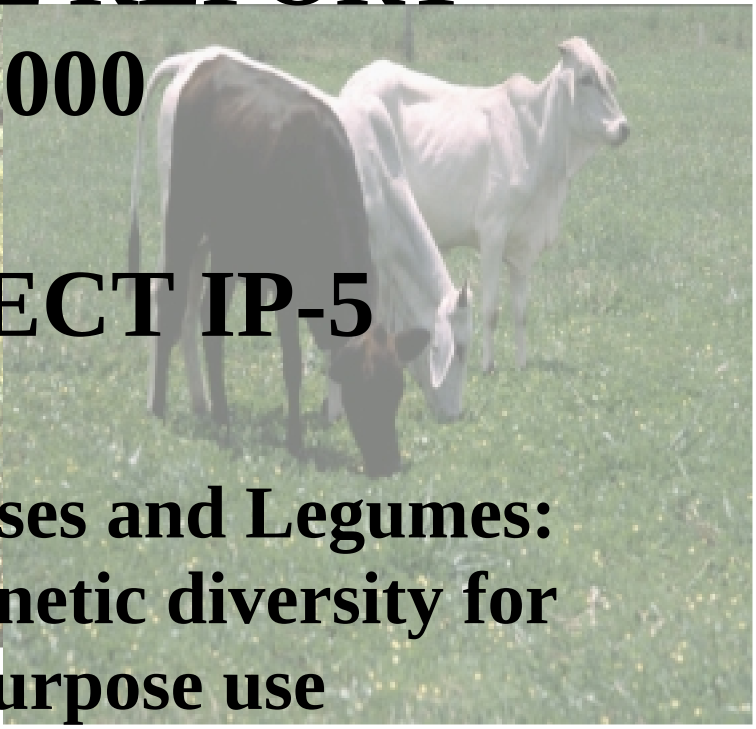

ANNUAL REPORT
2000

PROJECT IP-5

Tropical Grasses and Legumes: Optimizing genetic diversity for multipurpose use

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

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Tropical Grasses and Legumes: Optimizing genetic diversity for multipurpose use
(Project IP5)

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

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Publications

PROJECT OVERVIEW

IP5: 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 subhumid 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:

2001 New multipurpose legumes are available to NARS for use in crop/livestock systems.
Demonstrated under field condition benefits of endophytes in drought tolerance of *Brachiaria.*

2002 Defined potential of IPM components for managing spittlebug in lowland pastures.
Known animal production potential of *Brachiaria* hybrids with combined resistance to spittlebug, tolerance to Al, forage and seed quality.

2003 Methods and tools available to enhance targeting and adoption of multipurpose forage germplasm in small holder productions systems in the hillsides of Central America.
Brachiaria hybrids with combined resistance to spittlebug, tolerance to Al, forage and seed quality available to NARS.

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

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

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

CIAT Project linkages: Genetic resources conserved by SB-1 will be used to develop superior gene pools, using when necessary molecular techniques (SB2). Selected grasses and legumes evaluated in production systems (PE5) in collaboration with national partners (SN2).

PROJECT WORK BREAKDOWN STRUCTURE

Project Purpose

NARS use superior grasses and legumes to develop improved and sustainable livestock/crop production systems in humid and subhumid areas

OUTPUTS	**Grass and legume genotypes with high quality attributes are developed**	**Grass and legume genotypes with known reaction to pests and diseases and to interaction with symbiont organisms are developed**	**Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed**	**Superior and diverse grasses and legumes delivered to NARS partners are evaluated and released to farmers**
ACTIVITIES	• Selection of *Brachiaria* genotypes for high digestibility and other quality attribues • Assesment of quality and animal production potential of selected legumes • Assessment of quality and animal production potential of selected grasses	• Study the bioecology of spittlebug species in contrasting environments • Diagnosis of spittlebug for elaborating IPM components • *Brachiaria* genotypes resistant to spittlebug and other biotic stresses • Host mechanisms for spittlebug resistance in *Brachiaria* • Genetic control and molecular markers for spittlebug and reproductive mode in *Brachiaria* • Role of endophytes in tropical forage grasses • Interactions between host and pathogen in *Brachiaria*, and *Stylosanthes*	• Genotypes of *Brachiaria*, *Panicum*, and *Arachis* with adaptation to edaphic and climatic factors • Genotypes of grasses and legumes with dry season tolerance • Accessions of shrub legumes with adaptation to different environments • Accessions of *Brachiaria* and *Paspalum* with adaptation to poorly drained soils • Accessions of grasses for multipurpose use in different agroecosystems • Genotypes of *Arachis*, with broad edaphic and climatic adaptation	• Develop partnerships with NARS, NGO's, IARC's, ARIS and private sector in LAC, Asia and Africa to undertake evaluation and diffusion of a range of grasses and legumes for multipurpose use • Evaluation with farmer participation of multipurpose forages for crop and livestock systems • Forage seeds: reproductive biology, quality, multiplication, and delivery of experimental and basic seed • Expert systems for legume biodiversity by linking geographic information with biological data • Facilitate communication through Newsletters, Journals, Workshops and Internet

Revised Project Log-Frame
(2000)

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

Narrative Summary	**Measurable Indicators**	**Means of Verification**	**Important Assumptions**
Goal To contribute to the improved welfare of small farmers and urban poor by increasing milk and beef production while conserving and enhancing the natural resource base	New cultivars of grasses and legumes used by farmers and raise productivity of livestock/crops while protecting biodiversity and land in savannas, forest margins and hillsides	Statistics on income and natural resource conservation in smallholder livestock farms in LAC and SE Asia	Policies are put in place by governments to favor sustainable livestock/forage development in marginal areas occupied by small farmers
Purpose NARS use superior grasses and legumes to develop improved and sustainable livestock/ crop production systems in humid and sub-humid areas.	Demonstrated economical and ecological benefits of multipurpose grasses and legumes to livestock/ crop farmers in savannas, forest margins, and hillsides agroecosystems	• Range of variation in desirable traits • Performance of forage components in systems	• Support from traditional and non-traditional donors • Effective collaboration: • CIAT's Projects • AROs, NARS, NGOs
Outputs 1. Grass and legume genotypes with high quality attributes are developed.	• New Brachiarias accessions characterized for animal performance are available for release by NARS partners by 2000. • Known animal production potential of selected *Brachiaria* hybrids by 2000. • New shrub legumes for multipurpose use with known quality attributes are available to NARS partners by 2001.	• On-farm demonstrations • Scientific publications • Annual Reports • Theses	• Effective collaboration with: • CIAT Projects (PE2) • AROs, NARS, and Farmer Groups
2. Grass and legume genotypes with known reaction to pests and diseases and to interaction with symbiont organisms are developed.	• Demonstrated under field condition benefits of endophytes in *Brachiaria* on drought tolerance by 2001. • Defined potential of IPM components for managing spittlebug in lowland pastures by 2002. • *Brachiaria* hybrids with resistance to spittlebug, tolerance to Al and good seed quality available to NARS by 2002.	• On-farm demonstrations • Scientific publications • Annual Reports • Theses	• Effective collaboration with: • CIAT Projects (SB1, SB2) • AROs, NARS and Farmer Groups
3. Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed.	• New *Brachiaria*, *Paspalum*, *Desmodium*, *Arachis*, Leucaenas and *Calliandra* with known edaphic and climatic adaptation selected by farmers are scaled up in different production systems in Central America by 2001. • New multipurpose legumes are available to NARS for green manures in crop/livestock systems by 2001.	• On-farm demonstrations • Scientific publications • Annual Reports • Theses	• Effective collaboration with: • CIAT Projects (SB1, PE2, PE4, PE5) • AROs, NARS, NGOs, Farmer Groups
4. Superior and diverse grasses and legumes delivered to NARS partners are evaluated and released to farmers.	• Selected accessions of *Brachiaria* and *Cratylia* are released by NARS partners by 2001. • Demonstrated that improved grasses and legumes contribute to intensification of dual- purpose cattle system and to income of farmers in hillsides and forest margins by 2002.	Surveys on adoption impact of new grasses and legumes: • Seed sold • Area planted • Production parameters • Environmental/socio-economic indicators	• Effective collaboration with: • CIAT Projects (PE2, PE5, SN2, SN3, BP1 and Ecoregional Program) • NARS, NGOs and Farmer Groups

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Activities 2000	**Milestones (2000-2001)**		
1.1 Selection of *Brachiaria* genotypes for high digestibility and other quality attributes (CEL, JWM) 1.1.1 Calibration of NIRS for IVDMD 1.1.2 Effect of oven and freeze-drying on IVDMD of Brachiaria 1.1.3 Screening of selected *Brachiaria* accessions and hybrids for saponins 1.1.4 Analysis of digestibility data on a *Brachiaria* mapping population and selection of clones for QTL detection	• Efficient protocol using NIRS for assessment of forage digestibility in a Brachiaria hybrid population incorporated into the breeding program • Population of Brachiaria hybrids screened for in vitro digestibility	• List of *Brachiaria* hybrids with high digestibility • Annual Report	• Effective collaboration with the plant breeder • Genetic variability exists for digestibility in Brachiaria hybrid populations • Special funds from ACIAR are available for comparative mapping with pearl millet
1.2 Assessment of quality and animal production potential of selected legumes (CEL) 1.2.1 Characterization of a range of shrub legumes for quality attributes 1.2.2 Feed value of selected *Calliandra* accessions 1.2.3 Milk production of cows supplemented with selected shrub legume species	• Known utility of Calliandra as a protein supplement for ruminants • Known utility of Cratylia under direct grazing by milking cows	• Established grazing trials in CIAT's Quilichao research station • Annual Report	• Availability of milking cows in CIAT-Quilichao from neighboring farmer
1.3 Assessment of quality and animal production potential of selected grasses (CEL, JWM) 1.3.1 Milk yield with new accessions and hybrids of Brachiaria.	• Defined benefits in animal production of new accessions and hybrids of Brachiaria relative to commercial cultivars.	• Established grazing trials in CIAT's Quilichao research station • Annual Report	• Continued availability of cows in CIAT-Quilichao from neighboring farmer
2.1 Study the bioecology of spittlebug species in contrasting environments (DCP) 2.1.1 Comparative biology of Zulia spittlebugs 2.1.2 Detection of the Central American forage and cane pest, Prosapia simulans in South America 2.1.3 Biology and habits of Prosapia simulans 2.1.4 Characterization of substrate communication in adult spittlebugs 2.1.5 Population dynamics and phenology of spittlebugs in the Cauca Valley 2.1.6 Documentation of first generation population phenology in two lowland sites 2.1.7 Preoviposition determinants of egg diapause 2.1.8 Seasonal changes in the incidence and duration of egg diapause	• Defined variation in the biology and abundance of spittlebug species in Colombia	• Papers on biology/ecology of spittlebug submitted for publication • Annual Report	• PRONATTA funds made available • Security problems in Colombia do not increase

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
2.2 Diagnosis of spittlebug for elaborating IPM components (DCP) 2.2.1 Identity and distribution of spittlebugs associated with graminoids of Colombia and Ecuador 2.2.2 Evaluation of an artificial diet for maintenance of spittlebug adults 2.2.3 Identity, incidence and maintenance of spittlebug fungal entomopatahogens 2.2.4 Screening fungal entomopathogens for virulence to spittlebug adults 2.2.5 Evaluation methodology for measuring virulence of fungal entomopathogens to spittlebug nymphs 2.2.6 Economic impact of spittlebugs to animal production in *Brachiaria decumbens* (FH, DCP, CEL)	• IPM components relevant to spittlebug management in forage grasses and other graminoids better understood	• Papers submitted for publication • Annual Report	• PRONATTA funds made available • Research assistant funded through CIAT's SRF • Laboratory technician retained • Sufficient fund to hire full time worker
2.3 *Brachiaria* genotypes resistant to spittlebug and other biotic stresses (JWM, CC) 2.3.1 Development of new hybrid population for spittlebug screening using pollen from a resistant parent (Ap) and selected clones (Sx) as maternals (JWM) 2.3.2 Identify *Brachiaria* genotypes resistant to spittlebug 2.3.3 Greenhouse screening of *Brachiaria* accessions and hybrids for resistance to spittlebug 2.3.4 Field screening of *Brachiaria* accessions and hybrids for resistance to spittlebug 2.3.5 Adaptation of protocol for screening *Brachiaria* hybrids for Rhizoctonia foliar blight (SK, JWM)	• Sexual *Brachiaria* clones (50 or fewer) identified, propagated and recombined and seed harvested to initiate new breeding cycle • Screened selected sexual *Brachiaria* clones for spittlebug reaction under field conditions • Implemented an efficient protocol for screening *Brachiaria* hybrids for Rhizoctonia foliar blight resistance	• List of *Brachiaria* hybrids with resistance to spittlebug and adaptation to Al under grazing evaluation • Annual Report	• Additional funds to support the *Brachiaria* improvement program are identified
2.4 Host mechanisms for spittlebug resistance in *Brachiaria* (CC) 2.4.1 Studies on resistance to spittlebug species	• Defined reaction of *Brachiaria* hybrids to different species of spittlebug	• Papers submitted for publication on screening methodology on: - Field screening methodology for spittlebug resistance in *Brachiaria* - Mechanism of resistance of *Brachiaria* to spittlebug • Annual Report	• Effective flow of *Brachiaria* genetic recombinants for screening for spittlebug resistance • New funds are identified for testing the field screening method for spittlebug resistance • Additional funds are available to support work on antibiotic effects of *Brachiaria* to spittlebug
2.5 Genetic control and molecular markers for spittlebug and reproductive mode in *Brachiaria* (JWM)	• List of new *Brachiaria* hybrids (Sx x Ap) characterized for reproductive mode by progeny tests	• Seed of new *Brachiaria* hybrids • Annual Report	• *Brachiaria* genotypes for marking are available • Special funds from ACIAR are

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
2.5.1 Reproductive mode of new Brachiaria hybrids (Sx x Ap) 2.5.2 Identify candidate QTL's for spittlebug resistance in a Brachiaria mapping population (JT, CC, JWM) 2.5.3 Molecular fingerprinting of *Brachiaria* genotypes 2.5.4 Enhancing detail of molecular map of *Brachiaria*	• Identified tentative QTL's for spittlebug resistance in *Brachiaria*		available for comparative mapping with pearl millet • Continued and effective collaboration with SB2
2.6 Role of endophytes in tropical forage grasses (SK) 2.6.1 Endophyte seed transmission and artificial implications of endophytes in *Brachiaria* 2.6.2 Alkaloid profiles in endophytes isolated in *Brachiaria* 2.6.3 Cloning and characterization of endophyte-specific DNA fragment for quick detection 2.6.4 The role of endophytes in drought tolerance of *Brachiaria* species (with IMR) 2.6.5 Effect of endophytes on plant-pathogenic fungi in vitro 2.6.6 Screening of *Brachiaria* hybrids for endophyte presence 2.6.7 Studies on bacterial endophytes	• Defined effect of endophytes in Rhizoctonia foliar blight in *Brachiaria*. • Confirmed effect of endophytes on drought tolerance in *Brachiaria*	• Papers submitted for publication on: endophytes and their interaction with pathogens • Annual Report	• Effective collaboration with a US institution for determining alkaloids • Effective collaboration with the Breeder, Entomologist and Plant Nutritionist of IP5
2.7 Interactions between host and pathogen in *Brachiaria*, and *Stylosanthes* (SK) 2.7.1 Biodiversity studies on the anthracnose pathogen in *Stylosanthes: Colletotrichum gloeosporioides* 2.7.2 Epidemiology studies on the anthracnose pathogen in *Stylosanthes* 2.7.3 Characterization of transgenic *Stylosanthes* plants containing a rice chitinase gene 2.7.4 Bacterial wilt disease of *Brachiaria*: casual agent, inoculation methods, bacterial population dynamics, and host resistance	• Determined anthracnose pathogen diversity of Stylosanthes using AFLP. • Determined wilt disease causing bacterial multiplication in resistant and susceptible *Brachiaria* genotypes	• Paper submitted for publications on: - Transgenic Stylo and reaction to anthracnose - Arachis and anthracnose interactions • Annual Report	• Funds from ACIAR channeled through CSIRO for anthracnose research are made available to CIAT
3.1 Genotypes of *Brachiaria, Panicum*, and *Arachis* with adaptation to edaphic and climatic factors (IMR, JWM) 3.1.1 Improved tetraploid, sexual *Brachiaria* hybrid breeding population for resistance to edaphic factors and general environmental adaptation 3.1.2 Studies on mechanisms of acid soil	• Selected *Brachiaria, Arachis* and *Panicum* genotypes with superior adaptation to low soil fertility available to partners for evaluation	• Paper submitted for publication on mechanisms of aluminum tolerance in *Brachiaria* • Review paper on adaptation of forages to low soil fertility conditions • Book chapter on role of physiology in improving crop adaptation to abiotic stresses	• Continued collaboration with University of Göttingen, Germany • Funds are available to support PhD student in Germany • Effective collaboration with •CIAT Projects (SB2, PE2) •ARO's

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
adaptation in *Brachiaria* cultivars and development of screening methods 3.1.3 Studies on genotypic variation in *Arachis pintoi* for tolerance to low phosphorus supply 3.1.4 Determine N and P requirements of *Panicum maximum* genotypes		• Annual report	
3.2 Genotypes of grasses and legumes with dry season tolerance (IMR and JWM-Matazul; PJA-Atenas) 3.2.1 Field studies to determine genotypic variation in dry season tolerance in *Brachiaria* 3.2.2 Field evaluation of accessions of *C. argentea* in a subhumid environment 3.2.3 Agronomic characterization of a core collection of *Cratylia argentea* in acid soils 3.2.4 The effect of cutting height and frequency on production and quality of *Cratylia argentea*	• Identified *Brachiaria* accessions and hybrids with superior tolerance to drought relative to commercial cultivars • New *Cratylia* accessions characterized for agronomic performance	• Field trials with selected accessions of *Brachiaria, Arachis, Cratylia* and *Calliandra* in the Llanos of Colombia, and Costa Rica • Annual Report	• Continued input from Forage Agronomist in Costa Rica
3.3 Accessions of shrub legumes with adaptation to drought and cool temperatures (MP) 3.3.1 Genotypic variation in dry season tolerance in a core collection of *Calliandra calothyrsus* (Quilichao) (MP) 3.3.2 Agronomic characterization of a core collection *of Flemingia macrophylla* (Quilichao) (MP) 3.3.3 Agronomic characterization of a core collection of *Rhynchosia* (Quilichao) (MP)	• Known genetic variation in agronomic attributes in collections of *Rhynchosia* • Advanced to participatory evaluation of selected accessions of *Calliandra*	• Paper submitted for publication on strategy for forage germplasm development • Seed of selected shrub legume accessions distributed for on-farm participatory evaluation • Annual Report	• Continued input from Forage Agronomist in Costa Rica • Effective collaboration with U. of Hohenheim, Germany, MAG, Costa Rica and CIAT Projects (PE2, PE5)
3.4 Accessions of *Brachiaria* and *Paspalum* with adaptation to poorly drained soils (PJA, MP) 3.4.1 Field evaluation of *Brachiaria* and *Paspalum* genotypes established in poorly and well drained sites in Costa Rica (San Carlos and Atenas)	• Grazing trial with Paspalum atratum established in areas with poorly drained soils in Costa Rica	• Agronomic plots and grazing trial with selected accessions of Paspalum in Costa Rica with NARS partners • Annual Report	• Continued input from Forage Agronomist in Costa Rica • Effective collaboration with NARS in Costa Rica
3.5 Accessions of grasses for multipurpose use in different agroecosystems (MP) 3.5.1 Evaluation of a core collection of *Vigna unguiculata* for multipurpose uses (Quilichao) (MP)	• Characterized a core collection of *Vigna unguiculata* for use as a multipurpose legume	• Field trials with selected accessions of legumes for cover crops and green manures with NARS partners in Colombia and Central America • Annual Report	• Continue to have input from Forage Agronomist in Costa Rica • Continued collaboration with oil palm and rubber growers in the Llanos of

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
3.5.2 Evaluation of a core collection of *Lablab purpureus* for multipurpose uses (Quilichao) (MP)			Colombia
3.6 Genotypes of Arachis with broad edaphic and climatic adaptation (MP, PJA) 3.6.1 Genotype x Environmental interaction of a core collection of Arachis 3.6.2 Plant growth characteristics during the establishment phase of one cultivar and three accessions of Arachis pintoi in a subhumid environment of Costa Rica (PJA)	• Identified accessions of *Arachis* with broader adaptation than the commercial cultivar	• Paper submitted for publication on performance of *Arachis* in different environments	• Completion of PhD Thesis by A. Schmidt
4.1 Develop partnerships with NARS, NGO's IARC's, ARIS and private sector in LAC, Asia and Africa to undertake evaluation and diffusion of a range of grasses and legumes for multipurpose use 4.1.1 On-going collaboration with NARS, NGO's, IARC's and ARIS (IP5 Staff) 4.1.2 Development of new collaborative Research Proposals with NARS, NGO's, IARC's and ARIS (IP5 Staff) 4.1.3 Releases of grasses and legumes by NARS partners and Private Seed Companies (CEL, PJA)	• Developed new partnerships for forage evaluation in LAC and Africa • Selected *Brachiaria* genotypes released by NARS partners • Legume species available for cover crops in plantations (oil palm and rubber) in the Llanos	• Technical bulletins on grasses and legumes released by NARS • Concept Notes and research proposals on collaborative R&D with partners • Annual Report	• Effective collaboration with NARS that form part of TROPILECHE • NARS partners in Africa interested in forage evaluation • Funds from the Colombian Government available for work in Carimagua

4.2 Evaluation with farmer participation of multipurpose forages for crop and livestock systems
4.2.1 On-farm evaluation of forages for multipurpose use with farmer participation in Central America (MP, PJA)
4.2.2 On-farm evaluation of selected forages as feed resources in dual cattle systems of Central America through the Tropileche Consortium (FH, PJA)
4.2.3 Study on adoption of *Arachis* in Costa Rica (MP, PJA)
4.2.4 Diffusion of *Cratylia* in Costa Rica (PJA)
	• Trained personnel from different institutions and organized farmer groups for participatory evaluation of forages in Central America
	• Known adoption and constraints for diffusion of *Arachis* in Costa Rica
		• List of forage species selected by partners
		• Report on adoption of *Arachis* in Costa Rica
		• Annual report
			• Effective collaboration with:
			  • CIAT Projects PE2, PE3, PE4, PE5
			  • NARS, NGO and Farmer Groups
			• BMZ funds for participatory evaluation of forages become available

4.3 Forage seeds: reproductive biology, quality, multiplication and delivery of experimental and basic seed 4.3.1 Multiplication of selected grasses and	• Increased seed supply to farmers of new grass and legume options	• Papers submitted for publication • Annual Report	• New *Brachiaria* hybrids set seed

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
legumes in the Seed Units of Palmira and Atenas, Costa Rica (JWM, PJA) 4.3.2 Delivery of seed of selected forages to partners (JWM, PJA) 4.3.3 Reproductive biology of *Arachis* (JWM)			
4.4 Expert systems for legume biodiversity by linking geographical information with biological data 4.4.1 Development of a forage database with geographical interphase (MP, MAF) 4.4.2 Development of a DSS to target forages based on environmental adaptation (MP, GIS Staff)	• β version of CIAT's Forage database in Intranet and CD-ROM	• CD-ROM with forage data base • Annual Report	• CIAT establishes a policy on IPR on databases
4.5 Facilitate communication through Newsletters, Journals, Workshops and Internet 4.5.1 Development of a Forage Web Page (MP, BH) 4.5.2 Updating FAO's Grassland index (MP, RSK-U. of Hohenheim) 4.5.3 Methodologies for the evaluation and diagnosis of spittlebugs in graminoid crops (Proposal) (DCP) 4.5.4 Manual for the study of the biology and ecology of grassland spittlebugs (Proposal) (DCP) 4.5.5 Workshops (CEL, PJA, DCP)	• Developed a Web Page of CIAT's Forage Project • Defined with partners strategies needed for R&D of *Calliandra* in Central America	• Number of recipients of the Forage Newsletters • Number of published articles in Pasturas Tropicales • Annual Report	• Effective contributions of IP-5 members to the Newsletter • IP5 members and NARS partners continue to submit papers for publication in Pasturas Tropicales

Project Highlights

Output 1

- Milk production was higher with a recently released *Brachiaria* hybrid (CIAT 36061) than with commercial cultivars.
- Direct grazing system of *Cratylia argentea* resulted in more milk yield than a cut and carry system, which has implications in nutrient cycling and labor cost in livestock farms.
- The feed value of *Calliandra calothyrsus* was increased when the forage was sun dried and when plants were grown in fertile soils.

Output 2

- Continued to make significant genetic gains (28%) in breeding for spittlebug resistance in the *Brachiaria* improvement program.
- Identified molecular markers (microsatellites) associated with apomixis in *Brachiaria*, which can have a major impact on time and cost savings in the breeding program.
- Endophytes confer drought tolerance in *Brachiaria* by promoting production of fine roots, which in turn contribute to greater acquisition of minerals (K).
- Isolates of *Metarhizium* were found to be effective in controlling adults of spittlebug (*A. varia*).

Output 3

- Selected for the first time *Brachiaria* hybrids with moderate levels of aluminum resistance, high biomass yield under low nutrient supply and with tolerance to moderate drought.
- Two *Panicum maximum* accessions with superior root growth were identified for low fertility acid soils.
- Superior accessions of multipurpose legumes (*Calliandra calothyrsus, Rhynchosia schomburgkii*, and *Vigna unguiculata*) were selected for different environments and production systems.

Output 4

- Through on-farm collaborative work demonstrated the value of *Centrosema macrocarpum* as a protein supplement for milking cows and of *Arachis pintoi* to reclaim degraded pastures in hillsides of Central America and forest margins or Peru.
- Innovative farmers in Honduras selected grasses to test in their farms and more than 200 farmers are participating in the selection of improved forage options.
- A *Brachiaria brizantha* accession (CIAT 26110) and the first *Brachiaria* hybrid (CIAT 36061) were released as cultivars Toledo and Mulato, respectively, in Costa Rica and Mexico.

Summary of Research Progresss in 2000

Inputs

a) Principal Investigators

Lascano Carlos E, Project Manager and Animal Nutritionist: 70% in IP5 and 30% in PE5; Headquarters
Argel Pedro, Forage Agronomist: 50% in IP5 and 50% in PE5; San Jose, Costa Rica
Cardona Cesar, Entomologist (Host Plant –Resistance): 50% in IP5 and 50% in IP1; Headquarters
Kelemu Segenet, Pathologist: 100% in IP5; Headquarters
Miles John, Plant Breeder: 100% in IP5; Headquarters
Peck Daniel, Entomologist (Insect Ecology): 50% in IP5 and 50% in PE1; Headquarters
Peters Michael, Forage Biologist: 100% in IP5; Headquarters
Idupulapati M. Rao, Plant Nutritionist: 30% in IP5, 30% in IP1 and 40% in PE2; Headquarters
Schmidt Axel, Forage Agronomist (Systems): 1/3 IP5, 1/3 PE2, 1/3 PE3, Managua, Nicaragua

b) Main collaborators in CIAT

Debouck Daniel, SB1
Tohme Joe, SB2
Belloti Anthony, PE1
Thomas Richard, PE2
Barrios Edmundo, PE2
Holmann Federico, PE5
Kerridge Peter, PE5 (Laos)
White Douglas, PE5 (Pucallpa)
Sanz José I., PE3
Hyman Glenn, PE4
Thomas Oberthur, PE4
Jones Peter, PE4
Posada Rafael, SN2
Quiroz Carlos, SN3
Pachico Douglas, BP1
Rivas Libardo, BP1

c) Main collaborators outside CIAT

Forage Quality: Stewart Janet, OFI, UK; Theodorou Mike, IGER, UK; Jiménez Carlos, U. de Costa Rica, Costa Rica; Romero Frank, ECAG, Costa Rica

Genetic Improvement of *Brachiaria*: do Valle Cacilda B., EMBRAPA**,** Brazil

Pests (spittlebug) : Valerio Raúl, EMBRAPA, Brazil; Pérez Antonio, Universidad de Sucre, Sincelejo, Sucre, Colombia

Diseases (anthracnose): Chakraborty Sukumar, CSIRO, Australia; Changshun Jiang, CATAS, The People's Republic of China; Skinner Dan Z., USDA/Kansas State University, USA; Charchar Maria J., EMBRAPA, Brazil

Endophytes: White James F., Rutgers, USA ; Porter James, USDA/University of Georgia, USA

Adaptation to abiotic stress factors: Claassen N., University of Göttingen, Germany; R. Albert and Heberle-Bors E., University of Vienna, Vienna, Austria; Mayer J. E., CAMBIA, Canberra, Australia; Oberson A. and Frossard E., ETH, Zurich, Switzerland; Osaki M., and Hokkaido T. Tadano, University, Sapporo, Japan; Escobar C.J., CORPOICA, Macagual, Colombia

On-station and on-farm evaluation of forages: Restrepo Jose, FIDAR**,** Colombia; Velásquez Jaime, U de la Amazonia, Colombia; Rincón Alvaro, CORPOICA, Colombia; Hidalgo Carlos, MAG, Costa Rica; Lobo Marco, MAG, Costa Rica; Guillen Ricardo, MAG, Costa Rica; Burgos Conrado, DICTA, Honduras; Lara Daisy, FUNDAAM, Peru; Ibrahim Muhammad, CATIE, Costa Rica; Posas Marlen Iveth, SERTEDESO, Honduras; Mena Martin, INTA, Nicaragua; Latino Javier, PRODESSA, Nicaragua; Schultze-Kraft Rainer, University of Hohenheim, Germany; Hoffmann Volker, University of Hohenheim, Germany; Reiche Carlos, IICA-GTZ, Costa Rica

Outputs and verifiable indicators

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

Efficient protocol using NIRS for assessment of forage digestibility in a *Brachiaria* hybrid population incorporated into the breeding program and hybrid populations screened for in vitro digestibility. We have now confirmed that the NIRS calibration curve for IVDMD developed in CIAT's Forage Quality Laboratory is adequate for estimating in vitro digestibility of hybrids produced in the *Brachiaria* breeding program. However, during the last three years we screened a population of *Brachiaria* hybrids for in vitro digestibility and found that the correlation between samplings was very low, probably as a result of sampling and sample processing problems. Consequently, we still need to define a reliable sampling and processing method before we can routinely screen for digestibility in the breeding program using NIRS.

Known utility of *Calliandra* as a protein supplement for ruminants and of *Cratylia* under different forms of utilization. We continued with the characterization of quality attributes of selected shrub legumes and found that:

1. Intake of *Calliandra* was improved when the forage was fed dried as opposed to fresh, which is contrary to thinking of some extension people. However, the positive effect of feeding sun dried *Calliandra* on intake of the legume did not translate in higher DM digestibility or N absorption in sheep fed a low quality grass. In fact apparent N absorption was greater when fresh *Calliandra* was fed as a result of increased total N and bacterial flow to the duodenum, which suggest less protein degradation by rumen microbes. The feeding value of *Calliandra* was also significantly improved when the legume was grown in a fertile soil as opposed to an acid soil as reflected by higher legume intake and N flows to the duodenum and apparent N absorption.

2. Sowing *Cratylia* in pastures for direct grazing by cows resulted in more milk production as compared to the cut and carry system even during periods when rainfall is not limiting. Sowing *Cratylia* in mixture with the grass as compared to the cut and carry system being currently promoted in farms will undoubtedly provide additional benefits to farmers due to reduced labor and improved N cycling in the pastures.

Defined benefits in animal production of new accessions and hybrids of *Brachiaria* relative to commercial cultivars. The results from two grazing trials carried out this year confirmed that milk yield with *B. brizantha* of CIAT 26110, which had been selected by several NARS partners for potential release, was lower than that of other commercial *Brachiaria* cultivars due mainly to lower protein content in the forage on offer and forage consumed by grazing animals. However, one significant advantage of this accession over other *Brachiaria* cultivars is its higher biomass production, which translates into higher stocking rate capacity and thus production per hectare.

A very significant finding was the higher milk production potential of the *Brachiaria* hybrid (CIAT 36061) as compared to well known commercial *Brachiaria* cultivars, which appears to be associated with higher forage quality. This superior performance of the first *Brachiaria* hybrid that reaches cultivar status is a major accomplishment of CIAT's *Brachiaria* breeding program.

Output 2: Grass and legume genotypes with known reaction to pest and diseases, and to interactions with symbiont organisms are developed

Developed *Brachiaria* hybrids with high antibiosis resistance to spittlebug and defined resistance to different species of spittlebug. Significant progress continues to be achieved in developing *Brachiaria* hybrids with antibiosis resistance to spittlebug. A comparison of results obtained from screening of two cycles of a tetraploid, sexual population (1998 vs 2000) indicated a 28% genetic gain in resistance to *Aeneolamia varia*. Mean damage scores caused by the pest were 2.3 and 3.2 (scale: 1 =susceptible 5= resistant) in 1998 and 2000, respectively, which indicates that the *Brachiaria* breeding program is now producing hybrids with higher level of resistance than the commercial cultivar (*B. brizantha* cv Marandu) used as a resistant check.

We confirmed that *Brachiaria* hybrids selected for antibiosis resistance to *Aeneolamia varia* and *Mahanarva* sp. do not necessarily have antibiosis resistance to the *Zulia* complex (*Z. carbonaria* and *Z. pubescens*). We again observed a high correlation between resistance to *Aeneolami*a *varia* and *Mahanarva* sp in terms of visual damage score and nymph survival. However, with the exception of the *Brachiaria* hybrid CIAT 36062, all other hybrids exposed to the *Zulia* complex did not exhibit antibiosis resistance, even though some showed reduced visual damage. Thus there is a need to investigate the mechanisms of antibiosis resistance to certain species of spittlebug found in some selected genotypes of *Brachiaria* but not in others.

Molecular markers for spittlebug in and for reproductive mode of *Brachiaria* hybrids. Significant progress was made this year in collaboration with the Biotechnology group in: a) identifying QTL's (75 microsatellite loci identified) for spittlebug resistance on a parental and hybrid (full-sib) progeny population, and in b) identifying molecular markers associated with apomixis. With nine selected hybrids there was complete agreement between field (progeny testing) and marker data on the identification of sexual and apomictic lines. If this finding is reconfirmed in other tests, it will have a great impact on CIAT's *Brachiaria* breeding program in terms of cost and time efficiency.

Defined variation in the biology of species of spittlebug and development of IPM components for managing the pest. We completed comparative biological studies on three spittlebug species (*Zulia carbonaria, Zulia pubescens, Zulia* sp. nov.) and initiated studies on a fourth (*Prosapia simulans*). Some significant differences among species, that can have implications for pest management include: a) generation time varies 12 days among *Zulia* spp. and *P. simulans*; b) unlike most species studied to date, *Z. pubescens* and *P. simulans* prefer to lay eggs on the plant stem versus soil or leaf litter, and c) male courtship calls vary among the five species studied to date.

As a complement to our host-plant resistance strategy for dealing with spittlebug in *Brachiaria*, we continued to investigate complementary IPM components and alternative IPM tactics, particularly biological control. We made excellent progress on the collection, and evaluation of fungal entomopathogens of spittlebug. Our results showed that isolates of *Metarhizium* were more effective in controlling adults of *A. varia* than strains of *Paecilomyces* and *Fusarium*. However, these taxa were under represented in the population screened and thus should not be ignored in future studies. The next step of this research will be to determine the variation in virulence of selected fungal entomopathogens across different spittlebug species and life stages and to carry out field studies.

Defined utility of endophytes in tropical grasses. We continued to make progress in: a) artificial inoculation of endophytes in grasses, b) developing methods for identifying endophytes in grasses and c) defining the usefulness of endophytes for conferring resistance to pathogens and tolerance to drought in *Brachiaria*. Our results showed that with inoculation procedure used only few (1%) plants resulted with stable endophyte associations mainly due to heavy damage caused in the meristem tissue of seedlings. We identified promising PCR amplified DNA products, which are specific to isolates of endophytes in *Brachiaria* and which can now be used for quick endophyte detection in plant tissue. In vitro studies showed that the growth of *Rhizoctonia solani*, an important fungal pathogen in grasses, was reduced by as much as 30% in the presence of endophytes. We also reconfirmed that under green house conditions, endophytes confer tolerance to drought in *Brachiaria* through promoting greater production of fine roots, which in turn contribute to greater acquisition of minerals (K) thereby increasing not only leaf expansion, but also facilitating osmotic adjustment under severe water stress. We still need to define the alkaloid profile of endophytes isolated from *Brachiaria* and to determine under field conditions their effect on drought tolerance.

Defined biodiversity of the anthracnose pathogen in *Stylosanthes.* As part of on going collaborative project with other research groups in Australia, Brazil and China we determined the genetic diversity of 119 isolates of *Colletotrichum gloeosporioides* from Colombia, Brazil and Australia using RAPD and AFLP. Our results indicate no apparent association between the pathogenicity pattern and the geographic origin of the isolates. Furthermore, we found that none of the isolates was pathogenic on all differentials and that none of the differentials was immune or susceptible to all isolates. This information is key for developing effective plant breeding schemes for durable resistance of *Stylosanthes* to anthracnose.

Defined causal agent of bacterial wilt disease of *Brachiaria* and identified sources of resistance. A severe wilt disease was observed last year in *Brachiaria* plants grown in the glasshouse used to screen for spittlebug resistance and we determined that the wilt-causing organism was *Xanthomonas campestris.* Thus we were interested in identifying sources of resistance by screening 21 selected *Brachiaria* hybrids and 14 accessions. Our results indicated that bacterial multiplication was low in 9 hybrids and 9 accessions, which we now classify as sources of resistance to this wilt disease.

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

Improved *Brachiaria* hybrids with resistance to edaphic constraints. Successful *Brachiaria* hybrids should not only have resistance to spittlebug but should also be adapted to the acid infertile soil complex. Using a rapid and reliable root elongation screening procedure for aluminum (Al) resistance, this year we selected 4 out of 13 *Brachiaria* hybrids with moderate level of Al resistance. Among the hybrids selected, one is sexual thus offering a great opportunity to make further improvements in edaphic adaptation in the breeding program. We also evaluated the performance of selected *Brachiaria* hybrids to low nutrient supply and found that one hybrid (FM0503/S094/011) was outstanding in its adaptation to low P, K, Ca, Mg and S supply. We also found that the *Brachiaria* hybrid CIAT 36062, which is resistant to spittlebug and currently being evaluated under grazing in Caquetá, Colombia was also outstanding in biomass production under low nutrient supply. Unfortunately this hybrid does not produce seed and it is very susceptible to waterlogging.

Selected genotypes of *Arachis pintoi* with improved adaptation to different environmental conditions. One major limitation of the commercial cultivars of *Arachis pintoi* is their lack of adaptation to low fertility soils. Thus we continued with field evaluation of accessions of *Arachis* in order to identify genotypes that perform better under low soil nutrients than the commercial cultivars. Out of 10 accessions being evaluated in a forest margins site (Caquetá, Colombia), we identified CIAT 22159 as promising for soils low in Ca and P.

This year we also completed the analysis of a G x E data recorded with a core collection of *A. pintoi*. Results indicated that several accessions (CIAT 18748, 18747, 18751, 22159 and 22160) performed better than commercial cultivars (CIAT 17434 and 18744) across environments that ranged from lowlands to mid altitude hillsides and from subhumid to humid environments. However, results from an ongoing trial in a site with relative fertile soils and long dry season (Atenas, Costa Rica), indicated that *A. pintoi* CIAT 22159 and 22160 had similar yields as compared with the commercial cultivar (CIAT 18744) being promoted in Central America.

Selected genotypes of *Panicum* for low fertility soils. Last year we reported that the increase of forage yield per unit of N applied was superior in two CIAT *Panicum maximum* accessions (6177 and 16051) as compared to some commonly used cultivars such as Vencedor. We also report that the two CIAT accessions (16061 and 36000) were superior in root growth than the commercial cultivar and therefore could be better adapted to acid infertile soils. These CIAT selections will now be included in a seed multiplication program to allow field-testing in different regions with low fertility soils.

Identified grasses and legumes with dry season tolerance. A major objective of the Forage Project of CIAT is to identify grasses and legumes suited for areas with long dry seasons. Therefore we have ongoing work in two sites with moderate and severe dry seasons. In the Matazul site with moderate dry season (4 months) we selected the *Brachiaria* hybrid (FM9503-S046-024) due to its ability to produce more green leaf during the dry season regardless of fertilizer level, as compared to other hybrids and accessions tested, including the commercial *B. decumben*s cultivar.

The shrub legume *Cratylia argentea* has proven to be well adapted to locations with long dry seasons (5 to 6 months). In the 11 initial accessions evaluated we did not find significant variation in biomass production in the wet or dry seasons. However, we are currently evaluating 30-40 new accessions of *C. argentea* acquired from CENARGEN-EMBRAPA in two contrasting sites (Quilichao- acid soils and Atenas- long dry season) and have found a large variation in seasonal dry matter yields and other agronomic attributes. Of interest is the accession CIAT 22374 that yielded over 100 g DM/plant in both seasons of the year in the subhumid site, which is not the case with the accessions being currently promoted (CIAT 18516/18668). In the site with acid soils the accession CIAT 18674 produced almost two times more DM than the accessions being advanced for on-farm testing. These initial results indicate that there is great scope for selecting genotypes of *C. argentea* that outperform our more advanced accessions in areas with long dry seasons and in areas with acid soils.

Identified grasses for poorly drained soils. We continued with the agronomic evaluation of *Brachiaria* and *Paspalum* species in poorly drained (San Carlos) and well -drained (Atenas) sites in Costa Rica. Results indicate differences in forage production between sites and among species. In both sites the two *Paspalum* species (*P. atratum* and *P. plicatulum*) have performed better than the *Brachiaria* accessions being evaluated. However, it is has been interesting to observe that in San Carlos *B. brizantha* cv Toledo (CIAT 26110) and *Brachiaria* hybrid cv Mulato (CIAT 36061) are performing better than some *B. humidicola* accessions known to be well adapted to poorly drained soils. In the Atenas site, *B. humidicola* CIAT 26427 and a *B. brizantha* accession (no CIAT number) seems to be very productive in well-drained soils.

Selected shrub legumes for multiple uses in areas with acid soils and for mid-altitude hillsides. We have long recognized that there are very limited options of shrub legumes for multiple uses in acid soils and mid- altitude hillsides and consequently we have given priority to the evaluation of a range of species in our Quilichao Research Station. Currently we are evaluating core collections of *Flemingia macrophylla*, *Calliandra calothyrsus*, *Cratylia argentea*, and *Rhynchosia schomburgkii*.

We can report that the *C. calothyrsus* accession CIAT 22310 continues to produce more edible DM than other 12 accessions in both low and high rainfall periods and consequently it has been promoted for seed multiplication and for on-farm evaluation. Among the 13 accessions of *R. schomburgkii* being evaluated, CIAT 17918 has produced 2 times more DM as compared to the other accessions. We will now assess the fodder value of selected accessions of *R. schomburgkii*, which is could be an alternative for in mid-altitude hillsides with cool temperatures.

Selected legumes for multiple uses in crop/livestock systems. One of our objectives is to provide farmers with legume alternatives that could be used as fodder or to enhance soils in crop/livestock systems. Currently we are evaluating in the Quilichao research station a core collection (14 accessions) of *Vigna unguiculata* (cowpea) acquired from IITA and a core collection of *Lablab purpureus* (44 accessions) acquired form CSIRO, Australia, ILRI and CIAT's GRU.

Results with cowpea have shown that accessions being evaluated have established very quickly and that there are accessions (i.e. IT90K-277/2) that produce both high quality fodder production and high grain yield. Soil incorporation of the cowpea accessions as green manure resulted in maize yields similar to those obtained with 160 kg of N. We are currently multiplying seed of selected cowpea accessions for testing with farmers in hillsides of Central America.

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

Strengthen and developed new partnerships for forage evaluation in LAC and Africa. Development of strong partnerships within CIAT and outside CIAT is a major strategy of the Forage Project to deploy elite grasses and legumes to farmers. So far we have been successful in establishing collaboration with different organizations (NARS, Universities, NGO"S and ARIS) on participatory forage evaluation for different uses in Colombia, Peru, Costa Rica, Honduras, Nicaragua, Haiti and Canada. We have not yet been successful in establishing collaborative projects in Africa. However, we developed a collaborative proposal with ILRI, IITA and the U. of Bayreuth, Germany, to develop a Decision Support Tool that will facilitate targeting forages to Africa and Central America based on a better definition of the bio-physical and socio- economic constraints that can influence adoption of legumes. The following results from the on-going collaboration are highlighted:

- Through on-farm trials demonstrated the biological and economical benefits of *Centrosema macrocarpum* as a protein supplement for milking cows in smallholder farms in the forest margins of Peru.
- Confirmed in the field the high biomass yield and antibiotic resistance to spittlebug of the *Brachiaria* hybrid CIAT 36062, but found that it was susceptible to waterlogged conditions in the forest margins of Colombia.
- Found through collaborative work with the U. of Montreal, Canada a large variation in L-dopa in Mucuna germplasm, which has implications for therapeutic medicine.
- NGO and NARS partners in Central America trained in participatory selection of forages
- Completed a participatory diagnosis of problems and opportunities in three rural communities of Honduras.
- Innovative farmers in Honduras selected grasses to test on their farms and more than 200 farmers initiated selection of improved forage options.
- Demonstrated feasibility of making high quality *Cratylia* silage to replace expensive protein supplements for dry season feeding.
- Showed that reclamation of degraded pastures with *Arachis pintoi* in subhumid and humid areas of Central America and forest margins increases carrying capacity and milk yield.

One major task of the IP5 staff during this year has been the development with partners in CIAT and outside CIAT of new research proposals for submission to different donor agencies. A total of 6 research proposals were submitted to donors in Colombia, Ecuador and Germany for on-farm forage evaluation and for effectively targeting forages in Africa and Central America.

Selected *Brachiaria* accessions and hybrids released. A significant accomplishment of collaborative work with different partners was the release this year of a *Brachiaria* accession and hybrid. In Costa Rica, MAG (Ministerio de Agricultura y Ganaderia) released *B. brizantha* CIAT 26110 as cultivar Toledo in a well-attended field day. This same *Brachiaria* accession was released by a private seed company in Brazil (Matsuda) as cultivar MG5 Vitória. In Mexico, a private seed company released for the first time a *Brachiaria* hybrid (CIAT 36061) as cultivar Mulato based on the following agronomic merits:

1. Vigorous growth and stoloniferous growth habit.
2. More yield (25% more) than other commercial *Brachiaria* cultivars.
3. Good forage production under seasonal low light intensity and low temperatures found in certain parts of Mexico.
4. High quality as indicated by high protein content (12 to 16%) and in vitro DM digestibility (55 to 62%).

Known adoption and constraints for diffusion of *Arachis* in Costa Rica. Adoption of *Arachis pintoi* in Costa Rica was assessed and results showed a low adoption rate (4.5%), but great potential for widespread diffusion if some actions are taken by different organizations. Our results indicated that to increase the adoption of *Arachis* and possibly other forage legumes, there is a need to promote more contacts between farmers and local R & D institutions. Therefore training NARS and NGOs field staff in participatory methods is urgently needed in Costa Rica and other Central American countries.

Increased seed supply to farmers of new grass and legume options. The availability of experimental and basic seed of promising forage germplasm is the key for the evaluation, selection and eventual release of new forage cultivars to farmers. The Seed Units in Palmira and Atenas continued to multiply and deliver experimental seed of several grass and legume species to NARS and NGO’s for on-farm testing. In addition, we delivered seed of *Brachiaria brizantha* CIAT 26110 (released in Costa Rica) and of the shrub *Cratylia argentea* CIAT 18516/18668, which will be released next year in Costa Rica as cv Veraniega.

Defined with partners strategies needed for R&D of *Calliandra* in Central America. Participants in a consultation workshop held in Costa Rica this year agreed that the most appropriate strategy for *Calliandra* utilization was as fodder for cattle, but used at a moderate level and as one of a range of species in a system, rather than as the only supplement. Fed as one component of a mixture, its high tannin levels could even improve the utilisation of other species (e.g. *Cratylia argentea*) whose N may be too rapidly degraded in the rumen.

However, in view of the very limited utilisation of tree fodder in general in Central America, other uses of *Calliandra* should also be considered as part of a promotion strategy. In Costa Rica, for instance, there is currently much interest in agroforestry interventions for soil improvement and rehabilitation of degraded lands, and *Calliandra* has been identified as a valuable mulch/soil improvement species because of the slow rate of nutrient release from the leaves (due again to their high tannin content). The research on differences between Patulul and San Ramón provenances has relevance to this: San Ramón leaf has been shown by research at CIAT to have a slower rate of decomposition than Patulul, and therefore to be preferable for this use, whereas Patulul is superior for fodder. It was felt that these provenances should be tested in parallel for soil amelioration and fodder respectively.

CIAT’s forage database in Intranet and CD-ROM. A beta version of CIAT’s Forage database is now available in a CD-ROM in Spanish. After clearing copyright issues and doing the graphic layout for publication we intend to publish the database in CIAT's CD-ROM series and via the Internet. Linkage to other databases e.g. FAO – will also be evaluated.

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

Activity 1.1 Selection of *Brachiaria* genotypes for high digestibility and other quality attributes

Highlights

- Found good correlation between NIRS and laboratory analysis for determining digestibility of *Brachiaria* hybrids.
- Found that screening of *Brachiaria* hybrids for in vitro digestibility can be accelerated by oven drying of samples as opposed to freeze drying.
- Found variability in saponin activity in *Brachiaria* accessions and hybrids indicating that there is scope for selecting for these compounds associated with photosensitization in young cattle and sheep.

1.1.1 Calibration of NIRS for IVDMD

Contributors: N. Narváez, J. W. Miles and C. Lascano

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

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

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

Last year we indicated that to calibrate NIRS for IVDMD we had used a narrow – based equation and that resulting parameters had high precision as indicated by low SE of the calibration (0.98). In addition, estimates of IVDMD of few samples using NIRS had a high correlation (r= 0.89) with IVDMD values obtained with the two-stage in vitro procedure.

This year we tested the NIRS calibration curve developed last year with leaves of 176 *Brachiaria* hybrids that form part of a population used to develop molecular markers for digestibility. Results shown in Figure 1 indicate that a high correlation (r= 0.84) between values obtained from the in vitro procedure and those obtained using NIRS. One important aspect that we learnt this year is that sample processing is a key factor when using NIRS to estimate IVDMD in large populations of *Brachiaria* hybrids. Samples used to test the precision of the NIRS equation had to be reground and reanalyzed for IVDMD in order to obtain adequate estimates with NIRS.

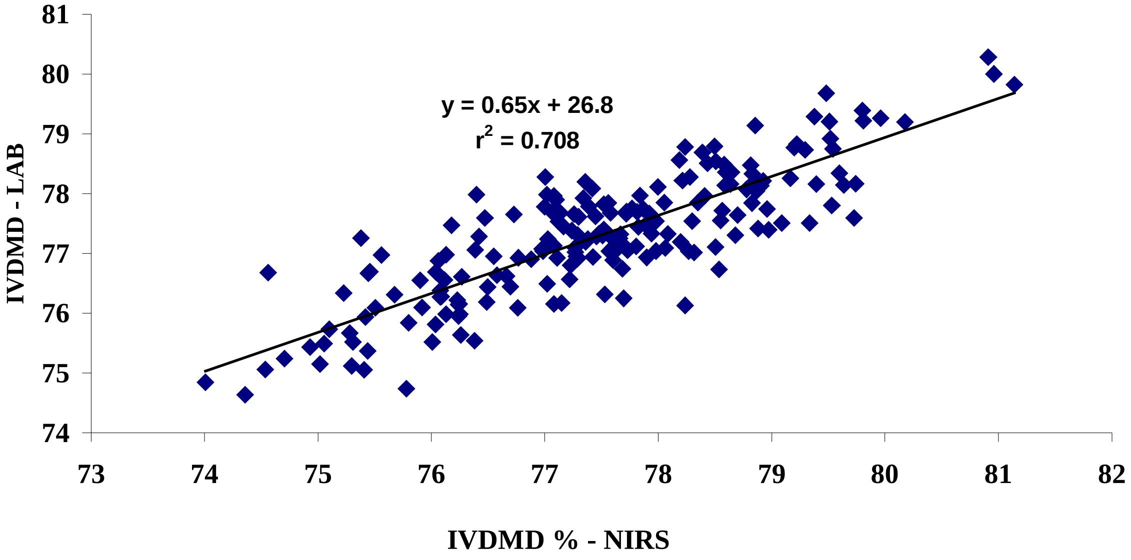


Figure 1. Relationship between in vitro digestibility values recorded in the laboratory (two-stage system) and NIRS

1.1.2 Effect of oven and freeze drying on IVDMD of *Brachiaria*

Contributors: N. Narváez, and C. Lascano

A bottleneck for selecting forages on the basis of digestibility is the processing of samples such as drying. It is well documented that exposing fresh forage samples to excessive heat during the drying process may result in artifact products, which are indigestible and result in underestimation of digestibility. Thus a common practice to overcome the use of heat for drying forage samples for subsequent quality analysis such as in vitro digestibility is to freeze dry the samples. However, freeze drying is a slow process given that samples have to be frozen before drying and in most cases the freeze driers available have low capacity, as is the case in the apparatus available in CIAT's Forage Quality Laboratory.

Given that one of the objectives of the *Brachiaria* breeding program in CIAT is to select hybrids with high digestibility, we were interested in determining to what extent forage samples could be dried in an oven using heat. Samples of leaves of 49 *Brachiaria* accessions from plants growing in CIAT Popayan were either subject to heat drying (60^0C for 48 hours) or to freeze-drying and then analyzed for in vitro digestibility using the Tilley & Terry two stage procedure.

Results presented in Figure 2 show a high and significant correlation (r= 0.94) between values of in vitro digestibility recorded with *Brachiaria* samples exposed to the two drying methods and a regression slope not different from 1. Thus in order to cope with the large number of *Brachiaria* lines generated in the breeding program samples in the future will be oven-dried at 60^0C and then ground in a Willey Mill fitted with a 1 mm screen before used in NIRS to estimate IVDMD.

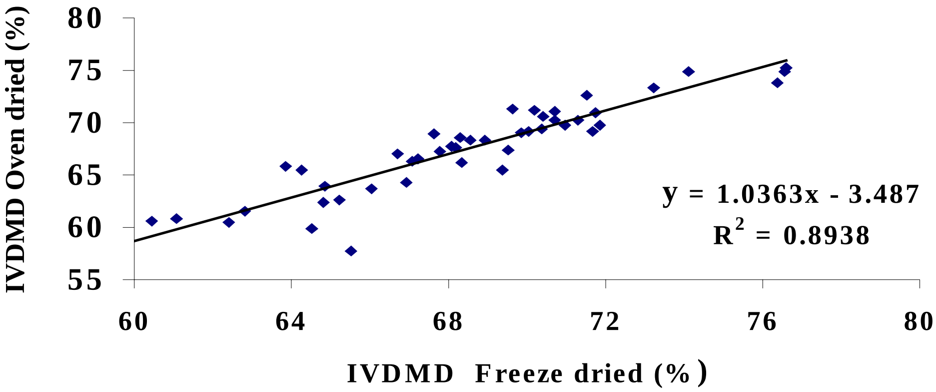


Figure 2. Relationship of in vitro digestibility (IVDMD) in *Brachiaria* determined in oven-dried and freezes dried samples.

1.1.3 Screening of selected *Brachiaria* accessions and hybrids for saponins

Contributors: N. Narvaez, and C. Lascano

A widespread, but sporadic, toxicity syndrome associated with *Brachiaria decumbens* is hepatogenous photosensitization, which can cause severe losses in LWG and in some cases death, particularly with young animals. This syndrome has been related to infestation of the grass by the saprophytic fungus *P. chartarum*, which produces spores that contain toxic sporidesmin. However, the cause – effect relationship between the fungus and photosensitization in *B. decumbens* has been challenged by some researchers with the argument that: a) strains of *P. chartarum* isolated in *Brachiaria* pastures where animals have shown toxicity do not produce sporidesmin and b) steroidal saponins were isolated from the rumen contents of poisoned animals fed with *Brachiaria* and that steroidal saponins have been identified in plants known to cause photosensitization.

Based on the assumption that saponins may be associated with photosensitization in *Brachiaria* we were interested in determining presence or absence of these compounds in accessions and hybrids of *Brachiaria.* The laboratory procedure used is one of hemolisis of red blood cells obtained from rabbits, with a solution extracted (80% aqueous methanol) from fat free samples (0.1 g) of test forages. A dilution of 1:20 was used to determine absorbance in a spectrophotometer in the Forage Quality Laboratory. Leaves of wheat and of the tree *Entorolubium ciclocarpum* (Orejero) were used as negative (low levels of saponins) and positive (high levels of saponins) controls in the assay, respectively.

Results shown in Table 1 indicate differences in saponin activity among the few *Brachiaria* accessions included in the assay. Saponin activity was very high in the commercial cultivar *B. decumbens* CIAT 606, which in fact was as high as that recorded in the positive control. In contrast saponin activity seemed to be low in *B. humidicola* and in *B. brizantha* cv Marandu, which is the source of spittlebug resistance in the *Brachiaria* breeding program. Saponin activity in the *Brachiaria* hybrids was also variable, ranging from very high (absorbance of 786 to 893) to low (absorbance of 230 to 344). However, among the hybrids, chosen at random, included in the assay we did not find any with very low saponin activity.

It would seem from the results that two of the parents (*B. decumbens* and *B. brizantha*) used in the *Brachiaria* breeding program have very contrasting levels of saponins and this is reflected in the variability of saponin activity measured in the hybrids included in the test. Unfortunately we did not include in the saponin test being reported the sexual tetraploid *B. ruziensis* also used in the breeding program as a parent. The high concentration of saponins in *B decumbens* is consistent with the observations of photosensitization in cattle and sheep fed with this grass. Thus from these results it would seem that there is justification to screen *Brachiaria* hybrids for saponins. However, before we embark on this screening we need to determine if the assay being used is adequate, particularly since it is time consuming and does not allow us to quantify the exact concentration of saponins present. Thus as a future activity will be to investigate alternative laboratory procedures for the determination of saponins that are more accurate and less time consuming than the qualitative method used.

Table 1. Saponin activity in *Brachiaria* accessions and hybrids

Classification	**Forage sample**	**Absorbance***
Very High	**“**Orejero” (positive control)	881-990
	B. decumbens	840-845
	Hybrids	
	1084-3	786-801
	1084-10	887-893
High	*B. ruziziensis* 26164	610-618
	Hybrids	
	1092-5	686-705
	1092-15	702-714
Intermediate	**Hybrids**	
	1092-14	453-466
	1092-2	475-480
	1092-3	453-466
	1092-15	531-512
	1092-11	530-550
Low	**Hybrids**	
	1092-4	230-233
	1092-13	253-236
	1092-1	344-367
Very Low	Wheat (negative control)	38-42
	B. humidicola 16871	57-51
	B. brizantha 6780	63-65

*Duplicates (dilution 1:20)

1.1.4 Analysis of digestibility data on a F1 *Brachiaria* mapping population and selection of clones for QTL detection

Contributors: J. W. Miles, N. Narváez and C. Lascano

This year a mapping population of >200 full sib individuals (tetraploid *B. ruziziensis* x *B. brizantha* cv. Marandu) was sampled (glasshouse-grown plants) for IVDMD analyses. We were particularly interested in comparing results on IVDMD (two-stage in vitro) recorded in three successive samplings in 1998, 1999 and 2000.

Results shown in Table 2 indicate a very poor correlation between samplings as indicated by low correlation coefficients. These disappointing results affect our current ability to relate microsatallites markers to digestibility, which is one of our main objectives.

Table 2. Correlation between values of IVDMD recorded in successive years in the same population of *Brachiaria* hybrids used for identifying molecular markers (No of samples 238)

Years of Sampling	**r**
1998 vs 1999	0.08
1998 vs 2000	0.37
1999 vs 2000	0.01

A careful analysis of the IVDMD data obtained using the two-stage in vitro system indicated not only lack of correspondence of IVDMD between years but also between duplicates in each run. We now believe that the main problem we are facing has to do with sampling of the material in the greenhouse and with processing of the harvested material. Individual plants have been harvested after 5 or 10 months regrowth and then freeze dried. Leaf is then separated from stem before grinding in Willey Mill fitted with a 1 mm screen. It is possible that separation of leaf from stem is not resulting in uniform material across samplings and that the grinding process is not producing samples with uniform particle sizes. Thus, we propose to change our sampling scheme as follows:

1. Sample only leaves from individual plants following a 3 month regrowth
2. Dry leaves in a oven at 60^{o} C for 24 hours
3. Grind leaf samples in small laboratory mill

In order to determine correspondence of IVDMD values between samplings, next year we will sample the *Brachiaria* population using the sampling and processing procedures described above.

Progress towards achieving milestones

- **Efficient protocol using NIRS for assessment of forage digestibility in a *Brachiaria* hybrid population incorporated into the breeding program**

We have now confirmed that the NIRS calibration curve for IVDMD developed in CIAT's Forage Quality Laboratory is adequate for estimating in vitro digestibility of hybrids produced in the *Brachiaria* breeding program.

- **Population of *Brachiaria* hybrids screened for in vitro digestibility**

During the last three years we screened a population of *Brachiaria* hybrids for in vitro digestibility and found values that ranged from 61 to 80%. Unfortunately, the correlation between samplings has been very low, probably as a result of sampling and sample processing problems.

Activity 1.2 Assessment of quality and animal productions potential of selected legumes

Highlights

- Determined that digestibility of woody legumes with tannins was more correlated to fiber than to condensed tannin fractions

- Found that differences in feed value of *Calliandra* offered to sheep as supplement was affected by provenance, by soil fertility and by post harvest management (fresh vs sun dried) of the forage harvested.

- Found that a system of direct grazing of *Cratylia* in association with a grass resulted in higher milk yield as compared to a cut and carry system

1.2.1 Characterization of a range of shrub legumes for quality attributes

Contributors: N. Narváez, and C. Lascano

We have documented that both climate and soil fertility have a significant effect on quality of herbaceous legumes with tannins such *Desmodium heterocarpon* subs. *Ovalifolium* (See IP-5 AR-1998 and 1999). To follow-up this initial studies we determined as part of an MS Thesis quality parameters of a range of woody legumes with tannins grown in contrasting sites in Colombia.

To carry out the study several N fixing and no- fixing legumes species were sampled in seven locations in Colombia with contrasting soils and climate. In each location we attempted to harvest uniform immature and mature forage by sampling during the wet season terminal (immature) and lower (mature) edible plant tissue (leaves and fine stems). Samples were freeze-dried for subsequent chemical analysis (crude protein, in vitro digestibility, neutral and acid detergent fiber, soluble and insoluble condensed tannins). An ANOVA including as sources of variation location, species, maturity and corresponding interactions was performed in the cases where two or more legume species were present in two or more locations.

To better define "niches" for targeting woody legumes, we were particularly interested in defining G x E interactions with well know species that have variable concentrations of condensed tannins and in defining with these species what chemical fractions were more correlated with in vitro digestibility. In this report we only highlight the results on quality parameters measured in *Leucaena leucocephala* and *Calliandra calothyrus* growing in two contrasting environments (Cauca valley and Cauca hillsides).

Results shown in Table 3 indicate a significant species and maturity effect on quality parameters measured. The in vitro digestibility of *L. leucaena* was considerably higher than of *C. calothyrsus* regardless of maturity and this was associated to lower fiber content and concentration of soluble and insoluble CT. A significant site effect was also observed, as indicated by the lower IVDMD and fiber fractions of both legume species grown in the less fertile soils in hillsides of Cauca as compared to the more fertile soils found in the valley. However, it was interesting to observe that the effect of site on digestibility was greater with *C. calothyrsus* than with *L. leucocephala,* which could be related to its higher concentrations of total condensed tannins.

As part of the study we performed a correlation analysis of different chemical fractions measured in woody legumes with variable levels of tannins with in vitro digestibility. Results indicated that in legumes species (8) sampled in 5 sites, the ADF fraction was the chemical fraction that showed the highest correlation (r= -0.71 to -0.99) with IVDMD followed by NDF (r= -0.62 to -0.96) and by the insoluble CT fraction (r=-0.53 to -0.97). Overall, 58, 36 and 32% of the observed variation in IVDMD in the 8 legume species grown in 5 sites was explained by the ADF, NDF and insoluble CT fractions, respectively, whereas the soluble CT fraction only explained 20% of the variation in IVDMD.

In general, our results suggest that digestibility of woody legumes is negatively affected by low soil fertility, but that the effect can be greater with species that have very high levels of CT as is the case of *C. calothyrsus* as compared to *L. leucocephala*. In addition, it is apparent that the low digestibility of woody legumes with tannins is not explained by high concentration of soluble CT, which is the chemical fraction most commonly associated with the low quality of these legumes. Rather it appears that fiber fractions are key plant factors affecting digestibility of woody legumes with variable levels of tannins.

Table 3. Effect of location and maturity on in vitro digestibility and chemical composition of the edible forage of two contrasting woody legumes.

Locations	Legumes	Maturity	Quality Parameter (% of DM)				
			IVDMD	NDF	ADF	SCT	ICT
Cauca Valley	*L. Leucocephala*	Immature	74.3	15.5	10.3	4.3	2.4
		Mature	65.9	22.2	16.2	4.5	2.1
	C. calothyrsus	Immature	38.0	27.2	20.5	18.2	5.0
		Mature	25.1	29.5	26.0	17.4	5.3
Cauca Hillsides	*L. leucocephala*	Immature	66.8	28.0	14.0	6.7	4.1
		Mature	60.6	31.0	17.7	5.6	3.4
	C. calothyrsus	Immature	17.9	36.6	31.0	12.3	7.3
		Mature	16.9	34.6	31.1	13.4	4.9
SEM			1.7	1.1	1.4	1.2	0.3
Significance < P							
Site			0.01	0.01	0.01	NS	0.01
Species			0.01	0.01	0.01	0.01	0.01
Maturity			0.01	0.01	0.01	NS	0.01
Site x Species			0.01	0.05	0.05	0.01	NS
Site x Maturity			0.05	0.05	NS	NS	0.01
Species x Maturity			NS	NS	NS	NS	NS

1.2.2 Feed value of selected *Calliandra* accessions

Contributors: N. Narvez, P. Avila, J. Stewart (OFI, UK) and C. Lascano

The evaluation and selection of shrub legumes as a feed resource is an area of interest in the tropics and one that is receiving high priority in CIAT's Tropical Forages Project. Over the last three years we have been evaluating the nutritional characteristics and feed value of two contrasting provenances *of Calliandra calothyrsus* as a part of a collaborative OFI, UK – CIAT, Colombia project funded by DFID.

It is well documented that *C. calothyrsus*, which is native to Central America, is adapted to acid-low fertility soils with high levels of Al saturation and produces high edible biomass rich in protein. However, one commonly cited limitation of *Calliandra,* as source of fodder is the high concentration of condensed tannins (CT) in the edible forage, which have been associated with low palatability, and digestibility. Results reported in the literature on the feed value of *Calliandra* are contradictory, given that some indicate that intake and digestibility of the edible fodder is higher when offered fresh to animals, while results form others indicate that drying *Calliandra* enhances its feed value. On the other hand, it has been postulated that the feed value of *Calliandra* fodder could be associated with climate and soil factors given that CT in the leaf and stem tissues can increase when the plants are grown under stressful conditions.

Initial chemical characterization of *Calliandra* provenances: To address some of the questions related to the feed value of *Calliandra*, we carried out together with OFI a detailed characterization of the chemical composition of the edible forage of two contrasting provenances (CIAT 22310 and CIAT 22316) harvested in the greenhouse (OFI) and in field locations (CIAT) (Quilichao and Palmira) with contrasting soil fertility (Table 4).

Table 4. Quality parameters in edible of forage of *Calliandra calothyrsus* provenances grown in contrasting locations (all samples were freeze-dried).

Location	Provenance CIAT No.	Quality parameter			
		Crude protein	Fiber		Soluble CT
			Neutral Detergent	Acid Detergent	
			(% of DM)		
Field Quilichao	22310	16.0	27.6 a	23.4 a	14.7 b
	22316	16.3	19.7 b	15.0 b	21.9 a
Field Palmira	22310	17.6	28.4 a	23.3 a	14.4 b
	22316	18.0	17.8 b	16.0 b	22.5 a
Greenhouse	22310	20.6	37.9 c	20.4 c	13.7 d
Univ of Reading	22316	24.3	30.8 d	16.5 d	14.5 c

a, b Values in the same column in plants grown in the field with the same letter are not different (P<0.05).
c,d Values in the same column in plants grown in the greenhouse with the same letter are not different (P<0.05).

Chemical analysis of forage samples harvested in the field showed a greater effect of provenance than location on quality parameters measured, as indicated by a higher concentration of soluble CT in CIAT 22316 than in CIAT 22310, but lower levels of fiber fractions (NDF and ADF) and insoluble CT in CIAT 22316 (Table 4). Similar results but with different magnitude were recorded in samples of the two *Calliandra* provenances grown in a greenhouse at the University of Reading, UK.

An interesting finding reported last year (see IP5 AR-1999) was that the chemical composition or structure (the ratio of procyanidin: prodelphinidin) of the soluble CT fraction varied between provenances regardless of location were plants were grown as shown in Table 4. Soluble CT in

CIAT 22316 were comprised mainly by procyanidin subunits, whereas the soluble CT fraction in CIAT 22310 was composed largely of prodelphinidin subunits. Similar results were observed in forage samples harvested in same the *Calliandr*a provenances grown in a greenhouse in the University of Reading, UK (Table 4).

The different tannin structure found in the two *Calliandra* provenances was associated with differences in astringency or ability of tannins to bind protein. In the studies carried out at CIAT using BSA (Bovine Serum Albumin) as a source of protein, we found that astringency was higher with soluble CT of CIAT 22310 (0.90 g protein/g of CT) than with tannins of CIAT 22316 (0.57 g protein/g of tannins), regardless of location used to grow the plants. Similar results were recorded with samples of *Calliandra* harvested from plants grown in a greenhouse in the U of Reading, UK but the differences between provenances in astringency was smaller (CIAT 22310-0.66 g protein /g of tannins; 22136 – 0.57 g protein/g of tannins), for reasons not understood. In temperate legumes the reactivity of CT with Rubisco was found to be higher with increased proportion of delphinidin relative to cyaniding, which is consistent with what we have found in the two *Calliandra* provenances evaluated. The challenge ahead is to define the biological significance of the different tannin chemical structure found in *Calliandra* provenances.

Methods used in the feeding trials using *Calliandra* as a supplement: Detailed chemical characterization of the two *Calliandra* provenances was followed by two feeding trials with sheep housed in metabolism crates and designed to evaluate the value of *Calliandra* forage as a protein supplement to a low quality grass fed as basal diet.

