nicaragua given that farmers participating in Tropileche are evaluating *C. argentea*. Results from on-farm work will be key in the decision by countries in Central America to release *Cratylia* in the near future.

Suboutput 4.2 Defined niches for selected grass and legume cultivars based on G x E interactions

An important objective of the Forage Project is to be able to define environmental niches within target ecosystems to grow selected grasses and legumes. To accomplish these objective multilocational trials with legumes (*Arachis pintoi* and *Desmodium ovalifolium*) were carried out in Colombia to define G x E interactions. In addition, there is an ongoing multilocational trial with *Brachiaria* genotypes in 13 locations in Colombia.

Last year we reported, on the basis of preliminary assessment of the results from multilocational trials with *Arachis*, that 10 promising accessions had been selected for seed multiplication and subsequent on-farm evaluation. The new accessions of *A. pintoi* have shown to be superior to the commercial cultivar (CIAT 17434) terms of faster establishment, and compatibility with aggressive grasses.

This year we completed the analysis of multilocational trials with *Desmodium* and summary of the main results is presented in this section. Results on the multilocational evaluation of *Brachiaria* are presented in another section of this report.

Analysis performance of *Desmodium ovalifolium* from multilocational trials (A. Schmidt, C. Lascano, Brigitte Maass, N. Narváez, R. Barahona, G. Ramirez and R. Schultze-Kraft)

Highlight

- New genotypes of *D. ovalifolium* with wide adaptation and superior quality will be available in 1999 for on-farm evaluation

Rationale: Current tropical forage research 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 environmental factors on quality and anti-quality components like tannins and possible interactions with genotypes. Yet the knowledge about genotype x environment interactions on quality components of tropical legumes is limited. An important example for a tropical legume, whose quality seems to be influenced by the environment is *Desmodium ovalifolium*.

The species shows high biomass production in acid soils and adapts to a wide range of conditions. However, forage quality is influenced by high condensed tannin (CT) content, which seems to be influenced by environmental factor as well as by genotypic variation. Genotypes with high nutritive values and low CT content will be of mayor interest for developing persistent grass/legume production systems in the humid tropics where at present only *A. pintoi* is promoted.

Results and Discussion: The *D. ovalifolium* genotypes CIAT 33058, 13105, 13651, and 23762 were selected and recommended for seed multiplication. The selection focused on high forage quality and rated biomass production as of minor importance since nearly for all genotypes biomass production was more than acceptable. The accession CIAT 350, which is the only commercial cultivar of the species at the moment, showed outstanding performance particularly in savannas where the genotype was selected years ago.

Grazing trials with the selected genotypes of *D. ovalifolium* will be initiated in collaboration with partners in Colombia to assess their pasture potential. These grazing trials will include other legumes like *A pintoi* and several *Brachiaria* species and will start at the beginning of the wet season 1999. Milk production and liveweight gain will be measured throughout the trials.

A resulting high quality *D. ovalifolium* cultivar would represent a significant technological breakthrough for grass/legume pasture establishment in the humid tropics where the only persistent herbaceous legume option at present is *A. pintoi*. The advantage of *D. ovalifolium* over *A. pintoi* is that is more adapted to conditions of low soil fertility and much easier and cheaper to establish.

Participatory evaluation and targeting multipurpose forage germplasm in the hillsides of Central America (M. Peters, P. Argel, and C. Burgos)

Highlight

- Formation of a multidisciplinary team for better targeting forages to hillsides of Central America

Rationale: Forage germplasm in its multiple uses - for example as feed, for the suppression of weeds, for maintenance and improvement of soil fertility, for erosion control etc. - 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 forage germplasm technologies with farmers, using a participatory approaches.

To address this issue, a multidiciplinary team was formed for strategic targeting of forage germplasm to environmental and socio-economic niches in hillsides of Central America using GIS tools. The work will also contribute to the development of an overall strategy to guide future research 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.

The work been undertaken is a true team effort involving several CIAT Projects. Inputs are presently coming from:

SN3: Ann Braun
PE4: Glen Hyman
PE2: Richard Thomas/Edmundo Barrios
IP5: Carlos Lascano
PE5 Peter Kerridge/Federico Holmann

The initial primary focus will be on hillsides in Yoro, Honduras, but an extension to sites in the Olancho and Atlantida regions of Honduras is planned.

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: Germplasm nurseries have now been established at four sites in Honduras and a strong collaboration within CIAT and with DICTA (Dirreccion de Ciencia y Technologiá Agropecuaria) Honduras has been formed.

Progress towards achieving output milestone:

- **List of grasses and legumes with well defined attributes suited for different agroecologies and production systems (1999)**

We are making progress in identifying new grass and legume accessions with superior plant attributes than commercial cultivars. We are also placing high priority on seed multiplication of the selected forage accessions for regional testing.

