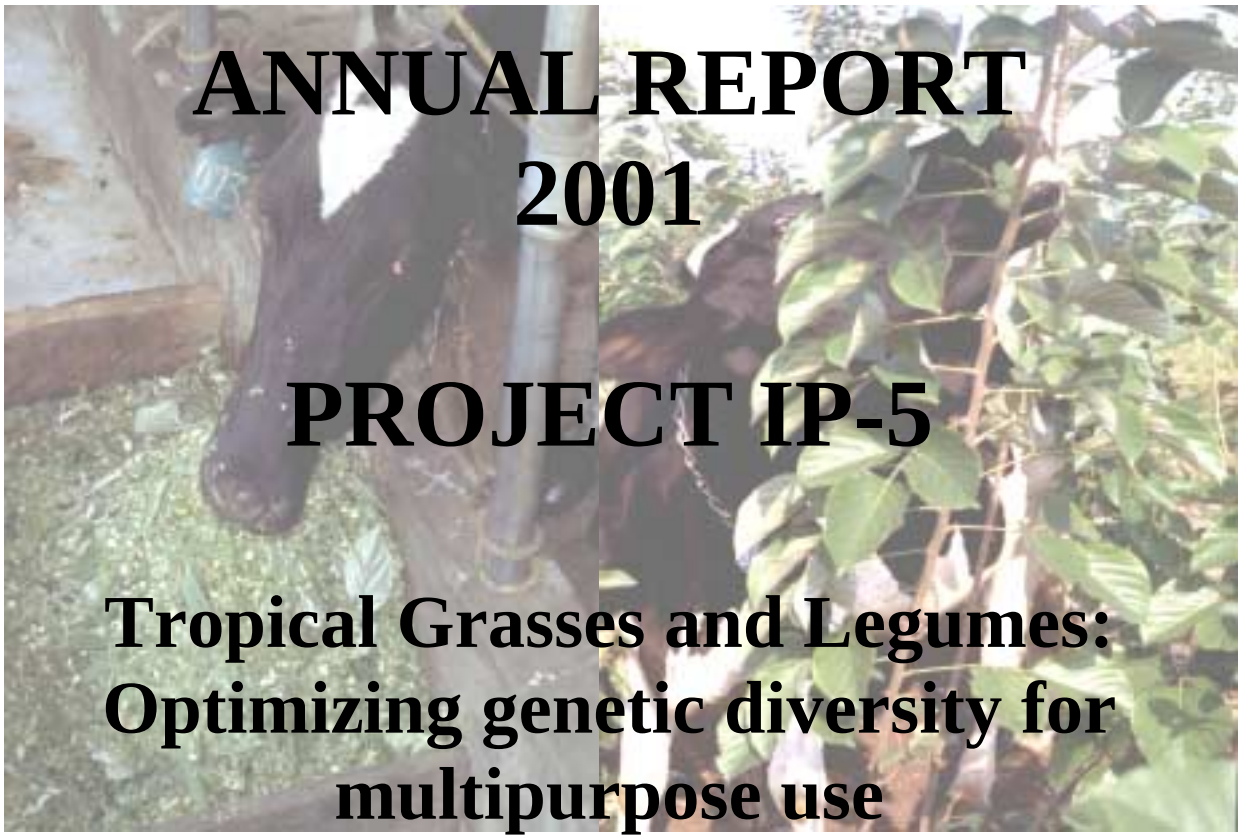

ANNUAL REPORT 2001

PROJECT IP-5

Tropical Grasses and Legumes: Optimizing genetic diversity for multipurpose use

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Tropical Grasses and Legumes: Optimizing Genetic Diversity for Multipurpose Use (Project IP5)

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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:

1. 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 grasses.
2. Selected grasses and a range of herbaceous and shrubby legumes evaluated with partners, available to farmers for ruminant production and for 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 supplies to children and cash flow for small dairy farmers, while conserving and enhancing the natural resource base.

Milestones:

2002 Defined potential of IPM components for managing spittlebug in lowland pastures. Known animal production potential of *Brachiaria* hybrids with resistance to spittlebug.

2003 Methods and tools available to enhance targeting and adoption of multipurpose forage germplasm in smallholder production systems in the hillsides of Central America. *Brachiaria* hybrids with resistance to spittlebug are released to farmers.

2004 Multipurpose legumes validated for use in priority crop livestock systems. Prototype field management systems designed for enhancing endophytes' role in drought tolerance of *Brachiaria* species.

Users: Governmental, nongovernmental, and farmer organizations throughout the subhumid and humid tropics who 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; SROs (Univ. of Hohenheim, Cornell Univ., IGER, OFI, CSIRO).

CGIAR system linkages: Enhancement & Breeding (20%); Livestock Production Systems (15%); Protecting the Environment (15%); Saving Biodiversity (40%); Strengthening NARS (10%). Participates in the Systemwide Livestock Program (ILRI).

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

PROJECT WORK BREAKDOWN STRUCTURE

Project Purpose

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 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 attributes • Assesment of quality and animal production potential of selected legumes • Assessment of the potential of saponin-rich tropical fruits to reduce methane in ruminants on grass diets • Assessment of quality and animal production potential of selected grass species • Adjustment of methods for the simultaneous evaluation of tropical legumes for feed and soil improvement	• Bioecology of spittlebug species in contrasting environments • IPM components for spittlebug management • *Brachiaria* genotypes resistant to spittlebug and other biotic stresses • Identify host mechanisms for spittlebug resistance in *Brachiaria* • Genetic control and molecular markers for spittlebug and reproductive mode in *Brachiaria* • Role of endophytes in tropical grasses • Define interactions between host and pathogen in *Brachiaria*, *Arachis*, and *Stylosanthes* • Antifungal compounds isolated from seeds of tropical forage legumes	• Genotypes of *Brachiaria*,*Panicum*, and *Arachis* with adaptation to edaphic and climatic factors • Genotypes of grasses and legumes with dry season tolerance • Shrub legumes with adaptation to drought and cool temperatures • Selection of legumes for multipurpose use in different agroecosystems	• Development of 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 in crop and livestock systems • Forage seeds: reproductive biology, quality, multiplication, and delivery of experimental and basic seed • Expert systems for forage biodiversity linking geographical information with biological data • Facilitate communication through Newsletters, Journals, Workshops and Internet

Revised Project Log-Frame
(2001)

CIAT
Area: **Genetic Resources Research**
Project: **IP-5 - Tropical Grasses and Legumes: Optimizing Genetic Diversity for Multipurpose Use** **Project 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. • Raised productivity of livestock and 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 Southeast Asia	Policies are put in place by governments to favor sustainable livestock and forage development in marginal areas occupied by small farmers
Purpose To help NARS use superior grasses and legumes to develop improved and sustainable livestock and crop production systems in humid and subhumid areas.	Demonstrated economical and ecological benefits of multipurpose grasses and legumes to livestock and crop farmers in savannas, forest margins, and hillsides	• Range of variation in desirable traits • Performance of forage components in systems	• Support from traditional and nontraditional donors • Effective collaboration: • IAT's Projects • AROs, NARS, NGOs
Outputs 1. Grass and legume genotypes with high quality attributes are developed.	• Utility of different accessions of *Cratylia* under direct grazing by milking cows known by 2002. • New *Brachiaria* genotypes with superior forage quality are made available to NARS for improved animal performance by 2003..	• 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 interaction with symbiont organisms are developed.	• Known diversity of *Colletotrichum gloeosporioides* is used by NARS to develop and select resistant genotpes of *Stylosanthes* by 2002. • Benefits of endophytes (biotic [against pests and diseases] and abiotic [against drought]) demonstrated under field conditions by 2002. • QTL's for resistance to spittlebug and high aluminum in the soil in *Brachiaria* are available for marker-assisted selection by 2003. • *Brachiaria* genetic recombinants with combined resistance to different species of spittlebug are available to NARS by 2003.	• On-farm demonstrations • Scientific publications • Annual Reports • Theses	• Effective collaboration with: • CIAT Projects (SB1, SB2) • AROs, NARS and farmer groups
3. Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed.	• New *Brachiaria, Paspalum, Leucaena, Calliandra, Desmodium,* and *Arachis* accessions with adaptation to major abiotic constraints (low fertility soils, drought, poor drainage, and cool temperatures) are available to NARS by 2002. • Improved accessions of *Vigna* and *Lablab* with adaptation and known value to farmers in hillsides of Central America are available to NARS by 2003. • *Brachiaria* genetic recombinants with resistance to high aluminum in the soil and with drought tolerance are available to NARS by 2004.	• On-farm demonstrations • Scientific publications • Annual Reports • Theses	• Effective collaboration with: • CIAT Projects (SB1, PE2, PE4, PE5) • AROs, NARS, NGOs, farmer groups
4. Superior and diverse grasses and legumes delivered to NARS partners are evaluated and released to farmers.	• New grass and legume cultivars released by NARS are available to farmers by 2002. • Improved multipurpose grasses and legumes result in increased on-farm milk, beef, and crop production in benchmark sites (hillsides and forest margins) by 2003.	Surveys on adoption impact of new grasses and legumes: • Seed sold • Area planted • Production parameters • Environmental/socioeconomic 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 (2001-2002)**		
1.1 Selection of *Brachiaria* genotypes for high digestibility and other quality attributes (CEL, JWM) 1.1.1 Calibration and utilization of NIRS to screen *Brachiaria* hybrids for digestibility 1.1.2 Screening of selected ***Brachiaria*** accessions and hybrids for saponins	• Efficient and reliable protocol for screening *Brachiaria* hybrids for digestibility	• List of *Brachiaria* hybrids with known digestibility • Annual Report	• Effective collaboration with the plant breeder and Biotechnology staff • Human and financial resources for developing markers are available in the Biiotechnology Unit
1.2 Assessment of quality and animal production potential of selected legumes (CEL) 1.2.1 Effect of tannins with different structure on degradation of rubisco 1.2.2 Effect of feeding *Calliandra* with diferent tannin structure on N utilization by sheep 1.2.3 Milk production of cows supplemented with sun-dried and fresh *Calliandra*	• Known effects of tannins with different chemical structure on N utilization by ruminants • Known value of *Calliandra* as a protein supplement for milking cows	• Established grazing trial in CIAT's Quilichao Research Station • MS Thesis • Manuscripts for publication • Annual Report	• Availability of milking cows from neighboring farm in CIAT Quilichao
1.3 Assessment of the potential of saponin-rich tropical fruits to reduce methane in ruminants on grass diets (Dhess, CEL) 1.3.1 In vitro evaluation of the potential of saponin-rich tropical fruits to manipulate rumen fermentation and to reduce methanogenesis 1.3.2 In vitro evaluation of the potential of semi-purified saponins from *Sapindus saponaria* to manipulate rumen fermentation and to reduce methanogenesis 1.3.3 In vitro evaluation of fruits of *Sapindus saponaria* in relation to semi-purified saponins and saponin-free diets on their effect on rumen fermentation and methane release 1.3.4 In vitro evaluation of the effect of varying proportions of *Arachis pintoi* in a basal diet of a low quality grass on rumen fermentation	• Known utility of saponin-rich plants and semi-purified saponins to reduce methane by ruminants	• Annual Report	• Availability of fruits of *Sapindus saponaria*
1.4 Assessment of quality and animal production potential of selected grass species (CEL) 1.4.1 Milk yield with new hybrids of *Brachiaria*	• Benefits in animal production of new *Brachiaria* hybrids relative to commercial cultivars	• Established grazing trial in in CIAT's Quilichao Res. Station • Apparatus for continuous in vitro fermentation (RUSITEC) in Forage Quality Lab • Annual Report	• Availability of milking cows from neighboring farm in CIAT Quilichao • Access to an atomic absorption equipment of the Soils group

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
1.5 Adjustment of methods for the simultaneous evaluation of tropical legumes for feed and soil improvement (K. Tscherning, EB, MP, RJT and CEL) 1.5.1 Assessment of the effect of species and drying method on aerobic and anaerobic decomposition of legumes	• Established correlation between in vitro anaerobic systems and aerobic soil-based systems in the decomposition of legumes with contrasting quality	• Greenhouse experiments on degradation of cuttings from different legume species • Annual Report	• Effective collaboration with staff in PE2 and IP5
2.1 Bioecology of spittlebug species in contrasting environments (DCP) 2.1.1 Biology and habits of *Mahanarva andigena* 2.1.2 Biology and habits of *Prosapia simulans* 2.1.3 Population dynamics and phenology of *Prosapia simulans* 2.1.4 Population dynamics and phenology of *Zulia carbonaria* 2.1.5 First generation population phenology in two lowlands sites 2.1.6 Preoviposition determinants of egg diapause 2.1.7 Seasonal changes in the incidence and duration of egg diapause	• Defined variation in the biology and abundance of spittlebug species in Colombia	• Manuscripts on biology and taxonomic description • Annual Report	• Collaboration maintained with CORPOICA (CI-La Libertad) with U. of Sucre • Conditions on security allow travel to sites in Colombia
2.2 IPM components for spittlebug management (DCP) 2.2.1 Artificial diet for maintenace of spittlebug adults 2.2.2 Maintenance of a ceparium for fungal entomopathogens of major forage grass and cassava pest 2.2.3 Variation in the virulence of fungal entomopathogens among spittlebug species 2.2.4 Characterization and formulation of select fungal entomopathogens isolates for field evaluation 2.2.5 Field evaluation of fungal entomo-pathogens in two contrasting regions	• IPM components relevant to spittlebug management in forage grasses and other graminoids better understood	• List of characterized fungal isolates • Papers submitted for publication • Annual Report	• Collaboration maintained with U. of Amazonia • Agreement formalized with BioCaribe • Condition on security allow travel to sites in Colombia
2.3 *Brachiaria* genotypes resistant to spittlebug and other biotic stresses (JWM, CC, SK) 2.3.1 Development of new hybrid population for spittlebug screening using pollen from a resistant parent (AP) and selected clones (SX) as maternals 2.3.2 Identify *Brachiaria* genotypes resistant to different species of spittlebug 2.3.3 Greenhouse screening of *Brachiaria*	• Produced new *Brachiaria* hybrids with selected sexual clones and different pollen parents. • Identified new *Brachiaria* hybrids for spittlebug screening based on field performance • Identified parental clones for field evaluation of recombined progeny • Identified *Brachiaria* hybrids with resistance to *Rhizoctonia* foliar blight	• List of *Brachiaria* hybrids with antibiotic resistance to spittlebug under glasshouse and field conditions • List of *Brachiaria* hybrids with resistance to Rhizoctonia foliar blight • Annual Report	• Continued collaboration of CORPOICA-Macagual for field screening of hybrids for spittlebug resistance • Additional funds to support the *Brachiaria* improvement Program identified • *Brachiaria* hybrids available for screening for Rhizoctonia reaction

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
accessions and hybrids for resistance to *Aeneolamia* species 2.3.4 Field screening of *Brachiaria* accessions and hybrids for resistance to spittlebug 2.3.5 Screening of *Brachiaria* hybrids for *Rhizoctonia* foliar blight			
2.4 Identify host mechanisms for spittlebug resistance in *Brachiaria* (CC, JWM) 2.4.1 Studies on resistance to spittlebug species	• Defined reaction of new *Brachiaria* hybrids to different species of spittlebug	• Manuscripts on mechanisms of resistance to spittlebug complex, and on improved methodology for field screening of genotypes • Annual Report	• Effective flow of *Brachiaria* genetic recombinants for screening for spittlebug resistance • New funds are identified for field screening for spittlebug resistance • Additional funds are available to support work on antibiotic effects of *Brachiaria* to spittlebug • Additional funds to support the *Brachiaria* Improvement Program identified
2.5 Genetic control and molecular markers for spittlebug and reproductive mode in *Brachiaria* (JWM, CC, JT) 2.5.1 Reproductive mode of new *Brachiaria* hybrids (SX x AP) 2.5.2 Construction of a molecular genetic map of *Brachiaria* and QTL analysis of spittlebug resistance	• Apomictic *Brachiaria* hybrid selections identified on the basis of progeny testing	• List of apomictic hybrids • Annual Report	• Funds and staff for developing molecular markers are available
2.6 Role of endophytes in tropical grasses (SK) 2.6.1 Endophyte seed transmission studies in *Brachiaria* 2.6.2 Synthesis of endophyte specific DNA fragment for quick detection of endophytes 2.6.3 Genetic diversity of isolates of endophytic fungi from *Brachiaria*, and search for new endophytes in hybrids of *Brachiaria* 2.6.4 Effect of endophyte on *Rhizoctonia solani* (in vivo)	• Isolated and characterized new isolates of endophytes • Synthesized and tested endophyte-specific primer • Defined effect of a new endophyte isolate on *Rhizoctonia solani*	• List of new endophyte ioslates • Annual Report	• Collaboration in NZ will deliver work on alkaloids • Enough plants containing endophytes will be obtained on time for drought studies in the field • Enough seeds will be collected from endophyte-infected *Brachiaria* for seed transmission studies
2.7 Define interactions between host and pathogen in *Brachiaria, Arachis* and *Stylosanthes* (SK) 2.7.1 Biodiversity studies on the anthracnose pathogen of *Stylosanthes* 2.7.2 Epidemiology studies on the anthracnose pathogen of *Stylosanthes* 2.7.3 Characterization of transgenic *Stylosanthes* plants with chitinase gene 2.7.4 Bacterial blight of *Brachiaria* caused by	• Determined *Colletotrichum gloeosporioides* diversity at a field in Quilichao using RAPD and AFLP • Defined the inheritance of a rice chitinase gene in *Stylosanthes guianensis* • Defined resistance of transgenic *S. guianensis* to *Rhizoctonia* foliar blight • Identified sources of resistance to *Rhizoctonia* foliar blight in *Brachiaria* hybrids	• Manuscript on Rhizoctonia bacterial blight of *Brachiaria*	• A replacement weather station arrives from Carimagua in good working condition for anthracnose epidemiology work • Proposal will be approved and funds will be made available for fruit anthracnose work • Collaborators at CORPOICA provide pathogen isolates

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Xanthomonas campestris pv. *graminis*: Isolate collection, pathogenicity evaluation and seed transmission			
2.8 Antifungal compounds isolated from seeds of tropical forage legumes (SK) 2.8.1 Screening of tropical forage legume collection for antifungal properties	• Detected antifungal compounds in seeds of tropical forage legumes	• List of legume species with antifungal properties • Annual Report	• Antifungal properties are present in legumes
3.1 Genotypes of *Brachiaria, Panicum,* and *Arachis* with adaptation to edaphic and climatic factors (IMR, JWM) 3.1.1 Development of 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 adaptation in *Brachiaria* cultivars and development of screening methods 3.1.2.1 Identification of Al-resistant *Brachiaria* hybrids 3.1.2.2 Identification of genetic recombinants of *Brachiaria* with tolerance to low nutrient supply 3.1.2.3 Field evaluation of promising hybrids of *Brachiaria* in the Llanos of Colombia 3.1.2.4 Screening accessiones of *Brachiaria humidicola* for suppression of nitrification and nitrous oxide emission from soil 3.1.3 Differences in phosphorus acquisition from less available phosphorus forms in an oxisol as determined by isotope exchange kinetics 3.1.4 Studies on genotypic variation in *Arachis pintoi* for tolerance to low phosphorus supply 3.1.4.1 Field evaluation of most promising accessions of *Arachis pintoi* in the Llanos of Colombia	• New *Brachiaria* sexual hybrids with Al resistance identified • *Brachiaria* hybrids with superior performance under low soil fertility identified • Ability to suppress nitrification and emission of nitrous oxide by *Brachiaria humidicola* quantified • Field experiment with promising accessions of *Arachis pintoi* established in the Llanos	• List of *Brachiaria* hybrids adapted to high Al • Annual Report	• Increased support from technician to carryout field trials in the Llanos • Additional funds to support the *Brachiaria* Improvement Program identified • Continued collaboration with the U. of Gottingen, Germany and with JIRCAS, Japan
3.2 Genotypes of grasses and legumes with dry season tolerance (IMR and JWM-Matazul; PJA-Atenas) 3.2.1 Determination of the genotypic variation in dry season tolerance in *Brachiaria* accessions and genetic recombinants in the Llanos of Colombia 3.2.2 Determination of the genotypic variation in dry season tolerance in *Brachiaria* and *Arachis* in Costa Rica	• *Brachiaria* accessions and hybrids with superior tolerance to drought relative to commercial cultivars identified • *Arachis* accessions with superior tolerance to drought identified • Advanced in the development of an improved screening method to evaluate drought tolerance in *Brachiaria*	• List of *Brachiaria* accessions and hybrids with superior drought tolerance • List of accessions of *A. pintoi* with superior adaptation to dry season tolerance • Annual Report	• Additional funds to support the *Brachiaria* Improvement Program identified • Germplasm import into Nicaragua is possible (agreement INTA-CIAT)

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
3.3 Shrub legumes with adaptation to drought and cool temperatures (MP, PJA, M. Andersson, JT, A. Schmidt, E. Barrios) 3.3.1 Characterization of a core collection of *Cratylia argentea* and *Leucaena* in a subhumid environment of Costa Rica 3.3.2 Genetic diversity in the multipurpose shrub legumes *Flemingia macrophylla* and *Cratylia argentea* 3.3.3 Agronomic characterization of a collection of *Rhynchosia schomburgkii*	• List of new accessions of *Cratylia argentea* and *Leucaena* species with known forage value • List of *Flemingia* acessions characterized for yield and quality	• List of accessions of *Cratylia* adapted to acid soils and with superior adaptation to severe drought • List of promising accessions of *Rhynchosia* • Annual Report	• Continued collaboration with the U. of Hohenheim • Effective collaboration with SB2 Project
3.4 Selection of legumes for multipurpose use in different agroecosystems (MP, PJA, AS) 3.4.1 Evaluation of a core collection of *Vigna unguiculata* for multipurpose uses in Colombia, Nicaragua and Honduras 3.4.2 Evaluation of a core collection of *Lablab purpureus* for multipurpose uses (Quilichao and Palmira)	• Suitability of *Vigna unguiculata* for acid and neutral soils defined • List of accessions of *Vigna unguiculata* for use as feed and/or green manure in Central America • Results on characterization of a core collection of *Lablab purpureus* in acid and neutral soils	• List of accessions of *Vigna* and *Lablab* with adaptation to contrasting soils • Annual Report	• Effective collaboration with MAG and ECAG • Germplasm import into Nicaragua is possible (agreement INTA-CIAT)
4.1 Development of 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 in forage evaluation with partners 4.1.1.1 Use of forages for recuperation of degraded areas in hillsides of Colombia 4.1.1.2 Evaluation of legumes as covers in plantations in the Llanos of Colombia 4.1.1.3 Evaluation of green manures in the Llanos of Colombia 4.1.1.4 On-farm evaluation of new grasses and legumes options for livestock systems in the Llanos of Colombia 4.1.1.5 Ex-ante analysis of the utility of *Cratylia argentea* in dual-purpose production systems of the llanos piedmont of Colombia 4.1.1.6 Analysis of intensification of milk production systems in Colombia 4.1.1.7 Participatory evaluation of forages for multipurpose use in Haiti 4.1.2 Releases and adoption by farmers of new forage species 4.1.2.1 Release of *Cratylia argentea* as cv. Veraniega by MAG in Costa Rica	• *Cratylia* released and available to farmers in Costa Rica • Suitable legume covers for plantations in the Llanos of Colombia defined • Selected grass and legume species for on-farm testing in Hillsides of Haiti	• Release Bulletin of *Cratylia* in Costa Rica • Annual Report	• Effective collaboration on forage evaluation with partners is maintained • Pronatta funds for on-farm evaluation of *Cratylia* in the Llanos Piedmont are available by mid year

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
4.1.2.2 Opportunities and constraints to adoption of *Cratylia argentea* in Costa Rica 4.1.2.3 Adoption of *Arachis pintoi* in Costa Rica 4.1.3 Development of new collaborative research proposals with NARS, NGO's, IARC's and ARIS 4.1.3.1 New initiatives for the evaluation and promotion of multipurpose forages, with focus in Africa			
4.2 Evaluation with farmer participation of multipurpose forages for crop and livestock systems (PJA, MP, AS, LAF) 4.2.1 Development of participatory methods to enhance adoption of forages as feed resources for INRM (MP) 4.2.2 Evaluation of forages for multipurpose use with farmer participation in Hillsides of Central America (MP) 4.2.3 On-farm evaluation of selected forages as feed resources in dual cattle systems of Central America through the Tropileche Consortium (PJA) 4.2.3.1 Grazing of *Leucaena leucocephala* by lactating cows and young calves in a dual purpose cattle system located in a sub-humid area of Costa Rica 4.2.4 Alternative strategies for on-farm forage seed production 4.2.5 On-farm evaluation of green manures in Hillsides of Nicaragua	• Model for participatory selection of forages developed • Known value of different legume green manures for crop production in Hillsides of Central America • Known value of *Leucaena* to supplement milking cows and pre-weaved	• List of grasses and legumes selected in participatory selection • List of grass and legume seed distributed to and by farmers in hillsides of Central America • Annual Report	• Unusual drought and problems with landlord at SOL-San Dionisio • Seed available • Agreement for renting of land and constant availability of animals
4.3 Forage seeds: reproductive biology, quality, multiplication and delivery of experimental and basic seed (JWM, PJA) 4.3.1 Multiplication and delivery of selected grasses and legumes in the Seed Units of Atenas and Palmira (JWM, PJA) 4.3.1.1 Seed Unit Atenas (PJA) 4.3.1.2 Seed Unit of Palmira (JWM) 4.3.1.3 The effect of storage conditions on viability and germination of acid-scarified and non-scarified seeds of *Brachiaria brizantha* cv. Toledo (CIAT 26110)	• Seed of selected forages delivered to partners and seed companies	• List of accessions of grasses and legumes multiplied and delivered • Annual Report	• Effective collaboration with CIAT Projects, NARS, NGO's nad farmer groups • Funding available from BMZ and Tropileche Projects • Adequate climate conditions to harvest seed in Costa Rica

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
4.4 Expert systems for forage biodiversity linking geographical information with biological data 4.4.1 Development of a forage database with graphical interphase (MP, MAF) 4.4.2 Incorporating Socio-economic data and Expert knowledge in representations of Complex Spatial Decision-Making (R. O'Brien, MP, GIS Staff)	• Forage database published in a CD-ROM and distributed • Developed a conceptual framework for a Decision Support System (DSS)	• CD-ROM with CIAT's Forage database • Annual Report	• Effective collaboration with Staff in GIS
4.5 Facilitate communication through Newsletters, Journals, Workshops and Internet 4.5.1 Development of a Forage Web Page (IP5 Staff) 4.5.2 Information and technology transfer for spittlebug management in graminoids (DCP) 4.5.2.1 Workshop in Guatemala on the Bioecology and Management of spittlebugs in graminoid 4.5.2.2 Reference collection and on-line bibliography of the Cercopoidea 4.5.3 Training course activities	• Research information produced by CIAT's Forage team made available to partners • During 2001 we carried out workshops and training courses in Guatemala, Haiti and Colombia on bioecology of spittlebug, principles of forage evaluation and progress made in forage development in the Llanos, respectively.	• Published numbers of Pasturas Tropicales • Annual Report	• Funds to continue funding Pasturas Tropicales are identified • Security in Colombia for carrying out field days

Research Highlights

- **Tannins in legumes with different chemical structure affect N utilization by ruminants**

To address questions related to the feed value of *Calliandra calothyrsus* we carried out detailed characterization of the chemical composition of the edible forage of two provenances (CIAT 22310 and CIAT 22316) harvested in two field locations with contrasting soil fertility. An interesting finding was that chemical composition (the ratio of procyanidin: prodelphinidin) of the soluble condensed tannin fraction varied between provenances regardless of location. Soluble tannins in CIAT 22316 were comprised mainly by procyanidin subunits, whereas the soluble tannin fraction in CIAT 22310 was composed largely of prodelphinidin subunits.

We initially associated the different tannin composition found in the two *Calliandra* provenances with differences in astringency or ability of tannins to bind protein. To further understand the biological significance of the different monomers found in the tannin fraction of provenances of *Calliandra* we carried out a feeding trial with sheep housed in metabolic crates. Results showed that estimates of absolute and relative values of escape dietary N in sheep were significantly greater with *Calliandra* 22310 than with *Calliandra* 22316, which is consistent with laboratory results.

Thus for the first time we have evidence that monomer composition of condensed tannins in a tropical legume can have an effect on the utilization of N by ruminants. The fact that our in vivo results were in close agreement with results on astringency of soluble CT extracted from *Calliandra* provenances is also a major finding, since they validate the utility of laboratory astringency tests for screening tropical legumes with tannins for quality traits.

- **Supplementing saponin-rich fruits from trees resulted in reduction of protozoa count and methane production in an anaerobic in vitro fermentation system**

The issue of global warming caused by anthropogenic greenhouse gases is of increasing concern. Ruminant animals have a considerable significance on global warming since they contribute 1/ 6 of the total atmospheric methane. Thus efforts to mitigate methane emissions from ruminants and other sources are urgent given that atmospheric methane concentration is increasing at a faster rate than CO_2. In addition, per molecule, methane is 21 times more potent as a greenhouse than CO_2, even though it has a relatively shorter half-life. To address the issue of methane production by ruminants we are participating in an SDC- ZIL- funded project that involves the Institute of Animal Science of the Swiss Federal Institute of Technology (ETHZ), Zurich, the U. Nacional in Bogotá and CORPOICA. The aim of the project is to develop feeding strategies based on locally available feed resources such as saponin -rich tropical fruits to reduce methane emissions from ruminants and simultaneously improve feed use efficiency in tropical smallholder livestock systems.

In vitro experiments were carried out to compare the effects of inclusion of tropical fruits from trees with saponins (*Sapindus saponaria*, *Enterolobium cyclocarpum*, *Pithecellobium saman*) on in vitro methane production. Results demonstrated that the fruits of the tropical tree *S. saponaria* when included at a level of 8 to 10% in forage-based diets reduced methane release by 10 to 20%. Since the depression of methanogenesis was also found in defaunated rumen fluid, it can be assumed that saponins or possibly other constituents of *S. saponaria* act directly against methanogens. The challenges ahead are to determine if saponins and/or other constituents in fruits of *S. saponaria* are responsible for depressing methane release and to define the effect of supplementing fruits of *S. saponaria* to ruminants in terms of methane production and N utilization.

- **The grass *Brachiaria humidicola* suppresses nitrification and nitrous oxide emissions by inhibiting the activity of ammonium oxidizing bacteria in the soil**

In a process known as nitrification, ammonium- N is transformed into nitrite- N and nitrate – N by soil microorganisms. Nitrification leads to substantial losses of applied N fertilizer through N_2O emissions and runoff/leaching of NO_3. Previous work carried out at CIAT had shown that *B. humidicola*, widely adapted lowland agroecosystems, has the ability to suppress nitrification in the soil, but for reasons not well understood. Through a collaborative project JIRCAS- CIAT, Japanese scientists undertook the task of determining the mechanisms through which *B. humidicola* suppresses nitrification.

Three grasses (*B. decumbens*, *B. humidicola* and *M. minutiflora*) grown in pots and fertilized with ammonium- N, where used to measure root exudates and to carry out soil nitrification studies. Results showed that ammonia-oxidizing bacteria (AOB) were nearly 10 times less in soils where *B. humidicola* had grown. In addition, N_2O emission during the nitrification study was 6 times less with *B. humidicola* than with the other two grasses. Thus our results strongly support the idea that *B. humidicola* has the ability to suppress nitrification by inhibiting the biological activity of AOB bacteria. The challenge ahead is to screen CIAT's collection of *B. humidicola* (62 accessions) to determine if there is genetic variability for inhibition of nitrification and reduction of nitrous oxide emissions.

- **Virulence of fungal entomapathogens isolates varied among spittlebug species**

A major challenge for the implementation of integrated management plan for grassland spittlebugs is the taxonomic diversity of species that contribute to this pest complex. We are obtaining new information on the biology and ecology of major species of spittlebug in contrasting ecoregions and this information is broadening our options to develop management strategies of the pest in different ecoregions.

This year we continued to make progress in the development of options to manage spittlebugs through IPM components. This research includes the screening entomopathogens for variation in virulence across spittlebug life stages and species. For three strains of *Metarhizium* selected for high virulence to *A. varia* (86-95% adult mortality), mortality was significantly reduced for *A. reducta* (42-62%), *Z. carbonaria* (20-31%) and *Z. pubescens* 16-30%).

These results confirm the need to continue documenting the pattern of variation among grassland spittlebugs given that effectiveness of control tactics such as insect pathogens may be more specific than anticipated. Significant variation in host/ plant resistance among spittlebug species is further corroboration of this need.

- **Selected new *Brachiaria* hybrids with resistance to different species of spittlebug**

Recent evidence had indicated that a significant genotype-species interaction existed for the reaction of *Brachiaria* to different spittlebug species. As a result screening *Brachiaria* hybrids developed in the Breeding Program with only one species (*A. varia*) of spittlebug may not be sufficient to characterize the performance in the field of a given genotype exposed to different species of the pest alone or in combination. Thus this year we screened 41sexual *Brachiaria* clones with high levels of resistance to *A. varia*, with 3 spittlebug species. Results showed that out of 41 *Brachiaria* hybrids included in the test, 15 (36 %) were resistant to 3 species of spittlebug (*A. varia, Z. carbonaria* and *Z. pubescens*) in terms of reduced damage level and that 3 hybrids showed antibiosis resistance (reduced nymph survival) to all spittlebug species tested.

In another experiment we evaluated the resistance of the same set of 41 sexual *Brachiaria* hybrids to *A. varia* and to *A. reducta*, which is the most important spittlebug species affecting grasses in the north coast of Colombia. Results showed that the 3 *Brachiaria* hybrids that had showed antibiosis resistance to *A. varia*, *Z. carbonaria* and *Z. pubescens* also showed high levels of antibiosis resistance to *A. reducta*. These are key results for defining future crosses in the Brachiaria Breeding Program.

- **Presence of endophytic fungus in a *Brachiaria* hybrid may contribute to resistance to *Rhizoctonia* foliar blight**

We had earlier found that *Brachiaria brizantha* CIAT 16320 had high and stable resistance to *Rhizoctonia* foliar blight. Subsequently, we isolated two endophytes from this *Brachiaria* accession Thus we were interested in evaluating the effect of eliminating with a fungicide (Folicur) the natural occurring endophytes in CIAT 16320 on reaction to *Rhizoctonia* foliar blight in the laboratory. Results showed that extracts from CIAT 16320 (+ endophytes) produced inhibition zones, while extracts from the treated CIAT 16320 (- endophytes) did not. Consequently, we now postulate that the high level of resistance of CIAT 16320 to *Rhizoctonia* foliar blight is due to presence of endophytes. Studies are in progress to assess the reaction of treated and non- treated CIAT 16320 live plants to inoculation with *Rhizoctonia.*

- **Selected sexual *Brachiaria* hybrids with greater level of Al resistance than the sexual parents**

Last year we implemented a screening procedure to identify Al- resistant *Brachiaria* hybrids with resistance to spittlebug. This year a total of 46 *Brachiaria* genotypes were screened for Al resistance and results showed that two sexual *Brachiaria* hybrids had higher level of Al resistance than the sexual parent. These sexual hybrids are currently being used in the breeding program to combine spittlebug resistance with high level of Al resistance.

The challenge ahead is to develop a screening method to identify *Brachiaria* hybrids with adaptation to low P supply in the soil. One option is to adapt a methodology used in a collaborative CIAT- ETH, Zurich project to assess the amount of P derived in grasses from applied carrier and from P derived from sources such as soil and seed. Using this methodology we found that *B. decumbens* cv. Basilisk, which is one of the parents in the breeding program, can acquire less available forms of P in the soil compared with *Agrostis capillaris* (control plant with no adaptation to low P).

- **Identified variability in seasonal yield and in quality in a core collection of *Flemingia macrophylla***

One major objective of the Forage Group in CIAT is to develop shrub legumes for regions with acid soils and long dry seasons. The legume *Flemingia macrophylla* is known to be well adapted to acid- low fertility soil, but the use of this legume as a feed resource is limited by low forage quality of the accessions that have been evaluated and promoted.

Currently we are investigating the genetic variability in a core collection (73 accessions) of *F. macrophylla* using conventional agronomic evaluation procedures and molecular markers (AFLP's). One initial result has been a map that shows that the potential natural distribution of *F. macrophylla* based on climate and latitude/longitude extends throughout vast areas of tropical Asia. In addition, agronomic data has shown large variation among accessions in dry matter yield in the dry (1 to 91 g/plant) and in the wet (8 to 184 g/plant) seasons. Finally, we found large variation among accessions of *F. macrophylla* in leaf in vitro digestibility (31 to 51 %) and crude protein (16 to 24%) content. Based on these results we selected the accession CIAT 21090 as one having the highest yields and digestibility for seed multiplication and regional testing.

- **Selected new legume and grass options for different production alternatives in the Llanos of Colombia**

Results this year confirm higher soil cover with *D. ovalifolium* CIAT 13651 in rubber and oil palm plantations and lower establishment and maintenance costs as compared with Arachis or Kudzu (the traditional cover used in the area). In addition, after two year of introducing small quantities (250 g of seed /ha) of *D. ovalifolium* CIAT 13651 in a degraded *Brachiaria* pasture, legume content is high (20%) and pasture productivity is high. Given the low cost and large benefits of *D. ovalifolium* to recuperate degraded pastures, some farmers have began to use the legume to recuperate degraded pastures in well-drained savannas and in the piedmont. The challenge ahead is to promote the use of *D. ovalifolium* CIAT 13651 by making seed available to private seed companies.

A number of new grass options are being evaluated with farmer participation in the Llanos of Colombia with the support of the Colombian Government. A total of 5 *Brachiaria brizantha* accessions are being evaluated under grazing in a well -drained acid savanna site. As a result of feedback from farmers who have evaluated the performance of the grasses, we pre- selected *B. brizantha* CIAT 26124 on the following positive attributes: a) high leaf content, b) soft leaves and c) fast recovery after grazing. The next step is to multiply basic seed of this accession to expand on-farm evaluation in the llanos.

- **Advanced on participatory evaluation of forages in Hillsides of Central America**

We continue to make progress in the participatory evaluation of forages in hillsides of Central America. A glossary of local terminology used by livestock farmers was completed to facilitate communication of farmers with technicians. From forage nurseries established in Honduras farmers (88) selected a number of grasses (*P. maximum* CIAT 16031, *B. brizantha* CIAT 26110), shrub legumes (*Cratylia argentea* CIAT 18668, *Leucaena leucocephala* CIAT 17263) and to a lesser extent herbaceous legumes (*Stylosanthes guianensis* CIAT 184, *Lablab purpureus*) for testing and seed multiplication. In Nicaragua, on farm trials with legume green manure were established in 8 sites where maize systems are predominant. In Costa Rica, we are evaluating *Cratylia argentea* planted as live barriers in steep hillsides to reduce erosion and to provide forage to feed confined livestock.

Output 1: Grasses and legumes genotypes with high quality attributes are developed

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

Highlights

- Confirmed that the NIRS equation developed in the Forage Quality Laboratory gives reliable predictions of in vitro digestibility in large *Brachiaria* hybrids population
- Found high correlation between saponin activity in *Brachiaria* samples harvested in two successive years.
- Found *Brachiaria* hybrids with high and low saponin activity indicating that there is scope for selecting for this attribute.

Progress towards achieving milestones

- **Efficient and reliable protocol for screening *Brachiaria* hybrids for digestibility**
  We calibrated the NIRS to measure digestibility in large number of hybrids generated in the Brachiaria Improvement Program and the resulting equation predicts in vitro digestibility with high precision. However, we have not been able to get consistent digestibility results between successive samplings of the same *Brachiaria* hybrid population and as a result selection for digestibility is still not part of the improvement program. Thus, we still need to define a sampling procedure in *Brachiaria* hybrids to improve the correlation between samplings.

1.1.1 Calibration and utilization of NIRS to screen *Brachiaria* hybrids for digestibility

Contributors: P. Avila, C. Lascano, J. W. Miles and G. Ramírez (CIAT)

Rationale

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

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

A justification for this strategy had been that with the current in vitro system in the Forage Quality Laboratory it 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.

In 1999 we developed a narrow – based NIRS equation and when applied found that the 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 Tilley and Terry in vitro procedure.

Validation of NIRS to predict in vitro digestibility

In 2000, we tested the NIRS calibration curve with leaves of 176 *Brachiaria* hybrids that form part of a population (tetraploid *B. ruziziensis* x *B. brizantha* cv. Marandu) used to develop molecular markers for digestibility. Results showed a high correlation (r= 0.84) between observed and values of IVDMD estimated using NIRS.

This year we were interested in determining the effect of age of plant material on the precision of the NIRS equation to estimate IVDMD in *Brachiaria* and in confirming the precision of the NIRS equation we developed. Thus we sampled the same population (144 entries) following a 7 and 10 week regrowth period and run the samples through a two- stage Tilley and Terry in vitro system. Samples we are also read with the NIRS and results correlated with the in vitro values obtained in the laboratory.

The in vitro digestibility values ranged from 68 to 80 % and from 71 to 83 % in samples of 51 and 71 days of regrowth, respectively, which indicate little effect of maturity on IVDMD. Similar correlations were observed between IVDMD values from the laboratory and values predicted with NIRS in the two sets of samples (Table 1).

Table 1. Correlation between IVDMD values of *Brachiaria* hybrids measured in the laboratory and values estimated using NIRS.

Sampling	No of Samples	Days of regrowth	r	SEP*
1	144	51	0.73	1.5
2	144	71	0.80	1.2

*Standard error of prediction

These results confirm that the NIRS equation we have developed to screen Brachiaria hybrids for IVDMD is adequate given the high correlation with IVDMD values measured in the laboratory and relatively low SE of predicted values.

Last year we reported a very low correlation between IVDMD values obtained in samplings of the same Brachiaria population in different times. These disappointing results affected our ability to relate microsatallites markers to digestibility, which is one of our main objectives.

We had postulated that the main problem we were facing had to do with sampling of the material in the greenhouse and with processing of the harvested material. Individual plants had been harvested after 5 or 10 months regrowth and then freeze dried. Leaves were then separated from stem before grinding in Willey Mill fitted with a 1 mm screen. Thus we thought that separation of leaf from stem was not resulting in uniform material across samplings and that the grinding process was not producing samples with uniform particle sizes.

The modified sampling procedure used was as follows:

1. Take samples (leaf and stem) from individual plants growing in large pots and fertilized with N following a 5 to 7 regrowth
2. Dry samples in a oven at 60^{o}C for 24 hours
3. Grind samples in small laboratory mill

This year, three successive samplings of the same mapping population used before were carried out using the new sampling scheme. All samples were analyzed for IVDMD using the Tilley and Terry method. Results showed higher correlations between samplings than reported previously, but still low (r= 0.4 to 0.5).

It is possible that a small change between samplings in leaf: stem ratio of individual plants is affecting the IVDMD values we are recording. Thus to obtain consist results between samplings we are going to probably need to sample the last expanded leaves in each plant, which is what we will do next year.

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

Contributors: P. Avila, C. Lascano, J. W. Miles and G. Ramírez (CIAT)

Rationale

A wide spread, but sporadic, toxicity syndrome associated with *Brachiaria decumbens* is hepatogenous photosensitization, which can cause severe losses in LWG and in some cases death, particularly young with young animals. This syndrome has been related to infestation of the grass by the saprophytic fungus *P. chartarum*, which produces spores thought to 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 show toxicity do not produce sporidesmin and b) steroidal saponins were isolated for the rumen contents of poisoned animals fed *Brachiaria* and that steroidal saponins have been identified in plants know to cause photosensitization.

Based on the hypothesis that saponins are responsible for photosensitization in *Brachiaria decumbens* last year we determined the presence or absence of these compounds in accessions and hybrids of *Brachiaria*. Results (See AR 2000) indicated 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 included in the assay was also variable, ranging from very high (absorbance of 786 to 893) to low (absorbance of 230 to 344). However, among the hybrids included in the assay we did not find any with very low saponin activity.

Thus this year were interested in verifying last years results and in determining saponin- like activity in *Brachiaria* hybrids chosen at random from a *B. brizantha* cv Marandu (apomictic) x *B. ruziziensis* (sexual) population used for developing molecular markers for spittlebug, apomixes and digestibility.

Materials and Methods

A large (>230 sibs), bi-parental (tetraploidized, sexual *B. ruziziensis*-x-*B. brizantha* CIAT 6294 [cv. Marandu]) F_1 hybrid population (full-sib family) was produced and propagated for determinations of saponin content. Unintentional maternal selfs were identified by isozyme analysis, and eliminated.

The laboratory procedure used to estimate saponin activity in the test forage samples is based on the 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 and Discussion

A high and significant correlation ($r = 0.96$; $P < 0.001$) was found between Absorbance value recorded in 2000 and in 2001. However, results shown in Table 2 indicate that the absorbance values recorded in the same accessions and hybrids of *Brachiaria* harvested in 2000 and 2001 were similar in magnitude in some groups but not in others.

Table 2. Saponin like activity in *Brachiaria* accessions and hybrids recorded in samples harvested in two consecutive years.

Classification	Forage Sample	Absorbance (2000)	Absorbance (2001)	Significance
	Entorolubium ciclocarpum (+ Control)	936	762	NS
Very High	*B. decumbens* 606			
	Hybrids:	843	695	**
	1084-3			
	1084-10	794	631	**
		890	808	**
	B. ruziziensis 26164	614	630	NS
High	Hybrids			
	1092-5	696	624	NS
	1092-15	708	520	**
	Hybrids			
Intermediate	1092-14	459	451	NS
	1092-2	478	491	NS
	1092-3	460	589	**
	1092-11	540	462	**
	1094-15		332	
	1094-19		478	
	Hybrids			
Low	1092-1	356	251	**
	1092-4	232	195	**
	1092-13	245	196	**
	1083-7		149	
	Wheat Straw (– Control)	40	28	**
Very Low	*B. humidicola* 16871	54	49	NS
	B. brizantha 6780	64	56	**
	Hybrids:			
	1078-31		28	
	1079-18		30	
	1080- 8		29	
	1081-25		31	
	1093- 31		31	
	1095- 4		29	
	1097- 6		33	

**P<0.01

Absorbance values were greater in samples harvested in 2000 and subjectively classified as very high, high and low, which was not the case for samples classified as intermediate and very low. The reason for this discrepancy is not known, but could be related to differences in maturity of the leaf tissues used for the assay since no attempt was made to harvest samples of the same maturity.

As reported last year, saponin activity was high in the commercial cultivar *B. decumbens* CIAT 606 but low in *B. humidicola* and in *B. brizantha* cv Marandu (CIAT 6780), which is the source of spittlebug resistance in the Brachiaria breeding program. Results from this year also showed that saponin activity in new *Brachiaria* hybrids was more variable than what was found last year. Out of 10 new hybrids included in the test, 7 had very low saponin activity and similar to what was recorded in one of the parents (*B. brizantha* CIAT 6789) used in the cross.

It would seem from the results of this and last year, 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. The high concentration of saponins in *B. decumbens* is consistent with the observations of photosensitization in cattle and sheep fed with this grass.

We conclude from these results that there is justification to screen *Brachiaria* hybrids for saponins. However, before we make this commitment we need to adapt a laboratory screening procedure that is fast, reliable and that allow us to quantify the exact concentration of saponins present. Thus a future priority is to establish collaboration with an advanced laboratory investigate the chemical nature of saponins in *Brachiaria* and to investigate alternative laboratory procedure for quantifying saponins that is more accurate and less time consuming than the qualitative method presently used. Meanwhile we will screen for saponins the elite hybrids selected on the basis of spittlebug resistance and adaptation to high soil Al.

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

Highlights

- Using Rubisco as a protein source in an vitro fermentation test we can estimate how the concentration of tannins in tropical legume species could affect the rate and extent of plant - protein degradation in the rumen.
- Differences in tannin structure in *Calliandra* provenances were associated with different proportion of N escaping degradation in the rumen and reaching the duodenum of sheep.
- Supplementing sun-dried or fresh *Calliandra* did not result in milk yield increments of cows grazing a well-managed pasture of *B. decumbens.*

Progress towards achieving milestones

- **Known effect of tannin with different chemical structure on N utilization by ruminants**
  Differences in monomer composition in two *Calliandra* provenances was shown to be associated with the proportion of N escaping degradation in the rumen and reaching the duodenum of sheep. A higher proportion of delphinidin relative to cyanidin in one *Calliandra* provenance resulted in a higher proportion of N consumed escaping the rumen and reaching the lower GI tract, which is consistent with what had been predicted using laboratory astringency assays. These results should now be validated in practical feeding systems where *Calliandra* forage is offered alone to ruminants as a protein supplement.

- **Known value of *Calliandra* as a protein supplement for milking cows**
  We completed a series of experiments with sheep supplemented with *Calliandra* and results indicate differences in quality between provenances, and large effect of soil fertility and drying on intake and digestibility of *Calliandra*. However, we were not able to demonstrate that supplementing fresh or sun-dried *Calliandra* to cows grazing a Brachiaria pasture resulted in more milk yield.

1.2.1 Effect of tannins with different structure on degradation of Rubisco

Contributors: N. Narvaez, C. Lascano and G. Ramírez (CIAT)

Rationale

Previous results had shown that the chemical structure of condensed tannins in tropical legumes could vary among species and that this in turn affected the biological activity of tannins. Purified tannins with high delphinidin: cyanidin ratio extracted form *Calliandra* provenances were more reactive (astringent) with protein than tannins with a high cyanidin: delphinidin ratio.

This year we were interested in defining the significance of different chemical structures of tannins in tropical legumes on N utilization by sheep. Results from a feeding trial are reported in the activity (1.2.3) that follows. In addition, we were interested in defining how in vitro fermentation parameters changed with tannins with different chemical structures and consequently be useful to screen tropical legume for quality.