In Trial 1, designed to study the effect of provenance and drying on the feed value of *Calliandra*, 8 African type wethers (BW) fitted with rumen and duodenal cannulas were assigned to one of four treatments in a replicated 4 x 4 Latin Square design: a) *Calliandra* CIAT 22310 fed fresh, b) *Calliandra* 22310 fed sun dried, c) *Calliandra* CIAT 2316 fed fresh and d) *Calliandra* CIAT 22316 fed sun dried. Animals housed in individual metabolism crates were offered daily 100 g DM / kg BW $^{.75}$ of a low quality grass hay (*Brachiaria dictyoneura*) mixed with 50 DM/kg BW $^{.75}$ of edible forage (leaves + fine stems) of fresh or sun- dried *Calliandra* in two meals (8:00 and 15:00). Animals were offered a mineral mix ad-lib and water 4 times a day and were fitted with fecal collection bags on day 7 of the adjustment phase. Sheep used in the trial were allowed to graze for one week between experimental periods, which had duration of 17 days, of which 10 were for adjustment and 7 for measurements.

Forage refused and feces were collected daily and one sub sample was frozen for subsequent freeze drying and chemical analysis and another oven-dried to determine DM content . A sub-sample of the forage refused was used to separate grass and legume and estimate intake of each component. On days 8 and 9 of the measurement period, samples of duodenal digesta were taken every 6 hours and on the last day rumen samples were collected every 3 hours to determine purines in rumen bacteria. Rumen and duodenal digesta samples were frozen for subsequent analysis.

Samples of legume offered were analyzed for condensed tannins (CT) using the butanol HCl method. In addition, forage and fecal samples were analyzed for N, and fiber (NDF and ADF). Concentration of indigestible acid detergent fiber (IADF) in the forage and feces was used as an internal marker to estimate flows of the solid phase of digesta to the duodenum. Purines were determined in duodenal samples to estimate microbial N and RNA from torula yeast type II-C from SIGMA (R-6875) was used as a standard. The ratio of N: RNA of bacteria flowing to the duodenum was estimated for each treatment from bacteria isolated from the rumen fluid by differential centrifugation. The ratio of rumen bacteria N: RNA equivalent was used to estimate proportion of bacterial N in duodenal samples. Data were subject to analysis of variance for a 4 x 4 Latin Square design and the Duncan's test was used to compare treatment means.

In Trial 2, designed to study the effects of provenance and growing location on the feed value of *Calliandra,* 8 African wethers (BW) fitted with rumen and duodenal cannulas, were assigned to one of 4 treatments arranged in a replicated 4 x 4 Latin Square design: a) *Calliandra* CIAT 22310 grown in acid soils (Quilichao), b) *Calliandra* CIAT 22316 grown in acid soils (Quilichao), c) *Calliandra* 22310 grown in fertile soils (Palmira), and d) *Calliandra* 22136 grown in fertile soils (Palmira).

The edible forage (leaves + fine stems) harvested at each site was sun- dried and stored in bags under shade before used to feed the animals. In this trial the sun dried legume supplement (40 g DM/kg BW$^{.75}$/day) was offered in three rations (8: 00, 12:00 and 15:00) alone and not mixed with the grass basal diet (100 g DM/kg BW$^{.75}$/day) as in Trial 1. All other procedures related to animal management, sampling, laboratory analysis and data processing were done as in Trial 1.

Results from feeding Trials 1 and 2: Results from the two trials indicated that there were differences between *Calliandra* provenances in chemical composition of the edible forage (Table 5).

Table 5. Chemical characterization of provenances of *Calliandra calothyrsus* fed to sheep as a supplement to a low quality grass[1].

Calliandra Provenances CIAT No.	Crude Protein	Neutral Detergent Fiber	Acid Detergent Fiber	Condensed tannins	
				Soluble	Insoluble
	(% of DM)				
Trial 1					
(Drying effect)					
22310 fresh-freeze dried	16.3	42.8	41.3	5.1 c	9.0
22310 sun-dried	15.0	39.2	32.9	12.6 b	8.0
22316 fresh-freeze dried	16.0	31.9	36.0	17.1 b	8.1
22316 sun-dried	15.5	32.1	24.4	33.8 a	5.2
SEM	0.4	1.3	2.1	1.7	0.4
Significance (P)<					
Provenance	NS	0.0001	0.01	0.0001	0.004
Drying treatment	0.07	NS	0.002	0.0001	0.002
Provenance x Drying treatment	NS	NS	NS	0.03	0.08
Trial 2					
(Location effect)					
22310-Quilichao	14.6 a,b	40.4	31.8	18.5 c	8.0
22310-Palmira	14.3 a,b	39.0	30.5	16.0 c	9.1
22316-Quilichao	13.4 b	32.6	24.8	34.5 a	6.2
22316-Palmira	15.8 a	33.4	22.4	25.8 b	5.5
SEM	0.6	0.6	0.8	1.2	0.7
Significance (P)<					
Provenance	NS	0.0001	0.0001	0.0001	0.05
Location	0.1	NS	0.05	0.001	NS
Provenance x Location	0.05	0.1	NS	0.02	NS

[1] Grass: 3% crude protein.

a,b, c Values within each trial, in the same column with the same letter are not different (P<0.05).

In the two trials the level of crude protein was similar in both provenances, but was higher in the forage that was offered fresh and in the forage harvested in Palmira. The tannin fractions measured were also affected by treatments imposed in the two trials. Soluble CT was higher in CIAT 22316 than in CIAT 22310, regardless of drying method (fresh- freeze dried and sun-dried) or location (Palmira or Quilichao), but the opposite was found with the bound CT fraction

Fiber content measured as NDF was higher in CIAT 22310 than in CIAT 22316, but was not affected by drying method or by location, which is in agreement with results found in the U of Reading with the same provenances (Table 5). In contrast, the ADF fraction was affected by both provenances, by drying treatment and by location, being higher in CIAT 22310, in the fresh forage and in the forage harvested in the site with acid soils (Quilichao).

Results on intake of grass and legume shown in Table 6 indicated significant provenance, drying and locations effects. In Trial 1 intake of *Calliandra* CIAT 22310 was 37.5 % higher than intake of CIAT 22316 and intake of sun dried *Calliandra* was 2.3 fold higher than the intake of the fresh forage, which is contrary to general belief. In Trial 2 the intake of *Calliandra* 22316 was also higher (44%) than the intake of CIAT 22310 and intake of *Calliandra* provenances grown in the higher fertility soil (Palmira) was 2 fold higher than the intake of provenances grown in the acid soil (Quilichao).

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

Calliandra Provenance CIAT No.	Intake Grass (g DM/kg BW/d)	Intake Legume (g DM/kg BW/d)	Digestibility DM (%)	Digestibility NDF (%)
Trial 1*				
(Drying effect)				
22310 fresh	19.2 a	5.8 c	53.6	67.6
22310 sun-dried	15.7 b	10.3 b	57.0	65.3
22316 fresh	19.9 a	6.3 c	59.1	69.6
22316 sun-dried	12.3 c	15.6 a	60.9	59.6
SEM	1.0	0.9	1.6	2.0
Significance (P)<				
Provenance	NS	0.03	0.01	NS
Drying treatment	0.0001	0.0001	NS	0.01
Provenance x Drying treatment	0.05	0.05	NS	0.07
Trial 2**				
(Location effect)				
22310-Quilichao	16.9	3.4 c	53.5	54.2
22310-Palmira	15.3	5.2 b	51.0	45.9
22316-Quilichao	16.4	3.1 c	53.8	53.6
22316-Palmira	15.0	9.3 a	51.9	47.6
SEM	0.5	0.6	1.8	1.4
Significance (P)<				
Provenance	NS	0.01	NS	NS
Location	0.01	0.001	NS	0.003
Provenance x Location	NS	0.002	NS	NS

[1] Grass: 3% crude protein

a, b, c Values within each trial, in the same column with the same letter are not different (P<0.05).

*Fresh or sun-dried legume offered mixed with grass in two daily rations and comprised 33% of forage offered (65 g DM/kg BW/d).

**Sun-dried legume offered alone in 3 daily rations and comprised 28% of forage offered (56 g DM/kg BW/d).

In Trial 1, the higher intake of CIAT 22316 was associated with higher in vivo DM digestibility as shown in Table 6, but this was not the case in Trial 2. Sun drying and location had no significant effect on in vivo DM digestibility of the legumes-based diets, but supplementing sun-dried *Calliandra* and *Calliandra* harvested in the fertile soil resulted in lower NDF digestibility as shown in Table 6. In the two trials grass intake was not affected by the *Calliandra* provenance used as supplement, but was higher with *Calliandra* offered fresh and with *Calliandra* harvested in Quilichao as a result of a substitution effect.

A major objective of the feedings trials carried out by CIAT in collaboration with OFI, UK was to determine the efficiency of N utilization by sheep on a low quality grass diet supplemented with *Calliandra*. The accuracy of nutrient flow measurements in ruminants depends on recovery of indigestible markers and upon valid calculations procedures for the liquid and solid phases of digesta. In our studies digesta flows were estimated using a particulate marker (IADF) and as a result flow values presented may be inaccurate. However, we consider that the flow data is still useful because relative differences between treatments may indicate important biological effects. Results from Trials 1 and 2 shown in Table 7 indicate that N utilization was not consistently affected by provenance but that drying and location had a significant effect on most of the parameters measured.

Table 7. Nitrogen utilization by sheep fed two provenances of *Calliandra calothyrsus* as supplement to a low quality grass [1].

Calliandra	Nitrogen					
Provenances CIAT No.	Intake	Flow to duodenum	Bacterial flow	NAN-MIC flow	Fecal	Absorbed
			(g/d)			
Trial 1						
(Drying effect)						
22310-fresh	11.8 bc	13.9	5.5	8.4	7.0	6.8 a
22310-sun-dried	12.9 b	8.2	2.9	5.3	5.4	2.8 c
22316-fresh	9.8 c	10.7	5.5	5.2	5.7	5.0 ab
22316-sun-dried	17.0 a	9.3	3.8	5.5	5.1	4.3 bc
SEM	0.8	1.1	0.9	1.0	0.5	0.8
Significance (P)<						
Provenance	NS	NS	NS	NS	NS	NS
Drying treatment	0.0001	0.01	0.02	NS	0.03	0.01
Provenance x Drying treatment	0.001	0.08	NS	NS	NS	0.05
Trial 2						
(Location effect)						
22310-Quilichao	9.1 c	10.1 b	1.8	8.2 b	4.7 c	5.3
22310-Palmira	11.2 b	12.4 b	2.9	9.5 b	5.9 b	6.5
22316-Quilichao	8.8 c	9.6 b	1.9	7.7 b	4.3 c	5.3
22316-Palmira	14.4 a	18.6 a	2.6	16.0 a	8.4 a	10.2
SEM	0.6	1.4	0.4	1.2	0.6	0.9
Significance (P)<						
Provenance	0.02	0.05	NS	0.02	0.07	0.06
Location: Quilichao, Palmira	0.0001	0.001	0.03	0.001	0.0002	0.004
Provenance x Location	0.007	0.03	NS	0.009	0.02	0.06

[1] Grass: 3% crude protein.

a,b, c Values within each trial, in the same column with the same letter are not different (P<0.05).

In Trial 1, there was no effect of provenance on N intake or N utilization, but supplementing sun dried *Calliandra* resulted in less N flow to the duodenum, in less bacterial N, in less fecal N and in less apparent N absorption as compared to supplementing fresh *Calliandra.* However, it was interesting to observe that the negative effect of feeding sun -dried *Calliandra* on N absorption was greater with CIAT 22310 than with CIAT 22316 (Table 7).

In Trial 2, N utilization was affected both by provenance and by the location where *Calliandra* plants grew (Table 7). Intake of N was higher when *Calliandra* CIAT 22316 and when *Calliandra* grown at Palmira were supplemented, but the effect of location was greater with CIAT 22136 than with CIAT 22310. The higher N intake with *Calliandra* CIAT 22316 and with *Calliandra* grown in the site with fertile soil (Palmira) was associated with more total N and non ammonia- no microbial N reaching the duodenum, with higher fecal N and with more apparent N absorption. As in Trial 1, bacterial N flow was not affected by provenance, but was greater with *Calliandra* grown in the Palmira location

Discussion: Results from the two feeding trials showed differences in potential feed value between the two *Calliandra* provenances evaluated and how drying and location can affect their utility as a protein source to ruminants fed a low quality grass.

Contrary to what was expected intake and digestibility of *Calliandra* provenances was not related to concentration of soluble CT in the edible forage as indicated by the higher intake of CIAT 22316 than CIAT 22310 in both trials. Form the evidence gathered it appears that intake of the two *Calliandra* provenance evaluated was associated with concentrations of insoluble CT, NDF and ADF, which were lower in CIAT 22316 as compared to CIAT 22310.

The higher intake of *Calliandra* CIAT 22316 by sheep did not consistently result in higher total N and non ammonia- non- microbial N flows to the duodenum, or in higher N apparent absorption when comparing results from the two trials. A possible cause for this lack of consistency of results between trials could be related to the way the legume was fed, which in turn resulted in different rumen digestion efficiencies. In Trial 1 *Calliandra* provenances were mixed with the basal diet whereas in Trial 2 they were fed and consumed alone in three rations throughout the day. Consequently in Trial 1, both grass and legume were consumed by sheep simultaneously, which may have resulted in more synchronized release of energy and N for efficient rumen function. This interpretation is supported by the higher mean values of rumen ammonia (Trial 1: 7.6 mg/dL vs. Trial 2 5.6 mg/dL), microbial N flow (Trial 1: 4.0 g/d vs. Trial 2: 2.3 g/d) and DM digestibility (Trial 1: 57.6% vs. Trial 2: 52.6%) recorded in Trial 1 as compared to Trial 2.

The intake of *Calliandra* was improved when the forage was fed dried as opposed to fresh, which is contrary to thinking of some extension people. However, the positive effect of feeding sun dried *Calliandra* on intake of the legume did not translate in higher DM digestibility or N absorption in sheep fed a low quality grass. In fact apparent N absorption was greater when fresh *Calliandra* was fed as a result of increased total N and bacterial flow to the duodenum, which suggest less protein degradation by rumen microbes. The feeding value of *Calliandra* was also significantly improved when the legume was grown in a fertile soil as opposed to an acid soil as reflected by higher legume intake and N flows to the duodenum and apparent N absorption.

From the evidence gathered in the two feeding trials carried out by CIAT it is not possible to clearly define what forage quality attributes are responsible for the improved intake and feed value of *Calliandra* when sun- dried or when grown in a fertile soil. One limitation of the study was the impossibility to compare quality parameters in the sun dried and fresh *Calliandra* offered to sheep, given that the fresh samples were freeze dried for subsequent chemical analysis.

However, studies in the U of Reading with the two *Calliandra* provenances indicated that air dried samples had higher concentrations of soluble and insoluble CT as compared to fresh- frozen samples. The sun dried *Calliandra* used in the feeding trials also had higher concentrations of soluble CT as compared to fresh-freeze dried samples obtained from the forage offered to sheep, but levels of insoluble CT were lower. In addition, we did not find any evidence of changes in NDF concentration due to oven or freeze drying, which is consistent with results from the U of Reading. In the case of quality parameters in *Calliandra* harvested in two contrasting locations, the only significant difference found in the forage offered was in soluble CT, which were lower in samples from *Calliandra* grown in the fertile soil (Palmira) as compared to the site with acid soils (Quilichao). These results are not in agreement with initial chemical characterization of *Calliandra* forage from the two sites, but do agree with the higher concentration of tannins recorded with other tropical legumes (i.e. *Desmodium ovalifolium*) when grown in acid low fertility soils.

Finally, the results of the two feeding trials do not provide any clues on how the different chemical structures of CT in the two *Calliandra* provenances affect their feed value. It was expected that protein degradation in the rumen of sheep supplemented with *Calliandra* would have been greater with CIAT 22316 than with CIAT 22310, given the higher astringency of the tannins found in the latter. However, we found no evidence of higher rumen ammonia concentrations when *Calliandra* CIAT 22316 was fed as compared to CIAT 22310. In all cases level of rumen ammonia recorded when *Calliandra* was fed at a supplement were below the requirements for maximal microbial growth.

1.2.3 Milk production of cows supplemented with selected shrub legume species

Collaborators: P. Avila, and C. Lascano

In several on-station and on-farm experiments it has been demonstrated that supplementing *Cratylia argentea* (*Cratylia*) to milking cows either alone or in combination with sugar cane or elephant grass results in milk yield increments, particularly during the dry season. In addition, work carried out in the Tropileche consortium in Costa Rica has shown the benefit of using Cratylia as silage to supplement cows in the dry season. However, studies on economical analysis have also shown that the use of *Cratylia* in cut and carry systems could be affected by high labor costs. Thus it was of interest to evaluate the use of Cratylia in direct grazing system.

Two experiments were carried out in the Quilichao research station to determine the effect of using Cratylia as a supplement to milking cows in a cut and carry system or grazed directly by cow when in association with *B. decumbens.* In an existing pasture of *Brachiaria* we introduced *Cratylia* in rows (1 m between rows and 1 m between plants within rows). In each experiment 6 cows were assigned to one of three treatments arranged in a 3 x 3 Latin Square design: T 1- Grass alone, T2 Grass + *Cratylia* cut and fed at milking (1.5 kg of DM /100 kg BW) and T3 – Grass + *Cratylia* in association with the grass grazed by cows. The two experiments were carried out during a period of adequate rainfall.

Results shown in Table 8 indicate that milk yield was 17 and 14 % higher when cows grazed *Cratylia* as compared to the grass alone in experiment 1 and 2, respectively. Feeding *Cratylia* at milking (cut and carry) did not increase milk yield relative to the no supplementation treatment, which is consistent with other results that have showed limited response to *Cratylia* supplementation during the wet season.

Table 8. Milk production and milk urea nitrogen (MUN) of cows fed *Cratylia argentea* in different systems

Feeding System	Experiment 1		Experiment 2	
	Milk Yield (l/cow/d)	MUN (mg/dL)	Milk Yield (l/cow/d)	MUN (mg/dL)
Grass alone	6.1 b	9.8 b	6.3 d	3.0 b
Grass + *Cratylia* Cut and Carry	6.7 b	33.6 a	6.6 d	11.6 a
Grass + *Cratylia* Direct Grazing	7.5 a	27.3 a	7.3 c	12.5 a

a, b Values in the same column with the same letters are not different ($P<0.05$).
c, d Values in the same column with the same letter are not different ($P<0.10$).

As expected in the two experiments, the values of MUN were higher in the treatments with *Cratylia* as compared to the grass alone (Table 8). However, MUN value in cows fed *Cratylia* were between 2 and 3 times greater in experiment 1 than in experiment 2, indicating higher legume intake possibly as a result of the lower green DM on offer in the *Brachiaria* pasture. In experiment 1 the actual amount of *Cratylia* consumed in the cut and carry system was greater than in experiment 2, which is again consistent with the higher MUN values recorded. Other milk quality parameters such as fat (3.6% and non-fat solids (8.2 %) did not differ among treatments within experiment or between experiments.

Progress towards achieving milestones

- **Known utility of *Calliandra* as protein supplement for ruminants**

We have made considerable progress in defining the feed value of the shrub legume *Calliandra* through experiments carried out with sheep. Our findings indicate differences in feed value between provenance of *Calliandra* associated to fiber and insoluble condensed tannin concentrations. Supplementing sun dried *Calliandra* did not negatively affect intake of the legume as previously thought, but it did not translate in improved N utilization when compared to feeding fresh *Calliandra*. We also found that the feed value of *Calliandra* improved when the legume was grown in fertile soil as compared to acid low fertility soils. Future studies will validate results obtained with confined sheep with grazing milking cows supplemented with fresh and sun-dried *Calliandra* provenances grown in locations with different soil fertilities.

- **Known utility of C*ratylia* under direct grazing by milking cows**

Our results indicate that sowing *Cratylia* in pastures for direct grazing by cows results in more milk production as compared to the cut and carry system even during periods when rainfall is not limiting, as was the case in the two experiments reported. Sowing *Cratylia* in mixture with the grass as compared to the cut and carry system being currently promoted in farms will undoubtedly provide additional benefits to farmers due to reduced labor and improved N cycling in the pastures.

1.3 Assessment of quality and animal production potential of selected grasses

Highlights

- Confirmed that milk production with *B. brizantha* (CIAT 26110) cv Toledo is lower than with other commercial *Brachiaria* cultivars.

- Found that milk production with the *Brachiaria* hybrid (CIAT 36061) cv Mulato, the first hybrid to reach cultivar status, was higher than in other commercial *Brachiaria* cultivars

1.3.1 Milk yield with new accessions and hybrids of *Brachiaria*

Contributors: P. Avila, J. W. Miles, and C. Lascano

Last year we reported that milk production had been 11% lower in cows grazing *B. brizantha* CIAT 26110 (now cv Toledo) as compared to *B. decumbens* cv Basilisk and *B. brizantha* cv Marandu and that this was probably associated with lower crude protein (CP) in the forage as indicated by low MUN values (5.4 vs 7.0 mg/ dL). Subsequent results confirmed that forage on offer in pastures of CIAT 26110 had lower CP content (3.9%) as compared to the forage on offer in Marandu and Basilisk (5.0%). However, we argued that an advantage of CIAT 26110 as compared to the commercial cultivars was its greater biomass production and fast rate of regrowth following defoliation.

To reconfirm results from last year, we carried out in 2000 a second trial (Latin Square design) during a period of adequate rainfall to compare milk yield of cows grazing *B. decumbens* cv Basilisk, *B. brizantha* cv Marandu and *B. brizantha* (CIAT 26110) cv Toledo.

Results shown in Table 9 indicate that milk yield was higher with *B. decumbens* cv Basilisk than with *B. brizantha* cv Marandu and cv Toledo (CIAT 26110), but was similar in the two *B. brizantha* cultivars.

Table 9. Milk production and milk urea nitrogen (MUN) of cows grazing commercial cultivars and a hybrid of *Brachiaria*

Pastures	Experiment 1		Experiment 2	
	Milk yield (l/cow/d)	MUN (mg/dL)	Milk yield (l/cow/d)	MUN (mg/dL)
B. decumbens cv Basilisk	7.0 a	3.6	7.6 a	4.1 b
B. brizantha cv Marandu	6.7 ab	4.2		
B. brizantha (CIAT 26110) cv Toledo	6.3 b	3.3	6.5 b	4.3 b
Brachiaria hybrid (CIAT 36061) cv. Mulato			8.1a	9.7 a

a, b values within experiments with the same letters are not different ($P<0.05$).

MUN values were not significantly different among treatments, but tended to be lower in *B. brizantha* cv Toledo, which was related to lower CP (5%) in the forage on offer as compared to CP values (6%) in the other two cultivars. The amount of forage on offer was higher in cv Toledo (2,200 kg green DM/ha) than in cv Basilisk (1600 kg green DM/ha) and cv Marandu (1200 kg green DM/ha), which again indicates that a major advantage of cv Toledo (CIAT 22610) is its high forage yield capacity, which translates into high stocking rate capacity and high animal productivity per unit of land.

This year we also compared the animal production potential of the *Brachiaria* hybrid CIAT 36061, which is now commercial and known as cv Mulato, with commercial check cultivars. Using a Latin Square design, six cows were assigned to one of the following treatments: T1: *B. decumbens* cv Basilisk, T 2 *B. brizantha* (CIAT 26110) cv Toledo and T3 *Brachiaria* hybrid (CIAT 36061) cv Mulato.

Results shown in Table 9 indicate that milk yield was 25% greater in Mulato than in Toledo and 7% higher than in Basilisk, but with this cultivar the difference was not significant. It was interesting to observe that MUN values were twice as high in cv Mulato as in the other two *Brachiaria* cultivars, suggesting a higher concentration of CP in the forage on offer. As in other experiments the amount of forage on offer in the pastures was greater in cv Toledo (4300 kg of green DM/ ha), followed by Mulato (3900 kg green DM/ha) and Basilisk (2300 kg of green DM/ha).

Progress towards achieving milestones

- **Defined benefits in animal production of new accessions and hybrids of *Brachiaria* relative to commercial cultivars**

The results from the two grazing trials carried out this year confirm that the forage quality of *B. brizantha* cv Toledo (CIAT 26110) is lower than that of other commercial *Brachiaria* cultivars due mainly to lower protein content in the forage on offer and forage consumed by grazing animals. However, one advantage of cv Toledo over the other *Brachiaria* cultivars is its higher biomass production, which translates into higher stocking rate capacity, which is a key factor for intensification of dual purpose cattle farms in subhumid and humid environments.

A very significant finding this year was the higher milk production potential of the *Brachiaria* hybrid (CIAT 36061) cv Mulato as compared to well known commercial *Brachiaria* cultivars, which appears to be associated with higher forage quality. This superior performance of the first *Brachiaria* hybrid that reaches cultivar status is a major accomplishment of CIAT's *Brachiaria* breeding program.

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

Activity 2.1 Study the bioecology of spittlebug species in contrasting environments

Highlights

- Completed comparative biological studies on three spittlebug species (*Zulia carbonaria, Zulia pubescens, Zulia* sp. nov.) and imitated studies on a fourth (*Prosapia simulans*).
- Detected and made preliminary assessment of the presence of the Central American forage grass and sugar cane pest, *Prosapia simulans*, in *Brachiaria decumbens* of the Cauca Valley, which is a first report of the species and genus in Colombia and South America.
- Further characterized substrate communication in spittlebug adults by describing the male courtship calls of three species and confirming significant differences in call structure among taxa.
- Gathered data on early season population dynamics of spittlebug nymphs and adults in three contrasting sites to measure the correlation between phenology and rainfall and to gauge potential to predict the timing of outbreaks.
- Established new studies on egg diapause including an experiment to test the effect of preoviposition conditions on diapause incidence and a study to document seasonal changes in diapause incidence among field populations in three contrasting sites.

Despite a high pest status and long history in the Neotropics, an effective and coordinated program for the integrated management of spittlebugs in forage grasses does not yet exist. Among the factors that contribute is (1) a tendency to over generalize among the diversity of species, genera and habitat associations, (2) a poor understanding of the natural history of the family Cercopidae, (3) little biological information for the majority of economically important species, (4) scarcity of detailed site-specific studies on ecology that offer the resolution necessary to guide advances in pest management, and (5) IPM tools that are rudimentary or absent.

In 2000 we continued studies to overcome these limitations, focusing on the (1) the acquisition of new bioecological information on this pest complex and the family Cercopidae, (2) development of contrasting ecoregions in Colombia as model sites for advancing the diagnosis and management of spittlebugs, and (3) development and evaluation of research methodologies and technologies to promote higher quality research from NARS.

2.1.1 Comparative biology of *Zulia* spittlebugs

Contributors: Jairo Rodríguez, Daniel Peck

Rationale: An inadequate understanding of the biology and behavior of most spittlebug species, plus a tendency to over generalize in those same aspects among species, has contributed to their ineffective management. Of the 15 species of spittlebugs associated with forage grasses in Colombia, only five have had their biology studied to any degree: *Aeneolamia lepidior, A. reducta, A. varia, Mahanarva* sp. nov., *Prosapia simulans* and *Zulia carbonaria*. To advance our understanding of the patterns of variation among taxa, we are examining the biology of three species in the genus *Zulia*: *Z. carbonaria, Z. pubescens* and *Zulia* sp. nov. With the exception of one study on *Z. carbonaria*, this genus has not yet been the focus of any biological or behavioral study and therefore aspects such as characterization and duration of the life stages, reproductive biology and oviposition sites are unknown and unavailable to guide advances in pest management.

Methods: Small-scale colonies were established to ensure availability of all life stages of the insect despite seasonality in the field. Source populations of *Z. carbonaria* and *Z. pubescens* were local in the department of Valle del Cauca while *Zulia* sp. nov. was collected on the Pacific coast in Nariño, its only known range in Colombia. Methods were based on previous biological studies carried out by CIAT emphasizing morphological characterization of the life stages, duration of the life stages and reproductive biology. With the aid of a stereoscope and ocular micrometer, certain aspects of the external morphology were measured for four developmental stages of the eggs, five nymphal instars, and both sexes of the adults. Adult specimens were obtained from the field, nymphs were obtained from either the field or colony, and eggs were obtained from ovipositing adults in the colony.

To measure the duration of the life stages, field conditions were replicated in the screenhouse for controlled observations of adults and nymphs (Photo 1). Teneral adults (<12 hours old) from the colony were confined in cohorts of four individuals under acetate sleeve cages over pots of *Brachiaria ruziziensis*; mortality was assessed daily. For the nymphs, recently eclosed first instars (<12 hours old) were placed singly in pots of *B. ruziziensis* established with abundant surface roots required as feeding sites; transformation from one instar to the next was determined by direct observation of the molted exuvia. The mean longevity of each life stage was based on 40 individuals. Duration of the egg stages was determined under controlled incubation conditions (27°C, 100% RH, total darkness). Recently laid eggs (<24 hours old) were maintained on moist filter paper in petri dishes and observed daily. The duration of each of the four generalized developmental stages was based on 100 individuals. To study oviposition sites as part of the description of reproductive biology, field conditions were replicated in the screenhouse. The soil surface was specially prepared with soil oviposition substrate dispersed on top with 2 g leaf litter (Photo 2).

Photo 1. Pots employed to determine the longevity of adults (A) and nymphs (B).

Photo 2. Pots for determining oviposition site preferences according to different oviposition substrates

Each pot was infested with 2 females and 2 males from the colony and once they died eggs were recovered from four oviposition substrates: uncovered soil, soil covered by leaf litter, leaf litter and the plant surface.

Results: Male and female *Z. carbonaria* were larger than *Z. pubescens* and *Zulia* sp. nov. in every morphological measure (width of head capsule and body; length of stylet, wing and body with and without wings) (Table 10). Males of *Zulia* sp. nov. were the smallest life stage. With the exception of only a few measures, females were significantly larger than males for each species.

Table 10. Morphological characterization (mm) of *Zulia* adults by sex (mean±S.E., range, n=40).

Species	Sex	Head capsule width	Stylet length	Body length with wings	Body length without wings	Anterior wing length	Body width
Z. carbonaria	F	2.69±0.12 a (2.43-1.87)	1.23±0.10 a (0.98-1.50)	11.22±0.37 a (9.21-11.43)	10.30±0.79 a (9.07-12.00)	9.11±0.52 a (8.21-11.50)	5.64±0.26 a (5.29-6.29)
	M	2.40±0.08 b (2.22-2.67)	1.16±0.06 a (1.07-1.28)	10.29±0.52 b (7.14-10.36)	9.36±1.07 b (7.29-11.93)	8.44±0.44 b (7.50-9.07)	4.87±0.26 b (4.29-5.29)
Z. pubescens	F	2.25±0.10 d (1.96-2.46)	1.03±0.07 c (0.89-1.28)	8.98±0.54 d (7.79-10.64)	8.57±1.03 dc (7.07-11.07)	7.10±0.27 e (6.29-7.50)	4.25±0.24 cd (4.14-5.36)
	M	2.14±0.06 d (1.99-2.25)	0.97±0.06 d (0.84-1.07)	8.74±0.50 de (8.93-10.71)	8.01±0.69 ed (7.00-10.00)	7.13±0.34 d (6.57-8.36)	4.41±0.26 d (4.00-5.36)
Zulia sp. nov.	F	2.35±0.08 c (2.16-2.49)	1.09±0.06 b (0.98-1.24)	9.72±0.47 c (8.93-10.71)	9.04±0.92 cb (6.93-10.93)	7.75±0.40 c (7.14-9.21)	4.65±0.30 c (4.00-5.21)
	M	2.08±0.08 f (1.99-2.25)	1.05±0.06 bc (0.96-1.16)	8.65±0.34 e (8.07-9.57)	7.073±1.08 e (5.93-9.64)	7.12±0.29 d (6.36-7.79)	4.04±0.20 e (3.64-4.36)

Within columns, means followed by different letters are significantly different (P<0.05).

Each species presented the four generalized developmental stages established for *A. varia* and other spittlebug species. In terms of size, *Zulia* sp. nov. eggs were smaller than the other two species during each developmental stages (Table 11). In phase S4, within 1-2 days of hatch, eggs of *Z. carbonaria* and *Z. pubescens* were 1.12 times longer than *Zulia* sp. nov. Despite significant differences in size between adult *Z. carbonaria* and *Z. pubescens,* no significant differences were detected in egg size with the exception of width in phase S2.

Table 11. Width and length (mm) of development stages of *Zulia* eggs (mean±S.E., range, n=68-100).

Species	S1		S2		S3		S4	
	Length	Width	Length	Width	Length	Width	Length	Width
Z. carbonaria	1.06±0.03 a (1.00-1.13)	0.30±0.02 a ((0.24-0.34)	1.07±0.03 a (0.99-1.13)	0.32±0.02 a (0.27-0.37)	1.09±0.02a (1.01-1.14)	0.33±0.02 a (0.29-0.39)	1.12±0.03 a (1.06-1.20)	0.38±0.02 a (0.34-0.43)
Z. pubescens	1.05±0.04 a (0.97-1.17)	0.29±0.02 a (0.24-0.30)	1.06±0.03 a (1.00-1.17)	0.31±0.01 b (0.27-0.33)	1.07±0.04a (1.00-1.19)	0.33±0.02 a (0.29-0.36)	1.12±0.04 a (1.00-1.24)	0.37±0.02 a (0.34-0.41)
Zulia sp. nov.	0.95±0.04 b (0.84-1.01)	0.28±0.01 b (0.24-0.31)	0.97±0.03 b (0.86-1.03)	0.29±0.01 c (0.27-0.31)	0.97±0.03 b (0.91-1.04)	0.32±0.02 b (0.29-0.34)	1.00±0.03 b (0.93-1.07)	0.34±0.01 b (0.31-0.36)

Within columns, means followed by different letters are significantly different (P<0.05).

For *Z. carbonaria* and *Z. pubescens*, there were significant differences from each instar to the next in all parameters measured (width of head capsule, length of body, anterior wing pad and stylet) (Table 12). There were also significant differences in life stages between the two species for most measures, and these differences increased with instar. *Zulia* sp. nov. nymphs were not available for measurements.

Table 12. Morphological characterization (mm) of nymphal life stages of *Zulia* (mean, n=40).

Species	Instar	Head capsule width	Body length	Anterior wing pad length	Stylet length
Z. carbonaria	I	0.39 a	1.57 a	-	0.32 a
	II	0.62 b	2.54 b	-	0.43 b
	III	0.97 c	4.01c	0.33 a	0.67 c
	IV	1.50 d	6.21 d	0.95 b	1.00 d
	Va	2.18 e	9.81 e	2.80 c	1.40 f
	Vb F	2.24 f	10.62 g	2.93 d	1.45 g
	Vb M	2.21 f	10.10 f	2.85 d	1.37 e
Z. pubescens	I	0.39 a	1.48 a	-	0.32 a
	II	0.58 b	2.89 b	-	0.40 b
	III	0.90 c	3.91 c	0.35 a	0.58 c
	IV	1.34 d	5.66 d	0.86 b	0.83 d
	Va	1.92 e	8.40 e	2.37 c	1.16 f
	Vb F	1.93 e	9.53 f	2.48 d	1.10 e
	Vb M	1.88 f	9.43 f	2.53 d	1.12 f

Within columns for each species, means followed by different letters are significantly different (P<0.05).

Mean adult longevity was 19.6, 18.4 and 14.1 days, respectively, for *Z. carbonaria*, *Z. pubescens* and *Zulia* sp. nov. Longevity for *Zulia* sp. nov. was significantly less. By gender, longevity varied from 12.9-20.1 days among the three species (Table 13). No differences in longevity were detected between sexes of the same species.

Table 13. Longevity (days) of *Zulia* adults by sex (mean, n=24-40).

Species	Sex	Longevity
Z. carbonaria	Female	20.4 a
	Male	18.4 a
Z. pubescens	Female	19.4 a
	Male	17.6 ab
Zulia sp. nov.	Female	14.9 bc
	Male	12.9 bc

Within columns, means followed by different letters are significantly different (P<0.05).

Total duration of egg development varied from 14.3-17.4 days among the three species (Table 14), with *Z. carbonaria* > *Zulia* sp. nov. > *Z. pubescens*. Although S2 was the shortest stage for all species, S4 was the longest for *Z. carbonaria*, S3 for *Z. pubescens* and S1 for *Zulia* sp. nov., representing 38.9, 33.0 and 41.9%, respectively, of the total egg development time.

Table 14. Duration (days) of *Zulia* eggs by development stage (mean±S.E., range, n=108-126).

Species	S1	S2	S3	S4	Total
Z. carbonaria	5.93±0.70 a (5-8)	1.07±0.25 a (1-2)	3.69±0.53 b (3-5)	6.77±0.54 a (5-8)	17.40±0.91 a (12-20)
Z. pubescens	4.14±0.35 b (4-5)	1.05±0.21 a (1-2)	4.73±0.44 a (4-5)	4.42±0.50 c (4-5)	14.34±0.51 c (13-16)
Zulia sp. nov.	6.11±0.59 a (5-8)	1.10±0.30 a (1-2)	2.92±0.78 c (2-4)	4.69±0.53 b (3-6)	14.57±1.49 b (10-18)

Within columns, means followed by different letters are significantly different (P<0.05).

Total duration of nymph development varied from 38.0-42.6 days among the three species (Table 15). Duration was significantly longer in *Z. pubescens* given statistically longer stadia in instars I – IV. For each species, instar V was longer than other instars, representing 30.6, 33.1 y 30.7% of total nymphal development time for *Z. carbonaria*, *Z. pubescens* and *Zulia* sp. nov., respectively. For *Z. carbonaria* and *Z. pubescens*, there were no differences between instars I, II and III, but duration was incrementally longer in instars IV and V. For *Zulia* sp. nov., there were no differences among instars I–IV.

Table 15. Duration (days) of *Zulia* nymphs by instar (mean, n=40).

Species	**Instar**					
	I	**II**	**III**	**IV**	**V**	**Total**
Z. carbonaria	7.48 a	7.20 a	6.38 a	8.33 a	12.98 a	42.35 a
Z. pubescens	6.65 b	6.28 b	5.63 b	7.08 c	12.57 a	37.95 b
Zulia sp. nov.	7.96 a	7.13 a	6.71 a	7.79 b	13.08 a	42.67 a

Within columns, means followed by different letters are significantly different (P<0.05).

Based on these studies, the complete life cycle of *Z. carbonaria*, *Z. pubescens* and *Zulia* sp. nov. was 69.6, 61.5 and 64.4 days, respectively (Table 16).

Table 16. Life cycle summary for three *Zulia* species.

Life stage		**Duration (days)**		
		Zulia carbonaria	***Zulia pubescens***	***Zulia* sp. nov.**
Egg	Sum	17.4	14.3	14.6
	S1	5.9	4.1	6.1
	S2	1.1	1.0	1.1
	S3	3.7	4.7	2.9
	S4	6.8	4.4	4.7
Nymph	Sum	42.4	38.0	42.7
	Instar I	7.5	6.6	8.0
	Instar II	7.2	6.3	7.1
	Instar III	6.4	5.6	6.7
	Instar IV	8.3	7.1	7.8
	Instar V	13.0	12.6	13.1
Adult	Half longevity[1]	9.8	9.2	7.1
	Female	20.4	19.4	14.9
Life cycle		69.6	61.5	64.4

[1] Mean longevity calculated from the Weibull distribution.

Each species demonstrated some degree of flexibility in oviposition preferences by using two or more oviposition substrates (Figure 3). However, there were marked differences among species in those preferences. Both *Z. carbonaria* and *Zulia* sp. nov. preferred uncovered soil, laying 72.7 and 73.5% of their eggs in that substrate. *Z. pubescens* preferred laying eggs on the plant surface where 59.2% of eggs were recovered.

Discussion: In terms of size, adult spittlebugs from the genus *Zulia* exhibit the same sexual dimorphism as other genera studied to date (*Aeneolamia*, *Mahanarva*, *Prosapia*) in which females are larger than males in most body size measures. Reduction in the stylet length from Instar V to adult in *Z. carbonaria* and *Z. pubescens* is consistent with results from other species (*A. lepidior*, *A. reducta*, *Mahanarva* sp. nov., *Prosapia* sp. nov.). Eggs of all three species passed through the four generalized developmental stages expressed in other species (*A. lepidior*, *A. reducta*, *A. varia*, *Mahanarva* sp. nov., *Prosapia* sp. nov.). In terms of egg size, there appear to be general trends across genera where *Prosapia* = *Mahanarva* > *Zulia* > *Aeneolamia*.

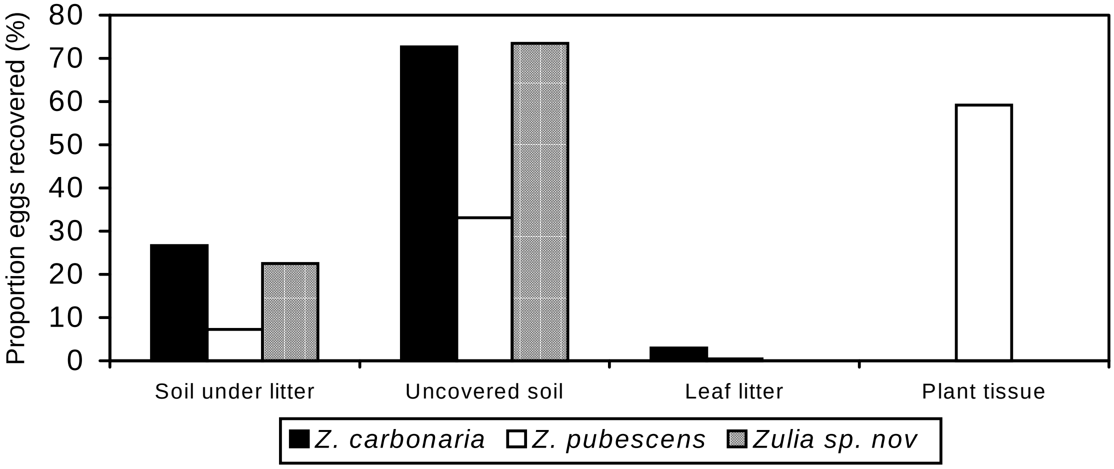


Figure 3. Oviposition site preferences determined in choice trials where eggs were recovered from four different substrates.

The physical parameters measured for the nymphs demonstrate morphologically distinguishable instars. Like in other species, little overlap in head capsule width among instars makes this character highly diagnostic of instar within species. When supported by other physical characters such as degree of sclerotization and size and form of the wing pads, it is possible to accurately distinguish instars. Early and late instar V (Va, Vb) are distinguished by physical characters other than size, such as visibility of adult structures, similar to other species of spittlebug studied previously.

Adult longevity in *Zulia* is considerable longer than most previously studied species (*A. lepidior*, *A. reducta*, *A. varia*, *Mahanarva* sp. nov.) with the exception of *Prosapia* sp. nov. Mean longevity of adult *Z. carbonaria* was 7-8 days greater than those obtained in a previous study while duration of the egg stage was similar (Arango & Calderón 1981). Nymphal longevity for *Zulia* was greater than that obtained for *Aeneolamia* but similar to *Mahanarva* sp. nov. Unlike *A. lepidior* and *Mahanarva* sp. nov., for these species of *Zulia* instar V was the longest. Overall generation time varied from 61.5-69.6 days among species. The generation time calculated for *Z. carbonaria* (69.6) was similar to that determined in one previous study (72 days)

Consistent with other species, *Zulia* lays eggs in the soil, leaf litter and on the plant stem. Species-specific preferences vary widely, however. Two species prefer soil substrate like the genus *Aeneolamia*, while *Z. pubescens* prefers to lay eggs on the plant as is being demonstrated for *P. simulans* (see activity 2.1.3). The methodologies established here have proven adequate for gathering biological information on previously unstudied spittlebug species. Continued studies will enable us to assess patterns of variation in spittlebug bioecology to help guide advances in spittlebug management.

2.1.2 Detection of the Central American forage and cane pest, *Prosapia simulans*, in South America

Contributors: Daniel Peck, Ulises Castro, Francisco López, Anuar Morales, Jairo Rodríguez

Rationale and Methods: *Prosapia simulans* is the most widely distributed species of grassland spittlebug, reported in the lowland tropics from Mexico to Panama. It is also a major pest of sugar cane in Central America. To our knowledge, this species and the genus *Prosapia* have never been reported in South America. Herein we report the first field detection of *P. simulans* in Colombia, quantitative measures of field abundance to make a preliminary assessment of population density and persistence, and an additional record from museum specimens collected in Venezuela. A manuscript has been submitted that includes a summary of the literature on geographic distribution, bionomics and pest status; diagnostic characters to distinguish it from other grassland spittlebugs in northern South America; and a discussion of its possible mode of introduction and pest status potential.

Results: Six populations of *P. simulans* were discovered in the Cauca Valley in 1999-2000. All specimens were identified using characteristics of the male genitalia and compared with type specimens at the Natural History Museum (BMNH), London. The first report was a single adult female obtained 2-VI-1999 during surveys of *Zulia carbonaria* populations in *Brachiaria dictyoneura* near Santander de Quilichao (Table 17). Despite additional surveys in surrounding pastures and sugar cane fields, and weekly surveys in the same site ever since, no more individuals were recovered.

Four additional populations were discovered subsequently (Table 17). Populations at these sites were persistent because *P. simulans* was detected in various visits over several months. Sites varied over a broad elevational range (1060-1620 m) and with the exception of Santander de Quilichao, the dominant forage grass at each site was *Brachiaria decumbens*. The greatest populations detected were in Santa Helena. A survey on 4-IV-2000 estimated densities at 46.8 nymphs/m^2 (n = 10, 0.25m^2 quadrats) and 190 adults/50 sweeps (n = 4 series of 50 sweeps). Although economic thresholds based on quantitative yield loss data have never been established for grassland spittlebugs, these levels are considered highly damaging in Mexico where >30 nymphs/m^2 and >25 adults/50 sweeps are designated as "severe" infestations.

Table 17. Populations of *Prosapia simulans* detected in the Cauca Valley, Colombia.

Department	Municipality	Vereda	Elev. (m)	First detection	Host plants
Cauca	Santander de Quilichao	Santander de Quilichao	1060	2-VI-1999	*Brachiaria dictyoneura*
Valle del Cauca	Calima del Darien	Diamante la Gaviota	1575	12-VI-2000	*Brachiaria decumbens*
Valle del Cauca	Calima del Darien	La Primavera	1621	12-VI-2000	*Axonopus micay* *Brachiaria decumbens* *Cynodon plectostachyus* *Hyparrhenia rufa*
Valle del Cauca	Yotoco	Cordobitas	1535	1-II-2000	*Brachiaria decumbens* *Cynodon plectostachyus* *Saccharum officinarum*
Valle del Cauca	El Cerrito	Santa Helena (a)	1155	2-VII-1999	*Brachiaria decumbens*
Valle del Cauca	El Cerrito	Santa Helena (b)	1100	6-VII-2000	*Brachiaria decumbens* *Saccharum officinarum*

Host plants of *P. simulans* in these sites included *Axonopus micay*, *B. decumbens*, *B. dictyoneura*, *B. ruziziensis*, *Hyparrhenia rufa*, *Cynodon plectostachyus* and *Saccharum officinarum*. A single adult was found feeding on sugar cane in Cordobitas while a single nymph was reported by CENICAÑA on cane in Santa Helena. An additional record of *P. simulans* in South America was discovered from museum specimens housed at CIAT's insect collection (2 specimens) and the Natural History Museum (7 specimens), London. This material was all collected 30-V-1980 by Gerardo Pérez Nieto from Venezuela, Bolivar State, La Vergareña, in pasture, calculated to be near 6.783^{o}N, 63.559^{o}W (Figure 1). No other South American specimens were found in the collections at Cornell University, the Universidad Nacional at Palmira (Cauca Valley) or the Universidad del Valle (Cauca Valley). *P. simulans* can be separated from the other 18 species associated with wild and cultivated graminoids in Colombia and Ecuador by dorsal color pattern: dark brown to black with one transverse band across the center of the pronotum and two across the tegmen (Photo 3). As the only known member of the genus in South America, *P. simulans* is also distinguished by the genus definition of Fennah (1949, 1953) and supporting male genitalia characters discussed by Hamilton (1977).

In Mexico and Central America, there is significant variation in the color and form of the transverse bands, ranging from yellow to pink/red to orange, broad to narrow, and distinct to completely obscured particularly in females. The Colombian populations displayed a particular subset of this color and pattern variation. Of 18 males examined, all had narrow pale yellow tegminal bands with some reduction of the posterior band. The color of the venter was predominantly pink (55%), but some individuals were yellowish brown (28%) or intermediate (17%). Background tegmen color was usually brown (94%) but sometimes black (6%). Unlike *P. simulans* from Costa Rica, males from the Colombian populations had black subgenital plates with black patches on the lateral sides of the abdominal sternites. Of 10 females examined, all had both tegminal bands greatly reduced to barely evident on a black background. Female venters were black with red (90%) to yellowish brown (10%) markings.

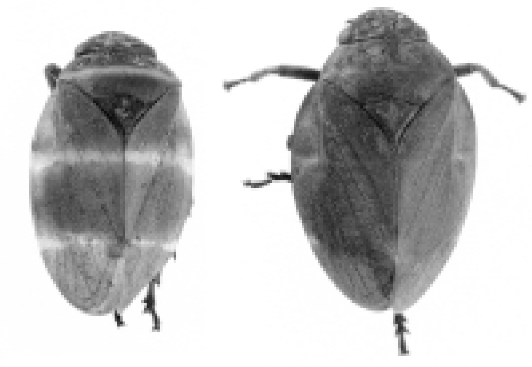


Photo 3. *Prosapia simulans*, Central American spittlebug newly reported from Colombia (Cauca Valley) and South America. Male (left) and female.

Discussion: The populations of *P. simulans* detected in *B. decumbens* are persistent, at economically damaging levels, and cover a broad elevational range (1000-1600 m). Wider surveys should be carried out to identify the distribution of *P. simulans* and monitor its spread in pastures and cane plantations of the Cauca Valley. At the spittlebug densities detected in this study, milk and beef cattle production will be negatively affected and the persistence of improved *B. decumbens* pastures will be compromised. Spread or introduction of this species to lowland regions of Colombia such as the Caribbean coast or the extensive eastern Llanos could have severe economic implications.

Up to now, *P. simulans* has not been reported on sugar cane beyond the observations noted above. Nevertheless, because the evidence suggests that *P. simulans* was introduced, cane producers should consider this species a potential threat. The menace may be heightened now as management shifts from preharvest burning to green production by the year 2005; elimination of burning may increase the susceptibility of sugar cane to this new insect pest. In Central America, this species is an injurious pest of cane in Honduras and Nicaragua. Up to now, the Cauca Valley of Colombia has been distinguished for the lack of spittlebug pests whereas essentially all other cane-producing regions of Central and South America have experienced major spittlebug pest problems.

Although the occurrence of *P. simulans* in Colombia and Venezuela could be attributed to low endemic populations only recently detected, we believe this is unlikely because *P. simulans* is an aposematic and economically important pest species. Furthermore, the Cauca Valley and Venezuela have been under relatively high surveillance over the last 20 years due to CIAT's activities and extensive fieldwork on grassland spittlebugs conducted in the 1950's across Venezuela. Our proposition is that *P. simulans* has been slowly invading from its known southern range in Panama and has advanced into the Cauca Valley from the Pacific Coast. Unfortunately, there are no known collections of cercopids from the remote areas of Chocó and the Darien to test this mode of introduction. Human-mediated introduction is one explanation for the Venezuelan reports. *Prosapia simulans* lays a majority of eggs on the plant stem (see activity 2.1.2), therefore arrival with infested vegetative material is a possibility .

Finally, these observations highlight the need for care in transfer of vegetative and soil materials associated with cercopid host plants. There is some other anecdotal evidence for regional introductions of grassland spittlebugs such as *Z. carbonaria* from the Cauca Valley into the Colombian Amazon, and an isolated report of the Central Brazil species *Notozulia entreriana* in the Colombian Llanos (see activity 2.2.1). One well-documented case is *Lepyronia coleoptrata* (Homoptera: Aphrophoridae), a Palearctic spittlebug with immigrant status in the United States (Hoebeke & Hamilton 1983). With the increasing movement of vegetative material throughout the Caribbean Basin and northward insect range expansion due to warming trends, sugar cane and forage grass production in the southern United States, like the Cauca Valley, would be threatened by the arrival of new spittlebug pests. The southeast United States already suffers from the native *Prosapia bicincta*, a damaging pest of forage grass, turf grass and ornamentals.

2.1.3 Biology and habits of *Prosapia simulans*

Contributors: Jairo Rodríguez, Ulises Castro, Anuar Morales, Daniel Peck

Rationale: The Central American forage and cane pest, *Prosapia simulans*, was recently reported for the first time in Colombia and South America (see activity 2.1.2). It is urgent to carry out a preliminary diagnosis of this pest in the Cauca Valley because several persistent populations over a broad elevational range (1100-1621 m) have been detected, some at economically damaging levels in pastures of *Brachiaria decumbens*. Moreover, this species poses a threat to sugar cane production especially with the future prohibition of burning (2005). This change in cultural practice is known to affect the status of insect pests in cane, and in Brazil it is known to have promoted previously unimportant spittlebug species to high pest status.

A literature review has shown that there is little known about the biology and ecology of this species despite being one of the most widely distributed spittlebugs in America, occurring from

Mexico to Panama. To gather biological information relevant to this species, and relevant to the conditions in Cauca Valley of Colombia, we launched studies on its bioecology.

Methods: A small colony of *P. simulans* was established to provide insects for study and overcome seasonality of field populations. Descriptive studies on biology were carried out according to previously established methodologies (see activity 2.1.1) focusing on three aspects: morphological characterization of the life stages, duration of the life stages and reproductive biology. The following is a summary of results obtained to date.

Results: For adults, mean lengths of six morphological measures were greater in females than males with the exception of the posterior wing (Table 18). For eggs, mean length and width increased with development phase (Table 19). Mean longevity of adults (n=80) was 16.5 days. No statistical difference was detected between males (15.3 days, n=40) and females (17.9 days, n=40).

Table 18. Morphological characterization (mm) of *P. simulans* adults by sex (mean±S.E., range, n=40).

Sex	Head capsule width	Stylet length	Body length with wings	Body length without wings	Anterior wing length	Body width
F	2.31±0.06 a (2.21-2.43)	0.98±0.07 a (0.89-1.16)	8.71± 0.33 a (7.29-9.29)	8.18±0.61 a (7.29-9.29)	6.80± 0.22 a (6.36-7.21)	4.63±0.15 a (4.36-5.07)
M	2.04±0.06 b (1.93-2.14)	0.89±0.03 b (0.82-0.94)	8.52±0.31 b (7.36-9.29)	7.23±0.32 b (6.57-8.14)	6.84±0.28 a (5.93-7.43)	4.16±0.14 b (3.79-4.43)

Within columns, means followed by different letters are significantly different (P<0.05).

Table 19. Width and length (mm) of development stages of *P. simulans* eggs (mean±S.E., range, n=75-100).

Parameter	Development stage			
	S1	S2	S3	S4
Length	1.16 ± 0.03 a (1.09 – 1.24)	1.18 ± 0.03 b (1.10 – 1.26)	1.21 ± 0.03 c (1.14 – 1.30)	1.25 ± 0.03 d (1.19 – 1.34)
Width	0.32 ± 0.02 a (0.29 – 0.36)	0.34 ± 0.01 b (0.31 – 0.37)	0.39 ± 0.03 c (0.30 – 0.47)	0.42 ± 0.01 d (0.39 – 0.46)

The duration of egg development differed between two collection sites, Santa Helena (El Cerrito, 1155 m elev.) and Cordobitas (Yotoco, 1535 m elev.). These two sites represented low and high elevation zones of the Cauca Valley. Mean egg development time in the lowland site was 50.5 days, significantly longer than the upland site with 18.0 days (Table 20). Eggs from the lowland site had an extended S1 and S2 stage, representing 71.5 and 19.0% of the total development time, versus 38.4 and 11.8% in the highland site. An extended S2 phase is evidence for egg diapause, however such an extended S1 stage has not been documented in grassland spittlebug before. Both stages may relate to an egg quiescence associated with unfavorable dry conditions.

During development, *P. simulans* eggs lacked certain externally visible features in particular development stages, namely appearance of a red pigment spot in S2 and red eye and abdominal spots in S3. Pigment spots were not visible until S4. For this reason it was hard to distinguish eggs of S1 and S2 (S2 is distinguished from S3 by a rupture in the chorion) and therefore an extended phase may have been erroneously attributed to S1.

Table 20. Duration (days) of *P. simulans* eggs by development stage and locality (mean±S.E., range, n=16-66).

Locality	Development stage				
	S1	**S2**	**S3**	**S4**	**Total**
Lowland: Santa Helena	36.14±4.52 (33-50)	9.60±7.30 (2-28)	3.63±1.15 (1-5)	5.57±1.02 (3-7)	50.53±3.58 a (45-57)
Upland: Cordobitas	6.90±1.09 (6-13)	2.13±1.69 (1-9)	3.98±0.77 (2-5)	5.18±0.58 (4-7)	17.99±1.27 (16-23) b

Means followed by different letters are significantly different (P<0.05).

Mean development time for nymphs was 45.6 days (Table 21). Instar V was the longest, representing 28.8% of total development time, followed by instars II and IV, and then instars I and II. From these studies, the life cycle of *P. simulans* is approximately 72.5 days (18.0+45.6+8.9, egg+nymph+1/2 adult). *Prosapia simulans* laid a majority, 82.6%, of eggs on the host plant stem, particularly the lower third. No eggs were recovered from the leaf litter while 3.6 and 13.8% were recovered from bare soil and soil under leaf litter, respectively.

Table 21. Duration of *Zulia* nymphs by instar (days) (n=40-55).

	Instar					
	I	**II**	**III**	**IV**	**V**	**Total**
Mean±S.E	6.75±1.16 a	7.54±2.16 a	9.30±2.79 b	10.04±2.26 b	13.14±2.70 c	45.59±5.45
Range	(5-11)	(4-13)	(5-17)	(5-14)	(10-20)	(35-57)

Means followed by different letters are significantly different (P<0.05).

Discussion: The methodology used here proved effective in rapidly assessing the biology of this previously unknown species. In common with most other grassland spittlebug studied to date, *P. simulans* is sexually dimorphic with adult females larger than males, instars can be reliably distinguished with certain morphological measures, and eggs increase in size with development. Observations on an extended S2 stage is evidence for egg diapause, common in most species.

Total life cycle of 72.5 days is longer than that reported from two Central American studies (58.4 and 58.0 days) and is comparable to *Z. carbonaria*, a species encountered in many of the same field sites in Colombia. The strong preference (82.6%) for oviposition on the plant surface is distinct from other Colombian species studied to date (*A. lepidior, A. reducta, A. varia, Mahanarva* sp. nov., *Z. carbonaria, Zulia* sp. nov.), with the exception of *Z. pubescens*, also found in the same field sites in Colombia, that lays 59.2% of eggs on the same substrate (see activity 2.1.1).

2.1.4 Characterization of substrate communication in adult spittlebugs

Contributors: Francisco López, Daniel Peck

Rationale: Substrate communication is a well-known mate recognition behavior in leafhoppers, planthoppers and treehoppers that has also demonstrated taxonomic utility for species differentiation. Until recently, this form of communication has not been examined in the froghoppers, or adult spittlebugs, and thereby represents a poorly understood yet fundamental aspect of behavior important to our basic understanding of this pest group. In 1998 and 1999 we developed recording and analysis methodologies to describe substrate communication for the first time in two spittlebug species, *Aeneolamia varia* and *Zulia carbonaria*. Structure of the male courtship calls were significantly different between these two taxa. To further characterize substrate communication in this insect family and gauge differences among species, male courtship calls were described in three new Colombian species: *Prosapia simulans*, *Zulia pubescens* and *Zulia* sp. nov.

Methods: All recordings were done with adult males obtained directly from the field or from small-scale colonies established at CIAT to support other biological studies. Only individual males were used in order to avoid disruption from responding females or interfering males. For recordings, males were placed on a stem of a preferred host plant with three leaves. This arrangement gave adults sufficient space and opportunity to feed and walk. *P. simulans* was offered *Brachiaria decumbens* as a host plant, *Z. carbonaria B. ruziziensis* and *Zulia* sp. nov. *B. mutica*.

Recordings were captured according to previously established methodologies. A ceramic crystal phonograph cartridge in contact with the plant stem converted vibrations into electrical signals followed by amplification and storage in a computer. Data were analyzed with software specialized for processing and analyzing sound files (CoolEdit 2000, Syntrillium Software Corporation). The following physical parameters were measured: call frequency, call duration, pulse duration and frequency of pulse repetition (FPR). Pulse was defined as the minimal unit of repetition within the call. If multiple calls were obtained for the same individual, these parameters were averaged to give an individual mean. Individual males were considered the units of repetition.

Results: A total of 11 recordings from 7 individuals was obtained for *P. simulans,* 1-2 calls per individual. Mean call duration was 5.41 sec. with a frequency of 456.22 Hz. Pulses were simple, with duration 141.40 msec. and frequency of pulse repetition (FPR) 13.36 pulses/sec. (Figure 4).

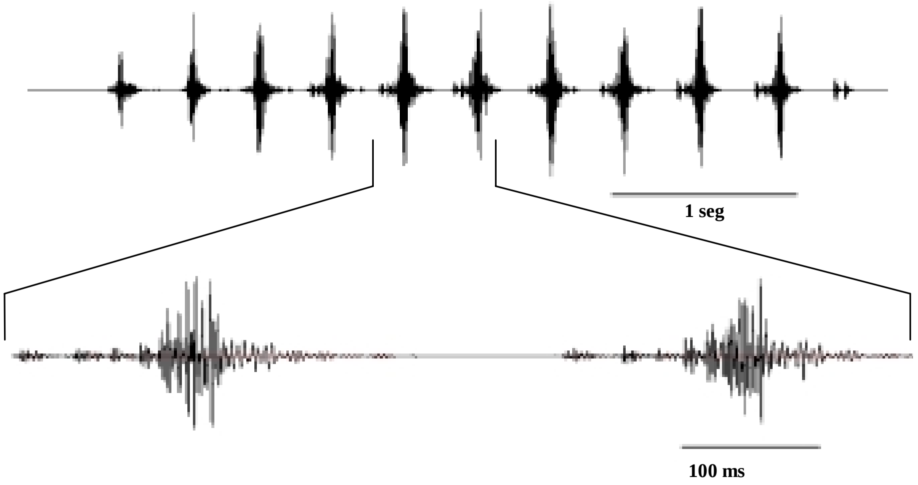


Figure 4. Courtship call of male *P. simulans* (above) with details of two pulses (below).

A total of 19 recordings was obtained from 10 individuals of *Z. pubescens*, 1-3 calls per individual. Mean call duration was 21.61 sec., much longer than any of the four other species studied to date. In certain occasions calls consisted of three well-defined phrases (Figure 5).

Mean call frequency was 376.10 Hz with pulse duration 147.44 msec. and FPR 4.98 pulses/sec. Unlike other species, pulses were not simple; they consisted of one large subpulse followed by 3 small subpulses.

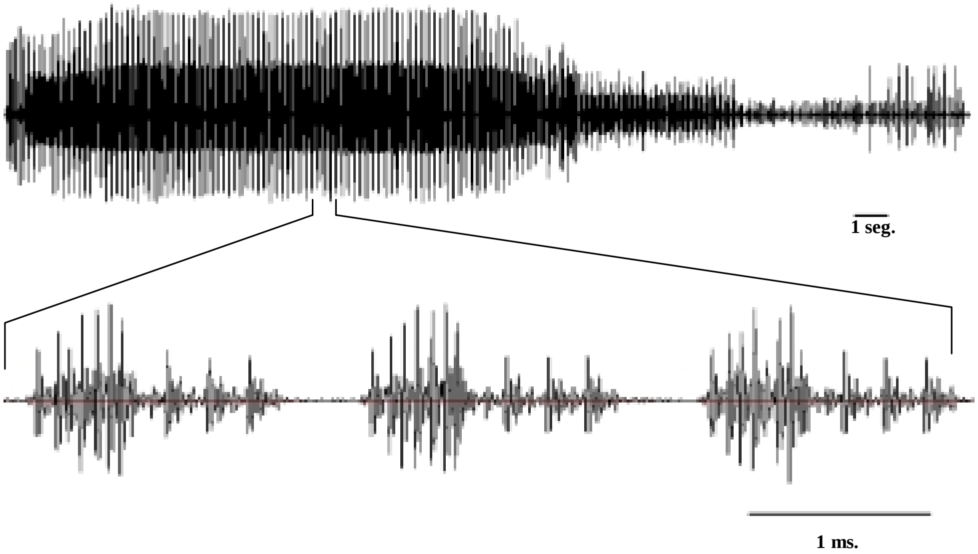


Figure 5. Courtship call of male *Z. pubescens* (above) with details of three pulses (below).

A total of 32 recordings from 9 individuals was obtained for *Zulia* sp. nov., 1-14 calls per individual. Mean call duration was 5.97 sec. with a frequency of 419.94 Hz. Pulses were simple, with duration of 63.90 msec. and FPR 8.12 pulses/sec. (Figure 6)

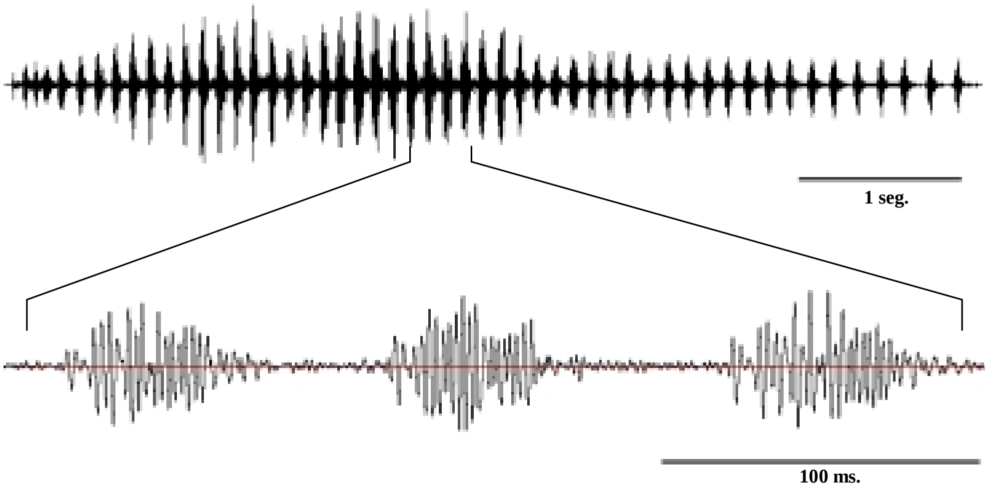


Figure 6. Courtship call of male *Zulia* sp. nov. (above) with details of three pulses (below).

There were differences among these three species in terms of all four parameters of call structure (Table 22). FRP was different for all three while call frequency, call duration, and pulse duration each had an indistinguishable species pair.

Table 22. Summary (mean±S.E., range) of physical components of male courtship calls in 5 spittlebug species (data for *Z. carbonaria* and *A. varia* obtained from CIAT 1999).

Parameter	***A. varia***	***Z. carbonaria***	***P. simulans***	***Z. pubescens***	***Zulia* sp. nov.**
Call duration	3.43±0.08	9.50±0.59	5.41±0.41 b	21.61±1.95 a	5.97±0.38 b
(sec.)	(3.35-3.51)	(8.91-10.09)	(5.00-5.82)	(19.66-23.56)	(5.59-6.35)
Call frequency	425.2±37.3	317.4±23.2	456.2±24.7 bc	376.1±19.4 a	419.9±18.8 ab
(Hz)	(387.9-462.4)	(293.9-340.9)	(431.56-480.91)	(359.2-393.0)	(401.1-438.8)
Pulse duration	22.43	57.16	141.7±8.0 b	147.4± 4.2 b	63.90±1.30 a
(msec.)	-	-	(133.72-149.58)	(143.05-151.39)	(62.60-65.20)
FPR	42.16±0.56	10.50±0.37	13.36±0.90 c	4.98±0.13a	8.12±0.25 b
(pulses/sec.)	(41.16-42.72)	(10.13-10.87)	(12.46-14.26)	(4.85-5.11)	(7.87-8.37)
N	5		7	10	8

For each parameter, means followed by different letters are significantly different ($P<0.05$); *A. varia* and *Z. carbonaria* were not included in the statistical analysis.

Discussion: Among the five species evaluated to date, the call structure of male courtship calls vary significantly in terms of all parameters measured. Whether these parameters vary with genus as well will depend on obtaining results from more species. In Colombia three species from each of three genera (*Aeneolamia*, *Mahanarva*, *Zulia*) are available for study to gather more information on the behavioral relevance of substrate communication. Additional studies will allow us to further characterize the behavior in the family Cercopidae, assess its utility as a taxonomic tool, and offer more characters to suggest patterns of relatedness among species.

2.1.5 Population dynamics and phenology of spittlebugs in the Cauca Valley

Contributors: Ulises Castro, Anuar Morales, Daniel Peck

Rationale: Spittlebug pest problems are apparently increasing in the hillsides and interandean regions of Colombia such as the Cauca Valley. This area has a bimodal precipitation pattern, thereby representing an environment for studying spittlebug seasonality that is distinct from previously studied lowland sites of the highly seasonal Caribbean coast, intermediate seasonal Orinoquia Piedmont, and the continuously humid Amazonian Piedmont. Moreover, the spittlebug complex has not yet been studied in the Cauca Valley. In this report we summarize second-year results from detailed population surveys of the spittlebug complex in the Cauca Valley.

Methods: Methods were the same as those used in previous population studies. The study farm (Las Palmas) was situated in Santander de Quilichao, Cauca, where surveys were initiated in January 1999 in pastures of *Brachiaria dictyoneura* associated with the legume *Centrosema* sp. Three 0.5 ha plots were established in separate pastures and divided into four subplots to facilitate sampling. Nymph surveys comprised counts in two $0.25m^2$ quadrats in each subplot while adult surveys comprised 50 sweeps of an insect net in each subplot. Each nymph was determined to instar, each adult was determined to sex and species, and all natural enemies were identified. Surveys were performed weekly. The following results are for the period January to May 2000.

Results: With the exception of one female, *Prosapia simulans*, (see activity 2.1.2), all adults collected in 1999 and 2000 were *Zulia carbonaria*. A total of 1062 nymphs and 550 adults were sampled over the period January-May. Total abundance of nymphs and adults varied 6.0 and 2.3 times, respectively between the plot of lowest (Plot 2) and highest (Plot 1) abundance.

Population fluctuation curves showed peaks that were less synchronous than 1999 (Figures 7, 8). Regardless, since all nymphs were determined to instar, it was possible to interpret population peaks as discrete generations based on recruitment from one life stage to the next. Although there was overlap in generations, an initial assessment was made of cumulative insect-days to calculate when each generation of nymphs and adults had accumulated 50% abundance (Table 23). Mean time between subsequent generations varied from 29.2-55.8 across the three plots. At the farm level (summed plots) generation time was calculated as 47.4 days. These calculations do not correspond well with generation time estimates (69.6 days) determined from screenhouse studies on the life cycle of *Z. carbonaria* (see activity 2.1.1). This incongruence supports the idea that the documented population peaks represent overlapping generations that did not give rise to the generation immediately following.