Through a new initiative we are now evaluating the role of forages species in hillsides of Central America using participatory approaches. This team effort should allow us to better target multipurpose forages based on farmer needs and preference, which in turn should result in faster adoption and impact.

Suboutput 4.3 Expert systems on forage biodiversity by linking geographic information with biological data

Highlight

- Formation of a working group with participants form different CIAT Projects to link forage databases with GIS

Linking information on genetic diversity with environmental adaptation to sites of origin (M. Peters, A. Franco, Luis Horacio Franco, Glen Hyman, Alexander Gladkov)

Rationale: For the conservation of bio-diversity and for planning of future germplasm acquisition and collection activities it is necessary to determine sites of high genetic diversity. In addition, it is of interest to define collection sites from which accessions of high agronomic interest have originated.

Methods: To carry out the work we will use GIS model developed by the Land Use Group of CIAT. With this model we can predict potential centers of biodiversity for specific forage species, based on passport data and description of collection sites.

Results: With the assistance of Project SB1 in delivering and preparing passport data, the original GIS model is in the process of being refined. The refined model will be available for use by CIAT scientists by early 1999 and will provide a powerful tool to match genetic diversity of forages with sites of origin.

The validity of the model could be determined through comparative studies on genetic biodiversity of selected forage species using molecular markers as indicators of genetic diversity. However, for these studies funds and human resources will need to be made available for the molecular studies. Initial contact have been made with possible collaborators in and outside CIAT to investigate possibilities to carry out this important work.

Use of GIS models for better targeting forage germplasm (M. Peters, Glen Hyman, Luis Horacio Franco, Luz Amira Clavijo, Arturo Franco, Bellisario Hincapie, Gerardo Ramirez, Alexander Gladkov)

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 enviromental conditions, the agro-ecological information is separated from socio-economic factors influencing forage germplasm adoption.

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 step-wise procedure for the development of the system.

1. Inclusion of the existing RIEPT (Red International de Evaluación de Pastos Tropicales) database – to start with the regional trials A+B – into the GIS –system to describe agro-ecological adaptation of forage germplasm in Latín America
2. Inclusion of supplementary information agro-ecological adaptation as existing in CIAT-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).

The GIS-program to be utilised is currently being identified, but discussions center around the use of *MAPINFO* or *PLANTGRO*.

The working group has inputs from the following CIAT Projects:

PE4: Glen Graham Hyman, Luz Amira Clavijo, Alexander Gladkov
PE5: Luis-Horacio Franco
IP5: Manuel Arturo Franco, Gerardo Ramirez, Bellisario Hincapié, Carlos Lascano and Michael Peters.

Results: In 1998 the workgroup agreed on the step-wise procedure as described above. Indicators of forage germplasm adaptation retrieved from RIEPT regional trials A and B have been identified and the data is currently organized for statistical analysis and for inclusion into the GIS system. During several consultations, it was agreed that the current system of ecosystem classification utilized in the RIEPT, is not suitable for the development of the GIS system and work has been initiated to for a different description of the ecosystems.

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:
1. Efficiency and client-orientation of future research will be enhanced, and
2. The dissemination of existing and future research results will be improved.

Converting forage database to a graphical platform (Martha Herrera, Arturo Franco, Carlos Lascano, Michael Peters)

Rationale: The Tropical Forage Program in CIAT has generated an information system for the 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 scientist.

For the actualization, inquiries and searches of forage data, an information system based on the fourth generation language ORACLE FORMS 3 was developed; this system is available via 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 magnetic means, it is important to convert this information system to a graphical, user-friendly and attractive platform.

Methods: At the present time the tropical forage database contains the following information related to the evaluation of germplasm:

- Collection and Exchange of germplasm (24047 accessions).
- Herbarium material (12455 accessions).
- Agronomic and morphological characterization of germplasm (7313 accessions in 140 projects).
- Evaluation of germplasm in small plots (1067 accessions in 26 projects).
- Agronomic evaluation of pastures (433 associations in 35 projects).
- Evaluation of pastures under grazing shepherding (multidisciplinary project CORE)
- Production and distribution of seeds (4192 seedlots)
- Insect and disease problems (4403 [accessions evaluated in 104 projects).
- Bank of funguses and bacterias (6611 funguses and 161 bacterias).
- Bank of Rhyzobium (4256 strains).
- Evaluation of lines of Rhyzobium (response of 431 strains in 41 projects).
- Description of projects (819 project proposals)
- International Network for the evaluation of tropical pastures RIEPT (Type A trials: 852 accessions in 67 sites, Type B trials: 685 accessions in 248 sites)
- International Network of pasture evaluation in Africa RABAOC (10 sites).