Materials and Methods

Four woody legume species (*Leucaena leucocephala, Calliandra calothyrsus, Clitoria fairchildiana* and *Bauhinia* sp.) were selected to carry out the study given that they had tannins with different monomer composition (Table 3).

Condensed tannins were extracted from leaf tissues of four legume species using a 70% aqueous acetone solution with ascorbic acid (0.1% w/v). Tannins in the liquid phase following centrifugation were extracted using diethyl ether and ethyl acetate. After evaporation of the organic solvent, samples were freeze-dried and the resulting solid phase was dissolved with 50% aqueous methanol. Tannins were then purified using a column (25 x 5 cm) with 60 ml of LH-20 Sephadex in suspension. A 50% aqueous methanol solution was used to wash out the column and purified tannins were recovered using a 70% aqueous acetone solution.

The ratio of pro-anthocyanidins was measured by hydrolyzing tannins of each legume with Butanol-Hcl (5%). An aliquot (1 ml) of Butane extracted tannins was evaporated and the resulting pro-anthocyanidins were put back in solution with pure methanol + 1% Hcl. The ratio of delphinidin: cyanidin: pelargonidin was then determined using an HPLC fitted with an 8 x 10 cm Nova Pack C 18 Column.

Table 3. Monomer composition of condensed tannins extracted from different tropical woody legumes

Legume	Monomer Composition (%)		
	Pro-Delphinidin	Pro-Cyanidin	Pro-Pelargonadin
Leucaena leucocephala	51.0	45.1	3.9
Calliandra calothyrsus	13.1	50.5	36.4
Clitoria fairchildiana	12.4	87.6	0
Bauhinia sp.	3.4	65.4	31.2

Rubisco was the protein used to form tannin complexes with tannins from extracted from the test legumes. To form the tannin – protein complex 0.1 g of Rubisco was placed in 100 ml centrifuge tubes with 2 ml of the tannins solution (McDougal Buffer) that corresponded to 5 or 10 % concentration. The Rubisco-Tannin mixture was left over night at 39^{o}C before adding 50 ml of the rumen liquor- artificial saliva solution used in the in vitro fermentation system. The rate of degradation by rumen microorganisms of

Rubisco-Tannin complexes was measured stopping fermentation at 6,9, 12, 24, 36 and 48 h. The extent of degradation of Rubisco-Tannin complex was measured weighing the residues after 48 h of incubation with rumen microorganisms.

Results and Discussion

The chemical composition of tannins extracted from the test legumes is shown in Table 3. With the exception of *L. leucocephala*, all species had tannins with more cyanidin relative to delphinidin. It was also interesting to observe that tannins in both *Calliandra* and *Bauhinia* had relatively large proportions of pelargonadin.

Differences in monomer composition of tannins should be associated with their biological properties, such as capacity to bind protein. Results on the effects of tannin monomer composition and tannin level on ammonia production and in vitro DM differences are shown in Table 4.

The addition of tannins from different legumes to Rubisco resulted in a significant reduction of dry matter disappearance and to a lesser extent ammonia N production relative to Rubisco alone (positive control), regardless of legume species or tannin level. A similar trend was observed with rate of DM disappearance and rate of ammonia production.

As expected, both the extent and rates of DM disappearance and ammonia N production were lower when the concentration of tannins was increased. However, it was interesting to observe that with *L. leucocephala* the effect of doubling the concentration of tannins was small in terms of DM disappearance at 48 h as compared to the other three legume species tested. This in turn was associated with a higher delphinidin: cyanidin ratio in L. *leucocephala* as compared to the other species.

Table 4. Extent and rate of in vitro ammonia N production and DM degradation of different Rubisco – Tannin complexes.

Legume	Level of CT (%)	Extent of Degradation of Rubisco-Tannin Complex (48 h)		Rate of Degradation of Rubisco-Tannin Complex	
		NH3-N Production (mg/l)	In vitro DM Disappearance (%)	NH3-N Production (mg/h)	In vitro DM Disappearance (%/h)
Rubisco (Positive Control)	0	24.8	83.5	0.35	1.16
Leucaena leucocephala	5	21.7	68.2	0.35	0.91
	10	20.9	66.5	0.30	0.72
	5	22.7	71.0	0.34	0.87
Calliandra calothyrsus	10	21.6	64.1	0.29	0.66
	5	23.5	80.1	0.36	0.90
Clitoria fairchildania	10	22.7	68.8	0.32	0.74
	5	22.6	73.3	0.35	0.77
Bauihnia sp	10	21.7	63.1	0.31	0.64
Significance					
Legume (Tannin Composition)		0.01	0.01	0.01	0.01
Tannin Level		0.01	0.01	0.01	0.01
Specie x Tannin Level		NS	NS	NS	NS

In *Calliandra calothyrsus* we found that the astringency of tannins was more related to monomer composition than to concentration. Tannins from provenances with higher delphinidin: cyanidin ratio were more astringent than tannins with higher cyanidin: delphinidin ratio. Thus our results suggest that astringency of tannins from L. *leucocephala* is more related to its high delphinidin: cyanidin ratio than to

concentration, which is not the case with the other legume species tested that had higher cyanidin: delphinidin ratios.

In general, our results indicate that using Rubisco as a protein source in an vitro fermentation test we can define how the concentration of tannins in tropical legume species can potentially affect the rate and extent plant - protein degradation in the rumen.

1.2.2 Effect of feeding *Calliandra* with different tannin structure on N utilization by sheep

Contributors: P. Avila, C. Lascano, G. Ramírez (CIAT) and J. Stewart (OFI, UK)

Rationale

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* (*Calliandra*) as a part of a collaborative OFI, UK – CIAT, Colombia project funded by DFID, UK.

It is well documented that *Calliandra*, 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.

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 two field locations (CIAT) (Quilichao and Palmira) with contrasting soil fertility. An interesting finding was that the chemical composition or structure (the ratio of procyanidin: prodelphinidin) of the soluble CT fraction varied between provenances regardless of location. 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

The different tannin composition 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 the location where the plants grew. 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 were 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 cyanidin, which is consistent with what we have found in the laboratory with the two *Calliandra* provenances evaluated.

In order to assess the biological significance of the different monomer composition found in the tannin fraction of two provenances of *Calliandra* grown in two sites with contrasting soil fertility (Quilichao with acid infertile soils and Palmira with Fertile soils), we carried out a feeding trial with sheep housed in metabolic crates.

Materials and Methods

African type sheep (6) fitted with rumen and duodenal cannulas were assigned to one of four treatments in an Unbalanced Simple Crossover Design, where:

T1: *Calliandra* CIAT 22310 grown in Quilichao,
T2: *Calliandra* 22316 grown in Quilichao,
T3: *Calliandra* CIAT 22310 grown in Palmira, and
T4: *Calliandra* CIAT 22316 grown in Palmira.

Animals housed in individual metabolism crates were offered daily 50 g DM / kg $BW^{.0.75}$ (1.7 % of body weight) of sun- dried edible (leaves + fine stems) *Calliandra* forage in two meals (8:00 and 15:00). The 6 animals used in the trial were distributed in the four treatments (T) and across experimental Periods (P) as follows:

Animals	Periods			
	P1	P2	P3	P4
3	T4	T2	T1	T3
5	T2	T4	T3	T1
2	T2	T3	T1	T4
6	T4	T3	T2	T1
4	T2	T1	T4	T3
1	T1	T3	T4	T2

Animals were supplemented via rumen cannula with 4 g/ kg of BW/ d of a high-energy source (extracted cassava meal) with negligible protein concentration and offered a mineral mix ad-lib and water 4 times a day. Sheep used in the trial were allowed to graze for one week between experimental periods, which had duration of 17 days, of which 7 were for adjustment and 10 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. 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 soluble condensed tannins (CT) using the Butanol Hcl method. In addition, forage (offered and refused) and fecal samples were analyzed for N, and fiber (NDF and ADF). Concentration of indigestible acid detergent fiber (IADF) in the forage (offered and refused) 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 an Unbalanced Simple Crossover Design with 4 periods and the Duncan's test was used to compare treatment means.

Results and Discussion

Chemical characterization of *Calliandra*: Results on chemical composition of the forage of *Calliandra* offered are shown in Table 5. The concentration of crude protein (CP) was lower in *Calliandr*a harvested

in Quilichao than in Palmira but on average did not differ between provenances. In contrast, fiber (NDF and ADF) concentration differed between *Calliandra* provenances, being higher in CIAT 22310 than in CIAT 22316, regardless of site.

In other studies carried out in CIAT, fiber content measured as NDF was also higher in CIAT 22310 than in CIAT 22316, and 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. The higher fiber concentration in CIAT 22310 was associated with lower in vitro digestibility (IVDMD) as compared with CIAT 22316. It was also observed that IVDMD of *Calliandra* was lower in the edible forage harvested in the site with acid soils (Quilichao) than in the site with fertile soil (Palmira), but these differences due to site were not explained by differences in fiber content.

Table 5. Chemical characterization of *Calliandra calothyrsus* provenances fed to sheep Housed in metabolism crates

Calliandra Provenances (CIAT No. and Site)	Crude Protein	In Vitro Digestibility	Neutral Detergent Fiber (% of DM)	Acid Detergent Fiber	Condensed Tannins Soluble	Condensed Tannins Insoluble
22310- Quilichao	15.5 b	20.1 c	35.0 a	32.1 a	28.3 b	6.3
22310- Palmira	17.0 a	32.2 b	36.7 a	29.6 a	18.0 c	4.4
22316- Quilichao	13.5 c	32.8 b	27.4 b	24.0 b	35.4 a	4.0
22316- Palmira	18.0 a	39.9 a	31.1 b	24.9 b	33.6 a	3.6
SEM	0.4	0.6	0.8	0.9	0.5	0.5
Significance (P) <						
Provenance	NS	0.0001	0.0001	0.0001	0.0001	NS
Site	0.0001	0.0001	0.01	NS	0.0004	NS
Provenance x Site	0.0036	0.004	NS	NS	0.003	NS

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

The concentration of soluble condensed tannins (CT) in the forage offered to sheep was higher in CIAT 22316 as compared to CIAT 22310, but the insoluble CT fraction did not change due to provenance (Table 5), which is in agreement with previous findings of CIAT and OFI. However, it was interesting to observe that soluble CT in CIAT 22310 was considerably lower in the edible forage harvested in Palmira as compared to Quilichao. Although the soluble CT fraction was higher in CIAT 22316, IVDMD was also higher with this provenance possibly as a result of having less fiber. Similar results were recorded in other feeding trials where the two provenances were used as supplements to sheep fed a low quality grass diet. These results are interesting since they confirm previous findings that suggest that digestibility of *Calliandra* forage is more related to fiber content than to the concentration of soluble CT.

Intake and digestibility: Results for intake and digestibility are presented in Table 6 for main effects given that the interaction of site x provenance was not significant for the variables measured. Intake of the two *Calliandra* provenances was very low when expressed as proportion of body weight (range 0.5 to 1.2 kg DM/ 100 Kg of BW). The amount of *Calliandra* consumed represented 41% and 63% of the amount offered daily of CIAT 22316 and CIAT 22310, respectively. It was also evident that intake of *Calliandra* was affected by location. Intake of forage harvested in Palmira (fertile soil) was 58 % higher than the intake from forage harvested in Quilichao (infertile soil).

On the other hand, DM intake of *Calliandra* CIAT 22130 was 45% higher than intake of CIAT 22316, which was an unexpected result. In previous feeding trials with sheep we had observed that intake of *Calliandra* CIAT 22316 was higher than *Calliandra* 22130 when fed in combination with a poor quality grass, and this was related to the lower fiber content in CIAT 22136. However, it should be noted that

intake of digestible DM was affected by site and not by provenance as a result of the higher digestibility of *Calliandra* CIAT 22316 as compared with CIAT 22130. The large site effect observed on DM intake of *Calliandra* in this study is consistent with previous results and points out the major influence that soil fertility has on the overall quality of *Calliandra*.

Table 6. Intake and digestibility of *Calliandra calothyrsus* fed to sheep housed in metabolism crates and supplemented with extracted cassava meal[1]

Item	Site Effect		Provenance Effect		SEM
	Quilichao	Palmira	CIAT 22310	CIAT 22316	
Intake of DM (g/kg of BW/d)	6.7 b	10.6 a	10.2 a	7.1 b	0.55
Digestibility of DM (%)	57.8	53.7	51.7 b	59.8 a	2.10
Digestibility of NDF (%)	50.1	50.9	45.5 b	55.6 a	3.60
Intake of Digestible DM (g/kg of BW/d)	3.6 b	5.4 a	5.1	4.0	0.37

a, b, c for each main effect, values in the same row with the same letters are not different (P<0.05)
[1]4 g/ kg of BW/ d of extracted cassava meal was fed via rumen cannula to each sheep

Digestibility (DM and NDF) values were influenced by provenance but not by site (Table 6). In both sites the digestibility of dry matter and fiber were higher with CIAT 22316 than with CIAT 22310, which can be explained by lower fiber content.

Nitrogen utilization: The main objective of running this feeding trial was to test the hypothesis that the higher proportion of delphinidin relative to cyanidin found in the soluble CT fraction of CIAT 22310 would result in greater protection of protein in the rumen (by-pass protein) as compared with CIAT 22316 with soluble CT comprised mainly by procyanidin subunits.

Results shown in Table 7 are again for main effects, given that we did not find a provenance x site interaction for the response variables measured. Total N intake was 44% higher with *Calliandra* CIAT 22310 than with CIAT 22316. In addition, N intake was 85% higher when *Calliandra* harvested in Palmira was fed. These differences are the result of the higher DM intake of *Calliandra* 22310 and of the forage harvested in the fertile soils of Palmira.

Flow of different N fractions to the lower GI tract was affected by provenance fed and by site. A higher amount of total N (solid phase) and non-ammonia non-microbial N (solid phase) reached the duodenum when CIAT 22310 and when *Calliandra* grown in Palmira were fed. On the other hand, estimates of absolute and relative values of escape dietary N were significantly greater when *Calliandra* 22310 was fed, which is consistent with in vitro results that had shown greater astringency of the soluble CT of this provenance relative to CIAT 22316. It could be argued that the greater amount of N reaching the duodenum when *Calliandra* CIAT 22310 was fed is the result of the higher N intake when this provenance was fed.

However, when the N reaching the duodenum was expressed as a proportion of N fed, results also showed more N from *Calliandra* CIAT 22310 by-passing or escaping the rumen as compared with CIAT 22316 (Table 7). The greater level of escape N observed with *Calliandra* 22130 was not only associated with more fecal N but also with more apparently absorbed N, which was associated with the higher N intake recorded when this provenance was fed. It is important to note that the proportion of N absorbed as a proportion of N intake was similar (76 % for CIAT 22136 and 88% for CIAT 22310) for the two provenances.

In previous feeding trials carried out in CIAT we had fed sheep the two *Calliandra* provenances used in this trial in combination with a low quality grass. Results from those feeding trials had shown 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. Specifically we found that intake of *Calliandra* CIAT 22316 was higher than intake of CIAT 22310 and that intake of *Calliandra* was improved when the forage was fed dried as opposed to fresh, which is contrary to common belief. The results from this feeding trial contradict previous results, given that DM and N intake of Calliandra CIAT 22310 when fed alone was greater than when CIAT 22316 was fed. However, it should be pointed out that intake of digestible dry matter was similar for the two provenances given the higher digestibility of CIAT 22316 associated with lower fiber content.

Table 7. Nitrogen (N) utilization by sheep housed in metabolic crates and fed two provenances of *Calliandra calothyrsus* grown in contrasting sites[1].

Item	Site Effect		Provenance Effect		SEM
	Quilichao	Palmira	CIAT 22310	CIAT 22316	
N intake, g/d	6.7 b	12.4 a	10.8 a	8.3 b	0.67
Duodenal N, g/d	12.5 b	18.6 a	18.6 b	12.5 a	1.26
NAMNmic- N [2], g/d	5.5 b	9.5 a	11.0 a	4.0 b	1.27
Fecal N, g/d	5.7 b	9.7 a	9.1 a	6.2 b	0.38
Absorbed N [3], g/d	6.9 b	8.9 a	9.5 a	6.3 b	0.10
Escape dietary N, g/d	5.5	9.5	11.0	4.0	1.30
Escape N, % of N intake	77.2	79.0	99.0 a	57.2 b	13.90

[1] 4 g/ kg BW/d of extracted cassava meal was fed via rumen cannula to each sheep
a, b for each main effect, values in the same row with the same letter are not different ($P<0.05$)
[2] NAMNmic- N = Non-ammonia non- microbial nitrogen
[3] Apparently absorbed N = Duodenal N – Fecal N
[4] Escape dietary N = N flow to the duodenum – (Bacterial N flow + Endogenous N)
where: Endogenous N = 2.2 g N/kg of DM intake

On the other hand, results from previous feeding trials did 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 more escape protein when *Calliandra* CIAT 22310 was included as protein supplement as compared to CIAT 22316, possibly as a result of Calliandra forage comprising only a small proportion of the diet fed and consumed by sheep. Thus we postulate that differences in astringency between the two *Calliandra* provenances, which resulted in different levels of by pass - protein when fed to ruminants could have implications in practical feeding systems only when the legume is fed alone, rather than as a protein supplement to a low quality basal diet

An interesting finding of this study is that for the first time it has been shown that the monomer composition of soluble CT in a tropical legume can have an effect on the utilization of N by ruminants. The fact that our in vivo results were in close agreement with results on astringency of soluble CT extracted from *Calliandra* provenances is also a major finding, since they validate the utility of the laboratory astringency tests for screening tropical legumes for quality traits.

The results of this feeding trial indicate that *Calliandra* CIAT 22310 was more consumed than *Calliandra* 22136 when offered to sheep as the only forage source. The higher intake of DM found with CIAT 22310 resulted in more N intake and N apparently absorbed in the small intestine, but not in more intake of

digestible DM given the higher digestibility of CIAT 22316. Thus it is not possible to conclusively infer that the feed value of *Calliandra* 22310 is higher than that of CIAT 22316. However, these results do indicate that differences in tannin structure of the two *Calliandra* provenances had an effect on nitrogen utilization by sheep fed the legumes alone.

1.2.3 Milk production of cows supplemented with sun-dried and fresh *Calliandra*

Contributors: P. Avila, C. Lascano and G. Ramírez (CIAT)

Rationale

Work carried out at CIAT as part of an OFI-CIAT collaborative Project had shown that voluntary intake of *Calliandra* fed to sheep housed in metabolism crates was improved when the forage was fed dried as opposed to fresh. 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.

Thus this year we were interested in determining if milking cows grazing a well-managed *Brachiaria decumbens* pasture consumed more of the sun-dried than fresh *Calliandra* when offered as a supplement and if this would result in higher milk yield.

Materials and Methods

A grazing trial was carried out in Quilichao during a wet period (April- May, 2001) to measure milk yield and composition of cows (3 Holstein and 3 crossbred Zebu) grazing a *B. decumbens* pasture stocked with 2 cows/ha. Three treatments (T1: Control; T2: Fresh *Calliandra calothyrsus* CIAT 22310 and T3: Sun Dried *Calliandra calothyrsus* CIAT 22310) were compared using a 3 x 3 Latin Square design. Each experimental period consisted of 7 days adjustment and 7 days measurement phase. At milking (AM and PM) cows were given the legume (leaves, fine stems) supplements at a level of 1.5% DM of BW/day.

Results and Discussion

Results shown in Table 8 indicate that there was no significant effect of supplementing fresh or dry Calliandra on milk yield and composition of cows grazing a well managed *B. decumbens* pasture. These results are interesting given that intake of fresh *Calliandra* was 3.5 times higher (2.4 vs 8.3 g DM/Kg BW/day) than the intake of sun-dried *Calliandra*, which is contrary to what was to be expected based on the results from feeding trials with sheep housed in matabolism cages (See AR 2000). Results with sheep also showed that 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.

Table 8. Effect of supplementing fresh or dry *Calliandra calothyrsus* on milk yield and composition of grazing a *B. decumbens* pasture during the wet season.

Treatment (Supplement)	Milk Yield (kg/cow)	Fat (%)	Non Fat solids (%)	MUN (mg/dL)
Control	5.4	3.9	8.3	12.6
Calliandra-Fresh[1]	5.6	3.9	8.0	14.7
Calliandra- Sun-Dried[1]	5.3	3.8	8.3	12.9

[1]Cows were supplemented with fresh or sun-dried *Calliandra* (leaf + fine stem) by offering 1.5% DM of BW in two separate meals/day during milking.

It is not known why sheep fed a low quality grass consumed more sun-dried and fresh *Calliandra* as compared to milking cows grazing a *B. decumbens* pasture. However, these conflicting results do point out the danger of extrapolating results from sheep to cattle and from grazing to confinement.

In conclusion, our results indicate that supplementing sun-dried or fresh *Calliandra* did not result in milk yield increments of cows grazing a well-managed pasture of *B. decumbens*. It is possible that cows grazing poorly managed (low forage availability an low forage quality) pastures would respond to supplementation of *Calliandra*, particularly if fed fresh. This hypothesis will be tested next year.

Activity 1.3 Assessment of the potential of saponin-rich tropical fruits to reduce methane in ruminants on grass diets

Highlights

- Out of three saponin-rich fruits evaluated, only *S. saponaria* significantly decreased protozoa count by 54% and daily methane release by 20% relative to the control.
- Defaunation suppressed methane by 43% on average of all diets, but the effect of *S. saponaria* against methane was even higher in defaunated (29%) than in faunated rumen fluid (14%).
- Depression of methane release was related to the proportion of *S. saponaria* in the diet. Methane release was reduced by 10% when the proportion of *S. saponaria* in the diet was increased from 0 to 8%. A further increase of the proportion of S. saponaria, up to 14%, had no further depressing effect on methane release.

Progress towards achieving milestone

- **Known utility of saponin-rich plants and semi-purified saponins to reduce methane by ruminants**
  We made considerable progress in defining the potential of saponin-rich tropical fruits to manipulate rumen fermentation through in vitro experiments carried out with the Rusitec-system. Our findings indicate a high potential for *Sapindus saponaria* to reduce methanogenesis and rumen protozoa populations. We also found a relation between daily methane release and the proportion of *S. saponaria* in the diet. The highest depression occurs in diets with approximately 8% *S. saponaria* in the DM. Future studies, will validate results obtained with the Rusitec-system in feeding trails with confined sheep.

1.3.1 In vitro evaluation of the potential of saponin – rich tropical fruits to manipulate rumen fermentation and to reduce methanogenesis

Contributors: Hess H.D. (ETH, Zurich), Soliva C (ETH, Zurich), Diaz T.E (CORPOICA, Colombia), Kreuzer M, and Machmuller A. (ETH, Zurich)

Rationale

The issue of global warming caused by anthropogenic greenhouse gases is of increasing concern. Although fossil- fuel based industrial development is the major cause of the environmental imbalance, agricultural practices cannot be ignored. Combustion of fossil energy and deforestation are primarily responsible for the increases in of atmospheric CO2. However, ruminant animals have a considerable significance on global warming since they contribute 1/6 of the total atmospheric methane. Thus, efforts to mitigate methane emissions from ruminants and other sources (wet lands, rice paddies, waste management etc) are urgent given that atmospheric methane concentration is increasing at a faster rate than CO2. In

addition, per molecule, methane is 21 times more potent as a greenhouse than CO2, even though it has a relatively shorter half-life.

In tropical countries, where the majority of the world's domestic ruminants are located, production systems are mostly based on forages and crop residues of low nutritive value. Under these conditions, the productivity of the animals is low and the production of methane from microbial fermentation of feed in the rumen represents a loss of 15-16% of the digestible energy consumed. Saponin-rich fodder plants have been shown to reduce protozoa population by 80%, and some methanogens are known to be closely associated with protozoa. There are a variety of tropical plants and fruits differing in contents and types of saponins, and their effect on ruminal fermentation processes and their efficacy against methane is still unexplored.

To address the issue of methane production by ruminants we joined an SDC- ZIL- funded collaborative research effort led by Prof. M. Kreuzer of the Institute of Animal Science in the Swiss Federal Institute of Technology (ETH), Zurich and by Dr. Dieter Hess (ETH) a former Visiting Scientist at CIAT, which includes as partners the U. Nacional in Bogotá and CORPOICA.

The aim of the project is to develop feeding strategies based on locally available forage components and ingredients such as saponins to reduce methane emissions per unit of edible animal product (beef and milk) and to simultaneously improve feed use efficiency in tropical smallholder livestock systems.

The project has three main research components: (a) in vitro screening of different feed and forage sources, (b) in vivo validation of promising feeding components and strategies and (c) assessment of the applicability of the experimental results at local and regional level and definition of diffusion strategies to promote the use of feeding systems leading to reduce methane production.

Two initial experiments were carried out in ETH, Zurich to compare the effects of the dietary inclusion of saponin-containing tropical fruits (*Sapindus saponaria*, *Enterolobium cyclocarpum*, *Pithecellobium saman*) and of increasing proportions of S. *saponaria* on in vitro rumen fermentation parameters including methane production.

Materials and Methods

Experiment 1

The daily control diet consisted of 9.30 g low quality hay, 3.72 g *Arachis pintoi* (a tropical pasture legume), 1.80 g barley straw and 0.18 g urea (on a DM basis). The other diets contained 10% *Sapindus saponaria*, 20% *Enterolobium cyclocarpum* or 20% *Pithecellobium saman*, corresponding to the dietary proportions described to be still applicable *in vivo*. Daily dietary DM supply and crude protein content (13%) were kept constant.

The four diets were evaluated simultaneously with faunated and defaunated (Synperonic) rumen fluid during 4 × 10 day periods in an eight-fermenter Rusitec system (n=4). Feed was incubated for 48 h. Daily rumen fluid samples were taken 4 h before feed introduction and were analyzed for pH, ammonia, VFA and protozoa count. Fermentation gases were collected in gas proof bags and chromatographically analyzed for methane. For statistical analysis mean values from days 5 to 10 were used.

Experiment 2

To follow-up this initial study we determined the effect of increasing proportions of *S. saponaria* fruits in the diet on rumen fermentation parameters. The daily control diet consisted of 14.67 g low quality hay and

0.33 g urea on a DM basis. The other diets contained 2, 4, 6, 8, 10, 12, or 14% of ground *S. saponaria* fruits.

Daily dietary DM supply and crude protein content (13%) were kept constant. The 8 diets were evaluated during 2 x 10 day periods in an eight-fermenter Rusitec system (n=2).

Results and Discussion

Experiment 1

Of the saponin-containing fruits only *S. saponaria* significantly decreased protozoa count by 54% and daily methane release by 20% relative to the control. Defaunation suppressed methane by 43% on average of all diets, but the effect of *S. saponaria* against methane was even higher in defaunated (29%) than in faunated rumen fluid (14%) (Figure 1).

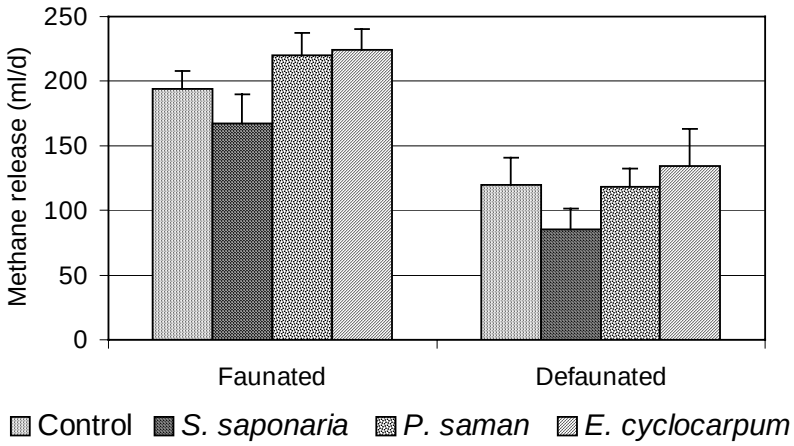

Figure 1. Daily methane release from Rusitec-fermenters supplied with a control diet or with diets containing 10% *Sapindus saponaria*, 20% *Enterolobium cyclocarpum* or 20% *Pithecellobium saman*.

When related to apparently fermented OM, the use of *P. saman* and *E. cyclocarpum* had no effect on methane release, but differences in daily methane release relative to the control remained similar to what was observed with *S. saponaria*. The results form this experiment demonstrated that the fruits of the tropical tree *Sapindus saponaria* (Photo 1) when included at a level of 10% in forage-based diets has the potential to reduce methane release from ruminal fermentation. Since the depression of methanogenesis was also found in defaunated rumen fluid, it can be assumed that saponins or other constituents of *S. saponaria* act directly against methanogens.

Experiment 2

Increasing the proportion of S. *saponaria* in the diet did not affect ammonia (averaged 10.4 mmol/l) content in rumen fluid. Total protozoa count was not affected when lower proportions of *S. saponaria* (2-6%) were included in the diet. However, with higher proportions (12 and 14%) protozoa count was reduced by over 50%. Daily methane release was related to the proportion of *S. saponaria* in the diet.

When the level of *S. saponaria* was increased from 0 to 8%, methane release was reduced by 10%. A further increase of the level of *S. saponaria*, up to 14%, had no depressing effect on methane release. On the contrary, methane release seemed to increase with the highest dose. The reasons for these unexpected

results are not clear, but could be related to other components of the fruit of *S. saponaria*, which, at higher levels, could affect rumen fermentation.

Although reduction of methane release was somewhat lower than in experiment 1, results confirm the potential of *Sapindus saponaria* fruits to reduce methane emissions from ruminal fermentation. The optimal level of *S. saponaria* seems to be around 8% of the daily diet dry matter.

Photo 1. Fruits of *Sapindus saponaria* (Courtesy: CIPAV)

1.3.2 In vitro evaluation of the potential of semi-purified saponins from *Sapindus saponaria* to manipulate rumen fermentation and to reduce methanogenesis

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Rationale

Saponins are a complex group of plant ingredients with highly diverse biological activity, but limited data are available on saponins from typical tropical plants like *Sapindus saponaria*. Therefore we extracted saponins from *S. saponaria* and used the extract to evaluate the effect of semi-purified saponins on rumen fermentation in vitro.

Materials and Methods

The daily basal diet consisted of 8.81 g of *Brachiaria dictyoneura* cv. Llanero hay (a low quality grass; 3% crude protein, 78% neutral detergent fiber, 41% acid detergent fiber) and 5.87 g of sun dried leaves of *Cratylia argentea* (a multipurpose shrub legume with medium forage quality; 18% crude protein, 65% neutral detergent fiber, 38% acid detergent fiber).

Daily dosage of semi-purified saponins (purity approximately 95%) was 0, 0.06, 0.12, 0.18, 0.24, 0.30, 0.36 or 0.42 g per day on a dry matter basis. The eight treatments were evaluated during 4 x 10 day periods in an eight-fermenter Rusitec system (n=4).

Results and Discussion

Due to the moderate forage quality of the basal diet used, ammonia concentration (3.19 mmol/l) was considerably lower than in the first two experiments. However, rumen ammonia decreased linearly from 4.02 to 2.40 mmol/l when the dosage of saponins was increased from 0 to 0.42 g. Lower doses of semi-purified saponins (0.06 to 0.24 g/day) had no effect on total protozoa count, which is in contrast to the 50% reduction in protozoa observed with the highest dose (0.42 g/d) (Figure 2).

The effect of saponins on daily methane release was not clear in this experiment. Since none of the saponin levels tested reduced methane release when compared with the control diet. However, methane release from the diet with 0.18 g saponins (1.2% in DM) was approximately 20% lower ($P<0.05$) than release from the diets with 0.24, 0.30 and 0.36 g (1.6%, 2.0% and 2.4% in DM).

Although differences between most of the treatments were not significant ($P>0.05$) it is interesting to note, that the methane release pattern observed in this experiment agrees well with the one from the experiment carried out with complete fruits of *Sapindus saponaria*. In that trial the lowest methane release was measured with proportions between 8 and 10 % of fruit in the diet and when the highest dose (14%) was used, methane release was increased. Taking into account the saponin content in the complete fruits (approximately 12%), 8 to 10% of fruit is equivalent to 1.0 to 1.2% of saponins, and 14% of fruits is equivalent to 1.7% of saponins in the diet.

Finally, results of this experiment suggest that a possible methane depressing effect of saponins from *Sapindus saponaria* (if there is any) would be independent of their potential to suppress rumen protozoa, which agrees with the observations made in the first study.

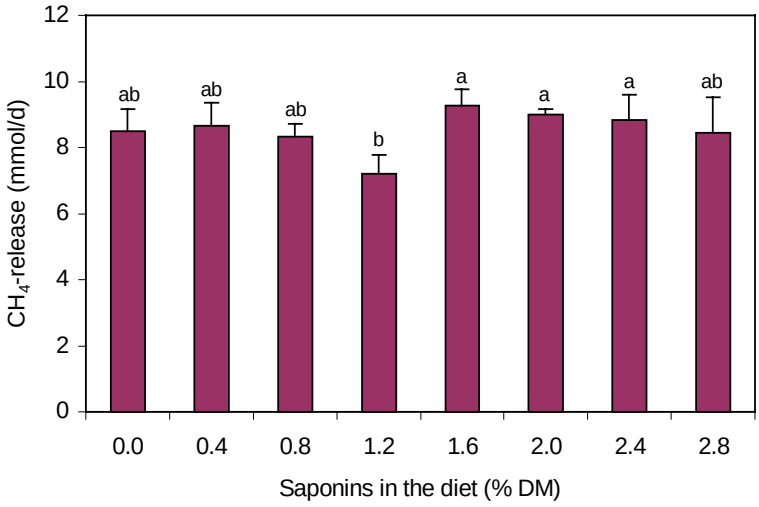

Figure 2. Daily methane release from Rusitec-fermenters supplied with a control diet or with diets containing 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 or 2.8% saponins.

1.3.3 *In vitro* evaluation of fruits of *Sapindus saponaria* in relation to semi-purified saponins and saponin-free diets on their effect on rumen fermentation and methane release

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Rationale

In earlier *in vivo* studies carried out by other groups to evaluate the potential of *S. saponaria* to manipulate rumen fermentation, only the pericarp of the fruit has been fed used. Separation of the pericarp from the residual fruit is quite laborious and time consuming and probably not practical under farm conditions. Thus it was of interest to investigate effects of whole fruits of *S. saponaria* in relation to pericarp and semi-purified saponins on methanogenesis.

Materials and Methods

The control diet in this experiment consisted of 8.8 g of low quality hay (*Brachiaria dictyoneura* cv. Llanero) and 5.9 g sun dried *Cratylia argentea* leaves. The other three diets contained either a) 8% complete and ground *S. saponaria*, b) 5% *S. saponaria*, pericarp or c) 1.2% semi-purified saponins. Daily dry matter supply was kept constant for the evaluation of the 4 diets during 2 x 10 day periods in an eight-fermenter Rusitec system (n= 4).

Results and Discussion

Ammonia concentration across all diets averaged 3.26 mmol/l. Although crude protein content in the diets was constant and the estimates for apparent CP degradability were similar for all diets (45.1%), ammonia concentration was considerably lower (-25%, $P<0.05$) with the fruit and the pericarp than with the control diet. This could indicate a more efficient use of nitrogen by the rumen microbes when the diets contained fruits or pericarp.

Total protozoa counts were not affected when 1.2% of semi-purified saponins were included in the diet, but were reduced by 50% ($P<0.05$) when the diet contained 8% of fruit or 5% of pericarp. The absence of a reduction in protozoa counts when semi-purified saponins were included in the diet could be due to: (a) saponins could have lost their anti-protozoal effect during the extraction process or (b) other constituents (different from saponins) are responsible for the anti-protozoal effect of fruits and pericarp of *S. saponaria*.

Daily methane release averaged 218.0 ml and did no vary with diet ($P>0.05$). These contrasts clearly with the results form the first experiment where 8% of fruits of *S. saponaria* reduced methane release by 14% in faunated rumen fluid. Possible explanation for these contrasting results could be that fiber content in the diets used in the present experiment was considerably higher than in the first one (72.1% vs. 60.8%) and CP content was slightly lower (10.8% vs.13.0%). Since interactions between forage quality and saponin-effects cannot be excluded, this could have contributed to the contrasting results.

Additionally, it is possible that rumen liquid from cattle grazing tropical pastures, as used in the present experiment, contains methanogens resistant to saponins. And finally, it is known that differences in saponin content and composition may be considerable between fruits from provenances or of different age and consequently their effect on rumen fermentation may also be different.

1.3.4 *In vitro* evaluation of the effect of varying proportions of *Arachis pintoi* in a basal diet of a low quality grass on rumen fermentation

Contributors: H.D. Hess (ETH, Zurich), J.E. Carulla (U. Nacional, Colombia), C.E. Lascano (CIAT)

Rationale

Considerable information has been generated on the effect of improved grass and legume species on animal production and soil fertility parameters. However, up to now little information is available on the effect of these improved forage alternatives on rumen methanogenesis. Thus a Rusitec-trail was carried out to evaluate the consequences of the inclusion of varying proportions of *Arachis pintoi* in a low quality diet on rumen fermentation parameters.

Materials and Methods

The daily control diet consisted of 15.0 g of low quality hay (*Brachiaria dictyoneura*; 3.0% CP, 76.1% NDF, 42.6% ADF). The other diets contained 33.3%, 66.7% or 100% *Arachis pintoi* (a high quality pasture legume; 17.0% CP, 50.5% NDF, 38.4% ADF). The four diets were evaluated simultaneously alone and with saponins (1.2% of DM). The 8 treatments were tested in 4 × 10-day periods (n=4).

Results and Discussion

None of the variables evaluated was affected by the addition of saponins (P>0.05), but most of the variables were clearly affected by the proportion of *A. pintoi* in the diet. The pH of rumen fluid decreased from 7.13 with the control diet to 6.86 with the diets containing *A. pintoi*. Ammonia concentration increased linearly from 0.45 to 10.32 mmol/l when the proportion of *A. pintoi* increased from 0 to 100%. Total protozoa as well as bacteria counts were significantly increase when *A. pintoi* was included in the diet and fiber degradation was increase by 100%.

All these results indicate that ruminal fermentation with the pure grass hay diet was strongly limited by the low quality of *B. dictyoneura* (i.e. its low CP content). A proportion of 33.3% of *A. pintoi* in the diet was sufficient to overcome these limitations and to increase microbial activity.

Daily methane release was lowest with the control diet (1.7 mmol/d), intermediate with 33.3% of *A. pintoi* (7.3 mmol/d) and highest with 66.7 and 100% *A. pintoi* in the diet (8.8 and 9.0 mmol/d, respectively). When related to apparently fermented OM the inclusion of *A. pintoi* increased methane release by approximately 150%.

Based on these results, one could argue that, in terms of methane release, pure *B. dictyoneura* hay would be the best diet. However, it has to be taken into consideration that animal production based on such a low quality diet would be very low or even negative (animals would probably lose weight), whereas with diets containing *A. pintoi*, animal production is usually high probably resulting in less methane produced / unit of milk or milk.

Activity 1.4 Assessment of quality and animal production potential of selected grass species

Highlights

- Confirmed that milk yield is higher with the *Brachiaria* hybrid cv Mulato than with the commercial *B. decumbens* cultivar.

- Demonstrated that with proper grazing management milk yield in the recently released *B. brizantha* cv Toledo can be higher than in the commercial *B. decumbens* cultivar

Progress towards achieving milestones

- **Benefits in animal production of new *Brachiaria* hybrids relative to commercial cultivars**
  This year we confirmed that cows grazing *Brachiaria* hybrid cv Mulato (first hybrid released) produce more milk than when grazing the commercial *B. decumbens* cv Basilisk, and that this is associated with a higher protein content in the forage on offer.

1.4.1 Milk yield with new hybrids of *Brachiaria*

Contributors: P. Avila, C. Lascano, J. W. Miles and G. Ramírez (CIAT)

Rationale

Last year we reported that milk yield with the commercial *Brachiaria* Hybrid Mulato was 25% greater than with *B. brizantha* cv Toledo and 7% higher than with *B. decumbens* cv Basilisk. It was interesting to observe that MUN values were two times greater in cv Mulato as compared to the other two *Brachiaria* cultivars, suggesting a higher concentration of CP in the forage on offer.

This year we completed an additional short-term grazing experiments to compare milk yield of the newly released cultivars (Mulato and Toledo) with the commercial *B. decumbens* cv Basilisk in the rainy season.

Materials and Methods

A grazing trial was carried out in October/November 2000 (rainy period) with 2 cows/ha. A total of 6 cows (3 Holstein and 3 Zebu Crossbreds in early-mid lactation) arranged in a 3 x 3 Latin Square were used to measure milk yield in pastures that were mowed 3-4 weeks prior to grazing. Each period was of 14 days of which 7 were for adjustment to the treatment and 7 for measurement of milk yield milk composition parameters and pasture attributes.

Results and Discussion

Our results did not show a significant interaction of cow group and pasture for milk yield, so mean values across cow types are presented in Table 9. Milk yield was higher in cows grazing *B. brizantha* cv Toledo and *Brachiaria* hybrid cv Mulato as compared to what was recorded in the widely used *B. decumbens* cv Basilisk.

As observed last year (See AR 2000), MUN was greater in cows grazing Mulato than the other two cultivars and this was associated with higher CP in the leaf tissue (8.5% in Mulato vs 7.3 % in Basilisk and 7.9 % in Mulato) in the forage on offer. Forage on offer expressed as green DM was also higher in the Mulato pasture (3200 kg/ha) than in the pasture with Basilisk (2000 kg/ha) and Toledo (2300 kg/ha), which is as reflection of the high production capacity under grazing of this Brachiaria hybrid.

Previous results had shown that milk yield in pastures of *B. brizantha* cv Toledo were lower than in *B. decumbens* (See AR 2000) and that this was due to lower protein content in the edible forage. We also suggested that the fast capacity of Toledo to grow following grazing could contribute to a rapid loss of quality if not properly managed. In the experiment being reported, all pastures were grazed after a 3-4 weeks regrowth periods using high stocking rate and as a result the forage on offer in the three pastures had high digestibility and was not limiting in protein.

Table 9. Milk yield of cows grazing contrasting *Brachiaria* pastures (Quilichao Research Station).

Pastures	Milk Yield (kg/d)	MUN (mg/dL)
B. decumbens cv. Basilisk	7.0 b	4.4 b
B. brizantha cv. Toledo	8.5 a	3.8 b
Brachiaria Hybrid cv Mulato	8.1 a	5.7 a

a, b, c, $P<0.05$

In general, these results confirm that *Brachiaria* hybrid released as cv Mulato has a high quality and milk yield potential when compared to other Brachiaria cultivars. In addition, it would seem that an additional advantage of Mulato is that it has the potential to produce more edible biomass than Basilisk and Toledo in the absence of N fertilizer and when grown in an acid soil with high OM, as is the case of Quilichao.

Activity 1.5 Adjustment of methods for the simultaneous evaluation of tropical legumes for feed and soil improvement

Highlights

- Showed that decomposition of legume plant material in the soil using the litterbag technique is highly correlated with DM disappearance using vitro anaerobic methods. The advantage of this finding is in terms of time and cost savings.
- Initial results indicate that decomposition of legumes in the soil and rumen is not a function of total cell wall in the plant but rather it is a function of indigestible fractions of the cell wall such as lignin alone or corrected for presence of condensed tannins

Progress towards achieving milestone:

- **Established correlation between in vitro anaerobic systems and aerobic soil-based systems in the decomposition of legumes with contrasting quality**
  We confirmed with legumes of contrasting quality that there is a high correlation in the decomposition of plant material using an anaerobic in vitro fermentation method and the aerobic litterbag technique.

1.5.1 Assessment of the effect of species and drying method on aerobic and anaerobic decomposition of legumes

Contributors: K. Tscherning (U. of Hohenheim, Germany), E. Barrios, (CIAT), R. Schultze-Kraft (U. of Hohenheim, Germany), and C. Lascano, M. Peters (CIAT)

Rationale

It is recognized that legume species are useful to enhance existing feed resources and to contribute to soil fertility in mixed livestock-cropping systems through their use in associated grass-legume pastures, as green manure or as mulch through prunings.

In mixed crop-livestock production systems legume quality is a key factor for obtaining maximum benefits in terms of rate and extent of N release in the rumen or soil. Consequently, Animal Nutritionist and Soil Scientist have been interested in defining plant quality parameters that are correlated with release of nutrients from topical legumes. However, research in quality of legumes as it relates to ruminants or soil has been carried out in an independent manner and consequently there has been very little information sharing on methodological aspects.

Microbial populations mainly mediate the decomposition of plant material in the soil with lesser effects from soil macrofauna. Decomposition is often studied using the litterbag technique whereby plant material is placed in or on the soil in series of nylon litterbag. Decomposition is determined by sampling the bags over time of usually several weeks or months and relating the results (DM disappearance and N release) to initial compositional factors of the plant material. This method is resource -and time- consuming but provides valuable data for comparing plant species in terms of their relative decomposition and nutrient release patterns.

Ruminants also decompose plant material through microbes that degrade plant protein and cell wall constituents to ammonia, amino acids and energy for the host animal. To assess the extent and rate of nutrient release from plant material used as a feed resource, samples are incubated with rumen microbes using in vitro systems or alternatively using in situ techniques, which follow the same principle of the soil nylon litterbag method.

It is recognized that soil and rumen processes involved in plant degradation have fundamental differences namely an anaerobic aqueous environment in the rumen, higher number of microbes and much faster degradation rates in the rumen compared with soil. Despite these differences, the extent and rate of nutrient release from plants in the two processes is greatly affected by compositional factors of the plant (i.e. N level, lignin, condensed tannins).

Thus we are interested in testing the hypothesis that similar plant chemical entities control decomposition and the release on nutrients in the rumen and soil and that in vitro values on rates and extents of digestion by rumen microbes can be used for predicting decomposition values of legume plant material in the soil.

To test these hypotheses we setup a research program, which involves three phases:

a) Laboratory studies to determine rates and extent of aerobic and anaerobic degradation of plant material from legumes with contrasting quality subject to different drying treatments and using different methods.
b) Laboratory studies to determine relationships between plant chemical entities and aerobic and anaerobic decomposition and release of nutrients in a range of legumes of contrasting quality.
c) Field studies using selected legumes as green manures and indicator crops to validate predictions of equations of nutrient release and in vitro anaerobic and aerobic results.

We hope that through this research we can produce the following outputs:

a) Know applicability of in vitro methods used to assess feed value of forages to define potential decomposition and release of nutrients from legumes used as feed resource or to improve soil fertility.
b) Known chemical entities in plant material that controls the extent and rates of decomposition of tropical legumes in the rumen and soil.
c) Guidelines for quick and reliable assessment of the value of tropical legumes as feed resources and to improve soils.

In this report we summarize results from the first series of laboratory studies in which measured anaerobic and aerobic decomposition of plant material from shrub legumes with contrasting quality and subject to different drying treatments.

Materials and Methods

The following woody tropical legumes were selected for the initial studies: a) *Indigofera constricta* (low tannin content), b) *Cratylia argentea* (medium tannin content) and c) *Calliandra* sp (high tannin content).

Plant material from the three legumes growing in a hillside site (Pescador, Cauca) was harvested after 6 weeks of regrowth and cuttings (leaf + fine stem) where subject to the following drying treatments prior to aerobic and anaerobic incubation: fresh, frozen, freeze-dried, oven-dried (60^{o}C) and air-dried.

All samples were subjected to the following chemical analysis: N, C, P, Fiber (NDF and ADF), lignin, soluble and bound condensed tannins and ash following standard protocols.

For measuring anaerobic degradation of DM we used two procedures:

a) **Tilley and Terry In Vitro method**, which comprises an incubation of the samples with rumen microorganisms followed by pepsin extraction and

b) **In Vitro Gas Production**, which involves the incubation of samples with rumen microbes and measurement of gas produced at regular intervals using a transducer.

For measuring aerobic decomposition and nutrient release two procedures were used:

a. **Litterbag-Technique:** A greenhouse decomposition trial was carried out to observe decomposition and disappearance-rate of the legume prunings. Litterbags (10 cm x 10 cm, mesh size 1 mm) were filled with 5 g dry matter and placed on the soil surface. Soil from the upper layer obtained in Pescador was air-dried and filled in pots of 17 cm diameter. Pots were arranged in a randomized block design with 5 replicates. Moisture content of the soil was maintained at 60 % of water holding capacity. Sampling of litterbags was done after 1, 2, 4, 8 and 20 weeks. Bags were oven-dried (40^{o}C) to constant weight with the plant material inside. Later plant material was manually cleaned from soil particles and weeds to determine dry weight and nutrient concentrations at different sample times.

b. **Leaching Tube Assay:** An aerobic leaching tube incubation method (Photo 2) was used to measure N-release rates from legume pruning. Glass tubes (5 cm diameter and 20 cm length) with a funnel bottom were filled (from the bottom to the top) with a fine layer of glass fiber wool, 10g of acid-washed sand, 90 g of soil/sand mixture (1:1), and 200 mg of the different legume samples. Tubes were arranged in a randomized block design with 5 replicates and kept in a dark room at 26^{o}C +/- 1^{o}C. Leaching will be performed 8 times (1, 2, 4, 6, 8, 12, 16 and 20 weeks) with 100 ml of leaching solution (1mM $CaCl_2$, 1mM $MgSO_4$ and 1mM $KHPO_4$). Leachates will be analyzed for NO_3^-, NH_4^+ and condensed tannin content. Results of this experiment will show amount and period of N-release of the different legumes during degradation.

Results on gas production and DM decomposition over time were fitted to appropriate regression models to estimate rates, which were then subject to an analysis of variance with drying treatment and legume species as sources of variation.

Results and Discussion

The effect of drying method on chemical composition of the three legumes used in the study is shown in Table 10. As expected, there were large differences among legumes in cell wall content, lignin and N, which could results in different decomposition rates when exposed to rumen and soil microorganism.