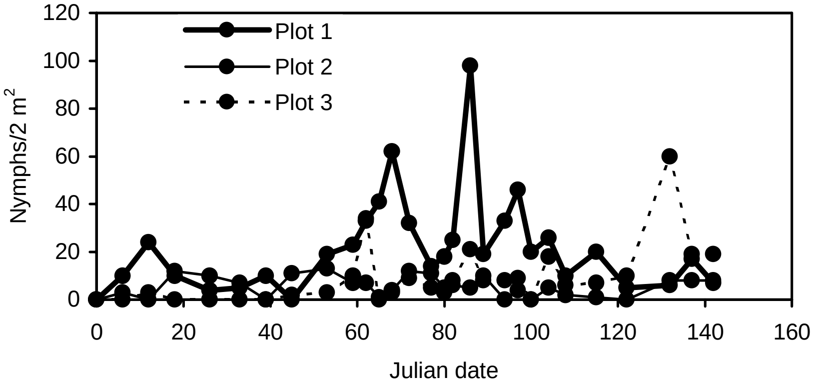


Figure 7. Fluctuation curves of *Z. carbonaria* nymphs in three plots of *B. dictyoneura*, Cauca, January-May, 2000.

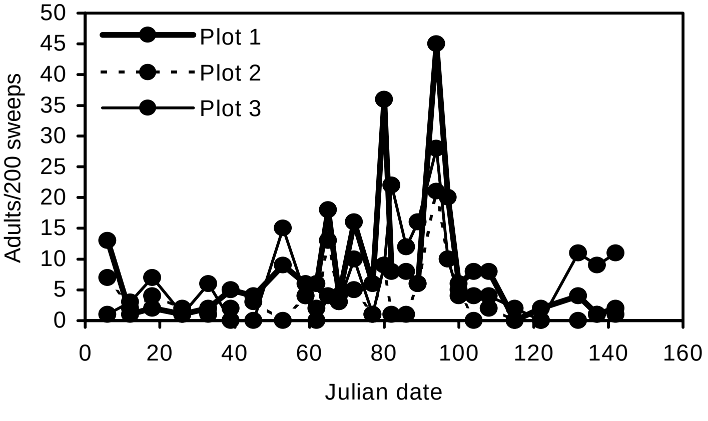


Figure 8. Fluctuation curves of *Z. carbonaria* adults in three plots of *B. dictyoneura*, Cauca, January-May, 2000**.**

The incidence of natural enemies was low compared to 1999. From January-May 2000 only 6 larvae of *Salpingogaster nigra* (Diptera: Syrphidae, predaceous on spittlebug nymphs) were sampled in spittle mass surveys, and 6 parasitic mites (Acari: Erythraeidae) were found on sampled adults. In 1999, a total of 74 larvae, 40 pupae and 9 adult *S. nigra* were sampled, as well as 79 parasitic mites.

Table 23. Population phenology of *Z. carbonaria* in Cauca expressed in terms of julian date of accumulation of 50% abundance for sequential generations.

Life Stage	Generation	Plot 1	Plot 2	Plot 3	Summed plots
Nymph	1	15.6	23.5	-	26.6
Adult	1	34.9	21.1	-	39.3
Nymph	2	66.0	54.7	60.3	63.9
Adult	2	73.3	67.8	56.8	69.5
Nymph	3	89.9	77.4	84.6	90.3
Adult	3	95.0	88.1	90.9	94.3
Mean generation time[1]		55.8	49.7	29.2	47.4

[1] Mean of time between subsequent nymph generations and adult generations (n=2 or 4)

Discussion: Future analyses will examine the correlation between population phenology and certain climatic variables such as rainfall. This site will be included in a comparative study along with Meta and Sucre to assess how well arrival of the early wet season generation can be predicted based on rainfall patterns at the end of the dry season (see activity 2.1.6). The apparent overlap of generations documented in early 2000 indicates a weaker seasonality of *Z. carbonaria* populations than expected. This may be caused by lack of a severe dry season that is expected to promote a synchronous population development upon return of the wet seasons rains. Alternatively, the diapause syndrome of *Z. carbonaria* may be distinct from other species studied to date and thereby lead to a weak correspondence between environmental seasonality and population phenology.

2.1.6 Documentation of first generation population phenology in two lowland sites

Contributors: Ulises Castro, Daniel Peck, Antonio Pérez (Universidad del Sucre), Guillermo León (CORPOICA)

Rationale: In regions with seasonal precipitation, spittlebug management may depend on suppression of the initial outbreaks before they adults colonize new areas or lay eggs that contribute to future generations. Effectively targeting control tactics therefore requires that we predict when and where the early season foci occur on the farm level. Given the correspondence between spittlebug abundance and the favorable conditions of the wet season, it may be possible to predict the arrival of the first generation of nymphs based on the precipitation patterns of the early wet season together with information on the determinants of diapause termination.

Methods: We documented the early season population dynamics of the spittlebug complex in three contrasting sites: the Caribbean coast (Corozal, Sucre), Orinoquia Piedmont (La Libertad, Meta), and the Interandean Region (Santander de Quilichao, Cauca). The data obtained from 2000 and 2001 will complement previous results from 1997 and 1998 giving repetitions over four seasonal cycles. The survey methodology in 2000 was identical to that of other population studies (see activity 2.1.5) only the survey period was limited to the early wet season, beginning one week after the first major rainfall and continuing for two months. Surveys were performed twice weekly with the collaboration of Universidad de Sucre (Sucre) and CORPOICA C.I. La Libertad (Meta). Results from Sucre and Meta follow while results from Cauca are described in activity 2.1.5.

Results: In Meta, 64 nymphs were sampled, but none were detected in Plots 2 and 3. The date (julian) of first detection was 108, with the first generation arriving at peak abundance (50% accumulated insect days) on day 117 (Table 24, Figure 9).

Table 24. Timing (julian date) of first generation spittlebug populations in two regions.

Region	Life stage	Date first detection			Date peak abundance		
		Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3
Meta	Nymphs	108	-	-	117	-	-
	Adults	104	101	101	121	122	122
Sucre	Nymphs	143	143	132	145	151	145
	Adults	143	143	143	155	161	158

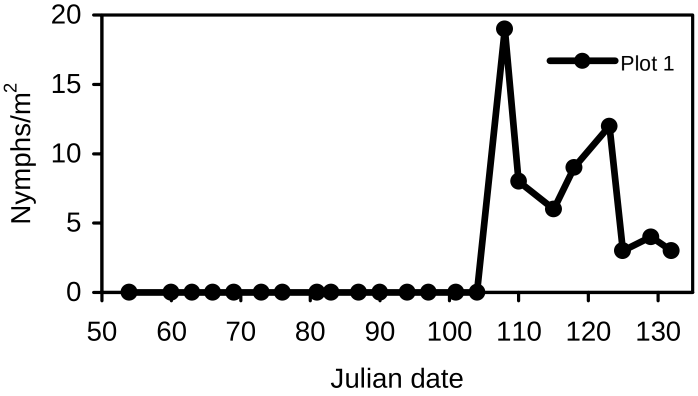


Figure 9. Population fluctuation of first generation spittlebug nymphs in Meta, 2000.

Species composition of the 566 sampled adults was 75.4% *A. varia*, 22.1% *A. reducta* and 2.5% *Z. pubescens*. Both *Aeneolamia* species were detected in all three plots while *Z. pubescens* was detected only in Plot 1. Across the three plots the proportion of *A. varia* ranged from 70.0-95.4% and *A. reducta* from 4.8-26.8% (Table 25).

Table 25. Adult abundance and species composition in three survey sites, Meta.

Species	Plot 1		Plot 2		Plot 3	
	Number	Proportion	Number	Proportion	Number	Proportion
A. varia	305	70.0%	62	95.4%	60	92.3%
A. reducta	117	26.8%	3	4.8%	5	7.7%
Z. pubescens	14	3.2%	0	0	0	0

The date of first detection of adults and the date of peak abundance varied by only 3 days across plots (Figure 10). For summed plots, the first generation adults peaked day 121, only 4 days after nymphs.

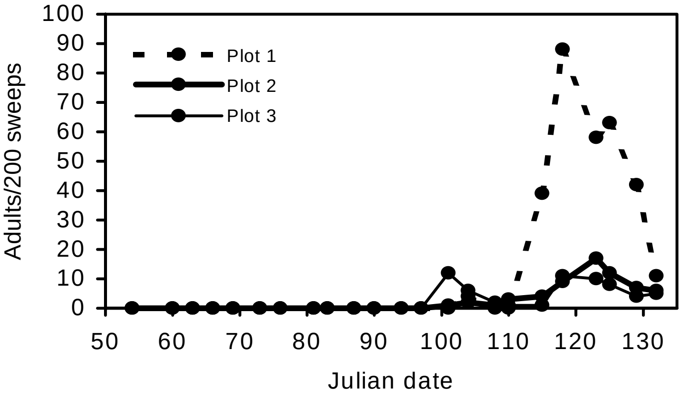


Figure 10. Population fluctuation of first generation spittlebug adults in Meta, 2000.

In Sucre, 93 nymphs were sampled. Dates of first detection across plots varied from 132-143 while dates of peak abundance varied from 145-151 (Table 24 Figure 11). Species composition of the 2953 sampled adults was 100% *A. reducta*. The date of first detection of adults was the same for each plot while the date of peak abundance varied from 155-161, or 10-13 days after the respective nymphal peak (Figure 12). For summed plots, the first generation adults peaked day 157, only 4 days after nymphs.

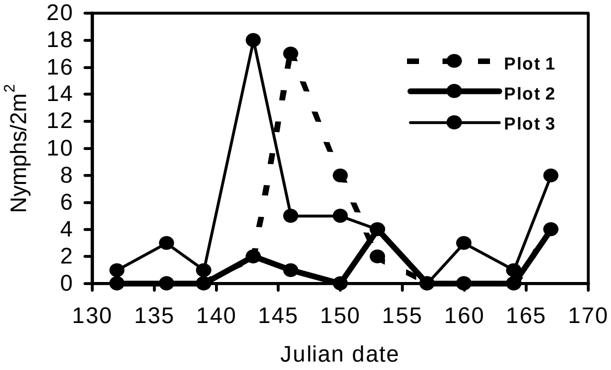


Figure 11. Population fluctuation of first generation *A. reducta* nymphs in Sucre, 2000.

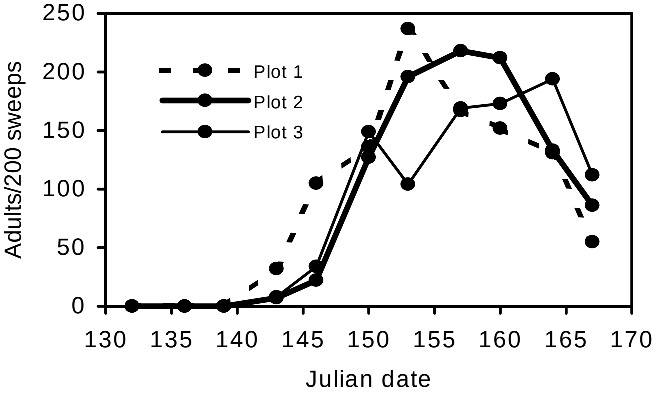


Figure 12. Population fluctuation of first generation *A. reducta* adults in Sucre, 2000.

Discussion: Precipitation data is being gathered from these sites to complement the early season population fluctuation curves. The combined data from 1997, 1998, 2000 and 2001 will be analyzed as four repetitions for Meta, Sucre and Cauca to test whether arrival of first generation spittlebugs is correlated with rainfall at the beginning of the wet season. The targeting of control tactics will depend on our ability to predict the arrival of early season outbreaks based on rainfall patterns and complemented by new information being gathered on the determinants of egg diapause (see activity 2.1.7).

2.1.7 Preoviposition determinants of egg diapause

Contributors: Ulises Castro, Daniel Peck

Rationale: Egg diapause permits synchrony between spittlebug populations and the favorable conditions of the wet season. It has been shown in some species that diapause incidence (i.e. the proportion of eggs that are not immediately developing) is lowest at the beginning of the wet season and highest at the end when late season females lay diapausing eggs that survive the harsh conditions of the dry season. An explanation for seasonal changes in diapause is hindered by little information on preovipositional cues that serve as token stimuli to the insect. In temperate zones photoperiod and temperature play important roles but in tropical areas other factors such as plant quality may be more important in the regulation of diapause induction. It is the immature that normally perceives the environmental cues that regulate diapause induction. In this phase of our continuing studies on the diapause in *A. varia* we assess the role of drought stress on the nymphal host plant.

Methods: Plants of *Brachiaria ruziziensis* were established in pots with a proliferation of surface roots necessary for nymph development. Each pot was infested with 20 *A. varia* eggs about to hatch. Four treatment combinations were established based on two factors: surface microclimate (with or without aluminum lids that maintain dark and humid soil surface conditions) and water stress (full field capacity or watering every other day at ¼ field capacity). It was predicted that poor host plant quality or extreme conditions would promote diapause induction.

The adults that emerged from each treatment were placed in separate large petri dishes for oviposition in moist filter paper. After 72 hours eggs were disinfected, incubated (27^{o}C, 100% RH, total darkness) and then scored for eclosion twice weekly. The effect of experimental conditions on diapause was measured as the proportion of eggs in diapause and time to eclosion. Nymph mortality, plant dry weight and quality were measured to confirm an effect of treatments on the host plants. Each treatment repetition consisted of 10 pots in a randomized block design with initiation date as blocks.

Results: To date two repetitions have been completed. Preliminary results show nymph survival ranging from 22-35% among treatments, diapause incidence 0 –0.2%, and time to eclosion for nondiapause and diapause eggs 19.7-20.2 and 39.9-40.1 days, respectively (Table 26). Of 4476 evaluated eggs, only 6 were diapausing.

Discussion: After two repetitions results showed no effect of these experimental adverse conditions on diapause. We are concerned that the experimental insect population is not appropriate for these studies. There is an extremely strong selection pressure in the CIAT colony against diapause because eggs that are not immediately developing are discarded and do not contribute to subsequent generations. Moreover, replenishment of the colony with field individuals has switched from Meta to Caquetá, from a seasonally dry site where diapause is advantageous to a continuously humid site where diapause may be a selective disadvantage. To address this dilemma, we are planning additional experiments using eggs obtained from females captured in the field in Meta.

Table 26. Influence of host plant quality on nymph mortality and egg diapause in *A. varia*.

Factor	No water stress		Water stress	
	With lid	**Without lid**	**With lid**	**Without lid**
Nymph survival	29%	22%	35%	23%
Eggs evaluated	1648	822	996	1010
Diapause incidence	0.24%	0.24%	0%	0%
Time to eclosion nondiapause	20.2	19.7	20.1	19.7
Time to eclosion diapause	40.1	39.9	-	-

2.1.8 Seasonal changes in the incidence and duration of egg diapause

Contributors: Ulises Castro, Daniel Peck, Antonio Pérez (Universidad del Sucre), Guillermo León (CORPOICA)

Rationale: Interpretation of the population fluctuations and phenology of spittlebugs depends on an understanding of egg diapause, which synchronizes the insect life cycle with seasonal environmental changes such as precipitation patterns. It has been shown in some species of spittlebugs that diapause incidence (i.e. the proportion of eggs that are not immediately developing) is lowest at the beginning of the wet season and highest at the end when late season females lay diapausing eggs that survive the harsh conditions of the dry season. We are examining seasonal changes in diapause incidence in three contrasting sites of Colombia to complement studies on population dynamics.

Methods: One year of field sampling began in January 2000 in three sites where ongoing surveys are documenting population fluctuations of different spittlebug species: Cauca (*Zulia carbonaria*), Meta (*Aeneolamia varia*) and Sucre (*Aeneolamia reducta*). This work was done in collaboration with CORPOICA C.I. La Libertad and the Universidad del Sucre. In three previously established study plots in each regions (see activities 2.1.5, 2.1.6), egg collections were made every 2 weeks. Two samples of field caught females were placed in separate large petri dishes for oviposition on moist filter paper. Given differences in availability according to site and date, groups consisted of 1-5 individuals for Cauca and 1-25 for Meta and Sucre. After three days, eggs were sent by express mail to CIAT, disinfected (2-3% solution of sodium hypochlorite for 3 min and rinsed thoroughly with distilled water) and incubated (27^{o}C, 100 % RH, total darkness). Twice weekly the groups were evaluated for egg eclosion, and eggs that eclosed after 30 days were considered diapausing.

Results: Of a total 18,753 viable eggs evaluated from January-August, only a very small proportion (0.18%) was diapausing: 0.27% of the total collected in Cauca, 0.06% in Meta and 1.39% in Sucre (Table 27). Over 16 collections in Cauca (n=1445 eggs), diapausing eggs were detected on only two sequential dates representing 2.1 and 5.7% of the total eggs. Over 9 collections in Meta (n=9408 eggs), diapausing eggs were detected only once (0.16%), and over 4 collections in Sucre (n=7900 eggs) a very small proportion of diapausing eggs (0.05 – 3.16%) was detected each date.

Mean time to eclosion for nondiapause eggs of *Z. carbonaria*, *A. varia* and *A reducta* was 18.2±2.6, 17.2±1.8, and 18.0±1.2 days, respectively. For diapausing eggs, mean eclosion was 48.2±12.5, 39.1 (6 eggs eclosed the same day) and 51.2±13.2 days.

Table 27. Incidence of egg diapause in field populations of three spittlebug species.

Cauca: *Z. carbonaria*		**Meta: *A. varia***		**Sucre: *A. reducta***	
Collection date	**Proportion Diapause (%)**	**Collection date**	**Proportion Diapause (%)**	**Collection date**	**Proportion Diapause (%)**
28 March	0	27 April	0	9 June	0.05
7 April	0	11 May	0	30 June	0.12
12 April	0	25 May	0	14 July	3.16
5 may	0	8 June	0	28 July	0.85
15 May	0	23 June	0		
17 May	0	5 July	0.16		
27 May	0	18 July	0		
5 June	0	1 August	0		
9 June	0	16 August	0		
16 June	0				
23 June	0				
2 July	2.08				
13 July	5.66				
25 July	0				
3 August	0				
21 August	0				

Discussion: In Cauca and Sucre, dates with diapausing eggs correspond to brief dry periods in the middle of the wet season (“veranillo de San Juán”), which may have prompted diapause induction in the small proportion of eggs. Meta did not experience a veranillo nor did eggs from that region show more than a barely detectable level of diapause. Over the study period completed to date, all three regions were in the rainy season during which high proportions of immediately developing eggs are predicted. We expect to document an increasing proportion of diapausing eggs laid by females at the end of the wet season when egg dormancy should be advantageous for dry season survival.

Progress towards achieving milestones

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

Previously established research methodologies were implemented to continue characterizing the natural history of the family Cercopidae, the comparative biology of the major spittlebug pests in Colombia, and the population ecology of the spittlebug complex in contrasting sites. The Interandean Region (Cauca Valley), with bimodal annual precipitation, was developed as a fourth model region that includes the Caribbean Coast (Córdoba and Sucre, highly seasonal for precipitation), Orinoquia Piedmont (Meta, intermediate seasonal) and Amazonian Piedmont (Caquetá, continuously humid). Development of these sites is crucial for linking bioecological information to improvements in pest management. Our research on the spittlebug complex in each of these regions is establishing the patterns of variation in biology, behavior and ecology, fundamental for advancing management by tailoring control tactics to the diverse habitats, regions and production systems where spittlebugs are economically important.

Activity 2.2 Diagnosis of spittlebug for elaborating IPM components

Highlights

- Confirmed 18 species and 7 genera of spittlebugs associated with graminoids of Colombia and Ecuador (15 species in Colombia, 9 in Ecuador, 6 in both countries), including 7 species for Colombia and 4 for Ecuador not yet reported in the literature, data on 27 host plants, and distribution data for 21 of 32 Colombian departments.
- Demonstrated the effectiveness of an artificial diet for maintaining spittlebug adults and thereby its potential as a tool to screen factors of interest to genetic transformation in *Brachiaria*.
- Strengthened the collection of fungal entomopathogens of spittlebugs, which now includes 71 strains, from 10 genera and 12 species of fungus, isolated from 4 genera and 7 species of Colombian spittlebugs collected in 6 departments.
- Screened 28 new fungal entomopathogen isolates to adults of *Aeneolamia varia*, obtaining high virulence measures of up to 95.1% adult mortality for *Metarhizium*, 62.8% for *Paecilomyces* and 53.5% for *Fusarium*.
- Developed and evaluated new methodology to screen fungal entomopathogens for virulence to spittlebug nymphs, obtaining up to 87.1% mortality compared to 24.6% in the control.

Efforts in spittlebug management have been compromised by difficult access to the literature, inappropriate research methodologies and lack of a model system for tailoring IPM to the contrasting regions and livestock systems where spittlebugs occur. In addition, the tools required to advance the integrated pest management of spittlebugs in forage grasses are rudimentary or absent.

Results from CIAT's group on Spittlebug Bioecology and IPM over the period 1997-2000 offer the most detailed information on this pest complex for any country. Through the development of contrasting ecoregions as model sites for advancing the diagnosis and management of this pest complex, these studies will serve as a template for other regions or countries confronting their own problems with this pest. Linking these results to advances in spittlebug IPM will depend on the transfer and diffusion of new information, diagnostic tools, and research methodologies and technologies.

In 2000 we continued to advance the diagnosis of spittlebugs through studies on diverse components of IPM. This research includes spittlebug identification, distribution and taxonomy; artificial diet; collection, evaluation and deployment of fungal entomopathogens; and preliminary evaluation of economic impact.

2.2.1 Identity and distribution of spittlebugs associated with graminoids of Colombia and Ecuador

Contributors: Daniel Peck

Rationale: Variation in the biology, habitat and taxonomy of neotropical spittlebugs seriously compromises their effective management given the tendency to overgeneralize the diverse insect/host/habitat associations. Despite certain broad generalities, there is considerable bioecological variation in aspects such as duration of the life stages, oviposition sites and number of generations per year.   In addition, cercopids are pests in diverse habitats because of their wide geographic (southeast U.S. to northern Argentina), altitudinal (0-3000 m elev.), habitat management (intensive to extensive grazing systems) and host plant (essentially all economically important genera of forage grasses, sugar cane, and occasionally other graminoid crops such as rice and turfgrass) range. These dimensions have implications for pest status and the tailoring of control strategies to particular sites.

Grassland spittlebugs are also a taxonomically diverse group. In the Neotropics there are dozens of native species associated with wild and cultivated graminoids, representing 11 genera: *Aeneolamia*, *Deois*, *Isozulia*, *Kanaima*, *Mahanarva*, *Maxantonia*, *Notozulia*, *Prosapia*, *Sphenorhin*a, *Tunaima* and *Zulia*. Relevant species in the genera *Monecphora*, *Phytozamia* and *Tomaspis* have been transferred to other genera. All these taxa belong to the subfamily Tomaspidinae, tribe Tomaspidini (sensu Fennah 1968).

Despite their economic importance, the taxonomy of this group is not very advanced. The complex presents a high degree of intraspecific variation and interspecific convergence that complicates species differentiation. In addition, very few cercopid species have descriptions of male genitalia, a key character for determination of genus and species. Published reports and studies on grassland spittlebugs in Colombia and Ecuador are scarce, documenting 7 species for Colombia (*Aeneolamia bogotensis, A. lepidior, A. varia, Sphenorhina rubra, Zulia birubromaculata, Z. carbonaria, Z. pubescens*) and 5 for Ecuador (*Isozulia minor, Mahanarva andigena, M. phantastica, S. rubra, Z. pubescens*).

Designing an effective IPM program for this pest group will depend on precise species determinations. Recent studies are demonstrating that the expression of host plant resistance, for instance, depends on the particular spittlebug species. It is therefore critical that management tactics consider the spittlebug/habitat/host relationships in detail.

The present study was undertaken to assess and summarize the diversity, classification and distribution of spittlebugs associated with wild and cultivated graminoids of Colombia and Ecuador. This work is considered timely because broadened research on this pest in the last four years by various regional collaborators has uncovered new species, distribution and host plant records, plus nomenclature clarifications and changes that should be disseminated as an updated taxonomic foundation.

Methods: Distribution information was obtained from fieldwork (1996-2000) and revision of museum collections (1999-2000). Distribution data were collected from visits to four institutions: The Natural History Museum (London, UK) (BMNH), Cornell University (Ithaca, US) (CU), CIAT's taxonomic reference collection (Cali, Colombia) (CIAT) and the Universidad Nacional at Palmira (Palmira, Colombia) (UNP). All adult specimens were identified to species and in the majority of cases this could be confirmed through examination of type specimens at BMNH. Certain characters of the male genitalia formed the basis for determinations while color and size served as secondary supporting characters.

Information from museum collections was complemented by reports and observations from the field. In particular, these included studies carried out over the last four years by CIAT and various national collaborators (Universidad de la Amazonia, Universidad de Sucre, CORPOICA C.I. Turipaná, La Libertad, Macagual, El Mira) in the Colombian departments of Caquetá, Cauca, Córdoba, Meta, Nariño, Sucre and Valle del Cauca.

The analysis of spittlebug diversity and distribution in Ecuador was more preliminary. No museums in Ecuador were visited and few specimens were available in the four collections examined. Information from field observations was obtained during a trip to Puyo (Prov.

Pastaza) in collaboration with SESA (Servicio Ecuatoriano de Sanidad Vegetal) and from material sent to CIAT for identification from three Ecuadorian entities.

Analysis was limited to cercopids associated with wild or cultivated graminoid hosts. This subgroup of species was determined by host record information in the collection data of museum specimens or from the literature. Geographic distribution data and host plant data were limited to the examined specimens; because of taxonomic errors, it was decided not to include data from published observations (the few exceptions are highlighted).

Results: From the four institutions, 2651 mounted specimens were examined from Colombia and 85 from Ecuador. Approximately 271, 99, 22 and 20 Colombian distribution records were obtained from CIAT, UNP, CU and BMNH, respectively, but only 5, 0, 5 and 17 for Ecuador. Museum and field data were acquired for 21 of the 32 Colombian departments and 9 of the 20 Ecuadorian provinces.

The presence of 15 species from 6 genera in Colombia and 9 species from 4 genera in Ecuador was confirmed for a total of 18 species from 7 genera: *Aeneolamia*, *Isozulia*, *Mahanarva*, *Notozulia*, *Prosapia*, *Sphenorhina* and *Zulia* (Table 28, 29 and 30). *Isozulia* was not reported in Colombia while *Aeneolamia*, *Notozulia* and *Prosapia* were not reported for Ecuador. All of these genera are known from the literature as graminoid pests, however *Notozulia* was reported for the first time for Colombia and *Prosapia* for the first time in South America (see activity 2.1.2).

Table 28. Diversity of spittlebugs associated with graminoids of Colombia and Ecuador.

Species	**Country**	
	Colombia	**Ecuador**
Aeneolamia bogotensis (Distant) (*Tomaspis*)	X	-
Aeneolamia lepidior (Fowler) (*Tomaspis*)	X	-
Aeneolamia reducta (Lallemand) (*Monecphora*)	X	-
Aeneolamia varia (Fabricius) (*Cercopis*)	X	-
Isozulia astralis (Distant) (*Tomaspis*)	-	X
Isozulia minor (*christenseni*) Fennah	-	X
Mahanarva andigena (Jacobi) (*Tomaspis*)	X	X
Mahanarva phantastica (Breddin) (*Tomaspis*)	X	X
Mahanarva sp. nov.	X	X
Notozulia entreriana (Berg) (*Tomaspis*)	X	-
Prosapia simulans (Walker) (*Sphenorhina*)	X	-
Sphenorhina rubra (L.) (*Cicada*)	X	X
Sphenorhina sp. 1	X	-
Sphenorhina sp. 2	-	X
Zulia birubromaculata (Lallemand) (*Monecphora*)	X	-
Zulia carbonaria (Lallemand) (*Monecphora*)	X	-
Zulia pubescens (Fabricius) (*Cercopis*)	X	X
Zulia sp. nov.	X	X

Seven new species reports were confirmed from Colombia: *M. andigena*, *M. phantastica*, *Mahanarva* sp. nov., *N. entreriana*, *P. simulans*, *Sphenorhina* sp. 1 and *Zulia* sp. nov. There were four new reports for Ecuador: *I. astralis*, *Mahanarva* sp. nov., *Sphenorhina* sp. 2 and *Zulia* sp. nov. The species list for Ecuador includes *I. minor* (*christenseni*) and *M. phantastica,* which were not examined in this study but were both originally described from Ecuadorian specimens. Six species were found in both countries: *M. andigena*, *M. phantastica*, *Mahanarva* sp. nov., *S. rubra*, *Z. pubescens* and *Zulia* sp. nov. Two species are confirmed as undescribed: *Mahanarva* sp. nov. (Amazonian Piedmont of Colombia and Ecuador, Coastal Ecuador) and *Zulia* sp. nov. (Pacific Coast of Colombia and Ecuador).

Table 29. Diversity and distribution of spittlebugs associated with graminoids in Ecuador.

Species	**Ecuadorian provinces**	**Geographic zone**	
		Amazonia	**Coast**
I. astralis	Pastaza	X	
I. minor (*christenseni*)[1]	Napo	X	
M. andigena	Chimborazo, Esmeraldas, Guajas, Pastaza, Tungurahua	X	X
M. phantastica[1]	Tungurahua		
Mahanarva sp. nov.	Napo, Pichincha, Sucumbios	X	X
S. rubra	Napo	X	
Sphenorhina sp. 2	Pastaza	X	
Z. pubescens	Cotopaxi, Napo, Pastaza, Pichincha, Sucumbios, Tungurahua	X	
Zulia sp. nov.	Esmeraldas, Pichincha		X

[1] Specimens not examined in this study but location cited in the literature

Table 30. Diversity and distribution of spittlebugs associated with graminoids in Colombia.

	COLOMBIAN DEPARTMENT																				
Species	Ama	Ant	Atl	Bol	Boy	Cal	Caq	Cas	Cau	Ces	Cór	Cun	Mag	Met	Nar	Qui	Ris	San	Suc	Tol	Val
A. bogotensis	-	-	-	-	-	-	-	-	-	-	-	**X**	-	**X**	-	-	-	-	-	-	-
A. lepidior	-	**X**	**X**	**X**	-	**X**	-	-	**X**[1]	**X**	**X**	-	**X**	-	-	-	-	**X**	**X**	-	**X**
A. reducta	-	-	**X**	**X**	-	-	-	-	-	**X**	**X**	-	**X**	**X**	-	-	-	**X**	**X**	**X**	-
A. varia	-	-	-	-	-	-	**X**	**X**	-	-	-	-	-	**X**	-	-	-	-	-	-	-
M. andigena	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**X**	-	-	-	-	-	-
M. phantastica	-	**X**	-	-	-	**X**	-	-	**X**[2]	-	-	-	-	-	-	**X**	-	-	-	**X**[2]	**X**
***Mahanarva* sp. nov.**	-	-	-	-	-	-	**X**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N. entreriana	-	-	-	-	-	-	-	-	-	-	-	-	-	**X**[2]	-	-	-	-	-	-	-
P. simulans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**X**
S. rubra	-	-	-	-	-	-	-	-	-	-	-	-	-	**X**[2]	-	-	-	-	-	-	-
***Sphenorhina* sp. 1**	-	-	-	-	-	-	-	-	**X**	-	-	-	-	-	-	-	-	-	-	-	-
Z. birubromaculata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**X**[2]	-	-	-	-	-	**X**
Z. carbonaria	-	**X**	-	-	-	**X**	**X**	-	**X**	-	-	-	-	-	-	-	**X**[2]	-	-	-	**X**
Z. pubescens	**X**[2]	**X**	-	-	**X**[1]	-	**X**	-	**X**	-	-	-	-	**X**	**X**	-	-	**X**[2]	-	**X**	**X**
***Zulia* sp. nov.**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**X**	-	-	-	-	-	-

[1] Gorgona Island
[2] Only one record

One major change in nomenclature was confirmed. The species previously known in Colombia as *Z. colombiana* is actually *Z. carbonaria*. Furthermore, *Z. colombiana* is a junior synonym of *Z. pubescens* and should therefore be retired from usage. Secondly, although certain Colombian specimens in CIAT were labeled as *A. flavilatera*, the presence of this species in Colombia could not be confirmed. It is thought that these specimens were confused with similar morphotypes of *A. varia*. *A. flavilatera* is known from Venezuela to Surinam.

Compared to other neotropical regions, Colombia has a relatively high spittlebug diversity. Costa Rica has 8 species from 3 genera reported, Venezuela 9 species from 5 genera and Brazil 16 species from 7 genera. Colombia shares *A. lepidior*, *A. reducta* and *P. simulans* with Costa Rica; *A. bogotensis*, *A. lepidior*, *A. reducta*, *A. varia*, *P. simulans* and *S. rubra* with Venezuela; and *N. entreriana* and *S. rubra* with Brazil. More detailed distribution surveys from Ecuador should significantly increase the number of species reported for this country. In a single visit to the Amazonian region (Puyo, Prov. Pastaza) a very high local diversity was encountered: 4 species from 4 genera (*I. astralis*, *M. andigena*, *Sphenorhina* sp. 2 and *Z. pubescens*)

Distribution data for Colombia indicate that the spittlebug complex varies in general terms among ecoregions. In the lowland tropics, *A. lepidior* and *A. reducta* are most important in the Caribbean Coast, *M. andigena* and *Zulia* sp. nov on the Pacific Coast, and *A. reducta* and *A. varia* in the Eastern Llanos. In the interandean regions *P. simulans, Z. carbonaria* and *Z. pubescens* predominate in the Cauca River Valley while *A. reducta*, *Z. carbonaria* and *Z. pubescens* are most common in the Upper and Central Magdalena River Valley. The predominant species in the Andean zone are *M. phantastica* and *Z. pubescens*, while *A. varia* and *Z. pubescens* are most important in the Amazonian Piedmont. The Colombian departments with the most diverse fauna (Cauca, Meta, Valle del Cauca) correspond to the regions where collection activity has probably been the highest due to the presence of CIAT and CORPOICA. It is therefore critical that further distributional surveys be carried out in other regions, particularly the 11 departments where no records were uncovered.

Spittlebugs were confirmed from 27 host plants in Colombia and Ecuador: *Andropogon gayanus*, *Axonopus compressus*, *A. micay*, *A. scoparius, Brachiaria plantaginea*, *B. brizantha*, *B. decumbens*, *B. dictyoneura*, *B. humidicola*, *B. mutica*, *Bothriochloa pertusa*, *Bothriochloa* sp., *Calopogonium* sp., *Cestrum* sp., *Cynodon plectostachys*, *Cynodon* sp., *Dichanthium aristatum*, *Dichromena ciliata*, *Digitaria decumbens*, *Homolepsis aturensis*, *Hyparrhenia rufa*, *Melinis minutiflora*, *Oryza sativa*, *Panicum maximum*, *Pennisetum clandestinum*, *Saccharum officinarum* and *Sorghum halepense*.

Discussion: Despite their economic importance in Colombia and other Latin American countries, new taxonomic, distribution, host plant and taxonomic information has been obtained. Correct taxonomic determinations and placing of voucher specimens are critical for augmenting the impact of research. Descriptions of male genitalia are important to distinguish species, however at the geographic level it should be possible to develop keys to sympatric species based on overall body size and color pattern.

Prosapia simulans in South America, *N. entreriana* in the Eastern Llanos and *Z. carbonaria* in the Amazonian Piedmont could represent invasions or range expansion from Central America, Brazil and Cauca Valley, respectively. Evidence suggests that introductions of exotic species constitute a risk for forage grass and sugar cane production in the new habitats. Care in transfer of vegetative host plant material is merited.

More detailed inventories and distributional surveys are also required, particularly in key regions such as the Chocó, Pacific Coast and Amazonia of Colombia. The summary for Ecuador is considered only a preliminary assessment. Information on the geographic distribution and identity of grassland spittlebugs in Colombia and other regions will serve to monitor range expansion and new species introductions. The determinants of distribution of grassland spittlebugs are poorly understood. Broadened distribution surveys and evaluation of museum material could lead to more detailed analysis of geographic range. With the aid of GIS software such as Flora-Map, range can be interpreted in terms of certain climatic variables such as temperature and precipitation and thereby used to construct probability maps of occurrence for assessing areas at risk for range expansion, outbreaks or introductions.

Results indicate that distribution varies depending on species and that different geographic regions support a distinct complex. Spittlebug management strategies should therefore be formulated according to the species composition of the local complex since there is significant variation among species in terms of biology and ecology.

2.2.2 Evaluation of an artificial diet for maintenance of spittlebug adults

Contributors: Ulises Castro, Claudia Flores, Rosalba Tobón, Daniel Peck, Zaida Lentini

Rationale: This activity contributes to the development of bioassays for the evaluation of proteins with potential insecticidal properties that could be incorporated into *Brachiaria* through genetic transformation. As a fundamental first step, we are investigating artificial diets for the maintenance of spittlebug adults. This diet will enable screening of potential proteins for insecticidal effects before the process of transformation. Potential factors include lectins, which are known to have deleterious effects on other sap-sucking Homoptera.

Methods: The diets evaluated in these preliminary studies were based on a published recipe prepared for *Aeneolamia varia saccharina*. Adult *A. varia* from CIAT's colony were presented with 500 µl liquid diet in parafilm sachets (3 x 3.5 cm) while housed in large petri dishes (15 cm diameter, 2 cm tall). In a first phase, longevity of adults on the original artificial diet was compared to longevity of adults feeding on *Brachiaria ruziziensis* stems (with bases in small vial of water) in the same petri dish environment (5 repetitions).

In the second phase, five alternative diets were prepared and evaluated against the original diet. Alterations were made to reduce costs, simplify preparation, and increase effectiveness. In the modified diets 2, 3, 4 and 5, yeast extract and casein hydrolysate replaced the various amino acids components of the original diet. Ribofavin was reduced from 43 to 0.25 ml/100 ml and B_{12} was replaced by P-aminobenzoic acid in diets 3, 4 and 5. Wesson salt was replaced by individual salt components in diets 2, 3 and 4, but diet 5 had Wesson salts plus individual salt components. $MgCl_2$ and KH_2PO_4 were added to diets 4 and 5. Each modified diet was compared with the original diet on separate study dates.

For all experiments, 2 adult males and 2 adult females were evaluated per petri dish. Mean adult longevity was calculated with a Weibull distribution and compared between treatments.

Results: Adult *A. varia* effectively acquired diet through the parafilm sachets. Mean adult longevity under experimental petri dish conditions and plant stems was similar to results obtained for adults kept on potted plants under acetate cages. Although statistical analyses have not yet been performed, mean adult longevity with the original diet was three days longer than the control of plant stems (Figure 13).

Effectiveness of the original diet decreased with time: only one batch was made and mean adult longevity decreased with subsequent trials of modified diets (Figure 13). None of the modification diets therefore surpassed the original diet in effectiveness at maintaining *A. varia* adults.

Discussion: Based on these preliminary results, the evaluation methodology is appropriate for assessing the longevity of *A. varia* adults on artificial diets. A diet originally published in1967 equals or surpasses the host plant in maintaining adults under these experimental conditions. Thus far, diets modified to reduce costs and simplify preparation do not work as well as the original diet. Overall, this diet and this methodology appears show promise for developing a bioassay for plant and fungus factors of interest to *Brachiaria* transformation. New modifications of the original diet will be sought to overcome the aforementioned limitations. An additional limitation has been the precipitation of product, which should be overcome to avoid loss of active ingredients of the diet or of the extracts being evaluated. A new experimental design will be used to evaluate several diets simultaneously, including a hot plant control.

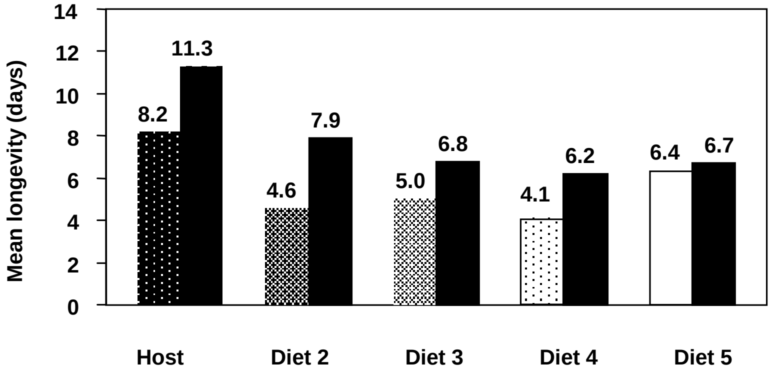


Figure 13. Mean longevity of adult *A. varia* comparing an original artificial diet (black bars) to the host plant and modified diets

2.2.3 Identity, incidence and maintenance of spittlebug fungal entomopathogens

Contributors: Anuar Morales, Rosalba Tobón, Daniel Peck

Rationale: Very few natural enemies of spittlebugs have been assessed for their potential as agents of biological control. Of the five different classes of spittlebug natural enemies in Colombia (parasitic flies, mites, nematodes; predaceous flies; fungal pathogens), fungal entomopathogens are the most diverse and widespread. As a component of IPM they have had no success in forage grasses, and have achieved only marginal, highly variable or poorly documented success in sugar cane.

One limitation is that past studies have focused on a narrow genetic diversity of isolates, largely limited to the species *Metarhizium anisopliae*. Ongoing studies on the spittlebug complex in contrasting regions of Colombia have allowed us to collect, isolate, propagate and store a diverse collection of fungal entomopathogens obtained from a broad range of spittlebug species and habitats. This ceparium is designed to serve as a source of pathogenic material for studies that focus on advancing the use of fungal entomopathogens as components for the integrated management of spittlebugs in pastures and cane fields.

Methods: Spittlebug nymphs and adults with evidence of mycosis were obtained during visits to the field. Fungal entomopathogens were isolated using two methods. If specimens were covered in mycelium or spores, and external contamination was limited, a sample was taken directly with a dissection needle for inoculation of culture medium in a petri dish. In cases where the insect was highly contaminated with little evidence of fungus, the specimen was sterilized in a test tube by vigorously agitating for 2-3 min in a solution of sodium hypochlorite (2%), rinsed 2-3 times with sterile distilled water, dried on sterile paper towel under a laminar flow hood, and divided into pieces for inoculation.

The culture medium for both isolation methods was Sabureaud agar modified with yeast extract (1%) and lactic acid (1%). Two to three days after inoculation, once the colonies measured about 1 cm diameter, the most promising were reisolated in culture medium and repeated as necessary to obtain a pure culture.

To prepare purified fungus for storage, 20-25 pieces (1 cm^2) of sterile filter paper were laid on modified Sabureaud agar in a petri dish. A small piece of the colony was taken from the vegetative growth zone and placed on each paper. After incubation and growth for 20-25 d, the pieces of filter paper were removed from the medium and dried in a new sterile petri dish under incubation for an additional 15-20 d. The dried paper and their fungus colonies were placed in labeled glycine envelopes for freezer storage in plastic boxes (–20°C).

Copies of isolates were periodically sent to the Collection of Entomopathogenic Fungal Cultures (ARSEF-USDA), Ithaca, USA for taxonomic identification by Richard Humber.

Results: The ceparium includes a total of 75 strains that have been isolated, propagated and placed under catalogued storage (Table 31). Of these, 14, 11, 40, and 10 were acquired during 1997, 1998, 1999 and 2000. A total of 71 have been obtained from spittlebugs, two from whiteflies (*Trialeurodes vaporariorum*), one from a planthopper (*Tagosodes orizicolus*) and one from a leaf miner. With collaboration of the ARSEF-USDA, 44 isolates have been identified to genus and 23 to species.

The strains isolated from spittlebugs belong to 10 different genera and 12 different species of fungus. *Metarhizium* is the most common genus with 16 isolates, all identified as *M. anisopliae*. *Fusarium* is represented by 15 isolates, none yet identified to species. *Paecilomyces* is represented by six isolates and is the most diverse genus with three species identified (*P. crustaceus*, *P. farinosis*, *P. lilacinus*), and one undetermined. The seven other fungus genera are *Aspergillus*, *Beauveria* (*B. bassiana*), *Curvularia*, *Dactylella*, *Penicillium*, *Sporothrix*, and *Trichoderma* (*T. viridae*).

Strains were isolated from both nymphal (11 isolates) and adult (60 isolates) life stages. Hosts include 4 genera and 7 species of Colombian spittlebugs: *A. reducta*, *A. varia*, *Mahanarva andigena*, *Mahanarva* sp. nov. *P. simulans*, *Z. carbonaria*, and *Z. pubescens*. Source regions include six Colombian departments (Caquetá, Cauca, Meta, Nariño, Sucre, Valle del Cauca) representing the Pacific Coast, Caribbean Coast, Amazonian Piedmont, Orinoquia Piedmont, and Interandean Region.

Discussion: The CIAT ceparium constitutes the largest collection of fungal entomopathogens isolated from grassland spittlebugs with the exception of a ceparium in Brazil that includes approximately 90 strains (CENARGEN, EMBRAPA, Brasilia). Based on the known fungal taxa, host taxa, and host ecoregions represented, the CIAT ceparium is a highly diverse collection of pathogenic material. This germplasm collection is a critical tool and resource for research on developing fungal entomopathogens as biological control agents of spittlebugs in major agroecosystems. In general, only a small number of strains of *M. anisopliae* and *B. bassiana* have ever been considered for the biological control of spittlebugs. It is anticipated that screening of this collection will identify isolates of *M. anisopliae*, *B. bassiana* and other fungal species with higher virulence than previously evaluated strains, and also identify those with enhanced quality attributes such as increased tolerance to solar radiation, low humidity and low water quality.

Table 31. Collection of fungal entomopathogens isolated from grassland spittlebugs (Homoptera: Cercopidae).

Isolate[1]		**Host**[2]	**Isolate**[1]		**Host**[2]
Accession	**Species**	**species**	**Accession**	**Species**	**species**
CIAT 001	*Metarhizium anisopliae*	*Z. pubescens*	CIAT 037	*Dactylella* sp.	*A. varia*
CIAT 002	*Metarhizium anisopliae*	*Z. pubescens*	CIAT 038	*Fusarium* sp.	*Z. pubescens*
CIAT 003	*Metarhizium anisopliae*	*A. varia*	CIAT 039	*Fusarium* sp.	*Z. pubescens*
CIAT 004	*Fusarium* sp.	*Z. pubescens*	CIAT 040	*Fusarium* sp.	*Z. pubescens*
CIAT 005	*Metarhizium anisopliae*	*Mahanarva* sp. n.	CIAT 041	undet.	*Z. pubescens*
CIAT 006	*Metarhizium anisopliae*	*Mahanarva* sp. n.	CIAT 042	*Metarhizium anisopliae*	*Z. carbonaria*
CIAT 007	*Metarhizium anisopliae*	*Z. pubescens*	CIAT 043	*Paecilomyces lilacinus*	*Z. carbonaria*
CIAT 008	*Metarhizium anisopliae*	undet.	CIAT 044	*Beauveria bassiana*	*Z. carbonaria*
CIAT 009	*Paecilomyces farinosis*	undet.	CIAT 045	undet.	*M. andigena*
CIAT 010	*Metarhizium anisopliae*	undet.	CIAT 046	*Fusarium* sp.	*M. andigena*
CIAT 011	*Paecilomyces* sp.	undet.	CIAT 047	undet.	*Z. carbonaria*
CIAT 012	*Paecilomyces lilacinus*	*Z. pubescens*	CIAT 048	undet.	*Z. carbonaria*
CIAT 013	*Sporothrix* sp.	undet.	CIAT 049	*Fusarium* sp.	*M. andigena*
CIAT 014	*Metarhizium anisopliae*	*A. varia*	CIAT 050	*Fusarium* sp.	*Z. carbonaria*
CIAT 015	*Metarhizium anisopliae*	*A. varia*	CIAT 051	undet.	*Z. carbonaria*
CIAT 016	*Trichoderma viridae*	*A. varia*	CIAT 052	*Paecilomyces crustaceus*	*Z. carbonaria*
CIAT 017	*Metarhizium anisopliae*	*A. varia*	CIAT 053	*Metarhizium anisopliae*	*Z. carbonaria*
CIAT 018	*Metarhizium anisopliae*	*A. varia*	CIAT 054	undet.	*A. varia*
CIAT 019	*Metarhizium anisopliae*	*A. varia*	CIAT 055	undet.	*A. varia*
CIAT 020	*Fusarium* sp.	*Z. carbonaria*	CIAT 056	undet.	*Z. pubescens*
CIAT 021	*Fusarium* sp.	*Z. carbonaria*	CIAT 057	undet.	undet.
CIAT 022	*Fusarium* sp.	*Z. carbonaria*	CIAT 058	undet.	*Z. pubescens*
CIAT 023	*Fusarium* sp.	*Z. carbonaria*	CIAT 059	undet.	*A. varia*
CIAT 024	*Fusarium* sp.	*Z. carbonaria*	CIAT 060	undet.	*A. varia*
CIAT 025	*Fusarium* sp.	*Z. carbonaria*	CIAT 061	undet.	*A. varia*
CIAT 026	*Metarhizium anisopliae*	*Z. carbonaria*	CIAT 062	undet.	*A. varia*
CIAT 027	*Penicillium* sp.	*Z. carbonaria*	CIAT 066	undet.	*Z. pubescens*
CIAT 028	*Fusarium* sp.	*Z. carbonaria*	CIAT 067	undet.	*Z. pubescens*
CIAT 029	*Penicillium* sp.	*Z. carbonaria*	CIAT 068	undet.	*Z. pubescens*
CIAT 030	*Metarhizium anisopliae*	*T. orizicolus*[3]	CIAT 069	undet.	leaf miner[3]
CIAT 031	undet.	*A. reducta*	CIAT 070	undet.	*T. vaporariorum*[3]
CIAT 032	*Curvularia* sp.	*Z. carbonaria*	CIAT 071	undet.	*T. vaporariorum*[3]
CIAT 033	undet.	*Z. pubescens*	CIAT 072	undet.	*Z. pubescens*
CIAT 034	*Aspergillus* sp.	*A. varia*	CIAT 073	undet.	*Z. carbonaria*
CIAT 035	undet.	*A. varia*	CIAT 074	undet.	*P. simulans*
CIAT 036	undet.	*A. varia*	CIAT 075	undet.	undet.

[1] Identifications made by Richard Humber, ARSEF-USDA, Ithaca, USA
[2] It is usually not possible to determine species of nymphal hosts
[3] Non-spittlebug host

2.2.4 Screening fungal entomopathogens for virulence to spittlebug adults

Contributors: Anuar Morales, Rosalba Tobón, Daniel Peck

Rationale: Fungal entomopathogens currently demonstrate more potential for spittlebug management than any other class of natural enemy. Despite reports of high virulence in the laboratory, however, effectiveness in the field (pastures) has never been demonstrated. Focus on a narrow diversity of isolates, lack of consideration of insect-pathogen interactions, poor formulation and application technologies, and inadequate field evaluation methodologies have compromised successful deployment. Exploiting and assessing this diversity for biological control depends on a dependable and rapid methodology for quantifying virulence in the laboratory and screening the collection of isolates. The following is a summary of investigations into a screening methodology for spittlebug adults and results of virulence screening of a diverse array of isolates.

Methods: Evaluation units were 30-day old plants (7-10 stems) of *Brachiaria ruziziensis* (CIAT 654) in pots (13 cm diameter) covered by acetate cylinders (15 cm diameter x 40 cm tall). These plants were infested with 10 adult tenerals (< 24 hours old) of *Aeneolamia varia* obtained from CIAT's colony. Two to three hours after infestation plants were sprayed with 5 ml of a concentrated conidial suspension (10^8 con /ml) with an airbrush and compressor (10 PSI). Ten repetitions (pots) were performed for each evaluated isolate, and every block (evaluation date) included a control consisting of water plus tween (0.05%). After spraying, plants and insects were maintained in a growth chamber (27°C ± 2°C, RH 80% ± 10%). The effectiveness of the treatments was evaluated 5 days later when all insects were scored as alive, dead, and dead with evidence of mycosis. Dead insects with no visible signs of fungus attack were stored in petri dishes with moist filter paper for 3-4 days to ascertain whether they were infected with fungus.

The following results pertain to 46 isolates evaluated with this methodology during 1999 and 2000, 28 of which were evaluated since last year. Of this group, 33 corresponded to *Metarhizium anisopliae*, 7 to unidentified species of *Fusarium*, 1 to *Paecilomyces farinosis*, 1 to *Paecilomyces lilacinus* and 4 undetermined. All isolates evaluated were previously reactivated on adults of *A. varia*; 35 were multisporic and 11 monosporic isolates.

Results: Overall mortality in the control was 25.1%, consistent with results from the previous year and an acceptable level for gauging efficiency. This evaluation method appears to be effective and appropriate for quantifying virulence against adults of *A. varia*.

Absolute adult mortality ranged broadly from 10.6–95.1% (Figure 14). Analysis of variance showed significant differences among isolates in virulence (P<0.0001) (Table32). Of the 46 total isolates, 17 obtained mortality scores >50%, 14 > 60%, 9 >70%, 3 >80% and 1 >90%. CIAT 054 was the most virulent, killing 95.1% of *A. varia* adults over the 5-day evaluation period. This strain, and the second most effective, CIAT 055, have not yet been identified but probably belong to the genus *Metarhizium*.

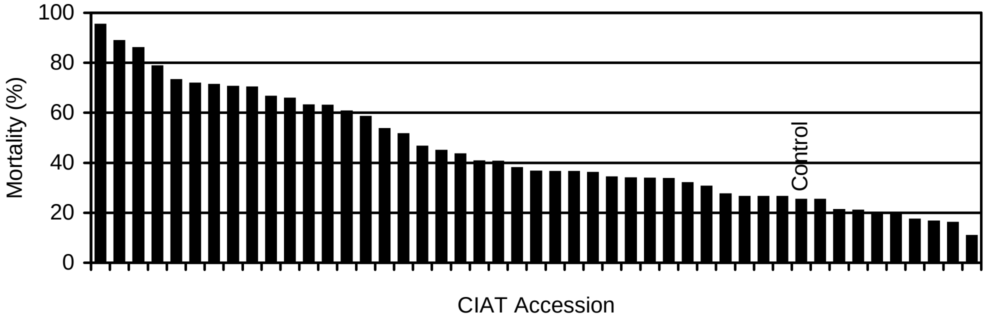


Figure 14. Virulence of 46 isolates of fungal entomopathogens to *A. varia* adults. The control was water plus tween.

Although 79% of the isolates achieving >60% mortality were *Metarhizium*, this genus was also the most represented in the evaluation group, comprising 72% of the total isolates evaluated. The most virulent isolates of other genera were *Paecilomyces lilacinus* (CIAT 009) at 62.8% and *Fusarium* sp. (CIAT 025) at 53.5%. All isolates in the most virulent group (>60% mortality) originally came from the Interandean Region (departments of Cauca and Valle) and the Amazonian Piedmont (department of Caquetá).

Table 32. Identification, origin and virulence (percent mortality) of select Colombian isolates of fungal entomopathogens on *Aeneolamia varia* adults.

Accession	**Species**	**Virulence**[1]	**Host**		
			Species	**Life stage**	**Origin**
			Aeneolamia varia	Adult	CIAT, Valle
CIAT 055	undet.	88.6 ab	*Aeneolamia varia*	Nymph	CIAT, Valle
CIAT 007-C	*M. anisopliae*	85.8 abc	*Zulia pubescens*	Adult	Albania, Caquetá
CIAT 003	*M. anisopliae*	78.5 abcd	*Aeneolamia varia*	Adult	Albania, Caquetá
CIAT O53	*M. anisopliae*	73.0 bcde	*Zulia carbonaria*	Adult	Quilichao, Cauca
CIAT 042	*M. anisopliae*	71.6 bcedf	*Zulia carbonaria*	Adult	Quilichao, Cauca
CIAT 007	*M. anisopliae*	71.1 bcedf	*Zulia pubescens*	Adult	Albania, Caquetá
CIAT 002-B	*M. anisopliae*	70.3 bcedf	*Zulia pubescens*	Adult	Albania, Caquetá
CIAT 010	*M. anisopliae*	70.0 bcedf	undet.	Nymph	Florencia, Caquetá
CIAT 007-B	*M. anisopliae*	66.3 cdefg	*Zulia pubescens*	Adult	Albania, Caquetá
CIAT 001	*M. anisopliae*	65.5 cdefg	*Zulia pubescens*	Adult	Florencia, Caquetá
CIAT 007-A	*M. anisopliae*	62.9 edfgh	*Zulia pubescens*	Adult	Albania, Caquetá
CIAT O09	*P. lilacinus*	62.8 edfgh	undet.	Nymph	Florencia, Caquetá
CIAT 002-A	*M. anisopliae*	60.4 edfghi	*Zulia pubescens*	Adult	Albania, Caquetá
CIAT 018	*M. anisopliae*	58.3 edfghij	*Aeneolamia varia*	Adult	CIAT, Valle
CIAT 025	*Fusarium* sp.	53.4 dfghij	*Zulia carbonaria*	Adult	Quilichao, Cauca
CIAT 006	*M. anisopliae*	51.4 fghijkl	*Mahanarva* sp. nov.	Adult	Albania, Caquetá
Control[2]		25.1 m			

[1] Means followed by different letters are significantly different ($P<0.05$).

[2] Control of water plus tween (0.05%).

Discussion: The evaluated isolates demonstrate a broad range of virulence against *A. varia* adults. The most effective strains also represent a broad host and geographic range of Colombia. Although strains of *Paecilomyces* and *Fusarium* were not as effective as *Metarhizium*, these taxa should not be ignored since they were relatively under represented in the screened population. Moreover, different taxa may have other biological attributes that are desirable once the isolates are brought to the field. Virulence to other spittlebug species and life stages, growth characteristics for propagation, and tolerance to adverse field conditions are some of the many factors important to suppressing pest populations in the field.

The next phase of this investigation will address variation in virulence across different spittlebug species and life stages. Based on the results of the present studies, five isolates have now been chosen for the next studies. These include the three overall most virulent isolates (CIAT 055, CIAT 054, CIAT 007-C: *M. anisopliae* and two *Metarhizium* sp.) and the best *Paecilomyces* (CIAT 009) and *Fusarium* (CIAT 025) isolates.

2.2.5 Evaluation methodology for measuring virulence of fungal entomopathogens to spittlebug nymphs

Contributors: Anuar Morales, Rosalba Tobón, Daniel Peck

Rationale: Advances in spittlebug management will depend on better knowledge of the nymphal life stage, which has been traditionally underemphasized relative to adults. For instance, nymphs are more difficult to survey in the field and manage in the lab, consequently CIAT's fungal entomopathogen collection has very few strains isolated from the immatures. Yet because nymphs account for about 70% of the generation time, requiring 5-7 weeks to complete development, there is a broader window of opportunity for certain management tactics. A rapid and reliable methodology now exists for screening fungal entomopathogens for virulence to spittlebug adults (see activity 2.2.4). In order to (1) obtain more information about the effectiveness of isolates in the laboratory before advancing to a field phase, and (2) to gauge variation in virulence between life stages, a methodology was developed and evaluated for screening fungal entomopathogens for virulence to spittlebug nymphs.

Methods: Evaluation units were the same small-scale PVC tubes (1.5" diameter) now standard for host plant resistance screening. At 6 wk after planting with *Brachiaria ruziziensis* (CIAT 654), sufficient surface roots were established for nymph development and egg infestation. Eggs of *Aeneolamia varia* about to hatch were prepared for treatments and infestation by placing 10 on each of 10 small pieces of filter paper in a petri dish that corresponded to one treatment. Concentrated conidial suspensions (10^8 conidias/ml) were prepared for four select isolates of *Metarhizium* (CIAT 005, CIAT 053, CIAT 054, CIAT 055) and one of *Paecilomyces* (CIAT 012) in sterile water with tween (0.05%). Each of these isolates was recently reactivated in adults of *A. varia*. Applications were made with an airbrush and compressor (10 PSI) at a volume of 1 ml for substrate and nymph applications and <1 ml for direct egg applications.

Four experimental treatments were evaluated: application to eggs (in petri dishes, followed by infestation), application to substrate (in PVC unit before egg infestation), application to eggs and substrate, and application to nymphs (in PVC units 4 days after infestation). Each treatment had a corresponding control with water plus tween (0.05%). There were ten repetitions of each treatment. The units were fertilized with urea (2 g/l water) before infestation and 15 day later. Half of the repetitions were evaluated for surviving nymphs 15 days after infestation and the other half at 34 days (3-4 days before adult emergence in the control treatments).

Results: Mean mortality in the control nymphs was 24.6 and 40.3% at the early (15-day) and late (34-day) evaluation periods, respectively (Table 33). The high mortality in the late evaluation is probably attributed to an overly heavy infestation level on the susceptible *B. ruziziensis* host. Although only five isolates were evaluated, remarkably high nymph mortality was achieved. When data were averaged across the early and late evaluation periods, the three most virulent treatment/isolate combinations achieved 84.3 (CIAT 055, egg+substrate application), 83.1 (CIAT 054, egg application) and 83.1% (CIAT 053, egg application) mortality. These three isolates are thereby considered equally promising for inclusion in future control trials.

For the early evaluation period, virulence varied from 19.4-87.1% for the egg application, 22.0-54.0% for the substrate application, 34.5-80.0% for egg+substrate, and 55.3-68.7% for the nymph application (4-day) (Figure 15). Although complementary results were obtained from the late evaluation (Figure 16), the high nymphal mortality in the control relative to the treatments makes interpretation difficult.

Among the four *Metarhizium* isolates averaged across the early and late evaluation periods, the egg application was highest for two isolates, while the combined egg+substrate application was highest for the other two. These results indicate that eggs about to hatch may be more a susceptible life stage than nymphs, and that direct application is more effective than application to the surrounding soil substrate.

Table 33. Virulence (% mortality) of different isolate/treatment combinations summed across the early and late evaluation periods to nymphs of *A. varia*.

Accession	Treatment	Virulence	Accession	Treatment	Virulence
CIAT 055	eggs+substrate	84.33	CIAT 055	substrate	65.00
CIAT 054	eggs	83.11	CIAT 005	substrate	64.69
CIAT 053	eggs	83.07	CIAT 005	nymphs	63.70
CIAT 005	egg+substrate	80.00	CIAT 012	nymphs	55.94
CIAT 053	nymphs	80.00	Control	egg+substrate	41.73
CIAT 054	egg+substrate	79.64	Control	substrate	34.33
CIAT 053	egg+substrate	77.67	CIAT 012	substrate	32.00
CIAT 055	nymphs	76.69	Control	nymphs	28.89
CIAT 053	Substrate	75.00	CIAT 012	egg+substrate	25.69
CIAT 055	Eggs	73.78	CIAT 054	substrate	22.00
CIAT 054	Nymphs	71.85	Control	eggs	20.00
CIAT 005	Eggs	70.19	CIAT 012	eggs	19.39

Discussion: Although analysis of these data is incomplete, the results suggest certain adjustments in this methodology for future studies. Egg infestation levels will be reduced to seven (vs. ten) to promote higher survivability of the control nymphs through the late evaluation period. Second, to begin to elucidate the role of the spittle mass as a shelter from conidia, additional 5-day and 10-day post egg infestation treatments will be added. It is suspected that direct egg application will be the most effective treatment for screening virulence to spittlebug nymphs.

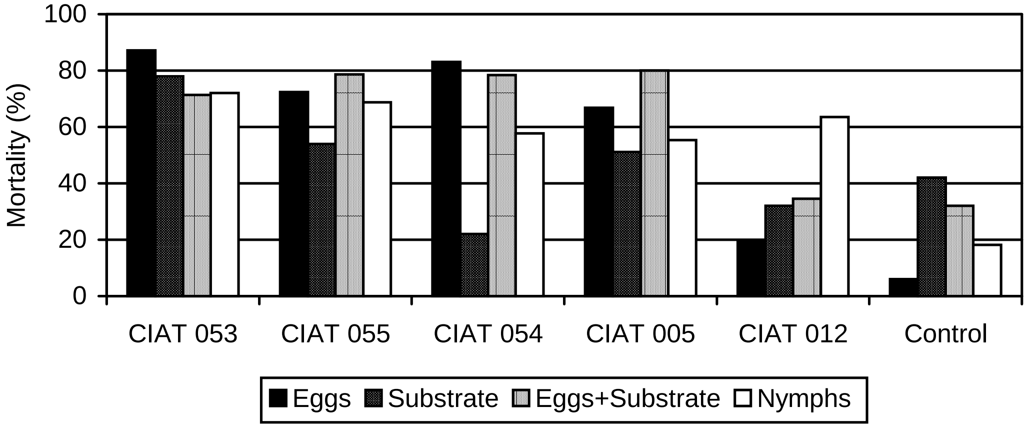


Figure 15. Virulence (% mortality) of five fungal entomopathogen isolates and four application treatments to nymphs of *A. varia* 15 days after application to eggs or substrate.

Evidence gathered in these experiments indicates that application of entomopathogens during the late egg stage is equally or more effective than application after egg eclosion. It is likely that when applied directly to the eggs, mortality is enhanced because emerging nymphs (1) more rapidly encounter conidia as they search for feeding sites, (2) are more susceptible to conidia establishment compared to early first instars that have already established spittle masses, and (3) are more rapidly affected by conidia than later instars due to smaller size and less protective integument. When the application is post eclosion, an effect of mortality might be delayed until later instars for the same reasons of differential susceptibility due to size and rate of contact with conidia.

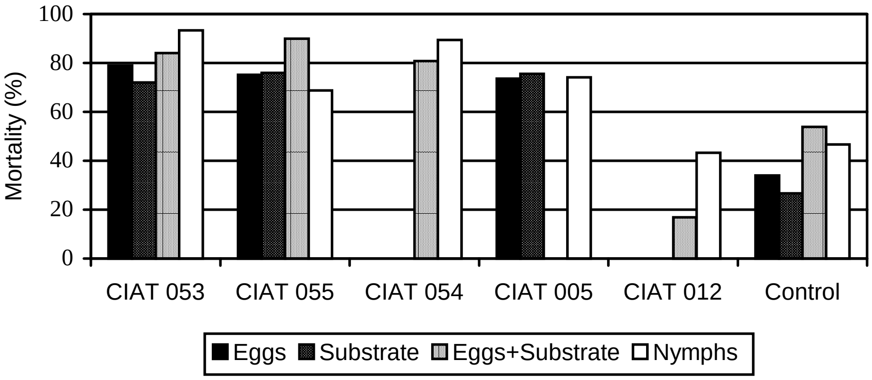


Figure 16. Virulence (% mortality) of five fungal entomopathogen isolates and four application treatments to nymphs of *A. varia* 34 days after application to eggs or substrate

These results indicate that in more seasonal environments, where early wet season emergence is relatively synchronous, predicating approximate time of egg eclosion will help target application of entomopathogen products. In less seasonal environments, where there is little population synchrony, entomopathogen strains should be sought that persist in the environment and thereby promote secondary contact between conidia and susceptible nymphal or adult life stages.

2.2.6 Economic impact of spittlebugs to animal production in *Brachiaria decumbens*

Contributors: F. Holmann, D. Peck, and C. Lascano

Background: *Brachiaria decumbens* cv. Basilisk is the largest grass species available in tropical America with an estimated 40 million ha, the majority located in Brazil, Colombia and Venezuela. The large adoption of this cultivar has been due to its good adaptation to acid, low-fertility soils. In addition, *B. decumbens* forms an aggressive, high-yielding pasture which resists intensive grazing and trampling. Likewise, *B. decumbens* is a palatable grass with good nutritional quality. However, its susceptability to spittlebug (Homoptera: Cercopidae) reduces its potential adoption in areas where this pest is important such as in the neotropical savannas. The spittlebug is considered the most important disease in pastures of tropical America due to its ample distribution and outbreak capacity. Dozens of spittlebug species have been reported which attact cultivated grasses from South of the United States to North of Argentina.

The objective of this study was to quantify the economic damage as a result of reduced milk and beef production under different degrees of spittlebug infestation on animals grazing *B. decumbens* using a simulation model.

To calculate the economic damage, the model was fed with information on three variables: (1) degree of infestation: low, medium, and high; (2) proportion of farm area infested: 0, 25, 50, 100%; and (3) type of ecosystem: dry tropics (infestation occuring only during the six-month rainy season), and humid tropics (infestation occuring throughout the year).

Results: Tables 34 and 35 contain the biological and economical impact from different degrees of spittlebug infestation in various proportions of farm area under infestation in a dual-purpose production system located in either the dry or the humid tropics ecosystems in Colombia.

Comparing the effect against a healthy grass, the spittlebug reduced the stocking rate and productivity of milk and beef per hectare 9%-34% with a low degree of infestation, between 38%-49% with a medium degree of infestation, and 53%-65% under a high degree of infestation. The spittlebug impact increased the production cost of milk and beef production by 3%-12% with a low degree of infestation compared to a healthy *B. decumbens*. Likewise, these costs increased 18%-29% with a medium degree of infestation, and by 36%-54% when it was evaluated with high levels of infestation. On the other hand, due to the spittlebug effects on reduced stocking rates and increased milk and beef production costs, the net income per hectare per year was signifficantly reduced by 19%-69% with low levels of infestation, by 78%-100% with medium infestation level, and by 106%-128% with a high infestation level.

Table 34. Biological and economical impact from different degrees of spittlebug infestation in various proportions of farm area under infestation in a dual-purpose production system located in the humid tropics of Colombia.

Level and proportion of farm area infested with spittlebug	Stocking Rate	Milk[1] Production	Beef[1] Production	Milk Production Cost	Beef Production Cost	Net Income
	(AU/ha)	(kg/ha/yr)	(kg/ha/yr)	($/kg)	($/kg)	($/ha/yr)
No infestation	1.16	390	95	0.123	0.61	32
10 individuals/m^2						
- 25%	1.14	382	94	0.124	0.62	31
- 50%	1.12	375	92	0.125	0.62	30
- 100%	1.08	360	88	0.126	0.63	27
25 individuals/m^2						
- 25%	1.06	357	87	0.128	0.63	26
- 50%	0.96	324	79	0.133	0.66	21
- 100%	0.76	258	63	0.142	0.70	10
50 individuals/m^2						
- 25%	0.72	243	59	0.146	0.72	7
- 50%	0.67	228	55	0.150	0.75	5
-100%	0.59	197	48	0.159	0.79	0

[1] Kilograms of fluid milk and kilograms of beef (live weight)

The economic damage at the regional scale was estimated at $7-25 million dollars per year in the 1,140,000 ha of pastures susceptible to spittlebug in the humid tropics and $33-118 million dollars in the 4,720,000 ha of pastures in the dry tropics asuming a low infestation level. This economic damage, assuming a medium level of infestation, was increased to $28-36 million dollars in the humid tropics and $132-175 million dollars in the dry tropics. Finally, with a high infestation level, the income reduction at the regional level reached $39-47 million dollars for the humid tropics and $228-273 million dollars for the dry tropics.

The estimated investment for the development of varietes resistant to spittlebug and adapted to low fertility soils is about US$ 6 million dollars with a 12-yr planning horizon. This investment, compared to the economic damage that the spittlebug does in Colombia, is low. Therefore, a large economic incentive exists for associations of livestock producers to invest in germplasm improvement by contracting research institutions with the capacity to succesfully develop a spittlebug-resistant grass.