Using of existent information, the programs developed in ORACLE FORMS 3 are being converted to ORACLE DEVELOPER 2000, which is a fourth generation tool that allows:

a) to present the data in an attractive graphic platform and
b) the possibility to share the information via Intranet or Internet, with the aid of the ORACLE WEB Server.

Expected Results:

a) An Information System available in the Intranet of CIAT
b) A CD-ROM with the forage database for use by NARS partners and
c) Integration and presentation of the outputs of forage germplasm evaluation of in the Home Page of CIAT.

Progress towards achieving output milestone:

- **Integrated forage data bases in a friendly user format (1999)**

A working group has been formed to assist in the integration of forage databases as a first step to link forage data with GIS.

The refined GIS model to match genetic diversity and sites of origin will be available for use by scientists by early 1999. The validity of the model could be refined with comparative studies of genetic biodiversity of selected species using molecular markers with predictions of genetic diversity using the GIS tool. However, these studies will require additional funds and human resources for the molecular studies. Initial contacts have been made with possible collaborators in and outside CIAT to investigate possibilities to carry out this important work .

Suboutput 4.4 Effective communication of research results through newsletters, journals and workshops

Highlight

- Continue to publish a Forage Newsletter and a Journal

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.

During 1998 we produced two newsletters: a) results on the multilocational evaluation of *Desmodium ovalifolium* in Colombia (A. Schmidt and R. Barahona) and b) literature review on agronomy and utilization of *Cratylia argentea* (P. Argel and C. Lascano). A third newsletter in 1998 will deal with endophytes in tropical grasses (S. Kelemu)

Pasturas Tropicales (C. Lascano and A. Ramirez)

During 1997 we continued publishing the Journal Pasturas Tropicales thanks to a one-time donation of COLCIENCIAS. Three volumes (18, No. 3, 19, No. 1 and 19, No. 2) were published. The themes covered in these volumes were: forage agronomy, nutritive value, pathology, animal production, pasture reclamation, and seed production. Contributions to the three volumes came Brazil, Colombia, Mexico, and Argentina.

During 1998, we published volume 20 , (1 and 2), which included eight papers submitted by forage researchers in the region (Table 50). It is interesting to note that the topics covered have ranged from germplasm evaluation to modeling and that contributors represent several countries in the region, with a high proportion coming from Brazil.

Table 50. Papers published in Pasturas Tropicales during 1998.

Theme	Number of papers		Institution	Countries
	Vol 20(1)	Vol 20(2)		
Ruminant Nutrition	--	2	FONAIAP	Venezuela
Forage germplasm evaluation	2	1	CIAT, CIAT-CR	Colombia/C.Rica
Methodology for forage evaluation		1	U. Vicosa	Brazil
Seed production	3	1	EMBRAPA/CPAC U. Corrientes	Brazil/Argentina
Fertilization	1	3	EMBRAPA/CNGL/CPAF	Brasil
Modeling	--	1	CIAT-U. Caldas	Colombia
Grass-legume associations	1	--	EMBRAPA-Cerrados	Brazil
Animal performance in improved pastures	1	-	IAPAR	Brazil
Total	8	8		

The demand for publication in Pasturas Tropicales is growing at a fast rate given that it is the only Journal in LAC dealing with forages that has a wide international distribution. This increased demand is illustrated by the large number of papers received and waiting for technical and editorial review. We have 16 papers under revision and in volume 20 (number 3), which we hope to publish in the next number.

Given the budgetary constraints we phase in the Forage Project we have the challenge of continuing the publications of Pasturas Tropicales in 1999 and subsequent years. To meet this challenge we are still debating the option of institutional advertisements.

Progress towards achieving output milestone

- **Number of institutions and individuals receiving/contributing to forage related publications (2000)**

Trough Workshops, Forages Newsletter and Pasturas Tropicales we are reaching a wide audience of researchers in LAC. Both publication 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 we face is to continue publishing high quality original research papers and most important to secure alternative funds to maintain or expand the number of subscribers, which is not an easy task.

PUBLICATIONS

Journal Papers

Argel, P. J. and Lascano, C.E. 1998. *Cratylia argentea* (Desvaux) O. Kuntze: Una nueva leguminosa arbustiva para suelos ácidos en zonas subhúmedas tropicales. Pasturas Tropicales (20(1): 37-42.

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Miles, J. W., B. L. Maass, and C. B. do Valle (eds.) 1998. *Brachiaria*: Biología, agronomía y mejoramiento (CIAT: Cali, Colombia y CNPGC/EMBRAPA: Campo Grande, MS, Brasil) 312 p.

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List of Donors

Donor/Project	Project duration
Australia – ACIAR	
Anthracnose in *Stylosanthes*	1998-2000
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Mechanisms of acid soil tolerance	1994-1997
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