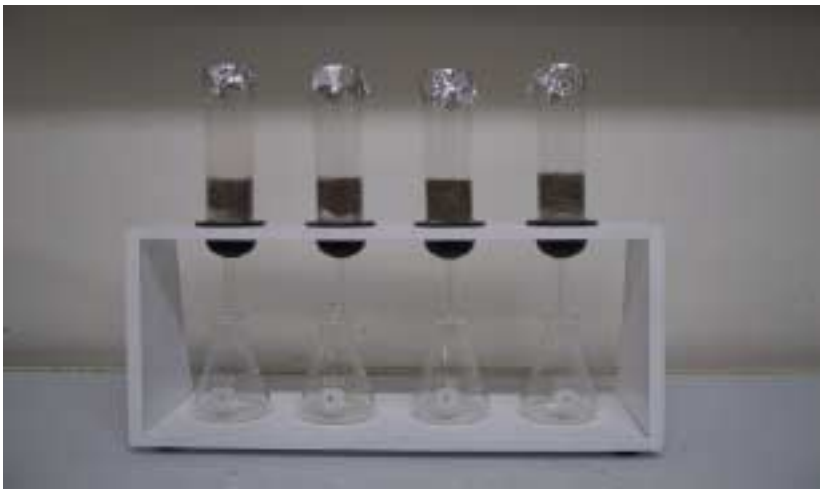

Photo 2. Leaching Tube Setup

The legume with the highest quality was *Indigofera constricta* (no condensed tannins) given its lower fiber and lignin concentration and higher N level when compared to the other two species. In the case of *Cratylia argentea* with low levels of condensed tannins, the main factor affecting its quality would seem to be the high and lignified cell wall fraction. In contrast, degradation of *Calliandra sp* could be more related to its high tannin content than to fiber and lignin.

Also as expected, drying treatment had a significant effect on the chemical composition of the three legumes included in the test. Results shown in Table 10 indicate that in all legume species, oven drying resulted in more fiber and lignin than freeze-drying or air-drying possibly as a result of artifacts formed by heat damage (Maillard reaction). However, this effect did not result in consistent reduction in the soil or rumen of DM degradation of the legumes under test as we had expected based on results in the literature.

The extent of DM decomposition of the three legumes by rumen microbes using two methods was highly correlated ($r = 98$; $P<0.01$) as has been shown in other studies. We also found a high correlation ($r = 0.87$; $P<0.0001$) between anaerobic in vitro DM loss and aerobic decomposition of DM in the soil, which had been shown in previous studies carried out in CIAT.

One important finding was that in vitro DM degradation by rumen microbes was more affected by legume specie than by drying method, regardless of the in vitro method used. The extent of degradation of *I. Constricta* was 1.5 times greater than *C. argentea* and almost 3 times greater than *Calliandra* sp, which is a reflection of the different chemical composition of the plant material used in the experiments.

Another parameter measured in the in vitro fermentation and litterbag trials was the rate of degradation of the three legumes subject to different drying treatments. Results indicated a positive correlation ($r = 0.49$; $P<0.05$) between anaerobic rate of in vitro gas production and aerobic rate of DM disappearance using the litterbag technique.

Table 10. Chemical composition of three tropical legumes subject to different drying treatments prior to aerobic and anaerobic incubation.

Treatment	NDF (%)	Lignin (%)	N (%)
*Infigofera constricta**			
Freeze-dried	27	5.0	4.58
Oven-Dried (60 ^{o}C)	**43**	**5.4**	5.04
Air-dried	30	4.5	5.3
*Cratylia argentea***			
Freeze–dried	57	12.0	3.65
Oven-Dried (60 ^{o}C)	**77**	**13.4**	3.75
Air-dried	67	12.6	3.89
Calliandra sp.***			
Freeze-dried	36	10.3	2.01
Oven-dried (60 ^{o}C)	**43**	**13.3**	2.71
Air-dried	35	8.5	2.27

*No tannins
**Low tannins (1-2 %)
***High tannins (17 to 22 %)

Results also showed that rates of aerobic and anaerobic rates of degradation were significantly influenced by legume species as shown in Table 11. However, the effect of legume species on rates of degradation was greater when samples were incubated under aerobic than under anaerobic conditions.

Table 11. Rates of anaerobic (gas production with rumen microbes) and aerobic (DM disappearance in litter bags) of three legumes (Data presented is as an average across drying treatments).

Legume Species	Anaerobic Conditions-Rumen Microorganisms Rate of in vitro gas production (% / h)	Aerobic Conditions-Soil Microorganisms Rate of DM disappearance (% /d)
Indigofera constricta	8.57 a	1.354 a
Cratylia argentea	6.16 b	0.334 b
Calliandra sp	2.51 c	0.190 c

The rate of DM disappearance of *I. constricta* under aerobic conditions was 4 times greater than *C. argentea* and 7 times greater than with *Calliandra* sp. However, under anaerobic conditions the rate of gas production of *I. constricta* when averaged across drying treatments was only1.4 times greater than with *C. argentea* and 3.5 times greater than with *Calliandra* sp.

One of the objectives of this work is to establish functional relationships between plant chemical components and decomposition and release of nutrients from legumes with contrasting quality in an anaerobic rumen system and in an aerobic soil system. Initial results indicate that cell wall content (ADF) was poorly correlated to DM loss in the anaerobic in rumen vitro system and in the aerobic soil litterbag system, but that negative and significant correlations were observed with ADF (cellulose + lignin) and lignin content (Table 12). By correcting the lignin fraction with condensed tannins and with N the correlations with observed DM decomposition under aerobic and anaerobic conditions significantly improved.

Table 12. Correlation between different plant chemical components and dry matter (DM) loss in an anaerobic in vitro gas production system and an aerobic soil litterbag system.

Plant Chemical Components	Anaerobic Conditions-Rumen Microorganisms DM loss (%) r	Aerobic Conditions-Soil Microorganisms DM loss (%) r
NDF	- 0.13 (NS)	- 0.28 (NS)
ADF	- 0.64 (P<0. 0045)	- 0.66 (P<0.0014)
Lignin	- 0.74 (P<0.0014)	- 0.78 (P<0.0002)
Lignin + Total Condensed Tannins	- 0.95 (P<0.0001)	- 0.91 (P<0.0001)
Lignin: N	- 0.98 (P<0.0001)	- 0.96 (P<0.0001)

In general, these results confirm that decomposition of legume plant material in the soil using the litterbag technique is highly correlated with DM disappearance using vitro anaerobic methods. The advantage of this finding is in terms of time and cost savings. While with the litterbag it takes 20 weeks to determine the extent and rate of decomposition of plant material in the soil with the in vitro anaerobic system it only takes 48 h to determine extent and rate of degradations of DM from plant material.

Finally, our results suggest that differences in plant quality attributes could be more important than sample preparation in determining the extent and rate of decomposition of plant material in the soil and rumen. Initial results indicate that decomposition of legumes in the soil and rumen is not a function of total cell wall in the plant but rather it is a function of indigestible fractions of the cell wall such as lignin alone or corrected for presence of condensed tannins.

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

Activity 2.1 Bioecology of spittlebug species in contrasting environments

Highlights

- Comparative biological studies completed for two spittlebug species (*Mahanarva andigena*, *Prosapia simulans*) and studies initiated to describe the population dynamics and phenology of *Prosapia simulans*, a newly detected *Brachiaria* pest in the Cauca Valley.
- Host plant water stress and age were found to have no detectable effect on the incidence and duration of diapause in *Aeneolamia varia* eggs.
- Documented an increase in the incidence of egg diapause towards the end of the dry season in field populations of spittlebugs from two seasonal sites with unimodal precipitation; incidence of diapause was minimal throughout the year in a site with bimodal precipitation.

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 challenges are (1) poor understanding of the natural history of the family Cercopidae, (2) diversity of insect/host/habitat associations, (3) lack of 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, (5) IPM tools that are rudimentary or absent, and (6) rapidly changing pest status.

In 2001 we continued studies to overcome these limitations, focusing on three entry points: (1) the acquisition of new bioecological information on this pest complex and the family Cercopidae, (2) development of five 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.

Progress towards achieving output 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. These five ecoregions are (1) the Interandean Region of the Cauca River Valley (Dept. Cauca and Valle del Cauca), highly seasonal, bimodal annual precipitation, (2) the Caribbean Coast (Dept. Córdoba and Sucre), highly seasonal, unimodal precipitation, (3) the Orinoquian Piedmont (Dept. Meta), intermediate seasonal, unimodal precipitation, (4) the Amazonian Piedmont (Dept. Caquetá), continuously humid and (5) the South Pacific Coast (Dept. Nariño), 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.

2.1.1 Biology and habits of *Mahanarva andigena*

Contributors: Jairo Rodríguez, Ulises Castro, Oscar Yela, and Daniel Peck (CIAT)

Rationale

Mahanarva andigena was detected for the first time in Colombia in 1999, augmenting the known diversity of spittlebugs associated with graminoids. Up to now, this species is only known in Colombia from the south Pacific coast of Dept. Tumaco at C.I. El Mira of CORPOICA (1º33'10.001 N, 78º42'05.849 W, 50 m elev.). Hosts in that region are *Sorghum halepensis* (Johnson grass) and *Saccharum officinarum* (sugar cane). *Mahanarva andigena* is also known from sugar cane in Ecuador where this spittlebug species is of increasing concern in cane production in the coastal and interior regions of the country.

No biological, behavioral or ecological studies have yet been carried out on this economically important species. We therefore studied certain aspects of the basic biology including description and recognition of the life stages, duration of the life stages and oviposition sites to obtain information on the habits of this species and thereby guide advances in pest management.

Materials and Methods

Biological studies were carried out according to methodologies previously established at CIAT emphasizing morphological characterization of the life stages, duration of the life stages and reproductive biology. To have access to all life stages, a small colony was established in the greenhouse with eggs collected from field-caught adults during a visit to C.I. El Mira. 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, both sexes of late instar V (Vb) and both adult sexes. Adult specimens were obtained from the field, nymphs from the colony, and eggs 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. For the adults, tenerals (<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 emerged first instars (<12 hours old) were placed in individual 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 or the nymph itself. The mean longevity of each life stage was calculated from observations of 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 mean duration of each of the four generalized developmental stages was calculated from observations on 100 eggs.

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. Each pot was infested with two females and two males from the colony and 10 days later eggs were recovered from four oviposition substrates: uncovered soil, soil covered by leaf litter, leaf litter and the plant surface.

Results

Mahanarva andigena eggs conformed to the four generalized developmental stages (S1, S2, S3, S4) established for *Aeneolamia varia* and other spittlebug species. Certain externally visible characteristics accompanied these stages. In S2 a spot of red pigment was visible. In S3 the chorion opened to expose the black operculum and the red spot was no longer visible. In S4 two pairs of red spots were visible, the posterior representing the Batelli glands of the abdomen and the anterior representing the eyes of the developing nymph. Each progressive stage was accompanied by a statistically significant increase in both

length and width (Table 13). Total development time was 16.4 days; S2 was the shortest development stage and S4 was the longest (Table 14).

Table 13. Width and length (mm) of development stages of *M. andigena* eggs (mean±S.E., range, n=93-100).

	Development stage			
Parameter	S1	S2	S3	S4
Length	1.22 ± 0.03 a (1.14-1.29)	1.24 ± 0.04 b (1.16-1.34)	1.26 ± 0.03 c (1.20-1.34)	1.30 ± 0.04 d (1.21-1.40)
Width	0.31 ± 0.01 a (0.29 -0.34)	0.33 ± 0.01 b (0.30-0.41)	0.35 ± 0.02 c (0.31-0.39)	0.39 ± 0.01 d (0.37-0.43)

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

Table 14. Duration (days) of *M. andigena* eggs by development stage (mean±S.E., range, n=96-105).

	Development stage				
	S1	S2	S3	S4	Total
Mean±S.E.	4.97 ± 0.46 c	1.57 ± 0.52 a	3.41 ± 0.61 b	6.44 ± 0.60 d	16.39 ± 0.85
Range	(4-6)	(1-3)	(2-5)	(5-8)	(15-19)

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

Nymphs increased in size from one instar to the next for each parameter measured. There was no overlap in head capsule width or stylet length among the five instars confirming these to be the most useful measures for instar determination (Table 15). Sexual dimorphism was observed in instar Vb (nymphs within a few days of molting to adults) where females were larger than males in all four parameters. Total development time was 46.5 days; instar I was the shortest and instar V was the longest representing 32.4% of the entire nymphal stage (Table 16).

Table 15. Morphological characterization (mm) of nymphal life stages of *M. andigena* (mean±S.E., range, n=15-40).

Instar	Head capsule width	Body length	Anterior wing pad length	Stylet length
I	0.42 ± 0.03 a (0.36-0.46)	1.80 ± 0.30 a (1.21-2.19)	---	0.30 ± 0.02 a (0.29-0.34)
II	0.67 ± 0.02 b (0.61-0.71)	2.90 ± 0.30 b (2.22-3.47)	---	0.39 ± 0.02 b (0.36-0.43)
III	1.01 ± 0.04 c (0.94-1.07)	4.25 ± 0.44 c (3.04-5.09)	0.39 ± 0.03 a (0.34-0.44)	0.59 ± 0.03 c (0.53-0.64)
IV	1.53 ± 0.05 d (1.45-1.63)	6.93 ± 0.80 d (5.14-8.79)	1.05 ± 0.07 b (0.89-1.19)	0.87 ± 0.04 d (0.80-0.95)
Va	2.12 ± 0.10 e (1.96-2.37)	9.57 ± 0.72 e (7.93-11.0)	2.52 ± 0.14 c (2.14-2.79)	1.20 ± 0.04 e (1.13-1.27)
Vb Female	2.27 ± 0.05 f (2.19-2.37)	11.28 ± 1.16 g (8.79-13.71)	2.79 ± 0.16 d (2.36-3.07)	1.26 ± 0.04 f (1.16-1.33)
Vb Male	2.09 ± 0.08 e (1.93-2.28)	10.70 ± 0.84 f (9.29-12.64)	2.57 ± 0.16 c (2.14-2.86)	1.19 ± 0.03 e (1.13-1.30)

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

The behavior of nymphs differed from other Colombian species studied to date because nymphs of all age classes sought feeding sites in the upper portions of the plant such as leaf axils. This behavior resulted in large cohabited spittle masses and increased aggregation of individuals. The behavior in laboratory is

similar to observations on the two known hosts in the field and to reports from sugar cane studies in Ecuador.

Table 16. Duration (days) of *M. andigena* nymphs by instar (mean, n=40).

	Instar					
	I	II	III	IV	V	Total
Mean±S.E.	6.35 ± 1.03 a	8.64 ± 1.10 c	8.18 ± 1.30 b	10.14 ± 1.50 c	15.05 ± 3.80 d	46.52 ± 9.85
Range	(5-8)	(7-10)	(8-11)	(8-13)	(9-23)	(41-54)

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

Adults were significantly larger than instar Vb nymphs of the same sex in terms of head capsule width and forewing length, but smaller in terms of body length without wings and stylet length (P<0.05). Sexual dimorphism was observed in the adults, expressed as the greater size of females in every parameter measured (Table 17). Overall adult longevity was 24.0 ± 11.1 days with 25.5 ± 12.9 (8-27) days for females and 20.6 ± 9.0 (8-27) for males (difference not statistically significant). Under the conditions of this study, duration of the life cycle of *M. andigena* was 74.9 d (=16.4+46.5+12.0, egg+nymph+½ adult).

Table 17. Morphological characterization (mm) of *M. andigena* adults by sex (mean±S.E., range, n=40).

Sex	Head capsule width	Stylet length	Body length with wing	Body length without wing	Anterior wing length	Body width
Female	2.54 ± 0.08 a	1.14 ± 0.06 a	10.97 ± 050 a	10.16 ± 0.92 a	8.61 ± 0.45 a	5.19 ± 0.28 a
	(2.29 – 2.64)	(1.05 – 1.28)	(9.71 –11.93)	(8.43 – 12.00)	(7.71 – 10.07)	(4.14 – 5.71)
Male	2.28 ± 0.09 b	1.03 ± 0.06 b	9.96 ± 0.46 b	9.05 ± 0.78 b	7.95 ± 0.32 b	4.72 ± 0.24 b
	(2.07 – 2.50)	(0.91 – 1.14)	(9.07 – 10.71)	(7.14 – 10.36)	(7.21 – 8.50)	(4.21 – 5.21)

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

Mahanarva andigena exhibited some flexibility in oviposition substrates. Most eggs (67.6%) were recovered from the soil with 51.2% from uncovered soil and 16.4% from soil under litter. Nearly a third of eggs (32.4%), however, was recovered from the plant surface. None were recovered from leaf litter.

Discussion

Like other graminoid spittlebugs studied to date, *M. andigena* eggs pass through four egg development stages that increase in size and are distinguished by externally visible characteristics; nymphs pass through five morphologically distinguishable instars best differentiated by width of the head capsule and stylet length; and adults exhibit sexual dimorphism expressed as greater size of females.

The life cycle of 74.9 d is longer than other spittlebugs studied to date with the same methodology, including *Aeneolamia* (45.3-52.6 d) and *Zulia* (61.5-69.6 d), and is 9.3 days longer than its congener *Mahanarva* sp. nov. *Prosapia simulans* is the only other described Colombian species with a life cycle >70 days.

A preference for laying eggs in the soil is common to most other species studied (*A. lepidior, A. reducta, A. varia, Mahanarva* sp. nov., *Z. carbonaria, Zulia* sp. nov.). The tendency of *M. andigena* to lay eggs (32.4%) on the surface of the plant stem is greater in *Z. pubescens* (59.2%) and *P. simulans* (82.6%).

2.1.2 Biology and habits of *Prosapia simulans*

Contributors: Jairo Rodríguez, Ulises Castro, Anuar Morales, and Daniel Peck (CIAT)

Rationale

The first detection of the Central American spittlebug, *Prosapia simulans*, in Colombia has serious economic ramifications for ranchers and cane producers of the Cauca River Valley. The insect has already reached economically damaging levels in *Brachiaria* pastures in the Dept. Valle del Cauca. In sugar cane, *P. simulans* represents a potential threat since it is the second most important spittlebug cane pest in Central America and since changing cultural practices in Cauca Valley cane production (prohibition of burning) may enable this species to get a foothold in cane fields. Up to now spittlebugs have not been present in sugar cane of this region.

We have previously documented that *P. simulans* occurs over a large elevation range, extending from the Cauca Valley floor (1100 m elev.) to just over the western cordillera of the Andes (1621 m elev.). Multiple visits to the same farms have shown *P. simulans* populations to be persistent especially in improved pastures of *Brachiaria decumbens*. Despite its importance in pastures and cane of Central America, little is known about this insect's biology and ecology. To support advances in management, we carried out initial biological studies of *P. simulans* in the Cauca Valley focusing on differentiation of the life stages, duration of the life stages and reproductive biology.

Materials and Methods

The biology of *P. simulans* was characterized using previously established methods. To differentiate among the life stages, these were characterized morphologically using different measures of body size. To quantify duration of the life stages, the development of individual eggs, nymphs and adults was observed under controlled conditions. To begin to describe the reproductive biology, oviposition site preferences were determined.

Results

The eggs conformed to the four generalized developmental stages (S1, S2, S3, S4) described in other spittlebug species. Both size and width of eggs increased from one stage to the next (Table 18). Total development time was 18.0 days; S2 was the shortest development stage and S1 the longest (Table 19). Diapause was not detected among individuals of the study population, however diapause during stage S2 was observed in eggs collected from a lower elevation site during the course of other studies (1100 m elev., Santa Helena, Dept. Valle del Cauca); maximum time to eclosion of these diapause eggs was 128 days.

Table 18. Width and length (mm) of development stages of *P. simulans* eggs (mean±S.E., range, n=75-100).

	Development stage			
Parameter	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)

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

Table 19. Duration (days) of *P. simulans* eggs by development stage (mean±S.E., range, n=66-100).

	Development stage				
	S1	S2	S3	S4	Total
Mean±S.E.	6.90 ± 1.09 d	2.13 ± 1.69 a	3.98 ± 0.77 b	5.18 ± 0.58 c	17.99 ± 1.27
Range	(6-13)	(1-9)	(2-5)	(4-7)	(16-23)

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

For the nymphs, each of the morphological parameters measured (head capsule width, body length, anterior wing pad length, stylet length) increased in size from one instar to the next (Table 20). There was no overlap in head capsule width among the five instars confirming this to be the most diagnostic character for instar determination. Total development time was 45.6 days; instar I was the shortest and instar V was the longest, representing 28.8% of the entire nymphal stage (Table 21).

Table 20. Morphological characterization (mm) of nymphal life stages of *P. simulans* (mean±S.E., range, n=40).

Instar	Head capsule width	Body length	Anterior wing pad length	Stylet length
I	0.45 ± 0.02 a (0.36-0.50)	1.66 ± 0.17 a (1.24-1.96)	-	0.36 ± 0.04 a (0.28-0.44)
II	0.65 ± 0.03 b (0.50-0.69)	2.33 ± 0.31 b (1.55-2.88)	-	0.47 ± 0.03 b (0.37-0.52)
III	0.96 ± 0.03 c (0.89-1.01)	3.16 ± 0.21 c (2.64-3.70)	0.32 ± 0.02 a (0.27-0.37)	0.62 ± 0.05 c (0.53-0.73)
IV	1.42 ± 0.06 d (1.28-151)	5.99 ± 0.57 d (4.91-7.52)	0.90 ± 0.08 b (0.71-1.10)	0.97 ± 0.04 d (0.89-1.04)
Va	1.92 ± 0.08 e (1.78-2.13)	7.79 ± 0.43 e (6.64-8.50)	2.23 ± 0.13 c (1.78-2.43)	1.16 ± 0.05 e (1.04-1.27)
Vb Female	2.01 ± 0.08 f (1.84-2.13)	8.12 ± 0.95 e (6.07-10.86)	2.27 ± 0.13 c (1.78-2.55)	1.30 ± 0.05 f (1.19-1.39)
Vb Male	1.92 ± 0.09 e (1.78-2.19)	7.99 ±0.65 e (6.43-9.43)	2.26 ± 0.09 c (1.99-2.43)	1.28 ± 0.06 f (1.04-1.42)

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

Table 21. Duration (days) of *P. simulans* nymphs by instar (mean, n=40).

	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).

Adults were larger than instar V in all parameters with the exception of male body length and male and female stylet length (shorter in adults). Sexual dimorphism was observed in the adults, expressed as the greater size of females in every parameter measured with the exception of forewing length (Table 22). Overall adult longevity was 17.8 ± 8.2 days with 19.9 ± 8.6 (6-32) days for females and 14.5 ± 5.1 (5-21) days for males (difference not statistically significant). Under the conditions of this study, duration of the life cycle of *P. simulans* was 72.5 days (=18.0+45.6+8.9, egg+nymph+½ adult).

Prosapia simulans exhibited a marked preference for laying eggs on the surface of the plant stem; 82.6% of eggs were recovered from this substrate. Only 17.4% was recovered from the soil with 3.6% from uncovered soil and 13.8% from soil under litter. No eggs were recovered from leaf litter.

Table 22. Morphological characterization (mm) of *P. simulans* adults by sex (mean±S.E., range, n=40).

Sex	Head capsule width	Stylet length	Body length with wing	Body length without wing	Anterior wing length	Body width
Female	2.31 ± 0.06 a (2.21-2.43)	0.98 ± 0.33 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)
Male	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)

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

Discussion

Prosapia simulans conforms to the developmental and morphological patterns established in graminoid spittlebugs (see section 2.1.1) including diapause expressed as an extended S2 egg stage. The life cycle of 72.5 days in the Cauca Valley is longer than what is reported from Central America (58.4 and 58.0 days). It is also longer than all other species studied to date in Colombia (*Aeneolamia lepidior, A. reducta, A. varia, Mahanarva* sp. nov., *Z. carbonaria, Zulia pubescens, Zulia* sp. nov.), comparable only to *M. andigena* from the south Pacific coast (74.9 days; see section 2.1.1). The marked preference for oviposition sites on the plant stem is different from other Colombian species studied to date which all prefer to oviposit in the soil. Only *Z. pubescens* has also been shown to lay a majority of eggs (59.2%) on the plant stem.

2.1.3 Population dynamics and phenology of *Prosapia simulans*

Contributors: Jairo Rodríguez, Ulises Castro, Anuar Morales, Oscar Yela, and Daniel Peck (CIAT)

Rationale

The graminoid spittlebug, *Prosapia simulans*, is a new arrival to the Cauca Valley of Colombia and has been detected in four municipalities to date: Santander de Quilichao (Dept. Cauca), Cerrito, Calima Darién and Yotoco (Dept. Valle del Cauca). In many of the sites where it has been detected, *P. simulans* shares pastures with *Zulia carbonaria* and *Zulia pubescens* particularly where *Brachiaria decumbens* is the dominant forage grass host.

In general, the management of graminoid spittlebugs has been compromised by a lack of bioecological information specific to the species and habitats of concern, and by a tendency to over generalize among the diverse insect/host/habitat associations in which these pests have economic impact. Advances in spittlebug management requires a detailed understanding of aspects such as differentiation and duration of the life stages, correspondence between population fluctuations and precipitation, habitat and host plant preferences, and the incidence of natural enemies.

Biological studies on *P. simulans* in the Cauca River Valley have been initiated. In this report we summarize recent phenological studies of *P. simulans*. This research was carried out to provide baseline data for field studies on fungal entomopathogens and assesses various components of population ecology including population fluctuation, diapause, natural enemies and precipitation.

Materials and Methods

Observation plots were established in *B. decumbens* pastures at Hacienda Piedechinche, Municipality El Cerrito, Dept. Valle del Cauca. Methods for surveying nymphs, adults, diapause eggs and natural enemies were modified from previously established protocols used in studies with similar objectives (CIAT Annual Report 1999, 2000).

Five 0.16-ha plots were established, each in a different paddock and under the same typical management regime of the farm in terms of grazing pressure, fertilization and weed suppression. To facilitate sampling, each plot was divided into 16 subplots in which nymphs were collected from two 0.0625m^2 quadrats to measure absolute density, and adults from two series of 10 sweeps of an insect net to measure relative density. All nymphs were determined to instar and adults to species and sex.

The abundance and incidence of natural enemies were measured as part of the same surveys. Surveys were carried out once weekly and were initiated 25 January 2001. The first 7 months (through 30 August 2001) are summarized in this report. Data were analyzed to determine patterns and variation at the farm level in population fluctuation, correspondence with precipitation, population synchrony, number of generations and incidence of natural enemies.

To help interpret phenology, parallel data were collected on the incidence and duration of egg diapause, a physiological condition that enables the insect to synchronize its life cycle with the humid environmental conditions necessary for development and reproduction. Every 15 days a group of females (1-10 individuals, depending on availability) was collected from each plot and allowed to oviposit for a period of 3 days in moist filter paper lining the bottom of a large petri dish. Petri dishes and their eggs were kept under incubation (27^{o}C, 100% RH, total darkness) and evaluated twice weekly for empty chorions (indicating nymphal emergence) and inviable eggs. The incidence and duration of diapause were quantified with eggs eclosing after 30 days considered diapausing.

Results

Over this 7-month period a total of 190 nymphs and 1465 adults were collected. Both life stages were found on every survey date until julian day 172 and 179 when nymphs and adults, respectively, were no longer detected. Abundance of these life stages coincided with the wet season, in particular March-May, historically the three wettest months of the year where 35.6% of the total annual precipitation falls. Nymphs and adults disappeared in the driest months of June-August when the insect presumably survives as diapausing eggs.

At the farm level (combined plot data), *P. simulans* exhibited one well-differentiated pair of nymph and adult population peaks (Figure 3). The major nymph peak occurred days 81-130 and was preceded and followed by periods of abundance days 25-67 and 158-164. The major adult peak occurred days 109-158 but was also preceded by the apparent tail end of a previous peak days 25-38.

These results suggest a pair of population peaks at the start of the new year just before initiation of the surveys. Although these population peaks probably represent discrete and consecutive generations a precise interpretation depends on future analysis according to nymphal life stages.

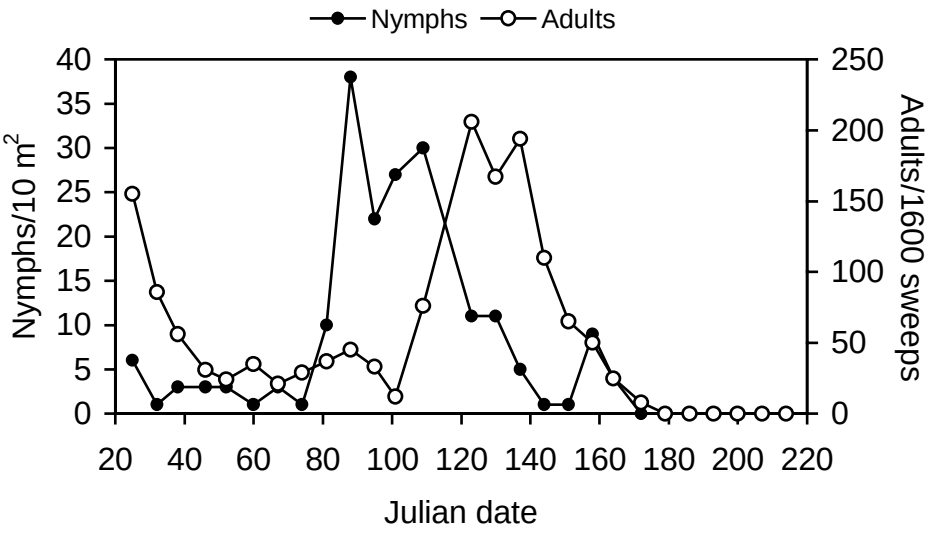

Figure 3. Population fluctuations of nymph and adult *P. simulans* populations in Piedechinche, Dept. Valle del Cauca in 2001.

Fluctuation curves for the five individual plots reveal the degree of on-farm variation in spittlebug phenology (Figures 4, 5). In terms of nymph abundance, the plot with the highest insect load (Plot 2) had 4.7 times more than the plot with the lowest (Plot 5), or 32.1% versus 6.8% of the nymph population. For the adult life stage Plot 1 ranked first and Plot 5 again ranked last with 7.4 times more adults in Plot 1, or 37.4 and 5.1% of the adults, respectively. All plots had the same general periods of peak abundance as in the overall farm fluctuation curves. However, some individual plots exhibited evidence of bimodal peaks in the period March-April. This included nymphs in Plots 2 and 4 and adults in Plots 1, 2 and 4. This suggests overlapping generations that are obscured in the overall farm fluctuation curve.

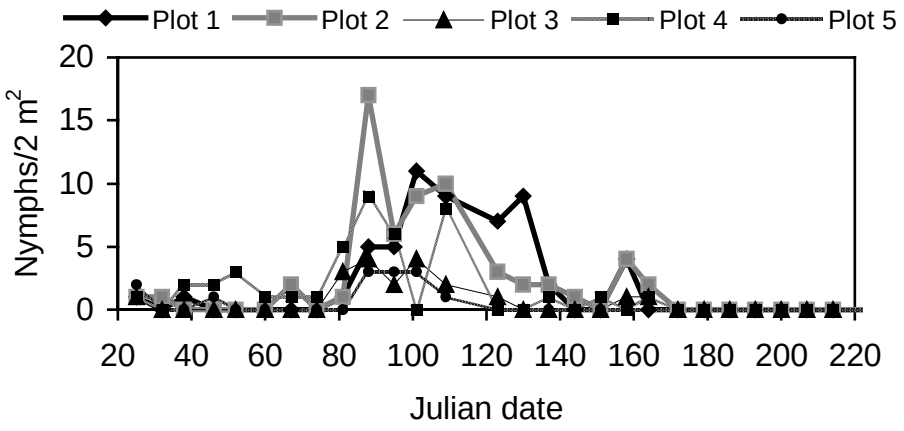

Figure 4. Population fluctuation of *P. simulans* nymphs in five *B. decumbens* pastures, Piedechinche, Dept. Valle del Cauca (2001).

Very few natural enemies were detected. Only parasitic mites (Acari: Erythraeidae) on adults were found over the survey period. Overall, 9.5% of adults had mites with a maximum of 10 mites per individual. For the entire population of adults mite load was 0.19 per adult or 0.20, 0.47, 0.21 and 0.40 per adult over

the months of March, April, May and June, respectively. Mite load according to sex was 0.19 per male and 0.16 per female.

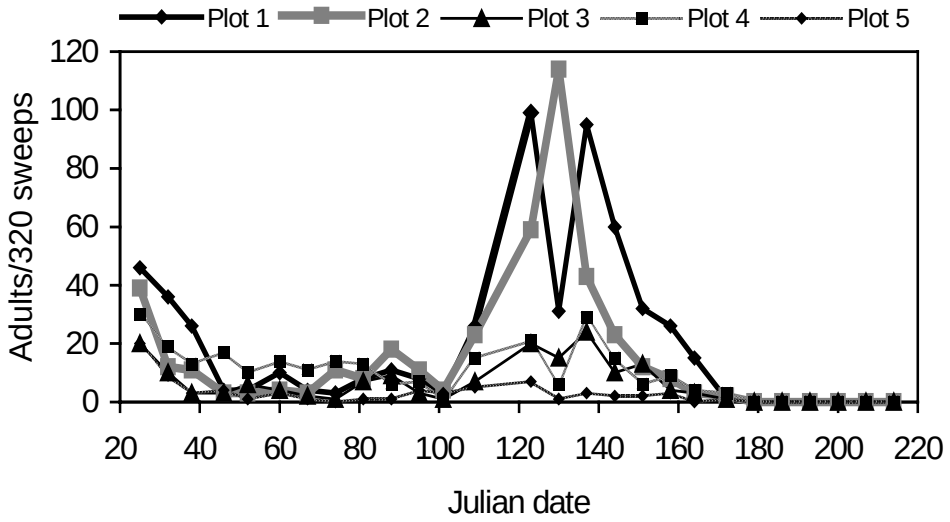

Figure 5. Population fluctuation of *P. simulans* adults in five *B. decumbens* pastures, Piedechinche, Dept. Valle del Cauca (2001).

For the 1249 eggs collected over eight dates during the first five months of the study (January-May), overall mortality was 5.5% and incidence of diapause eggs was 69.8%. With the exception of two dates (22 March, 31 May), the proportion of eggs in diapause exceeded 70% in each collection date (Table 23). There was a trend toward lower diapause incidence in the second half of the period. Eggs eclosed over a period of 18-128 days (Figure 6). Mean time to eclosion was 24.0 days for non-diapausing and 79.5 for diapausing eggs. There was a trend towards longer eclosion times for eggs in the second half of the period.

Table 23. Seasonal changes in the incidence and time to eclosion of nondiapausing and diapausing *P. simulans* eggs in Piedechinche, Dept. Valle del Cauca (2001).

Collection		Proportion (%)		Time to eclosion all eggs (days)	
Date	n	Nondiapause	Diapause	Mean ± S.E.	Range
22 Jan	204	6.4	93.6	43.0 ± 14.89	18-68
10 Feb	138	14.5	85.5	40.0 ± 11.98	20-60
23 Feb	84	3.6	96.4	53.0 ± 15.44	27-79
15 Mar	46	0.0	100.0	63.0 ± 2.16	60-66
22 Mar	69	100.0	0.0	23.0	---
5 Apr	190	29.5	70.5	74.5 ± 31.32	21-128
3 May	482	26.6	73.4	67.0 ± 28.72	18-116
31 May	36	61.1	38.9	48.5 ± 13.42	26-71
Overall	1249	30.2	69.8	18-29	30-128

Discussion

Prosapia simulans populations coincided with the wetter months of this initial survey period (January-May), and then declined and disappeared coincident with the dry season. The end of an initial generation and a complete second generation was documented based on nymph and adult peaks, but a precise

determination of generations depends on future analysis of the separate life stages of the nymphs. This further analysis will also shed light on apparent phenological differences observed among the five survey plots and help us measure the degree of on-farm variation.

The majority of eggs collected over this period were diapausing despite the apparently adequate humid conditions for population development. This differs from other species studied to date that exhibit very little diapause during the wet season. The relationship between the incidence of diapause and season for *P. simulans* is unclear and requires continued studies.

It is expected, for example, that we will document an even higher incidence of diapause in eggs collected from the field in June, start of the dry season. These methods should prove adequate for documenting the phenology of *P. simulans* populations in the field for the first time in Colombia. The new information should help us interpret the relationship between habitat and spittlebug presence and lead to predictions of the spatial and temporal arrival of outbreaks.

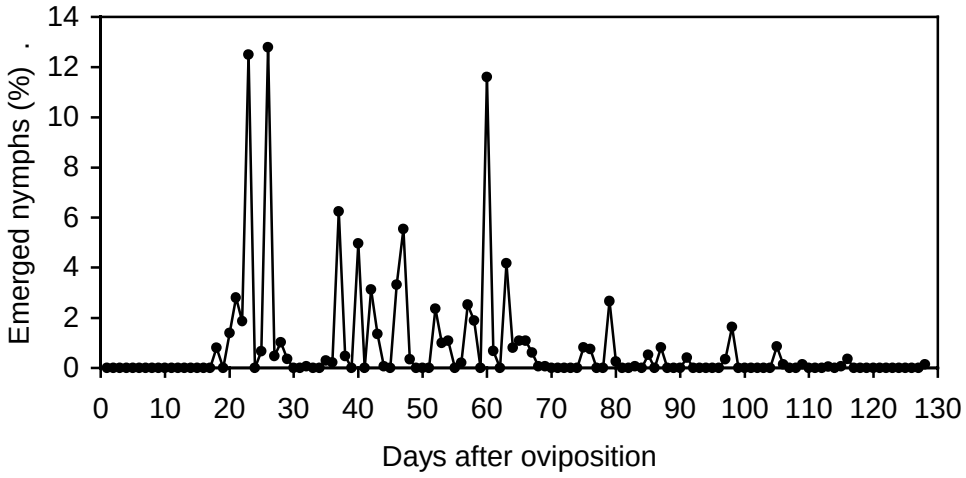

Figure 6. Pattern of eclosion of *P. simulans* eggs summed over eight collection dates (every two weeks) from January to May 2001 in Piedechinche, Dept. Valle del Cauca.

2.1.4 Population dynamics and phenology of *Zulia carbonaria*

Contributors: Ulises Castro, Anuar Morales, and Daniel Peck (CIAT)

Rationale

Over the past several years the impact of spittlebugs has apparently increased in forage grasses of the Interandean valleys and hillsides of Colombia, such as pastures of *Brachiaria* spp. in the Cauca River Valley. This area has a bimodal precipitation pattern and thereby represents an environment for studying spittlebug seasonality that is distinct from previously studied lowland sites of the highly seasonal Caribbean coast, intermediate seasonal Orinoquian Piedmont, and the continuously humid Amazonian Piedmont.

The first information on the phenology of the spittlebug complex in the Cauca Valley was presented in 2000 (CIAT Annual Report). In this report we summarize results from detailed population surveys of the spittlebug *Zulia carbonaria* over two complete years.

Materials and Methods

This study was carried out on a representative farm of the Cauca River Valley, Hacienda Las Palmas, Municipality Santander de Quilichao, Dept. Cauca. This site featured pastures of *Brachiaria decumbens* in association with the forage legume *Centrosema* sp. The methods were the same as in previously established protocols. Three 0.5-ha plots were established in separate pastures and divided into four subplots (0.125 ha) 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. Nymphs were counted and classified to life stage while adults were counted and classified to sex and species. Natural enemies were also recorded and identified.

Surveys were carried out weekly during two years (20 January 1999 to 19 January 2001). Data were analyzed to determine patterns and variation at the farm level in population fluctuation, correspondence with precipitation, population synchrony, number of generations and incidence of natural enemies.

Results

A total of 10,546 nymphs and 2,247 adults were collected during the course of this study. With the exception of one female *Prosapia simulans*, all adults were *Zulia carbonaria*. The abundance, or insect load, of *Z. carbonaria* varied greatly from one year to the next; there were 6.2 and 3.0 times more nymphs and adults, respectively, in 1999 compared to 2000 (Table 24).

Table 24. Variation in insect load of *Z. carbonaria* between years and among plots in Santander de Quilichao, Dept. Cauca.

		Insect load[1]	
Year	**Plot**	**Nymphs**	**Adults**
1999	Plot 1	760	253
	Plot 2	5704	754
	Plot 3	2612	676
	Sum	9076	1683
2000	Plot 1	703	251
	Plot 2	218	106
	Plot 3	549	207
	Sum	1470	547
Overall		10,546	2,247

[1]Measured as total number of individuals collected in surveys

There was also significant variation among the individual plots in total abundance. Insect load was 7.5 and 3.0 times greater for nymphs and adults, respectively, between the plot of lowest (Plot 1) and highest (Plot 2) abundance in 1999, and 3.2 and 2.4 times for 2000 (Plot 2 versus Plot 1). The plots of highest and lowest abundance were not consistent from one year to the next, in fact their ranking switched between 1999 and 2000.

Nymph and adult populations were most abundant during the first half of each year, coincident with the wettest months. *Z. carbonaria* essentially disappeared the second half of the year in 1999 after the two extremely dry months of June and July (Figure 7, Table 25).

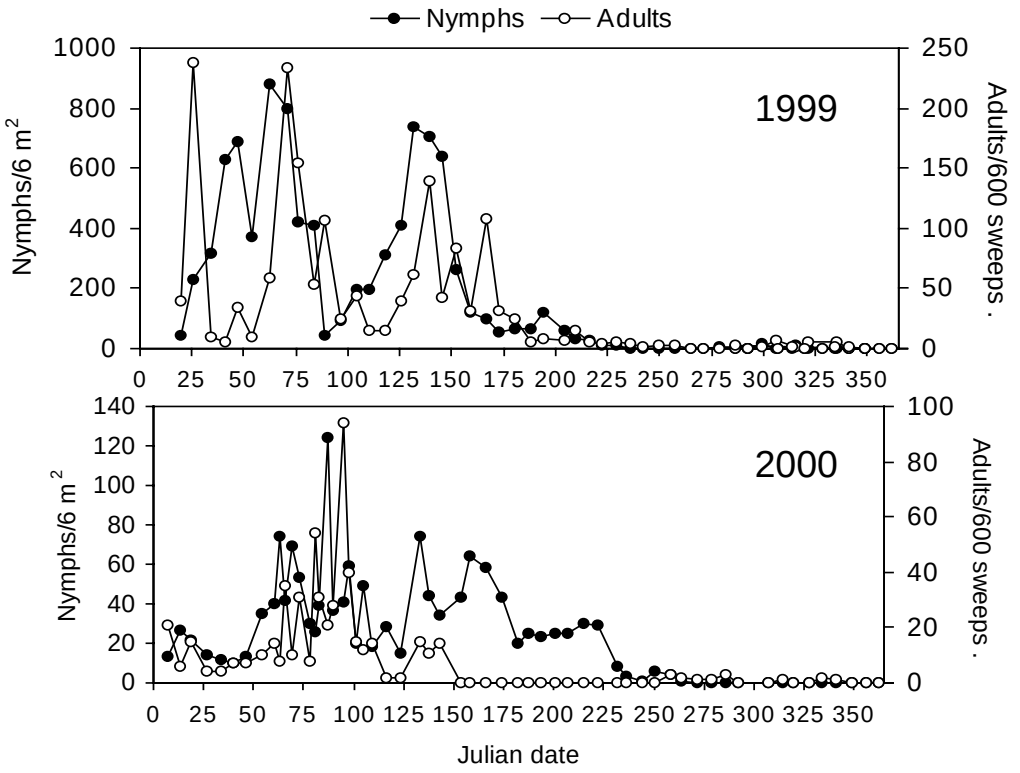

Figure 7. Population fluctuation of nymph and adult *Z. carbonaria* populations in Santander de Quilichao, Dept. Cauca over two years.

Table 25. Monthly precipitation in survey sites, Santander de Quilichao, Dept. Cauca.

	Precipitation (mm)			
Month	Mean (11 years)	1998	1999	2000
Jan	155.9	20.0	305.0	241.7
Feb	141.8	126.0	345.0	179.8
Mar	216.9	125.2	265.0	327.3
Apr	251.0	304.0	236.3	319.9
May	163.7	210.0	170.7	230.6
Jun	83.6	32.0	120.2	148.0
Jul	51.4	76.0	21.5	71.5
Aug	56.1	128.0	53.2	72.2
Sep	135.9	277.0	202.6	173.1
Oct	181.6	248.0	153.0	121.0
Nov	238.6	414.0	183.7	159.0
Dec	150.0	70.0	147.8	163.0

Populations did not recover until early 2000. Populations again declined severely after the dry months of June and July in 2001.

In 1999, population fluctuation curves revealed three well-defined peaks for each plot with a correspondence between nymph peaks and the subsequent adult peaks (Figure 8). In 2000, peaks were much less defined (Figure 9). To more precisely interpret these data and resolve different generations, population data were analyzed according to nymphal life stage. Recruitment patterns from one life stage to the next revealed three generations of *Z. carbonaria* in 1999 and four in 2000. Cumulative insect day calculations were used to quantify the arrival of discrete generations of nymphs and adults for each plot. Peak abundance was designated as the date of 50% accumulation of the insect days or the area under the population fluctuation curve. In 1999 there was little variation in the timing of generations across plots. The three generations of nymphs peaked at a mean of julian day 52.7, 132.0 and 194.0 while adults peaked at 66.0, 144.0 and 207.0 (Table 26). The time between subsequent nymph peaks and adult peaks is the generation time calculated as a mean of 70.6 (n=12, range 45-89) for the farm in 1999 with little variation among plots (Table 27).

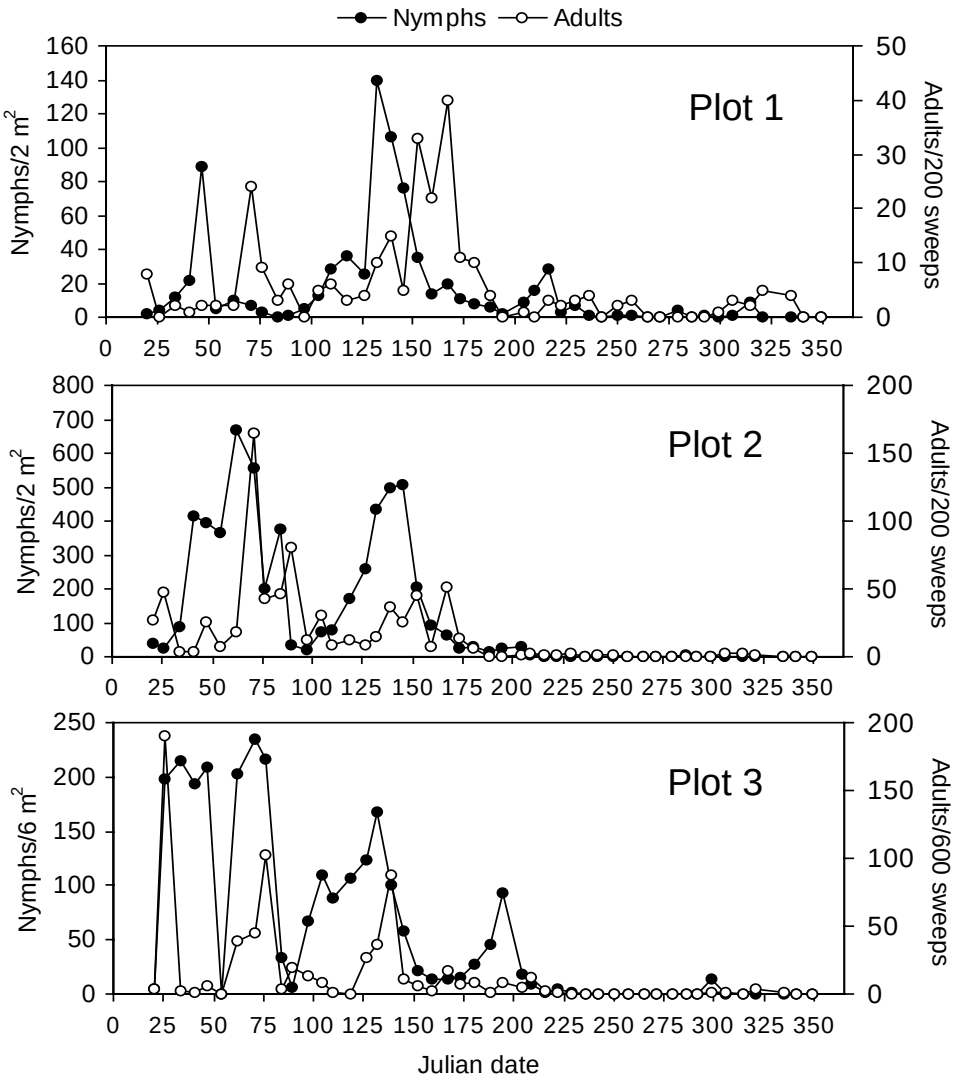

Figure 8. Population fluctuations of *Z. carbonaria* nymphs and adults in three survey plots in Santander de Quilichao, Dept. Cauca (1999).