Table 35. Biological and economical impact from different degrees of spittlebug infestation in various proportions of farm area under infestation in a dual-purpose production system located in the dry tropics of Colombia.

Level and proportion of farm area infested with spittlebug	Stocking Rate	Milk[1] Production	Beef[1] Production	Milk Production Cost	Beef Production Cost	Net Income
	(AU/ha)	(kg/ha/yr)	(kg/ha/yr)	($/kg)	($/kg)	($/ha/yr)
No infestation	0.93	529	84	0.116	0.57	42
10 individuals/m^2						
- 25%	0.91	519	82	0.116	0.57	40
- 50%	0.90	509	81	0.117	0.58	39
- 100%	0.86	489	78	0.119	0.59	36
25 individuals/m^2						
- 25%	0.85	484	77	0.120	0.59	35
- 50%	0.76	439	70	0.125	0.62	29
- 100%	0.62	350	55	0.133	0.66	17
50 individuals/m^2						
- 25%	0.57	328	52	0.137	0.68	14
- 50%	0.53	307	49	0.142	0.70	11
-100%	0.46	265	42	0.150	0.74	5

[1] Kilograms of fluid milk and kilograms of beef (live weight)

Progress towards achieving milestones

- **IPM components relevant to spittlebug management in forage grasses and other graminoids better understood**

Excellent progress was made on the collection, evaluation methodologies and screening of fungal entomopathogens of spittlebugs, providing the fundamentals required to carry out field evaluations in 2001. New information on the diversity, identity and distribution of spittlebugs in Colombia and Ecuador has established possibilities to use GIS-based software (Flora-Map) for assessing the determinants of distribution based on key climate variables, such as precipitation, that would lead to predictions of species range, and areas of potential outbreak, range expansion and invasion risk. A high priority is to obtain funding for addressing the next limits in advancing spittlebug IPM, namely quantitative damage estimates to establish economic thresholds, dispersion patterns to establish sampling schemes, and monitoring strategies to predict where and when localized outbreaks will occur to help target application of control tactics. These IPM tools would be addressed in the four contrasting model regions where bioecological information has acquired, ultimately leading to recommended IPM programs for field testing, followed by modification and impact assessment.

Activity 2.3 *Brachiaria* genotypes resistant to spittlebug and other biotic stresses

Highlights

- New hybrids with higher levels of resistance to *Aeneolamia varia* were identified
- Large genetic progress made in breeding *Brachiaria* for resistance to *A. varia*
- Field screening methodologies became fully implemented in Caquetá, and these were extended to cover all major species of spittlebug present in that region

2.3.1 Development of new hybrid population for spittlebug screening using pollen from a resistant parent (Ap) and selected clones (Sx) as maternals

Contributors: J. W. Miles

The objective is to identify superior, apomictic hybrids for advance towards cultivar status. Three hybrid populations were formed and approx. 1,700 individual hybrid seedlings were transplanted to field sites at CIAT-Quilichao and Matazul (Puerto López) during May 2000. Maternal parents of these progenies are sexual clones selected from previous cycle of the sexual population (in 1998). Apomictic parents are: CIAT 36061 (selected hybrid), CIAT 36062 (selected hybrid), and CIAT 26124 (germplasm accession). Periodic ratings of this material are being conducted. Pre-selections (<1,000) will be identified for spittlebug assessment by early next year. Preliminar observations indicate that progenies with CIAT 36062 lack vigor and are generally late flowering.

2.3.2 Identify *Brachiaria* genotypes resistant to spittlebug

Contributors: C. Cardona, J. W. Miles

Rationale: Assessment for resistance to spittlebug is an essential step in the process of breeding superior *Brachiaria* cultivars at CIAT. Routine screening in the greenhouse, coupled with field evaluation of selected hybrids should contribute to facilitate breeding efforts. As in 1999, large numbers of hybrids were screened in the glasshouse and in Caquetá.

Materials and Methods: Materials are screened in the glasshouse and in the field using the methodologies fully described in previous annual reports. Plants are infested with a known number of eggs of the respective spittlebug species and the infestation is allowed to proceed without interference until all nymphs are mature or adult emergence occurs. Plants (usually 10 per genotype) are scored for symptoms using a damage score scale. Percentage nymph survival is calculated. Materials are selected on the basis of low damage scores (<2.0 in a 1-9 scale) and percentage nymph survival (<30%). All those rated as resistant or intermediate are reconfirmed. All those susceptible are discarded.

Results and Discussion: This activity has been fully accomplished. Approx. 3,000 individuals from the latest cycle of the sexual Brachiaria breeding population were established in CIAT Quilichao and Matazul (Meta) early in 1999. A set of 928 "pre-selections" from these trials were propagated early in 2000, and infested with spittlebug eggs. Initial screening was with two reps, where 111 clones were identified.

These 111 were re-assessed using 10 replicates in a second phase. In total, 41 hybrid-derived, sexual clones equally or more resistant to *A. varia*. than the resistant cv. Marandu were identified. These have been propagated to an isolated crossing block to produce seed for next cycle population. Between now (August 2000) and next January's planting, we hope to reconfirm spittlebug reaction of these 41 selections and to acquire additional data on them regarding their reaction to Rhizoctonia foliar blight (artificial innoculation), in vitro digestibility, and Al-tolerance (in vitro). Additional clones can be culled based on these more complete data. These clones will be assessed for resistance to all four major species of spittlebug present in Caquetá using the field screening methodology developed there. Levels of resistance in some of the best selections are shown in Table 36.

Table 36. Levels of resistance to *Aeneolamia varia* found in selected *Brachiaria* genotypes. Greenhouse evaluation using 10 replications per genotype

Genotype	Damage scores[a]	Percentage nymph survival	Percentage poorly developed nymphs
SX99NO/2341	1.0	20.0	20.0
SX99NO/2857	1.0	12.5	10.0
SX99NO/2663	1.2	36.7	26.7
SX99NO/0236	1.2	4.0	4.0
SX99NO/1616	1.25	22.5	18.7
SX99NO/2030	1.25	21.7	11.7
SX99NO/3770	1.25	8.7	3.7
SX99NO/1370	1.25	26.7	13.3
SX99NO/0574	1.3	32.0	24.0
SX99NO/1260	1.3	10.0	8.0
CIAT 06294[b]	2.1	23.0	9.0
CIAT 06133[c]	4.3	76.0	0.0
CIAT 00606[d]	4.2	61.0	0.0
BR4X-44-02 [d]	4.9	77.0	0.0

[a] On a 1-5 damage score scale (1, no damage; 5, severe damage, plant killed)
[b] Resistant check
[c] Tolerant check
[d] Susceptible check

Data obtained from these screenings were used to measure progress made in incorporating resistance to *A. varia*. As shown in Figure 17 hybrid populations screened in 1998 showed a frequency distribution skewed towards susceptibility (higher damage scores), while populations developed later and screened in 2000 showed a frequency distribution skewed towards resistance. The mean damage score in 2000 was 28% lower than in 1998. This is a substantial gain that confirms the usefulness of the breeding scheme and the reliability of the screening methodology.

2.3.3 Greenhouse screening of *Brachiaria* accessions and hybrids for resistance to spittlebug

Contributors: C. Cardona, G. Sotelo, J. W. Miles

A mapping population formed from the cross tetraploid *B. ruziziensis* (susceptible) x *B. brizantha* cv. Marandu (resistant) was produced. A set of 232 hybrids from this mapping population were screened in the greenhouse for resistance to *A. varia*. The same set of genotypes were assessed in two separate (in time) trials, each with five replications. The combined analysis of variance shows no significant interaction between tests. There was a highly significant correlation (r= 0.892; P<0.001) between damage scores in test 1 and damage scores in test 2 (Figure 18). Similarly, a significant correlation (r= 0.826; P<0.001) was found when percentage survival in test 1 was compared with percentage survival in test 2 (Figure 19). These values confirm the reliability of the screening methodology that has been adopted to select for resistance.

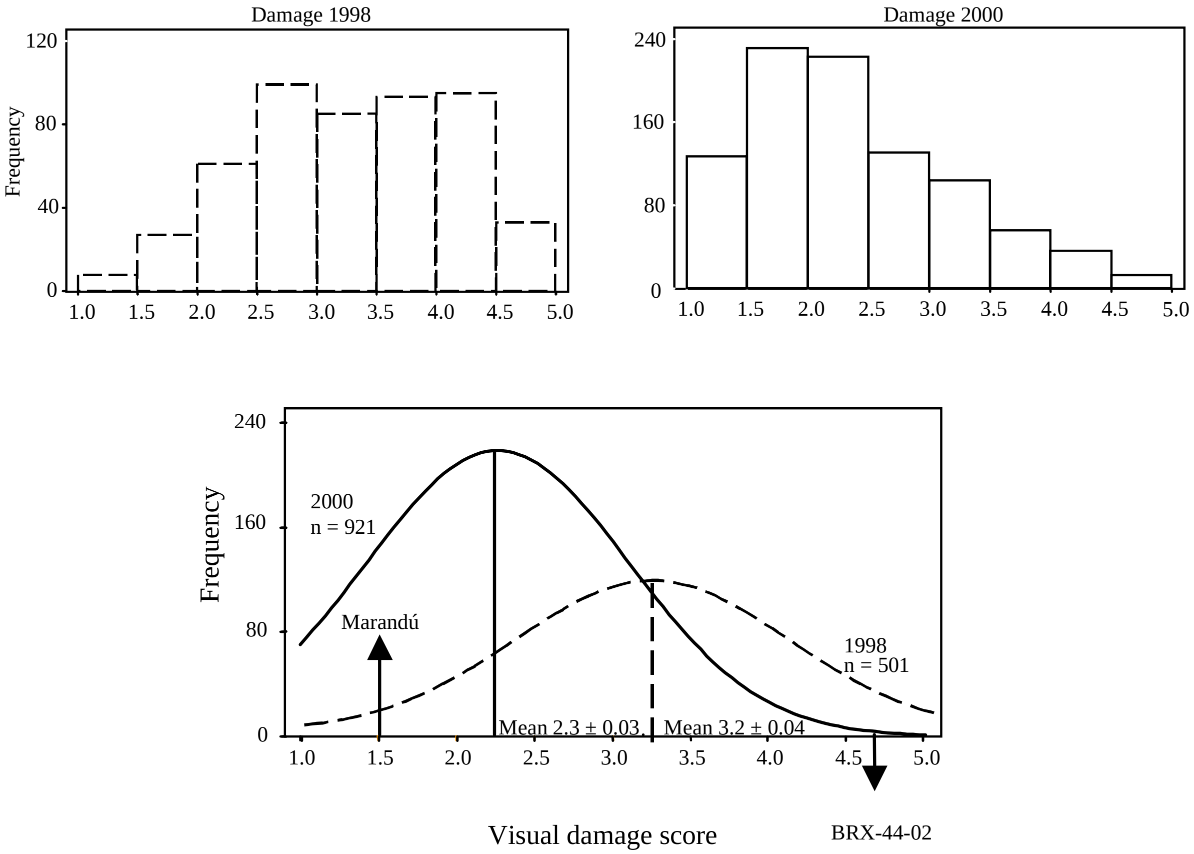


Figure 17 . Frequency histograms of damage scores in two populations of *Brachiaria* hybrids and 5 checks tested for resistance to *Aeneolamia varia* under greenhouse conditions.

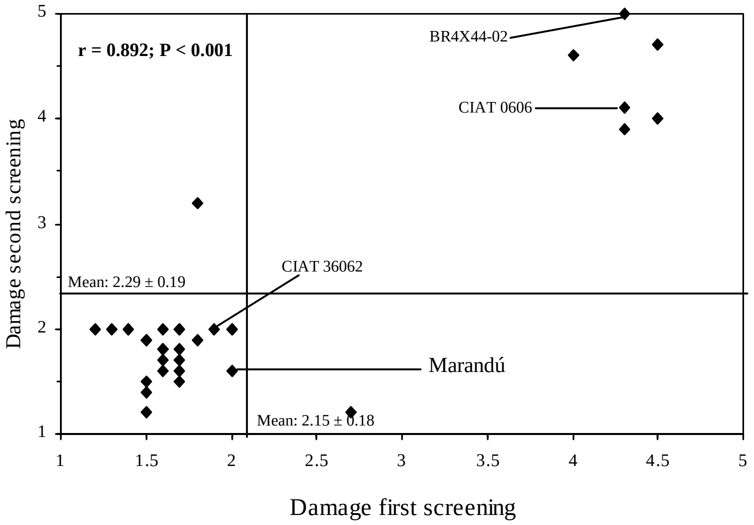


Figure 18. Damage scores in two identical, consecutive screenings of *Brachiaria* hybrids bred for resistance to spittlebug. Two resistant (CIAT 36062 and Marandú) and two susceptible (BR4X44-02 and CIAT 0606) checks are shown.

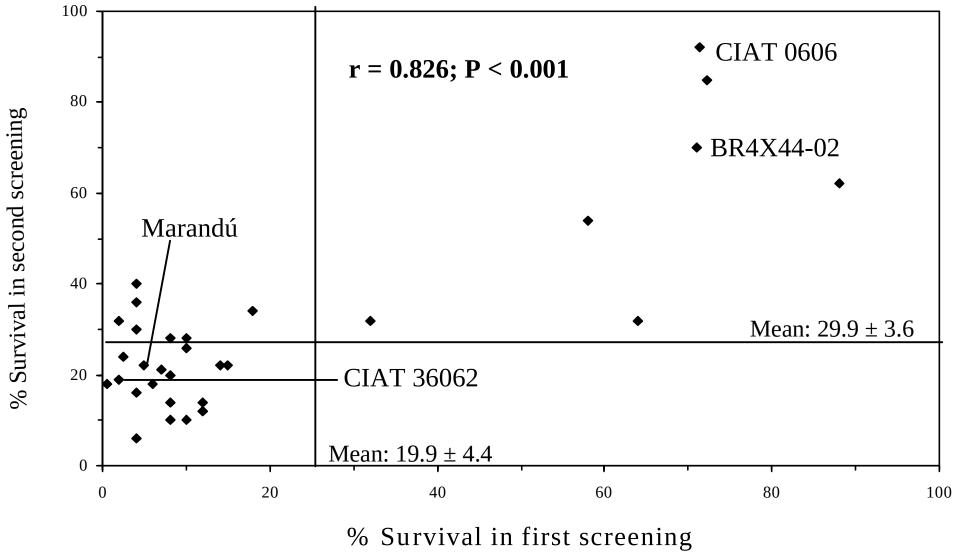


Figure 19. Percentage survival to fifth nymphal instar in two identical, consecutive screenings of *Brachiaria* hybrids bred for resistance to spittlebug. Two resistant (CIAT 36062 and Marandú) and two susceptible (BR4X44-02 and CIAT 0606) checks are shown.

There was a highly significant correlation between damage scores and percentage nymph survival (r= 0.886; P<0.001) in those genotypes that were selected for resistance (Figure 20). Genotypes falling in the lower left quadrant of the figure can be selected for resistance for having a low level of damage coupled with antibiotic resistance to nymphs (Table 37).

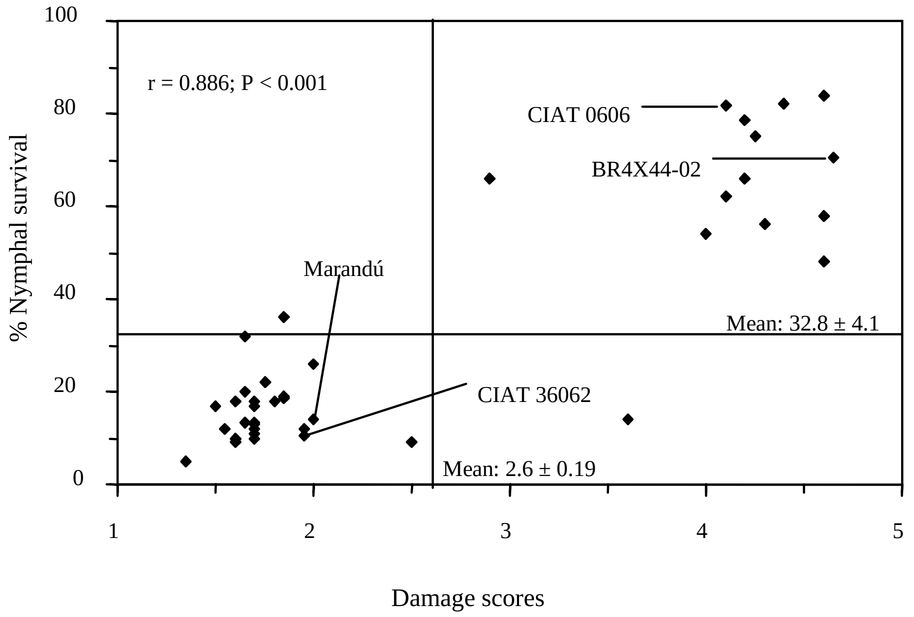


Figure 20. The relationship between damage scores and percentage nymphal survival in selected, resistant and susceptible *Brachiaria* hybrids bred for resistance to spittlebug. Two resistant (CIAT 36062, 'Marandú') and two susceptible (BR4X44-02 , CIAT 0606) checks are shown. Means of two tests, 10 reps per genotype per test.

Table 37. Damage and percentage nymph survival in selected *Brachiaria* hybrids bred for resistance to *Aeneolamia varia* (Means of 10 replications per genotype).

Genotype	Damage scores[a]			Percentage nymph survival		
	1st Screening	2nd Screening	Average	1st Screening	2nd Screening	Average
BRCRUC1080/0013	1.5	1.2	1.35	4.0	6.0	5.0
BRCRUC1094/0049	1.5	1.5	1.5	2.0	12.0	7.0
BRCRUC1094/0014	1.2	2.0	1.6	4.0	16.0	10.0
BRCRUC1078/0031	1.7	1.5	1.6	14.0	22.0	18.0
BRCRUC1078/0032	1.6	1.6	1.6	0.0	18.0	9.0
BRCRUC1092/0010	1.6	1.8	1.7	12.0	14.0	13.0
BRCRUC1093/0022	1.4	2.0	1.7	8.0	14.0	11.0
BR4X-44-02[b]	4.3	5.0	4.6	71.0	70.0	70.5
CIAT 00606[b]	4.3	3.9	4.1	71.3	92.2	81.7
CIAT 06133[b]	4.3	4.1	4.2	72.2	85.0	78.6
CIAT 06294[c]	2.0	2.0	2.0	7.0	21.0	14.0
CIAT 36062[c]	1.9	2.0	1.9	2.0	19.0	10.5

[a] 1, no damage; 5, very severe damage, plant killed.
[b] Susceptible check.
[c] Resistant check.

Another nursery evaluated this year was a set of 32 elite hybrids that were reconfirmed for resistance to *A. varia*. Very high levels of antibiosis resistance were found in 17 of these genotypes. Some of the best are shown in Table 38. It is interesting to note the relatively high proportion of poorly developed nymphs found feeding on resistant genotypes.

Table 38. Levels of resistance to *Aeneolamia varia* found in selected *Brachiaria* genotypes. Greenhouse evaluation using 10 replications per genotype.

Genotype	Damage scores[a]	Percentage nymph survival	Percentage of poorly developed nymphs
BR99NO/4138	1.2	5.0	13.3
BR99NO/4207	1.5	8.4	10.0
BR99NO/4278	1.7	2.0	0.0
BR99NO/4015	1.5	12.6	20.0
BR99NO/4076	1.7	4.0	16.7
CIAT 06294[b]	1.9	2.0	0.0
CIAT 36062[b]	1.8	3.0	8.1
CIAT 06133[c]	3.4	70.0	0.0
CIAT 00606[d]	4.6	68.6	0.0
BR4X-44-02[d]	4.8	62.5	0.0

[a] On a 1-5 damage score scale (1, no damage; 5, severe damage, plant killed)
[b] Resistant check
[c] Tolerant check
[d] Susceptible check

2.3.4 Field screening of *Brachiaria* accessions and hybrids for resistance to spittlebug

Contributors: C. Cardona, G. Sotelo, J. W. Miles

Field screening for resistance to spittlebug was fully implemented in 2000. The methodologies have been described in previous reports. We have reported before on the reliability of the system as judged by the high correlation between greenhouse and field resistance ratings. We have also reported on the possibility of adapting the methodology to all spittlebug species.

Six major screening trials (two with *A. varia*, two with *Z. pubescens*, and one each with *Z. carbonaria* and *Mahanarva* sp.) were set up in 2000. Pending final statistical analysis of most of them, we will highlight the results of evaluating 40 genotypes (24 accessions, 11 hybrids, and 5 checks) for resistance to *A. varia* in two consecutive identical trials. Damage scores between trials correlated well (r= 0.7235; P<0.0001). There was a significant, positive correlation (r= 0.894; P<0.001) between damage scores and percentage tillers killed by the nymphs (Figure 21).

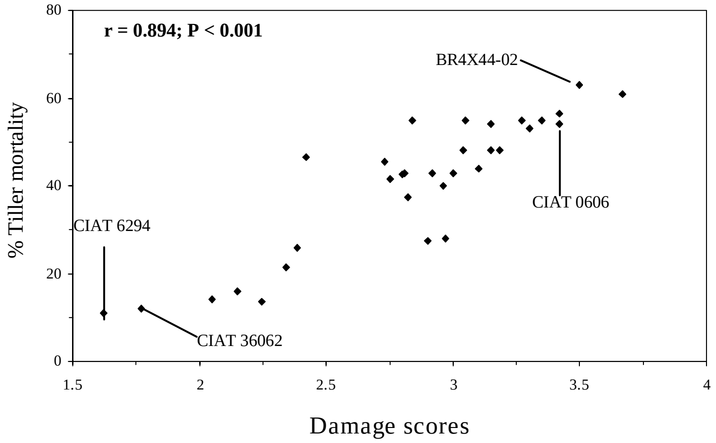


Figure 21. The relationship between visual damage scores and percentage tiller mortality in 33 *Brachiaria* genotypes evaluated for resistance to *Aeneolamia varia* under field conditions in Caquetá. Means of two trials, 10 replications per trial. Resistant (CIAT 36062, CIAT 6294) and susceptible (CIAT 0606, BR4X-44-02) checks are shown.

This means that field resistance expressed as reduced damage to the leaves may serve to predict losses due to nymphal feeding. The most susceptible genotypes tested, the checks CIAT 0606 and CIAT 0654 lost 54% and 61% of their tillers, respectively. In contrast, tiller mortality in the resistant checks CIAT 6294 ('Marandú') and CIAT 36062 was 11% and 12%, respectively.

2.3.5 Adaptation of protocol for screening *Brachiaria* hybrids for Rhizoctonia foliar blight

Contributors: C. Zuleta, X. Bonilla, J. W. Miles, S. Kelemu

Foliar blight of *Brachiaria* is caused by *Rhizoctonia solani*. It can cause substantial foliar damage on susceptible genotypes of a wide range of plant species. The fungus can survive for long periods in the soil or on plant debris as sclerotia. The use of resistant cultivars, when available, remains the cheapest and most effective method of controlling the disease.

Sclerotia of *R. solani* AG-1 were produced in a peptone sucrose yeast (PSY) broth containing 20 g peptone, 20 g sucrose, 5 g yeast extract and 1 l deionized water. Mycelial discs, 4 mm in diameter, were removed from a 4 to 5-day-old culture of *R. solani* grown on potato dextrose agar. One disc was added to each of several 250-ml Erlenmeyer flasks, each containing 30 ml PSY. The flasks were wrapped with aluminum foil and incubated as still culture at room temperature for 10 days. Sclerotia were harvested with sterile forceps that separated them from the mycelial mats. They were then air-dried overnight on sterile Whatman filter paper in a laminar flow hood. Dry sclerotia were placed in sterile glass tubes and stored at 4 C.

Plantlets of each of 60 *Brachiaria* hybrids separated from tillers were planted in sterile soil in cellular planting flats in the screen-house. Each plantlet was incoulated with one sclerotium placed on the soil surface in contact with the plantlet's stem. High humidity was maintained by placing inoculated plants in a plastic box with one side made of cheese cloth and immersed in a tray of water, until symptoms were fully expressed.

Experiments are in progress and data were not available at the time of this report writing.

Progress towards achieving milestones

- **Sexual *Brachiaria* clones (50 or fewer) identified, propagated and recombined and seed harvested to initiate new breeding cycle.**

Forty-one sexual clones selected and crossing block established (July, 2000). Recombined seed is being harvested to initiate next cycle in 2001. Between now and January, we will have additional information on the 41 selected clones regarding their tolerance to aluminum, reaction to Rhizoctonia foliar blight, seed yield and quality, and possibly digestibility. Up to 20 of the half-sib families currently being formed can be culled prior to planting early in 2001 on the basis of this additional information on the clones.

- **Screened selected sexual *Brachiaria* clones for spittlebug reaction under field conditions.**

Selected clones have been transferred to Caquetá for evaluation of spittlebug reaction under field conditions.

- **Implemented an efficient protocol for screening *Brachiaria* hybrids for Rhizoctonia foliar blight resistance**

The 41 selected clones are being assessed for reaction to Rhizoctonia foliar blight under glasshouse conditions of artificial inoculation.

Activity 2.4 Host mechanisms for spittlebug resistance in *Brachiaria*

Highlights

- Made further progress in understanding the mechanisms of resistance of *Brachiaria* hybrids to four species of spittlebug.
- *Brachiaria* hybrids with multiple resistance to two or more species of spittlebug were identified

2.4.1 Studies on resistance to spittlebug species

Contributors: C. Cardona, G. Sotelo

Rationale: There is need to ascertain that new *Brachiaria* hybrids produced by the Project do possess resistance to as many spittlebug species as possible. Full characterization of the mechanisms responsible for resistance to four species of spittlebug will contribute to the formulation of appropriate breeding strategies in the future. In 2000, we expanded this work to include new hybrids developed by the Project.

Materials and Methods: Several greenhouse and field tests were conducted. *Brachiaria* accessions and hybrids were infested following the methodologies described in previous annual reports. Screenings for resistance were conducted with *Aeneolamia varia, Zulia carbonaria, Z. pubescens* and *Mahanarva* sp. Test materials were compared with five checks fully characterized for resistance or susceptibility to *A. varia*. Damage scores and percentage nymph survival were used to classify the genotypes as resistant, intermediate, or susceptible.

Results and Discussion: A set of 13 genotypes was evaluated for resistance to four species of spittlebug. We included six new *Brachiaria* hybrids, two accessions, and five well-known checks. Highly significant differences among genotypes were found for visual damage scores and percentage nymph survival (Table 39).

Table 39. Levels of resistance to four species of spittlebug (*Aeneolamia varia*, Av; *Zulia carbonaria*, Zc; *Zulia pubescens*, Zp; *Mahanarva* sp., Ma) in selected *Brachiaria* genotypes. Means of 10 replications per genotype per species

Genotype	Damage scores[a]				Percentage nymph survival			
	Av	Zc	Zp	Ma	Av	Zc	Zp	Ma
BR97NO/0047	2.2ef	4.0abc	3.7bc	1.7d	29.2cd	44.3cd	43.5ab	8.6c
BR97NO/0082	2.9de	3.4bcde	3.3c	1.6d	57.1ab	49.4bc	58.2a	7.0c
BR97NO/0155	1.4f	3.7bcd	3.0cd	2.2cd	21.3de	54.7bc	50.4ab	11.7c
BR97NO/0402	3.6cd	3.0def	2.3de	3.4b	62.7a	65.4a	40.2b	46.0a
BR97NO/0457	3.8c	3.8abc	2.4de	3.5b	59.9a	60.5ab	52.5ab	42.2a
BR97NO/1143	1.6f	1.8h	2.4de	2.4c	32.8cd	42.2cde	39.7b	23.3b
CIAT 16844	2.6e	4.0abc	3.7bc	3.8b	42.6bc	44.7cd	40.0b	24.8b
CIAT 16314	3.5cd	2.7efg	2.9cd	3.5b	60.1a	42.9cde	48.6ab	39.8a
CIAT 00606[b]	4.2bc	4.1ab	4.1ab	4.1ab	66.9a	52.0bc	52.5ab	40.6a
BR4X-44-02[b]	5.0a	4.6a	4.5a	4.6a	67.1a	49.9bc	48.8ab	40.2a
CIAT 06294[c]	1.7f	2.6fg	2.9cd	1.6d	29.6cd	31.5e	45.6ab	10.8c
CIAT 36062[c]	1.5f	2.0gh	1.8de	1.7d	12.3e	32.7de	24.0c	7.0c
CIAT 06133[d]	4.7ab	3.3cde	3.3c	3.6b	65.2a	45.1cd	49.4ab	40.5a

[a] On a 1-5 damage scale (1, no damage; 5, severe damage; plant killed)
[b] Susceptible check
[c] Resistant check
[d] 'Tolerant' check
Means within a column followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test). LSD 5% value for comparing species means for damage scores in a row is 1.12. LSD 5% value for comparing species means for percentage survival in a row is 11.5%.

In general, *A varia* and *Mahanarva* sp. caused less damage than *Z. carbonaria* and *Z. pubescens*. Antibiosis resistance (expressed as reduced percentage survival) was more common to *Mahanarva* sp. than to the other species tested. In a number of genotypes, low damage scores with *Z. carbonaria* and *Z. pubescens* did not necessarily coincide with lower nymph survival suggesting that antibiosis to *Zulia* spp. may not be present in most of the genotypes tested.

To facilitate the interpretation of these data, genotypes were arbitrarily classified for visual damage scores as follows: 1-2, resistant; 2.1-3.0, intermediate; >3.0, susceptible. For percentage nymph survival the classification was as follows: resistant (<30.0 survival), intermediate (30-40%), and susceptible (>40%). Ratings are presented in Table 40.

Table 40. Differential levels of resistance to four species of spittlebug in *Brachiaria* genotypes.

Genotype	Damage scores				Percentage nymph survival			
	A. varia	*Z. carbonaria*	*Z. Pubescens*	*Mahanarva* sp.	*A. varia*	*Z. Carbonaria*	*Z. pubescens*	*Mahanarva* sp.
BR97NO/0047	I[a]	S	S	R	R	S	S	R
BR97NO/0082	I	S	S	R	S	S	S	R
BR97NO/0155	R	S	I	I	R	S	S	R
BR97NO/0402	S	I	I	S	S	S	S	S
BR97NO/0457	S	S	I	S	S	S	S	S
BR97NO/1143	R	R	I	I	I	S	S	R
CIAT 16844	I	S	S	S	I	S	S	R
CIAT 16314	S	I	I	S	S	S	S	S
CIAT 00606[b]	S	S	S	S	S	S	S	S
BR4X-44-02 [b]	S	S	S	S	S	S	S	S
CIAT 06294[c]	R	I	I	R	R	I	S	R
CIAT 36062 [c]	R	R	R	R	R	I	R	R
CIAT 06133[d]	S	S	S	S	S	S	S	S

[a] R, resistant; I, intermediate; S, susceptible.
[b] Susceptible check.
[c] Resistant check.
[d] 'Tolerant' check.

Several important conclusions can be drawn from our results:

a) There was a good correlation between resistance ratings for *A. varia* and *Mahanarva* sp. for both damage scores and percentage nymph survival. In general, those with antibiosis resistance to *A. varia* also showed antibiosis resistance to *Mahanarva* sp. These and previous results tend to suggest that the mechanisms underlying resistance to these two species may be the same.
b) Several genotypes showed reduced damage when exposed to *Z. carbonaria* and *Z. pubescens* but no evidence of antibiosis to *Zulia* spp. was found in the six new hybrids (labeled BR97NO/) nor in the two accessions tested (CIAT 16844 and CIAT 16314). Work is in progress to ascertain if the reduced damage caused by *Zulia* in genotypes that are resistant to *A. varia* is due to tolerance to the *Zulia* complex.
c) The only genotype showing antibiosis resistance to both *Zulia* species was the resistant check, CIAT 36062. This hybrid is the most resistant material ever tested for resistance to *A. varia*. Remains to be ascertained if this material possesses a mechanism of defense that works across spittlebug species.
d) The cultivar 'Marandú' (CIAT 6294), a well-known source of resistance to *A. varia*, was also resistant to *Mahanarva* sp. It was intermediate for damage when exposed to *Zulia* spp., showed intermediate levels of antibiosis resistance to *Z. carbonaria* but no antibiosis resistance to *Z. pubescens*. Work is in progress to measure tolerance to *Zulia* spp. in this genotype.
e) CIAT 6133 ('Llanero'), a cultivar that was released many years ago as tolerant to spittlebug was susceptible to all species tested.

Further testing and analysis with a few interesting genotypes (Table 41, Figure 22) confirmed that hybrids selected for antibiosis resistance to *A. varia* and *Mahanarva* sp., do not necessarily have antibiosis resistance to the *Zulia* complex.

Table 41. Percentage nymph survival and resistance ratings in five *Brachiaria* genotypes exposed to attack by nymphs of four spittlebug species. Means of 10 replications for each genotype-insect species combination.

Genotype	*Aeneolamia varia*		*Zulia carbonaria*		*Zulia pubescens*		*Mahanarva* sp.	
	% Survival	Rating [a]	% Survival	Rating	% Survival	Rating	% Survival	Rating
BR97NO/0047	26.0b	R	49.0b	S	48.0b	S	2.0c	R
BR97NO/0155	16.0c	R	67.0a	S	59.0a	S	7.0b	R
CIAT 06294[b]	28.0b	R	31.0c	I	51.0b	S	4.0bc	R
CIAT 36062[b]	5.5c	R	30.0c	I	18.0c	R	1.0c	R
BR4X-44-02[c]	68.0a	S	56.0ab	S	50.5b	S	36.0a	S

[a] R, resistant; I, intermediate; S, susceptible.

[b] Resistant check.

[c] Susceptible check.

Mean separation in a column by LSD at 5% level. LSD 5% value for comparing species means in a row is 10.6.

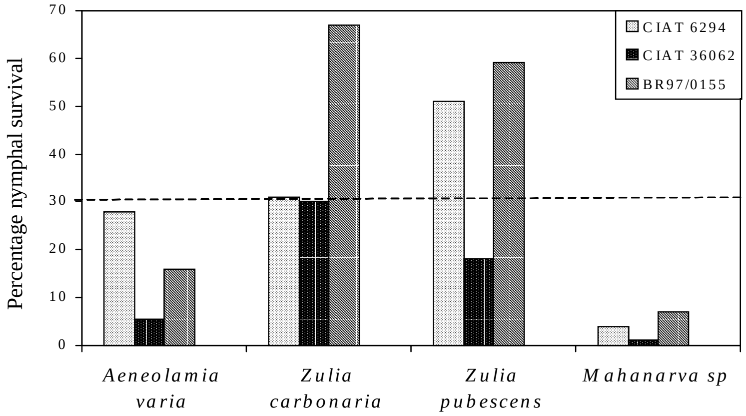


Figure 22. Levels of antibiosis to four species of spittlebug in 'Marandú' (CIAT 6294) and two *Brachiaria* hybrids bred for resistance to *Aeneolamia varia*. The dotted line represents the cut-off point for resistance rating. Means of 10 replications.

Progress towards achieving milestones

- **Defined reaction of *Brachiaria* hybrids to different species of spittlebug**

Progress continued to be made in defining the reaction of *Brachiaria* genotypes to different species of spittlebug. To implement appropriate breeding strategies to produce hybrids with broad and stable resistance to major spittlebug species, we need to understand the underlying mechanisms of antibiosis resistance to some spittlebug species.

2.5 Genetic control and molecular markers for spittlebug and reproductive mode in *Brachiaria*

Highlights

- Major progress in molecular mapping of *Brachiaria* using microsatellite and AFLP markers.
- Molecular markers (microsatellites) associated with apomixis in *Brachiaria* were identified.

2.5.1 Reproductive mode of new *Brachiaria* hybrids (SX x AP)

Contributors: J. W. Miles, J. Tohme

Nine highly promising new hybrids were assessed for reproductive mode by progeny test. Of the nine, three apomicts were confirmed. Uniformity of the nine open-pollinated progenies was assessed in the field and with molecular markers (microsatellites) (Photo 4). They were also assessed for presence of a putative tag of the apomixis locus. Agreement between field and marker data was perfect for this small set of hybrids. All three progenies identified as apomictic (based on uniformity among sibs for markers and morphological uniformity in the field) also had the putative tag of the apomixis locus.

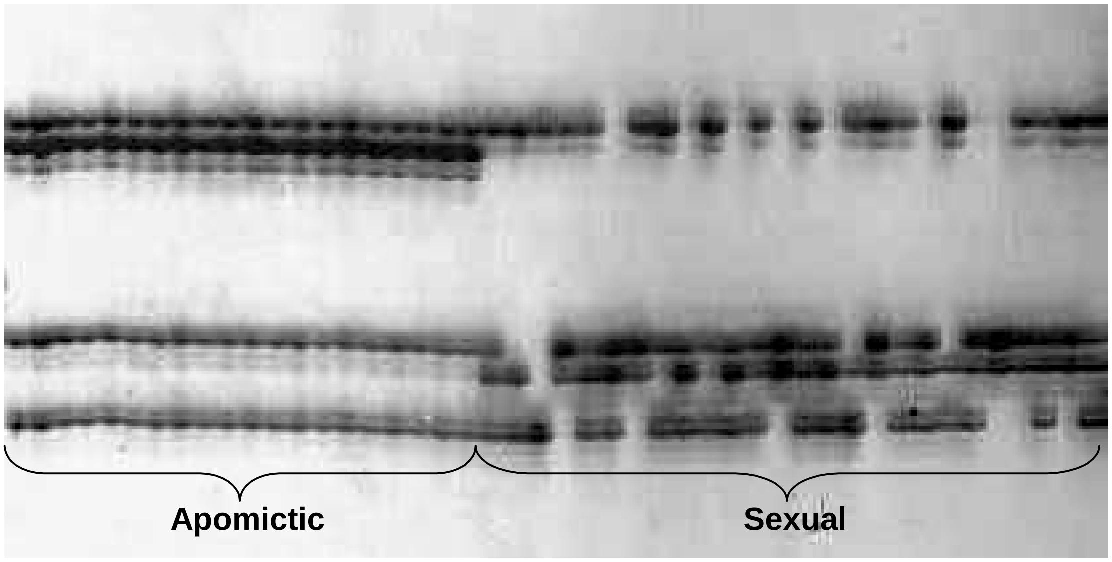


Photo 4. Identification of apomictic and sexual progenies using GM6 microsatellite.

2.5.2 Identify candidate QTL's for spittlebug resistance in a *Brachiaria* mapping population

Contributors: J. W. Miles, J. Tohme

Approximately 140 alleles at approx. 75 microsatellite loci have been assessed on parental and hybrid (full-sib) progeny (215 hybrid clones). AFLP data are also being gathered on these same genotypes. Mapping of marker loci is in progress. Spittlebug assessment on this hybrid family has been done.

2.5. 3 Molecular fingerprinting of *Brachiaria* genotypes

Contributors: J. W. Miles, J. Tohme

Sixty-seven *Brachiaria* genotypes (clones) were fingerprinted using microsatellite polymorphisms. These genotypes included: i) eight apomictic and two sexual parental genotypes, ii) nine agronomically promising accessions from CIAT's *Brachiaria* germplasm collection; iii) seven promising apomictic hybrids; and iv) 41 selections from the tetraploid sexual, hybrid-derived breeding population. A total of 118 alleles were revealed at 10 microsatellite loci on this set of genotypes. The genotypes tested showed considerable polymorphism (Photo 5). Using a correspondence analysis procedure for all the marker data, genotypes were classified into nine groups. This classification explains 99% of the total variability. The clones selected from the tetraploid sexual breeding population were similar and tended to be grouped into a single cluster, along with *B. decumbens* CIAT 606 (cv. Basilisk) and two tetraploid *B. ruziziensis* clones (Photo 5). The *B. brizantha* germplasm accessions evaluated were highly diverse. The genetic diversity documented by these markers may allow rational choice of candidate apomictic tester clone(s) for use in selecting the sexual population on heterotic interaction with the tester as well as directly on it's own attributes (e.g. spittlebug reaction, in vitro digestibility, etc.).

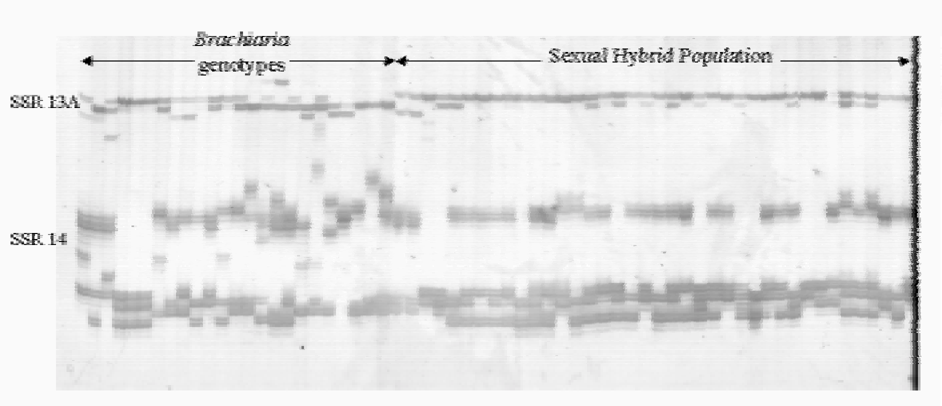


Photo 5. Silver stained polyacrylamide gel showing SSRs 13A and 14 in the 67 *Brachiaria* genotypes.

2.5.4 Enhancing detail of molecular map of *Brachiaria*

Contributors: J. W. Miles, J. Tohme

A comparative molecular genetic map of *Brachiaria* has been constructed with RFLP markers from rice, pearl millet, and sorghum. Additional loci are being added using 96 microsatellite markers and two combinations of AFLP markers on a mapping population consisting of 215 F1 hybrid progenies from the cross *B. ruziziensis* 44-03 x *B. brizantha* CIAT 6294 (cv. Marandu). Data processing is underway as this report is being prepared (17Oct00).

Progress towards achieving milestones

- **List of new *Brachiaria* hybrids (Sx x Ap) characterized for reproductive mode by progeny tests**

Three apomictic hybrid clones have been selected from a small set (nine) promising new selections based on progeny test using microsatellite markers to identify progeny uniformity, confirmed with field progeny test.

- **Identified tentative QTL’s for spittlebug resistance in *Brachiaria***

To date, and with a large number of molecular markers, no consistent association has been found between reaction to spittlebug and presence or absence of particular alleles. This result suggests either that resistance is owing to the combined effect of many genetic loci of individually small effect, or insufficient coverage of the Brachiaria genome with marker loci.

2. 6 Role of endophytes in tropical forage grasses

Highlights

- Identified promising PCR amplified DNA products specific to isolates of *Brachiaria* endophytes which can be used for quick endophyte detection in *Brachiaria* tissues.
- Confirmed antibiotic effects of endophytes against grass pathogens.
- The presence of endophyte (*Acremonium implicatum*) in *Brachiaria arrecta* improved root biomass production and contributed to greater tolerance to severe drought.

In temperate zones, endophytic fungi are widely used as biological protection agents for forage and turf grasses. They form nonpathogenic and intercellular associations with grasses and sedges, completing their entire life cycle within the plants' aerial parts. Our surveys and studies confirmed that various endophytic fungi, including *Acremonium* spp., also inhabit native savanna grasses and introduced tropical forage grasses. We are now determining the potentially symbiotic relationships between these fungi and C_4 tropical forages, specifically between the endophyte *A. implicatum* and *Brachiaria* grasses.

2.6.1 Endophyte seed transmission and artificial implications of endophytes in *Brachiaria*

Contributors: X. Bonilla, S. Kelemu

Endophyte hyphae can be detected in seeds, seedlings, leaf blades and leaf sheaths by staining. Mutualistic seed-borne associations between species of *Acremonium* and many cool-season grasses are the subject of considerable research.

Explanting aseptically excised embryos on an auxin-supplemented medium and examining resulting calli for the presence of endophytic fungi have been used to evaluate endophyte incidence in seed lots of cool-season grasses.

We have examined some seeds of naturally endophyte-infected *Brachiaria* plants using staining techniques described in earlier reports.

Endophytic fungi which reside in intercellular spaces of plants have the feature of maternal transmission through the ovule and seeds of their hosts which allows plant inoculations to be made only once and the grass-endophyte association becomes self- replicating. Although we have detected and identified the *Brachiaria* endophyte in *Brachiaria* seeds, its transmission through seeds to the next generations of plants is not known. Experiments are in progress to determine endophyte transmission through *Brachiaria* seeds.

Artificial introductions of endophytes in *Brachiaria* gentoypes

Inoculations of *Brachiaria* plants with endophytes to generate stable symbiotic associations are useful to further characterize endophytes, define, study and understand the endophyte-host associations, and generate novel materials. Successful endophyte-grass symbiosis often requires a compatibility between the endophyte genome and that of the plant. Not every endophyte/host association will result in mutually beneficial effects.

B. brizantha CIAT 6780, CIAT 26110, and *B. decumbens* CIAT 606 were inoculated with two isolates of *A. implicatum*. Methods of inoculation have been described in earlier reports (CIAT

IP5 Annual Report 1998, 1999). In short, endophyte inoculum was introduced into the apical meristem seedlings using a sterile fine entomological needle.

The inoculation procedure often causes damage to meristematic tissues of seedlings and as a result a number of inoculated plants died (Table 42). Of the surviving plants, only very few (approximately 1%) result with a stable endophyte association.

Table 42. *Brachiaria* plants inoculated with *Acremonium implicatum* isolate 6780.

	B. decumbens 606	*B. brizantha* 26110	*B. brizantha* 6780
No. of inoculated plants	218	73	86
No. of surviving plants	55	49	35
% survival	25	67	40

Inoculated plants with confirmed endophyte presence are being maintained for seed production in the green house.

2.6.2 Alkaloid profiles in endophytes isolated in *Brachiaria*

Contributors: X. Bonilla, J. Porter (USDA), S. Kelemu

Isolates of endophytes were cultivated in alkaloid media for several months as described in earlier reports (IP-5 Annual Report, 1999). For the benefit of the reader, we will indicate the methods used herein. Special alkaloid media were used to grow endophyte isolates. Flasks containing M102 medium (30 g sucrose, 20 g malt extract, 2 g bacto peptone, 1 g yeast extract, 0.5 g $MgSO_4$, 1 g KH_2PO_4, 0.5 g KCl and distilled water to 1L, pH 6.0) were inoculated with endophyte mycelium (20-40 pieces of 2 x 4 mm mycelial agar discs). The cultures were incubated on a rotary shaker (200 rpm) at 25 °C. Five ml of the two week M102 culture was used to inoculate 500 ml flasks containing 100 ml of SM medium (100 g sorbitol, 40 g glucose, 10 g succinic acid, 1 g KH_2PO_4, 0.3 g $MgSO_4$, 1 g yeast extract, 20 ml 50x Vogel's salts [150 g Na_2 Citrate, 250 g KH_2PO_4, 100 g NH_4NO_3, 10 g $MgSO_4$, 5 g $CaCl_2$, 5 ml trace elements, 5 ml 0.1 mg/ml biotin, 750 ml distilled water; pH 6.8], to 1L distilled water; pH to 5.6 with NH_4OH). The trace elements solution contained 5 g citric acid, 5 g $ZnSO_4.6H_2O$, 1 g $Fe(NH_4)2\ (SO_4)2.\ 6H_2O$, 250 mg $CuSO_4.5H_2O$, 50 $MnSO_4$, 50 mg boric acid, 50 mg $Na_2MoO_4.2H_2O$, and distilled water to 100 ml. The cultures were incubated for a week at 25 °C on the rotary shaker as earlier. At the end of one week, the cultures were moved to a stationary position where they remained undisturbed for about 4 months. A film of mat of mycelium is expected to form over the surface of liquid medium. At the end of the incubation time, the endophyte mycelium will be collected, freeze-dried and shipped for alkaloid analysis.

Unfortunately, the Toxicology and Mycotoxin Research Unit, USDA/University of Georgia, USA, apparently has so many samples for analysis that they were unable to provide us with alkaloid data at the time of this report writing.

2.6.3 Cloning and characterization of endophyte-specific DNA fragment for quick detection

Contributors: Y. Takayama (JICA, Japan), H. Guixiu (CATAS, The Peoples Republic of China), S. Kelemu

Methods of detecting endophyte hyphae in plant tissues such as leaf sheaths, meristems and seeds include tissue stains, immunology, culturing on media or a combination of these. Although these methods are useful, endophyte presence can be overlooked when their hyphae are few or sparsely distributed within the plant tissue. These methods are also time consuming. A sensitive and accurate method of detection is crucial for various experiments involving grass-endophyte symbiosis. The purpose of this study was to develop and evaluate a quick and accurate method for detecting endophytes in *Brachiaria* based on polymerase chain reaction (PCR).

Endophyte-infected (+E) and endophyte-free (-E) *Brachiaria* clones were generated using a systemic fungicide. The plants used in this study are as follows: *B. decumbens* CIAT 606 (+E), *B. decumbens* CIAT 606 (-E), *B. brizantha* CIAT 26110 (+E), *B. brizantha* CIAT 26110 (-E), *B. brizantha* CIAT 6780 Parcel 201 (+E), *B. brizantha* CIAT 6780 Parcel 201 (-E), *B. brizantha* CIAT 7680 Parcel 501 (+E), *B. arrecta* CIAT 16845 Parcel 909 (+E), *B. arrecta* CIAT 16845 Parcel 909 (-E), *B. arrecta* CIAT 16845 Parcel 904 (+E), *B. arrecta* CIAT 16845 Parcel 904 (-E). The 6 endophytic fungi were isolated from endophyte-infected plants mentioned above. The pathogenic fungi, *Rhizoctonia solani* CIAT 6780 and *Drechslera* sp., were isolated from infected *Brachiaria* plants.

Total genomic DNA was extracted according to standard procedures from fungal mycelia/conidia collected directly from agar medium. DNA amplifications were conducted using 84, ten-base oligonucleotic primers, programmed in a Programmable Thermal Controller with 45 cycles of a 1 min (2 min for the first cycle) denaturation step at 94 °C, annealing for 1 min at 35 °C, and primer extension for 1min (7min for the final cycle) at 72 °C. The amplification products were resolved by electrophoresis in a 1. 2 % agarose gel, stained with ethidium bromide, and photographed under UV lighting.

Photographs of the amplification products were examined for common bands in all the endophyte isolates. An approximately 500 bp common DNA fragment was recovered from agarose gel using Wizard® DNA Clean-Up System kit (Promega). The recovered fragment was analyzed on agarose gel. This common DNA fragment was ligated to linearlized pGEM®-T Easy Vector (Promega) plasmid. The recombinant plasmids were transformed into competent cells of *Escherichia coli* DH5α prepared using calcium chloride standard procedure (Sambrook et al., 1989. Molecular Cloning: A Laboratory Manual). Mini- plasmid preparations were done on ampicillin-resistant colonies to isolate recombinant plasmid DNA by alkaline lysis methods (Sambrook et al. 1989).

Total genomic DNA was extracted from fungal mycelia and plant tissues using Dneasy™ Plant Mini Kit (QIAGEN). Southern blot and dot blot hybridizations and probe labeling were performed using ECL™ direct nucleic acid labeling and detection system according to manufacturer's instructions (Amersham).

The approximately 500 bp common PCR fragment was obtained in 5 endophyte isolates amplified with primer AK10 (Photo 6). The recombinant plasmid was digested with *Eco*RI and electrophoresed in agarose gel (Phoyo 7). The fragment was recovered and used as a probe in Southern and dot blot hybridizations. Distinctive hybridization signals were observed in 5 endophyte isolates, but not in the total genomic DNA of plants (-E, +E) and in pathogenic fungi (*R. solani* and *Drechslera* sp.), and a non-pathogenic fungus isolated from *B. brizantha* CIAT 26110 (Photos 8, 9, 10).

These results are highly promising in that the probe hybridized specifically to DNA isolated from the endophyte isolates which are shown to be genetically similar (Kelemu et al., 2000), but not to DNA isolated from non-endophytic fungi or to DNA from *Brachiaria* plants. The fact that no hybridization signal was detected in either –E or +E *Brachiaria* plants does not necessarily indicate that the probe does not detect endophytes *in planta*. It is highly likely that the endophyte mycelia/DNA concentration in the plants is not high enough to be detected using Southern or dot blot analysis. However, it is likely that a PCR-based analysis may detect the presence of endophytes in *Brachiaria* plants. The usefulness of the probe is further confirmed by the fact that a recently isolated endophyte from seeds of *Brachiaria* by GRU seed pathology lab. tested positive in dot blot tests (Photo 8). This endophyte isolate has a number of morphological similarities to the other endophytic isolates (data not shown). We are in the process of sequencing the 500-bp probe in order to synthesize a specific primer.

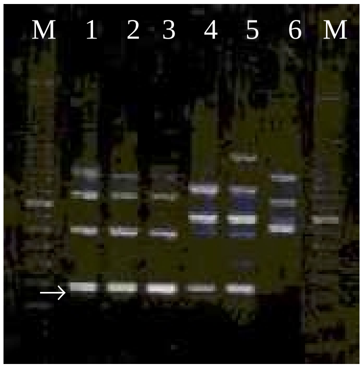


Photo 6. PCR patterns of isolates amplified with random primer (AK10). Lanes 1, DNA of isolate from *Brachiaria decumbens* 606 ; 2, from *B. brizantha* 6780 parcel 201 ; 3, from *B. arrecta* 16845 parcel 904 ; 4, from *B.brizantha* 6780 parcel 501 ; 5, from *B. arrecta* 16845 parcel 909 ; 6, non-endophytic isolate from *B.brizantha* 26110 ; M, marker 100bp ladder. The arrow indicates a 500-bp fragment used for cloning.

Photo 7. Electrophoretic analysis of restriction fragments of recombinant plasmid (pGEM®-T Easy Vector) DNA digested with *Eco*R I (Lane 1-4). Lane M is marker 100bp ladder. The arrow indicates the fragment (about 500bp) used as a probe for DNA hybridization. The picture shows successful cloning of the desired fragment.

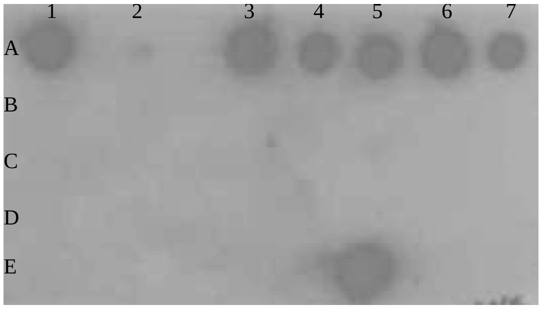


Photo 8. Dot blot hybridization of total genomic DNA extracted from endophytes, plants, and pathogenic and non-pathogenic fungi. Number A1, DNA of endophyte isolate from *Brachiaria decumbens* 606; A2, non-endophytic isolate from *B. brizantha* 26110; A3, endophyte from *B. brizantha* 6780 parcel 201 ; A4, endophyte from *B.brizantha* 6780 parcel 501 ; A5, from *B. arrecta* 16845 parcel 909 ; A6, from *B. arrecta* 16845 parcel 904; A7, DNA of an endopyte recently isolated from seeds of *Brachiaria*; B1, plant DNA from *B. brizantha* 6780 parcel 201 (-E); B2, from *Brachiaria decumbens* 606 (-E); B3, from *B. arrecta* 16845 parcel 904 (-E); C1, from *B. brizantha* 6780 parcel 201 (+E); C2, from *B. decumbens* 606 (+E); C3, from *B. arrecta* 16845 parcel 904 (+E); D1, fungal DNA from *Drechslera sp*.; D2, from *Rhizoctonia solani* CIAT 6780; E5, probe EB1.

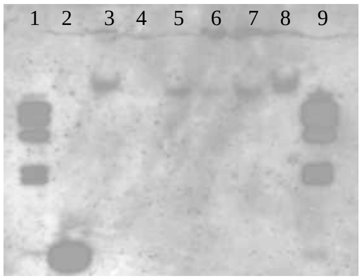


Photo 9. Southern hybridization of endophyte isolates. Lane 1,9, λ DNA digested with *Hin*d III; 2, probe EB1; 3, DNA of endophyte isolate from *Brachiaria decumbens* 606 ; 4, non-endophytic isolate from *B. brizantha* 26110; 5, from *B. brizantha* 6780 parcel 201; 6, *B.brizantha* 6780 parcel 501; 7, from *B. arrecta* 16845 parcel 909; 8, from *B. arrecta* 16845 parcel 904.

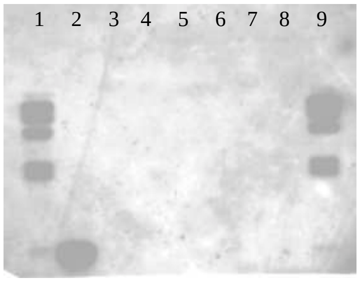


Photo 10. Southern hybridization of total genomic DNA of plants. Lane 1,9, λ DNA digested with *Hin*d III; 2, probe EB 1; 3, plant DNA from *Brachiaria decumbens* 606 (-E); 4, from *B. arrecta* 16845 parcel 904 (-E); 5, from *B. brizantha* 6780 parcel 201 (-E) ; 6, from *B. decumbens* 606 (+E); 7, from *B. arrecta* 16845 parcel 904 (+E); 8, from *B. brizantha* 6780 parcel 201 (+E).

2.6.4 The role of endophytes in drought tolerance of *Brachiaria* species

Contributors: A. C. Bolaños, J. Ricaurte, S. Kelemu, I. M. Rao

Rationale: Dry season tolerance and persistence are two major agronomic traits that determine the economic usefulness of tropical grasses in the subhumid regions. A glasshouse study was conducted to determine the role of endophyte (*Acremonium implicatum*) infection on drought tolerance of *Brachiaria arrecta*. Last year, we reported that the presence of endophyte had no significant effect on shoot growth characteristics of the grass at control and moderate drought stress levels. However, the endophyte infected plants maintained better leaf expansion and produced significantly greater leaf biomass under severe drought stress conditions. This year, we report further progress in testing the role of endophytes on root and shoot attributes that are linked to drought tolerance in *Brachiaria.*

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

Results and Discussion: The presence of endophyte maintained better leaf expansion and produced significantly greater leaf biomass under severe drought stress conditions (IP5 Annual Report, 1999). This improved ability of endophyte infected plants was found to be associated with significant increase in root biomass production at severe level of water stress (Table 43). Root production was relatively less affected by water stress than the shoot growth (IP5 Annual Report, 1999; Table 1).

Root length was also greater with endophyte infected plants that were subjected to water stress. Specific root length -- a measure of fineness of the root system -- was found to be greater with endophyte infected plants under water stress. The greater production of fine roots with endophyte infected plants contributed to greater acquisition of not only water but also nutrients, particularly potassium (K) thereby improving not only leaf expansion and leaf biomass production but also facilitating osmotic adjustment under severe water stress. Among the shoot attributes measured (leaf and stem nutrient contents, leaf and stem total nonstructural carbohydrates, and leaf and stem ash contents), stem ash content showed significant decrease with endophyte infected plants under water stress. Ash content is a measure of mineral status in the tissue.

The lower level of mineral status indicates efficient utilization of nutrients for growth during water stress. These results indicate that the presence of endophyte in tropical grasses such as *Brachiaria* can significantly improve dry season tolerance and contribute to greater production of meat and milk in the tropics. Further research work is needed to evaluate the role of endophytes in improving drought tolerance in *Brachiaria* under field conditions.

Table 43. Influence of endophytic fungus (*Acremonium implicatum*) infection on drought tolerance of *Brachiaria arrecta* CIAT 16845 grown in pots (4 kg soil) in the greenhouse at three levels of water stress.

Plant attributes	*Endophytic infection* Status	Water stress level*			LSD (P = 0.05)
		Control	Moderate	Severe	
Root biomass	+	1.60	1.57	1.33	
(g/pot)	-	1.38	1.44	1.07	NS
	LSD$_{(p = 0.05)}$	NS	NS	0.13	
Root length	+	234	129	120	
(m/pot)	-	256	92	97	107
	LSD$_{(p = 0.05)}$	NS	NS	NS	
Specific root length	+	147	129	120	
(m/g)	-	214	92	97	82
	LSD$_{(p = 0.05)}$	NS	NS	NS	
N uptake	+	251	218	125	
(mg/pot)	-	214	227	106	27
	LSD$_{(p = 0.05)}$	NS	NS	NS	
K uptake	+	757	509	187	
(mg/pot)	-	723	535	145	66
	LSD$_{(p = 0.05)}$	NS	NS	15	
Stem ash content	+	7.79	5.83	6.57	
(%)	-	7.50	7.86	7.61	NS
	LSD$_{(p = 0.05)}$	NS	1.41	0.16	

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

2.6.5 Effect of endophytes on plant-pathogenic fungi *in vitro*

Contributors: B. Pineda (SB1, CIAT), X. Bonilla, S. Kelemu

Several endophytic fungi associated with temperate grasses have been reported to inhibit the growth of plant-pathogenic fungi *in vitro*. We have reported the inhibitory properties of isolates of endophytes (CIAT, IP-5 Annual Report, 1999). A new endophyte isolate has been obtained from seeds of *Brachiaria* and purified in collaboration with GRU, CIAT. In this study, we used this new isolate to study its antifungal properties.

A method was used to determine the presence of antifungal activity of the endophyte when grown *in vitro*. PDA containing petri plates were inoculated with a colony of the endophyte on one side of each plate and incubated for 2 weeks. Two weeks later, an agar disk (4 mm diameter) of *Drechslera* sp. or *Rhizoctonia solani* removed from the actively growing edge was placed on the opposite side of each plate. The plates were further incubated and when *Drechslera* mycelia covered the control plates, measurements were taken as described above. All tests were replicated three times.

Results show that the growth of *R. solani* was reduced by as much as 30% in the presence of the endophyte compared to that of control plates. However, the effect on *Drechslera* sp. was less, with a reduction of up to 10%.

2.6.6 Screening of *Brachiaria* hybrids for endophyte presence

Contributors: X. Bonilla, J. Miles, S. Kelemu

Tissues of 60 *Brachiaria* genotypes provided by the breeding program were screened using two detection methods. Tissues (leaves or leaf sheaths) of *Brachiaria* were collected from visually symptom-free, healthy plants. Small pieces of the tissues were placed in tubes containing Carnoy's solution (6:3:1 ethyl alcohol : chloroform : 85% glacial acetic acid) for at least 24 h. They were then transferred to 70% aqueous ethyl alcohol twice, each for 24 h to remove chlorophyll. The tissues were further cut into small pieces no longer than 1.0 cm and stained with aniline blue (2:1 in 70% aqueous ethyl alcohol: 85% lactic acid) for 5-18 h, depending on the tissue's age and origin. The stained tissues were cleared by transferring them sequentially in solutions of 100% ethyl alcohol (2 × 60 min), 1:3 methyl salicylate : ethyl alcohol (60 min), 1:1 methyl salicylate : ethyl alcohol (60 min), 3:1 methyl salicylate : ethyl alcohol (60 min), and 100% methyl salicylate (60 min). The samples were then examined at ×200, using bright-field microscopy. Samples were also used for fungal isolation work.

Small pieces (5 mm) of tissues were surface sterilized in 3.25% NaOCl solution for 10 min, in 70% ethanol for 1 min, and rinsed three times with sterilized distilled water. Excess moisture was removed from the samples with sterilized filter papers. They were then plated on two different media—potato-dextrose agar (PDA, Difco) and corn meal agar (CMA, Difco)—and incubated for 4 to 6 weeks at 28°C.

At the time of this report writing, one *Brachiaria* sample tested positive in both tests for endophyte presence. This plant, Plant #47, is one of the 41 selections from the *Brachiaria* tetraploid, sexual breeding population. Complete identification of this endophyte will be conducted.

2.6.7 Studies on bacterial endophytes

Contributors: Viviana Pizo (Universidad del Cauca), S. Kelemu

Although many of the existing literature on endophytes mostly is on fungi, endophytic prokaryotes are also important. Symptom-less plants are often internally colonized by bacteria. Such bacterial colonization is thought to e mostly intercellular. To date, many reported endophytic bacteria belonged to the genera *Pseudomonas*, *Bacillus*, and *Azospirillum*. These bacteria are members of common soil bacteria. Many of these bacterial strains are known to have significant positive effects on plant growth. An important example is *Acetobacter diazotrophicus*, a nitrogen-fixing bacterial endophyte of sugarcane. Some bacterial endophytes are known to directly inhibit the growth of plant pathogens, or to induce systemic host resistance to pathogens.

Isolation, identification and characterizations

Green, vigorous plants of species of *Brachiaria* (23 accessions and hybrids) with no apparent disease symptoms of any kind were selected for bacterial isolations. Roots, leaf or stem tissues were used for bacterial isolations. Standard protocols were used for isolations and nutrient agar (Difco; bacto-beef extract 3 g, bacto-peptone 5 g, bacto-agar 15 g per L) was used for culturing.

Initial traits selected for characterization of predominant bacterial colonies were resistance to antibiotics and fungal inhibitory properties. The antibiotics chosen were: chloramphenicol (25 μg/ml), tetracycline (50 μg/ml), kanamycin (50 μg/ml), and streptomycin (50 μg/ml). Each antibiotic was incorporated into nutrient agar for culturing use. Bacterial growth on antibiotic-containing media was compared to control plates with no antibiotics. Methods for fungal inhibition studies have been described in Kelemu et al. 1995 (Australasian Plant Pathology 24: 168-172).

For identifications, colony morphology characteristics and biochemical test results were used. The most commonly isolated bacteria were tentatively identified as *Serratia*, *Enterobacter* and *Citrobacter*. However, further studies and confirmation are needed. Several distinct bacterial colonies with high levels of resistance to antibiotics were identified (Table 44). The bacterial colony with the strongest antifungal property was 16445-2 isolated from *B. brizantha* 16445.

Table 44. Bacterial colonies and their resistance to antibiotics.

Bacterial Colony Code	Plant Host	Antibiotic Resistance
16497-1	*Brachiaria decumbens* 16497	Chloramphenicol
1143-2	BR97/1143 (hybrid)	Chloramphenicol, tetracycline
16441-2	*Brachiaria brizantha* 16441	Streptomycin, kanamycin
383-1	BR97/383	Streptomycin
16327-2	*Brachiaria brizantha* 16327	Kanamycin
36061-2	Hybrid	Kanamycin
155-2	BR97/155	Kanamycin

Progress towards achieving milestones

- **Defined effect of endophytes on *Rhizoctonia***
- **Confirmed effect of endophytes on drought tolerance in *Brachiaria***

We have demonstrated that endophytes have negative effect on the growth of *Rhizoctonia in vitro*. In addition, we have demonstrated the presence of endophyte (*A. implicatum*) in *Brachiaria arrecta* improved root biomass production and contributed to greater tolerance to severe drought.

2.7 Interactions between host and pathogen in *Brachiaria,* and *Stylosanthes*

Highlights

- Determined the genetic diversity of 119 isolates of *Colletotrichum gloeosporioides* using RAPD and AFLP.
- Determined inheritance of the introduced chitinase gene in *Stylosanthes* progenies.
- Identified sources of resistance to a bacterial wilt disease identified in 1999 in genotypes of *Brachiaria*.

We continued our work on the population dynamics of the anthracnose pathogen and characterized more than 119 isolates collected in South America. We have adopted AFLP techniques for the first time. Some of the *Stylosanthes* population developed by the CIAT breeding program remained to be resistant to a wide range of *C. gloeosporioides* isolates. A new bacterial wilt disease of *Brachiaria* caused by *Xanthomonas campestris* has been further characterized. Sources of resistance have been identified in *Brachiaria*.

2.7.1 Biodiversity studies on the anthracnose pathogen of *Stylosanthes*: *Colletotrichum gloeosporioides*

Contributors: C. Giraldo, F. Muñoz, C. Garcia (Universidad Nacional, Bogota), Y. Takayama (JICA, Japan), G. Segura, S. Kelemu

Population studies to determine pathogen variations are important components of effective plant breeding for durable disease resistance. Traditionally, pathogen races are determined by artificially inoculating isolates of a pathogen on preferably genetically well defined genotypes of a host, and evaluating the phenotypes of the interaction. This provides valuable information for several host-pathogen system although it has shortcomings especially when the genetics of the host used is not well characterized. The use of molecular markers is important in determining genetic variation in pathogen populations. Random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP), and more recently, amplified fragment length polymorphisms (AFLP) have been used by plant pathologists to characterize plant pathogenic fungi. Understanding general disease development conditions is essential for overall disease management strategies.

The genetic diversity of 119 isolates of *Colletotrichum gloeosporioides* infecting *Stylosanthes guianensis* has been examined. The isolates were collected from 1993 to 2000, from four regions in Colombia (Palmira, Carimagua, Quilichao y Caquetá), Brazil and Australia (standard isolates). Two molecular techniques were used, random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP). Methodology details for the RAPD analysis have been described in previous progress reports and in various publications (Kelemu et al., 1997, 1999). AFLP analysis was initiated in 1999- 2000 to complement the information obtained with the RAPD analysis. AFLP technology is used to visualize genetic polymorphisms among isolates, generating fingerprints that can be used to assess the relatedness between isolates. The strength of this methodology is that it combines two technologies, the digestion of the DNA from the classical RFLP analysis (restriction fragment length polymorphism), followed by the amplification of the fragments from the PCR (polymerase chain reaction) technique, and the visualization of the polymorphism from RFLP.

The “AFLP Analysis System for Microorganisms” kit from Gibco BRL was used. Genomic DNA of each isolate was digested with the restriction enzymes *Eco*RI and *Mse*I. Double stranded DNA specific adapters were ligated to the *Eco*RI and *Mse*I ends of the fragments. On the next step, the fragments were amplified. Seven primers for the *Eco*RI and five primers for the *Mse*I ends were used for the amplification. The primers contained either zero, one or two selective nucleotides. To determine which primer combinations would give the most information on the genetic diversity, a preliminary evaluation was performed. All possible primer combinations were evaluated on four randomly chosen isolates. The primer pairs E-T/M-A, E-G/M-T, E-AC/M-A, E-AC/M-C, E-AC/M-G were the most polymorphic and were used to evaluate all other isolates.

The amplification was done on a MJ-Research PTC-100 thermal cycler. The amplification conditions were as described by Gibco BRL (USA). Amplification products were separated on a 6% denaturing polyacrylamide sequencing gel. Gels were electrophoresed for three hours at 100 watts. At the end of the electrophoresis period, gels were stained with silver nitrate. The resulting banding patterns were analyzed manually. Each band was codified either as 0 or 1, whether it was present or not, in any particular isolate. A matrix containing isolates and coded bands was generated. NTSYS-pc (Exeter Software, NY) was used for a similarity analysis using the Dice coefficient of similarity, then the program SAHN performed a cluster analysis to generate a similarity dendrogram. Pathogenic variability was established on 117 isolates by evaluating the virulence patterns on 12 *Stylosanthes* accessions. Methodology details have been described by Kelemu et al., 1996. A correlation analysis was performed on the groupings generated by the clustering of AFLPs and RAPDs with the pathogenicity data. The AFLP similarity analysis was done with four primers with a total of 519 bands. All primer combinations chosen gave banding patterns with sufficient number of bands to detect the uniqueness of isolates but also, there were sufficient common bands to detect the related organisms (eg. Photo 11).