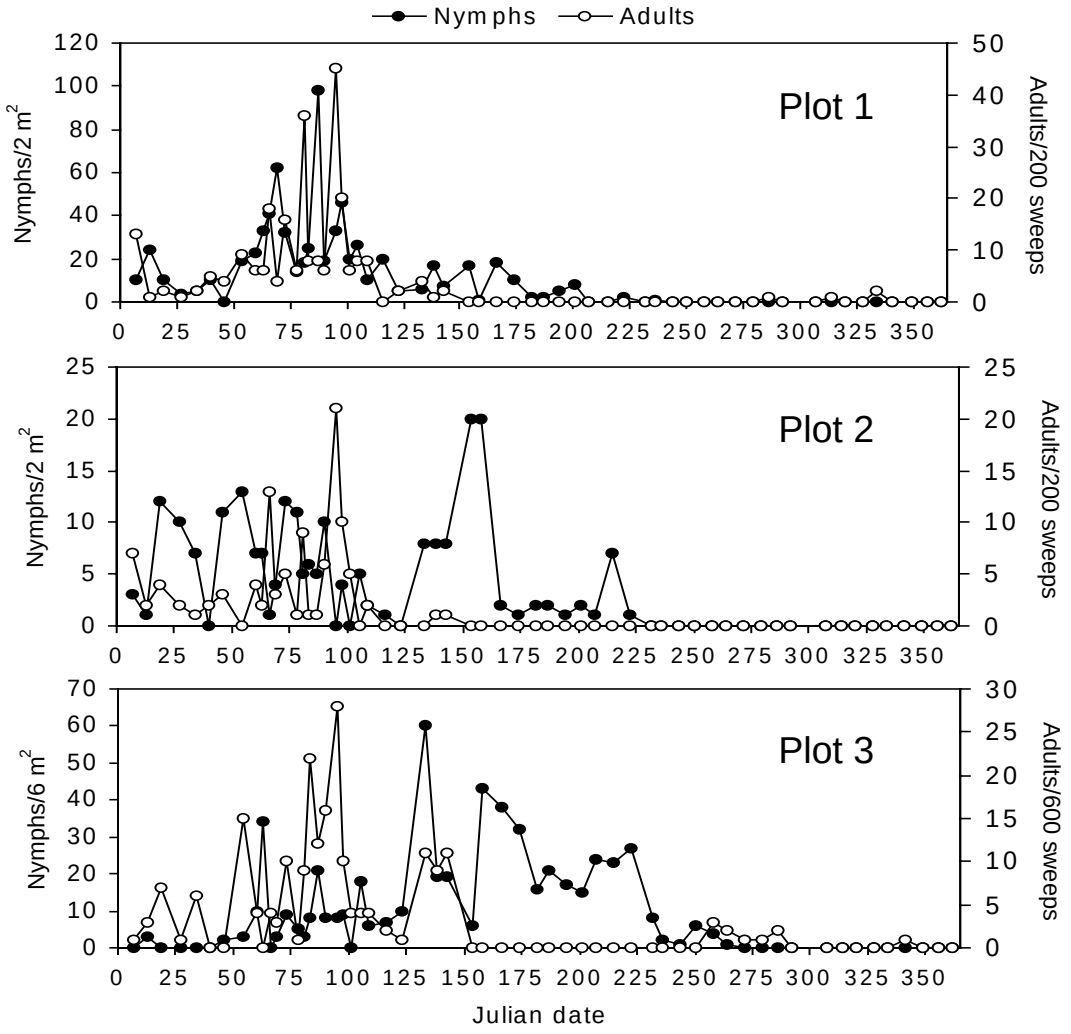

Figure 9. Population fluctuations of *Z. carbonaria* nymphs and adults in three survey plots in Santander de Quilichao, Dept. Cauca (2000).

In 2000, the phenology of spittlebug populations was best interpreted as two overlapping periods of emergence of the initial generation. The first outbreak of nymphs was day 17 leading to adults day 37. The next peak of nymphs was day 61, too early to represent progeny of the previous adult generation, and thereby probably representing the eclosion of an additional group of eggs that were late in contributing to the first generation. These dual peaks led to early and late groups of a second and third generation, and then lost any detectable separation in the fourth generation. With this interpretation, the mean generation time for 2000 was calculated as 63.9 (n=18, range 45-83) days (Table 27).

Over both years (1999 and 2000), mean generation time was 67.3 days (n=31), corresponding very well with the time determined from greenhouse biology studies (69.6 days).

Table 26. Time of arrival (calculated as 50% cumulative insect days) of *Z. carbonaria* populations in three survey plots, Santander de Quilichao, Dept. Cauca.

			50% cumulative insect days (julian date)			
Year	Generation	Life stage	Plot 1	Plot 2	Plot 3	Mean
1999	1	Nymph	47	60	51	52.7
		Adult	70	71	57	66.0
	2	Nymph	136	136	124	132.0
		Adult	156	140	136	144.0
	3	Nymph	205	185	192	194.0
		Adult	201	215	205	207.0
2000	1 a	Nymph	17	24	10	17.0
		Adult	48	38	25	37.0
	1 b	Nymph	67	53	63	61.0
		Adult	77	66	61	68.0
	2 a	Nymph	94	77	93	88.0
		Adult	97	94	92	94.3
	2 b	Nymph	142	106	133	127.0
		Adult	140	107	138	128.3
	3 a	Nymph	168	132	170	156.7
		Adult	---	140	---	140.0
	3 b	Nymph	195	154	215	188.0
		Adult	---	---	---	---
	4	Nymph	229	205	255	217.0
		Adult	---	---	267	---

Table 27. Generation time of *Z. carbonaria* calculated from population dynamics studies in three survey plots, Santander de Quilichao, Dept. Cauca.

	Generation time (days)			
Year	Plot 1	Plot 2	Plot 3	Overall
1999	72.3	67.3	72.3	70.6
N	4	4	4	12
Range	45-89	49-76	68-79	45-89
2000	65.2	50.4	76.0	63.9
N	6	7	6	19
Range	49.1-77.7	40.7-55.7	66.9-82.8	44.7-82.8
Overall	68.7	58.8	74.15	67.3
N	10	11	10	31

Discussion

Zulia carbonaria achieves 3-4 generations a year in *Brachiaria* pastures of the Cauca River Valley, increasing in abundance at the onset of the wet season, and decreasing with the dry season. Small populations were still detectable during the driest months indicating that the insect is capable of finding microhabitat suitable for maintenance of nymphs and adults despite the dry pasture conditions. The dramatic decline in population between 1999 and 2000 may have been caused by habitat alterations. Grazing bouts were more frequent and heavy in 2000 compared to 1999, degrading much of the pasture to turf-like conditions and offering poor spittlebug habitat.

Zulia carbonaria populations were shown to be highly synchronous, indicating a response to environmental variables such as mass eclosion of eggs upon return of the wet season rains. The very low frequency of diapause in eggs, however, means that *Z. carbonaria* may rely on quiescence to synchronize life cycle with humid conditions. These data will be analyzed further for the correspondence between precipitation and phenology and begin to clarify how *Z. carbonaria* phenology tracks bimodal precipitation in the Cauca River Valley.

2.1.5 First generation population phenology in two lowland sites

Contributors:: Ulises Castro, Daniel Peck (CIAT), Antonio Pérez (Universidad de Sucre) , Rafael Mora, Andrea Báez (Universidad de los Llanos), Juan Carlos Campo, Guillermo León (CORPOICA C.I., La Libertad)

Rationale

In seasonal pasture systems, the first generation of spittlebugs is of critical importance to forage production because it leads to subsequent generations (as many as six generations per year) and is the source of infestation of previously unaffected areas of the farm. Suppressing this initial outbreak depends on our ability to predict when and where focal populations of nymphs and adults will manifest on the farm and thereby more effectively target application of available control tactics. With information on environmental factors and population fluctuation of the first generation, we hope to generate a predictive model correlating the timing of initial outbreaks with precipitation patterns at the onset of the rainy season. In this report we summarize results of the first generation population phenology at two lowland sites of Colombia over two years.

Materials and Methods

The first generation population dynamics were documented in three contrasting ecoregions of Colombia over two years (2000, 2001). Survey methods were similar to those used in previous studies to document population fluctuations in forage grasses. The survey period was limited to two months starting at the beginning of the rainy season. Surveys were carried out twice weekly in three 0.5 ha focal plots each established in a separate pasture. These studies were carried out on the Caribbean Coast (pronounced seasonality, unimodal precipitation) at Finca Tarapacá, Corozal, Dept. Sucre with the collaboration of the Universidad de Sucre; in the Orinoquian Piedmont (intermediate seasonality, unimodal) at C.I. La Libertad, Villavicencio, Dept. Meta with the collaboration of CORPOICA; and the Cauca River Valley (pronounced seasonality, bimodal) at Hacienda Las Palmas, Santander de Quilichao, Dept. Cauca.

Results

The results from Cauca are summarized elsewhere.

In Meta, 64 nymphs and 566 adults were captured and assessed in 2000, 698 and 1883 in 2001 (Table 28). Populations of nymphs were very low in Plots 2 and 3 because no nymphs were detected there in 2000 and 97% of total nymphs came from Plot 1 in 2001. Adults were also much more abundant in Plot 1 where 77 and 76% of adults were recovered in 2000 and 2001, respectively. *Aeneolamia varia* comprised 75.5 and 97.8 of adult populations in the two years while *Aeneolamia reducta* comprised 22.0 and 2.2%, and *Zulia pubescens* 2.5 and 0.0%. The date of first detection of nymphs and date of peak abundance of the first nymph generation (date of 50% accumulated insect days) was julian day 108 and 117 in 2000, and ranged from 92-99 and 104-108 in 2001 across the three plots (Table 29, Figure 10). The corresponding dates for adults were 101-104 and 121-122 in 2000, and 99 and 115-117 in 2001. At the farm level over the two years, the first adult generation reached its peak 5 and 7 days, respectively, after the nymph generation.

Table 28. Comparative abundance of first generation spittlebugs surveyed in two regions over two years.

Site	Year	Life stage	Plot 1		Plot 2		Plot 3		Total
			No.	%	No.	%	No.	%	
Meta	2000	Nymphs	64	100.0	---	---	---	---	64
		Adults[1]	436	77.0	65	11.5	65	11.5	566
	2001	Nymphs	650	93.0	32	5.0	16	2.0	698
		Adults[2]	1423	76.0	270	14.0	190	10.0	1883
Sucre	2000	Nymphs	658	60.0	64	6.0	375	34.0	1097
		Adults[3]	20065	55.0	7318	20.0	9013	25.0	36396
	2001	Nymphs	157	41.0	123	32.0	104	27.0	384
		Adults[3]	7121	36.0	8418	43.0	4099	21.0	19638

[1] *A. varia+A. reducta+Z. pubescens*
[2] *A. varia+A. reducta*
[3] *A. reducta*

Spittlebug populations were much higher in Sucre where 1097 nymphs and 36,396 adults were captured and assessed in 2000, 384 and 19,638 in 2001. There were large populations across the three plots over each year. *Aeneolamia reducta* was the only species detected. The date of first detection of nymphs and date of peak abundance of the first nymph generation (date of 50% accumulated insect days) was julian day 132-143 and 145-152 in 2000, and 137-140 and 147-154 in 2001 across the three plots (Table 29, Figure 10). The corresponding dates for adults were 143-148 and 155-161 in 2000, and 140-148 and 158-162 in 2001. At the farm level over the two years, the first adult generation reached its peak 10 and 13 days, respectively, after the nymph generation.

Table 29. Time of arrival (calculated as 50% cumulative insect days) of the first spittlebug generation in two regions over two years.

Site	Year	Life stage	Date first detected			Date of abundance peak			
			Plot 1	Plot 2	Plot 3	Plot 1	Plot 2	Plot 3	Sum
Meta	2000	Nymphs	108	---	---	117	---	---	117
		Adults	104	101	101	121	123	122	122
	2001	Nymphs	92	99	99	108	104	104	108
		Adults	99	99	99	115	117	116	115
Sucre	2000	Nymphs	143	143	132	148	152	145	147
		Adults	143	143	143	155	161	158	157
	2001	Nymphs	137	140	140	147	154	154	147
		Adults	140	148	144	158	161	162	160

Discussion

In both survey sites there was little variation in the timing of the first generation among plots and between years. In Meta, the difference in arrival of nymph and adult populations between years was only 9 and 6 days. In Sucre the difference was only 8 and 5 days. From these results we predict that the timing of the return of the wet season rains was similar in 2000 and 2001 since post-diapause quiescent eggs in the soil continue their development and hatch in direct response to the return of humid conditions. These population data will be combined with data from previous years (1997-1998 in Meta and 1997-1999 in Sucre) as repetitions to establish a predictive model, based on precipitation patterns, of when the first generation of spittlebugs is expected to appear in pastures of these regions. Predicting when and where the first outbreaks occur is critical information for targeting spittlebug management tactics in highly seasonal ecosystems.

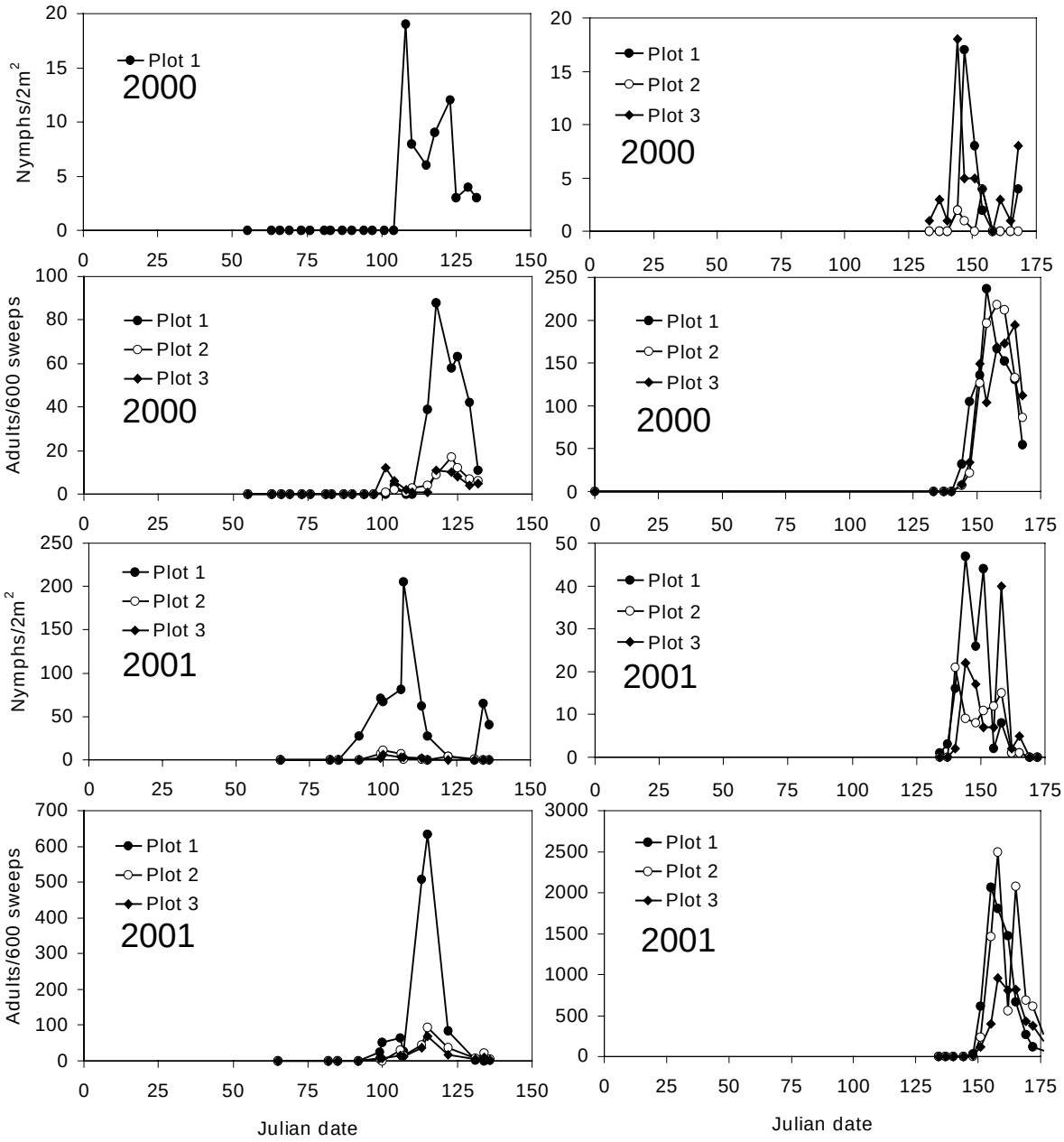

Figure 10. Population fluctuations of the first generations of spittlebug nymphs and adults in Meta (left column) and Sucre (right column) over two years.

2.1.6 Preoviposition determinants of egg diapause

Contributors: Ulises Castro, Oscar Yela, and Daniel Peck (CIAT)

Rationale

Female spittlebugs generally lay an increased proportion of diapausing eggs in response to the approaching dry season and the conditions unfavorable for spittlebug development and reproduction. The pest survives these adverse conditions of drought and high temperature as diapausing eggs that hatch upon

return of the rains in the subsequent wet season. The immature stage (nymph) is responsible for predicting the extreme conditions of the future by perceiving token environmental stimuli that induce diapause in the adult stage. In temperate zones, photoperiod and temperature are dominant stimuli involved in the induction and regulation of diapause in many insects. In the tropics, however, the precise token stimuli that induce diapause in graminoid spittlebugs remain unknown. Photoperiod probably does not play a role in Colombia due to its proximity to the equator. In this report we summarize advances in assessing the role of plant age, water stress and their combination in inducing diapause in *Aeneolamia varia*.

Materials and Methods

Plants of *Brachiaria ruziziensis* were established in wooden boxes (1.4 x 0.6 x 0.1 m) with a proliferation of surface roots required as feeding sites by the nymphs. This arrangement was described previously as a component of an improved mass rearing design (CIAT Annual Report 2000). Boxes served as units of repetition for four treatments based on combinations of two factors: host plant age (4 and 8 weeks after transplanting) and water stress (field capacity and stressed). Boxes at field capacity were watered daily at the rate of 6 l/m^2 while stressed plants were watered as the rate of 3 l/m^2 every 3 days. Each box was infested with eggs of *A. varia* collected from adults in the field in the Orinoquian Piedmont (C.I. La Libertad, Villavicencio, Dept. Meta). The CIAT colony was not used as the source for these eggs because the insects were not regarded as fully receptive to token stimuli; colony management selects strongly against diapause eggs and the most recent genetic addition to the colony were individuals from the Amazonian Piedmont (Dept. Caquetá) where conditions are continuously humid and diapause may not be important to species survival.

Each treatment had three repetitions. Boxes were infested with 1600 eggs and water treatments initiated one week later once first instars had emerged and established spittle masses. Once the adults began to appear the box was covered with an emergence cage (1.4 x 0.6 x 0.9 wooden frame covered in mesh) and individuals were collected with an aspirator and transferred to a separate small oviposition cage assigned to each repetition. After enough adults had emerged they were allowed 3 days to oviposit on fresh substrate following which eggs were extracted, disinfected and stored in petri dishes on humid filter paper under controlled conditions (27^oC, 100% RH, darkness). As more females emerged or stayed alive, a second batch of eggs was collected. Eggs were evaluated twice weekly to score chorions (emerged nymphs) and inviable eggs. Viable eggs remaining after 30 days were classified as diapausing. To confirm that treatments had an affect on the quality of the host plant, plant material in each repetition was assessed for dry weight and dry matter digestibility.

Results

Of a total 9277 eggs evaluated, 12.2% were considered diapausing. Among the four treatments, the incidence of diapause varied from 8.3-13.8 % and the time to eclosion 33.2-37.0 days (Table 30). For non-diapause eggs time to eclosion was 20.4-21.6 days and for diapause eggs 32.7-37.0 days. Analysis of variance did not detect an effect of water stress or plant age on diapause incidence. Mean dry matter (g), percent dry matter, and digestibility were measured to gauge differences in expression of the treatments on plant quality (Table 31). These means have not yet been statistically tested for differences.

Discussion

Under the conditions of these experimental treatments, no effect of host plant age or water stress was detected on the incidence or duration of diapause eggs in *A. varia*. Other factors are probably responsible for the documented increase in diapause incidence at the end of the wet season. The incidence of diapause of eggs from Meta populations was far higher than those previously examined in females from Caquetá

(0.24%), a continuously humid site. This reinforces the idea that diapause is more expressed in seasonal sites and that studies should use insects originating directly from these populations.

Table 30. Influence of host plant age and water stress on the incidence and duration (mean±S.E., range) of diapause eggs in *A. varia*.

	Eggs	Mean proportion (%)		Mean time to eclosion (days)	
Treatment	observed	Non-diapause	Diapause	Non-diapause	Diapause
Field capacity	2183	86.8 ± 0.5	13.2 ± 0.5	21.6 ± 1.4	37.0 ± 0.3
4-wk old		(86.5-87.2)	(12.8-13.5)	(20.5-226)	(36.8-37.2)
Field capacity	5017	86.5 ± 8.3	13.5 ± 8.3	20.6 ± 0.8	34.1 ± 3.5
8-wk old		(71.5-95.8)	(4.2-28.5)	(19.4-21.7)	(30.4-37.7)
Water stress	964	86.5 ± 4.7	13.5 ± 4.7	20.6 ± 1.4	33.2 ± 3.3.
4-wk old		(82.6-91.8)	(8.2-17.4)	(19.8-22.2)	(31.2-37.0)
Water stress	1113	91.7 ± 5.5	8.3 ± 5.5.	20.4± 1.9	34.9 ± 3.7
8-wk old		(83.7-96.1)	(3.9-16.3)	(18.3-22.7)	(31.4-38.2)

Table 31. Influence of host plant age and water stress on percent dry matter and in vitro digestibility of *B. decumbens*.

	Field capacity		**Water stress**	
Measure	**4-wk old**	**8-wk old**	**4-wk old**	**8-wk old**
Dry weight (g)	0.043	0.048	0.044	0.044
Dry weight (%)	60.8	63.8	64.4	54.0
Digestibility (%)	61.0	59.4	64.5	64.0

2.1.7 Seasonal changes in the incidence and duration of egg diapause

Contributors: Ulises Castro, Daniel Peck (CIAT), Antonio Pérez (Universidad de Sucre), Rafael Mora, Andrea Báez (Universidad de los Llanos), Juan Carlos Campos, Guillermo León (CORPOICA C.I. La Libertad)

Rationale

For various spittlebug species in seasonal environments it has been shown that the incidence of diapause eggs increases at the end of the wet season in anticipation of the unfavorable dry season. To complement studies on the population dynamics of spittlebug nymphs and adults in contrasting regions of Colombia, changes in the incidence and duration of diapause eggs was documented over the season in three contrasting ecoregions: the Caribbean Coast, the Orinoquian Piedmont and the Cauca River Valley.

Materials and Methods

One year of data has been analyzed for *Aeneolamia reducta* (Dept. Sucre, Caribbean Coast), *Aeneolamia varia* (Dept. Meta, Orinoquian Piedmont) and *Zulia carbonaria* (Dept. Cauca, Cauca River Valley). These data were collected from the same focal paddocks established in these sites for population dynamics studies. In each of the three plots per farm, two groups of females (1-5 for Cauca, 1-25 for Meta and Sucre, depending on availability) were caught with sweep nets, brought to the laboratory and confined to large petri dishes (2 cm tall, 15 cm diameter) lined on the bottom with moist filter paper that served as oviposition substrate. Females laid eggs over 3 days, the filter paper was disinfected for 2-3 min with Clorox and washed with distilled water, and petris were express mailed to CIAT for incubation under controlled conditions (27^{o}C, 100% RH, darkness). Collections were made every 2 wk over the season

when females were available in the field. Petri dishes were evaluated twice weekly for chorions (emerged nymphs) and inviable eggs.

Results

Of 38,088 eggs collected and evaluated from January to December 2000, 12.95% were diapausing (Table 32). The incidence of egg diapause in *Z. carbonaria* was extremely low throughout the year (Figure 11). Diapausing eggs were only detected on one date (July) and at very low incidence. The six diapause eggs eclosed 36, 36, 46, 46, 53 and 57 days after oviposition. In Sucre and Meta the incidence of diapause increased at the end of the year at the start of the dry season achieving rates as high as 85.0 and 49.5%, respectively. Overall, the time to eclosion for non-diapause eggs was 22.7 ± 5.5 for *A. reducta*, 18.2 ± 4.6 for *A. varia* and 18.0 ± 1.9 for *Z. carbonaria* (Table 32). The time to eclosion for diapausing eggs was 96.9 ± 43.6, 71.5 ± 32.7 and 48.2 ± 12.5 d, respectively. In Meta the time to eclosion of diapause eggs did not vary over the season, however in Sucre there was a noticeable increase in duration toward the end of the season.

Table 32. Seasonal changes in diapause incidence and time to eclosion of spittlebug eggs in three ecoregions of Colombia (bars indicate periods where no females were available).

Month	Incidence of diapause (%)			Time to eclosion (days)			n (eggs examined)		
(2000)	Sucre	Meta	Cauca	Sucre	Meta	Cauca	Sucre	Meta	Cauca
Jan	---	---	---	---	---	---	0	0	0
Feb	---	---	---	---	---	---	0	0	0
Mar	---	---	---	---	---	---	0	0	0
Apr	---	0.0	0.0	---	---	---	0	305	183
May	---	0.0	0.0	---	---	---	0	645	315
Jun	0.01	0.0	0.0	55	---	---	3781	1986	531
Jul	2.0	0.1	3.3	50	---	48	4274	2651	174
Aug	2.0	0.6	0.0	55	74	---	1916	6244	110
Sep	---	8.1	---	---	43	---	0	2991	0
Oct	57.0	11.2	---	137	89	---	1419	4953	0
Nov	72.0	2.7	0.0	127	53	---	3692	513	289
Dec	85.0	49.4	0.0	134	68	---	914	166	0

Discussion

In the Caribbean Coast and the Orinoquian Piedmont the incidence of diapause increased from zero at the beginning of the wet season to 85 and 50%, respectively, at the start of the dry season. In both of these sites the rainy season is highly seasonal with 2-4 months of extremely dry months annually and in accordance with our expectations, the principal spittlebug species in these regions use diapause as a means to survive the adverse conditions. In the Cauca River Valley, precipitation is bimodal and although dry periods are severe, they are comparatively brief.

These seasonality conditions may allow for different mechanisms for surviving the adverse conditions because no seasonal changes in the incidence of diapause or the time to egg eclosion was detected. In this region, *Z. carbonaria* may adopt a different strategy, such as maintenance of low population levels in localized humid areas with subsequent recolonization of pastures. Alternatively, instead of diapause *Z. carbonaria* could depend on drought-tolerant quiescent eggs where development is temporarily delayed in direct response to adverse conditions, and reinitiated once adequate humid conditions return. These possibilities will be explored in future studies.

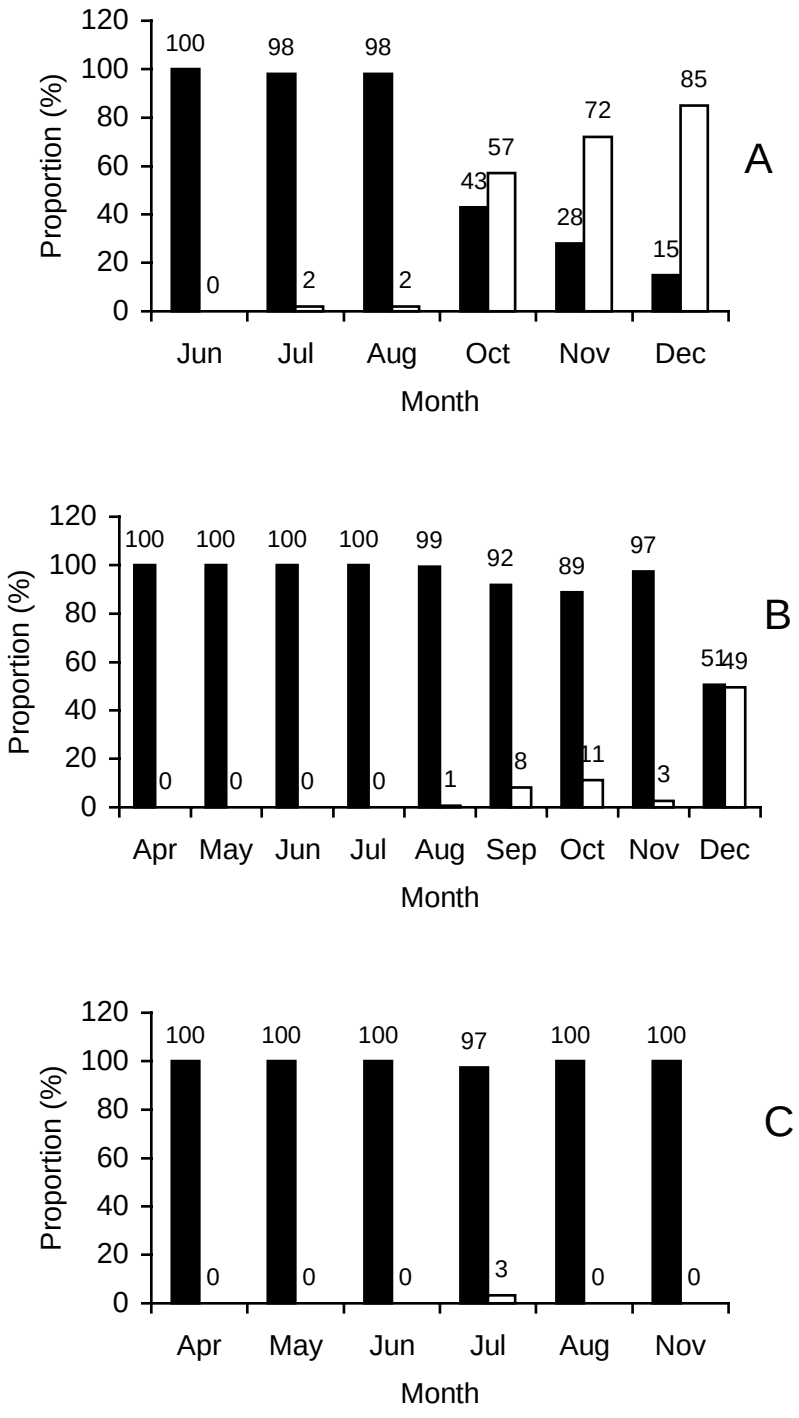

Figure 11. Seasonal changes in the incidence of spittlebug egg diapause in (A) the Caribbean Coast with *A. reducta*, (B) the Orinoquian Piedmont with *A. varia*, and (C) the Cauca River Valley with *Z. carbonaria* during 2000. Black bars are non-diapause and open bars are diapause eggs.

Activity 2.2 IPM components for spittlebug management

Highlights

- An artifical diet validated as effective for maintaining adult *Aeneolamia varia*.
- Strengthened the collection of fungal entomopathogens of major insect pests which now includes 73 isolates from cassava pests (burrower bugs, stem borers, whiteflies) and 77 from forage grass pests (spittlebugs)
- Eighteen fungal entomopathogen isolates were screened for virulence to burrower bug nymphs and adults showing high levels of virulence in certain strains.
- Determined that virulence of fungal entomopathogen isolates varies among spittlebug species.
- Protocols established for determining LC_{50} and LC_{90} of fungal entomopathogen isolates to spittlebug nymphs.
- Field studies initiated in two contrasting regions to test the number and timing of applications of a formulated fungal entomopathogen product to suppress spittlebug populations.

Efforts to control spittlebugs in forage grasses and other graminoid crops have been compromised by difficult access to the literature, inappropriate research methodologies, and rudimentary decision-support tools and other components of IPM. In addition, there is lack of a model system for tailoring IPM to the contrasting ecoregions and agricultural production systems where spittlebugs occur.

Results from CIAT's group on Spittlebug Bioecology and IPM over the period 1997-2001 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.

Progress towards achieving output milestones

- **IPM components relevant to spittlebug management in forage grasses and other graminoids better understood**
  In 2001 we continued to advance the management of spittlebugs through studies on diverse components of IPM. This research included evaluating artificial diets for the maintenance of spittlebug adults; strengthening and maintaining a ceparium of diverse fungal entomopathogen isolates; screening this entomopathogen collection for the most virulent strains; evaluating the variation in virulence across spittlebug life stages and species; and evaluating and deploying the most promising isolates in field trials. These and other IPM tools will be assessed withinin contrasting ecoregions serving as model systems where bioecological information is been acquired concurrently, ultimately leading to recommended IPM programs for field testing, followed by modification and impact assessment.

2.2.1 Artificial diet for maintenance of spittlebug adults

Contributors: Ulises Castro, Claudia Flores, Rosalba Tobón, and Daniel Peck (CIAT)

Rationale

The development of an artificial diet for maintenance of spittlebug adults would permit the evaluation of feeding deterrents or deleterious compounds such as lectins that could be incorporated into *Brachiaria*

though genetic transformation. Here we summarize results of three trials of a particular formula chosen as the best option from among a series of diets developed and assessed in 2000.

Materials and Methods

Trials consisted of three treatments: vial, plant and diet. In the plant treatment, adults were enclosed inside cylindrical acetate cages (40 cm tall, 15 cm diameter) over potted *Brachiaria ruziziensis* plants that provided a food source. In the other treatments adults were held in large petri dishes (2 cm tall, 15 cm diameter). In the vial treatment, the food source was stems of *B. ruziziensis* kept with their base in a vial of water. In the diet treatment, 500 µl of the liquid diet was sealed in parafilm sachets (3 x 3.5 cm). Thirteen repetitions were carried out over three trial periods. Each of the three trials and all their treatments were performed in an insect growth chamber with mean (± S.E.) temperatures of 23.8 ± 1.5, 22.5 ± 3.2 and 23.21 ± 1.4°C, respectively. Each treatment repetition had four adult *Aeneolamia varia* taken as tenerals (<12 hours old) from the CIAT colony (Table 33).

Table 33. Results from Probit analysis for three trial of three diets.

Treatment	Trial	n	X^2	Prob. X^2	b	L_{50}	L_{90}
Vial	1	585	7.6377	0.6642	6.7316	7.0661	10.9537
	2	780	30.0965	0.0027	7.6551	8.6623	12.7364
	3	728	44.5463	0.0000	5.1588	6.5599	11.6229
Diet	1	784	15.5965	0.2716	8.3733	9.9825	14.2001
	2	780	6.7797	0.8718	8.1406	8.7894	12.6295
	3	888	215566	0.0882	6.0587	7.5096	12.2219
Plant	1	550	43.3962	0.0000	7.0250	6.0452	9.2011
	2	714	9.4180	0.5834	14.7956	10.4654	12.7753
	3	901	5.2907	0.9813	10.6372	10.9781	14.4879

The composition of the diet was modified from Hagley 1967. To make the diet easier and cheaper to prepare, yeast and hydrolyzed casein were substituted as the sources of amino acids and p-aminobenzoic acid substituted as the source of vitamin B_{12}. The list of ingredients in the diet were (1) amino acids: yeast extract, hydrolyzed casein, (2) vitamins: biotin, calcium pantothenate, choline chloride, folic acid, inositol, nicotinic acid, pyridoxine, thiamine, riboflavin, ascorbic acid, ρ-aminobenzoic acid, (3) carbohydrates: sucrose, (4) salts: $MgCl_2$, KH_2PO_4, Wesson's' salts, and (5) lipids: cholesterol benzoate. Adult mortality was assessed daily. Mean adult longevity was determined with Probit analysis and differences among treatments were tested with an ANOVA.

Results

Mean longevity among repetitions varied from 6.0-11.0 days. Analysis of variance detected no significant differences among the three diet treatments with mean longevity of 7.4, 8.7 and 9.2 days for vial, diet and plant treatments, respectively. Probit analysis, however, indicated that more trials will have to be carried out due to low X^2 values in several individual trials.

Discussion

Replacement of certain components in the original diet (Hagley 1967) did not affect the maintenance and longevity of adults *A. varia*. Use of yeast extract and hydrolyzed casein as the sources of amino acids made preparation easier and could significantly reduce costs. Whether the diet is effective for maintenance of nymphs remains to be tested; Hagley's original diet was designed and tested only for

adults. Regardless, the current formulation should be adequate for future studies on substances of interest in adult feeding or toxicity.

2.2.2 Maintenance of a ceparium for fungal entomopathogens of major forage grass and cassava pest

Contributors: Anuar Morales, Rosalba Tobón, Mauricio Rendón, Irina Alean, and Daniel Peck (CIAT)

Rationale

Ongoing field studies on the major insect pests in diverse regions of Colombia have allowed us to collect, isolate, propagate and store a diverse collection of fungal entomopathogens. This ceparium was established last year (CIAT Annual Report 2000) and is designed to serve as a source of pathogenic material for studies on biological control. Maintaining and strengthening this collection is of utmost importance for advancing non-toxic alternatives to insecticides and other effective tactics as components of integrated pest management. In this report we summarize maintenance and diversity of the ceparium with a particular focus on the fungal entomopathogens of forage grass pests (spittlebugs) and cassava pests (burrower bugs, stem borers, whiteflies).

Materials and Methods

There are two main activities related to the ceparium. The first consists of the isolation, maintenance, propagation and storage of isolates based on previously established protocols (see CIAT Annual Report 2000). The second is the multiplication of isolates for reactivation and studies on virulence and pathogenicity.

Results and Discussion

The CIAT ceparium now houses a total of 150 different isolates of fungal entomopathogens. In the area of cassava pests, 34 new isolates of fungal entomopathogens were added to the 39 strains already purified and stored on filter paper (Table 34). Of the 73 isolates, 28 were reactivated on the burrower bug *Cyrtomenus bergi* (Heteroptera: Cydnidae) and 18 of these have been evaluated for virulence to nymphs and adults in laboratory studies. Once the efficiency of these isolates is calculated in comparison to mortality in the controls, the five most virulent isolates will be selected for future studies. The levels of control are highly promising with up to 100% mortality in nymphs and 58% in adults (Table 35).

Seven other isolates of diverse fungi including *Paecilomyces* spp. (CIAT 210, 211, 212, 216), *Verticillium lecani* (CIAT 215), *Beauveria bassiana* (CIAT 217) and *Cladosporium* sp. (CIAT 272) were reactivated on nymphs and adults of the whitefly *Aleurotrachellus socialis*. These isolates are currently in the multiplication phase to provide material for the first applications to determine pathogenicity and virulence.

In the area of spittlebug pests of forage grasses, the main activities were related to selection and characterization of isolates for field trials (see section 2.2.5) including (1) multiplication for virulence studies on different species of adult spittlebugs (see section 2.2.3), (2) multiplication for determination of LC_{50} and LC_{90} in nymphs (see section 2.2.4), (3) and quality control studies of formulated material developed in collaboration with BioCaribe, S.A. with the goal of achieving a product of higher quality.In terms of ceparium maintenance, viability tests of stored material are continuously carried out with the goal of reactivating on culture media the isolates that have lost vigorous growth characteristics. Three new isolates from spittlebugs were incorporated into the collection this year: CIAT 076 isolated from a nymph

collected in C.I. Macagual, Dept. Caquetá; and CIAT 077 and 078 isolated from an adult of *Mahanarva andigena* collected in Tumaco, Dept. Nariño.

Table 34. Accession, host and origin of fungal isolates entomopathogenic to different cassava insects.

Host species	CIAT accession numbers	Origin	
		Department	Municipality
Aleurotrachellus socialis	CIAT 215-217	undet.	undet.
Brassoly sp.	CIAT 246	Casanare	Villanueva
Chilomima klarkei	CIAT 249, 252-257, 263-267, 269	Tolima	Espinal
Chilomima klarkei	CIAT 274	Tolima	Ibague
Chilomima klarkei	CIAT 277	Tolima	Nataima
Coleoptera	CIAT 262	Cauca	undet.
Corinus sp.	CIAT 219	Valle	La Cumbre
Cosmopolites sordidus	CIAT 247	Valle	Jamundi
Cyrtomenus bergi	CIAT 200	Cauca	Timbio
Cyrtomenus bergi	CIAT 214, 224, 225	undet.	undet.
Cyrtomenus bergi	CIAT 226-243	Cauca	Popayan
Cyrtomenus bergi	CIAT 250, 251, 258-261, 268, 275, 276	Risaralda	Pereira
Erinnys ello	CIAT 218	undet.	undet.
Galeria melonella	CIAT 208, 213	Valle	Pradera
Galeria melonella	CIAT 270	Risaralda	Pereira
Galeria melonella	CIAT 271, 273	Tolima	Guamo
Galeria melonella	CIAT 278, 279	Cauca	Cajibio
Hymenoptera	CIAT 248	Valle	Palmira
Trialeurodes vaporariorum	CIAT 210-212	Valle	Pradera
Trialeurodes variabilis	CIAT 272	Tolima	Espinal
Whitefly	CIAT 244	undet.	Imbabura
undet.	CIAT 209	Valle	Palmira
undet.	CIAT 220-222	undet.	undet.

Table 35. Virulence (% mortality) of 18 fungal entomopathogen isolates to nymphs and adults of the burrower bug *C. bergi*.

Accession	Nymphs	Adults	Accession	Nymphs	Adults
CIAT 227	66.0	56.0	CIAT 230	89.0	53.0
CIAT 231	53.0	48.3	CIAT 237	81.0	50.0
CIAT 233	67.0	53.3	CIAT 261	74.0	49.0
CIAT 234	58.0	31.7	CIAT 224	100.0	47.0
CIAT 241	30.0	58.3	CIAT 245	100.0	47.0
CIAT 242	55.0	50.0	CIAT 239	76.0	33.0
CIAT 250	52.0	56.7	CIAT 228	55.0	23.0
CIAT 258	58.0	55.0	CIAT 238	50.0	20.0
CIAT 259	51.0	65.0	CIAT 240	74.0	21.0

In addition to these activities, "Access" software was used to establish a database to manage all information related to ceparium isolates. This program allows easy consultation of the information by interested scientists.

2.2.3 Variation in the virulence of fungal entomopathogens among spittlebug species

Contributors: Anuar Morales, Rosalba Tobón, Jairo Rodríguez, Ulises Castro, Oscar Yela, and Daniel Peck (CIAT)

Rationale

A major challenge for the implementation of an integrated management plan for graminoid spittlebugs is the taxonomic diversity of species that contribute to this pest complex. In Colombia, for instance, 15 species from six genera have been identified with graminoid host plants. Management is limited by the extent that a particular control tactic can be tailored to different species, further complicated by the presence of 2-3 species in the same local pastures. We are obtaining new information on the biology and ecology of major species in contrasting ecoregions of Colombia, and this information is broadening our understanding of the variation across this group and of the different strategies for their management in different habitats. From these results we predict that particular control tactics will also need to be tailored to the particular spittlebug species and habitat in which control is required. In the specific case of fungal entomopathogens as biological control agents, effectiveness of a given isolate may also vary across species. Advancing the use of fungal entomopathogens in an IPM program for spittlebug management will therefore depend on gauging the variation in virulence across different spittlebug species.

Materials and Methods

Methods were based on protocols established and described in 2000 (CIAT Annual Report). Evaluation units were 30-day old plants (7-10 stems) of *Brachiaria ruziziensis* (CIAT 654) in pots (15 cm diameter) covered by acetate cylinders (40 cm tall, 15 cm diameter). These plants were infested with 10 adult tenerals (<24 hours old) of *Aeneolamia reducta*, *Aeneolamia varia, Zulia carbonaria* and *Zulia pubescens* obtained from colonies maintained at CIAT. Two to three hours after infestation plants were sprayed with 5 ml of a concentrated conidial suspension (10^8 conidia/ml) with an airbrush and compressor (10 PSI). Four isolates were evaluated: CIAT 007C, CIAT 009, CIAT 054 and CIAT 055, identified as *Metarhizium anisopliae*, *Paecilomyces farinosis Metarhizium* sp. and *Metarhizium* sp. respectively. These isolates were selected from among 48 strains as the most virulent to *A. varia* adults (see CIAT Annual Report 2000).

For each spittlebug species, 10 repetitions (pots) were evaluated for each isolate and a control (water with tween at 0.05%). After spraying, plants and insects were maintained in a growth chamber (27°C ± 2°C, RH 80% ± 10%). Virulence 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. Differences were evaluated with an ANOVA and Tukey multiple range test.

Results

Mortality in the control varied from 3.9-28.5 among the four spittlebug species. The lowest mortality was experienced by *Z. pubescens* and the highest by *A. reducta*, corresponding to the species of longest and shortest adult longevity according to greenhouse biology studies.

Virulence of isolates varied significantly among species with *A. varia* being most susceptible, followed by *A. reducta*, then *Z. carbonaria* and *Z. pubescens* (Figure 12). As expected, control for all four isolates was significantly higher on *A. varia* since this species was used for preselecting the most virulent strains used in this study. Mortality ranged from 62.8-95.1%. Mortality in *A. reducta* ranged from 42.5-61.9%.

For *Z. carbonaria* and *Z. pubescens*, mortality ranged from 20.2-33.6 and 16.1-30.4%, respectively, and in most cases this was not significantly different than the control. In the case of *Z. carbonaria*, none of the isolates achieved higher mortality than the control. The *Paecilomyces* isolate (CIAT 009) achieved relatively higher mortality (33.6%) in *Z. carbonaria* compared to the *Metarhizium* isolates.

Control was also significantly higher in the genus *Aeneolamia* compared to *Zulia* for all four isolates evaluated. For instance, CIAT 054 achieved a mortality of 56.8 and 95.1% for *A. reducta* and *A. varia*, respectively but only 31.4 and 16.1 for *Z. carbonaria* and *Z. pubescens*. This suggests that some of the variance in virulence among spittlebug species may be expressed as differences at the genus level.

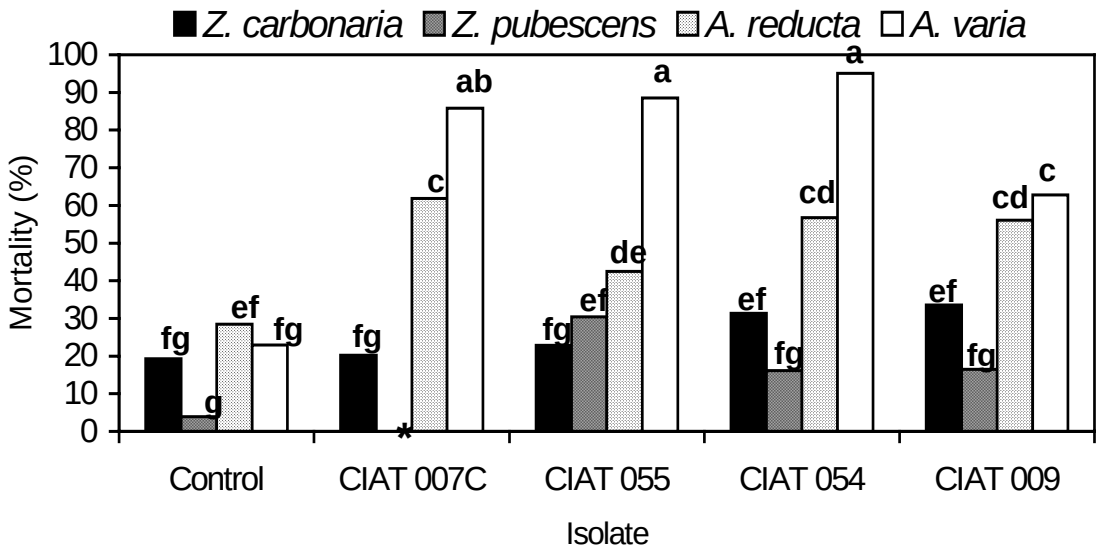

Figure 12. Mortality (absolute percent) of four isolates of fungal entomopathogens on four spittlebug species. Means followed by different letters are significantly different at P<0.05. * This isolate was not evaluated on *Z. pubescens*.

Discussion

Virulence of fungal entomopathogen strains varies among spittlebug species. Deploying these pathogens as agents of biological control therefore depends on an understanding of the species complex in the area where control is desired, selecting isolates specific to spittlebug species, and reassessing the broad effectiveness of commercial products. On the other hand, results indicate that the diverse collection of isolates in CIAT's ceparium probably has strains highly virulent to species other than *A. varia*, which up to this point has been used as the model species for developing evaluation methodologies. The most efficient screening process might therefore be evaluating a diversity of isolates to the particular spittlebug species of interest, rather than using preselection (with a model species such *A. varia*) with subsequent confirmation of high control on other species. One particular screening focus should be *Paecilomyces* isolates with *Z. carbonaria* since this fungus was relatively more virulent against this species than *Metarhizium*. At present there are three *Paecilomyces* strains in the ceparium that were originally isolated from *Z. carbonaria*.

These results confirm the need to continue documenting the patterns of variation among graminoid spittlebugs given that effectiveness of control tactics such as insect pathogens may be species specific.

Significant variation in host plant resistance among spittlebug species is further corroboration of this observation. Studies are under way to continue evaluating variation in virulence. Adults of *P. simulans* are under evaluation and variation between adults and nymphs are being explored with *A. varia*, *P. simulans*, *Z. carbonaria* and *Z. pubescens*.

2.2.4 Characterization and formulation of select fungal entomopathogen isolates for field evaluation

Contributors: Anuar Morales, Rosalba Tobón, Oscar Yela, and Daniel Peck (CIAT)

Rationale

Four isolates have been selected from CIAT's fungal entomopathogen collection for experimental field trials designed to test application techniques. These isolates are the three *Metarhizium* and one *Paecilomyces* strains screened from 49 isolates (see CIAT Annual Report 2000) as the most virulent to adults of *Aeneolamia varia* (Table 36). Before deploying in the field, these isolates must be characterized for their biological and virulence activity on different species and life stages of spittlebugs. Variation in virulence among adults of four species was described elsewhere. Here we summarize results of studies to determine the LC_{50} and LC_{90} on nymphs of *A. varia*.

Table 36. Identification and origin of fungal entomopathogen isolates selected for field trials.

	CIAT accession number			
	CIAT 054	CIAT 055	CIAT 007C	CIAT 009
Fungal isolate :				
Genus	*Metarhizium*	*Metarhizium*	*Metarhizium*	*Paecilomyces*
Species	sp. 1	sp. 2	*anisopliae*	*farinosis*
Spittlebug host:				
Genus	*Aeneolamia*	*Aeneolamia*	*Zulia*	undet.
Species	*Varia*	*varia*	*pubescens*	
Sex	Male	undet.	female	undet.
Life stage	Adult	nymph	adult	nymph
Department	Valle del Cauca	Valle del Cauca	Caquetá	Caquetá
Municipality	Palmira	Palmira	Albania	Florencia

Materials and Methods

Evaluation methods for nymphs were based on previously established protocols (see CIAT Annual Report 2000). Evaluation units were the same small-scale PVC tubes (1.5" diameter) now standard for host plant resistance screening. At 6 weeks after planting with *Brachiaria ruziziensis* (CIAT 654), surface roots were sufficiently 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. Nine different concentrations of conidial suspensions ($1x10^4$, $5x10^4$, $1x10^5$, $5x10^5$, $1x10^6$, $5x10^6$, $1x10^7$, $5x10^8$, $1x10^9$ conidia/ml) were prepared for three isolates (CIAT 007C, CIAT 054, CIAT 009) with a control (water and tween at 0.05%) (Table 37).

Applications were made on the substrate before infestation and on the eggs in petri dishes before infestation. An airbrush and compressor (10 PSI) were used at a volume of 1 ml for substrate and <1 ml for direct egg application. Plants were maintained in the greenhouse until evaluation of mortality 30-32 days after infestation. During this period, plants were fertilized twice (just before and 15 days after

infestation) with urea at 2g/l. There were ten repetitions per treatment. Mortality data were analyzed with Probit (SAS).

Table 37. Probit analysis of mortality caused by three fungal entomopathogen isolates to nymphs of *A. varia*.

Isolate	N	LC_{50} (95% CI)	LC_{90} (95% CI)	X^2	Prob X^2	B (S.E.)
CIAT 054	900	$8.0x10^6$ ($3.7x10^6$-$1.3x10^7$)	$8.9x10^7$ ($5.2x10^7$-$2.0x10^8$)	7.3	0.290	1.2 (0.19)
CIAT 009		-	-	22.3	0.66	2.4 (0.25)
CIAT 007C	900	$4.6x10^5$ ($1.6x10^4$-$3.0x10^6$)	$3.6x10^8$ ($6.7x10^7$-$4.4x10^9$)	12.2	0.057	0.44 (0.06)

Results

For CIAT 054 and CIAT 007C, *A. varia* nymph mortality increased with increasing conidial concentration as expected (Figure 13). For CIAT 009, however, the relationship between mortality and concentration was not clear, showing irregular activity along the concentration gradient.

Probit analysis showed low X^2 values and acceptable X^2 probability values for CIAT 054 and CIAT 007C. The LC_{50} and LC_{90} were $8.0x10^6$ and $8.9x10^7$ conidias/ml for CIAT 054, and $4.6x10^5$ and $3.6x10^8$ for CIAT 007C. Given the high X^2 value for CIAT 009, the calculated concentrations are inaccurate and the trial must be repeated.

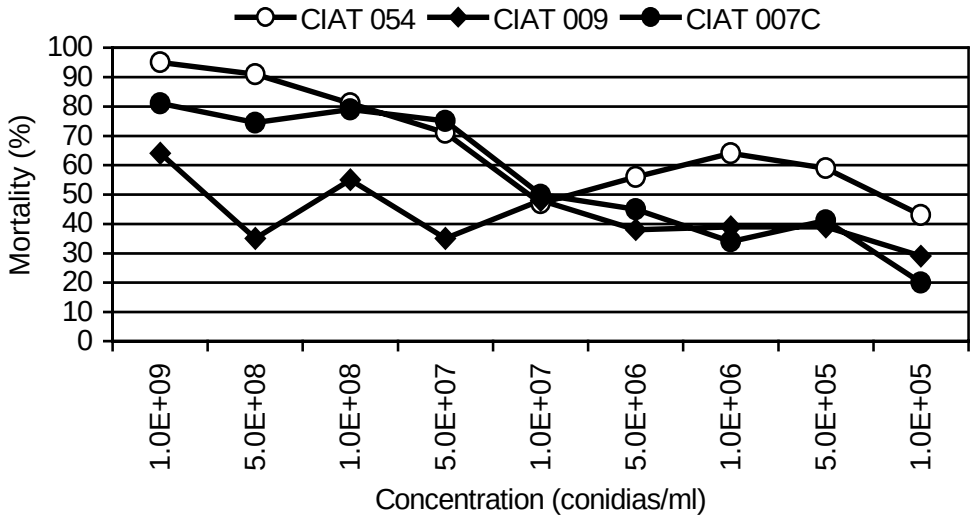

Figure 13. Mortality in *A. varia* nymphs caused by three fungal entomopathogen isolates at different concentrations.

Comparative studies are currently underway with the same three isolates and nymphs of *Prosapia simulans*, the major spittlebug species in one of the two field evaluation sites. An additional phase before field evaluation is the formulation of strains. This is being carried out by BioCaribe, S.A. in a formal agreement with CIAT. To confirm the quality of this material, studies are underway to compare the effectiveness of formulated versus unformulated product.

Discussion

Even though identical methodologies were used for the isolates, different LC_{90} were expected given the different origin of the strains. Applications of fungal entomopathogens in upcoming field trials will be based on the LC_{90} determined here to avoid the situations where too little material is applied to have an effect, or too much is added and material is wasted. Ongoing studies will corroborate this information and establish whether formulation has altered virulence. Plans are also underway to evaluated LC_{50} and LC_{90} on adults of *A. varia*.

2.2.5 Field evaluation of fungal entomopathogens in two contrasting regions

Contributors: Anuar Morales, Jairo Rodríguez, Ulises Castro, Oscar Yela, Daniel Peck (CIAT), Daniel Corradine, German Chacón, Orlando Narváez, Fabio Obregón (Universidad de la Amazonia)

Rationale

In general, previous attempts to evaluate the efficiency of fungal entomopathogens as biological control agents of spittlebugs in pastures have been focused on laboratory assays. The few that have gone to the field have demonstrated highly variable and low levels of control due to a variety of factors including poor evaluation and applications techniques. Aspects such as the number of applications and the timing of applications in relation to phenology of the life stages have received no attention. To seriously evaluate the potential of fugal entomopathogens as an alternative for managing pasture spittlebugs, we are combining a detailed knowledge of the biology and phenology of spittlebugs with a series of studies to collect, screen, characterize, and formulate select isolates for deployment in field trials. In this report, we summarize the field trials established in two contrasting ecoregions of Colombia, the Amazonian Piedmont and the Cauca River Valley.

Materials and Methods

The Amazonian Piedmont ecoregion is continuously humid, corresponding to presence of spittlebug nymphs and adults throughout the year with little population synchrony. In this site the number of applications required to achieve an effect will be evaluated. The Cauca River Valley ecoregion is a highly seasonal site with bimodal precipitation and here spittlebug nymphs and adults are present only during the rainy months and have a high population synchrony. In this site the timing of the applications in relation to the insect's life cycle will be evaluated. The premise is that the diverging environmental conditions of these two ecoregions will require different strategies and control tactics for management of spittlebugs in pastures.

Five plots each were established in Hacienda Piedechinche, Santa Helena, Dept. Valle del Cauca (1600 m^2) and C.I. Macagual of CORPOICA, Florencia, Dept. Caquetá (1200 m^2). Each plot is located in a separate pasture under the same fertilization, grazing and weed management regime established for the rest of the farm. Each plot was subdivided into subplots (100 m^2) for application of treatments. Applications began 7 September 2001 in Macagual and will begin 15 days after the start of the next wet season in Piedechinche, predicted to be around the third week of September. Treatments are summarized in Tables 38 and 39 and are in a completely randomized block design with 5 repetitions. The isolates CIAT 054 (*Metarhizium* sp.) and CIAT 007C (*Metarhizium anisopliae*) were selected from among 49 isolates as the most virulent to adult *A. varia*.

Weekly population surveys are being carried out before the treatments to establish baseline data that verifies and gauges presence of the insect, and for six months after application to measure treatment effect. Surveys consisted of nymph counts in two 0.0625 m^2 quadrats and adult counts in two series of 10

sweeps with an insect net. In the laboratory nymphs were determined to instar and adults to species and sex. Natural enemies were also collected as part of the spittle mass and sweep net surveys. Pretreatment surveys began 25 January 2001 in Piedechinche and 23 March 2001 in Macagual. The start of the experiment in Macagual was postponed due to a long delay in delivery of the formulated product based on technical difficulties related to production by our commercial collaborator BioCaribe, S.A. The start of the experiment in Piedechinche depends on start of the wet season.

To gauge field mortality due to entomopathogens, 5 adults will be collected from each plot repetition with a sweep net and confined to petri dishes lined with moist filter paper to assess mycosis after 5 days.

Table 38. Field treatments applied in Macagual, Dept. Caquetá.

Product	No. applications per month for six months
Entomopathogen (CIAT 054)	0.5
	1
	2
Entomopathogen (CIAT 007C)	0.5
	1
	2
Insecticide (Malathion)	0.5
	1
	2
Control	0

Treatment effects on spittlebug nymph and adult populations will be tested by measuring insect load, or the number of insects under the population fluctuation curves, using cumulative insect days analysis.

Table 39. Field treatments applied in Piedechinche, Dept. Valle del Cauca.

Product	Weeks after appearance of first generation outbreak
Entomopathogen (CIAT 054)	1
	2
	3
	4
	5
	6
Insecticide (Malathion)	1
	2
	3
	4
	5
	6
Control	0

Results

As confirmed in previous studies, three species occur in Macagual: *Aeneolamia varia*, *Zulia pubescens* and *Mahanarva* sp. nov. Of 1207 adults collected to date, 97.0% were *A. varia*, 2.6% *Z. pubescens* and

0.4% *Mahanarva* sp. nov. at overall mean relative densities of 2.87, 0.07 and 0.01 adults/10 sweeps, respectively. Over this same period a total of 795 nymphs and 8 adult tenerals were collected (Table 40).

Table 40. Number of nymphs per life stage and teneral adults collected in spittle mass surveys at two contrasting field evaluation sites.

Instar	Macagual	Piedechinche
I	151	10
II	190	25
III	167	52
IV	114	31
Va	113	29
Vb	60	38
Tenerals	8	5
Total	803	190

In Piedechinche, only *Prosapia simulans* (1465 adults) has been detected to date, although previous populations surveys before the start of this experiment detected the presence of *Zulia carbonaria* and *Z. pubescens* at lower abundance. Phenological analysis of these data is summarized elsewhere.

Discussion

Initial population data confirm that the selected sites have sufficient populations of nymph and adult spittlebugs for this field experiment. Application of treatments has only just begun in Macagual and will begin in Piedechinche at the start of the next rainy season.

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

Highlights

- For the first time selected *Brachiaria* hybrids that showed high levels of antibiosis resistance to *Aeneolamia reducta*
- New *Brachiaria* hybrids with high levels of resistance to one or more spittlebug species were identified
- New *Brachiaria* hybrids with resistance to Rhizoctonia foliar blight were identified.

Progress towards achieving milestones

- **Produced new *Brachiaria* hybrids with selected sexual clones and different pollen parents**
  A total of 15 crossing blocks, each including from 25 to 41 selected sexual clones were successfully established and F1 seed is currently being harvested.

- **Identified new *Brachiaria* hybrids for spittlebug screening based on field performance**
  After rigorous culling on poor seed fill, it is likely that fewer than 1000 clones will be judged to merit further testing for spittlebug reaction.

- **Identified parental clones for field evaluation of recombined progeny**
  Field evaluation of open pollinated progenies of 41 clones selected on resistance to *A. varia* is in progress. Only a small number of these clones is highly resistant to two or more spittlebug species. Final selections from the progeny population will be conditioned by additional information on maternal parents reaction to other species of spittlebug.

- **Identified *Brachiaria* hybrids with resistance to *Rhizoctonia* foliar blight**
  A total of 13 (out of 108 entries) *Brachiaria* hybrids were identified as resistant to *Rhizoctonia* foliar blight using as selection of criteria proportion of infected leaves and upward disease progress from the plant inoculation.

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 (CIAT)

Establishment of crossing blocks at CIAT-Popayán. Collaborative research agreements to develop new *Brachiaria* hybrids for spittlebug resistance and adaptation to drought stress required the generation of large populations of diverse hybrid genotypes formed by crossing sexual (SX) clones selected from our tetraploid, sexual breeding population with elite apomictic (AP) hybrids and accessions. A total of 41 SX clones, selected on agronomic performance and spipttlebug (*A. varia*) resistance (damage and nymphal survival as good, or better, than *B. brizantha* cv. Marandu) were identified in the 1999 cycle of the tetraploid, sexual breeding population (Table 41). Compact blocks of 15 different AP genotypes were available (as seed multiplication areas) or were established, at CIAT-Popayán. Vegetative propagules of the SX clones were transplanted into established field plots of each of the AP selections at approximately 3-5 m between SX plants; i.e., each SX plant was surrounded by plants of the respective AP genotype in each of the crossing blocks. (Some of the crossing blocks were not large enough to accomodate all 41 SX clones at sufficient distance between SX plants, and fewer SX clones were planted in these: A complete listing of the SX x AP combinations established is given in Table 1.) A space approx. 1 m diameter around each SX plant was maintained clear to reduce competition. By judicious defoliation of both SX and AP genotypes, synchronous flowering was sought, but was not always achieved owing to the wide differences in flowering date (or even failure to flower) among the SX clones.

Seed from open pollination is being harvested individually from each of the SX plants in each crossing block (Table 41). An inventory of hybrid seed harvested and processed is being prepared.

Propagation of plant material for spittlebug evaluations. In 2000, over 1,700 SX x AP seedlings were evaluated as unreplicated spaced plants in duplicate field trials established at CIAT-Quilichao and at the Matazul farm in Puerto López (Llanos Orientales). Following periodic visual assessment, 121 genotypes were pre-selected on vigor, leafiness, and general freedom from disease or nutrient deficiency symptoms. These plants were propagated for evaluation of spittlebug reaction and have been tested with three different spittlebug species. A very small set of new hybrids exhibit antibiotic resistance to more than one spittlebug species.

Preselect new sexual clones for spittlebug screening in 2002. Over 4,300 sexual progenies (obtained from the random intercrossing of 41 selected sexual clones included in the 2000 recombination block) were established during first semester 2001 as unreplicated spaced plants in duplicate field trials established at CIAT-Quilichao and at the Matazul farm in Puerto López (Llanos Orientales). Clones are being culled based on periodic visual assessment (vigor, leafiness, disease or nutrient deficiency symptoms). In addition, at CIAT-Quilichao, spikelet fill (caryopsis formation) is being assessed in some detail. Caryopsis formation will receive heavy weight in final selection. Preliminary observation suggests that, in general, the sexual breeding population has poor spikelet fill (as expected), but a small number of plants with good seed formation are being identified. Final "pre-selections" from this tetraploid, sexual breeding population will be identified before 01 December 2001, when propagation for subsequent evaluations (spittlebug, Rhizoctonia, Al tolerance, etc.) will begin.

Table 41. New *Brachiaria* hybrid cross combinations being formed at Popayán during 2000.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
SX99NO/	**606**	**6387**	**16113**	**16121**	**16212**	**16315**	**16316**	**16320**	**16322**	**16467**	**16488**	**26124**	**26318**	**26556G**	**26562**
29				√	√	√	√	√	√		√	√	√	√	√
164				√	√	√	√		√		√	√	√	√	
236	√			√	√	√	√	√	√		√	√	√	√	√
246				√	√	√	√	√	√		√	√	√	√	√
275	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
497	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
574	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
711	√				√	√	√		√		√	√	√	√	
731	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
823	√			√	√	√	√		√		√	√	√	√	
835		√	√	√	√	√	√	√	√	√	√	√	√	√	√
1145	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
1260	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
1345	√			√	√	√	√		√		√	√	√	√	
1370	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
1513				√	√	√	√	√	√	√	√	√	√	√	√
1616	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
1622		√	√	√	√	√	√	√	√	√	√	√	√	√	√
1630	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
1805		√	√	√	√	√	√	√	√	√	√	√	√	√	√
1833	√	√	√	√	√	√	√		√	√	√	√	√	√	√
2030	√				√	√	√		√		√	√	√	√	
2115		√	√	√	√	√	√	√	√	√	√	√	√	√	√
2162			√	√	√	√	√	√	√	√	√	√	√	√	√
2173				√	√	√	√	√	√		√	√	√	√	√
2200		√	√	√	√	√	√	√	√	√	√	√	√	√	√
2280		√	√	√	√	√	√	√	√	√	√	√	√	√	√
2341	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2349	√			√	√	√	√	√	√		√	√	√	√	√
2354	√				√	√	√		√		√	√	√	√	
2514				√	√	√	√	√	√		√	√	√	√	√
2606		√	√	√	√	√	√	√	√	√	√	√	√	√	√
2621		√	√	√	√	√	√	√	√	√	√	√	√	√	√
2663	√			√	√	√	√	√	√	√	√	√	√	√	√
2822	√				√	√	√		√		√	√	√	√	
2857	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
2927	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3488	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3564	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3690		√	√	√	√	√	√	√	√	√	√	√	√	√	√
3770	√			√	√	√	√	√	√		√	√	√	√	√

Sexual females are selections from tetraploid sexual breeding population. Apomictic males are CIAT accessions.
√ = Cross combination does exists. Other cross combinations not available.

2.3.2 Identify *Brachiaria* genotypes resistant to different species of spittlebug

Contributors: C. Cardona, J. W. Miles, and G. Sotelo (CIAT)

Rationale

Recent evidence suggests that a significant (and important) genotype-species interaction exists for reaction to different spittlebug pest species. Hence, evaluation of plants artificially inoculated with only one species (*A. varia*) is not sufficient completely to characterize a host genotype's possible performance in the field when exposed to different species, alone or in combination. Forty-one sexual clones selected on reaction to *A. varia* are being tested with additional insect species (*A. reducta, Zulia carbonaria*, and *Z. pubescens*). In 2001, intensive screening of selected hybrids was conducted under greenhouse and field conditions.

Materials and Methods

Screenings for resistance were conducted with *Aeneolamia varia, Zulia carbonaria, Z. pubescens, Mahanarva* sp., and for the first time, *A. reducta*. Test materials were compared with five checks fully characterized for resistance or susceptibility to *A. varia*. Plants were infested with a known number of eggs of the respective spittlebug species and the infestation was allowed to proceed without interference until all nymphs were mature or adult emergence occurred. Plants (usually 10 per genotype) were scored for symptoms using a damage score scale. Percentage nymph survival was calculated. Materials were selected on the basis of low damage scores (<2.0 in a 1-5 scale) and reduced percentage nymph survival (<30%). All those rated as resistant or intermediate were reconfirmed. All those susceptible were discarded.

Results and Discussion

As reported last year (see p. 55 of the 2000 Annual Report), a set of 41 hybrid-derived, sexual clones equally or more resistant to *A. varia* than the resistant cv. Marandú was identified in 2000. As planned, the reaction to *A. varia* was reconfirmed between August 2000 and January 2001. Subsequently, these clones were also tested for resistance to *Z. carbonaria* and *Z. pubescens* in order to detect materials combining resistance to two or more species. Some of these hybrids were outstanding for resistance to spittlebug showing low levels of damage and reduced nymph survival, in many cases outperforming the resistant cultivar 'Marandú' (Table 42).

Table 42. Levels of resistance to three species of spittlebug in a set of 41sexual *Brachiaria* hybrids previously selected for resistance to *Aeneolamia varia*

Genotype	Mean damage scores[a]			Mean percentage nymph survival		
	Aeneolamia varia	*Zulia carbonaria*	*Zulia. Pubescens*	*Aeneolamia varia*	*Zulia carbonaria*	*Zulia pubescens*
BRX 44-02[b]	4.8	3.7	3.0	88.3	78.3	63.3
CIAT 0606[b]	4.3	4.2	4.1	95.0	76.7	91.7
CIAT 6294[c]	1.2	2.0	2.0	18.3	56.7	53.3
Resistant hybrids	1.3	1.6	1.6	15.7	26.9	23.3
Intermediate hybrids	2.3	2.3	2.3	42.3	40.0	41.1
Susceptible hybrids	-	3.7	3.2	60.0	63.5	57.8

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

A number of materials combined resistance to two or more species of spittlebug. Thus, 15 were resistant to all three species tested (Table 43) in terms of reduced damage levels. More important, three hybrids (SX99NO/0164, SX99NO/0236, and SX99NO/0823) showed antibiosis resistance (reduced nymph survival) to all species tested. This is important because it means that the breeding process has been successful in recombining genes for antibiosis resistance to *A. varia* and to other species like *Z. carbonaria* and *Z. pubescens*.

Progenies derived from crosses between some of the best sexual hybrids and apomictic parents will be evaluated for spittlebug resistance early next year.Another nursery evaluated this year was a set of 121 hybrids coded BR developed in 2000. The 32 hybrids that were initially selected for resistance to *A. varia* were then screened for resistance to both *A. varia* and *Z. carbonaria*. As shown in Table 44, high levels of resistance to *A. varia* were present in this group of materials, but none of them combined antibiosis resistance to both spittlebug species (Table 45).

Table 43. Frequency distribution of resistance reactions to three species of spittlebug in a set of 41sexual *Brachiaria* hybrids previously selected for resistance to *Aeneolamia varia.*

Category	On the basis of damage scores				On the basis of percentage survival			
	A. varia	*Z. carbonaria*	*Z. pubescens*	All three species	*A. varia*	*Z. carbonaria*	*Z. pubescens*	All three species
Resistant	38	21	28	15	32	8	9	3
Intermediate	3	19	13	1	5	9	15	0
Susceptible	0	1	0	0	4	24	17	0

Table 44. Levels of resistance to two species of spittlebug in a set of 32 *Brachiaria 2*hybrids initially selected for resistance to *Aeneolamia varia* in 2000.

Genotype	Mean damage scores[a]		Mean percentage survival	
	Aeneolamia varia	*Zulia carbonaria*	*Aeneolamia varia*	*Zulia carbonaria*
BRX 44-02[b]	4.3	4.4	61.7	72.9
CIAT 0606 [b]	4.6	4.1	83.3	85.4
CIAT 6294[c]	1.6	2.9	20.8	75.0
Resistant hybrids	1.6	1.8	24.9	35.6
Intermediate hybrids	2.4	2.5	41.5	45.0
Susceptible hybrids	3.2	3.5	64.8	67.8

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

Table 45. Frequency distribution of resistance reactions to two species of spittlebug in a set of 32 *Brachiaria* hybrids initially selected for resistance to *Aeneolamia varia*

Category	On the basis of damage scores			On the basis of percentage survival		
	Aeneolamia varia	*Zulia carbonaria*	Both species	*Aeneolamia varia*	*Zulia carbonaria*	Both species
Resistant	26	8	5	7	0	0
Intermediate	5	18	4	14	5	1
Susceptible	1	6	0	11	27	-

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

Contributors: C. Cardona, G. Sotelo, and J. W. Miles (CIAT)

For the first time we conducted studies on the resistance of *Brachiaria* genotypes to *Aeneolamia reducta*, the most important spittlebug species affecting grasses in the Caribbean region of Colombia. This is an aggressive species that causes important losses in native savannas and that may have a significant impact on any new cultivar released by the project. In a split-plot design in which the spittlebug species was the main plot and the genotypes were sub-plots, we compared the resistance to *A. varia* and *A. reducta* of four well known checks and two hybrids previously selected for resistance to *A. varia*.

As shown in Table 46, *A. reducta* caused significantly higher levels of damage on all genotypes tested, irrespective of their resistance or susceptibility to *A. varia*. More important, survival of *A. reducta* was significantly higher on all *A. varia*-resistant genotypes, suggesting that there is no antibiosis to *A. reducta* in these genotypes. These are important results that should be taken into account in the development of

future breeding strategies. Further studies with *A. reducta* were conducted with a set of 41 hybrid-derived, sexual clones equally or more resistant to *A. varia* than the resistant cv. Marandú. This had been identified in 2000.

Table 46. Comparative levels of resistance to *Aeneolamia varia* and *Aeneolamia reducta* in six selected *Brachiaria* genotypes.

Genotype	Mean damage scores[a]		Mean percentage nymph survival	
	Aeneolamia varia	*Aeneolamia reducta*	*Aeneolamia varia*	*Aeneolamia reducta*
BRX-44-02[b]	3.5aB	4.6aA	55.8aA	65.8bA
CIAT 0606[b]	3.7aB	4.6aA	66.7aA	69.2bA
CIAT 6294[c]	1.3cB	3.0cA	13.4cB	60.8bcA
CIAT 36062[c]	1.3cB	3.0cA	1.2dB	58.3cA
FM9503/4624[d]	1.2cB	3.9bA	25.8bB	81.7aA
BR99NO/4132[d]	2.1bB	4.3abA	24.2bB	58.3cA

For each variable, means within a column followed by the same lower case letter are not significantly different. Means within a row followed by the same upper case letter are not significantly different; Fischer's protected LSD ($P<0.001$)

[a] On a 1-5 damage score scale (1, no damage; 5, severe damage, plant killed)

[b] Susceptible check

[c] Resistant check

[d] Previously selected as resistant to *A. varia.*

Surprisingly, five of the hybrids showed high levels of antibiosis resistance to *A. reducta* that are comparable to those detected with *A. varia* in this and previous trials (Table 47). Dry weight losses in resistant genotypes were low, a reflection of the high levels of antibiosis present in them. As in the previous trial (Table 46), CIAT 6294 ('Marandú') did not exhibit antibiosis resistance to *A. reducta*. These are important results as they show that combining resistance to *A. reducta* and *A. varia* is feasible.

Table 47. Comparative levels of resistance to *Aeneolamia varia* and *Aeneolamia reducta* in selected sexual *Brachiaria* hybrids previously identified as resistant to *A. varia.*

Genotype	Mean damage scores		Mean percentage survival		Mean percentage dry weight loss	
	Aeneolamia varia	*Aeneolamia reducta*	*Aeneolamia varia*	*Aeneolamia reducta*	*Aeneolamia varia*	*Aeneolamia reducta*
			Best hybrids			
SX99NO/0029	2.0	2.5	1.7	21.7	11.8	19.2
SX99NO/0164	2.8	2.7	10.0	17.0	34.7	23.9
SX99NO/0236	1.4	1.9	0.2	10.0	0.02	0.0
SX99NO/0823	1.8	1.9	15.0	29.9	3.2	6.5
SX99NO/2606	2.8	2.2	15.0	24.8	18.3	4.5
			Susceptible checks			
BRX-44-02	4.7	4.3	88.3	68.3	45.8	35.4
CIAT 0606	4.3	4.6	94.9	80.0	28.0	42.3
			Resistant check			
CIAT 6294	2.1	3.3	18.3	53.3	16.5	6.4

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

Contributors: C. Cardona, G. Sotelo, and J. W. Miles (CIAT)

Field screening for resistance to spittlebug continued in 2001. 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. Seven major screening trials (three with *A. varia*, three with *Z. pubescens*, and one with *Z. carbonaria*) were set up in 2001. In Table 48 we highlight the results of evaluating 50 genotypes (41 sexual hybrids, 3 apomictic hybrids, 4 resistant checks, and 2 susceptible checks). On average, resistant hybrids performed as well as the resistant checks and significantly outperformed the susceptible checks.

In general, resistance levels to *Z. carbonaria* were lower than those encountered with the other two species tested. Damage scores between trials correlated well ($r= 0.841$; $P<0.001$; $n= 1500$). There was a significant, positive correlation ($r = 0.876$; $P<0.001$) between damage scores and percentage tillers killed by the nymphs.

Table 48. Field resistance to three spittlebug species in selected sexual *Brachiaria* hybrids and checks. Means of three trials with *Aeneolamia varia*, three trials with *Zulia pubescens* and one trial with *Zulia carbonaria.*

Genotype	Mean visual damage scores[a]			Tiller ratio[b]		
	A. varia	*Z. pubescens*	*Z. carbonaria*	*A. varia*	*Z. pubescens*	*Z. carbonaria*
			Best hybrids			
SX99NO/2173	1.5	2.0	2.1	1.96	1.30	0.92
SX99NO/1630	1.5	2.1	2.1	1.96	1.43	0.96
SX99NO/2115	1.5	2.0	2.0	1.96	1.12	1.38
SX99NO/2663	1.5	2,1	2.1	1.80	1.59	1.11
SX99NO/0029	1.7	2.2	2.1	1.89	1.48	1.30
SX99NO/1370	1.4	2.1	2.2	1.85	1.17	1.11
SX99NO/2857	1.5	2.1	2.0	1.58	1.48	1.66
SX99NO/2822	1.4	2.2	2.1	1.40	1.35	1.18
SX99NO/3690	1.5	2.1	2.0	1.45	1.29	0.89
SX99NO/0835	2.1	2.2	2.0	1.89	1.55	1.49
SX99NO/0246	2.0	2.0	2.0	1.67	1.28	1.32
SX99NO/2349	1.5	2.1	2.2	2.23	1.70	1.02
Mean	1.6ab	2.1b	2.1ab	1.80a	1.39a	1.19a
			Susceptible checks			
CIAT 0606	4.1	4.1	3.7	0.39	0.37	0.52
CIAT 0654	3.5	4.1	3.3	0.62	0.54	0.54
Mean	3.8a	4.1a	3.5a	0.50b	0.44b	0.53
			Resistant checks			
CIAT 6133	1.7	2.0	2.0	1.61	1.25	1.44
FM9503/4624	1.4	1.9	2.0	1.79	1.20	1.02
CIAT 36062	1.1	1.3	1.7	1.72	1.72	1.15
CIAT 6294	1.0	1.4	1.1	1.67	1.63	1.31
Mean	1.3b	1.6b	1.7b	1.69a	1.40a	1.23a

Means within a column followed by the same letter are not significantly different ($P<0.05$) by Scheffe's F analysis of arbitrary linear contrasts

[a] On a 1-5 damage score scale (1, no damage; 5, severe damage, plant killed)

[b] Number of tillers per plant at the end of the infestation process/ Number of tillers per plant at the beginning of the infestation process.

This means that field resistance expressed as reduced damage to the leaves may serve to predict losses due to nymph feeding. The most susceptible genotypes tested, the checks CIAT 0606 and CIAT 0654 lost 48-63% and 38-48% of their tillers, respectively. In contrast, tiller mortality in the resistant checks CIAT 6294 ('Marandú') and CIAT 36062 was negligible. Most important, as shown in Table 48, a number of sexual hybrids showed high levels of resistance to all three spittlebug species studied.

2.3.5 Screening of *Brachiaria* hybrids for *Rhizoctonia* foliar blight

Contributors: C. Zuleta and S. Kelemu (CIAT)

Rationale

Foliar blight disease caused by *Rhizoctonia solani* can result in substantial foliar damage on a wide range of plants. Fungal sclerotia are formed as white masses on infected tissues which later turn brown as they mature. These sclerotia easily shed forming the primary source of inoculum. The use of resistant plant materials (when available) remains the cheapest and most effective method of managing the disease. Good levels of resistance have been identified in at least one accession of *B. brizantha* (CIAT 16320). In this study, we used a uniform and reproducible inoculation and screening method to assess the reactions of *Brachiaria* hybrids to *R. solani* including CIAT 16320 as a positive control.

Materials and Methods

A fresh mycelial disc of *R. solani* AG-1, removed from a 4-day-old potato dextrose agar (Difco) culture, was added to each of several 250-ml Erlenmeyer flasks containing 30 ml PSY broth (20 g peptone, 20 g sucrose, 5 g yeast extract and 1 l deionized water). The flasks were wrapped with aluminum foil and incubated as still culture at room temperature for 10 days. Sclerotia were collected using sterile forceps. They were air-dried overnight on sterile filter papers in a laminar flow hood. Dry sclerotia were stored in sterile containers at 4 C for further use as inocula.

Plantlets were separated from tillers of each *Brachiaria* genotype to be evaluated. Individual plantlets were planted in small pots. Two weeks after planting, each plantlet was inoculated with one sclerotium placed on the soil surface in contact with the plantlet's stem. These plantlets were placed in a plastic box with one side made of cheesecloth immersed in water, in order to maintain humidity at approximately 100%. Plants were evaluated for their disease reactions 2 weeks after inoculation.

Results and Discussion

One hundred eighty hybrids of *Brachiaria* provided by the *Brachiaria* Improvement Program were evaluated for their reactions to *R. solani* in two batches. Foliar blight symptoms are shown in Photos 3, 4.

Using percentage of infected leaves and upward disease progress from the plant inoculation point as evaluation criteria, *Brachiaria* hybrids with the following codes were identified as resistant materials: 36, 42, 49, 92, 221, 228, 599, 1227, 1501, 289, 49, 93, 172, and 1098 (Figure 14). These materials had up to 5-cm upward disease symptom progress from the inoculation point of the plant and up to 10 % infected leaves.

The materials identified as intermediate resistant are: 59, 144, 235, 438, 755, 1032, 1407, 1418, 1600, 17, 861, 519, 1278, 783, 1281, 1590, 1598, 1165, 1250. These had up to 5-cm upward disease symptom progress from the inoculation point of the plant and 10-20 % infected leaves (Figure 14). All remaining materials were rated as susceptible.

Photo 3. *Rhizoctonia* foliar blight disease symptom in *Brachiaria*

Photo 4. *Brachiaria* genotypes with resistant (left) and susceptible (right) reactions

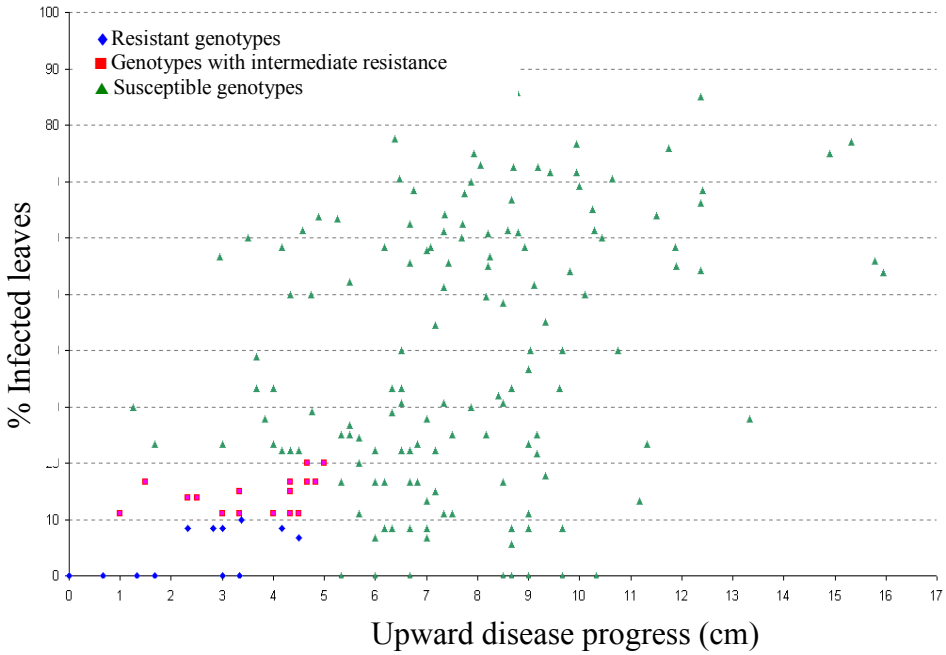

Figure 14. Evaluation of *Brachiaria* genotypes for their reactions to *Rhizoctonia* foliar blight using percentage of leaves infected and the upward progress of the disease from the inoculation points of the plants.

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

Highlights

- Progress was made in defining the mechanisms of resistance of *Brachiaria* to four species of spittlebug.

Progress towards achieving milestones

- **Defined reaction of new *Brachiaria* hybrids to different species of spittlebug**
  We have identified new *Brachiaria* hybrids with multiple resistance to two or more species of spittlebug. In addition, it would now seem that the main mechanisms responsible for resistance to *A. varia* and *Mahanarva* sp. in *Brachiaria* spp. is antibiosis. The resistance observed to *Z. pubescens* could be explained by a combination of tolerance with moderate levels of antibiosis. Resistance to *Z. carbonaria* seems to ne mostly tolerance.

2.4.1 Studies on resistance to spittlebug species

Collaborators: C. Cardona, G. Sotelo, P. Fory, and J. W. Miles

Rationale

There is need to ascertain that new *Brachiaria* hybrids produced by the Project do possess resistance to as many spittlebug species as possible. As reported previously, high levels of antibiosis resistance to *Aeneolamia varia* and to *Mahanarva* sp. have been detected in the resistant accession CIAT 6294, in the hybrid CIAT 36062, and in several other resistant hybrids. Although *Zulia carbonaria* and *Zulia pubescens* cause relatively little damage to these resistant genotypes, there are no indications of high

levels of antibiosis to the *Zulia* species. Tolerance might be an explanation for the lower levels of damage caused by the *Zulia* complex but we needed to ascertain this. Full characterization of the mechanisms of resistance is important in the formulation of appropriate breeding strategies. That is why a detailed study on the nature of resistance to four major spittlebug species was initiated in 2000. The main results are reported herein.

Materials and Methods

Studies on mechanisms of resistance were conducted with genotypes previously characterized for their resistance or susceptibility to *A. varia*. A susceptible check (CIAT 0654) and a resistant hybrid (CIAT 36062) were used to study antibiosis. These materials were infested following the methodologies described in previous annual reports. To determine mortality levels and the stage in the life cycle affected by the resistant hybrid CIAT 36062, 120 plants of each of the resistant and the susceptible genotypes were infested each with 6 eggs of each the spittlebug species per plant.

After egg hatching, a sample of 12 nymphs per species was taken daily and examined under a stereoscopic microscope in the laboratory. The fate of each individual (survival) was recorded. Nymphal instars and their duration were determined by measuring the widths of the head capsules of all nymphs recovered (dead or alive). This process continued until all surviving nymphs reached the adult stage.

We conducted two consecutive tests. To measure tolerance, we used a susceptible check (CIAT 0654) and two resistant genotypes (CIAT 36062 and CIAT 6294). For each spittlebug species we studied the effect of increasing levels of infestation: 2, 3, 5, 7, and 10 nymphs per plant. Uninfested controls were used for comparison and to calculate dry weight losses. Infestation was allowed to proceed until all nymphs were fully mature or adult emergence occurred. At this point plants were scored for damage, and survival rates and dry weight losses were calculated.

Results and Discussion

A very high level of antibiosis resistance to *Mahanarva* sp. was detected in the resistant hybrid CIAT 36062. All of more than 1,000 individuals studied failed to reach the second instar and the colony died out (Figure 15). The life cycles of *A. varia*, *Z. carbonaria* and *Z. pubescens* were delayed to a lesser extent by the resistant genotype.

Mortality of *Mahanarva* sp. on the resistant hybrid was so high that the population collapsed (Figure 16). The median survival time of the population on this genotype was 17 days, significantly lower than that calculated for the susceptible check (56 days). Mortality of *A. varia* was not so high, but still significantly different from that on the susceptible check. Apparently, the *Zulia* species were less affected.

Further analysis of the data by means of four different survival tests (Table 49) indicated that indeed the resistant variety CIAT 36062 had a significant impact on the survival of three of the species studied. The positive sign of Z, C, L, and Z (test statistics) in Table 49 indicate that the susceptible genotype (CIAT 0654) favors the survival of all spittlebug species.

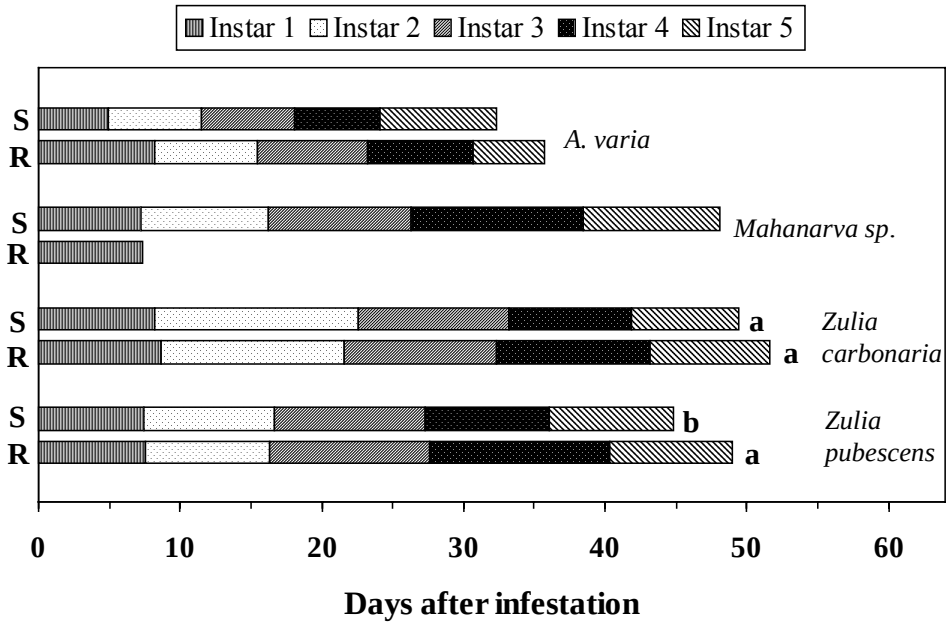

Figure 15. Duration of nymphal instars of four spittlebug species reared on a susceptible (S, CIAT 0654) and a resistant (R, CIAT 36062) *Brachiaria* genotype. Bars with the same letter are not significantly different (P<0.05). Pairwise comparison by *t* test within species. *Mahanarva* sp. was not analyzed due to total mortality of second instars. *A. varia* was not analyzed due to very high mortality of fifth instars in the resistant genotype.

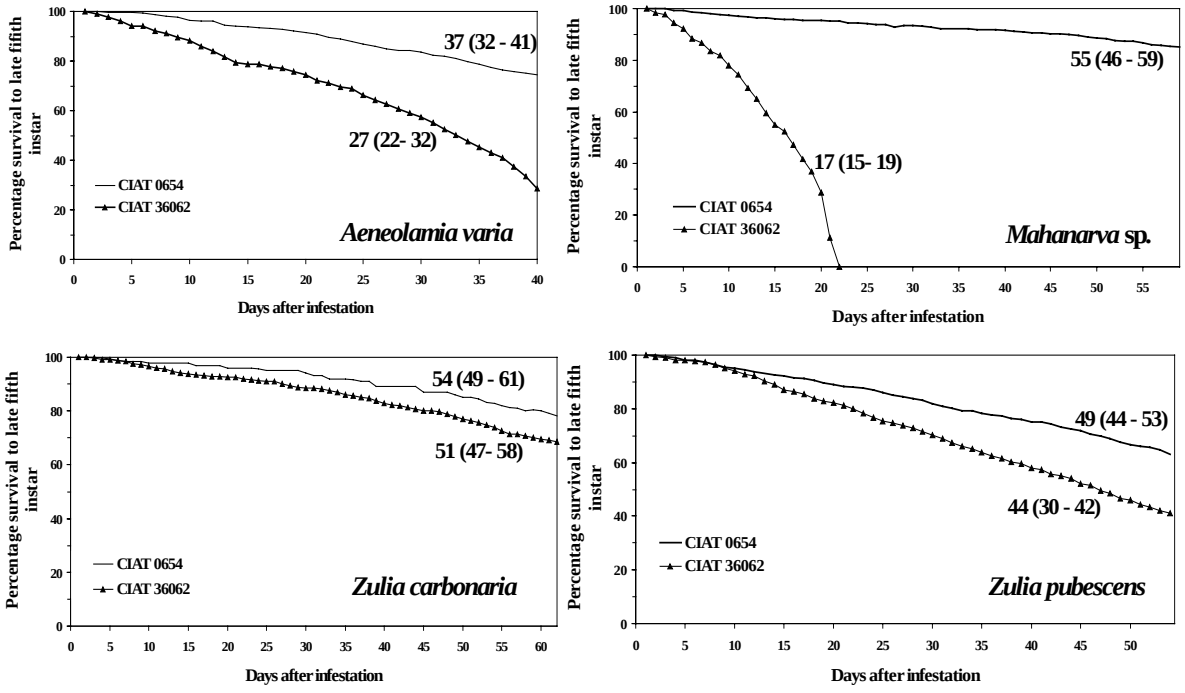

Figure 16. Survivorship curves for four species of spittlebug reared on susceptible (CIAT 0654) and resistant (CIAT 36062) *Brachiaria* genotypes. Values for median survival times (95% Confidence Intervals) are shown and were determined using the Kaplan-Meier survivorship test (Lee 1992). Values with overlapping confidence intervals are considered equal.

Table 49. Survivorship parameters for four spittlebug species reared on susceptible (CIAT 0654) and resistant (CIAT 36062) *Brachiaria* genotypes

Species	No. tested per trial per genotype	Two-sample survival tests			
		Z[a]	C[b]	L[c]	Z[d]
Aeneolamia varia	480	4.0**	4.8**	4.8**	4.3**
Mahanarva sp.	708	8.0**	9.7**	9.3**	8.2**
Zulia carbonaria	720	1.3ns	1.4ns	1.4ns	1.5ns
Zulia pubescens	648	1.8ns	2.2*	2.2*	2.2*

**Significant at the 1% level; *, significant at the 5% level; ns, not significant
[a]Gehan-Wilcoxon test; [b] Cox-Mantel test; [c] Logrank test; [d] Peto-Wilcoxon test

The higher the value of the coefficients, the higher the difference between the susceptible and the resistant genotype, that is to say the higher the level of antibiosis (mortality) caused by the resistant genotype. Highest values of the coefficients were calculated for *Mahanarva* sp. This means that this species is the one most affected by antibiosis, followed by *A. varia*. There was significance at the 5% level for *Z. pubescens*, suggesting low levels of antibiosis to this species. The lack of significance in the case of *Z. carbonaria* is a clear indication that there is no true antibiosis to this species. Furthermore, when we compared the survival of *Z. carbonaria* and *Z. pubescens* on the resistant genotype, we found that all test statistics were positive, meaning that CIAT 36062 was more favorable to *Z. carbonaria* than to *Z. pubescens*.

Antibiosis effects were also detected in terms of reduced dry weight of nymphs and adults. As shown in Figure 17, *A. varia* and *Z. pubescens* nymphs and adults reared on CIAT 36062 had significantly lower weights than those reared on the susceptible genotype. Antibiosis to *Mahanarva* sp. was so high that no nymphs developed beyond the third instar. There was no effect of the resistant genotype on the weight of *Z. carbonaria*. Further proof of differential antibiosis effects was obtained when the susceptible and the resistant genotypes were infested with increasing numbers of nymphs per plant (Table 50).

At all levels of infestation, survival of *Mahanarva* sp. nymphs was very low and significantly different from that of all other species studied. Significant differences in survival of nymphs at all levels of infestation also detected high and moderate levels of antibiosis to *A. varia* and *Z. pubescens*, respectively. No significant differences were found in the case of *Z. carbonaria*, meaning that antibiosis is not a mechanism of resistance to this species.

Highly significant differences were also found in terms of percentage dry weight losses (Table 51). Lower dry weight losses caused by *A. varia* and *Mahanarva* sp. are a reflection of high levels of antibiosis to these species; lower plant losses due to *Z. pubescens* may be due to a combination of moderate levels of antibiosis coupled with tolerance, whereas the only plausible explanation for resistance to *Z. carbonaria* is tolerance.

Similar results were obtained with the resistant accession CIAT 6294. At three levels of infestation highly significant differences among genotypes and spittlebug species were found for both percentage survival and percentage dry weight reduction (Table 52). CIAT 6294 was antibiotic to *A. varia* and to *Mahanarva* sp. but not to any of the *Zulia* species. Lower losses in CIAT 6294 due to *Z. carbonaria* and to *Z. pubescens* can only be explained as a manifestation of tolerance to the *Zulia* complex in this genotype.

Definite proof of tolerance as the main mechanism of resistance to *Z. carbonaria* was obtained when Functional Plant Loss Indexes for five levels of infestation were calculated (Table 53). Clearly, at all

levels of infestation tested, *Z. carbonaria* caused lower losses on the resistant genotypes CIAT 36062 and CIAT 6294 than on the susceptible genotype CIAT 0654. We conclude that the main mechanism responsible for resistance to *A. varia* and *Mahanarva* sp. in *Brachiaria* spp. is antibiosis. The resistance to *Z. pubescens* can be explained by a combination of tolerance with moderate levels of antibiosis. Tolerance is the mechanism underlying resistance to *Z. carbonaria*.

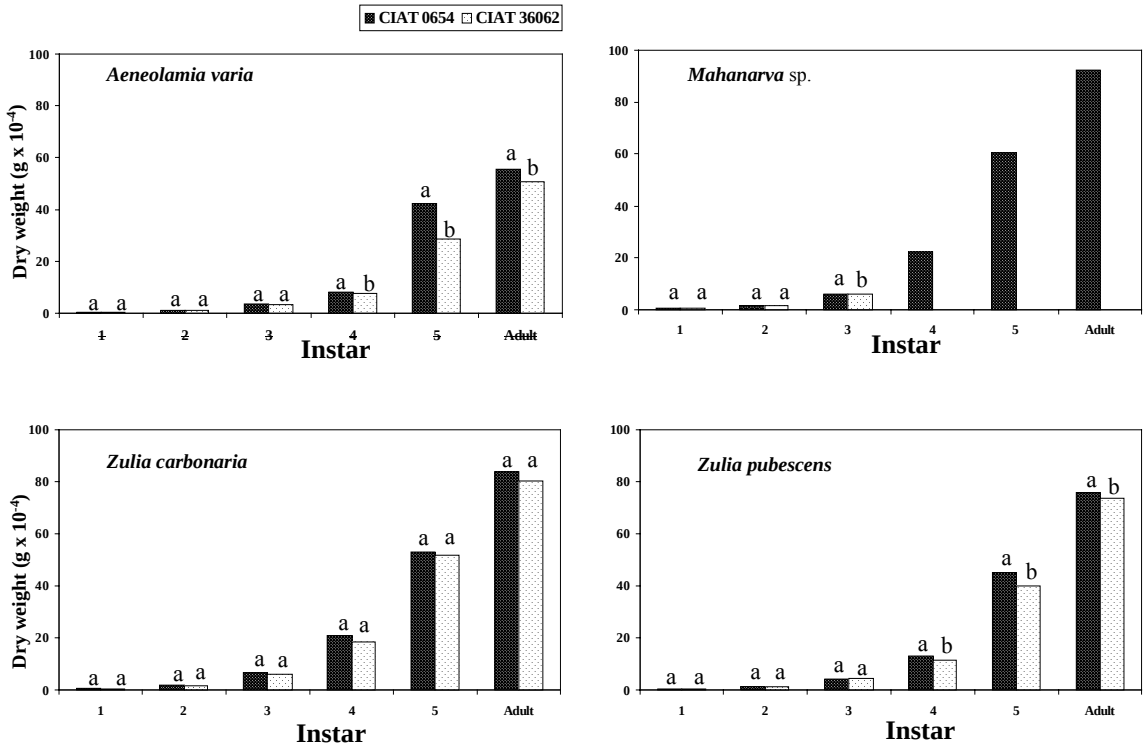

Figure 17. Effect of a susceptible (CIAT 0654) and a resistant (CIAT 36062) *Brachiaria* genotype on the dry weight of nymphs of four spittlebug species. Bars with the same letter are not significantly different ($P < 0.05$). Pairwise comparison by *t* test within instar.