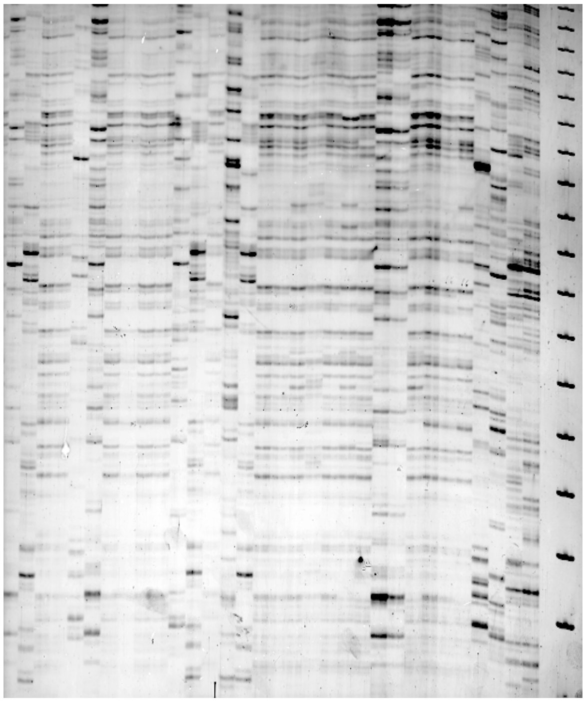


Photo 11. AFLP analysis (primer EGMT) of some isolates of *Colletotrichum gloeosporiodies* infecting *Stylosanthes guianensis*

DNA from some isolates did not amplify with a given primer, and when all the data were combined, only DNA from 73 isolates had amplified bands with all four primers used.The similarity analysis confirmed the genetic relatedness but also the complex variability of this pathogen. The dendrogram generated for the 73 isolates did not clearly separate groups, although some isolates had unique banding patterns so they separated as outliners (Figure 23).

All 73 isolates analyzed had unique morphotypes (unique banding patterns). Isolate 16011, collected in Palmira in 1993 separated from all others but the three other isolates collected at the same location on the same year grouped closely. A similarity analysis was done with 114 isolates. The DNA from these isolates were amplified with three of the primers (that means 41 isolates that did not have amplification data for one primer were among them).

The grouping generated was very similar to the one for the 73 isolates, the new 41 isolates distributed at random over the dendrogram. The cluster analysis for the primers E-AC/M-C and E-AC/M-G behaved similarly; they generated two groups of isolates, a large group that contains most of the isolates and a smaller group. The groups contain isolates from distinct origins and dates of collection. The dendrogram generated from the RAPD data was very similar to the above two AFLP primers. We are currently conducting principal component analysis and other detailed analysis to detect other possible relationships but the information is not complete yet.

During 1994 the isolate collection was intensified in Caquetá. Most of the isolates were collected from CIAT 184, the most widely used cultivar in Latin America and in China. Although these isolates were tightly close on the dendrogram, the analysis showed that they are also related to other isolates. During 1999 the collection was intensified in Quilichao, mostly because of the security problems in many parts of Colombia.

The isolates were collected from various *S. guianensis* genotypes. The RAPD analysis identified 238 polymorphic bands with 140 isolates. Most isolates had unique banding patterns but were not grouped into distinctive genetic groups. The dendrogram pattern was very similar to that generated with the AFLP data (Figure 24).

The pathogenicity analysis was done with 117 isolates. To know the pathogenic variability, disease expression on each of 12 *Stylosanthes* differentials was rated as resistant (0-3 on the Horsfall-Barratt scale) or susceptible (3-9 on the Horsfall-Barratt scale). Forty-five pathogenicity patterns were obtained. Most patterns were represented by one or two isolates showing that this phenotype is highly variable. A large group of isolates (37%) had low pathogenicity (caused disease ratings of 0-3) on all the differentials. All other isolates infected one to ten genotypes. A few isolates were highly pathogenic. The most virulent isolates of the collection were isolates number 16097 (collected from Caquetá, mean disease rating on all differentials, dr = 7.6), 16096 (collected from Caquetá, dr = 7.0), 16066 (collected from Carimagua, dr = 6.4), 16020 (collected from Palmira, dr = 5.9), 16313 (collected from Carimagua, dr = 5.6), 16072 (collected from Brazil, dr = 5.6), 16058 (collected from Palmira, dr = 5.4) and uq396 collected from Australia, dr = 5.1). ‘Endeavour’ was the most susceptible accession (mean disease rating for all isolates = 3.5) and the accession 1283 the least susceptible (mean disease rating for all isolates = 0.7).

There seems to be no apparent association between the pathogenicity pattern and the geographic origin of the isolate. A concordance analysis may have to be performed to see the relationships. None of the isolates was pathogenic on all the differentials, and none of the differentials was immune or susceptible to all isolates.

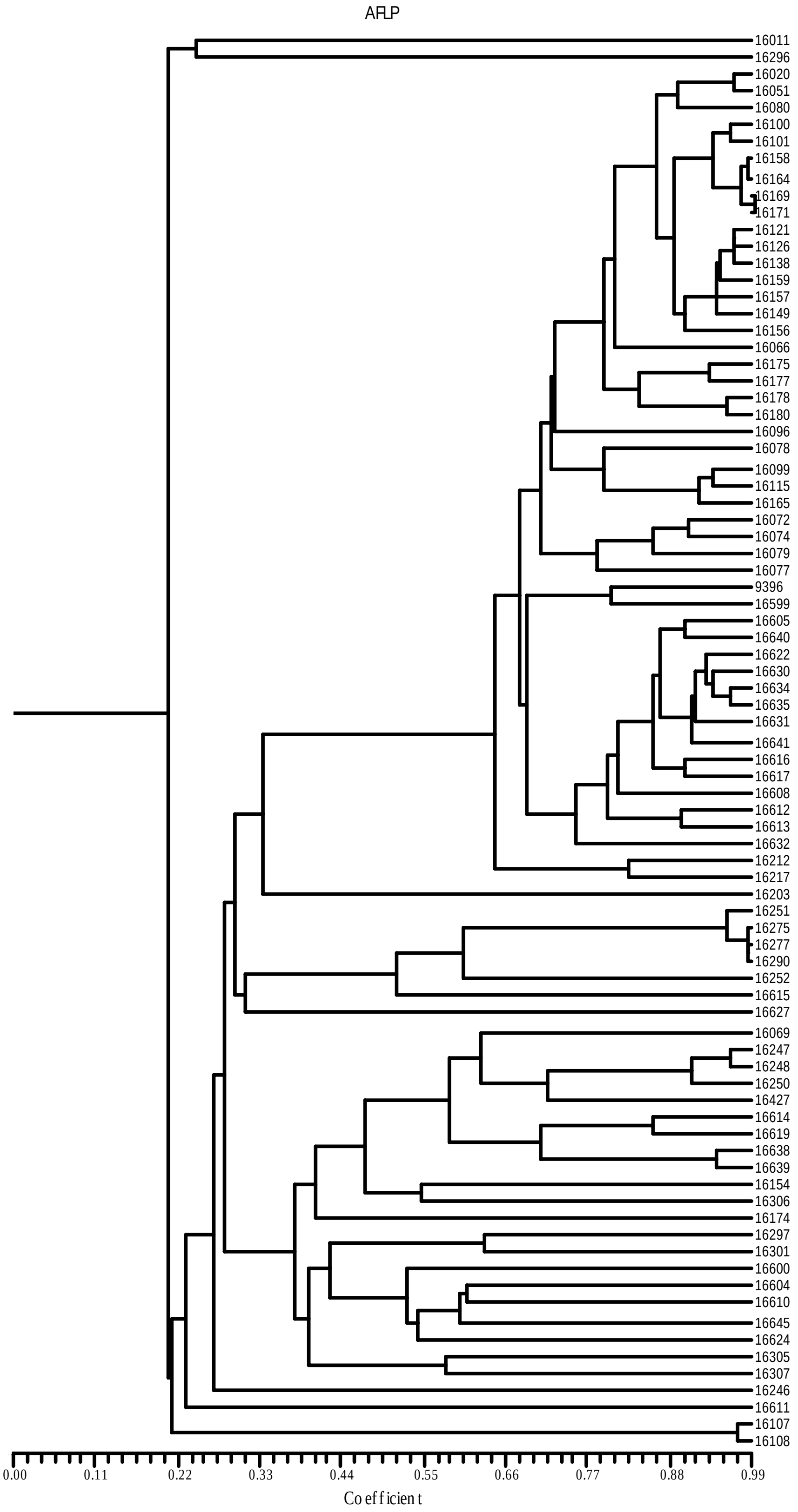


Figure 23. Cluster analysis of AFLP data of 73 *Colletotrichum gloeosporioides* isolates from *Stylosanthes guianensis*

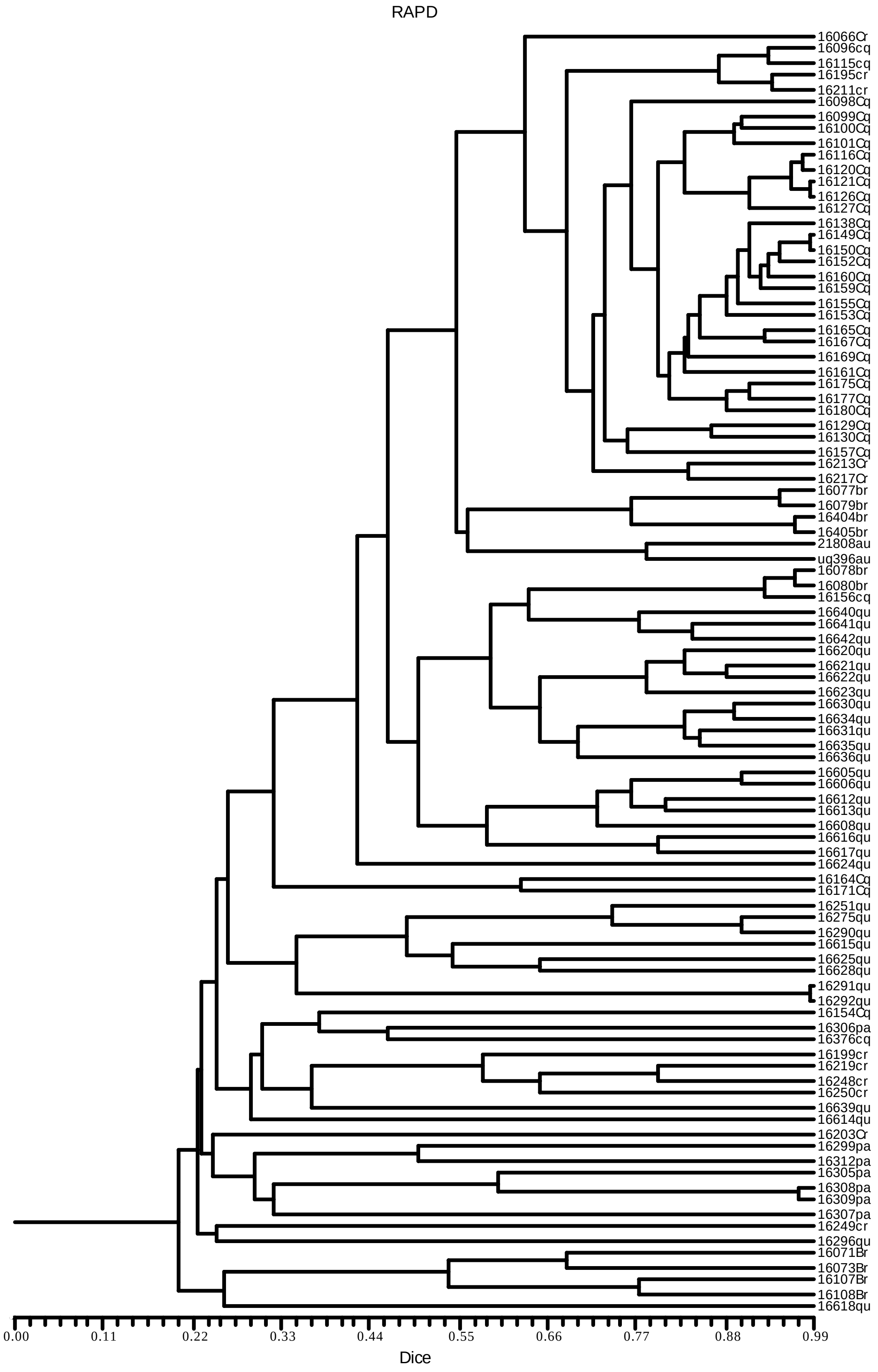


Figure 24. Cluster analysis of RAPD data of 96 *Colletotrichum gloeosporioides* isolates from *Stylosanthes guianensis*

2.7.2 Epidemiology studies on the anthracnose pathogen in *Stylosanthes*

Contributors: F. Muñoz, C. Giraldo, G. Segura, S. Chakraborty (CSIRO, Australia), S. Kelemu

Thirteen accessions and advanced hybrids/populations of *S. guianensis* and two accessions of *S. scabra* were planted at the CIAT Experiment Station in Quilichao, Department of Cauca (3^0 6' N, 76^0 31' W; altitude 1000 m; mean annual rainfall 1,700 mm with normally a bimodal distribution of March-June and September-December; mean annual temperature 24^0 C; ultisols) on 1999-03-30 (Table 45).

Table 45. *Stylosanthes* genotypes planted at Quilichao, Colombia, for epidemiology studies of *Colletotrichum gloeosporioides*.

Code	Species	Accession
A1	*S. guianensis*	CIAT 184
A2	*S. guianensis*	CIAT 136
A3	*S. guianensis*	cv. Mineirão (CIAT 2950)
A4	*S. guianensis*	Hybrid CIAT 11833 (source: CIAT breeding program)
A5	*S. guianensis*	Hybrid CIAT 11844 (source: CIAT breeding program)
A6	*S. guianensis*	Advance bulk populations FM9405 - 1 (source: CIAT breeding program)
A7	*S. guianensis*	Advance bulk populations FM9405 - 3 (source: CIAT breeding program)
A8	*S. guianensis*	Advance bulk populations FM9405 - 4(source: CIAT breeding program)
A9	*S. guianensis*	Advance bulk populations FM9405 - 5 (source: CIAT breeding program)
A10	*S. guianensis*	GC1578 (source: CIAT/EMBRAPA)
A11	*S. guianensis*	CIAT 2312
A12	*S. scabra*	cv. Fitzroy (source: Australia)
A13	*S. scabra*	cv. Seca (source: Australia)
A14	*S. guianensis*	CIAT 2340
A15	*S. guianensis*	cv. Endeavour (source: Australia)

There was an establishment period of six months before the first disease evaluation. Disease evaluation started on 1999-10-21. Five branches per plot were marked and each one rated on a scale of 0 to 9, according to the Horsfall-Barratt scale.

Evaluation has been done on a monthly basis up until May of this year, and will continue for at least 12 more months. Weather data were obtained from an automatic weather station (Monitor Sensors, Australia).

Continuous data was obtained for foliage temperature, relative humidity of the air and precipitation. Because of the inconsistency of the weather data, no statistical analysis could be performed on the relationship of the epidemic progress with weather.

On average disease severity increased steadily over time although, there was a clear reduction during January and May. There were marked differences on epidemic development depending on the *Stylosanthes* genotype (Figure 25). See Table 45 for code descriptions of *Stylosanthes* genotypes.

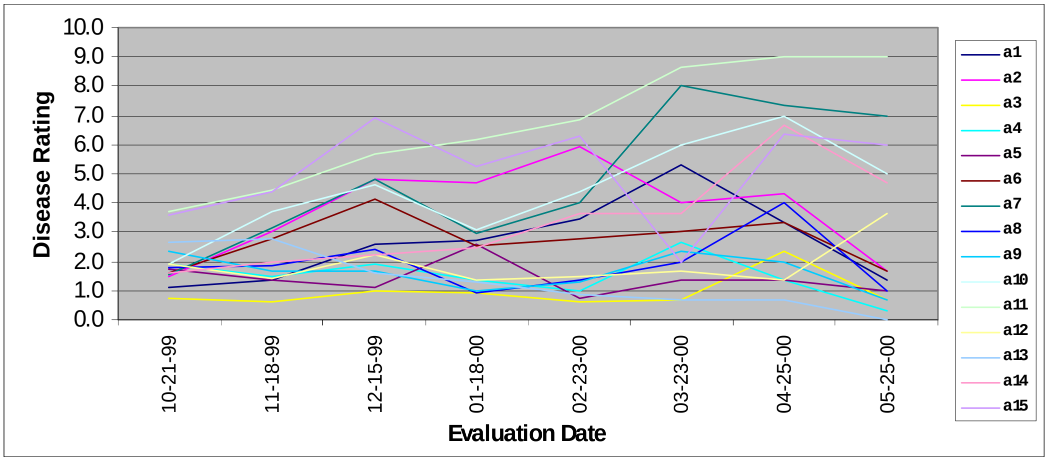


Figure 25. Anthracnose development on 15 *Stylosanthes* genotypes. Quilichao, Colombia October 1999-May 2000.

2.7.3 Characterization of transgenic *Stylosanthes* plants containing a rice chitinase gene

Contributors: H. Guixiu (CATAS, The Peoples' Republic of China) and S. Kelemu

Transgenic *Stylosanthes guianensis* CIAT 184 plants containing a rice chitinase gent have been developed (CIAT, IP5 Annual Report, 1999). Anthracnose, caused by *Colletotrichum gloeosporioides*, is a major disease of economic importance in species of *Stylosanthes*. Although sources of host resistance to anthracnose are available, the large pathogenic variability makes it necessary to look for alternative disease management strategies. The characterization of transgenic plants and their progenies is an essential part of this approach.

Chitinase-gene-expressing vector p*CAMBIACH*$_2$ was constructed as part of a graduate thesis by Jiang Changshun in our laboratory (CIAT IP-5 Annual Report 1999). The enzymes *Hin*d III, DNase-free RNase and reagents of PCR were purchased from Roche, Sigma and PE Company. DNeasy Plant Mini Kit, Wizard DNA Clean-up System, DIG High Primer DNA Labeling and Detection Starter Kit II were purchased from QIAGEN, Promega, and Roche Company, respectively. Gus gene (which encodes the enzyme β-glucuronidase) and marker gene *npt*II (neomycin phosphotransferase) primers were synthesized by Operon Technologies, Inc. Other biochemical reagents were purchased from Sigma, Gibco/BRL and Merck Company. All transformed *Stylosanthes* plants were created by Jiang Changshun as part of his graduate thesis work (plant numbers 18, 22, 31, 34, 34, 44, 62, 63, 64, 65, 66, 67, 69, 71, 72, 74, 75, 76, 79, 81, 82, 83, 84, 85, 86, 87, 88, 89, 91).

Genomic DNA was isolated from fresh leaves of *Stylosanthes* using an improved CTAB (cetyltrimethylammonium bromide) method (Huang, unpublished) or DNeasy Plant Mini Kit. DNA concentration was estimated using a Hoefer DyNA Quant 200 fluorometer (Pharmacia Biotech company).

The ~ 1.5 kb DNA fragment containing a rice chitinase gene was used as a probe (CH). The labeling of the probe and the labeling efficiency determination were conducted according to protocols supplied by the manufacturer.

All steps were carried out according to protocols supplied by the manufacturer. Ten-µg plant DNA samples were digested to completion with *Hin*d III, electrophoresed and transferred to nylon membrane for Southern blot analysis.

PCR amplifications were carried out in 25µl reaction mixtures containing 0.25mM dNTPs, 3.0 mM MgCl$_2$, 0.6µM primers (for *gus* gene primers; *npt*II gene primers using 0.12 µM), 1 unit of Taq DNA polymerase, 1 X PCR buffer and 250 ng of template DNA. Amplification conditions were: pre-denaturation at 94 °C for 1 min, and then 35 cycles of denaturation at 94 °C for 30 sec., annealing at 56 °C for 30 sec., and extension at 72 °C for 1.5 min, followed by a final extension at 72 °C for 7 min. Sequences of the primers were used are: 5'-CTGCGACGCTCACACCGATACC-3', 5'-TCACCGAAGTTCATGCCAGTCCAG-3' (*gus* gene primers) and 5'-ATCGGGAGCG-GCGATACCCTA-3', 5'- GAGGCTATTCGGCTATGACTG-3' (*npt*II gene primers).

DNA isolation from species of *Stylosanthes* has been reported to be a difficult task (C. J. Liu and J. M. Musial, Theor Appl Genet, 1995, 91: 1210 - 1213). However, we developed an improved CTAB method for isolation of good quality DNA. The DNA can be digested and successfully used for dot blot (Photo 12). DNeasy Plant Mini Kit can isolate good quality DNA in shorter time than the CTAB method.

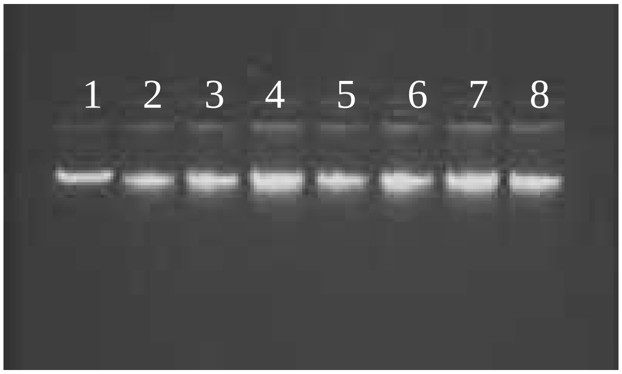
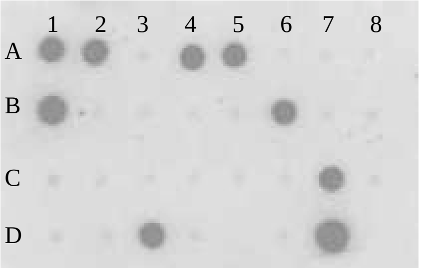


Photo 12. Electrophoresis analysis of total DNA extracted from *Stylosanthes* plants using an improved CTAB method on 0.8 % agarose gel. Lane 1 = λ DNA marker; lanes 2-8 = transformed plants # 18, 22, 31, 34, 44, 62, 63, respectively.

Dot blot results using CH (chitinase gene) as a probe showed positive hybridization signals with DNA samples of transformed plants number 18, 22, 34, 44, 65, 72, 85 and 89 (Photo 13).

Photo 13. Dot blot with DIG - labeled CH probe to DNA samples of transformed *Stylosanthes guianensis* CIAT 184 plants (5 µg DNA per sample). Plant numbers are: A$_1$ = 18; A$_2$ = 22; A$_3$ = 31; A$_4$ = 34; A$_5$ = 44; A$_6$ = 62; A$_7$ = 63; A$_8$ = 64; B$_1$ = 65; B$_2$ = 66; B$_3$ = 67; B$_4$ = 69; B$_5$ = 71; B$_6$ = 72; B$_7$ = 74; B$_8$ = 75; C$_1$ = 76; C$_2$ = 79; C$_3$ = 81; C$_4$ = # 82; C$_5$ = 83; C$_6$ = 84; C$_7$ = 85; C$_8$ = 86; D$_1$ = 87; D$_2$ = 88; D$_3$ = 89; D$_4$ = 91; D$_6$ = non-transformed control plant; D$_7$ = ~ 1.5 kb fragment containing chitinase gene (probe).



Southern blot analysis results were consistent with those of dot blot analysis in that plants number 18, 22, 34, 44, 65, 72, 85, and 89 gave positive hybridization signals (Photo 14).

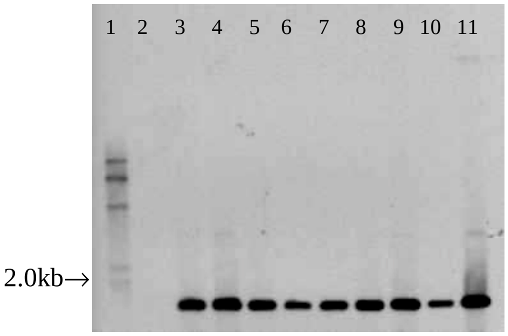


Photo 14. Southern blot hybridization pattern of genomic DNA from transformed plants and a non-transformed control plant (10 µg DNA per sample). DNA was digested with restriction enzyme *Hin*dIII. Lane 1 = λ DNA/*Hin*d III marker; lane 2 = non-transformed control plant; lanes 3-10 = plants number 18, 22,34,44, 65, 72, 85, 89, respecctively. Lane 11 = ~ 1.5 kb fragment containing chitinase gene (probe).

With the *gus* gene primer, transformed plants number 18, 22, 34, 44, 65, 72, 85 and 89 and the positive control (p*CAMBIACH*$_2$) gave an approximately 440-bp amplification product (Photo 15).

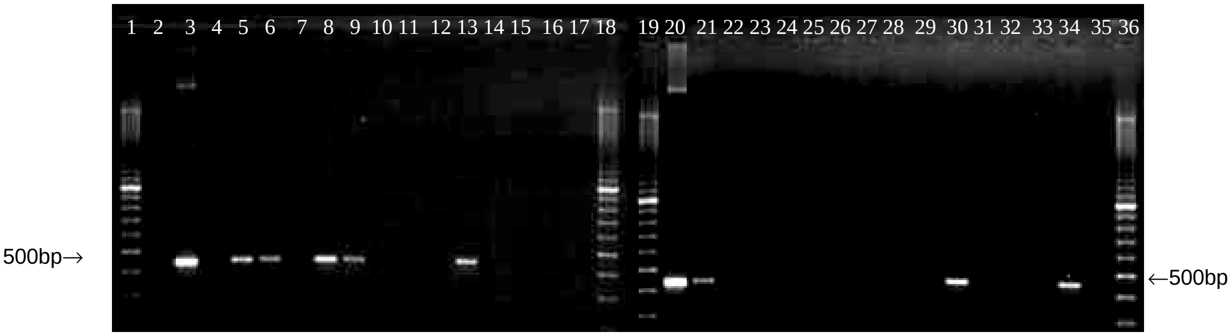


Photo 15. Amplifications of genomic DNA from transformed *Stylosanthes guianensis* plants and a non-transformed control plant with *gus* gene primers. Lane 1 = marker 100 bp ladder; lane 2 = negative control; lane 3 = positive control; lane 4 = non-transformed control plant; lanes 5-17 = plants number 18, 22, 31, 34, 44, 62, 63, 64, 65, 66, 67, 69, 71, respectively; lane 18 = marker; lane 19 = marker; lane 20 = positive control; lanes 21-35 = plants number 72, 74, 75, 76, 79, 81, 82, 83, 84, 85, 86, 87, 88, 89, 91, respectively; lane 36 = marker.

The *npt*II gene primer generated an approximately 700-bp amplification product with DNA from transformed plants number 18, 22, 34, 44, 65, 72, 85 and 89, and the positive control (p*CAMBIACH*$_2$) (Photo 16).

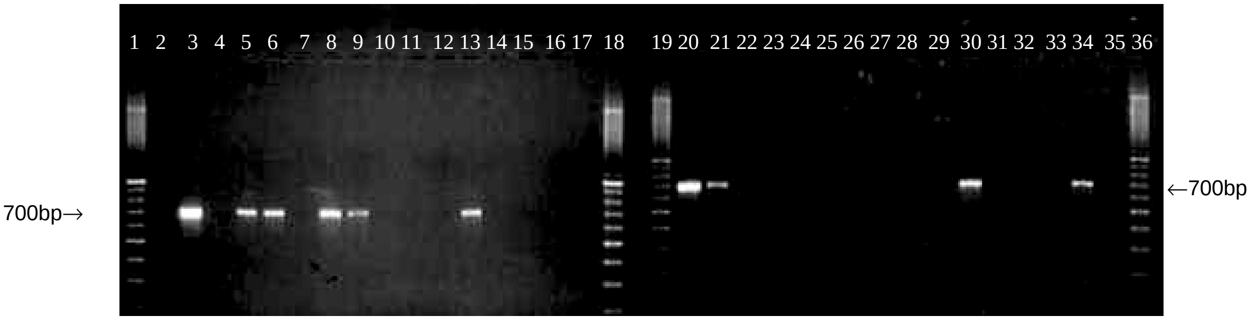


Photo 16. Amplifications of genomic DNA from transformed and non-transformed control plants with *npt*II gene primers. Lane 1 = marker 100 bp ladder; lane 2 = negative control; lane 3 = positive control; lane 4 = non-transformed control plant; lanes 5-17 = transformed plants number 18, 22, 31, 34, 44, 62, 63, 64, 65, 66, 67, 69, 71, respectively; lane 18 = marker; lane 19 = marker; lane 20 = positive control; lanes 21-35 = transformed plant numbers 72, 74, 75, 76, 79, 81, 82, 83, 84, 85, 86, 87, 88, 89, 91, respectively; lane 36 = marker.

Germinated seeds from selfed, transformed and non-transformed *S. guianensis* plants were transplanted to potted soil in the glasshouse to study segregation ratios of the introduced rice gene. Six weeks after transplanting, the seedlings were inoculated with spore suspensions of a *C. gloeosporioides* isolate. Leaf samples were also collected from the progenies to determine segregation of the gene(s) using dot blot or PCR reactions. The data were not complete at the time of this report writing.

Transgenic plants were also inoculated with sclerotia of *Rhizoctonia solani*. Sclerotia were produced in a peptone, sucrose and yeast (PSY) broth inoculated with *R. solani* agar discs and incubated as still culture for 10 days. Sclerotia were placed on the soil surface in contact with each plant's stem. Humidity was maintained at 100% by placing each plant in a plastic bag with one side made of cheese cloth which was immersed in a tray of water.

Preliminary data show that transgenic plants expressed resistance to *Rhizoctonia* foliar blight.

2.7.4 Bacterial wilt disease of *Brachiaria*: casual agent, inoculation methods, bacterial population dynamics and host resistance

Contributors: C. Zuleta (Universidad del Tolima, Ibague), S. Kelemu

A severe wilt disease appeared in *Brachiaria* plants in the greenhouse used by the *Brachiaria* breeding program. Infected plants initially appear wilting and then eventually die completely. This was particularly troublesome as the plants which expressed severe symptoms have been part of the breeding program. Work was initiated to determine the casual agent, to develop inoculation techniques, to identify sources of resistance and to study bacterial multiplication in susceptible and resistant *Brachiaria* genotypes.

Wilting leaf blades of *Brachiaria* CIAT 1015 (a result of a cross between *B. ruziziensis* 44-03 and *B. decumbens* CIAT 606) were cut to pieces of approximately one centimeter long and surface sterilized (1% NaOCl for 2 minutes, 70% ethanol for one minute with agitation, wash 3X with sterile distilled water). Once diseased tissues were surface sterilized, they were macerated in up to one ml of sterile distilled water with autoclaved mortar and pestle and the suspension was plated on nutrient agar medium (Difco, MI, USA; bacto-beef extract 3 g, bacto peptone 5 g, bacto agar, 15 g per L of distilled water). The plates were incubated at 28 C for 48 hours. Independent bacterial colonies were picked with sterile tooth-picks and propagated on fresh nutrient agar medium for further analysis.

Individual bacterial colonies isolated from diseased leaves were separately grown in tubes containing nutrient broth (Difco) and incubated with shaking at 200 rpm, 28°C, overnight. Bacterial cells were collected by centrifugation at 4,000 rpm for 20 minutes. The medium was removed and bacterial cells re-suspended in sterile distilled water and adjusted to an optical density of $OD_{600} = 0.1$. Sterilized scissors were immersed in the bacterial suspension and used to cut leaves of *Brachiaria* plants. Leaves of control plants were cut with scissors immersed in sterile distilled water. All plants were placed in humidity chambers maintained at 27°C and RH of 70% for 48 hours. They were then moved to a growth chamber at 28-30°C and photo period of 12 hours until symptoms expressed. Bacteria were re-isolated from diseased plants and the bacterial colony plated on fresh nutrient agar. Inoculum was prepared as described above and healthy plants were inoculated. The disease causing bacterial colony was re-isolated, purified and stored in 20% glycerol at –80°C for further analysis.

Identification of the disease causing bacterium was conducted using pathogenic bacteria identification protocols in: Laboratory Guide for Identification of Plant Pathogenic Bacteria (N. W. Shaad, ed., 1988. Bacteriology Committee of the American Phytopathological Society. St. Paul, MN). The methods included gram stain, growth on various media, and various biochemical tests.

An overnight culture of bacterial cells were plated on nutrient agar medium containing rifampicin (20 µg/ml) and incubated at 28°C for 48 h. Individual colonies which appeared on the medium were transferred on to freshly prepared medium containing the same concentration of rifampicin. The growing colonies were transferred on to freshly prepared medium containing 25µg/ml rifampicin. The same process was repeated until a mutant bacterium was obtained which grew on rifampicin-containing medium at a concentration of 35 µg/ml. Dilution series of the mutant bacterium were plated on nutrient agar medium with and without rifampicin to determine that the mutant grew equally on both media. Growth curves of the mutant bacterium were also conducted in nutrient broth media with and without rifampicin. The pathogenicity of the rifampicin-resistant mutant was determined in comparison with the original isolate from which the mutant was derived.

Two *Brachiaria* genotypes, BR 97/405 and CIAT 1015, were identified a resistant and susceptible, respectively. These were used to further study bacterial multiplication in their leaf blades. Forty-eight plants of each genotype were inoculated with the rifampicin-resistant mutant derived from the wilt causing bacterium using the method described above. The complete inoculated leaf of each plant (3 plants/incubation day) was macerated in one ml sterile distilled water. A dilution series was made and plated on nutrient agar containing rifampicin. The colonies were counted after 48 hours incubation at 28°C. The values were used to calculate the approximate number of colony forming units per leaf. The wilt-causing organism was identified as *Xanthomonas campestris* (CIAT, IP5 Annual Report 1999) using morphological and biochemical tests. Plants with initial symptoms showed rolled leaves (Photo 17) which resembled those associated with drought stress. The infected leaf and eventually the entire susceptible plant dies (Photo 18). Vegetatively propagated tillers from infected plants often expressed symptoms.

Photo 17. Bacterial wilt disease symptoms in *Brachiaria*. Note rolled leaves.

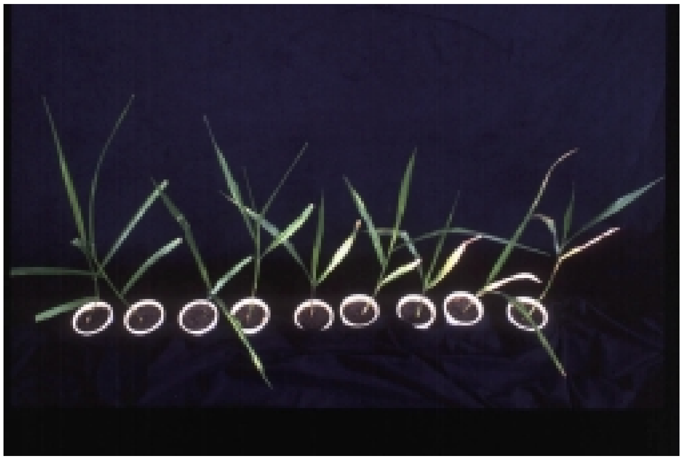


Photo 18. Bacterial wilt disease symptom progress in *Brachiaria* plants within ten days of artificial inoculation.

A *X. campestris* mutant resistant to the antibiotic rifampicin, at a concentration of 25-35 µg/ml, was created for bacterial multiplication studies in *Brachiaria*. The virulence of the mutant remained unaltered (data not shown). The growth of the mutant in culture was the same in the presence or absence of rifampicin (Figure 26).

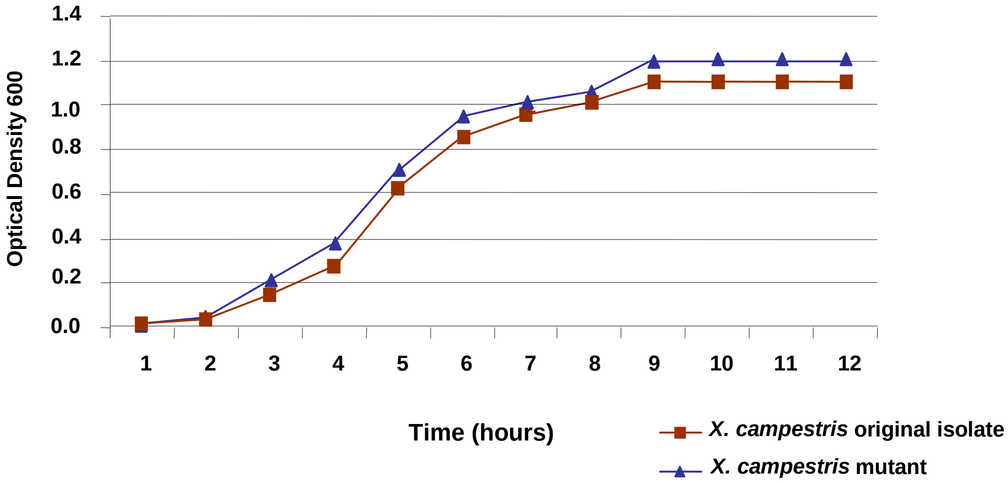


Figure 26. Growth of *Xanthomonas campestris* in nutrient broth (original isolate) and in nutrient broth containing 25µg/ml rifampicin (rifampicin-resistant mutant).

X. campestris multiplications in artificially inoculated resistant (BR97-405) and susceptible (CIAT 1015) plants of *Brachiaria* plants were determined. Bacterial numbers increased significantly over time in the susceptible plant leaves (Figure 27). These results are consistent with similar studies conducted in other host-bacterial pathogen systems.

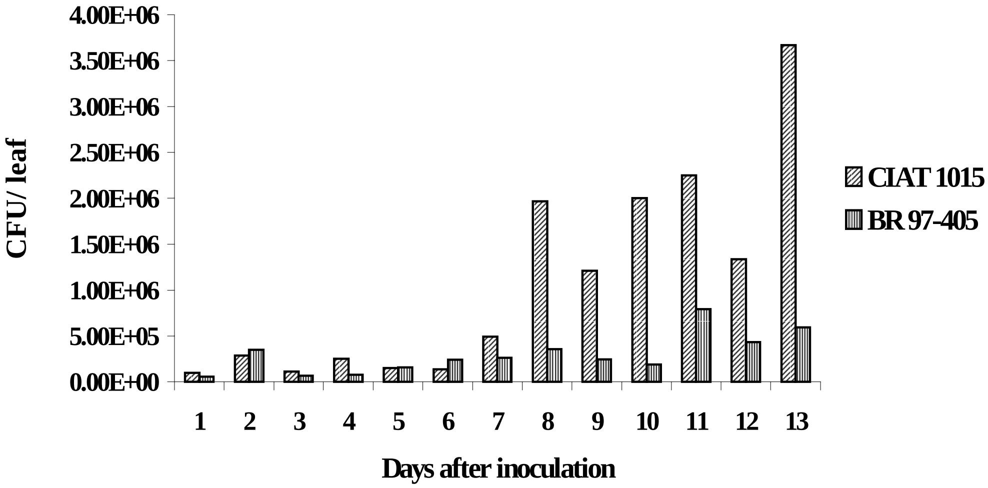


Figure 27. Bacterial multiplication determined as colony forming units/leaf (CFU/leaf) in susceptible (CIAT 1015) and resistant (BR97-405) plants of *Brachiaria* up to 13 days after inoculation.

A number of selected *Brachiaria* accessions and hybrids have been evaluated for their reactions to *X. campestris*. Plants which showed any visible wilt symptoms within 15 days after inoculations were rated as susceptible (S), and those which maintained "healthy" appearance were rated resistant (R) [Table 46].

Table 46. Reactions of *Brachiaria* hybrids and accessions to *Xanthomonas campestris*

***Brachiaria* Hybrids/accessions**	**Disease Reaction**	***Brachiaria* Hybrids/accessions**	**Disease Reaction**
BR 97 NO/ 0047	R	BR 99 NO/ 4138	R
BR 97 NO/ 0082	S	BR 99 NO/ 4278	S
BR 97 NO/ 0155	S	BRUZ4X/ 4402	S
BR 97 NO/ 0383	S	FM 9503/ S46/ 024	S
BR 97 NO/ 0402	R	CIAT 606	R
BR 97 NO/ 0405	R	CIAT 679	R
BR 97 NO/ 0457	R	CIAT/ 1015	S
BR 97 NO/ 1143	S	CIAT 6133	R
BR 97 NO/ 1173	S	CIAT 6294	R
BR 97 NO/ 2965	R	CIAT 6780	S
BR 98 NO/ 0709	S	CIAT 16322	R
BR 98 NO/ 0773	S	CIAT 16488	S
BR 98 NO/ 1251	R	CIAT 26110	R
BR 99 NO/ 4015	R	CIAT 26124	R
BR 99 NO/ 4016	S	CIAT 26318	R
BR 99 NO/ 4099	R	CIAT 26556-G	R
BR 99 NO/ 4132	S	CIAT 36061	S
		CIAT 36062	S

Progress towards achieving milestones

- **Determined anthracnose pathogen diversity infecting *Stylosanthes* using AFLP**
- **Determined wilt disease causing bacterial multiplication in resistant and susceptible *Brachiaria* genotypes**

We have adopted amplified restriction fragment length polymorphism (AFLP) technique and evaluated 119 isolates of *C. gloeosporioides*. AFLP technology is used to visualize genetic polymorphisms among isolates, generating fingerprints that can be used to assess the relatedness between isolates. Population studies to determine pathogen variations are important components of effective plant breeding for durable disease resistance.

The casual agent of a new bacterial wilt disease of *Brachiaria* has been characterized and sources of resistance identified. Bacterial multiplication is significantly reduced in some of the resistant *Brachiaria* plants.

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

Activity 3.1 Genotypes of *Brachiaria, Panicum* and *Arachis* with adaptation to low edaphic and climatic factors

Highlights

- Identified several physiological and biochemical plant traits as candidates for developing screening methods for P and N acquisition in *Brachiaria* hybrids.
- A rapid and reliable screening procedure to evaluate aluminum resistance of *Brachiaria* hybrids using stem-cuttings was implemented and hybrids with moderate level of Al resistance have been identified.
- Field evaluation of 10 accessions of *Arachis pintoi* resulted in identification of CIAT 22159 as a promising accession to target for infertile soils.
- Showed that phosphorus supply and tillage at establishment had minimal effects on *Arachis pintoi* persistence in degraded soils of Caqueta region of Colombia.
- Showed that 2 accessions of *Panicum maximum* (CIAT 16061 and CIAT 36000) were superior in root growth than the commercial cultivar Vencedor and therefore could be better adapted to acid infertile soils.

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

3.1.1 Improved tetraploid, sexual *Brachiaria* hybrid breeding population for resistance to edaphic factors and general environmental adaptation

Contributors: J. W. Miles, I. M. Rao

A 2-year selection and recombination cycle of the tetraploid sexual population is being completed this year. Clones selected on spittlebug resistance are being recombined in an isolated crossing block. These 41 clones will be submitted to solution culture assay of Al tolerance by year's end and some can be culled prior to next January's planting time.

3.1.2 Studies on mechanisms of acid soil adaptation in *Brachiaria* cultivars and development of screening methods

Contributors: P. Wenzl, J. Mayer (CAMBIA, Australia), J. W. Miles, G. M. Patiño, R. García, J. Ricaurte, I. M. Rao

Last year, we achieved significant progress in (*i*) the elucidation of physiological mechanisms underlying Al tolerance of *B. decumbens* and (*ii*) the modification of a simple Al tolerance screening procedure for application to vegetative cuttings instead of seedlings (IP5 Annual Report, 1999). This year the final outcome of our research efforts on elucidation of mechanisms and development of screening methods is summarized below.

Mechanisms of adaptation of *Brachiaria* cultivars to acid-soil stress

The adaptation of two *Brachiaria* cultivars to Al toxicity and deficiencies in P and N -- three major edaphic constraints in infertile acid soils -- has been studied. The two cultivars, *B. decumbens* cv. Basilisk and *B. ruziziensis* cv. Common, chosen for detailed studies are being used as parents in an ongoing breeding program at CIAT, which aims at combining acid-soil adaptation with spittlebug resistance. They represent the two extremes of the range of edaphic adaptation found in *Brachiaria* cultivars. The following are the most significant results from the detailed studies on mechanisms of acid soil adaptation.

Aluminum toxicity: 1) Aluminum resistance of *B. decumbens* is outstandingly high, when compared to the most Al-resistant genotypes of cereal crops; 2) *B. ruziziensis* is significantly less Al-resistant. The interspecific difference in Al resistance can be readily detected in 5-d old seedlings, using a root-elongation assay in simple nutrient solutions; 3) The differential Al resistance of the two cultivars coincided with an interspecific difference in root elongation, hematoxylin staining and callose concentrations of root apices under Al stress; 4) In solution culture, differential Al resistance becomes only apparent under nutrient-limited growth conditions mimicking soil solutions of highly weathered acid soils. The interaction between Al stress and nutrient supply suggests that Al resistance of *B. ruziziensis* but not of *B. decumbens* is affected by nutrient deficiency; 5) External mechanisms of Al detoxification such as organic-acid secretion commonly thought to be an important strategy of Al resistance are not responsible for the great Al resistance of *B. decumbens*. This is because its root apices secrete only small quantities of Al-chelating ligands. Instead, Al-toxicity symptoms indicated that root apices of *B. decumbens* may tolerate symplastic Al; 6) Correlative evidence indicates that organic acids detoxify Al within root apices of *Brachiaria* cultivars. Aluminum may be sequestered into apical vacuoles, where it might be stored, complexed by malate and citrate. Interspecific differences in the effect of Al toxicity on apical malate concentrations suggest that this mechanism may be more effective in *B. decumbens*; 7) Unidentified Al-chelators, different from the most common aliphatic organic acids, form complexes with Al in mature root zones; 8) Cation-exchange properties of root cell walls at the whole-root level are not related to differential Al resistance of the two cultivars; and 9) In contrast to *B. decumbens*, Al toxicity causes a dramatic decline in the P content of root apices of *B. ruziziensis*. This may either be the result of reduced meristematic activity or an inhibition of acropetal P translocation by Al.

Phosphorus deficiency: 1) *B. decumbens* has a finer root system than *B. ruziziensis*, irrespective of the P level in the growth medium. It produces longer roots per unit of leaf area. Because the rate of net photosynthesis depends critically on P supply, and phosphate is highly immobile in acid soils, this morphological trait may enhance growth on P-deficient acid soils; and 2) Roots of P-deprived *Brachiaria* cultivars accumulate two novel di-hydroxycinnamoylquinic-aicd esters: 1,3-di-feruloylquinic acid and 1-feruloyl-3-*p*-coumaroylquinic acid. These compounds might increase the root's lifespan by retarding tissue senescence and acting as pre-formed protectants against fungi. Accumulation of these two compounds was more pronounced in *B. ruziziensis* than in *B. decumbens*.

Nitrogen deficiency: 1) *B. ruziziensis* requires higher N concentrations than *B. decumbens* to support growth. This may be due to a more efficient N uptake by *B. decumbens* roots; 2) Differential adaptation to N deficiency is associated with interspecific differences in biomass partitioning. Under moderately N-deficient growth conditions, *B. ruziziensis* allocates a greater proportion of its biomass to roots than *B. decumbens*; and 3) Under N deficiency, roots of both *Brachiaria* species accumulate the same two di-hydroxycinnamoylquinic-aicd esters as under P deficiency. Roots of *B. ruziziensis* accumulate larger amounts than those of *B. decumbens*.

Acid-soil adaptation as an aggregate trait: Results obtained so far indicate that *B. decumbens* is better adapted to acid-soil stress than *B. ruziziensis*. In Table 47, the identified cultivar differences in physiological and morphological characters that are likely to contribute to differential acid-soil adaptation, are listed. These findings reinforce the notion that acid-soil adaptation is a physiologically and hence genetically complex aggregate trait.

Table 47. Relative ranking of *Brachiaria* cultivars with respect to identified characters relevant for acid-soil adaptation (well adapted > less adapted). The ranking in terms of acid-soil persistence is Bd > Bb > Br.

Stress factor	**Plant Character**			
Al toxicity	Degree of Al resistance	Al resistance unaffected by low nutrient supply	Internal Al detoxification by organic acids in root apices	Acropetal P translocation under Al stress
	Bd > Br[a]	Bd > Br	Bd > Br	Bd > Br
N deficiency	Growth at low N concentration	N uptake efficiency		
	Bd > Bb > Br	Bd > Bb > Br		
P deficiency	Fineness of root system			
	Bd > Bb > Br			

[a] Bb, *B. brizantha* cv. Marandú; Bd, *B. decumbens* cv. Basilisk; Br, *B. ruziziensis* cv. Common

Suggested procedures for screening *Brachiaria* cultivars for acid-soil adaptation

(i) Physiological traits suitable for mass screening: Physiological screening methods should be based on characters that can be measured easily in a large number of individuals. In the case of seedlings, they have to be non-destructive, or at least leave a sufficiently large portion of the plant to allow recovery. The following list of physiological and morphological characters fulfills these requirements to varying degrees:

Al resistance

- Root elongation of vegetative propagules in simple nutrient solutions containing Al
- Hematoxylin staining of Al-stressed root apices
- Concentrations of Al, callose, malate and P in root apices, or a combination of them

P acquisition

- Root thickness, irrespective of the P concentration in the medium
- Ratio of total root length to leaf area

N acquisition

- Root-weight ratio under moderate N deficiency
- N uptake efficiency per unit root surface area

Some interspecific differences identified are not suitable for screening. For example, accumulation of the two di-hydroxycinnamoylquinic acid esters in roots appeared to be associated with susceptibility to P and N deficiency (see chapter "Two Di-hydroxycinnamoyl-quinic Acid Esters from Roots of Nutrient-Deprived *Brachiaria* Species"). However, under N deprivation, *B. brizantha* Marandú accumulated significantly smaller amounts than *B. ruziziensis*, although growth was less inhibited than in *B. ruziziensis*. Measurement of interspecific differences in relative growth in nutrient solutions simulating acid-soil stress may be difficult to implement as this would require growing each genotype under both control and stress conditions to calculate growth parameters.

(ii) Screening procedure suggested for seedlings: Based on the characters listed above, a multi-tiered screening procedure was designed for evaluating acid-soil adaptation of *Brachiaria* seedlings (IP-5 Annual Report, 1998). It comprises two consecutive cultivations of seedlings, first in a $CaCl_2$ solutions containing Al (for 3 d), then in a nutrient solution, but containing only 100 μM NO_3^- and 10 μM NH_4^+ (for 13 d).

The first cultivation is designed to select for Al-resistant genotypes, based on the root-elongation assay, using the Al concentration that gave the greatest dynamic range (50 μM; that is $\{Al^{3+}\} = 32$ μM). This step should considerably reduce the number of genotypes being evaluated.

The second cultivation is designed to select for genotypes adapted to N and P deficiency. The concentration of N in the nutrient solutions is selected to fall in the range where interspecific differences in carbon partitioning in response to N deficiency are expressed. Plants, which under these moderately N-deficient conditions allocate less biomass to roots, are selected. Their root system is stained and scanned with a flatbed scanner. Root-image-analysis software is then used to select the genotypes with a small mean root diameter similar to that of *B. decumbens*, a trait likely to enhance P acquisition.

It is necessary to separate roots from shoots to measure carbon partitioning at the end of the second cultivation,. At this stage, plants have usually already 2 to 3 adventitious roots. Therefore, after separation of roots from shoots -- and pruning of some leaves to reduce evapotranspiration -- it should be possible to transfer plants to soil in a similar manner as this is routinely done with stem cuttings. Alternatively, the shortest adventitious root could be left attached to the stem, and selected individuals could be grown in nutrient solutions for an additional period to produce stem cuttings.

Although the individual steps of the suggested screening procedure are based on the results obtained in previous experiments, the procedure as a whole remains to be adequately tested. It has the advantage that a greater number of genotypes could be tested in parallel than that of the stem-cuttings procedure. However, it is more labor intensive than the latter. Notwithstanding, it may be more suitable than the stem-cutting procedure for selecting genotypes that possess individual component traits contributing to acid-soil adaptation.

(iii) Screening procedure suggested for stem cuttings: The ongoing *Brachiaria* breeding program has already produced a large number of hybrids that have been selected for spittlebug resistance, but not for acid-soil adaptation. Thus a screening procedure for stem-cuttings is to be prioritized over a procedure for seedlings at this stage. Fortunately, single-internode stem cuttings of *Brachiaria* species can be rooted easily in nutrient solutions.

A root-elongation assay distinguished well between Al-resistant and Al-susceptible seedlings of *Brachiaria* cultivars (IP5 Annual Report, 1999). Under simultaneous nutrient deficiency and Al toxicity, *B. decumbens* grew better than the other two cultivars. For these reasons, we tested whether root elongation in a nutrient-free, Al-containing solution could be used to discriminate between acid-soil-adapted and -susceptible stem cuttings of *Brachiaria* cultivars.

The results reported last year clearly demonstrated that measuring root elongation, using stem cuttings in a solution containing 200 μM $CaCl_2$, 200 μM $AlCl_3$ (pH 4.2), is a simple, but powerful method to discriminate between *B. decumbens* and the other two cultivars. Al toxicity markedly reduced root elongation of *B. brizantha*, while it had little effect in *B. decumbens*, confirming the high level of Al resistance of the latter. *B. ruziziensis* is significantly less Al-resistant than *B. decumbens*. However, this difference was not noticeable in the assay, because roots of *B. ruziziensis* hardly elongated in the solution lacking Al. Only roots of *B. decumbens* continued to elongate under simultaneous Al stress and nutrient deficiency.

To further reduce the number of genotypes, it might be desirable to screen for additional characters, such as apical P content of Al-stressed root apices (Figure 28).

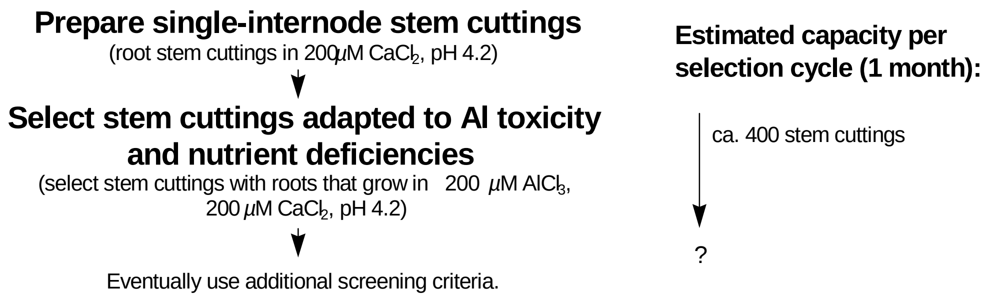


Figure 28. Proposed screening procedure for selecting acid-soil adapted *Brachiaria* hybrids using stem cuttings.

Insufficient supply of nutrients from internal reserves to elongating root apices is the likely reason for the poor elongation of *B. ruziziensis* roots in the Al-free assay solution. A detailed study, comprising addition of single nutrients, or combinations of nutrients, should identify the limiting nutrient(s). Previous results would suggest that N deficiency might be one of the reasons.

Research implications

(i) Important traits not investigated in this study: We focused on three individual factors of acid-soil stress -- Al toxicity, P deficiency and N deficiency. Aluminum toxicity was chosen because a root-elongation assay with seedlings indicated a marked difference in Al resistance between *B. decumbens* and *B. ruziziensis*. Phosphorus deficiency was selected because it is a major constraint to growth of *Brachiaria* species on acid soils. Nitrogen deficiency was selected because degradation of *B. ruziziensis* pastures tends to coincide with chlorosis of leaves, a typical symptom of N deficiency.

It is important to point out that acid-soil stress is considerably more complex than a combination of the three investigated factors. Typically, deficiencies of cations such as Ca^{2+}, Mg^{2+} and K^+ co-limit growth, none of which has been investigated in detail. However, the low cation concentrations in soil solutions of neotropical savanna soils have been taken into account in a general way, when designing low-ionic-strength nutrient solutions used for studying Al resistance, because adequate supply of these cations can ameliorate Al toxicity. Interspecific differences in adaptation to cation deficiencies may exist, as field experiments have suggested in the case of Ca^{2+} (K. Haussler, I. M. Rao and H. Marschner, unpublished results).

In addition to focusing on three individual stress factors, only a handful of physiological characters were investigated for each of these stress factors. This limitation has been particularly significant in the case of P deficiency, because plant adaptation to P deficiency is difficult to study in nutrient solutions. Using intermittently renewed nutrient solutions, it is impossible to maintain phosphate concentrations at realistically low levels ($\leq 1\mu$M). Therefore, differential growth in response to varying P levels in nutrient solutions cannot be studied, unless using flowing solution culture. More importantly, *Brachiaria* species, and *B. decumbens* in particular, are highly dependent of mycorrhizal association, and a considerable portion of P is taken up via the fungal symbiont. Interspecific differences in symbiotic efficiency exist, but have not been investigated in this study. In addition, it is known that *Brachiaria* species such as *B. decumbens* rely, at least in part, on associative N_2 fixation by rhizosphere bacteria. This aspect has not been addressed in this study either.

It is therefore important to keep in mind that screening procedures based on the results of this study are unlikely to take into account some important physiological characters that contribute to acid-soil adaptation.

(ii) Physiological screening criteria versus field screening: The classical method for screening for adaptation to acid soils is based on yield in the field. While there is no better way to test the genetic adaptation of plants to acid soils, than to grow them on acid soils, there are a number of reasons arguing against the use of field screening in the initial phase of a breeding program. First, problem soils, such as acid soils, present a complex challenge to plants, which is likely to require several genetically independent mechanisms of adaptation. Screening based on overall performance in the field may not be able to identify genotypes possessing individual components of adaptation, because they are likely to be scattered throughout the different genotypes of a segregating breeding population. Second, stress conditions reducing yield tend to increase the environmental component of variation, and thus reduce the heritability of yield. Soils are notoriously heterogeneous and the spatial variability could affect the efficiency of the selection process. Finally, field screening is expensive, time-consuming and thus puts a limit to the number of genotypes that can be evaluated.

Physiological screening criteria can be targeted to isolate individual traits from overall performance. This can help to circumvent the problem that plants possessing component traits might be lost due to the inadequate sensitivity of screening for overall performance. Whether the latter is also a limitation of the screening method proposed for stem-cuttings remains to be tested. Being quantitative in nature, physiological criteria may be more objective than visual assessment in the field. Physiological methods also tend to be more rapid, and generally allow the evaluation of larger numbers of genotypes. However, they are inherently limited by the degree to which physiological characters of parental genotypes have been investigated.

The contrasting strengths and limitations of field screening and physiological screening suggest that the two should be combined in a complementary way that maximizes their discriminatory power at different stages of the breeding program. Physiological methods may be most useful in the initial phase to rapidly discard a large number of non-adapted genotypes and to guarantee the survival of genotypes possessing component traits. Once these component traits have been reassembled in a reduced number of genotypes, field screening could be used to increase the stringency of selection and to select for uncharacterized physiological traits contributing to acid-soil adaptation.

(iii) Brachiaria decumbens as a model for cereal crops: Food production is based on a handful of graminaceous crops. For most of these species, growth conditions in acid soils are outside or marginal to their ecological range. In addition, useful genetic variation to deal with acid-soil stress that may have been present in ancestral races, is likely to have been lost during domestication. To adapt cereal crops to acid soils, novel genetic information may be required. "Wide crossing" -- in the absence of an understanding of underlying mechanisms -- can be used to introduce additional allelic variation from wild relatives. However, depending on the species, this may not always be a feasible strategy. Genetic engineering has been successfully used to improve traits that are well understood and genetically simple. However, complex aggregate traits such as acid-soil adaptation are unlikely to be tackled successfully in this way in the near future.

Comparative mapping of genomes has shown that the order of genes on the chromosomes of higher plants is well conserved. This genomic colinearity is particularly extensive in grasses. Although there may be exceptions to it at small scales, it has been argued that because of this colinearity, grasses can be considered as a 'single genetic system'. From this perspective, an understanding of gene functions and interactions relevant for acid-soil adaptation in any grass species should be useful for the genetic improvement of cereal crops. Identifying adaptive traits and their genetic basis in species with a long evolutionary history on acid soils -- followed by their transfer to agricultural species -- may thus be a more promising approach than genetic engineering based on oversimplified and reductionistic models.

In this sense, *B. decumbens* cv. Basilisk is an attractive model for enhancing acid-soil tolerance of cereal crops. It is a species that is well adapted to the individual stress factors of acid-soil complex. This may be because the genetic constitution of the natural accession has been preserved due to its apomictic mode of reproduction. Yet, it can be genetically recombined with poorly adapted, sexually reproducing, *B. ruziziensis*. Successful clones of apomictically reproducing species tend to be highly heterozygous and therefore hybrid populations are likely to segregate for individual physiological characters contributing to acid-soil adaptation. Preliminary observations seem to confirm this tendency in *Brachiaria* hybrid populations. These populations should provide an excellent tool to genetically and physiologically dissect a complex aggregate trait such as acid-soil adaptation. We believe that this is an achievable goal if we could combine this approach with recently developed microarray-based methods that allow a massively parallel detection of gene expression. A restricted core project funded by BMZ-GTZ of Germany will focus on these research objectives.

Identification of Al-resistant *Brachiaria* hybrids

Rationale: Last year, we adapted the root elongation method to stem-cuttings. This year we implemented this screening procedure to assay Al-resistant *Brachiaria* hybrids that had previously been preselected for spittlebug resistance.

Methods: A total of 16 genotypes including 3 parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294 and *B. ruziziensis* 44-02) were selected for evaluation of Al resistance. Among the 13 hybrids selected for screening, 8 were sexuals. All the hybrids except CIAT 36061 were highly resistant to spittlebug infestation (C. Cardona, personal communication). Stem-cuttings were rooted in a $CaCl_2$ (200µM) solution, selected for uniformity and transferred to a solution containing 200µ*M* $CaCl_2$ (pH 4.2) and exposed to 2 levels of $AlCl_3$ (0 and 200µM). The solution was replaced every third day, and the length of the longest root was monitored for each stem cutting during 21 days. Total root length, root weight and specific root length (root length to root weight ratio) were measured after 21 days.

Results and Discussion: Results on total root length and specific root length indicate that the parent *B. decumbens* CIAT 606 is outstanding in its level of Al resistance (Figure 29A). Among the 13 hybrids tested, 4 hybrids (BR99NO/4132, CIAT 36062, BR99NO/1015/50 and CIAT 36061) showed moderate level of Al resistance (Figure 29A). Among these 4 hybrids, BR99NO/1015/50 is sexual and it offers a great opportunity to genetically combine Al resistance with spittlebug resistance. Results on specific root length – a measure of fineness of the root system – indicate that among the hybrids BR99NO/4132 could be outstanding in the presence or absence of Al in solution (Figure 29B). This attribute is also very desirable not only to acquire P from infertile acid soils but also to improve dry season tolerance in *Brachiaria*.

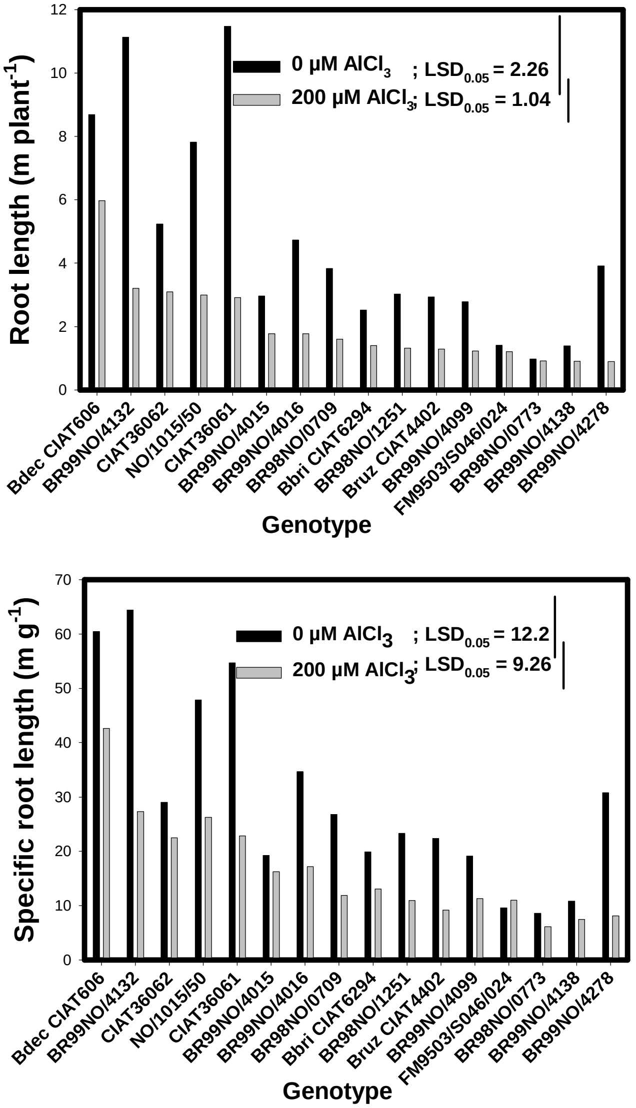


Figure 29. Screening for Al resistance among 16 genotypes of *Brachiaria*. Total root length (A) and specific root length (B) were measured after exposure to 0 or 200 M AlCl3 with 200 M CaCl2 (pH 4.2) for 21 days.

The relationship between root length and specific root length was very close suggesting that the measurement of total root length may be adequate to eliminate a large proportion of hybrids and to select a few promising hybrids with Al resistance (Figure 30). These can be subjected to the measurement of specific root length for further screening and identifcation of Al resistant *Brachiaria* hybrids.

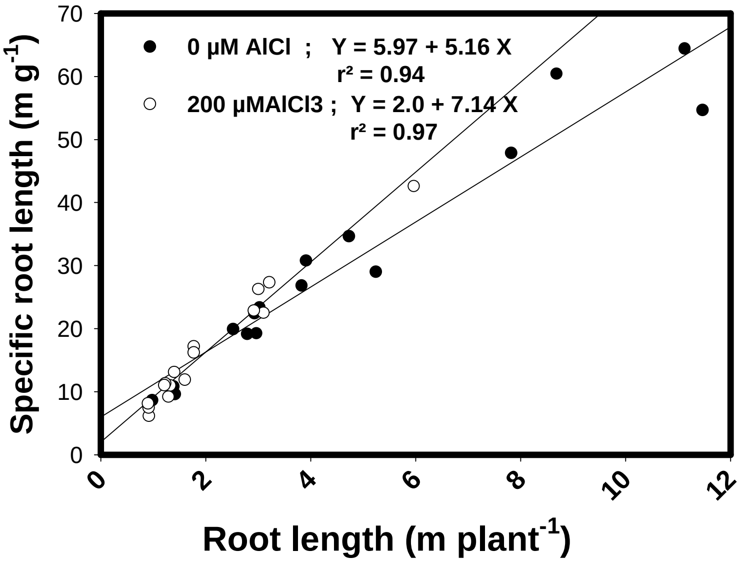


Figure 30. Relationship between total root length and specific root length of 16 genotypes of *Brachiaria*. Stem cuttings were exposed to 0 or 200 M AlCl3 with 200 M CaCl2 (pH 4.2) for 21 days. Specific root length was determined as length per unit weight.

Identification of genetic recombinants of *Brachiaria* with tolerance to low nutrient supply

Rationale: The breeding efforts of *Brachiaria* generate significant number of recombinants that are resistant to spittlebug. There is a need to test these promising hybrids for their tolerance to low nutrient supply in soil so that we can identify recombinants that combine spittlebug resistance of B. brizantha with the excellent edaphic adaptation of *B. decumbens*. A glasshouse experiment evaluated genotypic differences in tolerance to low nutrient supply among 20 genotypes of *Brachiaria* (15 genetic recombinants, 4 parents and 1 most promising accession). The CIAT accession *B. brizantha* 26110 was included because this accession showed outstanding adaptation to drought conditions in Costa Rica.

Methods: A sandy loam oxisol from Carimagua (Maquenque) of the llanos of Colombia was used to grow the plants (4 kg of soil/pot). Nutrients were applied before planting at two levels (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 (46 days of growth), several plant attributes such as forage yield, leaf area, leaf to stem ratio and nutrient uptake were determined.

Results and Discussion: Plant attributes were influenced by genotype and also the level of nutrient supply to soil (Tables 48 and 49). As expected, increase in nutrient supply improved forage yield by 2 to 3-fold as a result of stimulation of leaf area production. One of the parents, *B. decumbens* CIAT 606 was outstanding in its adaptation to low nutrient supply as well as its response to high nutrient supply. Among the genetic recombinants, FM0503/S094/011 was outstanding in its adaptation to low nutrient supply. One of the genetic recombinants, BRN093/1371, which is resistant to spittlebug infestation, was particularly outstanding in leaf area production and in leaf to stem ratio of the forage yield with low as well as high nutrient supply. Another spittlebug resistant hybrid, FM9503/S046/024, also showed greater ability in leaf area production. Values of leaf to stem ratio of several hybrids were markedly greater than those of the parents suggesting that breeding for leafiness while combining desirable attributes such as spittlebug resistance and adaptation to low nutrient supply is an achievable research objective.

Table 48. Genotypic variation as influenced by nutrient supply (low and high) in forage yield, leaf area and leaf to stem ratio of genetic recombinants, parents and 1 germplasm accession of *Brachiaria* grown for 46 days in pots (4 kg soil/pot) in a sandy loam oxisol from Carimagua (Maquenque), Colombia. LSD values are at the 0.05 probability level.

Genotype	Forage yield		Leaf area		Leaf to stem ratio	
	Low Supply	High Supply	Low Supply	High Supply	Low Supply	High Supply
	(g/pot)		(cm^2/pot)			
Recombinants:						
FM9201/1873	6.69	14.9	571	1534	1.40	1.93
BRN093/1371	5.38	14.2	622	1368	6.87	3.07
FM9503/S002/057	5.98	16.9	570	1378	2.45	1.63
FM9503/S015/010	6.60	15.2	554	1176	1.93	1.59
FM9503/S040/037	5.38	13.7	507	1389	2.63	2.61
FM9503/S046/024	5.45	12.2	575	1439	4.72	3.02
FM9503/S047/002	6.36	15.8	592	1525	2.47	1.86
FM9503/5057/014	6.09	14.7	550	1337	4.09	2.54
FM9503/S070/003	5.89	14.3	588	1447	4.02	3.16
FM9503/S070/016	6.29	15.8	540	1439	2.53	1.77
FM9503/S070/049	6.35	16.2	518	1195	1.79	1.42
FM9503/S075/028	5.73	15.0	612	1550	3.12	2.57
FM9503/S076/001	6.65	16.5	517	1378	1.48	1.36
FM0503/S076/013	5.79	16.0	586	1615	4.18	2.34
FM0503/S094/011	6.90	17.4	539	1435	1.22	1.43
Parents:						
CIAT 606	10.9	22.6	580	1366	0.54	0.51
BRUZ/44-02	6.18	13.4	389	987	1.39	2.10
CIAT 6294	7.24	17.2	595	1346	1.68	1.49
CIAT 26646	8.10	18.1	458	1230	0.96	0.78
Accessions:						
CIAT 26110	7.92	21.1	609	1539	1.81	1.29
Mean	**6.60**	**16.1**	**554**	**1384**	**2.56**	**1.92**
LSD *(P=0.05)*	0.95	1.42	87	236	1.36	0.55

Measurements of shoot nutrient uptake indicated that one of the parents, *B. decumbens* CIAT 606 was outstanding in its ability to acquire N (Table 49). Among the genetic recombinants, FM0503/S094/011 was superior in its ability to acquire nutrients, particularly Ca.