Table 50. Percentage nymph survival[a] of four species of spittlebug reared at varying levels of infestation on a susceptible (CIAT 0654) and a resistant (CIAT 36062) *Brachiaria* genotype

Genotype	Species	Level of infestation (nymphs per plant)				
		2	3	5	7	10
CIAT 0654	*Aeneolamia varia*	86.0 ± 5.7ab	77.3 ± 6.5bc	74.1 ± 5.1b	85.0 ± 3.3a	51.9 ± 4.9b
	Mahanarva sp.	97.5 ± 2.5a	95.0 ± 2.7a	94.0 ± 2.6a	74.5± 3.9ab	80.5± 5.0a
	Zulia carbonaria	84.2 ± 5.5ab	85.0 ± 4.5ab	70.0 ± 8.8b	78.0 ± 3.6ab	65.5 ± 3.7b
	Zulia pubescens	79.2 ± 5.8b	65.0 ± 7.4cd	64.0 ± 5.1b	66.7 ± 4.3b	56.5 ± 4.2 b
CIAT 36062	*Aeneolamia varia*	17.5 ± 6.6d	26.3 ± 5.6f	19.0 ± 4.8cd	1.4 ± 0.8d	14.6 ± 3.6d
	Mahanarva sp.	0e	1.7 ± 1.7g	0d	0d	3.6 ± 2.0e
	Zulia carbonaria	77.5 ± 5.7b	55.5 ± 7.9de	61.0 ± 6.2b	62.8 ± 4.9b	57.5 ± 5.7b
	Zulia pubescens	47.5 ± 9.9c	38.3 ± 8.5ef	28.0 ± 6.4c	37.7 ± 5.7c	20.5 ± 4.6c

[a] Means ± SEM of two trials, 10 replications per level of infestation per trial. Means within a column followed by the same letter are not significantly different by LSD. Each level of infestation analyzed separately.

Table 51. Percentage dry weight loss caused by four species of spittlebug reared at varying levels of infestation on a susceptible (CIAT 0654) and a resistant (CIAT 36062) *Brachiaria* genotype

Genotype	Species	Level of infestation (nymphs per plant)				
		2	3	5	7	10
CIAT 0654	*Aeneolamia varia*	21.6 ± 3.2a	23.8 ± 4.3ab	38.6 ± 6.2a	49.9 ± 5.4a	41.7 ± 3.7a
	Mahanarva sp.	26.1 ± 3.7a	30.6 ± 4.9a	25.7 ± 5.5bc	35.3 ± 3.1b	32.2 ± 3.6ab
	Zulia carbonaria	23.8 ± 3.4a	25.3 ± 3.1a	37.8 ±3.0ab	33.0 ± 3.3b	35.7 ± 2.7ab
	Zulia pubescens	24.3 ± 3.6a	28.2 ± 3.4a	27.6 ± 3.7ab	32.1 ± 3.5b	29.4 ± 4.3b
CIAT 36062	*Aeneolamia varia*	9.9 ± 2.8b	11.7 ± 3.1c	7.5 ± 2.5ef	3.5 ± 1.4d	11.6 ± 3.6de
	Mahanarva sp.	5.6 ± 1.9b	6.6 ± 2.0c	2.7 ± 1.3f	5.5 ± 1.9d	6.0 ± 2.3e
	Zulia carbonaria	12.6 ± 3.2b	12.7 ± 2.6bc	17.9 ±3.9cd	15.5 ± 2.7c	19.6 ± 3.2c
	Zulia pubescens	9.2 ± 2.6b	10.9 ± 1.6bc	14.8 ± 3.6de	17.1 ± 3.1c	13.0 ± 2.0cd

Means ± SEM of two trials, 10 replications per level of infestation per trial. Means within a column followed by the same letter are not significantly different by LSD. Each level of infestation analyzed separately.

Table 52. Response of susceptible (CIAT 0654) and resistant (CIAT 6294) *Brachiaria* genotypes to three levels of infestation with four species of spittlebug.

Genotype	Species	Percentage nymph survival			Percentage dry weight loss		
		3[a]	5	7	3	5	7
CIAT 0654	*A. varia*	81.0 ± 9.3ab	80.0 ± 6.7ab	79.1 ± 8.1a	27.5 ± 5.2a	36.3 ± 8.1ab	45.7 ± 4.4ab
	Mahanarva sp.	76.7 ± 7.4ab	76.0 ± 7.8ab	65.7 ± 11.5a	40.9 ± 6.4a	45.9 ± `4.5a	53.6 ± 6.2a
	Z. carbonaria	100.0 ± 0a	86.0 ± 6.7a	70.0 ± 8.4a	28.3 ± 6.1a	31.0 ± 2.9b	30.7 ± 3.9bc
	Z. pubescens	51.5 ± 8.4c	75.0 ± 8.0ab	78.6 ± 7.4a	30.0 ± 7.9a	38.3 ± 4.5ab	41.6 ±3.2ab
CIAT 6294	*A. varia*	16.7 ± 7.4d	24.0 ± 10.5c	31.4 ± 10.8b	6.0 ± 2.1b	8.0 ± 3.8c	19.0 ± 4.6cd
	Mahanarva sp.	0d	0d	0c	4.2 ± 2.3b	6.3 ± 2.7c	11.0 ± 3.0de
	Z. carbonaria	83.3 ± 7.4ab	62.0 ± 9.2bc	61.0 ± 7.1a	7.9 ± 2.8b	15.4 ± 3.1c	14.1 ± 3.7de
	Z. pubescens	66.7 ± 8.6bc	72.0 ± 6.1ab	75.7 ± 7.0a	10.6 ± 4.3b	6.2 ± 3.7c	10.6 ± 5.2e

[a] Nymphs per plant

Means ± SEM of two trials, 10 replications per level of infestation per trial. Means within a column followed by the same letter are not significantly different by LSD. Each level of infestation analyzed separately.

Table 53. Functional plant loss indexes (F.P.L.I.)[a] for susceptible (CIAT 0654) and resistant (CIAT 36062, CIAT 6294) *Brachiaria* genotypes exposed to five levels of infestation (nymphs per plant) with four species of spittlebug.

Levels	*Aeneolamia varia*			*Mahanarva* sp.			*Zulia carbonaria*			*Zulia pubescens*		
	CIAT 0654	CIAT 36062	CIAT 6294	CIAT 0654	CIAT 36062	CIAT 6294	CIAT 0654	CIAT 36062	CIAT 6294	CIAT 0654	CIAT 36062	CIAT 6294
2	73.6	24.9	35.4	69.3	21.6	28.0	70.7	47.4	46.0	67.0	37.9	25.6
3	80.3	30.5	26.0	70.4	22.6	26.0	65.5	48.1	48.6	65.3	40.1	36.0
5	84.2	25.2	35.4	72.5	18.5	26.0	76.2	48.2	64.8	76.2	43.3	38.4
7	84.3	19.2	43.9	76.8	17.7	37.4	77.5	49.5	50.0	74.8	49.2	40.5
10	91.6	15.0	43.0	74.6	17.1	31.8	80.5	56.8	62.9	72.7	41.2	37.0

[a] F.P.L.I. = 1 - [Dry weight of infested plants / Dry weight of uninfested plants] x [1 - damage score / 5] x 100

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

Highlights

- A new set of *Brachiaria* hybrids with known mode of reproduction is now available.

Progress towards achieving milestones

- **Apomictic *Brachiaria* hybrid selections identified on the basis of progeny testing**
  Using visual assessment of relative uniformity/heterogeneity among open pollinated siblings we selected 18 new apomictic *Brachiaria* hybrids which will now be evaluated in regional trials.

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

Contributors: J. W. Miles (CIAT)

A set of 121 "pre-selected" SX x AP hybrids was identified among over 1,700 hybrids evaluated in field trials during 2000. Reproductive mode of these hybrids is expected to be approximately 50% sexuals and 50% apomicts. In late 2000 and early 2001, open pollinated seed of 56 of these selections was harvested on the spaced plants in the field nursery established at CIAT-Quilichao in 2000. Open pollinated seed of the remainder of the set of 121 was subsequently obtained from pot-grown, vegetative propagules at CIAT-headquarters.

Open pollinated seed of 56 pre-selected hybrid clones was germinated and a maximum of 20 seedlings (where available) established. Seedlings were transplanted to the field at CIAT-headquarters in first semester, 2001, in 20-plant progeny rows. A vegetative propagule of the maternal genotype was established at the head of each progeny row. Reproductive mode of the maternal clone was assessed visually by the relative uniformity/heterogeneity among the OP siblings. A final assessment was made on 18 September (on approx. 3-mo-old plants). Of the 56 progenies, 13 were classified as sexual, 18 as apomictic, with 3 as facultative apomicts (progeny generally uniform with one or more off-types), and the rest more or less ambiguous. (Herbicide damage early in this trial lead to significant loss of plants as well as non-genetic heterogeneity among plants, which confused the results in some progenies.)

2.5.2 Construction of a molecular genetic map of Brachiaria and QTL analysis of spittlebug resistance

Contributors: O. X. Giraldo, J. Vargas, E. Gaitán, M.C. Duque, J. Miles, C. Cardona, and J. Tohme (CIAT)

Rationale

The genus *Brachiaria* Griseb. belongs to the tribe Paniceae, comprises aproximately 100 species, mostly of African origin. Some of these have found commercial use as forage in tropical America, with approximately four-five million hectares of *Brachiaria* pastures in Brazil alone (Valle and Miles 1992). The commercial species of B. *brizantha* and B. *decumbens* are tetraploid apomitic (Valle 1986), The Construction of a Brachiaria molecular map was initiated (BRU annual report pp 123-127, 2000), using a population of 215 F1 individuals derived from a cross between an autotetraploid spittlebug susceptible individual *B. ruziziensis* and a tetraploid spittlebug resistant individual *B. brizantha*. The objective of the study is to increase the saturation of the map using SCARs and SSRs developed at CIAT, AFLPs, RFLPs

probes from other grases species and tag the quantitative trait loci (QTLs) controlling spittlebug resistance in Brachiaria.

Materials and Methods

Plant Material: A sexual tetraploid *B. ruziziensis* (Swenne and Durjardin, 1981), susceptible to spittlebug (accession CIAT 44-3), was used as a female parent in a cross with natural and apomitic tetraploid genotype *B. brizantha* resistant to spittlebug (accession CIAT-6294).

DNA Extraction: DNA was extracted using the protocol described by Carlos Colombo (personal communication) with some modifications. 1g of tissue was dried at 48 °C for 20 hours and ground to fine power; 15 ml of extraction buffer (0.1M Tris-Hcl pH8.0, 0.05M EDTA pH8.0, 0.7 M NaCl, 4% CTAB and 1% ßMe) was added and incubated at 65 °C for 10 min; 15 ml of chloroform:isoamyl alcohol (24:1) was added and centrifuged at 3000 RPM for 30 min. The aqueous phase was transferred to a new tube and 8 ml of chloroform:isoamyl alcohol was added and centrifuged at 3000 RPM for 30 min, repeated twice. A volume of cold isopropanol was added to the supernatant and incubated over night at - 20 °C. The isopropanol mixture was centrifuged at 3000 RPM for 30 min at 4 °C. The DNA pellet was washed with cold 75% ethanol and dried at room temperature, and then resuspended in 300 ul of TE. Pancreatic RNAse was added to a final concentraction of 20 ug/ml. DNA was quantified on a DYNA QUANT 200 fluorometer (Hoffer Scientific Instruments, San Farancisco CA).

Microsatellites: The isolartion of the microsatellites and the methodology for PCR amplification and evaluation of polymorphism have been described previously in (BRU annual report pp 123-127, 2000). An additional set of 26 new SSRs were evaluated this year.

AFLP, RFLP RAPD: All 215 individuals were evaluated using the combination (E-ACG/M-CTA), The screening methodology was described in (BRU annual report pp 123-127, 2000). Protocols for RFLP and RAPD, markers in *Brachiaria* were described previously (BRU Annual report pp 105-110 1997).

Linkage Analysis: Segregation of markers as single dose restriction fragment (SDRF) markers according to the genetic model was determined by departure from the hypothesized 1:1 ratio by the Chi-square test. The data matrixes obtained for presence or absence of bands were analyzed with MAPMAKER v 3.0b for PC (Lander et al. 1987), using LOD score of 6.0 and recombination fraction 0.3. Recombination was translated to genetic distances using the Kosambi map function.

Results and Discussion

Phenotypic screening: The Tropical Forage Entomology section screened the population of the average damage of individual hybrid plant (C. Cardona et al., 1999). The results indicate that approximately 74.5% of the population can be classified as resistant on susceptible individuals to the spittlebug damage. The average damage values cover a continuous range from 1 to 5 suggesting a quantitative trait. Three different ranges were derived allowing the classification of the population as resistant, intermediate or susceptible individuals.

The Genetic Linkage Map: Sixty-eight SSRs, 5 combinations AFLPs (116 markers), and 35 RFLPs segregating in the male parent (CIAT-6294), were tested for linkage using MAPMAKER V.3.0b. Polymorphisms were scored for presence (H), and absence (A), and analyzed for dosage among F1 progeny using Chi-square tests ($P<0,01$). 45 SSRs, 67 AFLPs and 16 RFLPs markers, were found to define 22 linkage groups spanning 1079.031 cM, with an average marker density of 1 marker every 8.43 cM (Figure 18), map distance in centimorgans was calculated using the Kosambi mapping function. Linkage groups were organized according the number of markers presents in each group.

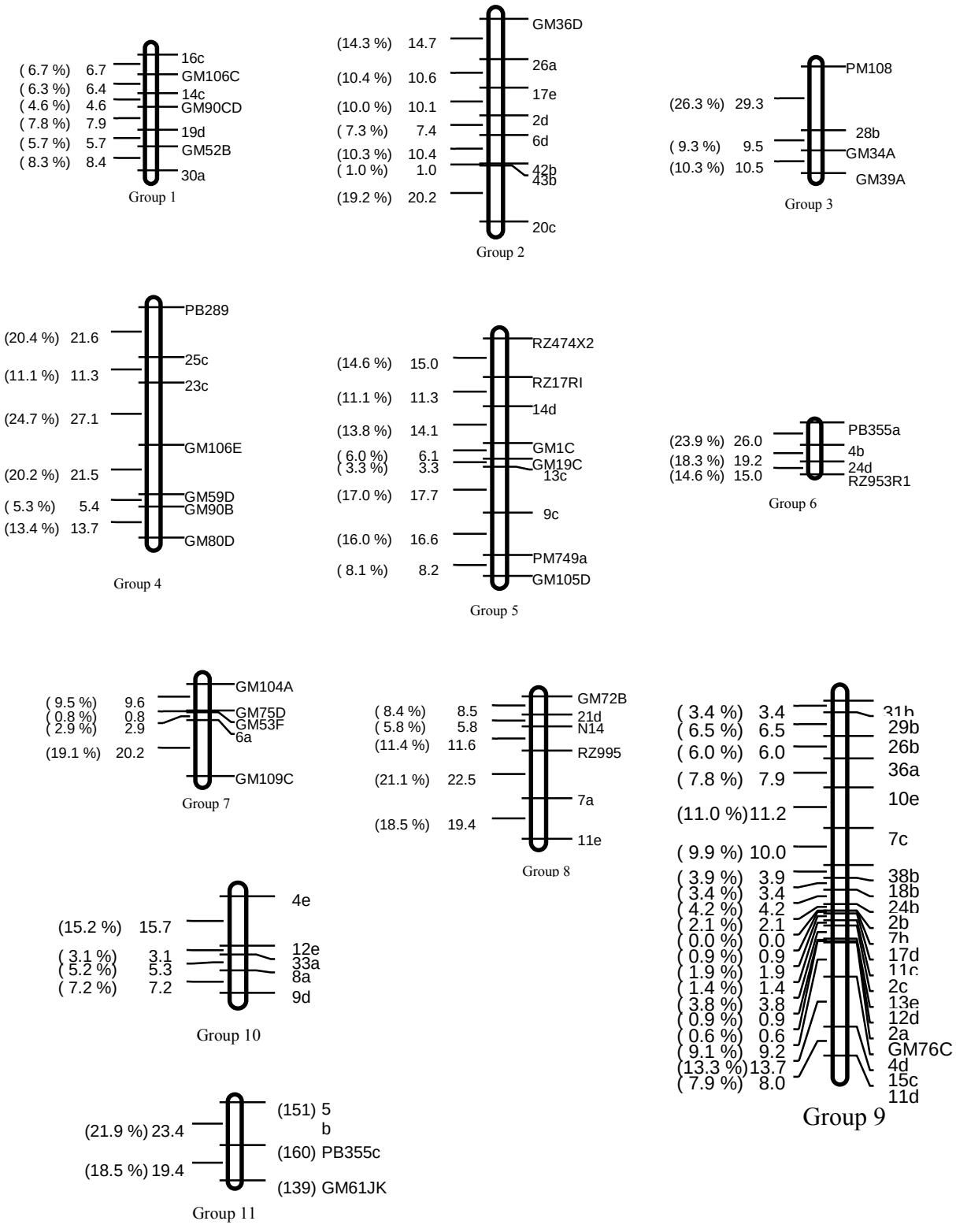

Figure 18. Preliminary *Brachiaria* framework map (LOD:6 Tetha: 0.3) (continues…..)

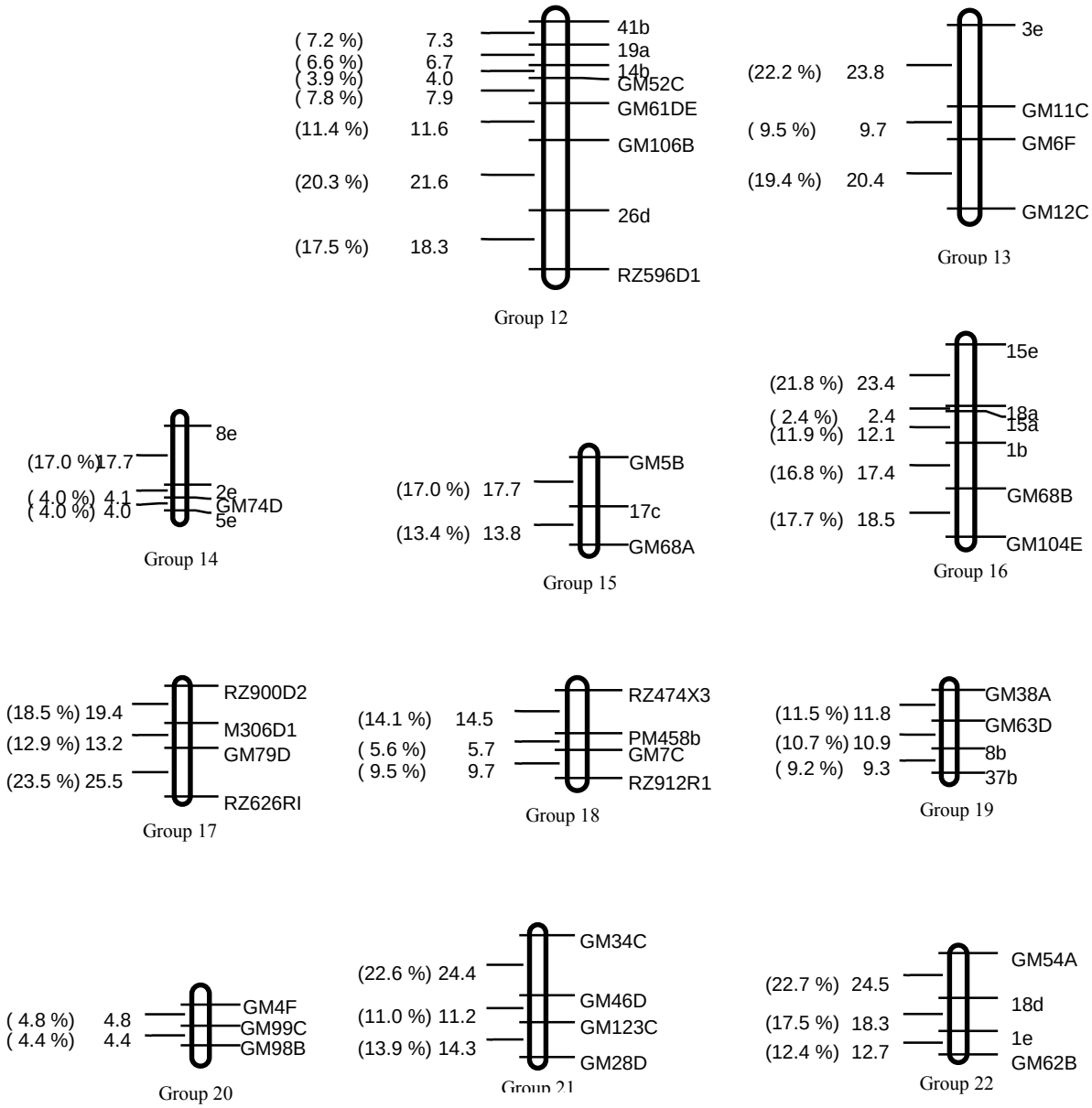

Figure 18. Preliminary *Brachiaria* framework map (LOD:6 Tetha: 0.3) (Continuation….)

The most densely populated linkage group 9 spanned 99.0 cM with 21 markers, follow by the linkage group 5 with 9 markers, spanned 92.306 cM, the groups 2 and 12 with 8 markers spanned 74.404 and 77.275 cM each one, while the least populated group was the linkage group 20 with 3 markers. The markers were grouped using a LOD = 6 and a recombination fraction of 0.3.Only the markers from linkage group 9 were grouped using a LOD=34 and recombination fraction of 0.3 (Figure 18).

Quantitative Trait Loci (QTL) analysis The average damage values from each genotype (F1) were analyzed with QTL Cartographer software using the map generated by Mapmaker. First, a search test was conducted, to find the association of segregant markers and the trait of interest, through lineal regression for each marker in relation to the quantitative trait and using the Composite Interval mapping Method

(CIM). Statistical significance levels of 0.01% were obtained by evaluation of F test in 6 markers of linkage group 2 (GM36D, 42b, 6d, 2d, 17e, 26a), and 3 markers of linkage group 16 (1b, 16a, 15a). These significance levels indicate a strong genetic linkage. Using the Composite Interval Mapping method, two major QTLs were found on linkage groups 2 and 16 with a LOD of 21 and 8 respectively (Figure 19) suggesting a strong evidence for the presence of QTLs for resistance to spittlebug. The most significant QTLs explained up to 37% and 15 % of the variance for QTL 1 and QTL2 respectively.

More makers will be placed on the Brachiaria map as they become available. The QTLs data will be integrated with the work on the isolation of Brachiaria resistance gene analogs (RGAs). We plan to saturate the region of the different QTLs with additional markers using AFLP and Dart to fine map the region of QTL1 and QTL2. Such markers could eventually be used for a marker assisted selection program. However we will need to confirm the QTL1 and QTL2 in a different background.

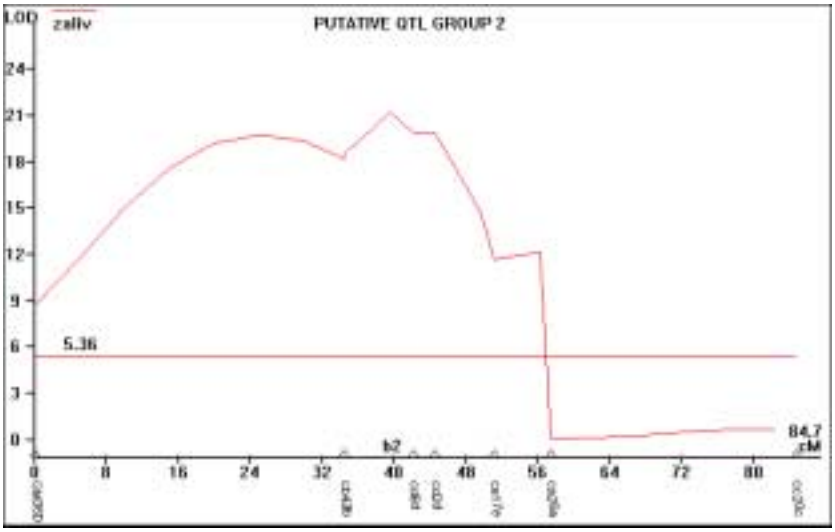

Figure 19. Putative QTLs associate with susceptibility to spittlebug in the linkage group 2.

Activity 2.6 Role of endophytes in tropical grasses

Highlights

- Endophyte isolates were successfully introduced into nine plants of two different accessions.
- DNA fragment common to most of the isolates of *A. implicatum* has been cloned, sequenced, and specific primers synthesized for use in detection of endophytes in species of *Brachiaria*.
- The genetic diversity of isolates of *Brachiaria* endophytes was determined.
- New endophyte isolates have been isolated and characterized.
- The presence of an endophytic fungus in a *Brachiaria brizantha* accession may have a major contribution to its *Rhizoctonia* foliar blight resistance.

Progress towards achieving milestones

- Isolated and characterized new isolates of endophytes
- Synthesized and tested endophyte-specific primer
- Defined effect of a new endophyte isolate on *Rhizoctonia solani*

We have isolated and characterized new isolates of endophyte from some *Brachiaria* hybrids and accessions. One of the accessions (*B. brizantha* CIAT 16320) which contained an endophyte has high levels of resistance to *Rhizoctonia* foliar blight. Elimination of the endophyte from the plant using a fungicide eliminates the antfungal properties of plant extracts. We have cloned and sequenced a RAPD fragment common to most isolates of the endophyte *A. implicatum*. A specific primer was synthesized based on the sequence data. The primer differentiates endophytic fungi from other fungi and can be used to detect endophytes in the plant.

2.6.1 Endophyte seed transmission studies in *Brachiaria*

Artificial introductions of endophytes in *Brachiaria* genotypes

Contributors: X. Bonilla, C. Zuleta, H. Dongyi and S. Kelemu (CIAT)

Rationale

Successful and efficient artificial inoculation methods are essential for studies of effects of endophytes on plant physiological processes. Beneficial endophytes, which have the feature of maternal transmission through the ovule and seeds, can then be introduced into the plant genotype of interest via artificial inoculations so that the grass-endophyte association becomes self-replicating as long as the infected seeds are stored under conditions which keep the fungus alive.

Materials and Methods

Seeds of *Brachiaria brizantha* CIAT 6780 and CIAT 26110 were surface-sterilized as follows: lemas and peleas were carefully removed without damaging embryos. These were washed with 70% ethanol for 2 minutes, in 2.5% NaOCl for 10 minutes, rinsed 5 times with sterile distilled water. The seeds were transferred to sterile filter papers to remove excess moisture, then planted onto magenta vessels (Sigma) containing plant growing medium (MS), 8-10 seeds/vessel, and incubated at room temperature. Seedlings were inoculated with selected isolates of *Acremonium implicatum* 15-20 days after germination. Fungal mycelium was introduced into meristem tissues with the use of an entomological needle under a stereoscope.

The wound area where the fungus was introduced was sealed with sterile vaseline. Inoculated plants were transplanted onto magenta vessels with MS medium and incubated for 10-15 days. Surviving plants were transplanted to pots containing sterile soil. Plants were checked for the presence or absence of the endophyte 2.5 -3 months after inoculations.

Results and Discussion

Of the 70 inoculated and surviving plants of *B. brizantha* CIAT 6780, only 6 plants tested positive for the presence of the endophyte. Forty-nine inoculated *B. brizantha* CIAT 26110 plants survived, of which only 3 tested positive for the endophyte. Examinations for endophyte presence were done 4-12 months after inoculations.

The plants which tested positive for endophytes were transplanted into big pots for further tillering and propagation. Half of these tillers were treated with the fungicide Folicur in order to create genetically identical clones of *Brachiaria* with and without the endophyte.

2.6.2 Synthesis of endophyte specific DNA fragment for quick detection of endophytes

PCR analysis and screening of *Brachiaria* genotypes
PCR analysis of other fungi (pathogens or non-pathogens) and new endophytes

Contributors: H. Dongyi, (South China University of Tropical Agriculture, The People's Republic of China), Y. Takayama (Tamagawa University, Tokyo, Japan), and S. Kelemu (CIAT)

Rationale

Both the benefits and harmful effects of endophytes make it important to examine their presence in forage grass *Brachiaria*. Microcopy and culture isolation methods had been established earlier. But these methods are time and resource consuming. PCR detection methods have been used to detect fungi in plants. A RAPD fragment common to six endophyte isolates (generated with primer OPAK10) had been cloned and sequenced (Figure 20). Specific primers were synthesized based on this sequence data. Two sets of primer pairs were tested for specific detection of endophytes.

These primer pairs were: P1 (5'-TTCGAATGATAAGGCAGATC-3') and P4 (5'-ACGCATCCACTGTATGCTAC-3'); P4 and P5 (5'-TGAGAAGACCTCTTGTTATG-3').

CAAGCGTCACGGAGTACATAGTTTCACCCGGTGTTCGAATGATAAGGCAGATCCGAG
GAGCACTTTGCGGATGAGAAGACCTCTTGTTATGCCGGAGGGGGGTTGTTGGAGTTA
CGCTGATCTCTATGAGCCCAGATTATTAGAGGAGGAGACGAGTTGGAAATTTGAGTC
CCGTCGCCCCTTCCCGACTTCTGACCAGACTGTGGTGACCACTTGCGCCTGCCATTGC
CACCTGGCGTCCCGACTGTTGCCAACCCAGTTTTCTTCCATGGCCGGCTATGCTCAGT
CTTGCATTTGTGAGAAAAGAAAGATCCTGCTCTCTATTCGTGCAGCGAAAAATGTTT
GCGGAACCCCCTTTCACCCCTTGGATGCCGATCCATGCGCGACTTCCTCTTGGTTGAT
CTAGCTGGTGTTATNCACATGTCCCGATCTCGCCTTCTTGTACTAGTAGTGACGAGAC
AGCTGGACCGTAGCATACAGTGGATGCGTCCTATACTCCTGTCATGACGGTGACGCT
TG

Figure 20. Sequence data of a RAPD fragment common to six endophyte isolates from species of *Brachiaria*.

This system can be used, without a doubt, to correctly identify endophytes of *Brachiaria in vitro*. However, because of the high sensitivity of the method, and a lack of completely endophyte-free control clonal plants, we have not yet been able to routinely use the method for endophyte detection *in planta*. Further improvements of this PCR method are currently in progress. These 2 sets of primer pairs could specifically amplify the target fragment (about 450-bp), using genomic DNA template of all endophyte isolates or endophyte-containing species of *Brachiaria*. With primer pair P1/P4, the genomic DNA of two major pathogens of *Brachiaria*, *Drechslera* spp. and *Rhizoctonia solani*, a very faint and insignificant band was amplified, whereas a strong specific band was amplified in genomic DNA of all endophyte isolates (Photo 5). Plants with codes of "P + numbers" are plants artificially inoculated with endophytes. P015 and P018 are *B. brizantha* 26110. P56, P63, P100 and P111 are *B. brizantha* 6780. Other codes (H + numbers) are *Brachiaria* hybrids. Endophytes were isolates on culture from hybrid plants H19, H32, H45 and H47, but not from H50. The endophyte used to inoculate the plants was successfully re-isolated from adult plants P015, P056, P63 and P111, but not from plants P018 and P100. These PCR and culture data are compatible indicating that the PCR detection method can be highly effective.

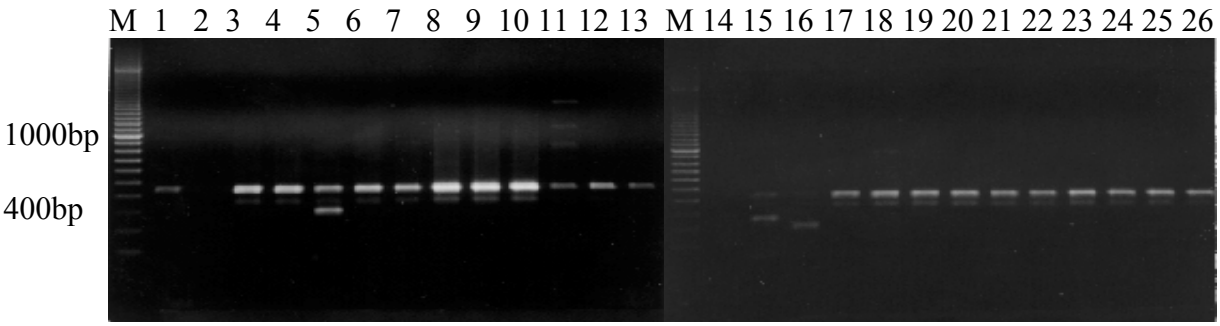

Photo 5. PCR amplified products with specific primer pairs of P1/P4. M, 00-bp marker.
Lanes 1-13 are amplifications of genomic DNA from *Drechslera* spp., *Rhizoctonia solani*, endophyte isolates EH45, EH19, EH47, EH32a, EH32b, EB606, EB6780 (P-201), EB16845 (P-904), EB6780 (P-501), EB16845 (P-909) and Epinedo, respectively. All of the fungi were isolated from species of *Brachiaria*.
Lanes 14-26 are amplifications of plant genomic DNA from P018, H50, P100, H32F', H32F, H19, H32, H45, H47, P015, P056, P063 and P111, respectively.

2.6.3 Genetic diversity of isolates of endophytic fungi from *Brachiaria*, and search for new endophytes in hybrids of *Brachiaria*

Contributors: H. Dongyi (South China University of Tropical Agriculture, The People's Republic of China), and S. Kelemu (CIAT)

Rationale

The search for new endophytes continued this year in new hybrids of *Brachiaria* species. We believe that the search for new isolates of endophytes may enable us find endophytes with various beneficial properties such as disease and insect control without other deleterious effects. Fungal endophytes were shown to be widely distributed in tropical forage *Brachiaria* through tissue staining and microscopy detection. Eleven endophyte isolates were isolated from different accession or hybrids of *Brachiaria*. Table 54 shows the list of isolates and their plant origin. Molecular methods such as RFLP (restriction fragment length polymorphism), SSR (simple sequence repeat, microsatellite), RAPD (random amplified polymorphic DNA) and AFLP (amplified fragment length polymorphisms) were successfully used to show the genetic variation among species or biotypes. In this study, we used RAPD and AFLP to measure diversity in the 11 endophyte isolates listed in Table 54. Both methods were used successfully to determine genetic diversity and relations among endophyte isolates from *Brachiaria*.

Table 54. List of endophytic isolates and their original plant hosts.

Endophyte isolate codes	Host grass of endophyte isolates	
EH45	*Brachiaria* hybrid # 45	
EH19	*Brachiaria* hybrid # 19	
EH47	*Brachiaria* hybrid # 47	
EH32a	*Brachiaria* hybrid # 32a	
EH32b	*Brachiaria* hybrid # 32b	
EB606	*Brachiaria* 606	
EB6780.201	*Brachiaria* 6780	(201)
EB16845.904	*Brachiaria*16845	(904)
EB6780.501	*Brachiaria* 6780	(501)
EB16845.909	*Brachiaria*16845	(909)
EPinedo	*Brachiaria* seeds (Santander de Quilichao)	

Materials and Methods

Endophyte isolates were cultured on potato dextrose agar for a period of 2-3 weeks to produce fresh mycelia for DNA extractions. Genomic DNA extractions were done accoring the manufacturer's instructions using Dneasy™ plant mini Kit (Qiagen).

RAPD analysis. PCR was carried out in 20 µl of reaction mix containing 2.0 µl of 10 x PCR buffer, 1.2 µl of 25 mM $MgCl_2$, 2.6 µl of 2 mM dNTPs, 1.0 µl of 10 µM Primer, 0.2 ul of 5 U/µl Taq DNA polymerase, 10.0 µl of dd H_2O and 3.0 µl of 10 ng/µl Template DNA (DNA of endophyte isolate), placed in a PTC-100™ Programmable Thermal Controller (MJ Research Inc.) programmed for initial denaturation at 94°C for 2 min, annealing at 28°C for 5min, 45 cycles of 94°C for 1 min, 92°C for 20sec, 35°C for 1 min, 72°C for 1 min, following a final extension at 72°C for 10 min. PCR products were resolved by running a 1.2% agarose gel, stained with ethidium bromide, and photographed under UV lighting.

After preliminary testing of 3 isolates with 30 arbitrary10-base oligonucleotide primers (Operon), 11 primers were selected and used to evaluate all 11 fungal endophyte isolates. About 20 different bands were amplified with every primer. Eleven primers generated a total of 220 bands. Comparisons of each banding profile for each primer were conducted on the basis of presence or absence (1/0) of RAPD products of the same size (Photo 6). Matrices were generated and analyzed using NTSYSpc, Dice coefficient and UPGMA clustering method to produce a similarity dendrogram.

Results of RAPD data. There were some differences in similarity analysis results of data matrix generated from amplified products by different primers. However, the trend was very similar. The similarity clustering diagram tree of UPGMA using the entire RAPDs data showed that EH45, EH19 and EH47 are identical (Figure 21).

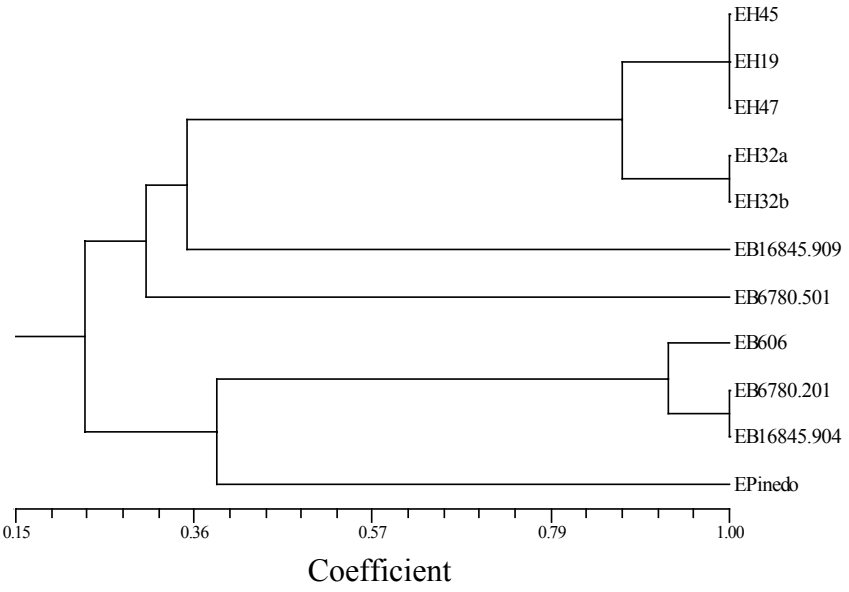

Figure 21. Similarity dendrogram of 11 endophyte isolates isolated from species and hybrids of *Brachiaria*, based on RAPDs data.

EH32a and EH32b are also identical. The two groups' similarity coefficient is above 0.8. EB6780 (201) and EB16845 (904) have same identity and very similar to EB606 with coefficient of over 0.9. EB6780 (501), EB16845 (909) and Epinedo are very different from each other and the former group, with less than 40% similarity. A single primer data analysis produced similar results and conclusions (eg. Figure 22).

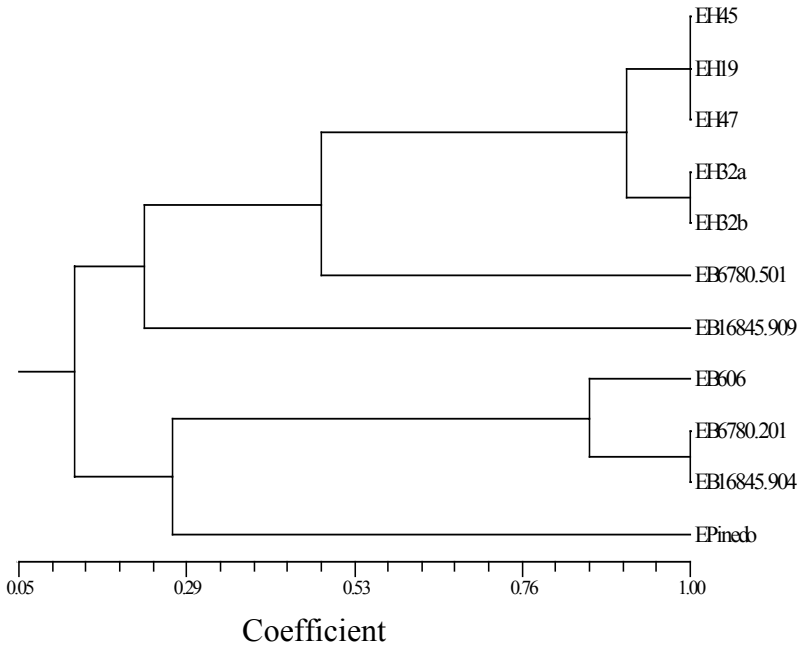

Figure 22. Similarity dendrogram of 11 endophyte isolates isolated from species and hybrids of *Brachiaria*, based on RAPDs data using primer AJ12.

AFLP analysis. In order to possibly differentiate those isolates that showed high similarity using the RAPD data, a further study was conducted using the AFLP method.

AFLP assays were performed with AFLP Analysis System for Microorganisms (Gibco) according to the manufacturer's instructions. Genomic DNA of each isolate was digested with the restriction enzymes *Eco*RI and *Mse*I, and ligated to their specific adapters. The restriction fragments were then amplified by PCR. Fifteen combinations of *Eco*RI and *Mse*I selective primers were first used to perform PCR of 2 very similar isolates (based on RAPDs data) in order to determine which primer pairs could give best results.

The primer pairs E-A/M-C, E-C/M-C, E-C/M-G and E-G/M-T were the most appropriate and were used to conduct PCR for all the 11 isolates. Amplification products were separated by electrophoresis on a 6% denaturing polyacrylamide gel. The gel was stained with silver nitrate after electrophoresis and developed on APC film. Methods of band reading, matrix preparation, and PCR conditions were the same as those used in RAPD analysis. Correlation of the matrices of RAPDs and AFLPs was conducted using MxComp program of NTSYSpc.

Results of AFLP analysis. Four hundred sixty-six bands of sizes between 100- and 330-bp were used in AFLPs similarity analysis. The results of AFLPs clustering analysis were very consistent with those of RAPDs (Figure 23). The correlation coefficient of matrices based on AFLPs and RAPDs was as high as 0.98. Each matrix from different selective primer pairs data also had high consistence, with a correlation coefficient value between 0.97 and 0.99. Both methods of RAPDs or AFLPs, and even one set of selective primer pairs in AFLP (eg. Figure 24), could reveal the relationship of these endophyte isolates.

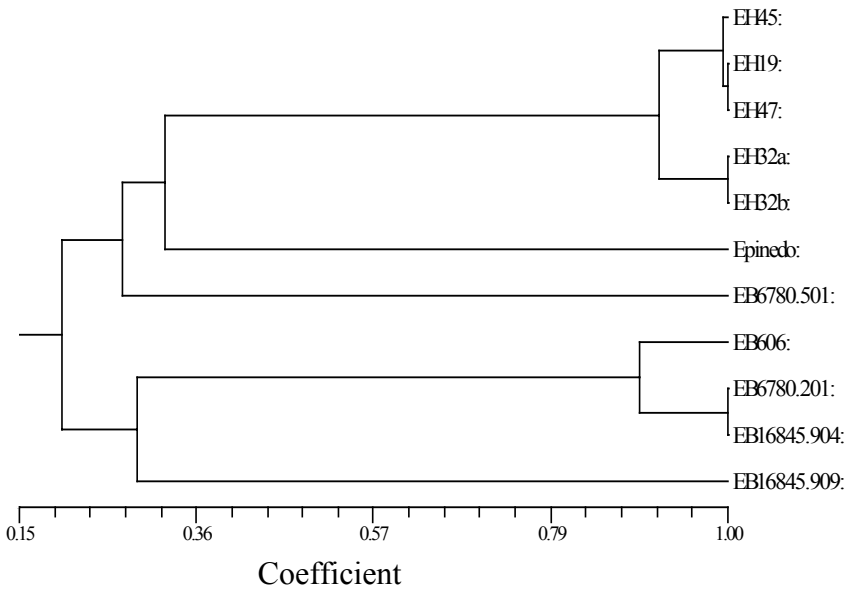

Figure 23. Similarity dendrogram of 11 endophyte isolates from species and hybrids of *Brachiaria* based on AFLP data.

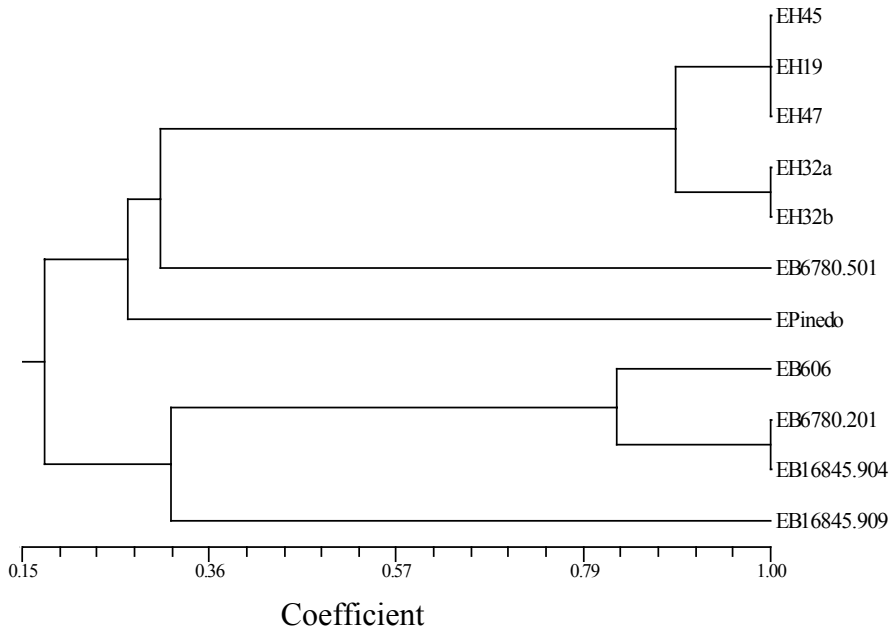

Figure 24. Similarity dendrogram of 11 endophyte isolates from *Brachiaria* based on AFLP data using primer pairs E-A/M-C

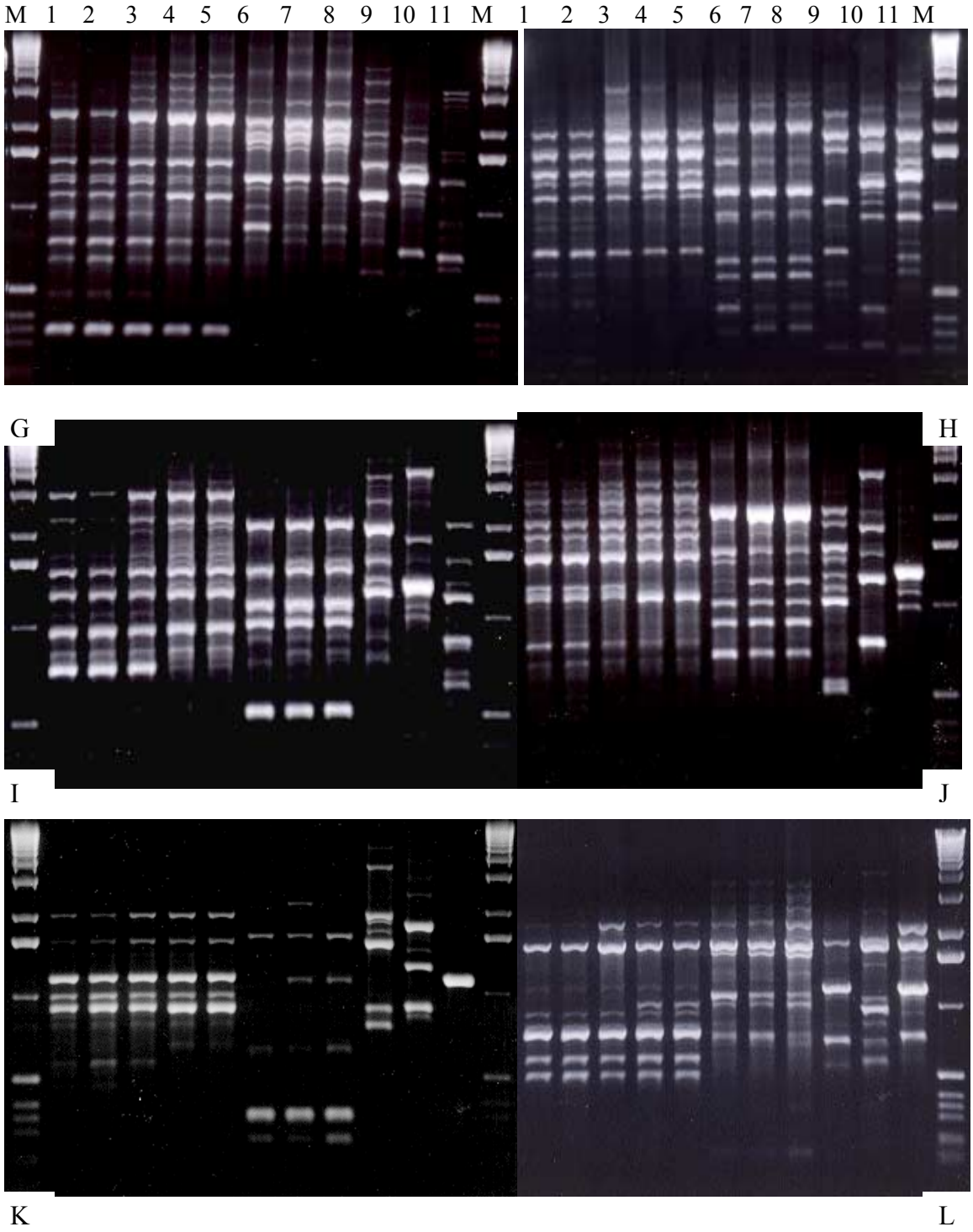

Photo 6. Electrophoresis gels of RAPD products of isolates of endophytic fungi. Gels G, H, I, J, K, L, with primers OPAK12 (5'-AGTGTAGCCC-3'), OPAK19 (5'-TCGCAGCGAG-3'), OPAJ01 (5'-ACGGGTCAGA-3'), OPAJ04 (5'-GAATGCGACC-3'), OPAJ12 (5'-CAGTTCCCGT-3'), and OPAJ13 (5'-CAGCCGTTCC-3'), respectively. Lanes 1-11 are endophyte isolates from *Brachiaria* hybrids #45, 19, 47, 32a, 32b, *Brachiaria* accessions 606, 6780 (P-201), 16845 (P-904), 6780 (P-501), 16845 (P-909), and endophyte Pinedo (isolated from seeds), respectively.

2.6.4 Effect of endophyte on *Rhizoctonia solani* (*in vivo*)

Contributors: H. Dongyi (South China University of Tropical Agriculture, The People's Republic of China) and S. Kelemu (CIAT)

Rationale

Previous results had shown that *B. brizantha* CIAT 16320 had high levels of resistance to Rhizoctonia foiar blight. It is interesting to note that 2 endophyte isolates (EH32a and EH32b) were isolated from CIAT 16320. We have demonstrated that endophytes of *Brachiaria* inhibit some fungal pathogens *in vitro* (IP-5 Annual Report, 1999, 2000) and *in vivo* (Kelemu et al., 2001). In this study, we examined the antifungal activities of clonal H32 plants with and without the endophyte.

Materials and Methods

Brachiaria material used in this study are: (1) CIAT 16320 *Brachiaria* plant naturally infected with an endophytic fungus; resistant to *Rhizoctonia* foliar blight, maintained in greenhouse. (2) Hybrid 30 *Brachiaria* plant, no endophyte detected, susceptible to *Rhizoctonia* foliar blight, maintained in greenhouse. (3) CIAT 16320F, which are tillers treated with 0.1 ml/L Folicur to eliminate endophytes (2 months old after treatment), maintained in greenhouse.

Pieces of surface sterilized leaf blades and sheaths were macerated in sterile distilled water (3-gm plant tissue in1-ml water). Plant debris was removed by centrifugation at 10,000 rpm for 5 minutes. The supernatant was filter sterilized and used to test its anti-fungal properties. Sterile filter paper discs were each soaked with 200 - 400 µl plant extract (supernatant) and placed at ends of plates containing potato dextrose agar with appropriate controls. A fresh sclerotium of *R. solani* was placed in the center of each plate. The plate was incubated at 28 C till measurements of mycelial coverage were taken Table 55. .

Table 55. Inhibition zone distance (mm) between filter paper disc treatments with extracts of *Brachiaria* hybrids and mycelial growth of *Rhizoctonia solani* on potato dextrose agar.

Treatment	CIAT 16320 (+E)*	CIAT 16320F (-E)	Hybrid 30 (ND-E)
200 µl water	0.00	0.00	0.00
200 µl extract	2.00	0.00	1.00
400 µl extract	6.33	0.00	1.00

* + E = naturally infected with an endophyte; -E = the endophyte eliminated using the fungicide Folicur; ND-E = endophyte not detected.

Sclerotia of *R. solani* were produced, and plants were inoculated as described earlier (Kelemu et al. 1995, Tropical Grasslands 29:257-262) to assess the level of resistance to the pathogen.

Results and Discussion

Three *Brachiaria* genotypes (codes FM9503/S046/024 [plants # 19 and 45], CIAT 16320 [plant # 32a, 32b], and SX99/2341 tested positive for endophytes. Of these, *B. brizantha* CIAT 16320 was highly resistant to *R. solani*. In order to determine whether the presence of an endophytic fungus was responsible for the resistance to *R. solani*, genetically identical lines of CAT16320 were created by treating half of the tillers derived from a single mother plant containing an endophyte with the fungicide Folicur while the remaining half were left untreated. Plant extracts from endophyte-infected and endophyte-free plants had differences in their growth inhibitory properties against *R. solani*. Results showed that extracts of CIAT 16320 produced inhibition zones while H30 (a hybrid with no detectable endophyte) and CIAT 16320F

(genetically identical clone to hybrid H32, but the endophyte was removed by treatment with the fungicide Folicur) did not (Photos 7, 8).

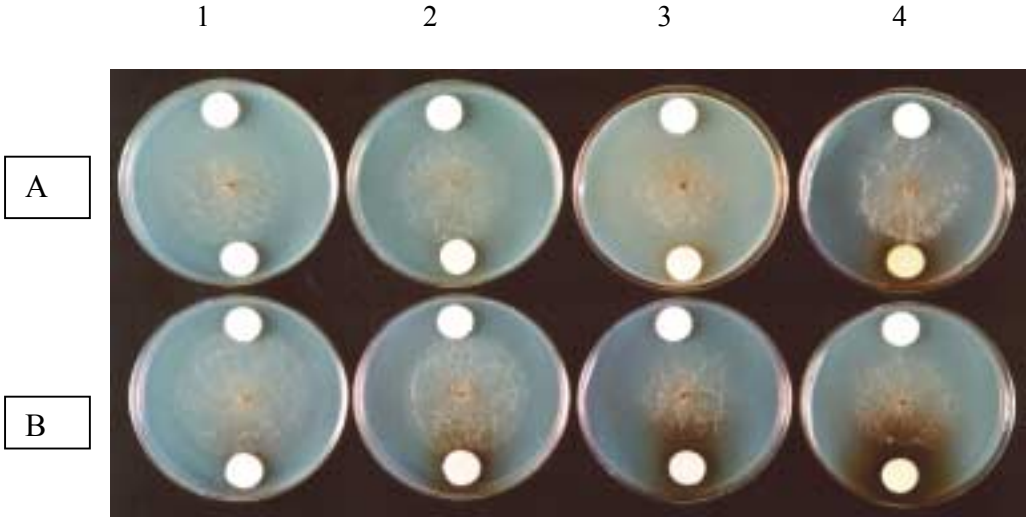

Photo 7. *Rhizoctonia solani* growth inhibition test by extracts from *Brachiaria brizantha* CIAT 16320 H32 and Hybrid 30. CIAT 16320 is naturally infected with an endophyte and is highly resistant to *R. solani*. H30 had no detectable endophyte and is susceptible to *Rhizoctonia*.
The filter disc in the upper part of each plate was treated with sterile water as control, whereas the disc in the lower part of each plate was treated with plant extracts.
Filter paper discs in plates A1 (200 µl), A3 (400 µl), B1 (200 µl) and B3 (400 µl) were treated with extracts from leaf sheaths, those in plates A2 (200 µl), A4 (400 µl), B2 (200 µl) and B4 (400 µl) were with extracts from leaf blades
Plates A1-A4 and B1-B4 had extracts from Hybrid 30 and CIAT 16320, respectively.

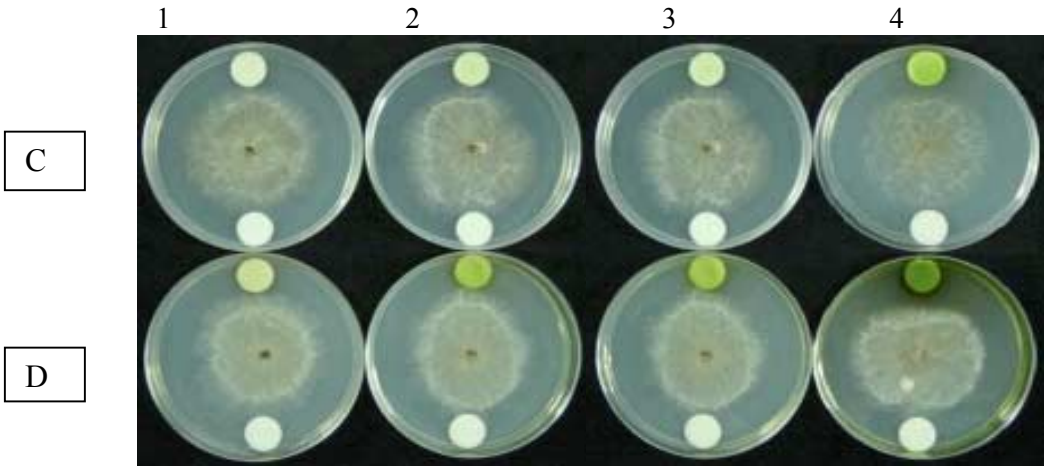

Photo 8. Growth inhibition test of *Rhizoctonia solani* by leaf and leaf sheath extracts from *Brachiaria brizantha* CIAT 16320 and CIAT 16320F. Extracts in plates C1, C2, D1 and D2 were from CIAT 16320F (tillers of CIAT 16320 treated with Folicur to eliminate the endophyte).
Extracts in plates C3, C4, D3 and D4 were from CIAT 16320 naturally infected with an endophyte. The filter disc in the lower part of each plate was treated with sterile water as control, whereas the disc in the upper part of each plate was treated with plant extracts. Extracts in plates C1 (200 µl), D1 (400 µl), C3 (200 µl) and D3 (400 µl) were from leaf sheaths. Extracts in plates C2 (200 µl), D2 (400 µl), C4 (200 µl) and D4 (400 µl) were from leaf blades

Since CIAT 16320 contained endophyte and had resistance to *Rhizoctonia*, and because elimination of the endophyte using a fungicide also eliminated the antifungal activities of the plant extracts, we concluded

that the fungal inhibition (and possibly the resistance) was from the endophye/plant interactions. Studies are in progress to assess the reactions of live plants (CIAT 16320 and CIAT 16320F) to *R. solani*. The compound (s) involved in the resistance will also be studied in the future.

2.7 Define interactions between host and pathogen in *Brachiaria, Arachis* and *Stylosanthes*

Highlights

- More than 200 isolates of *Colletotrichum gloeosporioides* infecting *Stylosanthes*
- *guianensis* have been characterized.
- A rice-chitinase gene confers resistance to *Rhizoctonia* foliar blight disease in transgenic *S. guianensis*. Segregation of resistance in the selfed progeny of a transgenic plant (118 resistant: 38 susceptible) suggest that resistance behaves as a single, dominant Mendelian factor.
- Sources of resistance to *Rhizoctonia* foliar blight have been identified in *Brachiaria*. It appears that the presence of endophytes contributes to this resistance.
- Sources of resistance to *Xanthomonas campestris* pv. *graminis* have been identified in *Brachiaria*.

Progress towards achieving milestones

- Determined *Colletotrichum gloeosporioides* diversity at a field in Quilichao using RAPD and AFLP
- Defined the inheritance of a rice chitinase gene in *Stylosanthes guianensis*
- Defined resistance of transgenic *S. guianensis* to *Rhizoctonia* foliar blight
- Identified sources of resistance to *Rhizoctonia* foliar blight in *Brachiaria* hybrids

We have determined the genetic diversity of more than 200 isolates of *C. gloeosporioides* collected at a field site in Quilichao, Colombia. The inheritance of a rice chitinase gene introduced into *S. guianensis* via *Agrobacterium*-mediated transformation was shown to be of Mendelian fashion. Transgenic *S. guianensis* plants were resistant to *Rhizoctonia* foliar blight. We have evaluated and identified some sources of resistance to *Rhizoctonia* foliar blight disease of *Brachiaria*.

2.7.1 Biodiversity studies on the anthracnose pathogen of *Stylosanthes*

Contributors: M. Rodriguez, G. Segura, X. Bonilla and S. Kelemu (CIAT)

Rationale

Anthracnose disease continues to be the most important limitation to *Stylosanthes* production. The pathogen population is diverse and complex. Knowledge on the pathogen population structure is crucial to effective disease management. The studies were conducted to advance understanding of anthracnose epidemiology and the population genetics of *Colletotrichum gloeosporioides* for improved disease management.

Materials and Methods

Fungal cultures, PCR analysis, plant inoculations, and pathogenicity evaluations, statistical data analysis were all as described before in various publications (Kelemu, et al., 1996, 1997 and 1999).

For evaluations of pathogenicity, we compared the commonly used visual evaluation method described by Horsfall-Barratt, with 4 control isolates and 12 differentials.

To construct dendrograms of RAPD and pathogenicity data, the presence and absence of RAPD bands was coded in a binary matrix with 1/0, respectively. The pathogenicity data was equally coded as 1 for disease evaluation values of 3-9 (Horsfall-Barratt scale) and 0 for values ≤ 3. Dendrograms were generated using SAHN of NTYS.

To determine the genetic and pathogenic variability, 217 isolates of *Colletotrichum gloeosporioides* were analyzed. DNA isolations, PCR reactions, plant inoculations and race determinations are as described earlier (Kelemu, et al. 1999. Genetic diversity in South American *Colletotrichum gloeosporioides* isolates from *Stylosanthes guianensis*, a tropical forage legume. European Journal of Plant Pathology 105:261-272). RAPD primers and their sequences used in the study are listed in Table 56.

Table 56. RAPD primers and their sequences used in this study.

Code	Sequence (5'……3')
OPA- 2	TGCCGAGCTG
OPA- 3	AGTCAGCCAC
OPA- 4	AATCGGGCTG
AJ- 4	GAATGCGACC
AJ- 6	GTCGGAGTGG
AJ- 8	GTGCTCCCTC
AJ- 9	ACGGCACGCA
AK- 4	AGGGTCGGTC
AK- 19	TCGCAGCGAG
D- 3	GTCGCCGTCA

AFLP analysis was initiated 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 (USA) was used according to the manufacturer's instructions. 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 picked isolates. The amplification was done with a MJ-Research PTC-100 thermal cycler. The amplification conditions were as described by the manufacturer of the kit (Gibco BRL). Amplification products were separated on a 6% denaturing polyacrylamide sequencing gel. At the end of the electrophoresis period, gels were stained with silver nitrate. The resulting banding pattern was analyzed manually.

For analysis of genetic similarity, each band was codified either as 1 or 0, whether it was present or not, in any particular isolate. The genetic similarity between two isolates was calculated based on Dice's coefficient with NTSYS-pc Version 1.8 (Exeter Software, NY). For each coefficient , the similarity matrix was used to construct dendrograms with the help of the unweighted pair grouping by mathematical averaging (UPGMA) methods using the SAHN and Tree programs in NTSYS. Multiple correspondence analysis was also employed to assign isolates to separate clusters.

Results and Discussion

AFLP. Four primers were used combined as follows: *Eco*RI primer E-AC with *Mse* I primer M-A, *Eco*RI primer E-AC with *Mse* I primer M-C, *Eco*RI primer E-AC with *Mse* I primer M-G, *Eco*RI primer E-T with *Mse* I primer M-A. In order to determine whether the information generated using these four primer combinations were complimentary or redundant, a correlation index between the similarity matrix generated using the four primers and that using only three of the primer combinations. The results showed a 97% correlation between the two matrices, which indicated that the information generated using three primer combinations (*Eco*RI primer E-AC with *Mse* I primer M-A, *Eco*RI primer E-AC with *Mse* I primer M-C, *Eco*RI primer E-AC with *Mse* I primer M-G) was sufficient to differentiate the isolates. A total of 371 bands were generated using these three combinations.

The analysis generated 9 groups of isolates (A-I). Group A is a heterogeneous group which contained 34 isolates from various geographic origins. This group has a 27% similarity with the rest of the groups.

Group B is the largest group with 153 isolates. There were three well-differentiated sub-groups within this large group. The first sub-group contained 75 isolates. Internal divisions within this sub-group showed a tendency for individual isolates to group based on their geographic origins. In general, the isolates from Quilichao and Caquetá formed consistent groups. The AFLP data showed that three isolates from Caquetá (isolate # 16116, 16120, 16160) were identical. This was interesting as the three isolates were collected from the same accession (*S. guianensis* CIAT 184) on the same date and at the same field. The remaining two sub-groups contained isolates collected in Quilichao from various host accessions between December 1999 and October 2000.

Group C had only four isolates from Quilichao collected from different accessions of *S. guianensis* within a month period. The similarity range within this group is between 59-82%, with an average of 68% similarity. The isolates 16203 (Carimagua), 16611 and 16296 (Quilichao) formed the AFLP groups D, H, and I, respectively. Interestingly, these three isolates were collected from three different resistant *S. guianensis* bred genotypes. The RAPD data did not differentiate these three.

Group E consisted of 13 isolates (12 of them from Quilichao). Interestingly, seven of these isolates isolated from a mixture of resistant hybrids 11833/11844 formed a sub-group. The second sub-group contained 5 isolates which originated from three resistant genotypes. The isolates in this group are most likely emerging pathogen genotypes.

Group F consisted of 8 isolates from Quilichao and Brazil.

Group G is a minor group with only two isolates collected in Carimagua, Colombia.

RAPDs. Analysis of the 217 isolates with the 10 random primers generated a total of 226 bands. A dendrogram constructed using this data generated 9 groups. As with the AFLP data, sub-groups of isolates were formed with a tendency based on their geographic origins. The second of these 9 major groups (group B) contained 69% of the isolates.

A closer look at AFLP and RAPDs groupings of the isolates and their original hosts revealed something interesting. Resistant host genotypes (eg. CIAT 2340, FM9405, CIAT 11833, CIAT 11844) provided isolates which belonged to more groups than very susceptible ones such as CIAT 2312 and Endeavour. For example, isolates collected from *S. guianensis* CIAT 2340 over time belonged to AFLP groups A, B, E and H; those from FM9405 clustered in AFLP groups A, B, C, E and F; whereas the highly susceptible genotypes like CIAT 2312 and Endeavour provided isolates which belonged to the predominant groups A

or B. These observations indicate that isolate sample collections for population studies should include as many diverse host genotypes as possible including those with high level of resistance to the pasthogen.

Pathogenicity studies. The isolates CIAT 16066 (Carimagua) and CIAT 1609 (Caquetá) were the most pathogenic infecting 11 of the 12 differentials. Eleven of the isolates did not cause any symptoms on any of the differentials used. Three of the differentials (CIAT 2312, Endeavour and 1875) were susceptible to more than 100 of the isolates. None of the differentials was susceptible to all the isolates tested. Two of the differentials (CIAT 2340, 1507) were infected by less than 30 of the isolates. When a matrix of 0 (resistant) and 1 (susceptible) was generated and a similarity dendrogram constructed, the groups formed did not show a clear association with location or collection date. The analysis showed no direct correlation between pathogenic groups and the genetic variability groups of AFLP and RAPDs data. However, the variability observed within the pathogen population in the three tests (AFLP, RAPDs and pathogenicity) was high.

Multiple Correspondence Analysis. Analysis of AFLP data using Multiple Correspondence Analysis (MCA) in three dimensions generated 7 groups with an average similarity of 58% (Figures 25, 26 different orientations of the same graph). The first dimension explains 47% of the variation within the isolates studied. This clearly separates group 1 and group 3. The second dimension differentiates groups 1, 2, 3, 6 and 7. It also separates groups 4 and 5 from the others. The third dimension divides the population in two clusters: the first forming the groups 3, 6 and 7; the second with groups 1, 2, 4 and 5.

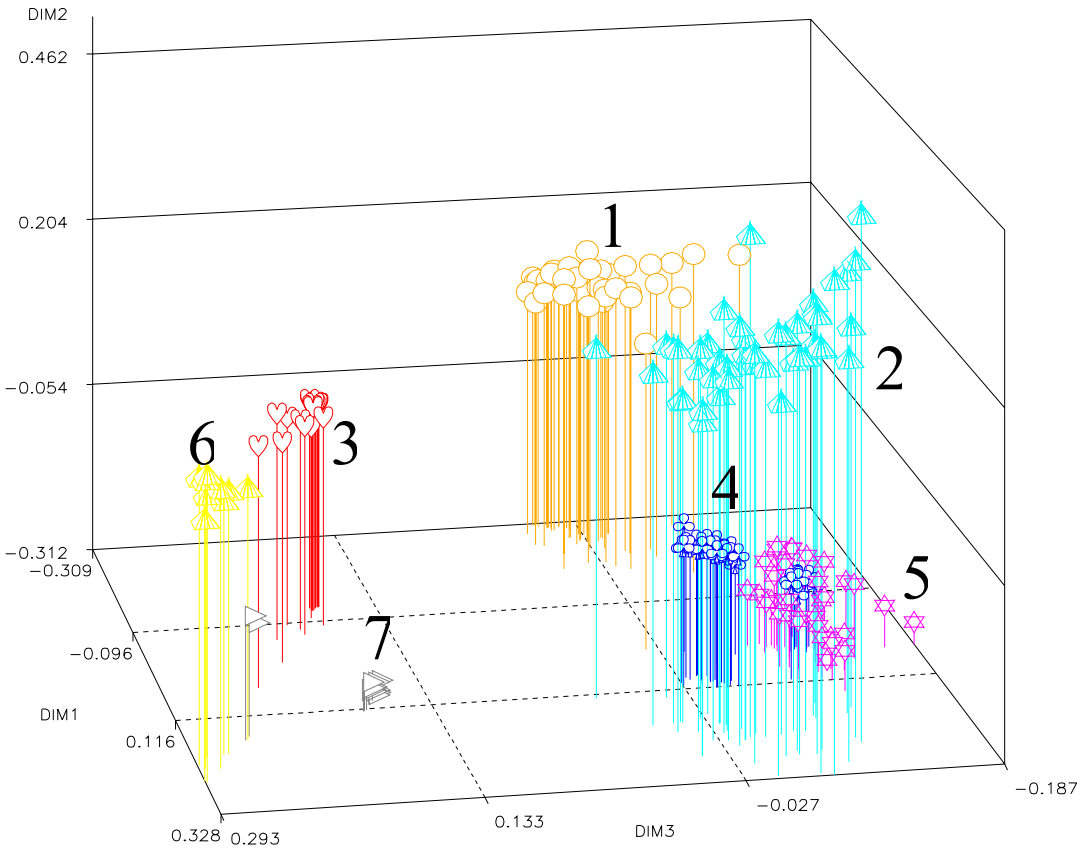

Figure 25. Cluster analysis of AFLP data on isolates of *Colletotrichum gloeosporioides*.

Group 1 consisted of 54 isolates with an average similarity of 35%. The isolates in this group together with those of group 3 formed one group (B1) using UPGMA analysis.

Group 2 contained 56 isolates. With the exception of some isolates, this group combines the groups A, E, F, H, and I formed using UPGMA. In general, this group had an average similarity index of 73%. This group is related to groups 4 and 5 in the third dimension with an average similarity of 56%.

Group 3 had 20 isolates with an average similarity of 52 %. As it appeared in group 1, two of the isolates were separated from the rest of the group.

Group 4 contained 39 isolates with an average similarity of 56%. Within this group, a sub-group of 12 isolates was formed in the third dimension which appeared more related to group 5 isolates. This same sub-group was formed in the UPGMA dendrogram.

Thirty-four isolates belonged to group 5. The UPGMA grouped 24 of these isolates in group B3, 4 in B2, 4 in C and 2 en group F. Similarity within this group is 76%. Groups 4 and 5 were all collected from Quilichao and are related to each other.

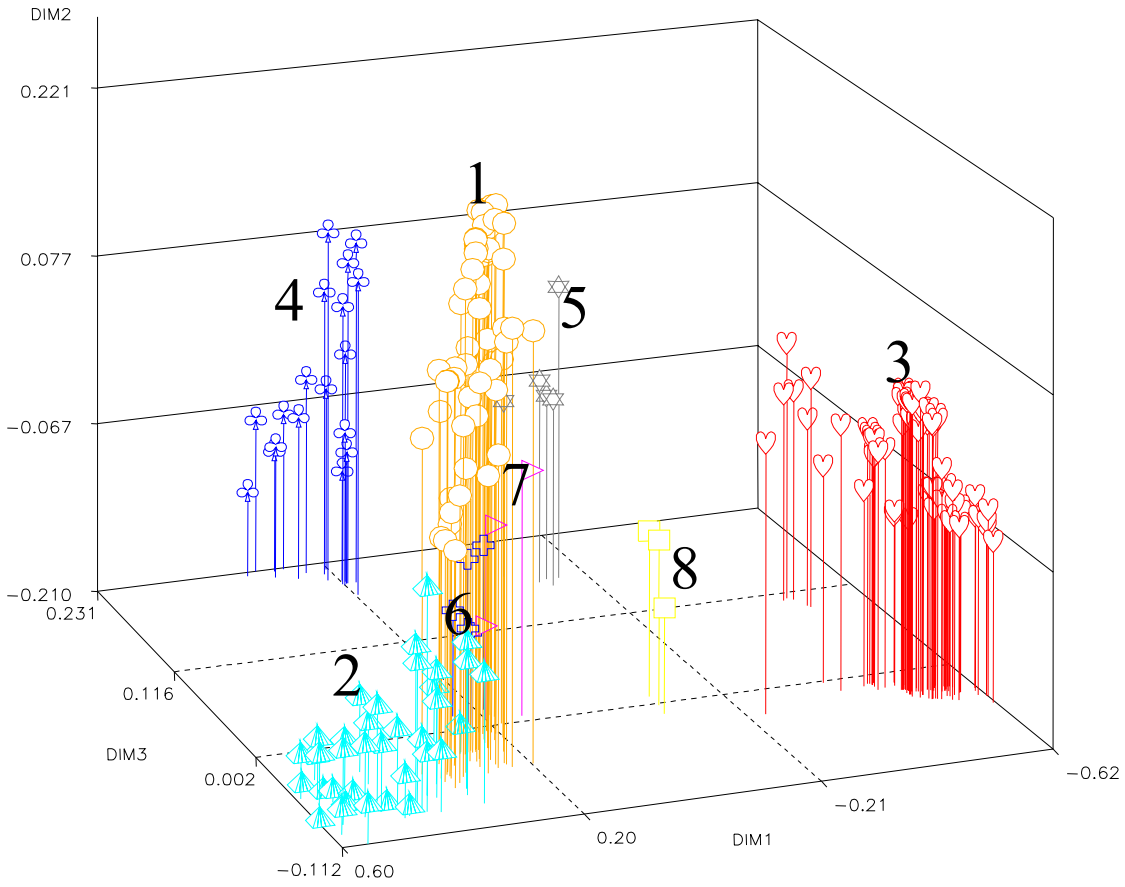

Figure 26. Cluster analysis of RAPDs data on isolates of *Colletotrichum gloeosporioides*.

The isolates of group 6 (9 isolates) had an average similarity of 88%. These isolates were dispersed in different groups using NTSYS. This group is related to groups 3 and 7. Isolates which formed group 7 are

very closely related to each other (average similarity of 91%) and were all collected from Quilichao. With the exception of few of the member isolates of this group, all were collected on the same date. This group of isolates was grouped together with RAPDs data and other statistical analysis.

The RAPDs data produced 8 groups of isolates with an average similarity of 54% (Figure 26). Group 1 had 60 isolates with an average of 45% similarity. The group is related to group 2 (76% similarity), and group 6 (55% similarity). This group of isolates is analogous to groups 1 and 3 formed in the AFLP data (Figure 25). Group 2 contained 36 isolates with an average similarity of 77%. Group is 3 has 87 isolates (all from Quilichao, years 1999, 2000 from various host genotypes) and is the most differentiated from the rest of the groups (Figure 26). Group 4 had 18 isolates with an internal average similarity of 83%. Groups 5 and 6 had 5 isolates each. With an average similarity of 70% and 52 %, respectively. Groups 7 and 8 had 3 isolates each. In general there was a good correlation between groups formed using UPGMA and those with multiple correspondence analysis. The results showed that isolates collected from Caquetá had less variability than those from Quilichao.

2.7.2 Epidemiology studies on the anthracnose pathogen of *Stylosanthes*

Contributors: Gustavo Segura, M. Rodriguez, and S. Kelemu (CIAT)

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. There was an establishment period of six months before the first disease evaluation (see field establishment problems on page 5). 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 accession.

2.7.3 Characterization of transgenic *Stylosanthes* plants with chitinase gene

Contributors: J. Changshun (CATAS, China), H. Guixi (CATAS, China), G. Segura and S. Kelemu (CIAT)

Rationale

Foliar blight, caused by *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* Donk), affects species of *Stylosanthes* in many parts of tropical America where the annual rainfall is more than 1500 mm (Lenné and Calderon, 1984, In: Stace, H. M. and Edye, L. A. (eds) Biology and Agronomy of *Stylosanthes*. Academic Press, North Ryde, Australia, pp. 279-194). The disease has also been reported in many parts of the world (O'Brien and Pont, 1977, Queensland Agricultural Journal 103:126-128; Lenné, 1990, Phytopathology Papers No. 31, CAB International, Wallingford, UK). The disease initially appears as water-soaked spots on infected leaves. Under favorable conditions such as prolonged humidity, these progress into rotting and extensive foliar death. The pathogen has an extensive host range and it can produce a substantial foliar damage on susceptible genotypes (Baker, 1970, In: Parameter, J. R. Jr (ed.) *Rhizoctonia solani*: Biology and Pathology. Pp.125-148. University of California Press: Berkeley), including various tropical and subtropical forage legumes and crops (Hepperly et al., 1982, Plant Disease

66: 256-257; Galindo et al., 1983, Plant Disease 67:1016-1021; Yang et al., 1990, Plant Disease 74: 501-504).

Sclerotia of the fungus can survive in the soil or on plant debris for long periods. Sclerotia first appear as white masses on infected plant tissues, and as they mature, they turn brown and loosely attached which then easily shed forming the primary source of inoculum.

Chitinases have been reported from a number of plants and various microbes (Punja and Zhang, 1993, J. Nematol 4:526-540; Ohme-Takagi et al., 1998, Mol Gen Genet 259:511-515). Both biotic and abiotic stresses can induce chitinase expression at relatively high level although many plant chitinases are often expressed constitutively at low levels. Chitinase catalyzes the hydrolysis of the β-1,4 linkages of the N-acetyl-D-glucosamine polymer chitin, a structural component in a number of organisms. However, no chitin-like substrates are present in plants (Boller et al., 1983, Plant Mol. Cell Biol. 5:145-174). In contrast, chitin constitutes 3-60% of the cell wall in fungi (Bartnicki-Garcia, 1968, Annu Rev Microbiol 22:87-108). Purified chitinases obtained from: (1) bean were effective against cell walls of *Fusarium solani* (Boller et al., 1983) and *Trichoderma viride* (Schlumbaum et al., 1986, Nature 324:365-367); (2) tomato (Young and Pegg, 1982, Physiol Plant Pathol 21:411-423), tobacco (Mauch et al., 1988, Plant Physiol 88:936-942), and pea (Sela-Buurlage et al., 1993, Plant Physiol 101:857-863) could inhibit fungal growth by lysis of fungal tips; and (3) *S. guianensis* leaves could kill *C. gloeosporioides* hyphae (Brown and Davis, 1992, J. Phytopathol 136:247-256). Chitinases have also been reported as pathogenesis-related proteins in cucumber (Metraux et al., 1988, Physiol Mol Plant Pathol 33: 1-9). These and other findings support the hypothesis that plant chitinases have antibiotic functions and thus probably constitute a defense mechanism in plants against pathogens.

Recombinant DNA techniques have allowed the isolation of specific genes and their introduction into plants which otherwise is not possible with conventional breeding. Complimentary DNA clones and genomic clones have been isolated, and the amino acid sequences deduced for several chitinases obtained from a number of plants (Van Damme et al., 1993, Physiol Plant 87: 177-186; Samac et al., 1990, Plant Physiol 93:907-914; Swegle et al., 1989, Plant Mol Biol 12:403-412; Broglie et al., 1986, Proc Natl Acad Sci USA 83:6820-6824; Metraux et al., 1989, Proc Natl Acad Sci USA 86:896-900; Huynh et al., 1992, J Biol Chem 267:6635-6640; Zhu and Lamb, 1991, Mol Gen Genet 226:289-296; Huang et al., 1991, Plant Mol Biol 16:479-480).

Some transgenic plants with chitinase-encoding genes such as canola (Benhamou et al., 1993, The Plant Journal 4:295-305), cucumber (Raharjo et al., 1996, Plant Cell Rep. 15:591-596; Tabei et al., 1998, Plant Cell Reports 17:159-164), rice (Lin et al., 1995, Bio/Technology 13:686-691), rose (Marchant et al., 1998, Mol Breed 4:187-194), tobacco (Broglie et al., 1991, Science 254:1194-1197), and tomato (Hironaka et al., 1993, In: Proc. Conference on "Molecular genetics of plant-microbe interaction", Rutgers, NJ. (Abstract no. 46) have also been generated to control fungal pathogens. A rice-chitinase gene, controlled by the CaMV 35S promoter and introduced into *Indica* rice, was shown to enhance resistance to *Rhizoctonia solani* (Lin et al., 1995). A rice-chitinase gene in a transgenic rose plant reduced the development of blackspot disease caused by the fungus *Diplocarpon rosae* Wolf (Marchant et al., 1998). Tabei et al. (1998) had shown that transgenic cucumber plants containing a rice chitinase gene had enhanced resistance to gray mold disease caused by *Botrytis cinerea*. Increased chitinase activity has also been reported to enhance anthracnose resistance in *S. guianensis* (Brown and Davis, 1992).

In this study, we introduced a rice-chitinase gene (Huang et al., 1991) into the widely grown *S. guianensis* CIAT 184 accession in order to enhance foliar blight resistance. This accession has broad adaptation to the humid tropics and has been released as a cultivar in various countries including Peru and southern China. It has also performed well in parts of Africa.

Materials and Methods

DNA manipulation. A 1.5-kb DNA fragment, containing a 1.1-kb rice chitinase gene and the CaMV 35S promoter, was recovered from the *Hind*III digested plasmid pBSKS-G11 (R) with the chitinase gene (provided by Dr. S. Muthukrishnan, Department of Biochemistry, Kansas State University), using a DNA recovery kit (BIO-RAD) according to the manufacturer's instructions. This DNA fragment was ligated to the *Hind*III site of pCAMBIA2301, a vector generously provided by the Center for the Application of Molecular Biology to International Agriculture (CAMBIA), Australia. The ligated DNA product was used to transform *Escherichia coli* DH5α. Two constructs, designated pCIATCH1 and pCIATCH2, with opposite orientations of the insert were selected. All recombinant DNA techniques were carried out with standard procedures (Sambrook et al., 1989).

Bacterial transformation. Bacterial strains and plasmids used are listed in Table 1. Competent cells of *E. coli* DH5α were prepared and subsequent transformations with plasmid DNA carried out according to the protocol described by Inoue (1990, Gene 96:23-28). Transformed cells were cultured on Luria agar medium (bacto-tryptone, 10 g; bacto-yeast-extract, 5 g; NaCl, 10 g; and agar, 15 g; per L of distilled water) with appropriate antibiotics (100 μg/mL ampicillin for pBSKS-G11(R), and 50 μg/mL kanamycin for pCAMBIA2301 and its derivatives) and incubated overnight at 37 °C. For α-complementation screening, 40 μL/plate X-gal and 4 μL/plate IPTG were used.

pCIATCH2 DNA was directly transformed into *Agrobacterium tumefaciens* strain LBA4404 (Jefferson et al., 1987, EMBO J. 6:3901-3907). Transformed cells were selected by plating them on Luria agar medium with 25 μg/mL, and 50 μg/mL kanamycin. Recombinant DNA was isolated from transformant *A. tumefaciens* cells and digested with *Hind*III for verification. All transformants were maintained at –80 °C in 20% glycerol.

Plant transformation and regeneration. Seeds of *S. guianensis* CIAT 184 were surface-sterilized with 3% NaOCl solution for 15 min and rinsed with sterile distilled water, then treated for 5 min with 70% ethanol and rinsed three times with sterile distilled water. Seeds were then germinated on a basal MS medium (Murashige and Skoog, 1962, Physiol Plant 15:473-479). Cultures were maintained under fluorescent light at 55 $\mu E/m^2$ per second at 24 °C, with a 12-h photoperiod. Segments were excised from leaves for transformation.

A. tumefaciens LBA4404, containing plasmid pCIATCH2, was incubated overnight at 28 °C, with shaking at 200 rpm, in 10 mL of Luria broth (LB) containing 100 μM acetosyringone, 25 μg/mL streptomycin, and 50 μg/mL kanamycin. For co-cultivation, cells were collected from the overnight cultures by centrifugation and re-suspended in fresh LB liquid medium.

Leaf-segment explants were inoculated by swirling for 2–5 min in the bacterial suspension, blotted dry on sterile filter paper, plated onto regeneration medium (basal medium with 1.0 μg/mL α-NAA and 4.0 μg/mL BAP) (Sarria et al., 1994), and incubated at 28 °C in the dark for 2 days. The explants were then washed in sterilized distilled water, blotted dry on sterile filter paper, and cultured on the regeneration medium, containing 15 μg/mL kanamycin and 250 μg/mL carbenicillin.

After 2 weeks, all growing calli were transferred to a fresh regeneration medium for further selection. The selected green calli were then transferred to basal medium, containing 0.01 μg/mL α-NAA and 4.0 μg/mL BAP for shoot induction. After shoots appeared, the regenerated plantlets were transferred to basal medium, containing 0.1 μg/mL α-NAA and 0.4 μg/mL BAP for elongation. Shoots were excised and cultured on basal medium with quarter-strength salt and 0.1 μg/mL α-NAA for rooting. Kanamycin and

carbenicillin were used in all regeneration steps. Regenerated plantlets were transferred to pots containing autoclaved soil and placed in a glasshouse.

Plasmid isolations. Medium-scale plasmid DNA isolations were done using the protocol described by Marko (1982, Annu Biochem 121:382). Mini-preparations of plasmids were made according to the protocol described by Birnboim and Doly (1979, Nucleic Acids Res 7:1513-1523). Highly purified plasmids were extracted with the QIAGEN plasmid kit (QIAGEN, USA).

DNA isolations. Genomic DNA was isolated from fresh leaves of *S. guianensis* using DNeasy Plant Mini Kit (QIAGEN). DNA concentration was estimated using a Hoefer® DyNA Quant® 200 Fluorometer (Amersham Pharmacia Biotech, USA).

Southern and dot blot hybridizations. For Southern blot analysis, 10 µg DNA sample was digested to completion with Hind III. For dot blot analysis, 5 µg DNA was used. A 1.5 HindIII DNA fragment containing the chitinase gene from pCIATCH2 was used as a probe. The probe was labeled using the DIG High Prime DNA Labeling and Detection Kit (Roche Molecular Biochemicals, Germany) according to the manufacturer's instructions. All hybridizations and detections were carried out according to the manufacturer's instructions.

Screening of transformed plants by PCR 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. The reaction was performed with 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 used in this study are 5'-CTGCGACGCTCACACCGATACC-3', 5'-TCACCGAAGTTCATGCCAGTCCAG -3' (Gus gene primers) and 5' - ATCGGGAGCG-GCGATACCCTA-3', 5'- GAGGCTATTCGGCTATGACTG-3' (NPT II gene primers). The primers were synthesized by Operon Technologies, USA.

Inoculum production. Sclerotia of *R. solani* AG-1 were produced in a peptone sucrose yeast broth (PSY; 20 g peptone, 20 g sucrose, 5 g yeast and 1 L deionized water). Mycelial discs (4 mm in diameter) were taken from a 4 to 5-day-old culture of *R. solani* AG-1 grown on potato dextrose agar (Difco). 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 cultures at room temperature (about 25 C) for 10 days. Sclerotia were harvested with sterile forceps that separated them from the mycelial mats. They were then air-dried overnight in a laminar flow hood. Dry sclerotia were placed in sterile glass tubes and stored at 4 C till use.

Plant inoculations and disease evaluations. Seedlings of both transformed and non-transformed *S. guianensis* plants were handled as described in Kelemu et al. (1999, European Journal of Plant Pathology 105:261-272). Ten weeks old plants were artificially inoculated with sclerotia by placing one sclerotium on the soil surface in contact with each plant's stem. Humidity was maintained at 100% by placing the inoculated plants in a plastic box with one side made of cheesecloth, and immersing the entire box in a tray of water until disease symptoms developed. Plants were evaluated for their reactions to R. solani starting six days after inoculation. The number of sclerotia produced per plant, diseased and defoliated leaves per plant, and disease upward progression per plant were measured.

Inheritance of resistance attributed to chitinase. Seeds were collected from selfed, transformed, and non-transformed plants and germinated in Petri dishes containing moist filter papers, and subsequently transplanted in potted soil in the glasshouse as described in Kelemu et al (1999). The transgenic plant (designated as plant No. 22) chosen for subsequent inheritance study in the progenies, showed high levels of foliar blight resistance, GUS activity, strong dot blot signal and positive PCR results with NPTII gene primer and GUS gene primer. One hundred fifty six selfed transgenic progeny seedlings and 20 selfed control progeny were transplanted each to a separate pot. Inoculations and disease evaluations were conducted as described above. The probability values for goodness of fit to expected ratios were calculated using the Chi-square analysis.

Results

Plant transformation and regeneration. Calli were induced from leaf segments infected by *A. tumefaciens* on selective medium, containing 15 mg/L kanamycin. Shoots were induced from kanamycin-resistant calli cultured on regeneration medium with half-strength salt. These shoots grown from kanamycin-resistant calli were elongated on regeneration medium, containing 20 mg/L kanamycin. Rooting occurred from shoots excised from calli. Fifty putatively transformed kanamycin-resistant plants were generated. Some of these showed strong GUS activity.

Southern and dot blot analysis. DNA isolated from the putative plant transformants were tested using dot blot analysis. DNA of those which showed positive signals were further analyzed using Southern blot analysis. Digestion of genomic DNA to completion with *Hind*III resulted in a band which hybridized to the probe. No hybridizing band was detected in DNA from untransformed control plants.

PCR analysis: DNA from randomly picked progenies of transgenic plants were quickly tested using NPTII or GUS gene primers in PCR reactions. These progenies segregated either generating an amplification DNA product or none (Photo 9). DNA from progenies of the control plant showed no amplification product.

Reactions of transgenic and control plants to *R. solani*. Transgenic *Stylosanthes* plants constitutively expressing the rice chitinase gene showed no visible differences in growth and vigor when compared with control plants. Results showed that transgenic plants had a higher level of resistance to *R. solani* than control plants (Photo 10). A larger number of sclerotia were produced on the infected adult control plant (137 sclerotia) than on the transgenic plant No# 22 (11 sclerotia). In addition, more leaves were infected and defoliated from control plants than from transgenic ones (data not shown).

These results have practical significance. Sclerotia produced on infected plants shed to the soil upon maturation. These sclerotia can survive in the soil for long periods of time and form sources of inoculum for the next disease cycle. Therefore, transgenic plants not only confer resistance to *Rhizoctonia* foliar blight disease, but also help drastically reduce the production of sclerotia and thus sources of inoculum and maintain healthier soil.

Inheritance of resistance to R. solani in selfed progeny. In order to determine the inheritance of resistance to *R. solani*, seeds were collected from selfed transgenic mother (T_0) plants. Foliar blight, seedling blight, root rot and brown-girdling are among the disease symptoms caused by *R. solani*, a chitinous soil-borne pathogen with a wide host range. The number of diseased and defoliated leaves, number of sclerotia produced on diseased tissues of each plant, and disease upward progression have been determined in each plant. There were 118 plants rated as resistant (showing either no symptoms or limited symptoms) and 38 plants rated as susceptible (heavily infected as control plants) .

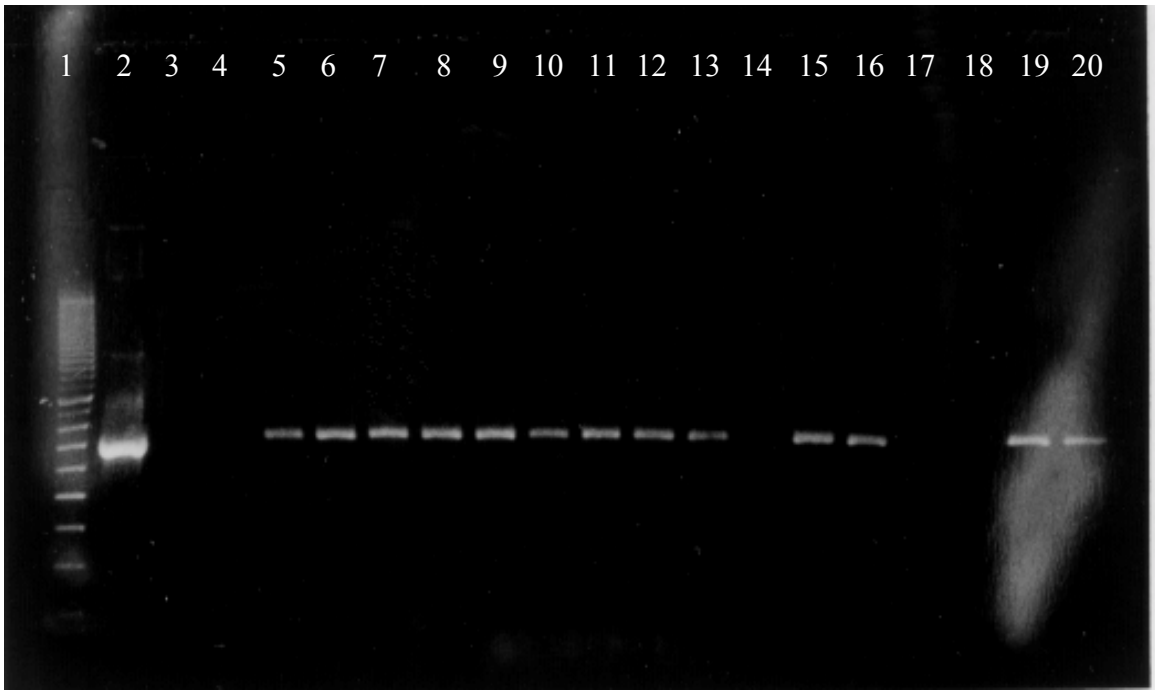

Photo 9. PCR amplifications of genomic DNA isolated from randomly picked selfed progenies and untransformed control plant with *npt*II gene primers. Lane 1 = marker 100-bp ladder; lane 2 = positive control; lane 3 = negative control; lane 4 = untransformed control plant; lanes 5-20 = selfed progenies of transformed plants. *Note:* All progenies lacking the amplified band were susceptible to *Rhizoctonia solani*.

Photo 10. Reactions of transgenic (left) and control (right) *Stylosanthes guianensis* accession CIAT 184 plants to *Rhizoctonia solani* AG-1 eight days after inoculations. Note the blighted leaves and stems on the lower part of the control plants.

This segregation in the progenies was in agreement with the expected Mendelian ratio of 3 resistant:1 susceptible, when analyzed by Chi-square test (Table 57). The number of infected and defoliated leaves per resistant progeny is significantly lower than that in each of the susceptible ones (Figure 27). Susceptible plants sustained more sclerotia per plant than the resistant ones (Figure 27). In addition, the

upward disease progression was significantly higher in susceptible segregants than the resistant ones (Figure 27).

The average root length and root dry weight were significantly higher in resistant segregants than those of susceptible ones. The average root length in each of the resistant progenies was 25 cm, and 21 cm in susceptible ones. The average root dry weight of resistant segregants was 0.228 gm. vs 0.156 gm of susceptible segregants. Disease development and progression over time on each of the resistant progenies was faster and greater than on susceptible ones (Figure 28).

These results confirm that the introduced rice chitinase gene in the transgenic *S. guianensis* plant was inherited in the progenies along with resistance to *Rhizoctonia* foliar blight disease.

Table 57. Inheritance of resistance to *Rhizoctonia solani* AG-1 conferred by a rice chitinase gene in progenies from a selfed transgenic *Stylosanthes guianensis* plant.

Plant Description	Observed Resistant	Observed Susceptible	Expected Resistant	Expected Susceptible	Chi-square value	P (%)
Selfed transgenic	118*	38♦	117	39	0.03418	85
Selfed control (accession CIAT No. 184)	-	20	-	20	-	-

*64 plants with no macroscopic symptoms of *Rhizoctonia* foliar blight, and 54 plants with some visible symptoms with upward disease progression of less than 6 cm.

♦38 progenies were as susceptible as the selfed control plants.