Hybrid clones from the cross of tetraploid sexual *B. ruziziensis* (intolerant) and *B. decumbens* cv. Basilisk (tolerant) will be used for molecular genetic markers and the identification of possible QTL's for Al tolerance.

Table 49. Genotypic variation as influenced by nutrient supply (low and high) in shoot nutrient uptake of genetic recombinants, parents and 1 germplasm accession of *Brachiaria* grown for 46 days in pots (4 kg soil/pot) in a sandy loam oxisol from Carimagua (Maquenque), Colombia. LSD values are at the 0.05 probability level.

	Shoot N uptake		Shoot P uptake		Shoot Ca uptake	
	Low Supply	High Supply	Low Supply	High Supply	Low Fertilizer	High Fertilizer
			(mg/pot)			
Recombinants:						
FM9201/1873	37.7	115	19.3	54.1	27.8	60.4
BRN093/1371	41.3	105	24.1	65.5	25.3	60.2
FM9503/S002/057	37.8	112	25.2	61.0	26.7	75.6
FM9503/S015/010	49.2	123	28.0	72.4	29.2	62.7
FM9503/S040/037	40.5	114	25.7	68.2	28.6	81.3
FM9503/S046/024	38.6	101	26.1	62.9	26.6	60.9
FM9503/S047/002	39.5	117	22.8	61.4	33.5	73.7
FM9503/5057/014	39.7	115	21.9	71.9	26.7	65.8
FM9503/S070/003	36.4	113	23.9	76.3	27.2	61.4
FM9503/S070/016	37.2	103	21.9	59.2	24.7	61.1
FM9503/S070/049	39.8	117	22.3	67.0	28.0	76.4
FM9503/S075/028	34.6	114	21.8	64.9	25.3	74.3
FM9503/S076/001	37.2	115	20.5	65.1	32.5	7.71
FM0503/S076/013	34.8	111	21.8	67.9	21.8	70.6
FM0503/S094/011	48.9	148	27.3	80.6	37.1	82.6
Parents:						
CIAT 606	56.3	188	21.7	70.6	32.7	72.2
BRUZ/44-02	37.2	122	20.4	64.5	20.8	49.4
CIAT 6294	40.2	126	21.7	70.3	26.1	64.9
CIAT 26646	54.2	150	26.0	60.1	16.9	36.0
Accessions:						
CIAT 26110	57.6	158	21.9	67.3	22.2	51.6
Mean	**41.9**	**123**	**23.2**	**66.6**	**27.0**	**65.9**
LSD *(P=0.05)*	6.5	12	5.5	9.1	5.2	11

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

Contributors: N. Castañeda, N. Claassen (Uu. Of Göttingen, Germany), A. Betancourt, R. García, J. Ricaurte, , I. M. Rao

Rationale: Last year, we reported the progress from the field and greenhouse studies that were aimed to determine genotypic differences among ten accessions of *Arachis pintoi* in P-acquisition and utilization from low P soil. This year we report the progress made on persistence of these ten accessions.

Identification of *Arachis pintoi* genotypes with adaptation to low P supply in the field

Methods: A field study is in progress at "La Rueda" ranch, Montañita, Caquetá (latitude 1º 25' N, longitude 75º 27' W and 180 m.a.s.l) (Photo 19). Plant growth was monitored since June 1998. The mean rainfall, temperature and relative humidity were 3500 mm/year, 25ºC and 75% respectively. The experiment was laid down in a split plot RCBD with three P levels [native P (NP), phosphate rock (PR), triple super phosphate (TSP)] as main plots and ten genotypes [CIAT 17434 (commercial), 18744, 18748, 22159, 18745, 18751, 22160, 18747, 22155, 22172] as subplots. The experiment was replicated three times. Application (kg P ha^{-1}) of PR and TSP was at 50 and 20, respectively. Plants were harvested at 20 months after establishment.

Photo 19. Overview of the field experiment at La Rueda ranch, Montañita, Caquetá.

Results and Discussion: Last year, we reported that CIAT 18747 was outstanding in terms of rapid establishment as determined by leaf area index, shoot biomass and shoot P uptake. Among the ten accessions tested, two accessions (CIAT 22159 and 22172) were found to be slow in establishment.

At 20 months after establishment and after a short dry spell, we evaluated the persistence of the same 10 accessions with 3 different sources of P applied at the time of establishment. Application of PR and TSP at establishment had only small effect on improvement of forage yield for only few accessions (Table 50). Live forage yield was greater for CIAT 18744. This was not due to better leaf production but due to a large biomass of stolons (Table 51). One of the accessions CIAT 22159 which was reported to be better for persistance in association with aggressive grass, *B. dictyoneura* cv. Llanero at Carimagua was also superior in its ability to produce greater leaf area development and therefore leaf biomass production. Dead forage yields were greater with the accession CIAT 18751.

The differences among the 10 genotypes in terms of leaf N and P contents were small when compared with leaf biomass production (Table 51). The content of N and P in green leaves was greater with the accession CIAT 18751.

Table 50 . Influence of P fertilizer source on genotypic differences in leaf area index, live forage biomass and dead forage biomass of *Arachis pintoi* grown in a low P soil at Montañita, Caquetá. Measurements were made at 20 months after planting. LSD values are at the 0.05 probability level. NS= not significant.

Plant	Source	Accessions of *A. pintoi*										$LSD_{0.05}$
attribute		17434	18744	18745	18747	18748	18751	22155	22159	22160	22172	
Leaf area index (m^2/m^2)	NP	0.28	0.27	0.23	0.17	0.27	0.19	0.15	0.40	0.21	0.39	NS
	RP	0.21	0.30	0.30	0.08	0.38	0.24	0.13	0.37	0.42	0.26	0.25
	TSP	0.23	0.41	0.34	0.22	0.36	0.32	0.09	0.48	0.21	0.26	0.17
Live forage yield (t/ha)	NP	1.38	2.18	1.22	1.51	1.51	1.59	1.82	2.14	1.15	2.20	NS
	RP	1.18	1.98	1.43	1.19	1.92	1.90	1.01	1.66	2.30	1.94	1.03
	TSP	2.40	2.26	1.54	1.80	1.66	2.40	1.02	1.93	1.13	1.68	0.85
Dead forage yield (t/ha)	NP	0.88	1.28	1.36	1.15	1.23	2.30	1.15	1.11	1.28	1.15	0.82
	RP	1.26	1.85	1.53	1.26	0.97	1.34	0.64	1.16	2.03	1.12	1.00
	TSP	1.30	2.02	1.16	1.20	1.59	2.48	0.75	1.84	1.05	1.25	1.04

NP= Native phosphorus (0 kg P/ha); RP= Rock phosphate (50 kg P/ha); TSP= Triple super phosphate (20 kg P/ha).

Table 51. Influence of P fertilizer source on genotypic differences in leaf biomass, leaf N content and leaf P content of *Arachis pintoi* grown in a low P soil at Montañita, Caquetá. Measurements were made at 20 months after planting. LSD values are at the 0.05 probability level. NS = not significant.

Plant	Source	Accessions of *A. pintoi*										$LSD_{0.05}$
attribute		17434	18744	18745	18747	18748	18751	22155	22159	22160	22172	
Leaf biomass (kg/ha)	NP	174	190	148	132	193	136	126	285	143	274	NS
	RP	149	216	209	68	234	170	98	266	303	204	197
	TSP	129	272	241	166	212	214	69	306	126	164	106
Leaf N content (g/kg)	NP	37.4	46.3	45.7	48.5	46.3	51.0	44.6	40.5	44.2	35.2	6.86
	RP	34.9	46.4	41.0	44.9	45.8	45.5	38.6	39.1	42.5	39.1	5.98
	TSP	41.7	45.8	47.8	51.8	44.7	51.2	45.1	41.8	43.4	39.4	7.38
Leaf P content (g/kg)	NP	2.82	3.69	4.08	3.84	3.39	4.33	3.63	3.43	2.36	2.65	0.81
	RP	3.14	3.91	3.64	3.87	3.67	4.24	2.78	3.02	3.30	3.27	0.92
	TSP	3.67	3.76	4.28	4.68	3.47	4.56	3.70	3.17	3.57	3.31	0.90

NP=Native phosphorus (0 kg P/ha); RP=Rock phosphate (50 kg P/ha); TSP=Triple super phosphate (20 kg P/ha).

Nutrient uptake, particularly P, N and Ca by green leaves during the dry spell was also greater by accessions, CIAT 22159 and CIAT 18744 (Table 52). Among these two accessions, CIAT 22159 was outstanding in its ability to acquire Ca particularly from RP treatment. Thus it appears from this field study that CIAT 22159 may be a better accession to target for infertile soils.

Table 52. Influence of P fertilizer source on genotypic differences in P, N and Ca uptake by green leaves of *Arachis pintoi* grown in a low P soil at Montañita, Caquetá. Measurements were made at 20 months after planting. LSD values are at the 0.05 probability level.

Plant attribute	Source	Accessions of *A. pintoi*										LSD$_{0.05}$
		17434	18744	18745	18747	18748	18751	22155	22159	22160	22172	
	NP	0.49	0.72	0.49	0.54	0.68	0.64	0.49	0.98	0.46	0.76	0.41
P uptake	RP	0.48	0.86	0.77	0.25	0.92	0.76	0.29	0.82	0.80	0.57	0.51
(kg/ha)	TSP	0.48	1.00	1.01	0.78	0.73	0.98	0.26	0.93	0.45	0.52	0.36
	NP	6.43	9.06	5.76	6.65	9.17	7.31	5.82	11.7	6.09	9.73	4.86
N uptake	RP	5.28	10.1	8.6	3.03	11.0	8.11	3.82	10.6	11.2	7.28	6.73
(kg/ha)	TSP	5.50	12.2	11.3	8.62	9.43	11.0	3.13	12.3	5.45	6.37	3.99
	NP	1.37	0.97	0.75	0.58	0.95	0.53	0.65	2.11	0.85	1.94	1.02
Ca uptake	RP	1.29	1.23	1.39	0.32	1.41	0.85	0.67	2.33	1.39	1.90	1.45
(kg/ha)	TSP	0.81	1.53	1.07	0.80	1.10	0.89	0.29	2.40	0.73	0.93	1.00

NP=Native phosphorus (0 kg P/ha); RP=Rock phosphate (50 kg P/ha); TSP=Triple super phosphate (20 kg P/ha).

Effect of tillage, fertilization, planting system and their interaction on establishment of *A. pintoi* genotypes

Methods: Field study is in progress at “La Esperanza” ranch, Morelia, Caquetá (latitude 1º 23’ N, longitude 75º 42’ W and 200 masl) (Photo 20). Plant growth was monitored since September 1998. The mean rainfall, temperature and relative humidity were 3500 mm/year, 25ºC and 85% respectively. The experiment was laid down in a split-split plot RCBD with a factorial (2 x 2) arrangement.

Photo 20. Overview of the field experiment La Esperanza” ranch, Morelia, Caquetá

Two planting systems (monoculture and grass-legume association) as main plots, two genotypes (CIAT 17434, 18744) as subplots and two P levels (native phosphorus (NP), and rock phosphate

(RP)) plus two tillage methods (without or with tillage) as sub-sub-plots in a factorial arrangement. Application of PR was at 50 kg ha^{-1}. The experiment was replicated three times.

Results and Discussion: The two accessions when grown in monoculture persisted but their production was markedly lower than that of the level produced at 6 months after establishment (IP5 Annual Report, 1999). Both accessions performed very poorly in association with the aggressive grass at 20 months after establishment. Initial application of RP and tillage had not improved the persistence of either accession.

Among the two accessions, CIAT 18748 performed better than the commercial material, CIAT 17434 in the absence of RP application and tillage (Table 53). Neither of the two accessions was very responsive to either RP application or tillage. However, initial application of RP and tillage had somewhat greater impact on leaf area production, green leaf biomass production and leaf P content of CIAT 18748.

Table 53. Influence of tillage and P application on the performance of two accessions of *A. pintoi* grown for 18 months either in monoculture or in association with *B. dictyoneura* (Bd). Leaf P uptake values were for green leaf biomass during the dry season. Legume yield in association was very poor. Therefore the values for the legume in association with the grass were not presented. LSD values are at the 0.05 probability level.

Plant attribute	Source	Accession of *A. pintoi*									
		Monoculture					Association with *B. dictyoneura*				
		17434		18748			17434		18748		
		-T	+T	-T	+T	LSD$_{0.05}$	-T	+T	-T	+T	LSD$_{0.05}$
Leaf area index (m^2/m^2)	NP	0.14	0.18	0.29	0.18		0.80	1.06	0.83	1.28	
	RP	0.23	0.23	0.21	0.26		0.75	1.14	0.96	1.17	
						0.17					0.36
Shoot biomass (kg/ha)	NP	276	375	497	440		1410	2008	1773	2288	
	RP	397	462	390	592		1515	1935	1597	2191	
						343					811
Leaf P uptake (kg/ha)	NP	0.18	0.25	0.33	0.25						
	RP	0.29	0.26	0.31	0.46						
						0.21					

NP = Native phosphorus
RP = Rock phosphate

- T = without tillage
+ T = with tillage

This field study indicates that CIAT 18748 is better adapted than the commercial cultivar to infertile soils of Caquta region but its persistence with aggressive grasses is not satisfactory. Therefore it may be possible to target this accession for other alternative uses such as cover legume in plantations in this region.

3.1.4 Determine N and P requirements of *Panicum maximum* genotypes

Contributors: I. M. Rao, P. J. Argel, J. Ricaurte, R. García

Rationale: Last year, we reported results from two separate glasshouse studies which were conducted with the objective of determining genotypic differences in N and P requirements of the selected accessions of *P. maximum* compared to commercial cultivars. We showed that increasing N supply improved shoot biomass production of all genotypes tested but the extent of increase per unit N supply was markedly superior for two CIAT accessions (16051 and 6177) compared to the commonly used cultivars (IP-5 Annual Report, 1999). The external N requirements of the same two accessions are also markedly lower than those of the other genotypes tested. Among the two cultivars, Vencedor had very high external N requirement. The critical N content of the youngest expanded leaf of accession CIAT 16028 was markedly lower than that of the other genotypes. Similar to external N requirement, the critical internal N requirement of cv. Vencedor was markedly greater than that of the other genotypes.

Increase in P supply markedly improved shoot biomass production of all the genotypes tested indicating that P supply in soil is a major limitation to forage yield. The external P requirement of the accession CIAT 16061 was markedly lower than that of the other genotypes, particularly cv. Vencedor which did not reach the asymptote even at 150 kg/ha of P supply. Internal P requirement of cv. Vencedor was also markedly greater than that of the other genotypes.

This year, we report the genotypic differences in root attributes in relation to changes in N or P supply to soil.

Methods: A sandy loam oxisol from Carimagua was used to grow the plants (4 kg of soil/pot). Basal nutrient supply (kg/ha) included 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo. For the experiment on N requirements a basal supply (kg/ha) of 50 P and seven levels of N (0, 10, 20, 40, 80, 160 and 320) were used.

For the experiment on P requirements, a basal supply (kg/ha) of 80 N and six levels of P (0, 10, 20, 40, 80 and 160) were used. At the time of harvest (50 days of growth), root attributes such as root biomass, root length and specific root length were measured for only the 3 promising genotypes (cv. Vencedor [CIAT 26900], CIAT 16061 and CIAT 36000).

Results and Discussion: Among the three genotypes tested, CIAT 16061 and CIAT 36000 produced more root biomass and root length with increase in N or P supply to soil (Figures 31 and 32). At 50 Kg/ha of N supply, root biomass levela of CIAT 16061 and CIAT 36000 were similar but root length of CIAT 16061 was markedly superior. Similar results were observed at 60 kg/ha of P supply indicating that the lower external P requirement for CIAT 16061 was due to its ability to explore greater volume of soil (Figure 32).

This accesion performed well in Central American hillsides agroecosystem and it could be better suited for cut and carry systems of forage production because of its better adaptation to low levels of N and P supply in soil. These results indicate that the cultivar Vencedor has a relatively smaller root system and therefore may be better suited for crop-pasture rotational systems where the input levels are usually much greater than those of the extensive pasture systems.

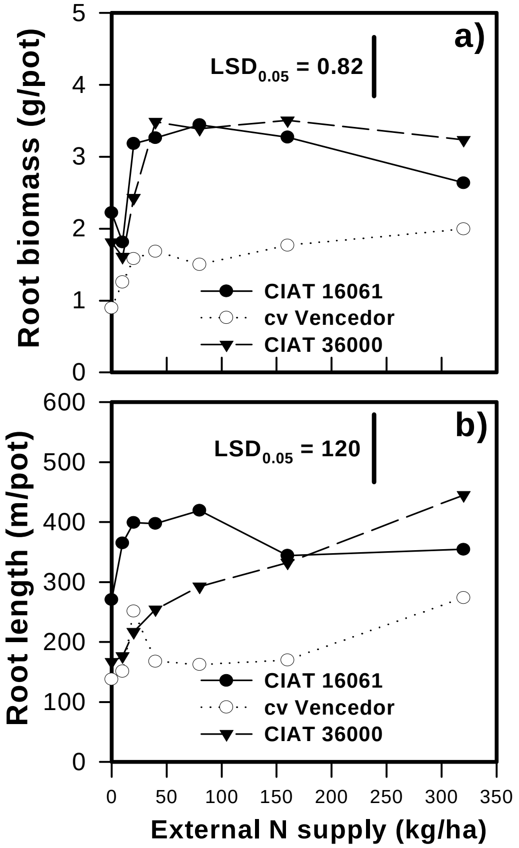


Figure 31. Differences in root biomass (a) and root length (b) production among three genotypes of *Panicum maximum* in relation to an increase in N supply to an oxisol. LSD values are at the 0.05 probability level.

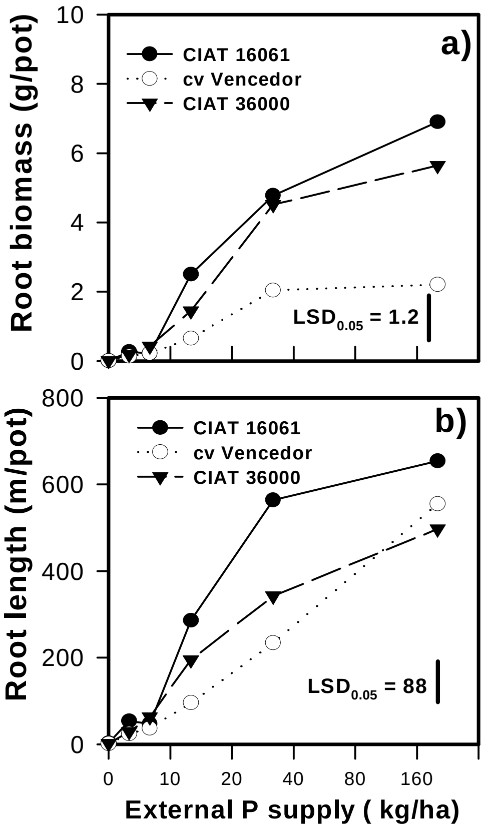


Figure 32. Differences in root biomass (a) and root length (b) production among three genotypes of *Panicum maximum* in relation to an increase in P supply to an oxisol. LSD values are at the 0.05 probability level.

Progress towards achieving milestones

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

We were successful in implementing a rapid and reliable screening method to evaluate aluminum tolerance of *Brachiaria* genetic recombinants. This method uses relative root elongation as a simple measure to identify aluminum sensitive genotypes. We adapted this method for vegetative stem cuttings and evaluated spittlebug resistant *Brachiaria* hybrids for their aluminum resistance. We are also using this method to evaluate a hybrid population of *B. ruziziensis* x *B. decumbens.* This will enable us to develop molecular markers for this trait.

We identified *Arachis pintoi* CIAT 22159 as a promising accession for targeting to infertile acid soils. This accession along with a few other accessions (CIAT 18744, 18748, 22160) and commercial cultivar (CIAT 17434) need to be further evaluated in relatively better soils of the Llanos of Colombia (Piedmont region) for their suitability as cover or forage legumes. We showed that two accessions of *Panicum maximum* (CIAT 16061 and CIAT 36000) were superior in root growth than the commercial cultivar Vencedor and therefore could be better suited to acid infertile soils.

Activity 3.2 Genotypes of grasses and legumes with dry season tolerance

Highlights

- Showed that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024 which maintained greater proportion of green leaves during dry season, was associated with lower levels K and N content in green leaves.
- Found significant differences among *Cratylia* accessions in DM production when grown in acid soils and under dry conditions.
- Identified a new sub-shrub of *Cratylia* with semi-prostrate growth habit that may be suitable for direct grazing under association with grasses.
- Defined that yield of *Cratylia* was significantly improved by cutting every 90 days, with no effect on forage quality.

3.2.1 Field studies to determine genotypic variation in dry season tolerance in *Brachiaria*

Contributors: I. M. Rao, C. Plazas, J. W. Miles, P. J. Argel, J. Ricaurte, R. García

Rationale: Quantity and quality of dry season feed is a major limitation to livestock productivity in subhumid regions of tropical America. A field study was conducted at Matazul Farm in the Llanos of Colombia. The main objective was to evaluate genotypic differences in dry season (4 months) tolerance of most promising genetic recombinants of *Brachiaria*.

Methods: A field trial was established on a sandy loam oxisol at Matazul farm in the Llanos of Colombia in July, 1999. The trial comprises 12 entries, including six natural accessions (four parents) and six genetic recombinants of *Brachiaria*. The germplasm accession -- CIAT 26110 – was identified from previous work in Atenas, Costa Rica as an outstanding genotype for tolerance to long dry season (up to 6 months). 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. Live and dead forage yield, shoot nutrient composition, and shoot nutrient uptake were measured at the end of the dry season (March 2000).

Results and Discussion: As expected, high fertilizer application improved forage yield and shoot uptake of nutrients. Live forage yield after 3 months of dry season with low fertilizer application ranged from 0.36 to 3.67 t/ha and the greatest forage yield was observed with one of the spittlebug resistant genetic recombinants FM9503-S046-024 (Table 54).

This was mainly attributed to its ability to produce green leaf biomass during dry season (Table 55). The leaf to stem ratio of this hybrid together with FM9301-1371 were markedly greater than the rest of the materials tested. One of the parents, *B. decumbens* CIAT 606 showed greater biomass of dead forage than other materials (Table 54).

Table 54. Genotypic variation as influenced by fertilizer application in live shoot biomass, dead shoot biomass and total forage yield of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 8 months after establishment (3 months of dry season). LSD values are at the 0.05 probability level.

Genotype	Live shoot biomass		Dead shoot biomass		Total forage yield	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
			(t/ha)			
Recombinants:						
BR97NO-0082	1.95	2.37	1.10	2.01	3.06	4.39
BR97NO-0383	1.70	1.98	0.93	1.76	2.63	3.74
BR97NO-0405	2.52	2.63	1.71	2.03	4.23	4.66
FM9201-1873	1.81	3.81	3.31	3.36	5.12	7.17
FM9301-1371	2.64	3.47	1.39	1.15	4.62	4.03
FM9503-5046-024	3.67	3.28	2.41	2.78	6.09	6.06
Parents:						
CIAT 606	1.78	2.55	4.18	3.95	5.96	6.50
CIAT 6294	2.85	3.79	1.61	3.50	4.46	7.29
BRUZ/44-02	0.36	0.44	0.33	1.29	0.84	1.73
CIAT 26646	2.68	2.47	2.28	2.36	4.96	4.83
Accessions:						
CIAT 26110	2.89	3.26	2.21	3.33	5.11	6.59
CIAT 26318	2.60	4.54	1.65	2.46	4.25	7.01
Mean	**2.45**	**2.72**	**2.01**	**2.43**	**4.46**	**5.15**
LSD *(P=0.05)*	1.68	1.85	1.34	2.08	2.70	3.80

Table 55. Genotypic variation as influenced by fertilizer application in leaf biomass, stem biomass and leaf to stem ratio of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 8 months after establishment (3 months of dry season). LSD values are at the 0.05 probability level.

Genotype	Leaf biomass		Stem biomass		Leaf to stem ratio	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
			(t/ha)			
Recombinants:						
BR97NO-0082	1.47	1.87	0.48	0.51	3.06	3.67
BR97NO-0383	1.11	1.22	0.59	0.75	1.88	1.63
BR97NO-0405	1.65	1.74	0.87	0.89	1.90	1.96
FM9201-1873	1.10	2.53	0.71	1.29	1.55	1.96
FM9301-1371	2.46	3.34	0.18	0.13	13.7	25.7
FM9503-5046-024	3.33	2.98	0.34	0.30	9.79	9.93
Parents:						
CIAT 606	1.07	1.56	0.71	0.99	1.51	1.58
CIAT 6294	2.14	2.77	0.71	1.02	3.01	2.72
BRUZ/44-02	0.33	0.28	0.02	0.16	16.5	1.75
CIAT 26646	1.55	1.54	1.12	0.93	1.38	1.66
Accessions:						
CIAT 26110	1.83	1.79	1.06	1.47	1.73	1.22
CIAT 26318	1.33	2.76	1.26	1.78	1.05	1.55
Mean	**1.73**	**1.91**	**0.72**	**0.81**	**4.80**	**4.58**
LSD *(P=0.05)*	0.93	1.07	0.87	0.99	-	-

Shoot N uptake with low fertilizer application was greater for two accessions (CIAT 26110 and 26318) was greater than that of the other materials (Table 56). The hybrid, FM9503-S046-024 was superior in its ability to acquire P, Ca and Mg from low fertilizer application (Tables 57 and

58). Among the parents, *B. brizantha* CIAT 6294 was superior in P, Ca and Mg acquisition from low fertilizer application.

Table 56. Genotypic variation as influenced by fertilizer application in leaf N content, stem N content and shoot N uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 8 months after establishment (3 months of dry season). LSD values are at the 0.05 probability level.

Genotype	Leaf N content		Stem N content		Shoot N uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(%)		(%)		(kg/ha)	
Recombinants:						
BR97NO-0082	1.29	1.19	1.82	1.55	28.3	29.6
BR97NO-0383	1.49	1.17	1.87	1.34	26.7	23.7
BR97NO-0405	1.10	1.18	1.33	1.51	33.8	34.6
FM9201-1873	1.07	0.96	0.88	1.27	21.3	40.8
FM9301-1371	1.02	1.01	0.93	0.96	27.1	35.2
FM9503-5046-024	0.95	0.99	0.92	1.19	33.4	33.4
Parents:						
CIAT 606	1.20	1.39	1.36	1.13	22.1	28.4
CIAT 6294	0.96	1.11	0.94	1.04	26.5	41.0
BRUZ/44-02	1.36	1.43	1.73	1.34	8.13	10.3
CIAT 26646	1.01	1.07	0.95	1.20	26.7	28.2
Accessions:						
CIAT 26110	1.42	1.20	1.83	1.42	46.7	43.2
CIAT 26318	1.17	1.25	0.83	0.83	26.8	48.4
Mean	**1.17**	**1.15**	**1.29**	**1.24**	**30.0**	**31.7**
LSD *(P=0.05)*	0.30	0.45	0.56	NS	23.3	24.4

NS = not significant.

Table 57. Genotypic variation as influenced by fertilizer application in leaf P content, stem P content and shoot P uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 8 months after establishment (3 months of dry season). LSD values are at the 0.05 probability level.

Genotype	Leaf P content		Stem P content		Shoot P uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(%)		(%)		(kg/ha)	
Recombinants:						
BR97NO-0082	0.054	0.055	0.049	0.059	1.04	1.30
BR97NO-0383	0.052	0.058	0.059	0.060	0.94	1.16
BR97NO-0405	0.050	0.047	0.046	0.053	1.21	1.17
FM9201-1873	0.057	0.050	0.042	0.047	1.27	1.86
FM9301-1371	0.067	0.062	0.075	0.074	1.80	2.20
FM9503-5046-024	0.064	0.065	0.048	0.071	2.26	2.13
Parents:						
CIAT 606	0.068	0.067	0.070	0.071	1.22	1.76
CIAT 6294	0.055	0.050	0.047	0.053	1.53	1.89
BRUZ/44-02	0.092	0.103	0.071	0.057	0.19	0.61
CIAT 26646	0.049	0.049	0.044	0.053	1.17	1.24
Accessions:						
CIAT 26110	0.048	0.059	0.053	0.075	1.36	2.02
CIAT 26318	0.049	0.043	0.063	0.049	1.41	2.05
Mean	**0.057**	**0.058**	**0.054**	**0.060**	**1.39**	**1.58**
LSD *(P=0.05)*	0.011	0.014	0.021	NS	0.78	1.07

NS = not significant.

Table 58. Genotypic variation as influenced by fertilizer application in shoot K uptake, shoot Ca uptake and shoot Mg uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 8 months after establishment (3 months of dry season). LSD values are at the 0.05 probability level.

Genotype	Shoot K uptake		Shoot Ca uptake		Shoot Mg uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(kg/ha)					
Recombinants:						
BR97NO-0082	16.3	20.7	5.38	8.05	3.43	4.91
BR97NO-0383	15.8	17.3	3.82	5.02	3.80	4.09
BR97NO-0405	24.0	24.9	6.02	6.05	4.85	4.64
FM9201-1873	16.7	30.7	5.02	8.34	4.63	7.09
FM9301-1371	25.0	34.6	9.74	16.1	8.90	11.8
FM9503-5046-024	21.1	23.7	13.2	11.2	9.82	7.47
Parents:						
CIAT 606	16.2	22.6	3.15	5.21	3.67	5.68
CIAT 6294	15.8	22.2	5.59	7.16	5.36	6.70
BRUZ/44-02	2.37	7.25	0.49	1.42	0.37	1.14
CIAT 26646	12.1	10.9	3.85	4.16	4.25	4.28
Accessions:						
CIAT 26110	25.6	29.8	4.02	5.57	3.91	5.70
CIAT 26318	14.6	27.7	4.28	6.34	4.46	7.00
Mean	**19.0**	**21.9**	**5.79**	**7.00**	**5.21**	**5.76**
LSD *(P=0.05)*	4.2	3.5	4.21	3.47	3.26	3.28

The superior performance of the hybrid, FM9503-S046-024 which maintained greater proportion of green leaves during dry season (visual observation) was associated with lower levels K and N content in green leaves (Table 59). It is important to note that forage stem yield with low fertilizer application showed a significant negative relationship (-0.38**) with leaf Ca content (results not shown). This observation is consistent with previous observation made from the work on long dry season tolerance at Atenas, Costa Rica where total forage yield was negatively correlated with low Ca content in forage.

Table 59. Correlation coefficients (r) between green leaf biomass (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low or high fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer	High fertilizer
Live forage yield (t/ha)	0.91***	0.87***
Total forage yield (t/ha)	0.71***	0.72***
Dead biomass (t/ha)	0.25	0.48***
Stem biomass (t/ha)	0.47***	0.26
Leaf N content (%)	-0.41**	-0.37*
Leaf P content (%)	-0.21	-0.22
Leaf K content (%)	-0.42**	-0.07
Leaf Ca content (%)	0.33*	0.27
Stem N content (%)	-0.37*	-0.04

*,**,***Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

3.2.2 Field evaluation of accessions of *C. argentea* in a subhumid environment

Contributors: Marco Lobo di Palma and Vidal Acuña [Ministry of Agriculture and Livestock (MAG) Costa Rica]; Pedro J. Argel and Guillermo Pérez

Background. A blended line of the shrub *Cratylia argentea* is presently registered in Costa Rica as cv. Veraniega. The shrub is well adapted to a wide range of climates and poor but well drained soils of the subhumid and humid tropics. It establishes easily by seed and although dry matter (DM) production is not very high during the year of planting, the DM yields increase as the plant grows. The shrub has good foliar retention during the dry season and responds well to cutting regimes, for this reason it is considered an appropriate alternative feed supply for milking cows during dry periods, and the shrub is being successfully used to supplement poor quality grasses and replace costly concentrates at that time of the year.

Cultivar Veraniega derived from the evaluation of eleven accessions of *C. argentea* that demonstrated little variation among them in terms of DM production and adaptation to biotic and abiotic factors. However, in recent years field collections have been carried out in the center of origin of the shrub in South America, mainly in Brazil, and a large number of accessions are available that need to be evaluated and characterized.

Results and Discussion. Thirty new accessions of *Cratylia* where planted in single rows of five plants, and replicated two times in a complete randomized experiment in the subhumid environment (5 to 6 months dry period) of Atenas, Costa Rica. Evaluation cuttings were carried out at a cutting height of 50 cm every 8 weeks during the dry period and every 8 to 12 weeks during the wet season for a period of two years. The foliage harvested was considered as edible and expressed as DM yields. Results showed significant variation in DM yields between accessions both during the wet and dry seasons; mean total DM yield was significantly high ($P<0.05$) during the wet period (95.6 g/plant) than during the dry season (77.3 g/plant).

However, Table 60 shows that the accession CIAT 22391 yielded more during the dry period than during the wet season, an observation of interest since we are searching for plants that can produce reasonable yields in subhumid sites with prolonged dry periods.

C. argentea accessions CIAT 22374, 22386, 22379 and 22382 had a total DM mean yield over 100 g/plant, meanwhile that accessions such as BRA 000841, CIAT 22396, 22377 and 22376 had significantly ($P<0.05$) less total yield. The accession identified as Yacapani (has no CIAT number yet) is a sub-shrub that produces vigorous lateral stems close to the ground, and it is very likely that this line is not a *C. argentea* species. This line had similar DM yields for both the dry and wet season, indicating the good tolerance to dry conditions; also because the growth habit of this plant it might be associated with stoloniferous or erect grasses. However, forage feeding qualities of this plant are unknown up to date.

Apart from the Yacapani line, there was no much variation in plant morphology within this collection, and some minor differences in leaves size and degree of leaf pubescence and color were observed. It seems that this core collection is very similar to other lines evaluated previously.

Table 60. Dry matter yields of *Cratylia argentea* accessions established in the subhumid environment of Atenas, Costa Rica

CIAT No.	Mean dry matter yields		
	Wet	Dry	Total
		(g/plant)	
22374	120.5	105.5	114.5
22386	118.4	97.1	109.9
22379	116.8	91.2	106.6
22382	117.4	84.8	104.4
22381	104.0	93.3	99.7
22375	106.4	85.2	97.9
22389	107.1	81.5	96.9
(BRA 000621)	104.5	85.1	96.7
22391	87.8	105.5	94.9
(BRA 000876)	97.7	89.0	94.2
22393	98.4	87.3	94.0
22378	106.2	74.0	93.3
(Yacapani)	92.1	93.9	92.8
22380	99.3	78.4	90.9
22385	96.4	82.1	90.7
22383	93.6	85.5	90.4
22384	96.9	70.4	86.3
22395	87.3	77.5	83.4
22373	89.7	72.7	82.9
22390	88.9	73.7	82.8
22392	79.5	76.0	78.1
22387	88.6	60.8	77.4
22394	81.6	69.1	76.6
(BRA 000884)	85.0	62.5	76.0
22388	80.7	68.4	75.8
(BRA 000604)	77.0	59.2	69.9
(BRA 000841)	67.5	62.2	69.9
22396	68.0	53.4	62.1
22377	65.4	46.3	57.8
22376	64.5	46.8	57.4
LSD ($P<0.05$)	51.2	47.9	29.6

Means of 8 cuts every 8 weeks during the wet season and 4 cuts every 8-12 weeks during the dry period**.**

3.2.3 Agronomic characterization of a core collection of *Cratylia argentea* in acids soils

Contributors: M. Peters, L. H. Franco, B. Hincapié, P. Avila

Background: *Cratylia argentea* has been identified as one of the key forage species for dry season feeding developed and propagated by CIAT; some accessions are currently being adopted by farmers, mainly in Central America and Colombia. However, the genetic resource base so far is limited and hence activities to acquire novel germplasm of *Cratylia argentea* is of high priority.

Methods: A range of new germplasm of *Cratylia argentea* had been received from EMBRAPA/ CENARGEN in Brazil. These were combined with accessions available in the CIAT collection. In total 41 accessions of *Cratylia argentea* were sown in the Palmira greenhouse (25-03-99). The collection was planted as a Randomized Complete Block design with three replications at Santander de Quilichao (06-05-99); each replication consists of 7 plants per accession. Characterization of these materials will concentrate on productivity and nutritive value aspects as well as seed proliferance. An additional replication with 33 accessions was sown for seed production and morphological observations.

Results and discussion: During the establishment phase the collection suffered an attack of nematodes. Though controlled, this attack led to slower establishment of *Cratylia.* However plants recovered rapidly. In the production phase the shrubs were affected by leaf-sucking insects, but only minor damages were encountered. The preliminary data -9 week regrowth- indicates phenotypic and agronomic variation in the available collection of *Cratylia* (Table 61).

From the initial results accessions CIAT 18674, 22408, 22406, 18671 and 22409 had the highest dry matter yields, which also were substantially higher than yields of the material advanced for cultivar release in Costa Rica - an accession mix of 18516/18668, whichs show quite similar performance. The trial is on-going and quality analyses are pending but preliminary results indicate the potential to identify materials of superior performance to accessions 18516/18668.

In the future it will be important to monitor if performance could be linked to origin and genetic characterisation. From the presented data and experiences obtained on-station and on-farm it may be that accessions 18516 and 18668 are closely linked.

Photo 21. *Cratylia argentea* accessions at Quilichao

Table 61. Agronomic evaluation of a collection of *Cratylia argentea* in Quilichao. Preliminary data.

Treatment No. CIAT	Height (cm)	Diameter (cm)	Regrowing points (No.)	Dry Matter > 0.5mm (g/pl)	Dry Matter < 0.5mm (g/pl)	Total Dry Matter (g/pl)
18674	123	122	12	41	181	222
22408	131	108	10	62	124	185
22406	128	118	11	65	119	184
18671	122	120	12	52	108	160
22409	130	119	10	53	106	159
18957	127	107	9	41	106	147
22375	137	97	12	43	100	143
22400	134	113	10	33	108	141
22374	132	106	11	29	108	137
18668	111	113	9	24	107	131
18676	115	103	8	29	101	130
18516	120	110	13	14	116	130
22407	125	97	10	29	98	127
18667	121	103	9	23	104	126
22379	118	92	9	43	82	126
22386	120	94	8	33	85	118
22373	124	92	10	35	81	116
22387	134	98	8	46	69	115
18675	116	100	10	27	87	114
22405	125	100	9	9	103	112
22382	123	96	7	18	91	109
22393	132	95	9	10	99	109
22410	123	91	7	30	76	106
22394	126	89	8	24	80	105
22412	126	88	6	32	71	104
22384	124	105	5	30	73	103
22391	112	103	7	23	79	102
22383	100	99	6	38	63	100
22390	111	104	7	39	59	98
22392	126	94	8	17	75	92
22411	114	93	7	24	65	90
22381	125	89	6	18	71	89
18672	110	76	6	17	72	89
22399	106	90	7	20	68	88
22380	112	100	8	18	67	85
22404	116	85	8	19	66	85
22378	111	85	7	16	68	84
22396	107	77	5	29	53	82
22376	100	67	5	10	42	52
Mean	120	98	8	30	88	118
Range	11-190	25-190	0–25	0–222	0–340	0–420

3.2.4 The effect of cutting height and frequency on production and quality of *C. argentea*

Contributors: Marco Lobo di Palma and Vidal Acuña [Ministry of Agriculture and Livestock (MAG) Costa Rica]; Pedro J. Argel and Guillermo Pérez

Background. The cv. Veraniega of *C. argentea* is not readily consumed by grazing animals during the wet season, therefore farmers used this forage shrub mainly under cutting to supplement sugar cane or king grass during the dry period or for making silage. In any case the plant is allowed to growth from the beginning to more than the middle of the wet season, then the plants are cut back and the re-growth used three to four months later. Previous observations indicate the good regrowth capacity of cv. Veraniega during prolonged dry conditions, but much of the observations have been made at a cutting frequency and height of 8 weeks and 70 cm respectively. There is also little information on the effect of this management on the forage quality of cv. Veraniega. Thus, it is necessary to know more on the effect of cutting frequency and height on dry matter (DM) yields and quality to define more efficient management practices of the shrub at the farm level.

Results and Discussion. One year old plants of cv. Veraniega planted at 1.0 x 0.5 m were selected from a plantation established on a farm located in a subhumid environment of Costa Rica. The soil is a clay loam ultisol (pH 5.4) with low Al content (0.2 meq/100 g), medium P content (6.0 ppm) and medium percentage of organic matter (3.1). Fifteen plants were assigned to each treatment consisting of a cutting frequency of 60 and 90 days, and cutting heights of 30, 60 and 90 cm. DM yields, height of regrowth, number of shoots, crude protein percentages (CP), acid and neutral detergent fiber (ADF and NDF), and lignin content were measured at each evaluation cut (11 cuts for the 60 days frequency and 8 cuts for the 90 days frequency). The experiment was evaluated for two consecutive dry and wet seasons.

Both cutting frequency and cutting height affected the growth parameters measured. As expected height of regrowth, number of shoots and DM yields increased as the cutting frequency increased from 60 to 90 days (Table 62).

Within each cutting frequency height of regrowth was little affected by cutting height, however it was interesting to observe that the number of shoots and DM yields increased as the cutting height increased from 30 to 90 cm for each cutting frequency. The largest number of shoots recorded was 17.1 and observed at both re-growth ages and at 90 cm cutting height, meanwhile DM yields were much higher when plants were harvested every 90 days at a cutting height of 90 cm (494.9 g/plant). However, at this cutting height and age plants tended to yield less during the dry period, perhaps because older regrowths suffer some degree of defoliation compared to young shoots during this time of the year.

Table 62. Mean dry matter yields, regrowth height and number of shoots of *Cratylia argentea* cv. Veraniega harvested at three cutting heights and two cutting frequencies during 1998-2000 in a subhumid environment of Costa Rica.

Cutting Frequency		Cutting height (cm)			Mean
		30	60	90	
	Re-growth height (cm)	0.5	0.5	0.6	0.5
	No. of shoots	10.5	12.3	17.1	13.3
60 days*	DM yields (g/plant)	89.7	110.6	178.1	126.1
		(40.0)	(43.4)	(44.5)	(42.6)
	Re-growth height (cm)	0.9	0.8	0.9	0.9
90 days**	No. of shoots	11.7	15.0	17.1	14.6
	DM yields (g/plant)	256.9	304.9	494.9	352.2
		(27)	(34)	(28.3)	(29.9)

*Mean of 11 cuttings, ** Mean of 8 cuttings. Within brackets DM yield percentages during the dry period.

For any cutting height, forage quality was not affected when plants were harvested at 60 days of re-growth, and CP content ranged around 17 %. However, lower values of CP (15 %), and higher values of NDF (59 %), ADF (45 %) and lignin (16 %) were observed at the cutting frequency of 90 days, particularly at cutting heights of 60 and 90 cm. The lower values of CP may be associated with less nitrogen content in the tissue of older plants, as it has been reported before with *C. argentea;* meanwhile, since the woody component of the plant increases as the plant grows, so it is expected the fiber and lignin content will increase as in this experiment.

The values of CP reported in this study are slightly lower than those reported for the same specie elsewhere. However, values of CP above 14 % as in this case, are considered appropriate for milking cows. Therefore, in terms of management it may be adequate to harvest established *C. argentea* cv. Veranera plantations every 90 days at a cutting height of 90 cm.

Progress towards achieving milestones

- **Identified *Brachiaria* accessions and hybrids with superior tolerance to drought relative to commercial cultivars**

One hybrid of *Brachiaria* (FM9503-S046-024) was identified as promising for areas with moderate drought stress in regions with acid soils.

- **New *Cratylia* accessions characterized for agronomic performance.**

Our results show that variability exists in forage yield among accessions in a new collection of *C. argentea* being evaluated in a site with acid soils and in a site with long dry season. The more advanced accessions of this important shrub tolerate very well the dry season and retain a high proportion of green leaves; however, within the new collection some accessions produce similar DM yields in the dry and wet season.

The most promising accessions so far are CIAT 18674 for acid soils and CIAT 22374 for areas with long dry seasons. It was also interesting to observe a new subshrub of *Cratylia* with a semi-prostrate growth habit that may suite this plant to associate with stoloniferous or erect grasses for direct grazing.

3.3 Accessions of shrub legumes with adaptation to different environments

Highlights

- Identified *Calliandra calothyrsus* CIAT 22310, 22312 and 22316 as promising accessions, and were advanced to farmer participatory selection in Central America.

3.3.1 Genotypic variation in dry season tolerance in a core collection of *Calliandra calothyrsus* (Quilichao)

Contributors: M. Peters, L. H. Franco, B. Hincapié, P. Avila, A. Pottinger (OFI, UK)

Background. *Calliandra calothyrsus* is a shrub legume with potential use as dry season cut and carry supplement for livestock. Thus a collection of 13 accessions was 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 randomized 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: Though differences between drier and wetter periods are not very well pronounced at the Quilichao site, the results in Table 63 give an indication of performance of *Calliandra calothyrsus* in environments with 3-4 dry (<50 mm rain/month) months. There was only a minor effect of season on performance of *Calliandra*, with about 20% lower productivity in the dry season ($P<0.05$) compared to more wet conditions. Percentage of edible material was not affected by season ($P<0.05$). No significant ($P<0.05$) interactions between treatments and season were observed meaning that ranking of species was not affected by season. The accession with the highest yield ($P<0.05$) in the extremely acid soil (pH around 4.0) was CIAT 22310.

Table 63. Dry matter yield of edible (<5mm) and non edible (>5mm) material in g/plant of *Calliandra calothyrsus* during Maximum and Minimum precipitation in Quilichao, Cauca.

	Season					
	Maximum precipitation			Minimum precipitation		
Treatment	<5 mm	>5mm	% material <5 mm	<5 mm	>5mm	% material <5 mm
	g/plant			g/plant		
Cc 22310	247	343	60	208	345	65
Cc 22320	90	242	73	63	197	76
Cc 22312	31	211	88	38	185	83
Cc 22309	78	179	70	81	136	63
Cc 22318	76	177	73	59	141	74
Cc 22315	105	170	62	82	139	63
Cc 22314	51	153	75	42	108	73
Cc 22313	60	148	71	66	122	66
Cc 22317	43	140	79	26	112	82
Cc 22316	53	135	72	49	97	67
Cc 22319	121	130	53	103	108	52
Cc 22308	97	126	56	67	98	60
Cc 22311	43	104	78	36	83	75

Best nutritional quality among the higher yielding accessions was measured for CIAT 22312 (Table 64). Therefore this accession may be targeted for ruminant feeding while CIAT 22310 – with a low digestibility and likely slower decomposition - may be targeted for soil conservation and melioration.

To test *Calliandra calothyrus* for dry season tolerance selected accessions or the whole collection would need to be tested in an environment with a pronounced dry season, i.e. Atenas. Preparations are underway.

Photo 22. *Calliandra calothyrsus* accessions CIAT 22310 and 22311 at Quilichao.

Table 64. Forage quality of a collection of *Calliandra calothyrsus* evaluated during Minimum and Maximum precipitation at Santander de Quilichao, Cauca, Colombia.

Treatment	Season							
	Minimum				Maximum			
	IVDMD	CP	Tannins		IVDMD	CP	Tannins	
			Soluble	Bound			Soluble	Bound
*Cc*22311	31.5	19.8	14.8	7.5	26.1	15.3	18.2	4.6
*Cc*22319	26.2	20.6	26.8	5.3	23.1	15.5	17.1	4.3
*Cc*22308	26.0	21.3	19.5	7.2	19.0	14.6	17.1	3.7
*Cc*22316	25.9	20.9	18.1	5.7	21.2	13.8	20.4	4.5
*Cc*22312	25.4	19.9	18.1	5.9	26.9	17.5	16.5	4.3
*Cc*22317	24.6	18.6	18.9	6.8	21.7	14.1	21.5	4.0
*Cc*22315	24.4	20.0	15.9	6.6	16.0	14.2	16.9	3.5
*Cc*22309	24.4	20.5	15.4	8.4	19.2	15.3	16.1	4.1
*Cc*22314	23.9	21.5	20.1	6.3	20.3	15.3	16.1	3.3
*Cc*22310	23.8	18.1	17.4	0.8	15.1	15.5	20.3	7.0
*Cc*22318	23.6	21.1	31.8	4.5	21.7	15.3	21.7	4.4
*Cc*22313	21.7	19.3	19.8	7.5	19.1	16.1	21.7	4.3
*Cc*22320	20.7	19.4	23.1	3.1	18.3	15.2	18.7	3.7

3.3.2 Agronomic characterization of a core collection of *Flemingia macrophylla*

Contributors: M. Peters, B. Hincapie, P. Avila

Background: Assessment of genetic diversity of tropical forage legumes has hitherto consisted mainly of morphological, physiological and agronomic germplasm characterization. It is increasingly complemented or substituted by laboratory studies in the form of isozyme electrophoresis and, more recently, by DNA markers. As far as large germplasm collections of any given species are concerned, because of continuously increasing limitations regarding research resources such as for the management of collections (assessing genetic diversity, documentation, seed multiplication and periodic rejuvenation, *ex situ* conservation in cold-storage rooms, etc.) a core collection concept has been suggested by which a large collection is reduced to a smaller size that can be easier and more cost-efficiently managed, whose diversity, however, is, *per definitionem*, representative of the total collection. Concepts are required regarding the most appropriate and resource-efficient way(s) of identifying a core collection for tropical wild legumes, including the consideration of using molecular markers and Geographic Information Systems (GIS) tools.

We intend to utilize *Flemingia macrophylla* (Willd.) Kuntze ex Merr. (syn. *F. congesta, Moghania macrophylla*) as a test species to evaluate the concept of core collection. In the case of *F. macrophylla*, documentation of genetic diversity is insofar particularly challenging as (i) many accessions, independent from their original collection sites, look quite similar; (ii) the issue of the value of (naturalized?) African and South American material awaits clarification; and (iii) the extent of variability in tannin content needs to be explored. Beyond this basic research, we have an interest in the agronomic performance of Flemingia macrophylla as a multipurpose shrub species – feed, soil cover, fuelwood, shade plant, erosion barrier, mulch, nematizide etc. - adapated to extremely acid, low fertility soils in the humid and subhumid tropics and drought tolerance. Particular attention would be given to quality parameters, both in respect to use as feed and for Natural Resource conservation.

Photo 23. *Flemingia macrophylla* accessions at Quilichao

Methods: Germplasm of *Flemingia macrophylla* was obtained from CSIRO and ILRI. Together with the accessions from CIATs gene bank we were able to plant 74 accesiones (Table 65), about 90% of available germplasm world-wide - of *F. macrophylla* in jiffy pots in the green house and tarnsplanted them to the field in Quilichao. A Randomized Complete Block Design with 3 replications and 9 plants of each accesions per replication. Phenotypic and agronomic parameters are collected. The genetic characterisation is in preparation and would be combinded with a GIS (FLORAMAP) based assessement of origin information. 72 materials were planted for seed multiplication.

Table 65. Passport data of a *Flemingia macrophylla* collection planted at Santander de Quilichao.

Number	Country	Location	km	D	City	Latitude			Longitude			Altitude msnm	Prec. mm
801	XXX												
7184	XXX												
17400	THA	Khon Kaen	28	N	Khon Kaen	16	N	41	102	E	48	160	1210
17403	THA	Chumphon	52	S	Chumphon	10	N	4	99	E	4	40	1870
17404	THA	Surat Thani	40	N	Surat Thani	9	N	25	99	E	11	50	1940
17405	THA	Surat Thani	106	N	Thung Song	8	N	59	99	E	23	70	1750
17407	THA	N.Si Thammarat	13	S	N.Si Thammarat	8	N	18	99	E	55	50	2410
17409	MYS	Perlis	5	NE	Kangar	6	N	39	100	E	17	70	2190
17411	MYS	Pahang	15	S	Temerloh	3	N	20	102	E	26	110	2030
17412	MYS	Terengganu	6	O	Jerteh	5	N	46	102	E	25	50	2660
17413	MYS	Kelantan	6	NE	Pasir Mas	6	N	3	102	E	11	30	2710
18048	CHN	Hainan	96	NE	Dongfang	19	N	23	109	E	6	150	1600
18437	IDN	West Sumatra	24	NO	Sijunjung	0	S	29	100	E	52	190	2430
18438	THA	Rayong	56	NE	Rayong	12	N	47	101	E	44	40	2390
18440	THA	Trat	5	NO	Trat	12	N	17	102	E	29	30	3000
19453	PNG	Morobe	67	O	Lae	6	S	57	146	E	34	1100	1660
19454	PNG	Morobe	83	O	Lae	7	S	13	146	E	35	650	1680
19457	PNG	East.H'lands	15	NO	Goroka	6	S	2	145	E	22	1630	1930
19797	IDN	West Sumatra	23	NO	Iolpanti	0	N	34	100	E	1	180	3820
19798	IDN	West Sumatra	32	NE	Solok	0	S	44	100	E	50	190	2370
19799	IDN	Jambi	36	O	Bangkok	2	S	8	102	E	2	150	2980
19800	IDN	South Sumatra	6	NE	Lahat	3	S	47	103	E	36	140	3250
19801	IDN	South Sumatra	40	SO	Lahat	3	S	57	103	E	25	520	3240
19824	IDN	West Sumatra	26	NO	Sijunjung	0	S	39	100	E	36	130	2990
20065	IDN	E.Nusatenggara	35		Isla Rote							70	
20616	IDN	Aceh	46	S	Bireuen	4	N	44	96	E	45	690	1730
20617	IDN	Aceh	49	S	Bireuen	5	N	1	96	E	42	560	1630
20618	IDN	Aceh	61	SE	Banda Aceh	5	N	24	95	E	29	250	1720
20621	IDN	Aceh	133	SE	Takengon	3	N	42	97	E	37	1100	2390
20622	IDN	North Sumatra	24	SE	Sidikalang	2	N	34	98	E	32	1350	2330
20624	IDN	North Sumatra	19	N	Sibuhuan	1	N	11	99	E	44	30	2460
20625	IDN	West Sumatra	13	NE	Simpangempat	0	N	15	100	E	5	270	4200
20626	IDN	North Sumatra	31	SE	Pematangsiantar	2	N	46	99	E	15	250	2440
20631	IDN	North Sumatra	35	SO	Bangunpurba	3	N	6	98	E	42	550	2300
20744	COL	Tolima	4	O	Chaparral	3	N	43	75	W	32	930	2890
20972	CHN	Hainan	52	NE	Wang Lin	18	N	55	110	E	26	50	2300
20973	CHN	Hainan	6	O	Wang Lin	18	N	47	110	E	20	70	2400
20975	CHN	Hainan	30	NE	Le Dong	18	N	49	109	E	17	230	1600
20976	CHN	Hainan	2	SE	Tongzha	18	N	45	109	E	30	330	1800
20977	CHN	Hainan	8	NE	Mao Yang	18	N	55	109	E	28	220	1800
20978	CHN	Hainan	17	SE	Baisha	19	N	10	109	E	28	250	1800
20979	CHN	Hainan	6	NO	Baisha	19	N	14	109	E	23	370	1800
20980	CHN	Hainan	165	SO	Haikou	19	N	23	109	E	6	200	1800
20982	CHN	Hainan	112	SO	Haikou	19	N	30	109	E	34	140	1800
21079	THA	Suphan Buri	32	SO	Dang Chang	14	N	46	99	E	27	250	1220
21080	THA	Lamphun	17	NO	Thoen	17	N	39	99	E	8	370	1090
21083	THA	Mae Hong Son	63	S	Mae Hong Son	18	N	40	97	E	56	620	1300
21086	THA	Chiang Mai	6	O	Chiang Mai	18	N	50	98	E	52	490	1250
21087	THA	Chiang Mai	10	O	Chiang Mai	18	N	51	98	E	52	700	1250
21090	THA	Chiang Rai	74	SO	Chiang Rai	19	N	12	99	E	31	550	1350
21092	THA	Chiang Rai			Mae Sai	20	N	26	99	E	54	500	1570
21241	COL	Meta	5	O	Granada	3	N	32	73	W	46	400	2920
21248	GHA	Kumasi											
21249	XXX												
21519	USA	Hawaii				22	N		158	E		20	1380
21529	IDN	Java			Isla Java	7	S	45	110	E	20	750	1500
21580	CMR	Centre			Yaounde	3	N	52	11	E	31	760	1680
21982	VNM	Hue	7	O	Hue	16	N	24	107	E	32	70	2900
21990	VNM	Quang Nam	10	SO	Tam Ky	15	N	28	108	E	22	80	2210
21991	VNM	Quang Ngai	99	SE	Da Nang	15	N	24	108	E	50	40	2200
21992	VNM	Quang Ngai	8	SO	Pho Van	14	N	51	108	E	52	40	2170

To be continued…….

Table 65. Passport data of a *Flemingia macrophylla* collection planted at Santander de Quilichao……(continuation)…..

Number	Country	Location	km	D	City	Latitude			Longitude			Altitude msnm	Prec. mm
21993	VNM	Binh Dinh	86	NO	Qui Nhon	14	N	24	109	E	0	150	1900
21994	VNM	Gialai Kontum	28	NE	Pleiku	14	N	0	108	E	13	700	2250
21995	VNM	Dac Lac	50	SO	Ban Me Thuot	12	N	25	107	E	46	800	1840
21996	VNM	Dac Lac	183	NE	Ho Chi Minh	11	N	56	107	E	24	450	2350
22082	THA	Nong Khai	64	NE	Nong Khai	18	N	14	103	E	10	190	2230
22087	THA	Nong Khai	12	SE	Bung Kan	18	N	20	103	E	41	160	3110
22090	THA	Nakhon Phanom	80	NO	Nakhon Phanom	17	N	53	104	E	15	170	2700
22285	VNM	Khanh Hoa	17	NO	Ninh Hoa	12	N	33	108	E	59	80	
22327	VNM	Song Be	54	N	Ho Chi Minh	11	N	5	106	E	36	80	
22058	THA	Phetchabun	40	N	Lom Sak	17	N	4	101	E	12	370	1270
J001	COL												
I-15146	CRI				Golfito	8	N	38	83	W	10	5	
C-104890					Gympie								

3.3.3 Agronomic characterization of a collection of *Rhynchosia schomburgkii*

Contributors: M. Peters, P. Avila, L.H. Franco, B. Hincapié

Rationale: From the evaluation of a range shrub legumes with tolerance to cool temperatures and work of Project PE-5 *Rhynchosia schomburgkii* has emerged as one of the most promising accessions for the higher altitude hillsides. Though forage attributes still need to be studied, the plant has also potential as a soil cover plant.

Methods: 13 accessions of *Rhynchosia schomburgkii*, mostly originating from Colombia, have been planted at Quilichao. Plants were transplanted into single-row plots, with 4 replications. Dry matter yield, plant persistence, drought tolerance and forage quality are the main parameters to be evaluated.

Photo 24. *Rhynchosia schomburgkii* CIAT 918, at Quilichao

Results and discussion. Variations in dry matter yields between accessions were highly significant ($P \leq 0.01$), with CIAT 17918, 22134, 918 and 19235 showing the best performance (Table 66). Among the most promising accessions in terms of dry matter production are CIAT 918, 17918, 19235 and 22134. 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 will depend on palatability of accessions. Earlier results indicate intraspecific variation in palatability *Rhynchosia schomburgkii*, important for the final assessment of the potential of *Rhynchosia* for feed and soil fertility maintenance.

Table 66. Agronomic parameters of a collection of *Rhynchosia schomburgkii* Santander de Quilichao, Cauca, Colombia.

Treatment NCIAT	Height (cm)	Diameter (cm)	Regrowth points (No.)	DM[1] (g/pl)
17918	87	147	40	208
22134	68	106	37	123
918	84	98	34	118
19235	65	109	37	104
20800	53	77	25	61
20456	40	80	36	58
18490	53	81	27	57
7389	63	86	25	57
8215	66	86	25	53
7810	57	79	25	53
8582	47	75	22	46
21777	48	67	7	16
21775	50	56	5	13
DMS				59

[1] Average of 10 cuts cut at an interval of 9 weeks

Progress towards achieving milestones

- **Known genetic variation in agronomic attributes in collections of *Rhynchosia***

We selected superior accessions (CIAT 17918, 22134, 918 and 19235) of *Rhynchosia schomburgkii* and will now determine their feed value.

- **Advanced to participatory evaluation of selected accessions of *Calliandra***

Selected accessions of *Calliandra* have advanced to Farmer Participatory Project in Honduras, Nicaragua and Costa Rica. We are in the process of obtaining basic seed of accessions CIAT 22310, 22312 and 22316 from OFI and ICRAF for multiplication in Costa Rica (with CATIE) and Colombia, with the aim to test material under drier conditions in on-farm testing in Central America.

3.4 Accessions of *Brachiaria* and *Paspalum* with adaptation to poorly drained soils

Highlights

- Found good adaptation of *P. atratum* and *P. plicatulum* to both poorly and well drained soils
- Confirmed the good adaptation of *B. brizantha* cv. Pasto Toledo to poorly drained soils

3.4.1 Field evaluation of *Brachiaria* and *Paspalum* genotypes established in poorly and well drained sites in Costa Rica (San Carlos and Atenas)

Contributors: Norman Mora Segura and Milton Villarreal (MAG-ITCR, Costa Rica); Pedro J. Argel , Guillermo Pérez

Background. There are not many high quality forage options for poorly drained soils in the tropics. Farmers rely on few species of *Brachiaria, Echinochloa polystachya* (pasto Alemán), and native poor quality species of *Hymenachne* and *Paspalum fasciculatum.* The situation is even more critical in sites with heavy clay soils that saturate during the wet season but lose water and crack during the dry period. This type of soils are well extended along the Central American isthmus, and the reality is that presently few forage species are available for these conditions.

The genus *Paspalum* is native to the American tropics, and many species growth in a diversity of climate and soil conditions, including poorly drained soils, but to the present little attention has been given to characterized and select species/lines of good forage quality with a wide range of adaptation. Similarly, the diversity of the genus *Brachiaria* has not been fully characterized in term of adaptation to cracking/waterlogged soil conditions.

The present experiment was established in two contrasting sites in 1999 (see IP5 Annual Report 1999). Evaluations ended in the poorly drained conditions of San Carlos, and are still underway in Atenas for an additional growing season. At San Carlos we are now in the phase of establishment a grazing trial with *P. atratum* in poorly drained conditions.

Results and Discussion. Significant differences in DM yields ($p<0.05$) were observed among the *Brachiaria* species evaluated in poor drained conditions of San Carlos, but no differences were observed for the two CIAT lines of *Paspalum* (Table 67). At this site, cv. Pasto Toledo significantly over-yielded ($p<0.05$) cvv. Marandú and Mulato, as well as the accessions of *B. humidicola*, an indication of the good adaptation of this Cultivar to poorly drained conditions, which confirms similar observations reported elsewhere.

Preliminary results at Atenas (5 months dry, 7 months wet) indicate larger DM yields either for the *Brachiaria* or the *Paspalum* species at this site compared to San Carlos. However, no statistical differences ($p<0.05$) in DM yields are reported within each genus, but it is interesting to observe the high DM yields of *B. humidicola* CIAT 26427, followed by *B. brizantha* Mixe (has not CIAT number yet) and *B. brizantha* CIAT 26124. Cultivars Pasto Toledo and Mulato are not being evaluated in this trial, they were evaluated before in a similar experiment at the same site; in this case DM yields were respectively 2912.0 and 2023.0 kg/ha/cut (see IP5 Annual Report 1999).

Table 67. Mean dry matter yields of *Brachiaria* and *Paspalum* species established in San Carlos (poorly drained soils) and Atenas (well drained soils), Costa Rica.

Species	CIAT No.	Mean DM yields (kg/ha/cut)	
		San Carlos	Atenas
B. brizantha (Toledo)	26110	1674.5 a*	-
B. brizantha	16113	1550.3 ab	1823.3**
B. brizantha	26318	1503.5 ab	1791.2
B. brizantha (Marandú)	6780	1445.1 bc	1517.3
B. hybrid (Mulato)	36061	1408.5 bcd	-
B. brizantha	26124	1251.6 cde	1991.9
B. humidicola	26427	1228.2 ed	2370.6
B. humidicola	26159	1122.5 e	1766.6
B. brizantha (Mixe)	NA	-	1934.1
B. brizantha	16305	-	1663.7
P. plicatulum (seed)	26989	2075.5 A	-
P. plicatulum (seedling)	26989	1765.5 B	2379.2
P. atratum	26986	2018.6 A	2261.9
P. atratum (Suerte)	NA	1670.6 B	-
P. guenoarum	28985	-	1559.6
		(P<0.05, Duncan)	(ns, P<0.05)

*Mean of 6 evaluation cuts every 4 weeks

**Mean of 4 evaluation cuts every 5 weeks during the wet period and 2 cuts every 8 weeks during the dry period

At San Carlos, *P. plicatulum* plots established by seed produced significantly (p<0.05) more DM yields than those established by seedlings, the reasons for this are not well known, but it seems that transplanted plants suffered some kind of stress during establishment. On the other hand this species produces abundant seed of high quality and the propagation by seed seems to be an appropriate way of planting. In this trial cv. Suerte of *P. atratum* (an introduction from Florida) produced significantly less DM yield (p<0.05) than *P. atratum* CIAT 26986.

Progress towards achieving milestones

- **Grazing trials with *Paspalum atratum* established in areas with poorly drained soils.**

Our results indicate good adaptation of *Paspalum plicatulum* and *P. atratum* accessions both to poorly drained and well drained soils. Arrangements are underway to establish grazing trials with *P. atratum* CIAT 26986 in San Carlos, Costa Rica, and in poorly drained site and in well drained site in Atenas, Costa Rica.

Activity 3.5 Accessions of grasses and legumes for multipurpose use in different agroecosystems

3.5.1 Evaluation of a core collection of *Vigna unguiculata* for multipurpose uses (Quilichao)

Contributors: M. Peters, Luis H. Franco, P. Avila, G. Ramírez, B. Hincapié, B.B. Singh (IITA, Nigeria)

Highlights

- Cowpea as green manure resulted in a 2 fold increase in maize grain yield as compared to the unfertilized crop and on a 50% yield increase as compared to the crop fertilized with N (80).

Rationale: Cowpea (*Vigna unguiculata*) is utilized in the subhumid/semi-arid tropics of West Africa and India as a source of food and feed. Work of CIAT with a limited number of accessions indicated potential of cowpea for soil improvement. However, distribution of cowpea in Latin America so far is limited.

Methods: A core collection of 15 cowpea accessions was been obtained from B.B. Singh, cowpea breeder of IITA. These have been planted at CIAT's Quilichao site on acid soils and are evaluated for their grain and forage potentials and there value as green manure for a succeeding maize crop. Using the maize crop the nitrogen equivalents for the incorporation of cowpea will be measured. Subsequently it is planned to repeat the experiment on alkaline soils to test the ability of cowpea to adapt to diverse soil types, an important feature in particular for Central America with high variability of pH.

Photo 25. *Vigna unguiculata* in grass production phase at Quilichao

Results and Discussion: The accessions established quickly, showing a cover of more than 70% 2 months after seeding; at the time of incorporation into the soil as green manure all accessions were fully established. In the preliminary analysis, no significant (P<0.05) differences between accessions were encountered in biomass production during the establishment phase, but differences at time of soil incorporation were significant (P<0.05), with IT90K-277/2, IT95K-1088/2, IT93K-573/5, IT86D-715 and IT96D-733 showing the highest dry matter yields (Table 68).

The accessions with the highest production of cowpea grains were 1088/4IT95K, IT90K-277/2 and IT96D-740 with yields of more than 2 t/ha. Grain production did not seem to effect herbage production and effect as green manure, important for dual-purpose use as grain/green manure or grain/forage. Interactions between accessions and repetitions were found for some accessions affecting some of the initial statistical analysis.

Quality of cowpea varied strongly between accessions as expressed in protein and lignin concentrations and digestibility ($P \leq 0.01$) and P and Ca concentrations ($P \leq 0.05$). With protein concentrations of 14-21 % and a digestibility of dry matter of 80% or more cowpea is also an excellent fodder for livestock (Table 68).

Table 68. Dry matter yield (kg/ha) of cowpea green manure herbage and grain before soil incorporation and grain and dry matter yield of a following maize crop.

Treatment	Cowpea		Maize	
	Grain	Herbage	Grain	DM Total
	(kg/ha)			
IT86D-716	1723	2549	4583	13218
IT6D-733	1880	2955	4323	12712
IT90K-277/2	2113	4629	4222	12393
IT93K-573/5	1380	3814	4215	11201
IT95K-1088/2	1570	3988	4205	12234
IT86D-715	1590	3764	4032	12088
IT96D-740	2000	1902	3973	11298
IT86D-719	1680	2844	3962	10813
IT90K-284/2	1383	1782	3917	11100
IT89KD-288	1820	2742	3806	9811
IT93K-637/1	1267	1964	3013	9630
160N	-	-	2978	7852
IT89KD-391	1437	2254	2902	8441
80N	-	-	2746	6632
IT95K-1088/4	2173	2125	2621	10792
40N	-	-	2614	6594
IT93K-503/1	1693	2815	2461	6913
0N	-	-	1480	4080
LSD ($P<0.05$)	661	1752	2541	6608

The majority of accessions sustained a maize yield without fertilization above the yields encountered in plots fertilized with 80 kg N/ha (Table 69). Of interest are the high yields of maize after IT96D-740, which is an accession that has low biomass but good cowpea grain production. The utilization of cowpea as a green manure led to large increases in maize yield, confirming earlier results with other cowpea accessions in Cauca hillsides.

The value of cowpea to small farmers can be not only as green manure but also as a source of fodder for livestock and grain for human consumption, provided, it is acceptable to people in target areas (i.e. Central America).

Table 69. Fodder quality of accessions in a collection of *Vigna unguiculata* (Caupi, cowpea) grown in Santander de Quilichao.

Treatment	Protein	IVDMD	Lignin	P	Ca
			%		
IT86D-715	21	80	4.5	0.14	2.1
IT90K-277/2	19	82	2.5	0.12	2.1
IT93K-573/5	19	82	2.7	0.13	1.5
IT96D-740	18	83	5.9	0.13	1.6
IT90K-284/2	18	81	2.4	0.13	1.6
IT96D-733	17	84	4.4	0.12	1.5
IT86D-719	17	83	4.2	0.11	1.8
IT93K-673/1	17	85	2.7	0.13	1.5
IT93K-503/1	17	83	2.1	0.12	1.3
IT95K-1088/2	16	85	4.5	0.11	1.4
IT89KD-391	16	82	1.7	0.10	2.1
IT95K-1088/4	16	84	3.4	0.14	1.6
IT89KD-288	15	85	2.6	0.09	1.4
IT86D-716	14	86	5.6	0.10	1.3
LSD ($P<0.05$)	**3.1**	**2.66**	**1.2**	**0.02**	**0.61**

3.5.2 Evaluation of core collection of *Lablab purpureus* for multipurpose uses (Quilichao)

Contributors: M. Peters, L. H. Franco, B. Hincapié, P. Avila

Background: *LabLab purpureus* is a free seeding, fast growing annual or short-term perennial forage legume, with widespread use through the tropics as a fodder plant; in Africa use for human nutrition is also common. Cultivars were released in Australia. The origin of the *Lablab* germplasm sown is mainly Eastern/Southern Africa and Asia (Table 70). *Lablab purpureus* is best adapted to lower altitudes and a rainfall regime of 750 –2000 mm/year.

Photo 26. Seed of *Lablab purpureus* at Quilichao

However it is highly drought tolerant. *Lablab* grows in a variety of soils, pH for growing is reported to be between 5.0 and 7.5. ILRI and CSIRO have advanced studies with collections of *Lablab*. CIAT obtained the collection available at ILRI/CSIRO to follow characterisation for the Latin American continent, with partcilar emphasis on tolerance of various pH, hence we intend to plant Lablab at two sites of contrasting pH (4.0 and 8.0). The assessment in acid to alkaline soils is important to define niches of Lablab mainly as green manure and fodder plant (especially for hay and silage or deferred feed), with emphasis on Central America with its soils highly variable in pH. Because of its potential as a green manure crop contribution in N-equivalents to a succeeding maize crop will be evaluated.

Methods: 44 accesions of *Lablab purpureus* (Table 70) were sown on an extremely acid soil (pH 4.0) in Santander de Quilichao for seed multiplication, to then plant the collection in soils of varying acidity.

Preliminary results and discussion: The accessions established very well and at the moment seeds have from most accession has been collected. The adaptation of *LabLab* to the very acid soil is of particular interest as it may point to an even wider adaptation to soil acidity as has been reported in the literature.

Table 70. Passport data of a collection of *Lablab purpureus* received from CSIRO (John Donelly) and ILRI (Jean Hanson) during 2000, complemented with accessions available in CIA's genebank.

Accession	Origin	Accession	Origin
CPI 100602	KEN	ILRI 11613	exMLI
CPI 106494	IND	ILRI 11615	BKF
CPI 106500	IND	ILRI 11630	MLI
CPI 106548	IND	ILRI 11632	exMLI
CPI 29398	ex IDN	ILRI 14411	KEN
CPI 30702	Burma	ILRI 14437	ZWE
CPI 34777	IND	ILRI 14441	SEN
CPI 35894	ex DAN	ILRI 14442	SDN
CPI 36903	ex Ukrai	ILRI 6533	exETH
CPI 51564	ZMB	ILRI 6536	exETH
CPI 52437	ZAF	ILRI 6930	exTH
CPI 52508	MOZ	ILRI 7072	exETH
CPI 52535	ex IND		
CPI 52544	IND	CIAT17189	XXX
CPI 60216	UGA	CIAT17192	MWI
CPI 67639	ex IND	CIAT17193	ZWE
CPI 69498	ZWE	CIAT17194	ZWE
CPI 76996	ZMB	CIAT17195	MWI
CPI 76998	ZMB	CIAT17196	XXX
CPI 81626	IND	CIAT17197	XXX
CPI 96924	CHN	CIAT21602	XXX
CPI 99985	Banglad	CIAT21603	PER
CQ2975	XXX	CIAT22183	COL
CPI 30212	ex KEN		
CPI 16883	KEN		

Progress towards achieving milestones

- **Characterized a core collection of *Vigna unguiculata* for use as a multipurpose legume.**

Our results confirm the value of cowpea as an excellent annual multipurpose legume for soil improvement when used as green manure, for fodder and for grain production, which may be attractive to small farmers in hillsides of Central America that operate mixed crop livestock systems. The accessions IT90K-277/2, IT95K-1088/2, IT93K-573/5, IT86D-715 and IT96D-733 are so far the most promising for seed increase.

3.6 Genotypes of *Arachis* and *Desmodium* with broad edaphic and climatic adaptation

Highlights

- Several *Arachis pintoi* accessions were identified as highly productive forage legumes with high stability across different agro-ecosystems. These accessions were superior to the commercial cultivar 'Mani forrajero' in all environments evaluated.

3.6.1 Genotype x Environmental interaction of a core collection of *Arachis pintoi*

Contributors: E. Cárdenas (U. Nacional, Bogotá), B. L. Maass (U. Stuttgart, Germany), M. Peters

Rationale*:* 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.

Methods*:* Following initial multiplication, two sets of multi-locational trials were established, one in 1994 (35 *Arachis* spp. accessions at six sites) and one in 1995 (39 *A. pintoi* accessions at seven sites) (See previous annual reports). Seven accessions were common to both sets of trials, with the commercial variety CIAT 17434 serving as a control. Each set of trials was planted at six sites, ranging from hillside ecosystem in the Cauca Department of Colombia to the lowland Amazon piedmont of Caquetá.

A seventh site (at CIAT-Palmira) was included in the 1995 trials. Twenty-square-meter plots were planted in association with *B. dictyoneura* or *B. decumbens*, and managed under periodic defoliation - either by clipping or mob grazing (depending on the availability of grazing animals). Data on forage yield, botanical composition, and seed production was taken over two years. Data was then subjected to a Genotype x Environment analysis to define performance of germplasm across different agro-ecosystems and to identify accessions with stable performance across sites but which respond to better environmental conditions.

Results and discussion: Figures 33 to 41 show the results of the Genotype x Environment Analysis for a number of productivity and quality and reproduction parameters. Dry matter productivity of accessions CIAT 22160, 18751, 18747, 18748, 22159, 18746 and 18752 of the

1994 collection was substantially more stable across environments, and at higher level of productivity than the commercial accession CIAT 17434.

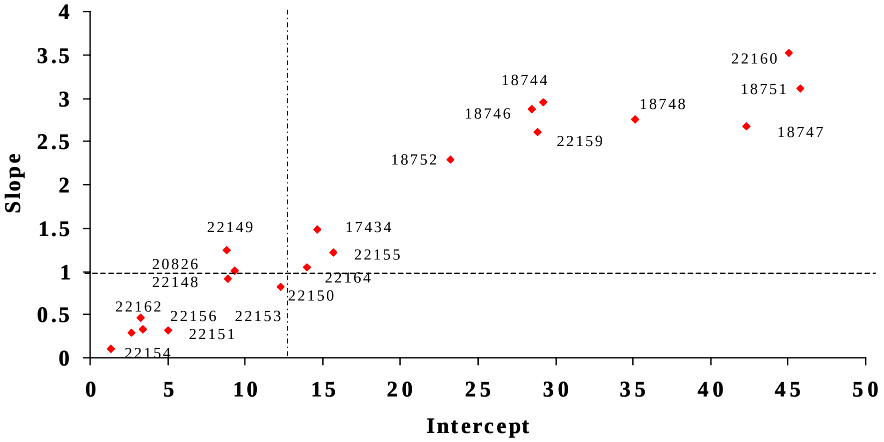


Figure 33. Results on stability analysis of dry matter (DM) productivity in a core collection (1994) of *Arachis pintoi* evaluated in contrasting locations.

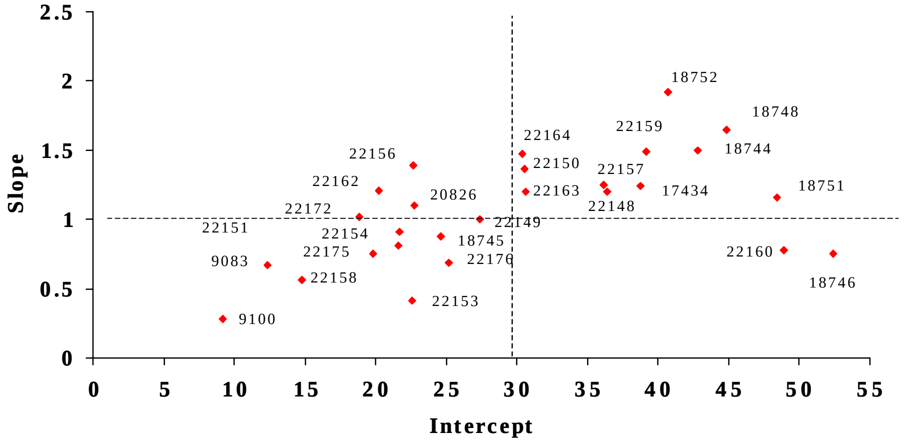


Figure 34. Results on stability analysis of soil cover in a core collection (1994) of *Arachis pintoi* evaluated in contrasting locations.

Results on the ability of accessions to rapidly covering the soil as an important characteristics for potential cover crops identified with accessions CIAT 18752, 18748, 18744 and 18751 of the 1994 a similar set of accessions with superior performance and higher stability than CIAT 17434. Though the latter proved to be among the best suited accessions as a cover crop. CIAT 18747, 18751, 18746, 18752 and 18744 had an average ability to maintain CP contents in different environments, at a low to average level. However, these accessions were superior to CIAT 17434, which had a low stability at a low level.

The best accessions in terms of CP content across environments were CIAT 22164 and 22165. CIAT accessions 18744 and 18746 proved to be the best accessions in terms of stability over diverse environments and high values. CIAT 17434 was as stable as 18744, but at a much lower level.

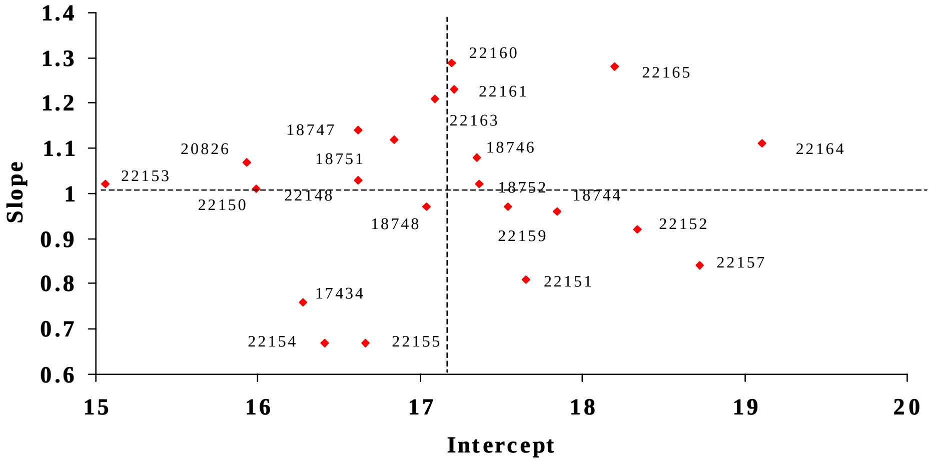


Figure 35. Results on stability analysis of Crude Protein (CP) contents in a core collection (1994) of *Arachis pintoi* evaluated in contrasting locations.

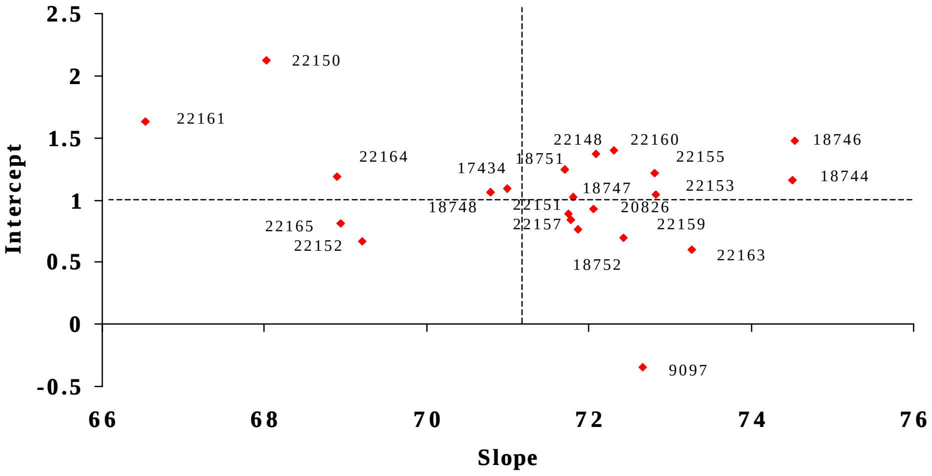


Figure 36. Results on stability analysis of *in vitro* dry matter digestibility (IVDMD) in a core collection (1994) of *Arachis pintoi* evaluated in contrasting locations.

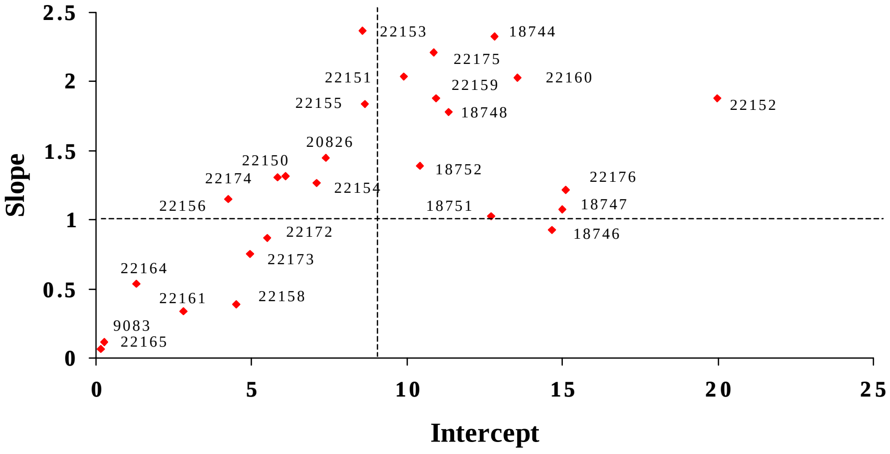


Figure 37. Results on stability analysis of number of flowers in a core collection (1994) of *Arachis pintoi* evaluated in contrasting locations.

CIAT 18744, 22153, 22175, 22151, 22159 and 22160 were the most predictable accessions in number of flowers across environments, an important aspect for artesenal seed production. We did not have cross-environment data for CIAT 17434 available. CIAT 22238, 22241 and 22236 had the highest dry matter yields, independent of location. However these accessions produce very few seeds. Other promising accessions with high yields across environments include CIAT 22268, 22257, 22259, 18744 and 22233. All these accessions, except CIAT 22233 produce seeds.

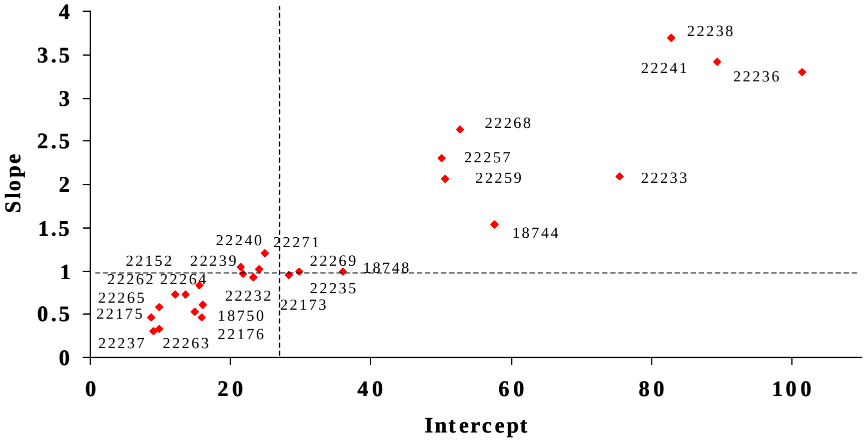


Figure 38. Results on stability analysis of dry matter (DM) productivity in a core collection (1995) of *Arachis pintoi* evaluated in contrasting locations.

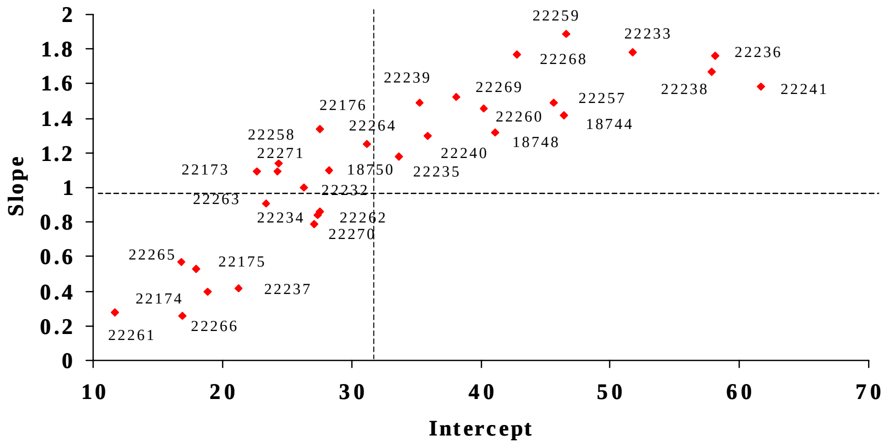


Figure 39. Results on stability analysis of soil cover in a core collection (1995) of *Arachis pintoi* evaluated in contrasting locations.

In their ability to cover soil, CIAT accessions 22233, 22238, 22236 and 22242 were the most stable, at a high level, across environments. The other outstanding accessions in terms of stability across environment, with a good soil cover, were CIAT 22259, 2268, 22257, 18744, 22269, 22260 and 18746.

None of the accessions was outstanding. CIAT 22160 was the most stable at a high level; CIAT 22268 had the highest CP contents but was more variable across sites. CIAT 17434 had the lowest stability and sub average CP contents. The best accessions in terms of proliferance and stability were CIAT 18759, 22160, 22152, 22240 and 22176. CIAT 18744 also was among the best accessions while CIAT 17434 had an average flower production at a high variation across environments.

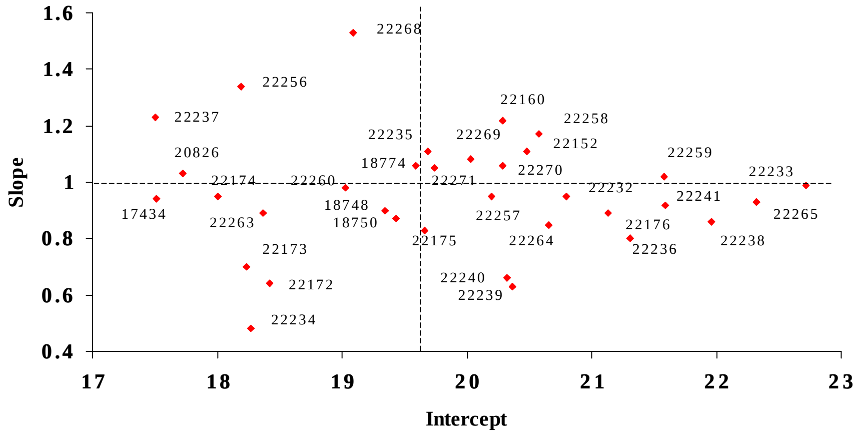


Figure 40. Results on stability analysis of Crude Protein (CP) contents in a core collection (1995) of *Arachis pintoi* evaluated in contrasting locations.

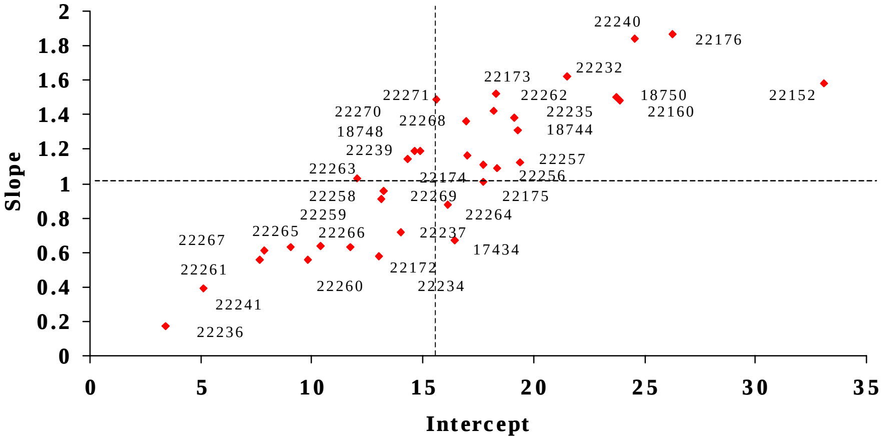


Figure 41. Results on stability analysis of number of flowers in a core collection (1995) of *Arachis pintoi* evaluated in contrasting locations.

Conclusion: Preliminary revision of data identifies accessions CIAT 18744, 18746, 18747, 18748, 18751, 18752, 22159, 22160, 22257, 22259 and 22268 as the most promising accessions across environments in terms of dry matter production and ability to cover the soil. Superior to this group of accessions in terms of dry matter productivity and soil cover are accessions 22233, 22236, 22238, 22241, all originating from one specific area in Brazil. However, these latter accessions are limited by low seed production, relying on vegetative propagation. The differences in forage quality of accessions are of lesser importance as generally all accessions have a high level. Except for the vegetative accessions the selected accessions also all have satisfactory flower and seed production. Of interest is high stability in the ranking of a number of accessions across sites, making it feasible to recommend accessions for a wide variety of environments.

3.6.2 Plant growth characteristics during the establishment phase of one Cultivar and three accessions of *Arachis pintoi* in a subhumid environment of Costa Rica

Contributors: Marco Lobo Di Palma, Beatriz Sandoval, Vidal Acuña [Ministry of Agriculture and Livestock (MAG), Costa Rica], Pedro J. Argel, Guillermo Pérez

Background. Two commercial cultivars of *A. pintoi* have been released in Costa Rica during the last years, cvv. Porvenir (CIAT 18744) and Maní Mejorador (CIAT 17434). The legume is presently utilized widely for multipurpose uses such as forage, cover crop, soil conservation plant and as ornamental. However, new promising lines have been collected more recently and need to be evaluated and characterized, particularly in relation with drought tolerance and other agronomic characteristics in order to select new forage options for different production systems and environments, particularly for sites with prolonged dry seasons.

Cultivar Porvenir is well recognized for adaptation to a wide range of environments including sites with 5 to 6 months dry, and because it is highly stoloniferous, good seeder and compatible with aggressive stoloniferous grasses. The new accessions CIAT 22160 and CIAT 22159 have been reported as promising because tolerance to dry conditions and good DM production;

meanwhile CIAT 18744 A is an off-type line -perhaps a natural hybrid- characterized by larger leaves, taller plants and reduced flowering and seed set.

With the exception of CIAT 18744 A, cv. Porvenir and the other two lines of *A. pintoi* were planted by seed at the beginning of the rains at a distance of 0.5 x 0.5 m between plants in 12 m^2 plots, replicated three times in a complete randomized plot design. Four months after planting 9 central plants from each plot were harvested and DM and other plant characteristics measured.

Results and Discussion. The off-type accession CIAT 18744A produced significantly more taller plants ($p < 0.05$) followed by CIAT 22160 (Table 71). However, plant cover percentages, DM yields, and number of primary and secondary stolons were not statistically different ($p < 0.05$) between cv. Porvenir and the other lines of *A. pintoi* during the establishment phase, although there is a clear tendency of cv. Porvenir to cover more rapidly the soil, which is associated with larger number of primary stolons/plant. This cultivar has other plant characteristics similar to accession CIAT 22160, but with a clear tendency to over-yield the accessions CIAT 22159 and CIAT 18744A with relation to length of primary and secondary stolons and number of nodes per stolons.

Table 71. Plant growth characteristics during establishment of one cultivar and three accessions of *Arachis pintoi* in a sub-humid environment of Costa Rica.

	Plant Height	Cover	DM yields		Primary stolons/plant			Secondary stolons/plant	
			Stems	Leaves	No.	Length	Nodes	No.	Length
	(cm)	(%)	(g/plant)			(cm)	(No.)		(cm)
Porvenir (18744)	5.8 a*	69.0	12.8	15.5	16.1	24.5 a	9.7 a	17.1	11.4 a
22160	8.2 a	52.0	11.1	17.9	14.2	25.4 a	9.7 a	19.2	11.0 a
22159	6.0 a	52.0	7.8	13.6	11.2	20.3 a	8.8 a	13.1	8.3 b
18744A	11.9 b	48.4	5.8	20.7	13.1	12.6 b	5.3 b	9.8	6.2 b
		(ns)	(ns)	(ns)	(ns)			(ns)	

*Within a column means followed by the same letter are not statistically significant ($P<0.05$).

It was interesting to observe that DM distribution between stems and leaves varied significantly among *A. pintoi* lines. Cultivar Porvenir produces similar proportions of leaves and stems while that CIAT 18744A concentrates DM yields of leaves. This plant produces hollow stems and this may be the reason for the low DM contribution of the stems. In Costa Rica, because the upright growth habit of this accession and the high foliage production, it is considered an adequate plant for making hay; the condition of having hollow stems may help in the process of a more efficient drying of the material.

Progress towards achieving milestones

- **Identified accessions of *Arachis* with broader adaptation than the commercial cultivar**

Data analysis and statistical analysis to investigate GxE interactions in a core collection of *Arachis pintoi* was completed. We were able to identify a range of accessions that produce sexual seed (CIAT 187478, 18748, 18751, 22160) with superior performance across environments when compare with the commercial cultivar (CIAT 17434).

Photo 27. Multilocational trial of *Arachis* spp. at Palmira

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

4.1 Develop partnerships with NARS, NGO's, IARC's, ARIS and private sector in LAC, Asia and Africa to undertake evaluation and diffusion of a range of grasses and legumes for multipurpose use

Highlights

- Strengthen on-going collaborative projects with partners and developed new partnerships with different organizations in Colombia, Costa Rica, Nicaragua, Honduras, Haiti and Canada to undertake strategic research and on-farm evaluation of forages for different uses
- Through on-farm trials demonstrated the biological and economical benefits of *Centrosema macrocarpum* a s a protein supplement for milking cows in smallholder farms in the forest margins of Peru
- Confirmed in the field the high biomass yield and antibiotic resistance to spittlebug of the *Brachiaria* hybrid CIAT 36062, but found that it was susceptible to waterlogged conditions in the forest margins of Colombia
- Found through collaborative work with the U. of Montreal, Canada a large variation in L-dopa in Mucuna germplasm, which has implications for therapeutic medicine
- A *Brachiaria* accession (CIAT 26110) and a hybrid (CIAT 36061) were released in Costa Rica and Brazil and Mexico, respectively

4.1.1 On going collaboration with NARS, NGO's, IARC's and ARIS

1. Use of forages for the recuperation of degraded areas in Hillsides of Colombia

Contributors: FIDAR (NGO lead partner), PE-4, PE-2, UMATA, Comite Cafeteros, REVERDECER, CVC, Alcaldia de Restrepo, Universidad de Valle, Comite de Cafeteros, University of Hohenheim

Background: The study zone located in the north of the 'Valle de Cauca' was characterized by a high natural diversity and richness of natural resources. However inappropriate land use has led to degradation of the natural resource base, threatening social, economic and environmental sustainability of the region. The deterioration of natural resources is leading to loss of faunal and floral biodiversity, lack of vegetative cover and resulting high erosion, and reduced yields. Communities at the lower end of the watershed and beyond face an increased risk of natural disasters; companies utilizing water for electricity and for human consumption at the downstream of the water lines have increased costs in maintenance of plants due to increased sediments as a result of erosion, increasing electricity costs and posing at risk the availability of water of high quality. Hence there are multiple effects on well -being and environmental quality as a result of the environmental degradation.

In the past the recuperation of such fragile areas was addressed by isolated activities by public and private institutions, often without incorporating the communities themselves and without long-term follow up. Often the costs of the suggested solutions were high, reducing the possibility for wider application and maintenance by the community.

The present collaborative project aims to develop a concerted effort, including the different actors in the region and most importantly to include the community. In the project, the Fundación para la Investigación y el Desarrollo Agrícola (FIDAR), the University of Hohenheim and CIAT try to offer sustainable alternatives based on multipurpose grasses and legumes (herbaceous, shrubs, trees), and on development of an evaluation system that incorporates the community.
The expected outputs of the project are:

- Development methods for participatory planning, monitoring and evaluation for recuperating and stabilizing fragile soils
- Stabilization of degraded zones through vegetative covers an mechanical barriers
- Economic and agronomic evaluation of different vegetative covers and mechanical options for recuperation and conservation of degraded lands, with focus on cost-effectiveness
- Farmer groups with the means and tools to continue recuperation and conservation of soils for wider application
- Develop and validate DST for the recuperation and conservation of soils, with focus on the adaptation of existing tools

Major activities and summary of results

In the first 6 months of the project the following activities were carried out:

Methods for planning, monitoring and participatory evaluation for soil stabilization and recuperation

a) Identification and prioritization of local indicators for soil stability and soil quality: Several meetings and workshops were held with farmers and local indicators identified with the following characteristics: easy and practical for field use, economical and easy to interpret, and should integrate physical, chemical and biological soil properties. The local soil indicators will be complemented with standard methods
b) Participatory mapping of natural resources in the micro watershed: Key informants have been identified, the activity is underway
c) Simulation models: The SWAT (Soil and Water Assessment Tool) model is being utilized. At the moment information is compiled and the model calibrated for use in the Aguamona watershed. Alternative Spatial analysis methods are evaluated.
d) Identification and planning of problem solving with the community: In meetings with the producers the principal problems and needs were identifies. Subsequently management alternatives to reclaim degraded areas such as cover crops and mechanical barriers were presented and adapted to the specific site conditions with farmers. 2 demonstrative plots – each 1 ha – as cover were established.
e) Plan for Monitoring and Evaluation elaborated: With farmer groups and technicians, evaluation parameters were identifies according to the interest of each of the parties. As basis the indicators mentioned in a) were used. A committee was formed, with one farmer per ‘Vereda’ participating, a UMATA technician, a REVERDESER member and a FIDAR technician selected as representatives. This committee meets every 3 months to take decisions for planning and evaluation of activities

Cover crops and barriers to stabilize degraded areas

For the establishment of the cover crops, chicken manure, mycorrhizas and mineral fertilizers were utilized. Cover crops were established on 2 ha. These were complemented with barriers such as Guadua and Vetiver, Axonopus and Citronella in the severely eroded areas. Of these two hectares one is severely, one moderately eroded. Cover crops – grasses and legumes – are established with farmers. Selected material will be multiplied

Farmer Organizations, farmers and technicians strengthened, by action plans for the stabilization degraded soils

Information meetings were held and field visits with farmer groups and organizations involved in the project were organized. Well-conserved areas of the watershed were visited, as were areas with severe degradation. A visit to the Cauca hillside was also organized to demonstrate experiences with soil conservation and recuperation in areas, which were affected by degradation due to intensive short-cycle crops. The formation of a CIAL (local research committee) was initiated.

2. On farm evaluation of forages for milk production in forest margins of Peru

Contributors: F. Holmann (CIAT), Daisy Lara (FUNDAAM, Peru), K. Reategui (DEPAM, Peru) and Dante Wong (CTAR, Peru)

This section is divided in two parts: (1) The effect of using *Centrosema macrocarpum* on milk yields in the Alto Mayo region of the Peruvian Amazon, and (2) Effect on milk yield of the association of *Brachiaria brizantha* with *Arachis pintoi* in the Alto Mayo region of the Peruvian Amazon.

A) The effect of using *Centrosema macrocarpum* as a supplement to dairy cows in the Alto Mayo region of the Peruvian Amazon.

The Alto Mayo region covers 630,735 ha of which 346,452 ha are suitable for grasslands but only 60.2% are in use (INEI,1994). A survey carried out in March of this year indicated that the Alto Mayo region has potential for livestock activities. Additional surveys directed toward cattle owners indicated that *Brachiaria decumbens* pastures are predominately used for maintaining livestock. The area planted to *B. brizantha* and to cut-and-carry grasses has also increased, and *Centrosema* is being evaluated in protein banks. The survey also indicated that more than 50% of the interviewees plan to improve their pastures and that, among their priorities for improving the system is the improvement of the forage base, stables, and breeding herd. *Centrosema macrocarpum* is being adopted by livestock producers as a cut-and-carry forage that reduces or eliminates the need to purchase agroindustrial supplements.

The objectives of this experiment were to (1) Measure the increase in milk production and the weight gain in calves attributed to the use of *Centrosema macrocarpum* to feed dairy cows; and (2) Compare the farmers' traditional system vs a system based on *Centrosema* in terms of economic merit.

Materials and Methods: Two 1-ha plots were planted on two farms located in Nueva Cajamarca and Soritor during September 1999. Planting density of *Centrosema* in both plots was high, with 0.40 m x 0.40 m between plants and 8 kg seed/ha. The evaluation was conducted in a completely randomized design with 3 treatments and 7 cows/treatment, over a 10-week period. Treatments were as follows: T1 = Cut-and-carry grass (king grass) restricted to milking; T2 = 50% king grass; and 50% of *Centrosema* restricted to milking; and T3 = 100% *Centrosema* restricted to milking.

Milking was done manually, once a day with calf on foot. After milking, calves were allowed to suckle for 5 minutes and then transferred to pens. Crossbreed dairy Gyr x Holstein cows were used and left to graze on the pasture 6 hours/day, after which they were taken to a pen where they received chopped king grass. For milking, cows were distributed according to treatments, beginning with the control treatment and followed by the 2 experimental treatments.

Control variables were: (1) Consumption of supplementary forage (kg DM/cow/d); (2) Milk production (kg/cow/d); and (3) Costs of producing supplementary feed. The following evaluations were carried out to calculate total volume of forage/ha/year (*C. macrocarpum* and king grass):

a. Forage availability (kg DM/ha). Random sampling was carried out at the beginning of each grazing cycle. A composite sample was taken to determine % DM.
b. Time of pasture recovery. In an area recently submitted to cutting, 1 m^2 was marked and visual evaluations of coverage conducted at 20, 45, and 60 days. Total recovery of the area was observed.
c. Biological-economic evaluation. Economic merit was evaluated using the following formula:

$$\text{E.M.} = \frac{\text{Cost of feeding/cow/period}}{\text{Milk production (kg)}}$$

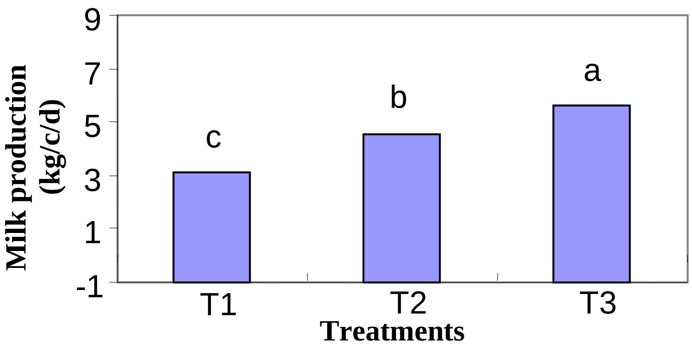
Results

Intake of forage based supplements. Average forage intake was 0.87, 0.96, and 0.90 kg DM/cow/d for T1, T2, and T3 respectivel.y. This amount includes the forage consumed at milking, the additional forage consumed during grazing (*Brachiaria*), king grass supplied in closed pens during the day and in the afternoon. A trend to consume more grass + legume mixture was observed so it can be inferred that the inclusion of the legume increases consumption by 23% in T2 and 11% in T3.

Milk production. Average milk production per treatment is summarized in Figure 42. Milk production was higher in cows supplemented exclusively with *Centrosema* (6.6 kg./c/d) as compared with the group supplemented with *Centrosema* + king grass (5.6 kg./c/d). Milk production was lowest in cows that only received king grass (4.1 kg/c/d). When production gains were calculated in terms of efficiency, feeding *Centrosema* increased production by 10% and 20% when fed in combination with king grass and when fed alone, respectively. When cows were grouped according to number of parturitions, there were no significant differences in milk production between cows with 2 and 3 parturitions (4.7 kg/c/d) and cows with 4 and 5 parturitions (4.4 kg/c/d). However, significant differences were observed, between the former 2 groups and first-calf cows (3..0 kg/c/d).

Economic merit. The cost per kilogram of dry matter for each alternative was taken into account, which included the depreciation in a six-year period, maintenance costs, and harvest costs. For the case of *Centrosema*, an establishment cost of $380/ha was used. In addition, $40/ha of annual maintenance costs and a harvest cost equivalent to 0.6 man-day per each 50 kg of fresh biomass daily was used. Annual productivity was estimated in 15 tons of DM during 5 harvests/yr. Thus, total cost per kilogram of dry matter was estimated at US$ 0.105. For the case of king grass, estimated establishment cost was $295/ha with annual maintenance costs of $110/ha and harvest costs equivalent to 1.4 man-day for each 100 kg of fresh, daily-cut biomass.

Annual productivity was estimated at 25 tons of DM during 4 harvests. Therefore, total cost per kilogram of dry matter was about $0.184. The economic merit for each feeding alternative is shown in Table 72.



T1= 100% cut-and-carry grass; T2 = 50% cut-and-carry grass;
T3 = 50% *Centrosema*; T4 = 100% *Centrosema*

Figure 42. Milk production with cows consuming different forage-based supplements in Moyobamba, Peru

Table 72. Economic merit of different feeding alternatives in Moyobamba, Peru.

Milk production/treatment	Economic merit
T1 Milk production = 4.1	0.037
T2 Milk production = 5.6	0.035
T3 Milk production = 6.6	0.027

Although milk production improved in T2 (50% king grass + 50% *Centrosema*) as compared with T1 (only king grass), the economic merit of this treatment did not improve as result of the high intake and thus cost recorded when *Centrosema* was included in the supplement. On the other hand, the economic index improved relative to the other supplementation treatments when only *Centrosema* was fed at milking. In general, results indicate that supplementation with *Centrosema* had a positive biological and economical impact on milk production when offered at milking to crossbred Gyr x Holstein cows adapted to prevailing conditions of the Alto Mayo region of Peru.

B) Effect on milk yield of the association of *Brachiaria brizantha* with *Arachis pintoi* in the Alto Mayo region of the Peruvian Amazon.

The inclusion of legumes grass alone pastures such as *Brachiaria decumbens* and *Brachiaria brizantha*, predominant species in the Alto Mayo region, is considered an alternative to increase the biomass productivity and milk production . Therefore, the objective of this study was to evaluate the effect of the forage peanut *Arachis pintoi* in association with *Brachiaria* pastures in terms of milk production.

Materials and Methods: The treatments evaluated were: T1: *Brachiaria* spp. in monoculture; and T2: *Arachis pintoi* associated with *Brachiaria* spp. Plots of the associated pasture, 4 ha in size, were established on one farm from a small farmer in Soritor. Initial availability of forage, milk production and establishment costs were recorded. Crossbred Gyr x Holstein cows were used in this evaluation. These same animals grazed lots of *Brachiaria* spp. (in monoculture) and associated pastures of *A. pintoi/Brachiaria* spp.

The control area (grass alone) had 4 ha of *B. brizantha*, similar to the grass/legumepasture, and was divided into 2 paddocks each. Animals grazed, on average, 7 days in every paddock. Milk production was measured in each paddock as of day 3. The evaluation was carried out during maximum precipitation . Forage availability and botanical composition of pasture were measured with random samplings in each paddock at the beginning of the grazing cycle.

Results: During the experimental period the producer controlled the resting period, the grazing period, number of animals grazing, and paddock area. The stocking rate of each pasture was calculated on the basis of these indicators. A stocking rate of 2.07 AU/ha was determined on pastures of *Brachiaria* spp. and 4.13 AU/ha in the associated *A. pintoi/Brachiaria* spp pasture. Measurements in the vegetation, showed that DM in the associated pasture (*A. pintoi/Brachiaria* spp) was 7,600 kg/ha at the beginning of the grazing period, with legumes accounting for 6% and weeds for 11% of the forage on offer. The grass alone pasture (*Brachiaria* spp) produced 6,300kg DM/ha out of which 9% were weeds. Results on milk production (Figure 43) indicated that cows grazing the *A. pintoi/Brachiaria* spp. pastures yielded 6 kg/cow/day, while those on *Brachiaria* spp. pasture yielded 5 kg/cow/day, wich represents a 20% difference in daily milk yield per cow.

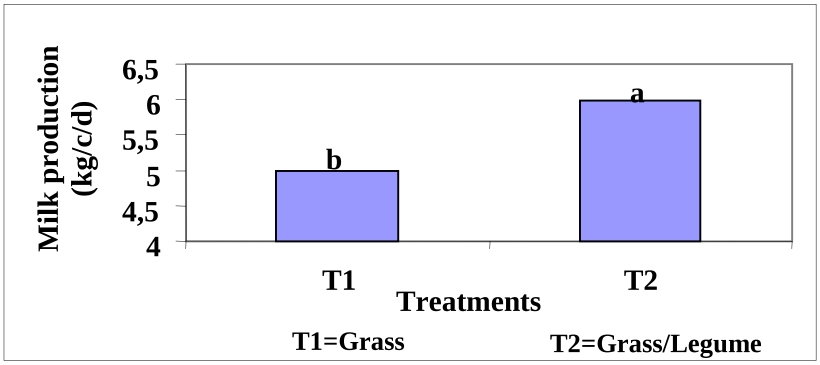


Figure 43. Milk production of cows grazing pastures of *Brachiaria* spp. alone and associated with *A. pintoi* (Moyobamba, Peru).

In general, the expectations of small producer in Moyobamba, Peru are high with the use *Arachis*-based pasture, given that the stocking rate of the associated pasture doubled that of the grass alone pastures and milk production increased by 20%. Thus, the potential impact of *Arachis* on livestock productivity in this region of the Peruvian Amazon can be significant.

3. Evaluation under grazing of new *Brachiaria* accessions and hybrids in forest margins of Colombia

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Background: The piedmont of Caqueta (forest margin) is an ideal site to evaluate *Brachiaria* genotypes developed by CIAT for resistance to spittlebug and for adaptation to poorly drained soils. Thus in collaboration with the U of Amazonia we established last year in the "La Rueda" farm a grazing trial to evaluate performance of selected accessions of *B. humidicola* (CIAT 16180 and 16888) and *Brachiaria* hybrid (CIAT 36062) with and without *Arachis pintoi* (CIAT 22233 + 18744)

The two *Brachiaria* accessions were selected for evaluation under grazing due to high biomass production whereas the hybrid was selected because of high antibiotic resistance to spittlebug. The materials were established in the first quarter of 1999 and measurements in the vegetation and on animal performance began in August and November of the same year, respectively.

Results and Discussion. After 10 months of grazing we have recorded differences among pastures on grass and legume on offer, reaction to heavy spittlebug attacks, tolerance to poorly drained conditions and animal live weight gain. The amount of forage on offer has consistently been greater with the *Brachiaria* hybrid than with the two accessions of *B. humidicola* regardless of presence or absence of legume (Table 73). In addition, it was interesting to observe that *A. pintoi* CIAT 22233, which does not produce seeds, has outperformed the accession CIAT 18744 (produces seed) when in association with grasses.

During the peak of the rainy season (May-July) there was a heavy attack of spittlebug and damage was recorded in pastures with the two *B. humidicola* accessions but not in the *Brachiaria* hybrid.

Table 73. Grass and legume on offer in *Brachiaria* pastures with and without legumes in forest margins (Caqueta, Colombia)

Pastures	Grass on Offer* (kg DM/ ha)	Legume on Offer* (kg /DM/ ha)
B. humidicola CIAT 16180	1088	
B. humidicola CIAT 16888	2587	
Brachiaria hybrid CIAT 36062	5759	
Brachiaria humidicola CIAT 16180 + *Arachis*	1507	264
Brachiaria humidicola CIAT 16888 + *Arachis*	1606	596
Brachiaria hybrid 36062 + *Arachis*	2182	520

* Sampling in August, 2000 (10 months under grazing)

Results on animal performance shown in Table 74 indicate that LWG recorded so far have been greater in the *Brachiaria* hybrid + *Arachis* pasture and that the relatively low animal performance in the hybrid without legume is most likely the result of poor grass quality associated with damage caused by water logging (Photo 28). Thus it would seem that animals compensated the poor quality of the grass affected by poor drainage by consuming more of the high quality legume.

Table 74. Liveweight gain of steers grazing *Brachiaria* pastures with and without legumes in forest margins (Caqueta, Colombia.

Pastures	LWG (g/a/day)*
B. humidicola CIAT 16180	322
B. humidicola CIAT 16888	492
Brachiaria hybrid CIAT 36062	307
Brachiaria humidicola CIAT 16180 + *Arachis*	328
Brachiaria humidicola CIAT 16888 + *Arachis*	366
Brachiaria hybrid 36062 + Arachis	524

*270 days of grazing

Photo 28. Pasture of *Brachiaria* Hybrid CIAT 36062 in forest margins of Caqueta; Recently established grass (left); Grass damaged by poor soil drainage (center: initial damage; right: advanced damage)

The effect of legume on LWG is still not evident on the *B. humidicola* pastures as it is in the Hybrid, but we expect this to change over time as the quality of the grass increases in the pastures with *Arachis.*

These results confirm the high antibiotic resistance of the *Brachiaria* hybrid CIAT 36062 as shown in glasshouse and field screenings carried by the Entomology Group (See IP5 AR 98 and 99). Unfortunately it does not produce seed and has shown to be very susceptible to the poorly drained soil of the site (Meson) where it was established (Photo 28), but with good recovery once the rainfall is less. The *Brachiaria* hybrid has caught the attention of producers visiting the grazing experiment and vegetative seed has been distributed through Nestle with instructions that it should be established in well -drained soils. In addition, next year vegetative material of the hybrid will be sent to the Llanos piedmont for evaluation under good soil drainage conditions.

4. On-farm evaluation of a pasture of *Brachiaria* spp. alone and in association with *Leucaena leucocephala* and its effect on milk production of dual purpose cows in a subhumid environment of Costa Rica

Contributors: Marco Lobo Di Palma, Beatriz Sandoval, Vidal Acuña (MAG), Pedro J. Argel, Guillermo Pérez

Background. There is very little used of forage trees in the tropics of Latin America, despite the fact that a great deal of research has been conducted on species selection and animal utilization. This paradox is even more conspicuous if we have in main that the referred tropics are the center of origin of very important multipurpose trees such us *Leucaena, Gliricidia* and *Calliandra* that play important roles in different production systems elsewhere, including grazing systems.

Part of the limited use of forage trees is due to farmer lack of knowledge on the importance and management of the trees. Tropileche is a project aimed at the improvement of animal feed supplies with emphasis on forage legumes with farmer participation, which has allowed the selection and utilization of forage trees and shrubs such as *Leucaena leucocephala* and *Cratylia argentea* in dual-purpose farms located in subhumid environments of Costa Rica.

In one farm located in Miramar, *L. leucocephala* CIAT 17263 was planted by seed in double rows separated 0.7 m between them and 0.5 m between plants to form strips that were in turn separated 5 m apart, to give a plant density of 1840 Leucaena trees/ha. Once the trees were established the grass *B. decumbens* (Basilisk) was planted to form an associated paddock of 4.0 ha.

Measurement of milk production of dual-purpose cows is underway in the associated paddock and it is being compared with a nearby non-associated paddock of *B. decumbens/brizantha.* Sixteen cows are being used for the evaluation using a Latin square crossover design under a grazing system of 10 days grazing and 35 days rest, to give an estimated stocking rate of 4.3 AU/ha. Changes in the soil will also be monitored in this experiment.

Results and Discussion. A first cycle of controlled grazing indicated that cows produced significantly more milk per day ($p<0.05$) in the associated grass (6.4 kg milk/cow/day) compared to the pure stand (5.6 kg milk/cow/day). Forage availability indicates 1165 kg DM/ha for the *L. leucocephala.* Cows consumed around 8.8 kg of green foliage/day of *Leucaena* in an initial grazing; the trees were browsed up to a mean height of 1.5 m, above which there was little grazing.

This experiment will be evaluated for another growing season and final results will be presented later.

5. Participatory evaluation of forages for multipurpose use in Haiti

Contributors: Levael Eugene (CIAT), Elliassaint Magloire (ORE), Joseph Andrefoine (ORE), P. Argel (CIAT), M Peters (CIAT) and C. Lascano (CIAT)

Background: This year we initiated a collaborative project in Haiti through the HGRP (Hurricane George Recovery Program), which is a program financed by USAID and administrated by the Pan American Development Foundation (PDF), an ONG based in USA, and which in turn has subcontracted with other ONGs based in Haiti, such as ORE (Organization for Rehabilitation of the Environment) and with CIAT for the recovery of the country after the devastation caused by hurricane George in 1998.

Haiti is a country with 27,500 km^2 and holds an estimated population of 8 million people, of which around 67 % live in the countryside, but with a high rate of rural migration to the cities; thus the population density is approximately of 275 persons per km^{2,} one of the highest in the world. The country is subject to tropical climates with a predominant bimodal pattern distribution of rains (rains normally from April to June and from August to November); temperatures range from a daily mean of 22ºC in January to a maximum 35ºC during the dry period. It is considered that around 80% of the country is on hillsides; the soils are mainly of calcareous origin, shallow depth and highly eroded. The pressure for crop land is high with an estimated land tenure between 0.5 to 1.0 hectare per family; thus agriculture and livestock is basically a subsistence activity in Haiti, based on annual and perennial crops (rice, sorghum, vegetables, tobacco, roots, coffee and banana), and on livestock (mainly bovines and goats).

Farmers combine crop activities with cattle raising in the same areas, but a high percentage own more than 3 to 5 either cows or goats. The common practice is to feed the animals by direct grazing in small paddocks, to tether them along roadsides or to use annual crop residue, usually of very poor quality. For this reason animal productivity is very low and insufficient to satisfy the national demand of milk and beef (it is estimated that the country spends annually around USD 10 millions each on imported beef and milk).

The low efficiency of livestock production in Haiti is mainly due to poor genetic potential of animals and inadequate/insufficient feed resources. Given that most of Haiti's land is degraded hillsides, there is recognition that forest, pastures and other perennial plant cover (i.e. coffee, cacao) should be emphasized as major land use options. Of the available perennial plant intervention options, pastures/livestock offer the most advantageous in terms of short term and probably mid-term returns to farmers and NR improvement.

Objective and expected outputs: The short-term objective of CIAT’s work on forages in Haiti is to select with farmer participation grasses and legumes well adapted to soils and climate of target hillsides and to determine seed production potential of selected species.

Thus the expected Outputs from the forage work in Haiti during the next 15 months are:

1. Selected grasses (prostrate for cover and erect for cut and carry) and legumes (herbaceous for cover and green manure and shrubs for cut and carry) based on initial assessment of environmental adaptation, seed production potential and farmer preference.
2. Trained Professional from ORE on Forage Agronomy and Seed Multiplication.
3. Defined strategy for scaling- up forage work in Haiti (i.e. choice of species for local seed multiplication, on-farm trials and diffusion mechanisms).

Activities: To accomplish these outputs we carried out this year the following activities:

1. Reviewed available rainfall and soils data in target areas and previous experiences with introduction of grasses and legumes (we now have some data and more will be sent to us)
2. Reviewed CIAT Forage database and selected grass and legume species that could be adapted to target areas
3. Delivered seed to Haiti through ORE for planting in August-October 2000 to establish selected grasses and legumes species (Table 75) in small plots in replicated trials in three sites (Camp Perrin, Debron and) to assess seed multiplication potential and farmer preference
4. Prepared research protocols to ORE’s staff for the evaluation of forage germplasm for multipurpose use (feed resource and covers**)**
5. Provided short-term training to a staff from ORE on Forage Agronomy in CIAT- Palmira and Costa Rica

Three contrasting sites were chosen for the evaluation activity: Marigot (south-east of the country), and Camp Perrin and Derón (south of the country). In Camp Perrin (flat areas) the rainfall is bimodal with a total of 2114 mm (mean of the last 6 years); soils are of calcareous origin and the predominant crops are maize, sorghum, tobacco and vegetables. The other two sites are located in hillsides with different slopes. The experiment located in Camp Perrin was established in August with the participation of Agronomist from ORE and CIAT. ORE personnel established the trial in Debron in September. Due to excessive rainfall following planting, the germination of small seed grasses was poor in some case and additional seed was sent for replanting this year and to complete the quantity necessary to establish the third trial in Marigot. We will follow-up the establishment of the different forage species in Haiti and provide backup on the evaluation with farmer participation that should take place in the first semester of next year.

Table 75. Multipurpose grass and legume species being tested for environmental adaptation and farmer preference in Haiti.

Grasses	Herbaceous Legumes	Shrub Legumes
B. brizantha CIAT 26110	*A. pintoi* CIAT 22160	*C. calothyrsus* CIAT 22310
B. decumbens CIAT 606	*A. pintoi* CIAT18744	*C. calothyrsus* CIAT 22316
B. dictyoneura CIAT 6133	*C. pubes*cens CIAT 15160	*C. argentea* CIAT 18516/18668
B. humidicola CIAT 26427	*C. macrocarpum* CIAT 25522	*C. ensiformis* CIAT 715
B. humidicola CIAT 679	*C. ternatea* cv Tejuana	*F. macrophylla* CIAT 17403
P. maximum CIAT 16028	*D. ovalifolium* CIAT 33058	*L. leucocephala* CIAT 17263
P. maximum CIAT 16031	*N. wightii* CIAT 204	*L. macrophylla* subsp. nelosnii OFI 47/85
P. maximum CIAT 16051	*M. pruriens* CIAT 9349	
	S. guianensis CIAT 11844	
	P. phaseoloides CIAT 7182	
	V. unguiculata cv Verde Brasil	
Total: 8	**10**	**8**

6. Evaluation of legumes for cover crops in plantations in the llanos

Contributors: M. Peters (CIAT), C. Plazas (CIAT) and Oil Palm and Rubber Growers of the Colombian Llanos

Background: There is a need in the plantation industry of the Llanos of Colombia to find sustainable ways to reduce weed infestation, to maintain and improve soil fertility, to control erosion and increase the micro fauna biomass. There is currently a trend to promote plantation systems in the Llanos. In the rubber plantation the target group for this promotion are small to medium size farmers who want to diversify there farming operations. In the oil palm plantations plots of up to 5 ha are rented out to landless farmers to manage the oil palms for the oil palm industry. Both trials therefore are seen to have beneficial effect on the welfare of resource poor farmers.

In 1999 a range of legume accessions of the species *Arachis pintoi*, *Desmodium heterocarpon* subsp. *ovalifolium* and *Pueraria phaseoloides* have been sown under shade and no-shade conditions in the Meta department of Colombia. Based on results in 2000 this work was amplified by studying different establishment procedures for the most promising accession *Desmodium heterocarpon* subsp. *ovalifolium* CIAT 13651, again in comparison with the standard management of the standard cover *Pueraria phaseoloides* (Photo 29).

Methods: About 80 m^2 plots of legumes were established in commercial young and old rubber and oil palm plantations in the Altillanura and Piedemonte areas of the Llanos. A Randomized Block Design with three replication is utilized. Agronomic parameters and insect incidences are mediated. The following treatments were sown: *Arachis pintoi*: 17434, 18744, 18748, 22159, 22160 (seed rate 10 kg/ha); *Desmodium heterocarpon* subsp. *ovalifolium*: 350, 13105, 13110, 13651, 23762 (0.5 kg/ha); *Pueraria phaseoloides*: 8042, 9900 (3 kg/ha). Additionally a mixture of *Arachis pintoi* CIAT 18744 and *Desmodium ovalifolium* CIAT 13651 was sown.

Photo 29. Plantations of *Desmodium heterocarpon* subsp. *ovalifolium* CIAT 13651 cover crops including rubber

Results and Discussion: During the first 4 months of establishment the cover of legumes was slow, with the exception of *Pueraria phaseoloides* (Table 76).

Table 76. Soil cover of different forage legumes under shade conditions in plantations at two sites in the Llanos de Colombia.

Treatments	**Rubber**		**Oil Palm**	
	Young plantation	Old plantation	Young plantation	Old plantation
		%		
Desmodium heterocarpon subsp. *ovalifolium* 13105	58	25	55	.
Desmodium heterocarpon subsp. *ovalifolium* 13110	68	7	53	.
Desmodium heterocarpon subsp. *ovalifolium* 13651	72	28	72	.
Desmodium heterocarpon subsp. *ovalifolium* 23762	67	17	58	.
Desmodium heterocarpon subsp. *ovalifolium* 350	63	13	62	.
Arachis pintoi 17434	25	3	47	.
Arachis pintoi 18744	33	7	65	.
Arachis pintoi 18748	32	6	53	.
Arachis pintoi 22159	18	8	60	.
Arachis pintoi 22160	28	7	80	.
Pueraria phaseoloides 8042	33	17	65	.
Pueraria phaseloides 9900	55	5	75	.
Association Ap/Dh	67	20	77	.

However, 6 months after planting, the legumes were well established, with higher covers achieved in the young plantations of oil palm than rubber, which was particularly true for *Desmodium*, which performed better than *Arachis.*

Due to shading effects, legume cover was low in shaded areas of rubber plantations – i.e. old trees or directly beneath the trees while it was impossible to establish legumes under existing oil palms.

In the production phase high weed populations of more than 30 % were measured in all treatments in oil palms. Highest legume yields and percentages were achieved with *Dh* CIAT 13651, 13105, *Ap* CIAT 22160, the mixture of *Desmodium* and *Arachis* and *Pp* CIAT 9900, 8042 (Table 77). Under rubber the *Desmodium* accessions and mixture performed best, with more than 70% and 28% cover for CIAT 13651 in young and old plantations, respectively.

Initial results indicate the potential of *Desmodium* and *Arachis* as a complement to *Pueraria* as cover crops for newly established rubber and oil palm trees. In older plantations, legume cover was much lower, but weed pressure was also low.

The trials to evaluate legume covers in plantation in the llanos is an on-going activity, but from the results so far it would seem that *Desmodium heterocarpon* subsp. *ovalifolium* CIAT 13651 is an attractive cover option, especially considering lower seed costs (much lower planting densities needed than for *Pueraria phaseoloides*).

Our hypothesis is that the advantage of *Desmodium* as a cover will further increase over time, given that it establishes extremely slow, but once established is very persistent and productive. Thus *Desmodium* could be an excellent cover until plantations are fully established and weeds are suppressed by shade. It will be also interesting to observe how the legume covers being evaluated develop under older rubber trees and how established legumes persist under higher shading conditions.

Table 77. DM yield in kg/ha of different cover legumes and weeds in rubber and oil palm plantations at two sites in the Llanos de Colombia.

Treatment	**Rubber**						**Oil Palm**	
	Young Plantation				Old Plantation		Young Plantation	
	Open (light)		Shade					
	Legume	Weed	Legume	Weed	Legume	Weed	Legume	Weed
				kg/ha				
Dh 13105	608	769	927	0	267	289	1453	544
Dh 13110	1180	69	1187	52	57	0	980	833
Dh 13651	1064	0	537	88	319	52	1575	1003
Dh 23762	896	67	440	20	586	0	1237	623
Dh 350	1690	163	1104	0	425	0	1357	741
Ap 17434	361	315	360	109	40	93	247	937
Ap 18744	671	164	577	75	167	0	895	637
Ap 18748	537	155	669	21	396	0	727	1051
Ap 22159	396	161	725	68	231	60	1314	817
Ap 22160	791	181	637	0	229	48	1488	983
Pp 8042	824	236	739	179	309	61	1490	619
Pp 9900	675	103	323	28	116	17	1745	836
Aso*Ap/Dh*	156	61	255	21	67	0	408	581
Aso*Dh/Ap*	435	61	499	21	225	0	967	581
Control		393		283		180		1531

7. On-farm evaluation of new grass and legume options for livestock production in the Llanos of Colombia

Contributors: C. Plazas, A. Rincón (CORPOICA), J. W. Miles, C. E. Lascano

Background: One major limitation for beef and milk production in Neotropical savannas is degradation of introduced grasses, as a result of nitrogen deficiencies and overgrazing. Thus CIAT's Forage Project (IP-5) has been developing improved grasses and legumes that can contribute to reclaim large areas of degraded pastures in tropical regions where livestock is a major land use system.

In collaboration with PE-5, and CORPOICA we initiated in 1998 evaluation of new grasses and legumes in representative farms of the llanos of Colombia. A total of four farms (two in the well-drained savannas and two in the piedmont) were initially selected to evaluate new grass and legume alternatives .Selected farms were representative of the two sub-ecosystems and have large areas of degraded pastures. In addition, farmers participating in the Project indicated their willingness to cover some of the cost of the work done in their farms.

Results and Discussion: The introduction of *Arachis* in degraded pastures in well- drained savanna sites was not successful, regardless of ecotype used or planting density. Even though the establishment of the legume was adequate, soon after the initiation of grazing the proportion in the vegetation dropped significantly as results of competition with the grass. We now feel that the use of *Arachis* to reclaim degraded pastures in well drained sites in the llanos will require high use of management and fertilizer inputs an, but it is unlikely that farmers would be willing to pay the extra cost. The alternative is the use of *Desmodium heterocarpon* subs *ovalifolium*, which is better adapted to acid-low fertility soils. In farms were *Arachis* failed we have successfully introduced *Desmodium* and are currently monitoring the vegetation.

On the other hand, the introduction of *Arachis* to reclaim degraded *Brachiaria* pastures in the piedmont of the llanos has been successful. Results shown in Table 78 indicate an adequate legume content in the pasture, which has been associated with high LWG. In one farm located in a well- drained savanna site with acid -low fertility soils we introduced two new *B. brizantha* accessions (CIAT 26110 and 26318). The performance of the two accessions during the dry season was not good (1300 to 1770 kg DM/ha) but recovery during the wet season was excellent, particularly with CIAT 26110 (7000 kg DM/ha). However, one limitation of B. brizantha CIAT 26110 has been low concentration of crude protein in the dry season (2.6 to 3.5%), which is consistent with results obtained in the Quilichao research station.

Table 78. Botanical composition and liveweight gain of steers grazing pastures reclaimed with *Arachis pintoi* in farms of the piedmont in the llanos.

Farm (Pasture)	Botanical Composition* (%)			LWG** (g/a/d)
	Grass	Legume	Weeds	
1 (*B. humidicola*)	72	18	10	566
2 (*B. decumbens*)	50	31	19	NA

*16 months after establishment

**12 months of grazing

Thus from the initial results on evaluation of new *B. brizantha* accessions, it would seem that CIAT 226110 (cv Toledo) is marginally adapted to the very acid-low fertility soils of the llanos, which would not be the case for the piedmont with better soils. We will continue to monitor these pastures for at least one more year before making any recommendations on the use of *B. brizantha* cv Toledo in the llanos.

8. Study on the distribution of secondary compounds and assessment of the agronomic potentials of species of the genus *Mucuna* (Fabaceae-Papilionoidae) in Central America

Collaborators: Luc St Laurent (U of Montreal, Canada), M Peters, C. E. Lascano

Background: The Leguminosae (Fabaceae) constitute one of the most important higher plant families with more than 650 genera and 18000 species. Many of those species, such as the common pea (*Pisum sativum*), the beans (*Phaseolus* spp.) and the soybean (Glycine max), are widely cultivated and give this family a considerable economic importance. Many other species, less known in temperate countries, are nonetheless important in tropical agricultural practices. For instance, the cowpea (Vigna spp.) is an important staple food in many tropical countries. Legumes have also the property of fixing atmospheric nitrogen, acting in fact as green manure.

Among the lesser-known species of legumes, the genus *Mucuna* is being studied more and more. Those are fast-growing species used traditionally to fertilize soils, as cover crops or as fodder. Particularly, some species are used in West Africa and India for human consumption and in Central America as a cover crop in rotation with maize. The potential economic advantages of *Mucuna* are several given that there are species known to produce L-dopa (L-3, 4-dihydroxyphenylalanine), a chemical found mainly in the seeds, which is used in pharmacology to treat Parkinson's disease. However, this compound, as well as others found in *Mucuna*, can cause severe health problems when ingested.

Many international research centers, focusing on research and the implementation of *Mucuna* in agricultural practices in developing countries, are beginning to work actively on the subject. IITA, CIMMYT and CIAT are among the leaders in the field.

A problem that arises when working with *Mucuna* is that the exact number of species and varieties is poorly known, particularly among the cultivated species. Moreover, the distribution of secondary compounds among the species of *Mucuna* is unknown. Thus an in-depth systematic study of the genus would allow for a better understanding of the diversity within the genus and to use the species and varieties corresponding to the requirements of farmers in developing countries. For instance, low L-dopa varieties could be cultivated for human or animal consumption while on the opposite, high L-dopa varieties could prove interesting for pharmacological uses.

We are collaborating on a project involving the systematics of the whole genus *Mucuna* from SE Asia, Africa and tropical America, that should facilitate a new classification of the species based on molecular, morphological and chemical markers.

The project described here aims at reaching three objectives:

1. Survey the diversity of wild and cultivated species of Mucuna in Honduras, Nicaragua and Costa Rica.
2. Preliminary quantify L-dopa concentrations in each species by HPLC

Activities, results and discussion

Collection: Collection took place in Central America (Honduras, Nicaragua and Costa Rica) from April 3 to May 25 2000. Several countries were visited in Central America and a total of 27 specimens belonging to the genus *Mucuna*, representing five species, were collected (Table 79). Given the fact that *Mucuna pruriens* var. *utilis* was obtained from CIAT, a total of six taxa out of eight present in the area were available for the studies.

Table 79. Origin and number of specimens of *Mucuna* collected in Central America and Colombia

Countries	Number of specimens of *Mucuna*	Collected species
Honduras	10	*M. Pruriens* *M. Sloanei*
Nicaragua	4	*M. pruriens* *M. holtonii*
Costa Rica	6	*M. pruriens* *M. holtonii* *M. mutisiana* *M. urens*
Colombia	7	*M. holtonii*
Total	**27**	5 species*

*Six species when taking account of *M. pruriens* var. *utilis* from CIAT.

Chemical analyses of L-dopa concentration in seeds: The HPLC analyses of quantification of L-dopa in the seeds were performed on representative samples of the five species collected in Central America and on 10 accessions from the Genetic Resources Unit (GRU) of CIAT, representing four different taxa (Table 80).

Mean L-dopa concentration (as percentage of dry weight) in the seeds varied from 2.38% for Mucuna *pruriens* var. *utilis* (CIAT 21883) to 9.58% for *M. holtonii* (collection # 00-30) collected in Nicaragua (Table 80). The relatively low standard deviations associated with those results make them reliable. Identical analysis performed on previously analyzed seed samples (St-Laurent et al. 2000) produced similar results, suggesting that the method used is valid. However, the high standard deviations associated with samples 00-20, 00-24 and 00-28 suggest a high variation in L-dopa concentration among the seeds of those specimens (belonging to species *M. sloanei* [00-20 and 00-24] and *M. holtonii* [00-28]). It would probably have required more seeds to estimate the correct mean L-dopa concentration in those specimens but this was impossible due to the limited number of seeds available.

The value of 9.58±0.59% obtained for sample 00-30 (*M. holtonii*) is particularly interesting, given that it is among the highest values ever recorded for a species of Mucuna and could potentially be useful as a source of L-dopa.

Future plans

The results obtained here prompt for more work. First, it would be of course interesting to increase the number of species of *Mucuna* analyzed here by including species from South America and the rest of the world. Other species might prove to produce even more L-dopa than *M. holtonii* as was shown. Next, a formal GxE trial should be initiated with the samples collected as well as with many of the accessions from CIAT. The characteristics of low and high L-dopa species and accessions should be assessed in many contrasting environments. This will make possible in the long run to select the accessions that would be most useful to local farmers in developing countries, either for food or as an eventual source of L-dopa. This will involve making those accessions available to the farmers and to promote its use.

Table 80. Specimens used for L-dopa quantification analyses in the seeds, L-dopa concentration expressed as percentage dry weight, standard deviation and number of replicates analyzed. Numbers preceded by 00 are from Luc St. Laurent's collection. Other numbers correspond to CIAT identification numbers

Number	Identification	% L-dopa	S.D.	N
00-2	*M. pruriens* var. *pruriens*	4.57	0.86	2
00-5	*M. pruriens* var. *pruriens*	4.32	1.06	3
00-20	*M. sloanei*	4.67	3.4	3
00-23	*M. pruriens* var. *pruriens*	6.31	0.59	4
00-24	*M. sloanei*	5.98	2.33	3
00-25	*M. pruriens* var. *pruriens*	5.62	0.48	5
00-26	*M. pruriens* var. *pruriens*	3.94	0.29	4
00-27	*M. pruriens* var. *pruriens*	4.38	0.67	4
00-28	*M. holtonii*	5.43	1.61	4
00-30	*M. holtonii*	9.58	0.59	3
00-36	*M. holtonii*	6.16	-	1
00-37	*M. holtonii*	3.49	0.05	2
00-38	*M. pruriens* var. *pruriens*	5.78	1.16	3
00-39	*M. holtonii*	-	-	-
00-40	*M. mutisiana*	6.53	-	1
00-41	*M. urens*	-	-	-
8833	*M. pruriens* var. *utilis*	3.94	0.21	3
9349	*M. pruriens* var. *utilis*	2.96	0.69	5
18245	*M. mutisiana*	5.24	1.14	3
19088	*M. pruriens* var. *utilis*	3.75	0.33	5
19370	*M. sloanei*	7.46	1.37	3
19837	*M. pruriens* var. *utilis*	3.59	0.68	5
20171	*M. pruriens* var. *utilis*	2.71	1.06	5
21262	*M. pruriens* var. *utilis*	3.04	1.02	3
21883	*M. pruriens* var. *utilis*	2.38	0.76	5
22033	*M. pruriens*	3.89	1.15	4

4.1.2 Development of new collaborative Research Proposals with NARS, NGO's, IARC and ARIS

One major activity of staff in IP5 during the last years has been the development of new research proposals for submission to different donor agencies. The following proposals were prepared and submitted during 2000:

1. Utility of *Cratylia argentea* in dual-purpose cattle systems in the piedmont of the Colombian Llanos. Submitted by CIAT in collaboration with CORPOICA, U de los Llanos and Umatas to Programa Nacional de Transferencia de Tecnologia Agropecuaria, Colombia (PRONATTA). **Responsible in CIAT: C. Lascano**
2. On-farm evaluation of new forage alternatives in dual-purpose cattle systems in coastal areas of Ecuador. Submitted by INIAP, Ecuador and CIAT to Programa de

Modernización de los Servicios Agropecuarios, Ecuador (PROMSA)**. Responsible in CIAT: Federico Holmann**

3. Evaluation of forages for multipurpose use in hillsides of Colombia: Submitted by FIDAR (NGO) in collaboration with CIAT to Programa Nacional de Transferencia de Tecnologia Agropecuaria, Colombia (PRONATTA). **Responsible in CIAT: M. Peters**
4. Characterization of *Prosapia simulans* , a new spittlebug pest in forage grasses of Colombia. Submitted by CIAT in collaboration with U del Pacifico, U Nacional and Umatas to Programa Nacional de Transferencia de Tecnologia Agropecuaria, Colombia (PRONATTA). **Responsible in CIAT: Daniel Peck**
5. Distribution patterns of grassland spittlebugs for gauging range expansion and invasion risk. Submitted by Roosevelt University in collaboration with CIAT to USDA (Foreign Agricultural Service, 2001 Scientific Cooperation Research Program): **Responsible in CIAT: Daniel Peck**
6. Effectively Targeting Forage Technologies to Intensify Land Use: An Integration of Disciplinary and Scalar Information: Submitted as a Concept Note to BMZ in collaboration with IITA, ILRI and the U of Bayreuth, Germany: **Responsible in CIAT: D. White, Th. Oberthur and M. Peters**

4.1.3 Releases of grasses and legumes by NARS partners and Private Seed Companies

Contributors: P. Argel (CIAT), Personnel of MAG, Costa Rica, Personnel of MATSUDA, Brazil and Personnel of PAPALOTLA, Mexico.

A major objective of CIAT's Forage work is to facilitate the release by NARS and private Seed Companies of elite grasses and legumes developed by the Project. During 2000, *B. brizantha* CIAT 26110 was released by MAG (Ministerio de Agricultura y Ganaderia) in Costa Rica and by MATSUDA (Seed Company in Brazil) as cultivars Toledo and MG-5 Vitoria, respectively.

The official release of cv Toledo in Costa Rica ,an event organized by MAG, took place in one of the farms participating in Tropileche. A total of 225 persons attended the field day out of which 60 were milk producers. The official release of cv MG-5 Vitoria took place in Sao Paulo, Brazil with the participation of different institutions (IAC, IAPAR and EMBRAPA).

It is foreseen that tons of seed of CIAT 26110 will be in the market in early 2001 in many countries of the region.

This year, for the first time a *Brachiaria* hybrid (CIAT 36061) derived from CIAT's breeding program was released as cv Mulato by a seed company (PAPALOTLA) operating in Mexico and Brazil. The main attributes of this hybrid are:

1. Vigorous growth and stoloniferous growth habit
2. More yield (25% more) than other commercial *Brachiaria* cultivars
3. Good forage production under season al low light intensity and low temperatures found in certain parts of Mexico
4. High quality as indicated by high protein content (12 to 16%) and in vitro DM digestibility (55 to 62%)

Seed of cv Mulato is being multiplied in Mexico and there are plans to initiate seed multiplication in Brazil. We expect tons of seed to be available in the market by early 2001.

Progress towards achieving milestones

- **Developed new partnerships for forage evaluation in LAC and Africa**

The development of partnerships within CIAT and outside CIAT is a major strategy of the Forage Project to deploy to farmers elite grasses and legumes. So far we have been successful in establishing collaboration with different organizations (NARS, Universities, NGO"S and ARIS) on forage evaluation for different uses in Colombia, Peru, Costa Rica, Honduras, Nicaragua and Canada. On the other hand, we have not yet been successful in establishing collaborative projects in Africa. However, we have developed a collaborative proposal with ILIRI, IITA and the U of Bayruth, Germany for submission to a donor, which aims at developing a better understanding of the socio economic factors influencing adoption of legume in Africa and Central America.

- **Selected *Brachiaria* genotypes released by NARS partners**

Key steps for the diffusion of forages developed by CIAT is that elite grasses and legumes be released as commercial cultivars and that seed is multiplied. We are happy to report that this year two *Brachiaria* lines were released: a) the accession *B. brizantha* (CIAT 26110 as cv Toledo in Costa Rica and as cv MG-5, Vitoria in Brazil) and the *Brachiaria* Hybrid (CIAT 36061) as cv Mulato in Mexico.

- **Legume species available for cover crops in plantations (oil palm and rubber) in the Llanos**

Desmodium heterocarpon subsp. *ovalifolium* CIAT 13651 identified as a potential cover crop option and based on preliminary results could be recommended for use in plantations, when sown at plantation establishment, as is the current practice for cover crops in plantations in the Llanos of Colombia.

4.2 Evaluation with farmer participation of multipurpose forages for crop and/livestock systems

Highlights

- NGO and NARS partners in Central America trained in participatory selection of forages
- Completed a participatory diagnosis of problems and opportunities in three rural communities of Honduras
- Innovative farmers in Honduras selected grasses to test on their farms
- More than 200 farmers initiated selection of improved forage options
- Research collaboration with NARS, NGO and Development project partners established and a Technical Committee formed
- Number of farmers planting *C. argentea* has increased in Costa Rica during the last year. A total of 164 kg of experimental seed has been distributed lately; farmers from the localities of Puriscal and San Juan de Acosta are the pioneers' adopters.
- Demonstrated feasibility of making high quality *Cratylia* silage to replace expensive protein supplements for dry season feeding.
- Showed that reclamation of degraded pastures with *Arachis pintoi* in subhumid and humid areas of Central America and forest margins increases carrying capacity and milk yield.

4.2.1 On-farm evaluation of forages for multipurpose use with farmer participation in Central America

Contributors*:* M. Peters, P.J. Argel, LA. Hernández, L.H. Franco, C. Burgos, H. Cruz F., M.I. Posas, W.Sebillón T. Reyes, M. A. Mena, F.J. Latino, R. Guillén M., J. Bustamante, C. Reiche, V. Hoffman, and R. Schultze-Kraft

Forage germplasm in its multiple uses - for example as feed, for the suppression of weeds, the maintenance and improvement of soil fertility and for erosion control - could play an important role in improving the well being of the small and medium size farmers in Central American hillsides. However, adoption - particularly of forage legumes - has been limited, possibly due to lack of direct interaction with the farmers. Therefore it is necessary to develop forage germplasm technologies with farmers, using a participatory approaches.

Photo 30. Farmers, technicians and scientists discussing in the field new San Dionisio, Nicaragua

To address this issue, CIAT in collaboration with NARS, NGO's and farmer groups identifies germplasm preferred by farmers. GIS (Geographic Information Systems) -tools are being developed for strategic targeting of forage germplasm first to environmental and later to socio-economic niches in hillsides of Central America. The work is anticipated to 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. As a spin-off an on-farm experiment to test the ability of improved forages to reclaim land in valley areas affected by Mitch is underway. It is anticipated that appropriate forages can revegetalise land without incurring the high costs of other land reclamation practices.

A combination of agronomic evaluation techniques, participatory technologies, soil indicators, socio-economic studies and GIS tools is 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.

To initiate the process of participatory selection of improved forage options several training and planning activities with involved partners were executed. These include:

- A course on Methods and Techniques of Participatory Research was carried in collaboration with CIAT's Participatory Research Group (SN3) in February/March 2000. 12 participants from several institutions from Honduras, Nicaragua, Costa Rica and Colombia were capacitated and are now using participatory methods in collaborative activities with CIAT.

- In May 2000 with collaboration from SN-3, Research and Development personnel from various national and international Research institutions and NGO's in Honduras (participants from CIAT, DICTA and SERTEDESO), Nicaragua (CIAT, INTA and PRODESSA) and Costa Rica (MAG and Fundación Ecotropica) were trained in methods for stakeholder analysis. These methods are now applied as part of the diagnosis in the study sites.

- A major activity in 2000 was a planning workshop for the project held in Comayagua, Honduras. 15 officials from institutions in Honduras (DICTA, SERTEDESO, and CIAT), Nicaragua (INTA, PRODESSA, and CIAT), Costa Rica (MAG, IICA-GTZ, CIAT, and ECOTROPICA), Colombia (CIAT) y and Germany (University of Hohenheim) involved in the project participated; staff from IICA-GTZ, Costa Rica facilitated the course.

Letters of Agreement with the institutions involved in the project were prepared and are in the process for ratification.

Several types of trials for the agronomic evaluation and simultaneous participatory selection of improved forages as agreed in the planning workshop were established in Honduras, Nicaragua and Costa Rica. Multipurpose germplasm including grasses, herbaceous legumes, shrub legumes and cover and green manure legumes are offered to farmers.

Table 81 describes the trials established in Central America. The sites were selected based on the diagnosis and in some cases complimented by approaches from interested farmers themselves. The initial plant material selected is as follows (in future years this list could be altered according to perceptions and demand by farmers as well as performance).

Table 81. Trials for the Participatory Selection of Forages established in 2000.

Country	Locality	Grasses	Herbaceous Legumes	Cover Legumes	Shrub Legumes
Honduras	Las Cañas	1	1	1	1
	Sol Luquigue	1	1	1	1
	Jícaro	1	1	1	1
	Ayapa			1	
Nicaragua	Sol San Dionisio	1	1	1	1
	Piedra Larga				
	El Corozo	1	1	1	1
		1	1	1	1
Costa Rica	Santa Marta	1	1	1	1
	Mastatal	1	1	1	1
	La Gloria	1	1	1	1
Total	10	9	9	10	9

The different forage options being evaluated in the different sites in Costa Rica, Nicaragua and Honduras are:

Grasses: *Andropogon gayanus* CIAT 621; *Brachiaria brizantha* CIAT 6780, 26110, 26646, 16322, 36061; *Brachiaria dictyoneura* CIAT 6133; *Panicum maximum* CIAT 16031; 'King grass'

Herbaceous Legumes: *Arachis pintoi* CIAT 18744, 22160; *Centrosema pubescens* CIAT 15160; *Centrosema plumieri* DICTA; *Desmodium ovalifolium* 33058; *Stylosanthes guianensis* CIAT 11844; *Clitoria ternatea* cv. Tejuana

Green Manure and Cover Legumes: *Pueraria phaseoloides* CIAT 7182; *Mucuna pruriens* IITA-BENIN; *Mucuna pruriens* DICTA; *Mucuna deerengianum* DICTA; *Canavalia brasiliensis* CIAT 17009; *Lablab purpureus* DICTA

Shrub Legumes: *Calliandra calothyrsus* CIAT 22310, 22316; *Cratylia argentea* CIAT 18516/18668; *Leucaena leucocephala* CIAT 17263; *Leucaena macrophylla* OFI47/85; *Gliricidia sepium; Erythrina verteruana; Erythrina poepigiana*

Each of the 10 trial sites corresponds to a group of 10-15 farmers. A total more than 100 farmers are so far involved in the project.

Advances during 2000

The establishment of the different grass and legume options was hampered in some of the sites by dry conditions and in others by extremely wet conditions. However, in the majority of sites, in after reseeding we have now acceptable plant populations. All plantings were done together with groups of small to medium farmers, including mixed crop-livestock farmers and farmers focusing on crop or livestock production. The farmers were identified in previous diagnosis studies (see below some examples), in some cases complemented with stakeholder analyses. These farmers are expected to continue in the process of participatory selection of forage options.

Results from preliminary work in Honduras with similar species are shown in the following tables, for legume covers and green manures, for grasses and for shrub legumes. Results from the evaluation of green manures and covers (Table 82) indicate that Cowpea (*Vigna unguiculata*) – and to a lesser extent the Lablabs and Mucunas show good performance in Honduras and in other sites were they are being evaluated across sites and thus have a high potential for wide-spread adoption by farmers in different regions.

Results on agronomic performance of grasses (Table 83) indicate *Brachiaria brizantha* 26110 (cv. Toledo) and the *Brachiaria* hybrid 1873 as the most stable accessions across the two sites in Honduras).

They also have excellent cover and consequently low weed populations. Together with other characteristics as appearance and texture these were criteria for farmers to positively select these two accessions (data not presented).

Results shown in Table 84 indicate that *Leucaena leucocephala* is outperforming *Cratylia argentea* in one of the sites in Honduras. Knowing the excellent performance of *Cratylia argentea* in the dry season and its growing acceptance by farmers in Costa Rica, these results indicate that with legumes one needs to view the particular niches in terms of environmental and systems adaptation.

Table 82. Agronomic performance of a range of cover and green manure legumes at three sites in Yorito, Yoro, Honduras

Locality	Accession	Stem DM kg/ha	Leaf DM kg/ha	Total DM kg/ha	Cover %	DM of pods kg/ha	Number of pods
Ayapa	Ce 715	323	167	490	8	167	1
	Chr 8990	607	498	1105	42	.	.
	Dl 1	233	82	315	7	0	0
	Dl 2	37	23	60	2	0	0
	Mp cv R	273	247	520	12	1020	21
	Mp cv T	290	212	502	12	823	4
	Vu cvVB	360	170	530	27	313	11
San Antonio	Ce 715	3380	1383	4763	47	923	11
	Chr 8990	0	0	0	0	0	0
	Dl 1	4210	830	5040	95	0	0
	Dl 2	4413	590	5003	95	0	0
	Mp cv R	950	283	1233	50	2528	91
	Mp cv T	3097	800	3897	100	458	98
	Vu cvVB	2690	307	2997	50	968	75
San Jerónimo	Ce 715	540	430	970	22	1947	6
	Chr8990	273	183	456	17	.	.
	Dl 1	1443	513	1956	37	5	1
	Dl 2	1340	433	1813	33	30	10
	Mp cv R	207	240	446	14	128	12
	Mp cv T	0	0	0	0	0	0
	Vu cvVB	473	473	946	30	219	80

Ce – *Canavalia ensiformis*; Chr - *Chamaecrista rotundifolia*; D - *Desmodium ovalifolium*; MP – *Mucuna pruriens*, Vu – *Vigna unguiculata*

Table 83. Agronomic performance of a range of grasses at two sites in Yorito, Yoro, Honduras.

Locality	Accession	Cover %	DM kg/ha	Grasses %	Broadleaf Weeds %	Other %
San Antonio	Bb 26110	62	3173	98	.	2
	Bb hi1873	57	2800	98	.	2
	Bh 6133	73	1213	83	.	17
	Pm 16028	77	3827	93	.	7
	Pm 16031	68	3173	93	.	7
	Pm 16051	48	2147	83	.	17
San Jerónimo	Bb 26110	52	2520	100	.	.
	Bb hi1873	53	2987	100	.	.
	Bh 6133	43	1493	57	36	7
	Pm 16028	38	2147	75	25	0
	Pm 16031	48	2147	90	10	0
	Pm 16051	23	1027	37	56	7

Bb – *Brachiaria brizantha*; Bh – *Brachiaria humidicola*; Pm- *Panicum maximum*

Table 84. Agronomic performance of a range of shrub legumes San Jerónimo, Yorito, Honduras

Evaluation	Accession	DM leaf material g/plant	DM stem material g/plant	Height cm	Number of regrowing points/plant
1	Ca 18516	8	0	28	2
	Ca 18668	6	0	28	2
	Ll 17263	102	30	53	5
2	Ca 18516	27	1	41	2
	Ca 18668	20	0	45	2
	Ll 17263	47	8	35	4
3	Ca 18516	59	1	62	6
	Ca 18668	63	1	62	7
	Ll 17263	165	33	89	12

Ca – *Cratylia argentea*; Ll – *Leucaena leucocephala*

Participatory diagnosis in three communities in the Department of Yoro, Honduras

A total of 59 persons, of whom 45% were women, took part in the participatory diagnosis. The age composition was very diverse, with participants ranging from 18 years to approximately 75 years. Livestock farmers as well as crop farmers and mixed farmers responsible for various activities in the field and at the homestead were involved.

The diagnosis included plenum and small-group activities, leading to the identification and prioritization of problems as related to agriculture and Natural Resource Management, with emphasis on forages. The diagnoses were done in three sites in and around CIAT's reference site Yorito, i.e. El Jícaro in Victoria, Las Cañas in Sulaco and Luquigue in Yorito.

The sites are characterized by an undulated topography with slopes of 30 to 50%. Altitudes ranges from 50 to 650 m asl. Annual rainfall is 1200 to 1500 mm, with 5 wet, 5 dry and 2 moderately dry months; Conifers characterize the forest. Soils are moderately acid to neutral clay soils Temperature is between 24 to 30 °C. The main agronomic activities include maize and beans cultivation and small-scale livestock production; in livestock production is extensive, with a tendency toward beef production.

In all diagnosis the participation of women was significant except for Luquique where only 18% of the participants were women. Common Problems identified across the 3 communities include:

- Erosion, lack of water, burning, deforestation, low productivity of the resource base, and low fertility
- Free roaming of animals in the dry season, lack of wood (poles and firewood), lack of land, and lack of green material in the dry season for conservation and as feed.
- Lack of technology options, lack of technical support, lack of seed and planting material
- Interestingly, one group mentioned the lack of interest of themselves to improve their situation as a problem

Most problems were closely related with the lack of feed for animals, in particular during dry periods, soil degradation and lack of firewood. Results of the prioritization of these problems were heterogeneous, but tended to emphasize the problem of lack of suitable planting material and lack of livestock feed in particular during the dry season. There was a clear perception of the communities of the degradation of soils and deforestation and the inherent negative effects of these on the communities.

The objective of farmers to participate in the project is to address the mentioned problems and thus to improve family income, family and community nutrition.

Photo 31. Pasture evaluation and system trials at San Dionisio, Nicaragua

Uptake of farmers of improved forage material

Based on earlier work with a group of about 15 farmers and after 1.5 years of collaboration, 8 farmers have established the improved grass *Brachiaria brizantha* La Libertad on their farms. One farmer established *Stylosanthes guianensis* as feed for rabbits. As expected, farmers have started with the uptake of grasses, but we anticipate this could be an entry point for legumes.

4.2.2 On farm evaluation of selected forages as feed resources in dual cattle systems of Central America through the Tropileche Consortium (CIAT and ILRI)

Contributors: F. Holmann, P. J. Argel, M. Lobo (MAG, Costa Rica), V. Acuña (MAG, Costa Rica), C. Jiménez (U. de Costa Rica), F. Romero (ECAG, Costa Rica)

This section of the report is divided in three parts: (1) Use of additives for making Cratylia argentea silage; *(2)* Effect of Supplementing Fresh and Ensiled *Cratylia argentea* cv. Veraniega on Dual-Purpose Cows in the Subhumid Tropics of Costa Rica*;* and (3) Milk production in dual-purpose cows grazing *Brachiaria brizantha* cv. La Libertad alone and associated with *Arachis pintoi* cv. El Porvenir in the subhumid tropics of Costa Rica.

A) Use of additives for making *Cratylia* silage

Background: In the dry tropics of Costa Rica (annual rainfall, 1200-1800 mm; 6 months of dry season, and altitudes ranging from 0 to 800 meters above sea level), *Cratylia argentea* has been successfully introduced into dual-purpose farms to solve the problems of low forage availability during summer months. Mixing *Cratylia* forage and chopped sugarcane has proved useful to maintain milk yields of 6-6.5 kg per cow/day, in crossbred cows Zebu x European dairy cows.

Producer's value *Cratylia*'s as a supplement for cows during the dry season, but they also consider that surplus forage in the wet season should be ensiled for use during times of feed shortage. Thus this collaborative project with the U of Costa Rica and ECAG aims to evaluate the process of making silage from the legume *Cratylia*, using several fermentative and nutritive additives. Overall, legumes are more difficult to ensile than grasses because they have a high buffer capacity that hinders adequate acidification of anaerobic media, in addition to its low soluble carbohydrate content.

Materials and Methods: The foliage of 90-day regrowth was manually harvested and chopped to a 16-mm bite size. Microsilos made of double polyethylene plastic bags, with approximately 1.5 kg capacity, were used. *Cratylia* was mixed with three additives: cane molasses (M), pineapple pulp (PP) and chopped sugarcane (CSC). Each additive was incorporated at three levels: M, at 10%, 20%, and 30%; PP and CSC, at 25%, 50%, and 75%, all on fresh basis (w/w).

Cratylia foliage was obtained from an experimental plot at the Escuela Centroamericana de Ganadería, located in Atenas, Alajuela, at 460 m asl. Pineapple pulp was collected at the Del Oro plant in Santa Cecilia de la Cruz, Guanacaste, and the sugarcane was gathered at a private farm located in the Hojancha canton, also in Guanacaste.

The silos were left to ferment for 60 days and, upon opening, pH and organoleptic characteristics (odor and color) were assessed. Part of each sample was oven-dried at 60 °C and a subsample was frozen to later analyze ammonium nitrogen. Laboratory tests were conducted to determine (a) dry matter (DM), organic matter (OM) and crude protein (CP) contents; (b) ammonium nitrogen (Nam); and (c) rumen degradability of dry matter ( ). Treatments were arranged in a completely randomized design with three replicates.

Results and Discussion: To evaluate the Cratylia silage produced with different additives we used a conventional classification scheme shown in Table 85.

Results of pH value and organoleptic characteristics (scale from 1 to 3) of the Cratylia silages are shown in Table 86. All silages presented a strong lactic odor, except for those with high CSC content (75%), which presented an alcoholic odor with a lactic essence. The silage had a brown color with high levels of CSC and 25% PP.

Table 85. Organoleptic properties of silages.

Organoleptic characteristics	1 (Poor)	2 (Intermediate)	3 (Good)
Odor	Butyric (rancid) Degraded amino acids or N sub-products (spoiled)	Acetic Alcoholic	Lactic Slightly acetic or alcoholic with lactic essence
Color	Dark brown Black Moldy	Light brown Yellowish	Original green Light green

The pH correlated highly with lactic and acetic acid contents. A pH lower than 4 is considered a good indicator of superior fermentation in moist substrata such as the ones used in this project and, accordingly, of a fairly stable, good-quality end product. When carbohydrate levels are very high or very low, fermentation is altered, yielding final products and pH values that fall outside the optimal range. In the case of 75% CSC, the high levels of soluble carbohydrates (SC) induced fermentation tending more toward an alcoholic base, with a pH of almost 5. In the case of 25% PP, because of the high moisture content of this substratum, its SC contribution in dry basis was small, causing lactic acid production to be poor. pH was accordingly almost 4.5.

Table 86. Odor, color, and pH of *Cratylia argentea* silage using three additives (M = Molasses; PP= Pineapple pulp; CSC= Shopped sugar cane).

Treatment			Odor	Color	pH
No.	Additive	% w/w			
1	M	10	3	3	4.12
2	M	20	3	3	4.00
3	M	30	3	3	4.05
4	PP	25	3	2.33	4.45
5	PP	50	3	3	3.90
6	PP	75	3	2.83	3.45
7	CSC	25	3	2.5	3.37
8	CSC	50	3	2.5	4.00
9	CSC	75	2.83	2.33	4.73

The “buffer” effect was evident in *Cratylia*, especially in Treatment 1 with 10% M. In grasses, for example elephant grass (*Pennisetum purpureum*), the addition of 10% M increases the dry matter SC content too more than 11%. Fermentation is also optimal with pH values close to 3.5. Furthermore, buffer capacity could limit carbohydrate use and lactic fermentation and, accordingly, high pH values in several other treatments, especially those with higher/lower SC contents.

Based on available data, the following conclusions and recommendations can be made:

a. The addition of 10% molasses should be sufficient to obtain a good final product. It is not necessary to use higher levels of this expensive additive. Additional research projects should be conducted to reduce the use of this additive. One possibility is to simultaneously work with lower amounts of molasses and inoculate the substratum with lactobacillus.

b. Pineapple pulp added at levels higher than 25% will improve fermentation, although transportation costs should be evaluated because it is such a watery material. Water content has an adverse effect on fermentation; therefore the convenience of draining the product upon leaving the plant and prior to ensiling should be evaluated.

c. Chopped sugarcane, added at 25%, should contribute sufficient soluble carbohydrates for good lactic fermentation; at higher levels, there is a risk of promoting an alcoholic process. It would be interested to evaluate the effect of inoculating the substratum with lactobacillus in the future.

B) Effect of supplementing fresh and ensiled *Cratylia argentea* on dual-purpose cows in the subhumid tropics of Costa Rica

Background: Costa Rica covers 51,023 km^2 of land of which 25,000 km^2 (49.2%) are under farming and cattle raising systems. Cattle are raised on 51,000 farms, with an estimated population of 2,150,000 heads. The main problems affecting cattle development in Costa Rica include the low meat and milk productivity, low reproductive indexes, nutritional deficiency—especially because of the low availability and quality of forage during the dry season—and sanitary problems.

The aim of the study was to evaluate the use of ensiled *Cratylia argentea* in terms of milk production and to define the economical benefits of using this technology. Two experiments were carried out on two farms located in Costa Rica's subhumid tropical Central Pacific region.

Materials and Methods: A Latin square design was used, with three treatments and two animals/treatment. The trials lasted 30 days, during which animals were rotated in each treatment over a 10-day period, which was divided into 7 days of adjustment and 3 days for measurement of milk production.

Experiment 1: Six cows were selected from a herd of 45 milking cows that presented broad genetic variation, on a farm located in Barbudal de Barranca, Miramar. All cows were in their second month of lactation, third parturition, and produced, on average, 5.5 kg milk/day.

Treatments imposed were as follows: T1: Control (only grazing); T2: 12 Kg of sugarcane, 8 Kg Cratylia, 0.6 Kg of rice bran and 0.5 Kg of molasses; T3 12 kg of sugarcane, 3 Kg of chicken manure, 0.6 Kg of rice bran and 0.5 Kg of molasses.

The *C. argentea* used had approximately 4 months regrowth; with an average nutritive value of 16% crude protein and 33% dry matter.

Experiment 2: Six cows were selected from a group of 11 milking cows of third parturition, on a farm located in San Miguel de Barranca, Esparza. All cows had similar breed characteristics (3/4 Swiss Brown and 1/4 zebu), and were in their second month of lactation and producing, on average, 5.5 kg milk/day.

Evaluated treatments were as follows: T1: 12 kg sugarcane, 6 kg Cratylia silage, 0.6 kg rice bran; T2: 12 kg sugarcane, 6 kg fresh Cratylia, 0.6 kg rice bran; T3: 12 kg sugarcane, 3 kg chicken manure, 0.6 kg rice bran

In Treatment 2, Cratylia plants were manually cut at 4 months regrowth for ensiling using a chopper coupled to a tractor. Molasses was gradually added at 10% weight basis of material for ensilage as the silage was being compacted by the tractor. Silage was first used 4 months after being ensiled and its quality was determined by measuring pH, percentage of crude protein, dry matter content, and several organoleptic characteristics such as odor and color.

Results and Discussion

Experiment 1: In Table 87 we show average values of milk yield and quality, and cost/benefit ratio for the treatments evaluated. Milk production differed significantly between treatments (p = 0.076), with the highest production being recorded when chicken manure was offered as protein supplement. However, no significant differences in milk yield were observed when chicken manure was replaced with fresh Cratylia.

Milk production of cows not receiving a protein supplement was relatively high and this could be attributed to the high availability of fruits of the cohune palm (*Acrocomia viniferous*), which could contribute a significant amount of nutrients to the diet, as well as to the consumption of several other sources of feed available in paddocks during the dry season.

Table 87. Average production, milk quality, and cost/benefit ratio of dual-purpose cows receiving supplements of *Cratylia argentea* and chicken manure.

Treatment	Milk production (kg/cow)	Total solids (%)	Fat (%)	Diet Cost ($/kg)	Milk Income ($/kg)	C/B ratio
1	5.5 b	11.2	3.1	-	0.235	-
2	5.9 a b	11.5	3.2	0.109	0.241	2.2
3	6.3 a	11.2	2.9	0.217	0.235	1.1

Waller-Duncan test (p = 0.076).
T1= Control; T2 = Fresh *Cratylia*; T3= Chicken manure

A statistical comparison was not performed for milk quality, but minimum variations in averages of total solids and percentage of fat were observed among the three treatments.

The results of greatest interest are reflected in the economic indicators such as cost of supplements, income obtained from the sale of milk, and the cost/benefit ratio. Replacing chicken manure with *Cratylia* reduced the cost of supplementation from $0.22/kg to $0.11/kg, thus reducing supplementation costs by half.

Experiment 2: Table 88 shows mean values of milk yield and quality of cows supplemented with fresh and ensiled *C. argentea* and with chicken manure. Significant differences were found in milk production among treatments (p = 0.08), with production being highest when fresh *C. argentea* was offered as compared to chicken manure. Milk yields was similar for cows supplemented with chicken manure and ensiled *Cratylia*.

The silage obtained was highly palatable and of good quality (pH 4.5, 16.5% crude protein, and 36% dry matter); these data were similar to those found by Romero and González (unpublished data). Based on trial results, silage quality can be ranked as excellent in terms of dry matter and pH. Test animals rejected neither fresh nor ensiled *Cratylia*, with the rejection being approximately 10% (mainly woody stems) of the material offered.

Table 88. Average milk production in dual-purpose cows supplemented with fresh and ensiled *Cratylia argentea* and with chicken manure.

Treatment	Milk production (kg/cow)	Total solids (%)	Fat (%)	Diet cost ($/kg)	Milk Income ($/kg)	C/B ratio
1	5.1 b	12.3	3.6	0.164	0.260	1.6
2	5.5 a	12.2	3.4	0.109	0.256	2.3
3	5.3 a b	11.7	3.0	0.217	0.245	1.1

Waller-Duncan test (p = 0.08).
T1= *Cratylia* silage; T2= Fresh *Cratylia*; T3= Chicken manure

In general our results indicate that the use of *Cratylia* as a protein supplement during the dry season can substitute chicken manure completely, thus reducing the farmer's dependency on off-farm resource. One additional benefit of supplementing *Cratylia* is the improved quality of the milk produced by cows in terms of fat and total solids.

Profits were higher when milk production involved fresh and ensiled *Cratylia* than when chicken manure was used given that production costs were lower and the income obtained by sale of milk was higher due to better quality of the milk.

C) Milk production in dual purpose cows grazing *Brachiaria brizantha* cv. La Libertad alone and associated with *Arachis pintoi* cv. El Porvenir in the subhumid tropics of Costa Rica

Background: Among the main problems affecting beef cattle development in Costa Rica are low meat and milk productivity, low reproductive rates, nutritional deficiencies (especially because of the low forage availability and quality during the dry season), and health problems. Forage is the most available and inexpensive resource of feed for livestock in Costa Rica and othe central american countries; however, a high percentage of pastures are now degraded, reducing forage availability and quality.

The improvement of both the quality and quantity of forage resources through the recovery of degraded pastures is important to increase overall livestock productivity. The study aims to evaluate milk production of dual-purpose cows grazing a recovered pasture of *Brachiaria brizantha* cv. La Libertad alone and associated with *Arachis pintoi* cv. El Porvenir and *Centrosema brasilianum*.

Materials and Methods: A grazing experiment is bein carried on a farm located in San Jerónimo de Esparza, in the subhumid tropical central Pacific region of Costa Rica, at an average altitude of 250 m.a.s.l. Four hectares of *Brachiaria brizantha* cv. La Libertad were planted in a paddock where a degraded pasture of *Brachiaria ruziziensis* existed. The area was divided in half and 2 hectares were planted in association with *A. pintoi* CIAT 18744 cv. Porvenir and *C. brasilianum* CIAT 5234. The region is characterized by having well defined dry (December-May) and rainy (June-November) seasons. Average temperature is 26 °C and average annual precipitation, 2500 mm.

Forage availability (dry basis) and botanical composition of both pastures (alone and associated) were determined using the Botanal method. A group of 26 milking cows of different breeds—predominantly Brown Swiss—and weighing, on average, 450 kg were used; a Brahman bull was also included. This group grazed the 2 hectares of grass alone pasture for 4 consecutive days and the grass/legume pasture for 5 days. Milk production was measured on days 1 and 4 in the grass alone pasture and on days 1, 3, and 5 in the legume-based pasture. Milk production from both groups of cows was compared with that obtained by cows grazing pastures sown to *Brachiaria ruziziensis*, the grass traditionally used on farms.

During the rainy season, pastures were rotated; the grazing cycle consisted of 4 to 5 days of occupation and 27 days of rest. During the dry season, grazing was continuous. The stocking rates of the local pasture, the grass alone pasture, and the legume -based pasture were calculated using the method proposed in the RIEPT, which takes into account animal units and grazing system.

Results: In Table 89 we show forage availability (dry basis) and botanical composition of both pastures during the rainy season over three consecutive periods. The percentage of *Centrosema* *brasilianum* found in the associated pasture was so low that it was not included in the percentage of legumes present in the associated pasture.

Table 89. Forage availability and botanical composition of a pasture of *Brachiaria brizantha* cv. La Libertad alone and associated with *Arachis pintoi* and *Centrosema brasilianum* during the rainy seasons of 1997, 1998, and 1999.

Year	DM availability (kg/ha)									
	Grass alone pasture					Legume-based pasture				
	kg/ha	Grass (%)[a]	*A. pintoi* (%)[b]	Legume (%)[c]	Weeds (%)[d]	kg/ha	Grass (%)	*A. pintoi* (%)	Legume (%)	Weeds (%)
1997	4,113	62	0	22	16	5,105	57	18	13	12
1998	4,483	67	0	18	15	5,408	58	24	4	14
1999	6,306	88.2	0	8.5	3.3	7,155	52.7	41.9	4.7	0.7
Mean	4,967	72.4	0	16.2	11.4	5,889	55.9	27.9	7.2	8.9

a. Mainly *B. brizantha* cv. La Libertad and *B. ruziziensis*.
b. *Arachis pintoi* cv. Porvenir
c. *Calopogonium mucunoides*, *Zornia* spp, and *Aeschynomene* spp, among others, in addition to *C. brasilianum*.
d. Mainly *Mimosa modest*, *Amaranthus* spp., *Borreria* spp., and others.

At the beginning of the trial, an average value of forage on offer (3000 kg DM/ha) in the local pasture was lower than that in the improved grass and grass/legume pastures.In addition, forage availability increased in both improved pastures over time as shown in Table 89. However, the associated pasture produced, on average, 19% more biomass that the grass alone pasture. This situation is reflected in the average stocking rate estimated for the pastures. In the case of local pastures, the estimated stocking rate was 1.5 AU/ha, whereas it was 2.0 AU/ha for the grass alone pasture and 2.4 AU/ha for the legume-based pasture.

Average milk production/animal on the local pasture, and improved pastures sown during the rainy seasons are shown in Table 90. No significant differences in milk yield per cow were observed among pastures. However, when the average production obtained over the three years on the local pasture was compared with that obtained on the associated pasture, differences in milk yield were significant.The benefit of the improved pastures as compared to the local pastures was also evident in terms of milk production per hectare given there higher carryn capacity.

Table 90. Milk yield of cows grazing contrasting pastures in a farm in Costa Rica

Year	Local pasture[b]	Grass alone pasture (kg/cow/ per day[a)])	Legume-based Pasture
1997	8.6	9.0	9.3
1998	7.5	7.7	7.9
1999	7.6	7.8	8.1
Average	7.9 a	8.2 a	8.4 b

a. Average of 26 milking cows.
b. Native grasses, mainly *Brachiaria ruzziziensis*.
Comparison of averages using the Student's (t) test, p = 0.2271.

In general, our results indicate that the reclamation of degraded pastures through the establishment of improved grasses not only increases milk production per cow but also milk production per hectare due improved carrying capacity. A further benefit is obtained with the introduction of legumes, such as *A. pintoi*, both in terms of milk production and carrrying capacity.

4.2.3 Study on adoption of *Arachis* in Costa Rica

Contributors: T. Wuenscher (University of Hohenheim), M. Peters, P. Argel, L. Rivas, F. Holmann, D. Pachico (CIAT)

Background: In Costa Rica, *Arachis pintoi* is in the early phase of adoption by farmers as component of grass-legume pasture associations and – to a lesser extent - as a cover crop in plantations. The present study attempted to identify the present state of distribution, the economic benefit and limitations and potentials for wider adoption of this multipurpose legume.

Methods: Out of 7138 livestock farmers registered for the zone 112 farmers were randomly selected for a survey. On top of these 42 farmers who have tried *Arachis pintoi* as a component of pastures (28 adopters, 6 non-adopters) and cover crop (5 adopters in orange plantations, 3 non adopters, 2 orange, 1 coffee) were selected.

Results and Discussion: In the random interviews an adoption rate of 4.5 % was found. However, nearly 90% of farmers knew *Arachis,* of which 77% were aware of its value as animal feed and/or cover crop, 54% thought that *Arachis* could be a beneficial component of their farming systems, and 28% did not know its potential utility. Nearly 80% of adopters have introduced *Arachis* based on institutional recommendations and most farmers were satisfied with the results achieved with the legume.

About 2/3 of interviewers indicated that major constraints to animal production were quality of available feeds in the dry and wet seasons and lack of fertilizers. On the other hand, the main solution envisaged by farmers to improve their livestock systems, is intensification through the use of improved forage varieties, thus offering the potential of wider adoption of improved grasses and legumes such as *Arachis*. A simulation exercise based on existing data predicted an increase of area under *Arachis* from presently 1.6 % in the northern zone of Costa Rica to more than 25% in 2005.

The main reasons identified for the relative slow adoption of *Arachis pintoi* in Costa Rica include:

1. Time lag in adoption of forages in general,
2. Legumes as a new forage component in contrast to grasses,
3. Aggressiveness and hence weed potential of *Arachis* and
4. Lack of experience and information on the legume

The results point out the paramount importance of institutional contacts for the adoption of *Arachis* and possibly forage legumes in general. Therefore more interaction with farmers through participatory approaches and extension services is needed. This includes further training of field staff of NARS, Extension Services and NGOs on participatory methods. Continued institutional support in promoting *Arachis* is essential to ensure a wide adoption rate in Costa Rica and in other countries of Central America.

4.2.4 Diffusion of *Cratylia* in Costa Rica

Contributors: C. Jiménez (U. of Costa Rica); R. Guillén, L. Mesén (MAG, Costa Rica); P. J. Argel

Background. A great deal of experimental results related to the utilization of *C. argentea* in Costa Rica, have been generated with farmer participation. The outstanding attribute of this shrub is the capacity to re-growth and retain foliage during the dry season. This particularity has made *C. argentea* a useful source of protein to supplement poor quality grasses and replace costly concentrates during that time of the year. Additionally, the surplus growth of the shrub during the wet period is made into silage of good quality that assists farmers to overcome the scarcity of animal feed during the dry period.

By means of field days, a video on the use of *Cratylia* and technical presentations, both farmers and technicians have become aware of the potential use of *C. argentea* in Costa Rica. The species is presently registered as cv. Veraniega and it is planned to officially release the shrub as a new forage variety early in 2001, in the mean time a process of spontaneous adoption is underway.

Results and Discussion. *C. argentea* is planted exclusively as a protein bank and used in cut and curry systems during the dry period, although some small beef producers are planing to use the shrub the whole year in semi-confined feeding systems. These systems predominate in the localities of Puriscal and San Ignacio de Acosta in Costa Rica, a very hilly country with serious problems of soil erosion. Presently 13 small beef/dual purpose cattle farmers have planted forage banks of *C. argentea* either by direct seed planting or from nurseries established on their farms.

C. argentea is also being planted by farmers in other parts of Costa Rica such as the subhumid areas of the Pacific coast. An idea of its adoption is given by the 164 kg of experimental seed distributed under request from August 1999 to August 2000 by the Seed Unit of Atenas. If we estimate that approximately 4 kg of seed are needed to plant 1 ha at a distance of 1.0 x 1.0 m between plants, then we can say that for the period mentioned, around 35 new ha of *C. argentea* have been planted. We need to recognize that the adoption of forage legumes is a very slow process. Farmers do not have the tradition and the extension service very often promotes the use of legumes. *C. argentea* is being adopted because the alternative uses during the dry period and the facility to establish the plant by seed. It is expected that in the near future particularly small and medium size dual-purpose cattle farmers will use this forage legume more widely.

Progress towards achieving milestones

- **Trained personnel from different institutions and organized farmer groups for participatory evaluation of forages in Central America**

The institutional framework and partnership with farmers for a success of the project on participatory selection of forages for multipurpose used has been established. Forage options are in the field and Participatory Selection by farmers should start end of 2000/early 2001. The value of grasses as an entry point for forages has been confirmed.

- **Known adoption and constraints for adoption of *Arachis* in Costa Rica**

Adoption of *Arachis pintoi* in Costa Rica were assessed and results showed a low adoption rate (4.5%), but great potential for wide spread adoption if some actions are taken by different organizations. Our results indicated that to increase the adoption of *Arachis* and possibly other forage legumes, there was a need to promote contacts between farmers and R & D institutions. Therefore more interaction with farmers through participatory approaches and extension services is recommended. This includes training of field staff of NARS, Extension Services and NGOs in participatory methods.

4.3 Forage seeds: reproductive biology, quality, multiplication and delivery of experimental and basic seed

Highlight

- Basic seed of *Brachiaria brizantha* CIAT 26110 (released as cv Toledo), and *Cratylia argentea* (pre-released as cv Veraniega) in Costa Rica was delivered to NARS and private seed companies.

4.3.1 Multiplication of selected grasses and legumes in the Seed Units of Palmira and Atenas, Costa Rica

Collaborators: J. W. Miles, P. J. Argel, A. Ortega

Atenas Seed Unit: Seed multiplication activities continued in the Atenas Seed Unit (Costa Rica) in collaboration with the Escuela Centroamericana de Ganadería (ECAG). The seed produced is to support advanced evaluations of promising forage germplasm both by CIAT's projects and regional research/development institutions in Central America and the Caribbean. From August 1999 through August 2000 a total of 882.8 kg of experimental and basic seed was either produced or procured from collaborator farmers. The bulk of the seed was formed by *Cratylia argentea* (278.3 kg), *Brachiaria* spp. (165.2 kg), *Arachis pintoi* (400.0 kg), *Leucaena* spp. (13.4 kg), *Centrosema* spp. (2.1 kg), *Desmodium heterocarpon* subsp. *ovalifolium* (7.2 kg) and *Paspalum* spp. (16.0 kg). Small quantities of experimental seed were produced of *Desmodium velutinum, Chamaechrista rotundifolia* spp. *grandiflora, Clitoria ternatea, Calliandra* spp. and *Stylosanthes guianensis.*

Palmira Seed Unit: The following seed multiplication activities took place during 2000:

1. **Multiplication of seed of *S. guianensis* (early flowering advance population).** Seed production plots (1,200 m^2) were established at CIAT-Quilichao, by transplanting seedlings, of a mixture of three early-flowering, bulk-advance populations (Table 91).

2. **Multiplication of seed of promising *Brachiaria* hybrids.** Seed harvests on established plots at CIAT-Popayán continue. Seed was harvested from plots established on rented land until expiration of rental contract in April this year. Seed continues to be harvested at the CIAT-Santa Rosa station. Seed volumes harvested in 2000 to date are presented in Table 91.

One advanced apomictic *Brachiaria* hybrid was established on 3,100 m^2 at CIAT-Popayán to produce basic seed for grazing trials (2001) and to advance seed multiplication. Smaller plots (approx. 50 m^2) were established at CIAT-Popayán of three additional promising apomictic hybrids. Small observation rows of these three hybrids are also established at CIAT headquarters and seed harvest is anticipated shortly (late August 2000) (Table 91).

Table 91. Seed multiplication at the CIAT-Quilichao, CIAT-Popayan, and CIAT-Palmira experimental stations. (January-September 2000).

CIAT No.	Genus	Species	Production (kg)	CIAT No.	Genus	Species	Production (kg)
16113	*Brachiaria*	*brizantha*	16.000	13086	*Desmodium*	*heterocarpon*	2.400
16116	*Brachiaria*	*brizantha*	18.500	13089	*Desmodium*	*heterocarpon*	13.400
16121	*Brachiaria*	*brizantha*	79.000	13105	*Desmodium*	*heterocarpon*	10.100
16316	*Brachiaria*	*brizantha*	15.600	13110	*Desmodium*	*heterocarpon*	2.260
16322	*Brachiaria*	*brizantha*	196.000	13651	*Desmodium*	*heterocarpon*	35.300
16327	*Brachiaria*	*brizantha*	1.050	23665	*Desmodium*	*heterocarpon*	24.700
16467	*Brachiaria*	*brizantha*	41.200	23762	*Desmodium*	*heterocarpon*	5.450
26110	*Brachiaria*	*brizantha*	14.000	33058	*Desmodium*	*heterocarpon*	11.800
26124	*Brachiaria*	*brizantha*	24.200	CPI-46562	*Desmodium*	*rensonii*	0.420
26159	*Brachiaria*	*brizantha*	34.000	ISP-1	*Indigofera*	sp.	0.630
26318	*Brachiaria*	*brizantha*	104.000	21245	*Leucaena*	*leucocephala*	2.340
26556G	*Brachiaria*	*brizantha*	83.000	21888	*Leucaena*	*leucocephala*	11.000
36060	*Brachiaria*	Hybrid	10.000	17503	*Leucaena*	*diversifolia*	0.270
36061	*Brachiaria*	Hybrid	38.000	17263	*Leucaena*	*leucocephala*	5.500
36062	*Brachiaria*	Hybrid	2.180	19235	*Rhynchosia*	*schomburgkii*	0.420
16867	*Brachiaria*	*humidicola*	0.680	20800	*Rhynchosia*	*schomburgkii*	0.135
16871	*Brachiaria*	*humidicola*	0.480	Parcela 4 (avance masal)	*Stylosanthes*	*guianensis*	0.115
26425	*Brachiaria*	*humidicola*	1.000	Parcela 5 (avance masal)	*Stylosanthes*	*guianensis*	0.040
26427	*Brachiaria*	*humidicola*	14.500	Parcela 6 (avance masal)	*Stylosanthes*	*guianensis*	0.069
16212	*Brachiaria*	*lachnantha*	0.920	288	*Vigna*	sp.	0.600
20400	*Calliandra*	*calothyrsus*	0.610	391	*Vigna*	sp.	1.080
21252	*Calliandra*	*calothyrsus*	0.510	715	*Vigna*	sp.	1.030
22320	*Calliandra*	sp.	0.120	716	*Vigna*	sp.	0.350
715	*Canavalia*	*ensiformis*	13.000	719	*Vigna*	sp.	1.750
17009	*Canavalia*	*brasiliensis*	2.700	733	*Vigna*	sp.	0.560
5924	*Centrosema*	*pascuorum*	0.380	740	*Vigna*	sp.	0.402
5126	*Centrosema*	*pubescens*	10.450	759	*Vigna*	sp.	0.360
20692	*Clitoria*	*ternatea*	6.280	1088-2	*Vigna*	sp.	0.730
18516	*Cratylia*	*argentea*	15.770	1088-4	*Vigna*	sp.	0.670
18668	*Cratylia*	*argentea*	6.655	277-2	*Vigna*	sp.	0.410
18672	*Cratylia*	*argentea*	14.890	284-2	*Vigna*	sp.	1.240
18674	*Cratylia*	*argentea*	6.770	503-1	*Vigna*	sp.	1.020
18675	*Cratylia*	*argentea*	2.480	573-5	*Vigna*	sp.	1.070
18676	*Cratylia*	*argentea*	3.370	637-1	*Vigna*	sp.	1.270
18957	*Cratylia*	*argentea*	4.930	VB-1	*Vigna*	sp.	22.000

13 genera; 70 distinct genetic materials (accessions).

4.3.2 Delivery of seed of selected forages to partners

Contributors: J. W. Miles, P. J. Argel, A. Ortega, G. Perez

Palmira Seed Unit: Our small seed multiplication unit based at CIAT-Palmira continues to serve a vital role in moving improved forage germplasm off the experimental station and on to farms. Seed volumes harvested during the reporting period, for 70 distinct accessions, are given in Table

92. A total of 1613 kg of seed was distributed during the first nine months of 2000 from the Seed Unit in Palmira. Seed of *Arachis pintoi* represented three-quarters of this total, but over all, seed of 19 different genera was distributed to 17 different countries (Table 92). In addition, a total of 56 kg of *Brachiaria* seed (three accessions) and smaller volumes of 11 additional legume accessions were delivered to CIAT personal stationed in Villavicencio for on-farm trials in the well-drained savanna and piedmont region.

Atenas Seed Unit: This Seed Multiplication Unit in Costa Rica continues also plays a vital role in moving improved forage germplasm off the experimental station and on to farms. Seed volumes harvested during the reporting period, for 70 distinct accessions, are given in Table 93. During the period August 1999-August 2000 a total of 942.71 kg of experimental and basic seed were delivered by the Seed Unit of Atenas (Costa Rica).

Table 93 shows that seventy seed requests were received from 14 countries, where more than half of the requests came from Costa Rica, the host country of the forage project. A large amount of seed was also delivered to Nicaragua, a country that is actively collaborating with the Tropileche project.

Table 92. Seed distribution (number of samples and weight (kg) during the first nine months of 2000***** (Palmira Seed Unit).

Genus	No. of Sample	Volume (kg)
Aeschynomene	1	0.02
Andropogon	2	8.20
Arachis	61	1202.24
Brachiaria	90	242.82
Calliandra	14	2.56
Canavalia	9	8.00
Centrosema	41	17.54
Codariocalyx	1	10.00
Cratylia	31	53.75
Desmodium	37	36.61
Flemingia	1	0.70
Leucaena	14	13.99
Melinis	1	0.20
Mucuna	4	1.54
Neonotonia	1	0.12
Panicum	10	0.42
Pueraria	14	2.74
Stylosanthes	35	12.22
Zornia	5	0.10

*These samples were dispatched to 17 countries [Germany (9); Argentina (14); Brazil (1); China (1); Costa Rica (14); Cuba (27); Ecuador (7); Philippines (1); Haiti (12); Holland (2); Honduras (9); Jamaica (1); Japan (4); Nepal (4); Nicaragua (11); Venezuela (6) and Colombia (250)] and five types of institutions: [CIAT (71), CORPOICA (23), Particular (77), University (59), Others (20)].

Table 93. Number of seed requests, country and amount of experimental/basic seed delivered by the Seed Unit of Atenas (Costa Rica) during the period August 1999 – August 2000.

No. of Requests	Country	Amount (kg)
45	Costa Rica	
9	Nicaragua	519.50
3	Honduras	35.38
1	Guatemala	0.40
1	Puerto Rico	25.00
1	Colombia	40.00
1	Belize	36.00
2	Haiti	0.90
1	Perú	0.16
2	Brazil	0.54
1	England	0.10
1	Philippines	0.50
1	El Salvador	6.00
1	Mexico	1.00
	Total	942.71

4.3.3 Reproductive biology of *Arachis*

Contributor: J.W Miles

Segregation of izozyme markers and flower color marker in a F2 *Arachis* population.

Crossing is being attempted between *Arachis pintoi* accessions of standard flower color and two off-type, flower-color-mutants (very light yellow, and dark yellow). Standard genetic analysis (segregation rations) will be conducted in the F_1 and F_2 generations, at least.

We are increasing the F_2 population size for an isozyme marker identified in 1998, to confirm Mendelian segregation.

A small field plot of the two contrasting isozyme phenotypes was planted to produce open-pollinated (OP) seed for assessment of natural outcrossing rate. A similar plot was established in 1998, but it was subsequently discovered that one of the parental phenotypes had been mis-identified. In the recent planting, F_2 clones were used, several per isozyme phenotype (genotype), so as to achieve a degree of uniformity of vigor and flowering between the two homozygous marker genotypes. This plot is well established and flowering. Harvest of OP seed will probably be not until early next year.

Progress towards achieving milestones

- **Increased seed supply to farmers of new grass and legume options**

The availability of experimental and basic seed of promising forage germplasm is the key component for the selection and release of new forage Cultivars to farmers. During the present year the species *Brachiaria brizantha* CIAT 26110 was released in Costa Rica as cv. Toledo, and the shrub *Cratylia argentea* CIAT 18516/18668 was registered as cv. Veraniega in the same country. In both cases basic seed was multiplied and delivered to NAR's and seed companies.

4.4 Expert systems for legume biodiversity by linking geographical information with biological data

Highlights

- Beta Version (In Spanish) of user-friendly forage database is available in CD-ROM and Intranet (selected users).
- Staff (Pre-Doc) identified for advancing work on DSS to target forages. The Pre-Doc will commence in January 2001.

4.4.1 Development of a forage database with geographical interphase

Contributors: F. Barco, A. Franco, C. Lascano, L.H. Franco, G. Ramírez and, M. Peters

Background: The Tropical Forage Program in CIAT has generated a great deal of information on the evaluation of germplasm, right from collection or exchange to the release of cultivars by national institutions. A great part of this information had been entered into an ORACLE database, which at present is available for CIAT scientists.

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 research results with partners through the Internet or via magnetic means, it is important to convert this information system to a graphical, user-friendly and attractive platform.

Methods: The programs in ORACLE FORMS 3 were converted to a tool based on Microsoft Access for utilization via CD-ROM and based on Microsoft Visual InterDev for the Internet Version, to ensure wide access of information. Target groups for the database are mainly NARS and NGO's, with initial emphasis on Latin America. In the future an English version of the database is planned.

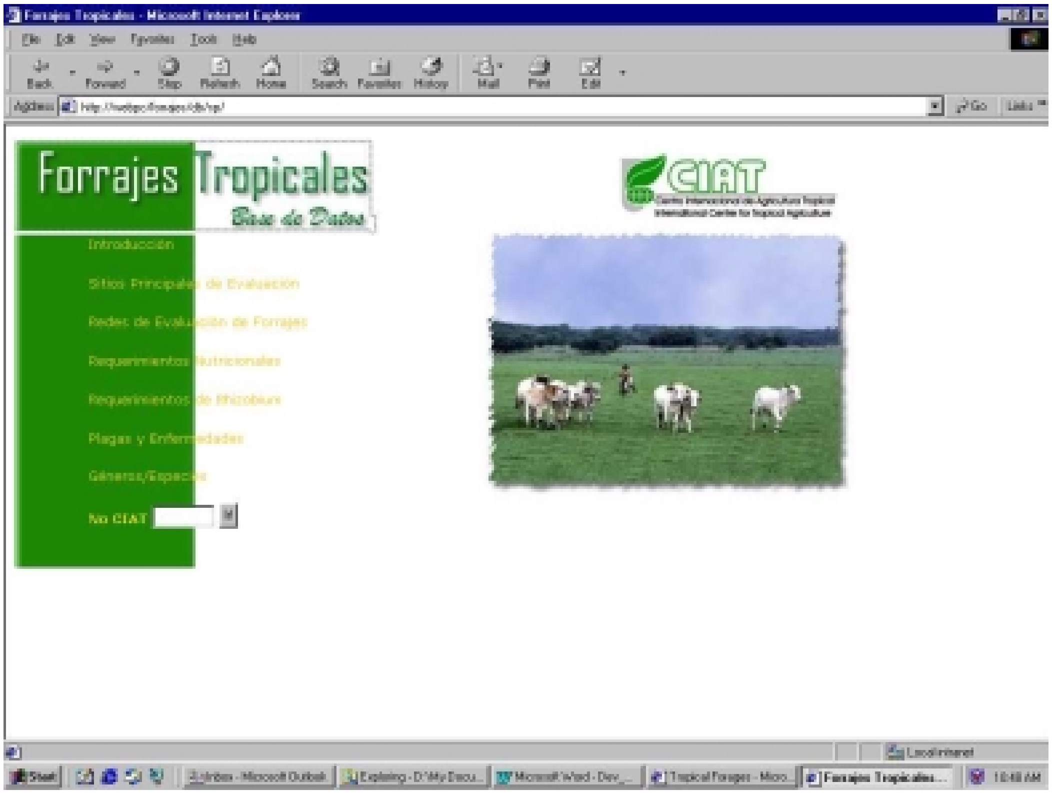

Photo 32. Screenshot of tropical forages database

Results and discussion*:* The information incorporated into the database includes information on characterization and adaptation of different forage accessions collected over the past 20 years in CIAT's main research reference sites and through networks (RIEPT).

The database contains information on agronomic characterization of 5374 accessions carried in Santander de Quilichao and Carimagua, Colombia and 2209 accession evaluated in 8 evaluation sites in the savannas, forest margins and hillsides. In addition the database includes data from over 230 sites from the RIEPT, RABAOC and *Centrosema* networks as well as data from Genotype x Environment trials of *Arachis pintoi* and *Desmodium ovalifolium.*

4.4.2 Development of a DSS to target forages based on environmental adaptation (M. Peters and GIS colleagues)

Contributors: G. Hyman, T. Oberthur, L.H. Franco, A. Franco, B. Hincapie, G. Ramirez, and M. Peters

Rationale*:* The overall approach, which intends to integrate agro-ecological, economic and social information, is based on the following two main assumptions.

1. A wealth of information on the agro-ecological adaptation of forage germplasm is available in CIAT's-held forage databases. However, the access and hence utilization of this information needs to be improved.

2. In previous evaluations of forage germplasm adaptation, to environmental conditions, the agro-ecological information is separated from socio-economic factors influencing forage germplasm adoption.

Based on these assumptions, the targeting of forage germplasm will enhance the utility of existing information and, in future, facilitate the integration of environmental adaptation of forage germplasm and of socio-economic data. 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 Latin 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).

Results and Discussion: Parameters for the description ecological adaptation and agronomic performance of accessions across environments were identified. Currently the group is in the process of revising the classification of agro-ecosystems to be utilized for the database, as the classification of agro-ecological zones developed by Thomas T. Cochrane may not be suitable for use in the GIS tool to be developed.

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.

Progress has been made in data preparation of the database underlying the GIS model and some trials have been run. For better targeting to climatic and soil conditions the classification of agro-ecosystems is being revised.

Progress towards achieving milestones

- **version of CIAT's forage database in Intranet and CD-ROM**.

A beta version in CD-ROM is now available in Spanish language. After clearing copyright issues and doing the graphic layout for publication with Nathan Russell it is intended to publish the database in CIAT's CD-ROM series and via the Internet. Linkage to other databases – i.e. FAO – will be evaluated.

4.5 Facilitate communication through newsletters, journals, workshops and internet

Highlights

- Trial version of forage web page developed
- Established link with FAO for exchange on information of forage databases, enabling access of wider audience

4.5.1 Development of a forage web page

Contributors: B. Hincapie and M. Peters

Background: It is becoming more and more important to quickly exchange information with partners and the community interested in research. We therefore embarked on developing a web page for the Tropical Grasses and Legumes Project as a source of information and an entry point for partnerships.

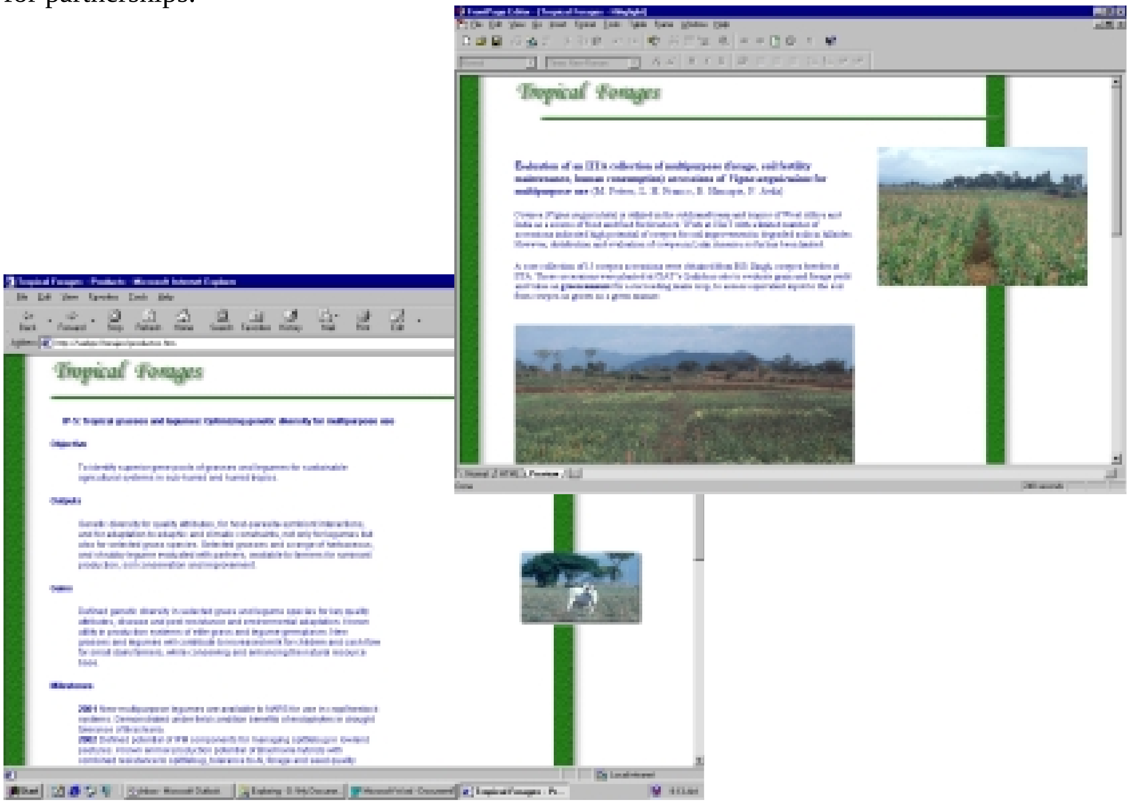

Photo 33. Screens showing a description of one Output of IP5

Methods: In 2000 we developed an attractive graphic platform, which should allow the user to obtain information on the 'What, How, and Who' of Project IP5.

The work that will follow is divided into three phases:

1) Development of a general framework established containing History, Personnel, Highlights and Products of the project. For the latter, we made some advances on a short overview of Forage Germplasm work in Output 3 of the project as a model for discussion.

2) Definition and discussion with all members of the project on the content and design of the page. Each member is than expected to provide the scientific and organizational input of the respective research area. Other potential areas for the web page include Annual reports in PDF format, a list of publications and an up-to date logframe.

3) Establishing a link to the newly developed Forage Data Base and linkages to sites of collaborators. Also to establish links to published information respective to the outputs of the project.

4.5.2 Updating FAO's Grassland index (M. Peters and Rainer Schultze-Kraft, University of Hohenheim)

Contributors: R. Schultze-Kraft (University of Hohenheim), S. Reynolds, C. Batello (FAO)

Background: The information for the existing FAO's Grassland Index was taken from two FAO publications on 'Tropical Grasses' (1990) (P.J Skerman and F. Riveros) and 'Tropical Legumes' (1988) (P.J. Skerman, D.G. Cameron and F. Riveros). Thus it was considered necessary to update this information and make information available to a wider audience via the World Wide Web. CIAT, in collaboration with the University of Hohenheim, was invited to contribute to the update of this information. The Grassland Index is accessible through FAO's web site. Links to other databases is envisaged

The U of Hohenheim and CIAT will be responsible for updating the following species: *Flemingia macrophylla, Pueraria phaseoloides, Cratylia argentea, Arachis pintoi, Desmodium ovalifolium, Centrosema pascuorum, C. brasilianum, C. macrocarpum, C. pubescens, C. acutifolium, Aeschynomene histrix, Calopogonium mucunoides, Calopogonium caeruleum* and *Mucuna pruriens.*

4.5.3 Methodologies for the evaluation and diagnosis of spittlebugs in graminoid crops (Proposal)

Contributors: Daniel Peck (CIAT)

An effective program for the integrated pest management (IPM) of spittlebugs does not yet exist, partially owing to difficult access to literature and inappropriate research methodologies. Stimulating and comparing research results on grassland spittlebugs in diverse systems will depend on the establishment and diffusion of the most recent and effective methodologies. In the last five years dramatic advances have been made in methodologies such as population sampling, rearing, and the evaluation of host plant resistance and entomopathogens.

Thus we are seeking special funds to elaborate and diffuse the newest methodological and diagnostic tools for the management of grassland spittlebugs through publication of an edited book, product of an international workshop of leading spittlebug researchers. Specialists will be united in an international workshop to discuss and establish the most effective research methodologies. The state-of-the-art will be summarized, published and diffused in an edited book. This first international conference will also serve to stimulate collaboration among diverse advanced research institutions that have contributed to improved spittlebug management. The detailed discussions will produce a methodologies book that is broad-based and includes results of the most recent research programs.

Possible themes include: biology and behavior, damage quantification, egg diapause, fungal entomopathogens, ornamentals and turfgrass, population dynamics, rearing, varietal resistance. Potential contributors represent the following institutions: CENARGEN, CNPGC, DIECA, Pontificia Universidad Católica, Roosevelt University, University of Georgia and USDA.

Outputs will provide producers, extension experts and researchers with the foundation required to advance management, and offer the materials needed to establish IPM programs in the diverse systems where spittlebugs compete with livestock or damage sugar cane. The stimulation of new research of greater quality, impact and applicability is anticipated as is the consolidation of national and regional research programs and collaborations among them. Outputs will also help researchers identify the frontiers of our understanding of this diverse group of forage pests and the barriers to overcome in achieving their integrated management.

4.5.4 Manual for the study of the biology and ecology of grassland spittlebugs (Proposal)

Collaborators: Daniel Peck (CIAT)

Difficult access to basic information and reference lists limit research advances on grassland spittlebugs. There is no book that summarizes the basic information at the family level. In addition, existing guides on grassland spittlebugs are not current, contain imprecise information, or do not consider the insect in the context of its close relatives.

Thus our objective is to seek special project funds that will allow us to broaden and publish a study guide originally developed for four workshops given in Colombia and Ecuador. The guide's focus will be bioecology, not management per se, to provide students, extensionists and researchers with the information foundation necessary to launch and carry out high impact studies.

4.5.5 Workshops

Workshop on the Bioecology and Management of Grasslands Spittlebugs

Contributors: Daniel Peck

Despite the impact of spittlebugs in forage grasses, sugar cane and other graminoid crops in the New World, there is little expertise on their biology and management outside of CIAT and EMBRAPA. Access to information is also extremely limited because there is no text that summarizes our knowledge of the family Cercopidae and existing guides to grassland spittlebugs are outdated, imprecise and ignore family level bioecology. To partially fill this gap, four workshops on the Bioecology and Management of Grassland Spittlebugs have been carried out from 1997 to 2000, three in CIAT and one in Ecuador.

The fourth workshop took place 23-27 October 2000 at CIAT. Like past events, the objective was to unite new direct collaborators of CIAT and other researchers, professors and agronomists working in forage grasses, sugar cane, or entomology. The workshop was five days of intensive lectures, labs and discussions to provide a theoretical and practical foundation on spittlebugs as insects so that they can be better interpreted as pests.

At the time of preparation of this report, the fourth workshop had confirmed participation of 21 people from 12 institutions: CENICAÑA (Centro de Investigación de la Caña de Azùcar de Colombia), CIAT, CINCAE (Centro de Investigaciones sobre la Caña de Azúcar del Ecuador), CORPOICA (C.I. Carimagua, C.I. Tibaitatá), In Cauca, S.A. (commercial sugar cane company), Laverlam, S.A. (commercial biologicals company), Universidad de la Amazonia, Universidad de Cauca, Universidad del Pacífico at Buenaventura, Universidad de Sucre, and Universidad Nacional at Medellin and Palmira.

Workshop on use and utility of C*alliandra* in Central America

Contributors: C. E. Lascano, P. J. Argel, J. Stewart (OFI, UK)

Summary: A three- day workshop was held this year (May 17 to 19, 2000) in the ECAG facilities in Atenas, Costa Rica to discuss and disseminate the research results from a CIAT- OFI collaborative Project mainly among members of the CIAT Tropileche consortium, and others working with fodder trees in Central America (Bolivia was also represented). The Pacific (seasonally dry) Central America is seen as the most suitable niche for this species within the region. However, there is no traditional use of tree fodder in this area, and research is still at the stage of on-farm testing of various species. Future activities would continue this process; it is too soon to promote Calliandra widely as its utility to farmers is not yet proven. The extent of its drought tolerance is a major concern and this was the area identified as needing further research before *Calliandra* can be confidently promoted as dry season fodder for Central America. CIAT and CATIE also undertook to scale up seed production of the best provenances in preparation for wider future dissemination of *Calliandra*. The situation in Bolivia (Santa Cruz) is different, with a much longer history of on-farm fodder tree research and adoption. This was the only country represented where dissemination, uptake studies etc would be appropriate at this time.

Workshop Structure: The workshop comprised three parts: presentations by participants about their experiences with *Calliandra* (day 1); a field visit to two farms participating in the Tropileche project (day 2); and working groups to define necessary actions for the future (day 3). Instead of the planned formation of two working groups to discuss, respectively, appropriate dissemination activities and future research needs, working groups were formed on a country-by-country basis, and concentrated on on-farm research and promotion. The reasons for this are discussed below.

Use of trees for fodder in Central America: Several important points emerged during discussions on the first day of the workshop. First and foremost was the current very low level of utilization of *Calliandra*, or indeed any woody legume, as fodder in Central America, despite past and present activities in this area by a range of institutions and projects. The participants felt that it was important to distinguish between humid and seasonally dry areas. CATIE's efforts in Costa Rica have been largely limited to the humid (Atlantic) zone, where human and animal population densities are relatively low, systems are extensive, and fodder shortage is not widely perceived as a problem. Tropileche works specifically in the dry (Pacific) zone, and sees the use of woody legumes as a strategy specifically to alleviate perceived dry season fodder shortages.

Dry season tolerance of *Calliandra*: Given the consensus that the potential for woody legumes in forage production lies mainly in the drier parts of Central America, the next issue of concern was the drought tolerance of *Calliandra*. Most natural populations occur in areas with only four months (or less) dry season, and moreover many populations are riverine, so probably have access to a high water table. The areas where woody legumes are thought to have most potential as fodder are those with a harsh dry season of up to 5-6 months. It is likely that most *Calliandra* provenances would not tolerate this level of drought, or at least would not maintain useful levels of production under these conditions. Only one accession of *Calliandra calothyrsus*, of unknown provenance, has been tested in the CIAT forage germplasm plots at ECAG (where the dry season lasts 5-6 months), and this died out after two years. In view of this there is a need to revisit the OFI provenance trial network data and look for evidence of drought tolerance in trials. There was also interest in further testing of *C. houstoniana* and *C. grandiflora* since they may have more drought tolerance than *C. calothyrsus*.

Forage Quality of *Calliandra*: The quality (nutritive value) of *Calliandra* leaves, as fodder remains a controversial issue, with opinion divided among researchers regarding the nutritional benefit of including Calliandra in ruminant diets. Low and variable values have been reported for in vitro dry matter digestibility (IVDMD), as well as problems with nitrogen utilization, probably due to very high levels of condensed tannin. Important findings from the research conducted by CIAT are reported in another section of this Annual Report (Output 1; Activity 1.2.2)

Progress towards achieving milestones

- **Developed a Web page of CIAT's Forage Project**

A format of a web page had been elaborated, which will be discussed and refined by all members of CIAT's Forage Project. Assigning responsibilities to members of the forage team on information gathering for inclusion in the web page will follow this consultation process. We will adapt the web page to a CIAT agreed common format once available.

- **Defined with partners strategies needed for R& D of *Calliandra* in Central America**

Participants in a consultation workshop held in Costa Rica agreed that the most appropriate strategy for *Calliandra* utilization was as fodder for cattle, but used at a moderate level (around 25-30% of the diet), and as one of a range of species in a system, rather than as the only supplement. Fed as one component of a mixture, its high tannin levels could even improve the utilisation of other species (e.g. *Cratylia argentea*, whose N may be too rapidly degraded in the rumen).

However, in view of the very limited utilisation of tree fodder in general in Central America, other uses of *Calliandra* should also be considered as part of a promotion strategy. In Costa Rica, for instance, there is currently much interest in agroforestry interventions for soil improvement and rehabilitation of degraded lands, and *Calliandra* has been identified as a valuable mulch/soil improvement species because of the slow rate of nutrient release from the leaves (due again to their high tannin content). The research on differences between Patulul and San Ramón provenances has relevance to this: San Ramón leaf has been shown by (non-FRP) research at CIAT to have a slower rate of decomposition than Patulous, and therefore to be preferable for this use, whereas Patulul is superior for fodder. It was felt that these provenances should be tested in parallel for soil amelioration and fodder respectively.

Publications

Journal Papers

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List of Donors

Donor/Project	**Project duration**
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