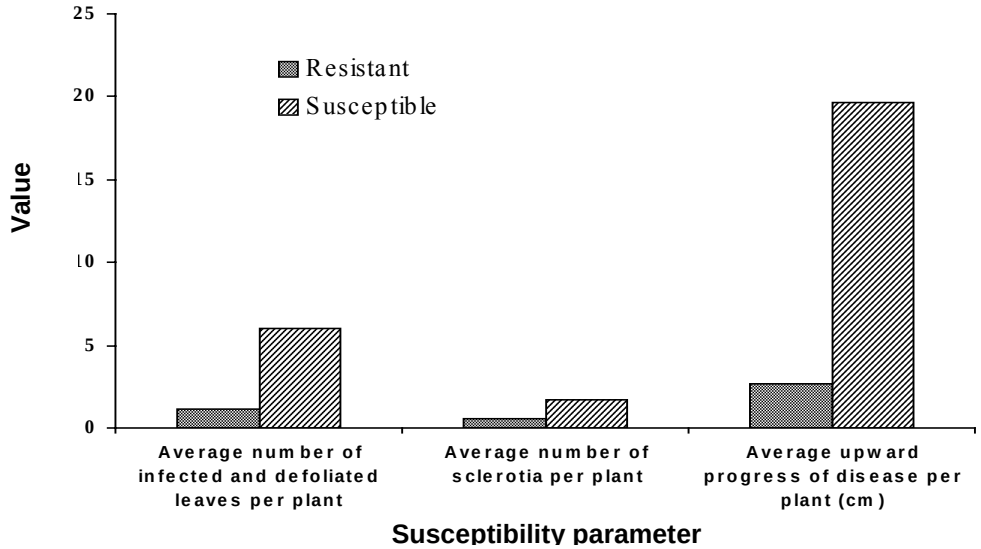

Figure 27. *Rhizoctonia* foliar blight disease development in selfed progenies of transgenic plant No. 22 according to the number of defoliated and infected leaves, number of sclerotia formed in infected plant tissues and disease progress.

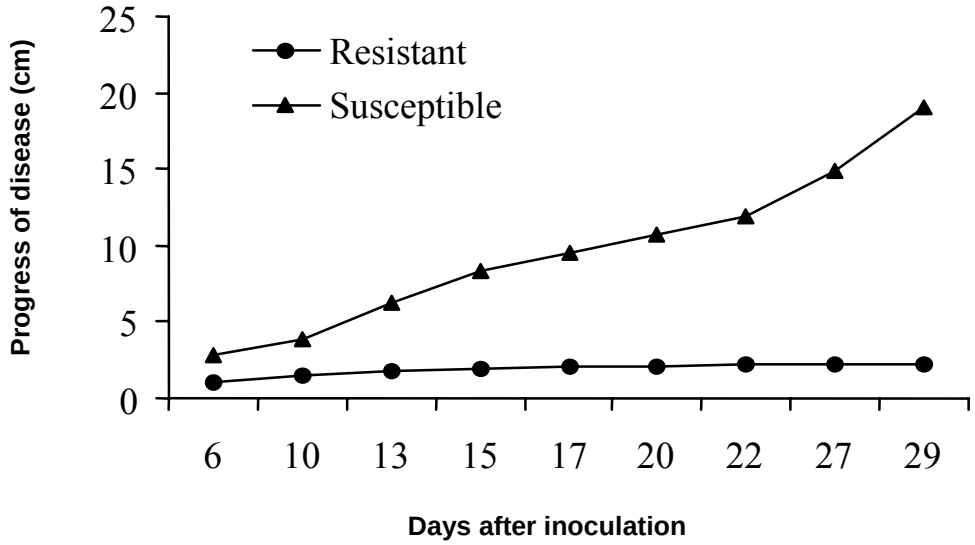

Figure 28. Progress of *Rhizoctonia* foliar blight disease in selfed progenies of transgenic plant No. 22 over time.

Discussion

Chitinases have no known function and have no known endogenous substrate in higher plants, but because its substrate, chitin, is a major component of the cell walls of many filamentous fungi with the exception of Oomycetes, it has been speculated to have a plant protection function. Plants naturally respond to microbial invasion by activating a number of defenses such as antibiotic synthesis, stimulation of enzymes, reinforcement of their cell walls and so on (Dixon and Lamb, 1990, Ann. Rev. Plant Physiol. Plant Mol. Biol. 41:339-367). Chitinases are among a group of proteins called pathogenesis-related proteins that are induced in response to attack by plant pathogens and some abiotic stresses (Boller, 1988, Plant Mol. Cell Biol. 5:145-174).

In this report, we have shown that the presence of a rice chitinase gene under the control of CaMV 35S-promoter in transgenic *S. guianensis* accession CIAT No. 184 confers resistance to foliar blight disease caused by *R. solani*. This resistance is manifested by a significant reduction in the upward progression of the disease, lower number of infected and defoliated leaves, greater root biomass and length, and fewer number of fungal sclerotia produced in transgenic plants than control plants. *Stylosanthes* is a perennial forage legume and the production of significantly fewer fungal sclerotia on transgenic plants than on control plants has important implications on curtailing the disease cycle through reductions of sources of inoculum. Constitutive production of chitinase in transformed tobacco plants has been shown to reduce plant mortality in soil infested with *R. solani* (Broglie et al., 1991; Vierheilig et al., 1993).

The segregation of selfed progeny fitting a ratio of 3:1 (resistant: susceptible) is consistent with a single locus of an active chitinase gene. The level of resistance expressed in most of the segregating resistant progeny (T_1) is comparable or similar to that observed in the transgenic mother plant, although some expressed a much higher resistance. Some of the progeny showing higher levels of resistance than the mother plant have been selected for further seed productions and evaluations of T_2 and T_3 generations.

Several studies have been reported on transgenic plants containing chitinase genes. For example, tobacco and canola transgenic plants expressing bean chitinase gene conferred resistance to *R. solani* (Broglie et al., 1991); rice plants transformed with a rice chitinase gene under the 35S promoter were shown to have resistance to the sheath blight disease caused by *R. solani* (Lin et al., 1995); transgenic cucumber plants containing a rice chitinase gene expressed an enhanced resistance to gray mold disease caused by *Botrytis cinerea* (Tabei et al., 1998); rose plants transformed with a rice chitinase gene had a reduced blackspot disease, caused by the fungus *Diplocarpon rosae*, severity (Marchant et al., 1998). However, our work is the first report on transgenic *S. guanensis* containing a rice chitinase gene for control of *Rhizoctonia* foliar blight disease.

This study indicates that there are benefits associated with the introduction of naturally existing defense related genes, which are automatically triggered in plants upon attack by pathogens or injury by abiotic stresses. The transfer of a gene from rice to a legume forage plant *Stylosanthes* is not possible using traditional breeding methods. Recombinant DNA techniques made this possible thus generating a genetic diversity for disease resistance. As more cloned plant defense genes and effective promoters become available, it is likely that more disease resistant, environmental friendly plants would be created. The challenge ahead would be to educate the general public and the media on the ever-growing debate of transgenics, their potential benefits and possible unknown draw-backs.

2.7.4 Bacterial blight of *Brachiaria* caused by *Xanthomonas campestris* pv. *graminis*: Isolate collection, pathogenicity evaluation and seed transmission

Contributors: C. Zuleta and S. Kelemu (CIAT)

Rationale

A bacterial wilt disease of *Brachiaria*, its casual agent, artificial inoculation methods and bacterial population dynamics in resistant and susceptible genotypes of *Brachiaria* have been described (IP-5 Annual Report, 2000; Zuleta et al. 2001). *Xanthomonas campestris* pv. *graminis* infects a number of cultivated forage grasses. Some of the first symptoms are chlorotic/necrotic stripes along the leaves. As the disease advances, the whole leaf may die. Under severe conditions, the whole plant may turn yellow and die. Another typical symptom is wilting and curling of leaves without any discoloration or lesions, which result in quick plant death. Isolates have been collected from three sites in Colombia to determine pathogenicity and distribution.

Materials and Methods

Infected tissues were collected from various accessions and hybrids of *Brachiaria*. After surface sterilization, these tissues were macerated in sterile distilled water and plated on nutrient agar. Single bacterial colonies were isolated 48 hours later. Bacterial cells originating from individual colonies were used for pathogenicity tests. Inoculation methods were as described by Zuleta et al.(2001). For seed transmission tests, seeds collected from diseased susceptible plants in the field were surface sterilized (in 1% NaOCl solution for 5 minutes, in 70% ethanol for 1 minute, and rinsed three times with sterile distilled water). The samples were transferred to sterile filter papers to remove excess moisture. These seed samples were then divided into two: 1) planted onto magenta vessels (Sigma) containing plant growing medium (MS), 9 seeds/vessel. These were maintained in a growth camber with 14 hours light period at 28 C; 2) plated on nutrient agar and incubated at 28°C.

Results and Discussion

Eighty seven percent of the isolates collected from Popayán and Santander de Quilichao were pathogenic on the most susceptible *Brachiaria* CIAT 36062. Disease symptoms typically appeared 15 days after inoculations. The disease was more prevalent than the previous years. Approximately 10 % of the seeds germinated, all of which developed bacterial wilt-like symptoms. Of these, we were able to isolate the wilt-causing causing bacterium from only 4% of the plants. These results indicate that the bacterium is seed transmitted. Approximately 7% of the seeds plated on nutrient agar produced colonies of the casual agent (Photos 11, 12) which were pathogenic on the susceptible *Brachiaria* CIAT 36062. In addition to seed transmission, the disease is transmitted via vegetative propagation and tools used for cutting.

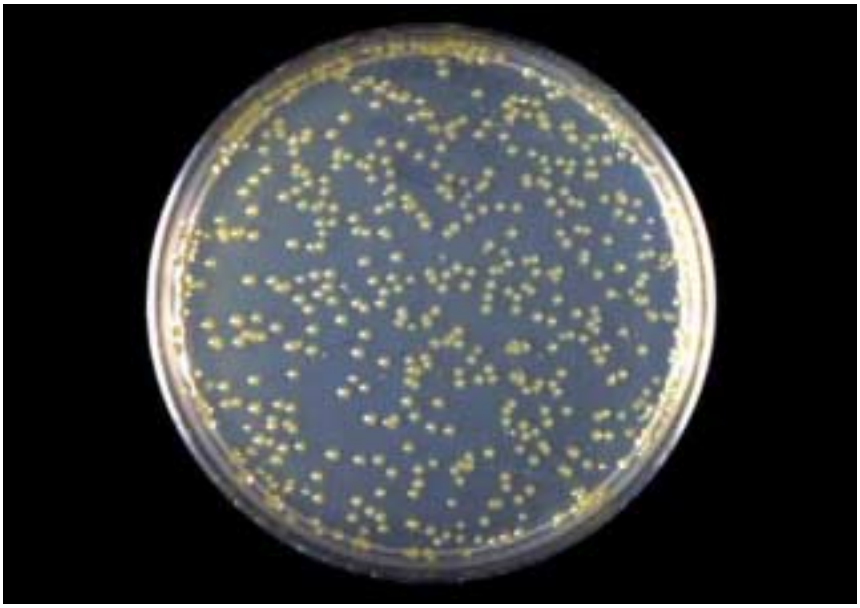

Photo 11. Bacterial colonies on nutrient agar

Photo 12. Symptoms of bacterial wilt disease at its initial (left) and later (right) stages after inoculations

2. 8 Antifungal compounds isolated from seeds of tropical forage legumes

2.8.1 Screening of tropical forage legume collection for antifungal properties

Contributors: W. Kunxin (South China University of Tropical Agriculture, The People's Republic of China), G. Segura and S. Kelemu (CIAT)

Highlights

- Seeds of several tropical forage legumes contained compounds with anti-fungal properties.

Progress towards achieving milestones

- **Detected antifungal compounds in seeds of tropical forage legumes**
  We have examined seeds of 239 different tropical forage species for anti-fungal properties. It was demonstrated that using the methods we applied, some of these clearly contained compounds with properties which inhibit fungal growth.

Rationale

Plants have developed a variety of defense systems to protect themselves from potential pathogens. Plants produce various anti-microbial compounds. Some are constitutive while others are induced upon pathogen infection. Anti-microbial proteins and peptides have been realized to play important roles in plant defense systems and have been detected in a wide range of plants and plant tissues.

Seed germination is probably one of the most vulnerable periods for pathogen attack. Many anti-microbial proteins or peptides have been identified in seeds, such as plant defensins (Osborn *et al.*, 1995, FEBS Lett.368: 257-262; Terras *et al.*, 1992, J. Biol. Chem. 267:15301-15309), proteinase inhibitors (Joshi *et al.*, 1998, Biochem. Biophys. Res. Comm. 246:382-387), chitin-binding proteins (Broekaert *et al.*, 1992, Biochemistry 31:4308-4314), lipid-transfer proteins, knottin-type peptides, and several 4-cysteine-type peptides (reviewed by Broekaert *et al.*, 1997, Crit. Rev. In Plant Sci. 16:297-323), chitinases (Schumbaum *et al*., 1986, Nature 324: 365-367). These anti-microbial proteins or peptides showed potent anti-microbial activity *in vitro*. The expression of certain anti-microbial peptides is induced both locally and systemically by pathogen attack. The expression of an antifungal protein (defensin) in transgenic potato plants has been shown to provide resistance to the fungal pathogen *Verticillium dahliae* in the greenhouse and in the field (Gao *et al*, 2001, Nature Biotechnology 18:1307-1310).

Materials and Methods

Rhizoctonia solani CIAT 5596 was used as a test fungus. Seeds were kindly provided by the germplasm bank at CIAT.

Intact seeds were surface sterilized as follows: 4 min in 70% ethanol, 15 min in 2.5% NaOCl solution, and 6 washes of 5 min each in sterile distilled water.

An incision was made with a scalpel in the seed coat. The incised seeds were soaked overnight in sterile distilled water (10 ml dH_2O per gram seeds) at 4°C. The seeds/dH_2O were ground with mortar and pestle. The solution was filtered through 4 layers of cheese cloth to remove plant debris. The filtrate was centrifuged at 15,000 rpm for 20 min. The suspension was sterilized with a 0.2-µm pore-size nylon membranes (Sigma). Ten-ml filter-sterilized suspension was concentrated by lyophilization. The dry material was re- dissolved in 1 ml sterilized deionized water and used to test its anti-fungal properties.

R. solani inhibition assays were conducted by placing an agar disc containing fungal mycelium at the center of a potato dextrose agar (Difico) containing plate (diameter 9 cm). The plate was then incubated at 28 °C for 1 day, after which two autoclaved 1.5 cm diameter filter disks were placed at opposite ends of the plate. One of the disks was soaked with 400-µl seed extract while the second one was treated with sterile deionized water as control. The plate was re-incubated at 28 °C for 2 days before measurements of mycelial expansion were taken.

Seed extracts (200 µl) of each plant species which showed anti-fungal activity were digested with pronase E (Sigma, P-6911) in reaction buffer (10 mM NaCL, 10mM Tris HCL, PH 7.5) by incubating at 37 °C for 16 h. The suspension was used to test whether the anti-fungal activity was lost.

Results

About 239 forage species were examined, and Table 58 shows those with some anti-fungal properties. Anti-fungal substances which can inhibit the hyphal growth of *R. solani* were detected, but treatment of samples with pronase E did not destroy the anti-fungal properties.

Table 58. List of tropical forage species seeds with anti-fungal properties.

Species	Measurement of zone of inhibition (mm) (two plates average value)	
	With pronase E	Without pronase E
Acacia glauca	4.6*	5.0
Calliandra calothyrsus	9.0	10.0
Calliandra houstoniana	6.5	7.0
Calliandra magdalenae	5.5	6.0
Antifungal protein (control)	2.0	12.5
Leucaena diversifolia	7.0	7.5
Leucaena diversxleucoc	6.3	6.8
Leucaena esculenta	5.5	6.0
Leucaena lanceolata	7.0	6.8
Leucaena leucocephala	6.6	7.0
Leucaena leucocxpulver	6.4	7.0
Leucaena macrophylla	5.7	6.2
Leucaena pallida	6.5	7.0
Leucaena pulverulenta	5.8	6.2
Leucaena shannonii	7.0	6.5
Leucaena retusa	6.8	6.4
Leucaena trichodes	6.6	7.0
Tephrosia africa	5.5	5.0
Tephrosia cinerea	6.4	7.0
Tephrosia pescador	5.5	6.0
Tephrosia polystachya	7.0	6.0
Tephrosia sinapou	6.0	5.5
Tephrosia vogelii	5	5.4
H_2O	0	0

*Values are the average of data from two plates

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 edaphic and climatic factors

Highlights

- Found that two sexual *Brachiaria* hybrids (SX 2349 and SX 497) had greater level of Al resistance than sexual parent (BRUZ/44-02).
- Showed that the superior performance of the *Brachiaria* hybrid (FM9503-S046-024) after establishment was associated with its ability to acquire greater amounts of nutrients from low fertility soil.
- Showed that *B. humidicola* suppresses nitrification and nitrous oxide emission by inhibiting the activity of ammonium oxidizing bacteria in the soil.
- Using phosphorus isotope exchange kinetics technique, found that *Brachiaria decumbens* CIAT 606 acquires P from less available P forms in an oxisol.
- Field evaluation over 3 years showed that *Arachis pintoi* CIAT 22159 may be better than the commercial cultivar (CIAT 17434) in terms of persistence with no P fertilizer input.

Progress towards achieving milestones

- **New *Brachiaria* sexual hybrids with Al resistance identified**
  We were successful in identifying 2 sexual hybrids (SX 2349 and SX 497) with greater level of Al resistance than that of the sexual parent, BRUZ/44-02. These sexual hybrids are currently being used in the on-going breeding program to combine spittlebug resistance with high level of Al resistance.

- ***Brachiaria* hybrids with superior performance under low soil fertility identified**
  We selected a *Brachiaria* hybrid, (FM9503-S046-024) for its excellent adaptation to low fertility soils, which was associated with its ability to acquire greater amounts of nutrients from the soil as compared to other hybrids. Using phosphorus isotope exchange kinetics technique in collaboration with scientists from ETH, Zurich Switzerland, we showed that *Brachiaria decumbens* CIAT 606 acquires P from less available P forms from low P oxisol. Further research work is needed to evaluate the ability of most promising *Brachiaria* hybrids for this unique ability to acquire less available P forms from low fertility oxisols.

- **Ability to suppress nitrification and emission of nitrous oxide by *Brachiaria humidicola* quantified**
  Collaborative research with JIRCAS scientists from Japan showed that *B. humidicola* suppresses nitrification and nitrous oxide emission by inhibiting the activity of ammonium oxidizing bacteria in the soil. Further research work is in progress to determine genotypic variation in this ability of *B. humidicola*.

- **Field experiment with promising accessions of *Arachis pintoi* established in the Llanos**
  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) are being evaluated in relatively better soils of the Llanos of Colombia (Piedmont region) for their suitability as cover or forage legumes.

Low supply of nutrients and aluminum (Al) toxicity are major limitations to tropical forage production on acid soils. Previous research indicated that 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 environment. Identification of those key plant attributes is fundamental to develop more efficient screening procedures for germplasm evaluation and/or improvement.

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

Contributors: I. M. Rao, J. W. Miles, P. Wenzl, R. García and J. Ricaurte (CIAT)

The first criterion for selection in the tetraploid, sexual *Brachiaria* breeding population is performance in field trials on acid, Al-toxic soils at CIAT-Quilichao and the Matazul farm in Puerto López (Llanos Orientales). Over 4,300 progeny plants were established during 2001 in duplicate, space-planted field trials. Plants are being culled on visual assessment of vigor and freedom of obvious deficiency symptoms as well as other attributes. A manageable set of fewer than 1000 clones will be identified by 01 December 2001 and propagated for in vitro assessment of Al tolerance (and other attributes) during first semester of 2002, to identify a small set of parental sexual clones to be intercrossed in isolation to produce the subsequent cycle population.

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

3.1.2.1 Identification of Al-resistant *Brachiaria* hybrids

Contributors: I.M. Rao, J. W. Miles, P. Wenzl, R. García and J. Ricaurte (CIAT)

Rationale

Last year, we implemented screening procedure to identify Al-resistant *Brachiaria* hybrids that were preselected for spittlebug resistance. As part of the restricted core project funded by BMZ-GTZ of Germany, this year we used this screening method to identify most promising sexual *Brachiaria* hybrids that combine Al resistance with spittlebug resistance.

Materials and Methods

A total of 46 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 43 hybrids selected for screening, 41 were sexuals and two hybrids, CIAT 36061 and CIAT 36062 were apomicts. 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 total root length and root biomass were measured after 21 days of Al treatment.

Results and Discussion

As observed before, results on total root length indicate that the parent *B. decumbens* CIAT 606 is outstanding in its level of Al resistance (Figure 29). Among the 43 hybrids tested, 2 apomictic hybrids (CIAT 36061, CIAT 36062) and 2 sexual hybrids (SX 2349 and SX 497) showed moderate level of Al

resistance (Figure 29). The level of Al resistance of these two sexuals was markedly superior to that of the sexual parent, BRUZ/44-02. Among the hybrids, CIAT 36061 showed greater level of Al resistance than that of the other hybrids. The relationship between root length with no Al and root length with high Al showed that the extent of total root length of the apomictic *Brachiaria* hybrid cv. Mulato (CIAT 36061) was similar to that of the outstanding parent *B. decumbens* CIAT 606 with no Al in solution (Figure 30). But in the presence of high level of Al in solution this hybrid did not perform as well as CIAT 606 but markedly superior to the rest of the hybrids. The hybrids that were identified in the upper box of the right hand side (Figure 30) are being utilized in the on-going breeding program.

The root apex (root cap, meristem, and elongation zone) accumulates more Al and attracts greater physical damage than the mature root tissues. Scanning electron microscopic observations revealed differences in Al toxicity effects on root tips of two parents and one hybrid (Photo 13). The extent of deformation caused by Al toxicity was minimum with *B. decumbens* and was marked with *B. ruziziensis*. The hybrid, CIAT 36061 showed relatively less deformation of root tips compared with *B. ruziziensis*.

Conclusions

We have identified 2 sexual hybrids (SX 2349 and SX 497) with greater level of Al resistance than that of the sexual parent, BRUZ/44-02. We are also in the process of evaluating a hybrid population of *B. decumbens* x *B. ruziziensis.* This will enable us to develop molecular markers for Al resistance in *Brachiaria*.

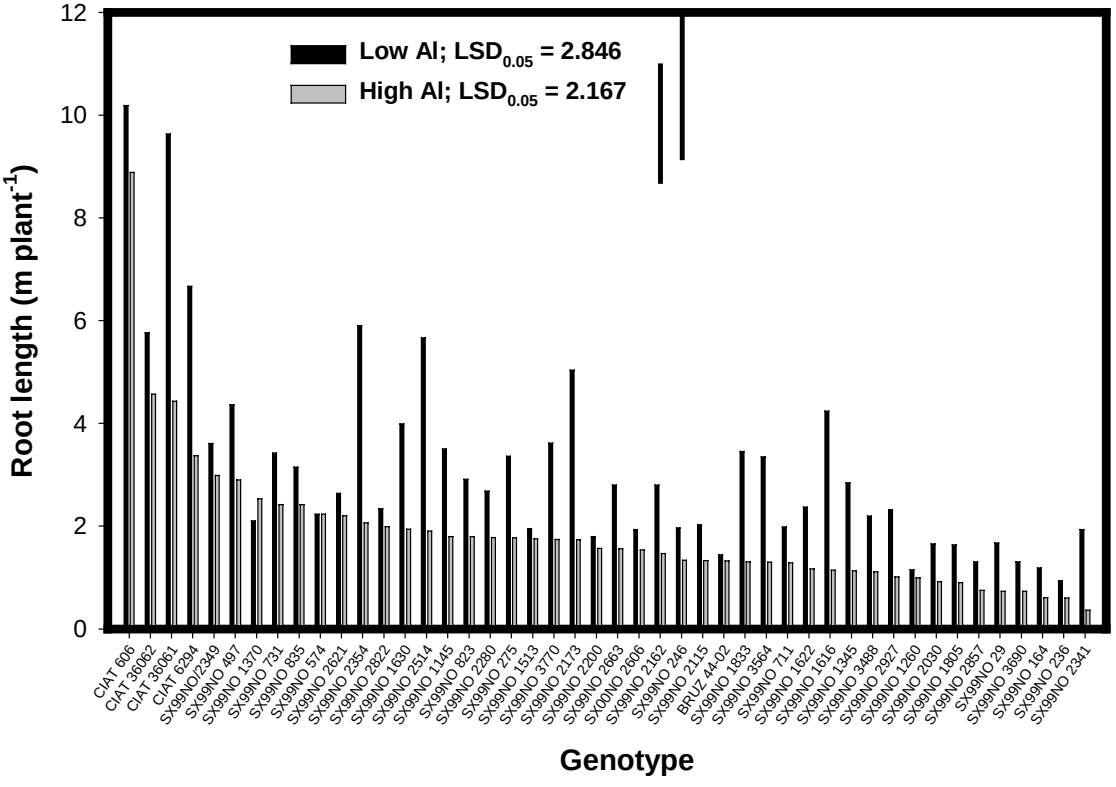

Figure 29. Screening for Al resistance among 46 genotypes of *Brachiaria*. Total root length was measured after exposure to 0 or 200 µM $AlCl_3$ with 200 µM $CaCl_2$ (pH 4.2) for 21 days.

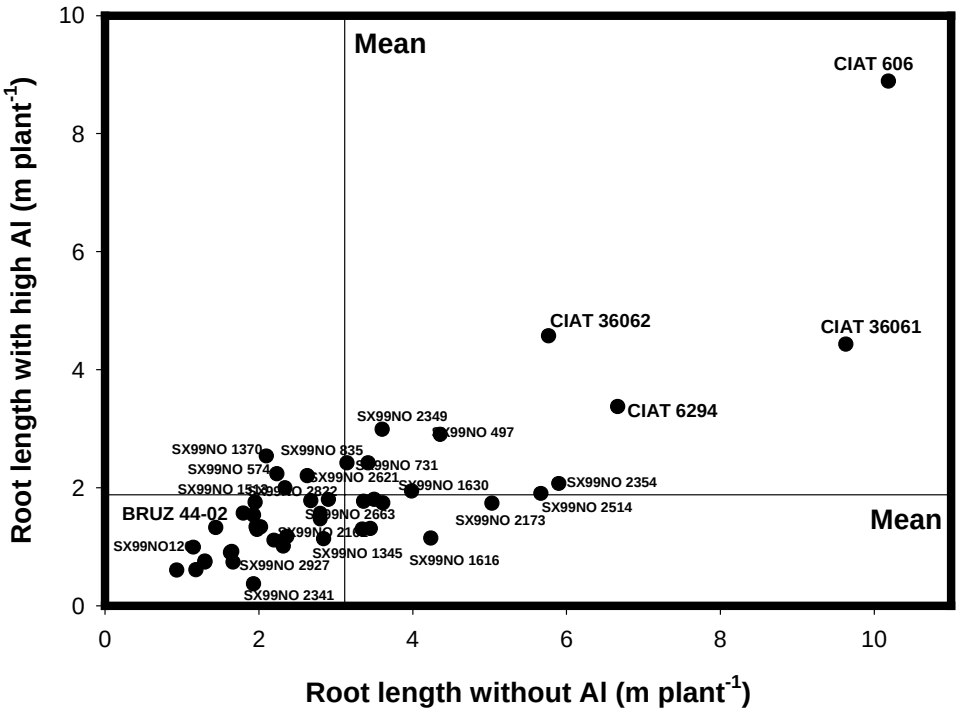

Figure 30. Identification of Al resistant *Brachiaria* hybrids. Hybrids that were superior in root length with no or high Al in solution were identified in the upper box of the right hand side. Total root length was measured after exposure to 0 or 200 µM $AlCl_3$ with 200 µM $CaCl_2$ (pH 4.2) for 21 days.

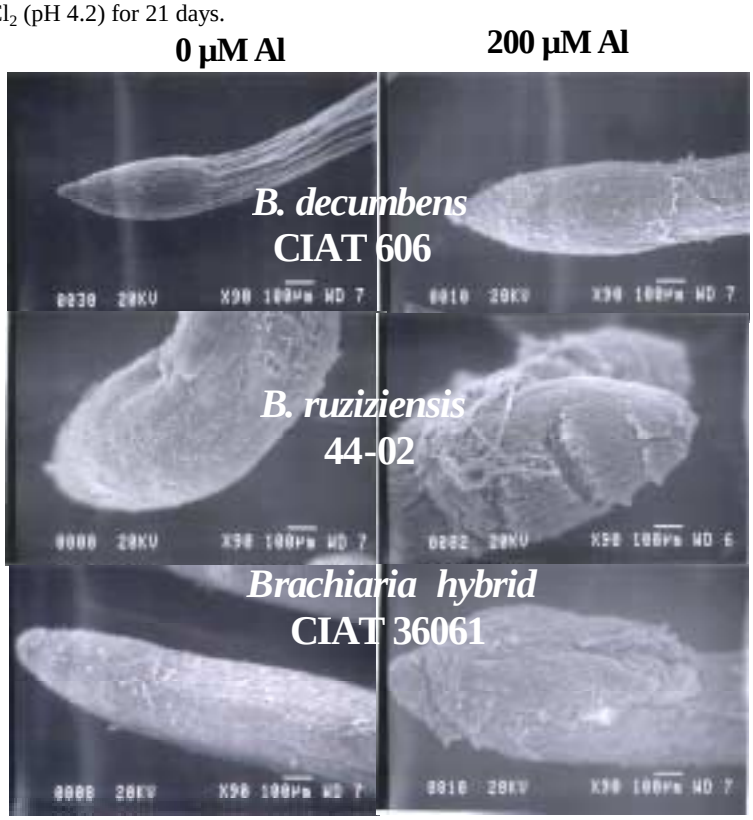

Photo 13. Scanning electron micrographs of root tips of two parents (*B. decumbens* and *B. ruziziensis*) and one hybrid (CIAT 36061) of *Brachiaria* exposed to either 0 or 200 µM of $AlCl_3$ for 21 days.

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

Contributors: I.M. Rao, J. W. Miles, C. Plazas, J. Racaurte and R. García (CIAT)

Rationale

A field study was conducted at Matazul Farm in the Llanos of Colombia. The main objective was to identify genetic recombinants of *Brachiaria* with tolerance to low nutrient supply and evaluate plant attributes that contribute to superior adaptation.

Materials and 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 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 wet season (October 2000).

Results and Discussion

Initial application of high amounts of fertilizer did not improve forage yield of most of the genotypes compared with low fertilizer application (Table 59). This indicates that the initial application of high amounts of N and K fertilizer at the time of establishment had very little residual effects into the second year. At 15 months after establishment, live forage yield with low fertilizer application ranged from 0.27 to 2.39 t/ha and the high values of forage yield were observed with two germplasm accessions (CIAT 26110 and CIAT 26318) and one spittlebug resistant genetic recombinant, FM9503-S046-024 (Table 59). As expected, the performance of one of the parents, BRUZ/44-02 was very poor compared with other parents and genetic recombinants.

One of the genetic recombinants, cv. Mulato (CIAT 36061), had more dead biomass with both levels of fertilizer application (Table 59). The gretest amount of total forage yield was obtained with one of the germplasm accessions, CIAT 26318 with both levels of fertilizer application. Two recombinants, cv. Mulato (CIAT 36061) and FM9503-S046-024 showed greater amount of dead biomass similar to one of the parents, CIAT 6294 with both levels of fertilizer application. These two recombinants were also superior in production of greater amount of green leaf and stem biomass (Table 60). Results on leaf and stem N content indicated that BRUZ/44-02 had greater amount of N per unit dry weight but its ability to acquire N (shoot N uptake) was lowest compared with other parents and genetic recombinants (Table 61). Shoot N uptake with low fertilizer application was greater for two accessions (CIAT 26110 and 26318), one parent (CIAT 6294) and one genetic recombinant (FM9503-S046-024). This genetic recombinant was also outstanding in its ability to acquire greater amounts of P, K, Ca and Mg from low fertilizer application when compared with parents, accessions and other genetic recombinants (Tables 62 and 63). Among the parents, *B. brizantha* CIAT 6294 was superior in P, K, Ca and Mg acquisition from low fertilizer application. It is important to note that live forage yield was associated with lower contents of not only essential nutrients but also Al in stems with both low and high fertilizer application. (Table 64). Live forage yield with low fertilizer application showed a significant negative relationship (-0.45**) with stem N content. This observation indicates that genotypes that are efficient in uitilization of N for the production of green forage is an important mechanism for superior performance with low fertilizer application.

Table 59. 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 15 months after establishment (October 2000) 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
	(kg/ha)					
Recombinants:						
BR97NO-0082	793	1125	385	427	1178	1552
BR97NO-0383	934	1376	375	516	1308	1892
BR97NO-0405	1230	1061	518	537	1748	1598
cv. Mulato (CIAT 36061)	1419	1824	1378	1650	2797	3474
CIAT 36062	1145	1355	415	761	1560	2116
FM9503-5046-024	2082	1712	1429	814	3511	2527
Parents:						
CIAT 606	907	1204	361	215	1267	1419
CIAT 6294	2022	2429	1030	1580	3052	4010
BRUZ/44-02	274	268	244	212	518	480
CIAT 26646	1194	1854	865	709	2060	2563
Accessions:						
CIAT 26110	2390	2231	1364	777	3755	3007
CIAT 26318	2379	2568	1618	1287	3996	3856
Mean	1397	1584	832	791	2229	2374
LSD *(P=0.05)*	800	812	1138	1195	1559	1745

Table 60. 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 15 months after establishment (October 2000). LSD values are at the 0.05 probability level.

Genotype	Leaf biomass		Stem biomass	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	(kg/ha)			
Recombinants:				
BR97NO-0082	578	741	215	384
BR97NO-0383	476	485	457	890
BR97NO-0405	629	523	601	538
cv. Mulato (CIAT 36061)	785	1014	634	810
CIAT 36062	808	1013	337	342
FM9503-5046-024	1356	1036	726	676
Parents:				
CIAT 606	571	779	335	425
CIAT 6294	1287	1184	735	1245
BRUZ/44-02	125	172	149	96
CIAT 26646	833	1185	361	668
Accessions:				
CIAT 26110	1371	1486	1019	744
CIAT 26318	1430	1412	948	1156
Mean	854	919	543	136
LSD *(P=0.05)*	440	428	415	522

Table 61. 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 15 months after establishment (October 2000). 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	0.970	0.920	0.83	0.61	7.32	9.35
BR97NO-0383	1.050	0.960	0.91	0.62	8.75	9.61
BR97NO-0405	0.990	0.990	0.88	0.63	11.15	8.14
cv. Mulato (CIAT 36061)	0.740	0.830	0.86	0.56	10.88	13.06
CIAT 36062	0.880	0.910	0.65	0.53	9.08	11.06
FM9503-5046-024	0.980	0.900	0.90	0.54	19.86	12.53
Parents:						
CIAT 606	0.850	0.840	0.80	0.47	7.37	8.58
CIAT 6294	0.890	0.750	0.67	0.65	16.50	16.54
BRUZ/44-02	1.290	1.210	1.08	0.80	2.98	3.49
CIAT 26646	0.860	0.770	0.69	0.56	9.50	12.96
Accessions:						
CIAT 26110	0.900	0.720	0.40	0.51	16.41	13.75
CIAT 26318	0.770	0.700	0.65	0.48	16.99	14.98
Mean	0.930	0.870	0.76	0.58	11.40	11.33
LSD *(P=0.05)*	0.188	0.164	0.32	0.22	6.81	4.74

NS = not significant.

Table 62. 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 15 months after establishment (October 2000). 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	0.154	0.141	0.127	0.075	1.11	1.34
BR97NO-0383	0.149	0.160	0.088	0.072	1.08	1.30
BR97NO-0405	0.146	0.166	0.119	0.079	1.55	1.31
cv. Mulato (CIAT 36061)	0.153	0.149	0.118	0.068	2.04	1.98
CIAT 36062	0.169	0.190	0.110	0.074	1.73	2.20
FM9503-5046-024	0.179	0.155	0.118	0.076	3.12	2.07
Parents:						
CIAT 606	0.162	0.147	0.180	0.106	1.49	1.57
CIAT 6294	0.153	0.151	0.112	0.086	2.75	2.84
BRUZ/44-02	0.193	0.166	0.110	0.087	0.42	0.45
CIAT 26646	0.142	0.129	0.117	0.067	1.56	2.00
Accessions:						
CIAT 26110	0.126	0.132	0.060	0.098	2.33	2.42
CIAT 26318	0.126	0.135	0.101	0.075	2.69	2.64
Mean	0.154	0.151	0.113	0.080	1.82	1.87
LSD (P=0.05)	0.049	0.041	0.050	NS	1.06	0.78

NS = not significant.

Table 63. 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 15 months after establishment (October 2000). 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	9.67	11.87	3.23	5.40	3.19	5.51
BR97NO-0383	11.97	9.49	2.79	4.64	3.06	4.71
BR97NO-0405	15.59	11.25	3.72	3.45	4.00	4.02
cv. Mulato (CIAT 36061)	15.27	21.76	4.96	6.08	5.93	6.41
CIAT 36062	16.59	13.73	4.69	6.31	6.13	8.45
FM9503-5046-024	27.52	18.32	9.48	7.07	10.27	9.07
Parents:						
CIAT 606	13.34	15.13	4.00	4.34	4.96	6.23
CIAT 6294	24.98	21.09	7.04	7.01	8.65	10.66
BRUZ/44-02	3.07	3.12	0.98	1.39	1.07	1.60
CIAT 26646	14.94	18.14	3.42	5.12	4.72	8.64
Accessions:						
CIAT 26110	23.11	19.92	6.40	7.06	7.35	11.24
CIAT 26318	28.61	18.65	6.65	7.10	9.25	12.06
Mean	17.05	15.46	4.78	5.50	5.71	7.51
LSD *(P=0.05)*	11.46	5.80	2.90	2.89	3.13	4.11

Table 64. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low or high intial fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer	High fertilizer
Total (live + dead) shoot biomass (t/ha)	0.88***	0.89***
Dead shoot biomass (t/ha)	0.56***	0.59***
Leaf biomass (t/ha)	0.96***	0.88***
Stem biomass (t/ha)	0.93***	0.87***
Leaf N content (%)	-0.31*	-0.56***
Leaf P content (%)	-0.31*	-0.27
Leaf K content (%)	-0.14	-0.31*
Leaf Ca content (%)	-0.16	-0.44**
Leaf Mg content (%)	-0.10	-0.02
Leaf Al content (%)	-0.21	-0.37*
Stem N content (%)	-0.45**	-0.43**
Stem P content (%)	-0.34*	-0.33*
Stem K content (%)	-0.13	-0.17
Stem Ca content (%)	-0.33*	-0.46**
Stem Mg content (%)	-0.28	-0.30*
Stem Al content (%)	-0.30*	-0.23

Conclusions

Results from this field study indicated that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024 at 15 months after establishment was associated with its ability to acquire greater amounts of nutrients from low fertility soil.

3.1.2.3 Field evaluation of promising hybrids of *Brachiaria* in the Llanos of Colombia

Contributors: I. M. Rao, J. W. Miles, C. Plazas and J. Ricaurte (CIAT)

Rationale

Based on the data collected from greenhouse and field screening of a large number of *Brachiaria* hybrids, we selected 4 hybrids for further field testing to evaluate persistence with low nutrient supply in soil at Matazul farm of the altillanura.

Materials and Methods

A field trial was established at Matazul farm on 31 May this year. The trial included 4 *Brachiaria* hybrids (1251; 4015; 4132; 4624) along with 2 parents (*B. decumbens* CIAT 606 and *B. brizantha* CIAT 6294). 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 with 3 replications. The plot size was 5 x 2 m.

Results

A number of plant attributes including forage yield, dry matter distribution and nutrient uptake are being monitored.

3.1.2.4 Screening accessions of *Brachiaria humidicola* for suppression of nitrification and nitrous oxide emission from soil

Contributors: T. Ishikawa (JIRCAS, JAPAN), and I.M. Rao (CIAT)

Rationale

Ammonium-N is transformed into nitrite-N and nitrate-N by soil microorganisms (Figure 31), a process known as nitrification. Nitrification leads to substantial losses of applied fertilizer N through nitrous oxide (N_2O) emission and runoff/leaching losses of nitrate from agricultural production systems. This is often associated with nitrate pollution of ground water and aquatic bodies. Nearly 50 to 70% of the applied fertilizer N is lost because of nitrification, causing enormous environmental pollution problems and also inefficiency in N utilization. Nitrous oxide, one of the greenhouse gasses, is emitted from the soil because of nitrification. Preliminary estimations indicate that N_2O emissions from the fertilizer N range from 7.3% of applied N for field crops such as maize to 12.0% of applied N for grasslands. By controlling nitrification in soils, it will be possible in future to reduce N fertilizer inputs into agricultural production systems and also minimize nitrate pollution in aquatic systems and ground water.

We found that a tropical grass, *Brachiaria humidicola* that is widely adapted to lowland agroecosystems (savannas) of humid and subhumid tropics, particularly in South America has the ability to suppress nitrification in soil and emission of N_2O to the atmosphere.

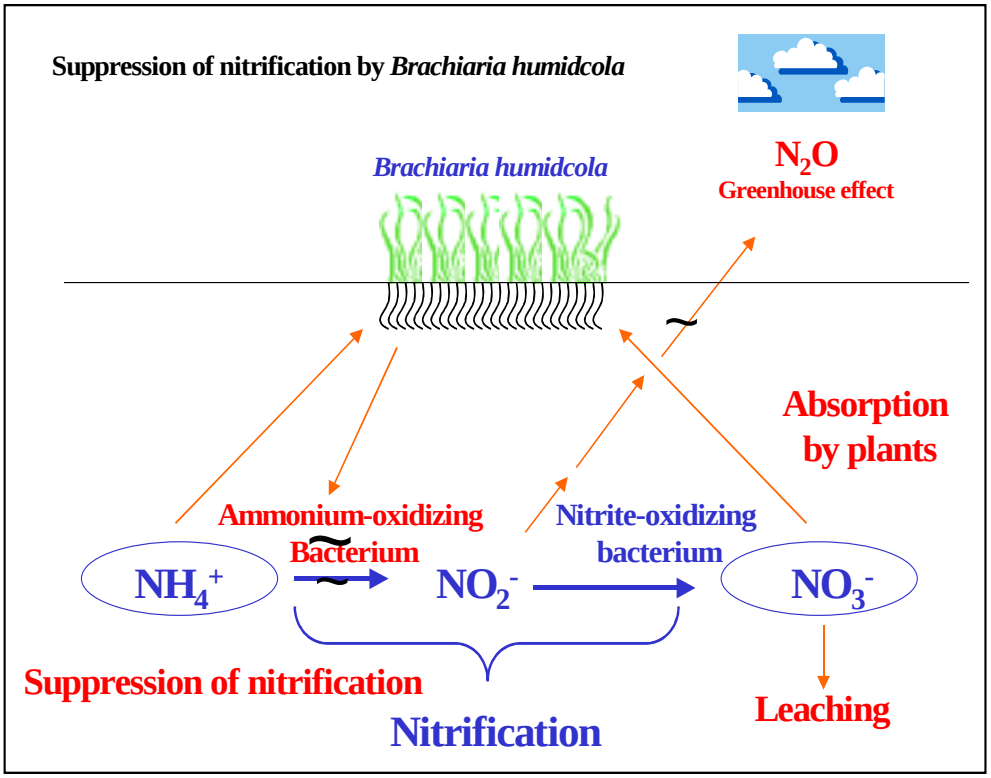

Figure 31. Mechanism of nitrification and nitrification suppression by *Brachiaria humidicola*

Materials and Methods

Three tropical grasses, *Brachiaria decumbens*, *Brachiaria humidicola* and *Melinis minutiflora* were grown in Wagner pots filled with Typic Hapludands [pH (H_2O) 6.0 and C/N ratio 11.68)]. Plants were grown in growth chamber with day/night temperature regimes of 30 ºC and 28 ºC, respectively with a 14 h photoperiod. Six weeks after sowing (WAS), 1.422 g of ammonium-nitrogen was supplied as ammonium sulfate. At 8 weeks after sowing, plants were separated from the soil. Root exudates were collected from these plants by keeping the plants in distilled water (1L pot^{-1}) for 24 h, plants were then harvested and dried for dry weight measurements. Soils were sampled for chemical analysis and for the initiation of nitrification study that followed. The effect of root exudates on the multiplication of ammonium oxidizing bacteria (AOB) was measured by MPN (most probable number) method.

Nitrification Study: Nitrification experiment was initiated to test the residual effect of the three grasses on soil nitrification during a 24 day period. Ammonium-N was applied (1.422 g NH_4 SO_4) to each of the sampled soil (2.3 kg pot^{-1}) and incubated for 24 days. Soil water content was maintained at 50% water holding capacity during this period (i.e. 24 day period after the harvest of plants at 8 WAS). Soil extract was made and its effect on multiplication of AOB was measured by MPN method. Soil samples were collected at various intervals and analyzed for NO_3 and NH_4 forms of nitrogen. Nitrous oxide emission from sampled soil was monitored by collecting the air samples at periodic intervals and N_2O levels were analyzed by gas chromatography.

Results and Discussion

Ammonia oxidizing bacteria (AOB) were nearly 10 times higher in soils where *B. decumbens* and *Melinis minutiflora* were grown at 8 WAS compared to soils where *B. humidocola* was grown (10,000 *vs* 1000 g^{-1} dry soil). Residual effect of *B. humidicola* on suppression of AOB lasted for about 12 days after the plants were harvested, but subsequently the AOB began to increase and reached levels similar to *B. decumbans* and *M. minutiflora* treatments at 24 days after plants were harvested (results not shown). For nitrite-oxidizing bacteria, however there were no significant differences among *B. decumbans*, *B. humidicola* and *M. minutiflora* treatments (results not shown).

Results on ammonium-N in soils were presented as the percentage of initial amount of ammonium-N applied (Figure 32). Nearly 50% of the ammonium-N was lost by 12 days after the initiation of nitrification treatment in soils where *B. decumbens* and *M. minutiflora* were grown (Figure 2). However, in soils where *B. humidicola* was grown, there was no significant change in ammonium-N levels up to 12 days but subsequently declined.

By 24 days after the nitrification study was initiated, NH_4-N in soils of *B. humidicola* treatment was similar to that of *B. decumbens* and *M. minutiflora*. Most of the applied ammonium-N was converted into nitrate-N or was lost as N_2O in all the three treatments by 24 days after the plants were harvested. Thus, the residual effect of *B. humidicola* on AOB has lasted only for about 12 days after the plants were harvested.

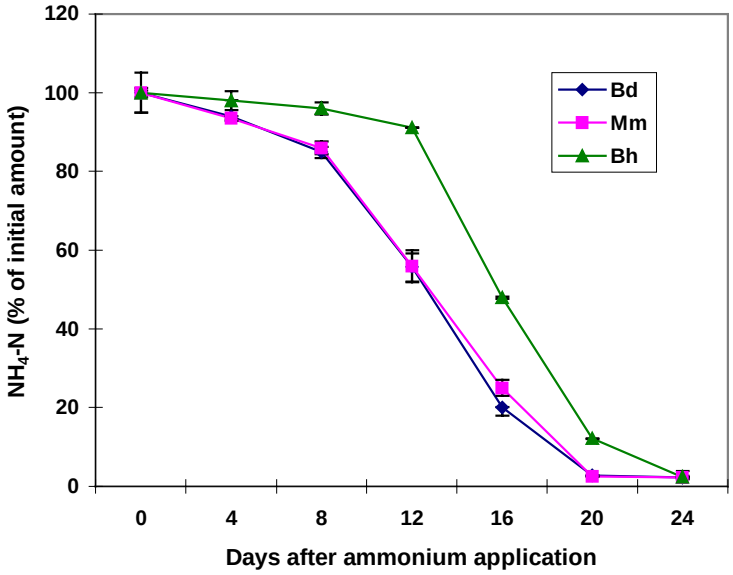

Figure 32. Percent of intial amount of ammonium-N in soil in relation to days after ammonium application

Nitrous oxide emission during the nitrification study was substantially higher for *B. decumbans* and *M. minutiflora* (31.0 and 29.3 µg-N m^{-2} hr^{-1}) compared to *B. humidicola* (5.0 µg-N m^{-2} hr^{-1}) treatment (Figure 33). For *B. decumbans*, and *M. minutiflora* treatments, N_2O emission reached the highest levels between 8 and 12 days after the initiation of nitrification study, which is similar to control pots (i.e. no plants). Soil

extracts and root exudates of *B. humidicola* treatment suppressed AOB, whereas no such effect was observed for *B. decumbans* or *M. minutiflora* treatments (results not shown).

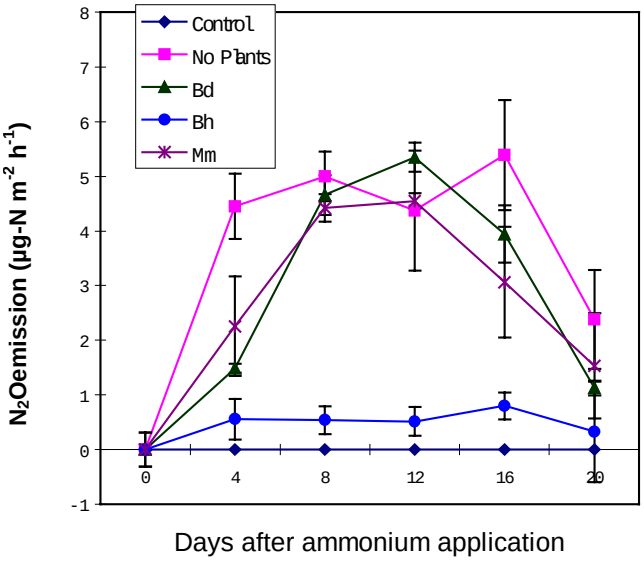

Figure 33. Nitrous oxide emission from soil in relation to days after application of ammonium. Control pots received no ammonium application while pots with no plants received ammonium application.

Conclusions

Our results strongly support the notion that *B. humidicola* has the ability to suppress nitrification by inhibiting the biological activity of ammonium oxidizing bacteria (AOB) in the soil. This was demonstrated by substantial decrease in AOB populations in soils where *B. humidicola* was grown. Also, nitrous oxide emissions, which is an indicator of the AOB biological activity was very low for *B. humidicola* treatment. Our results support the hypothesis that *B. humidicola* suppress nitrification and N_2O emission by inhibiting the activity of AOB in the soil. This may be achieved by secreting organic compounds from the roots that have the inhibitory effect on these AOB bacteria. Also, we have demonstrated that the residual effect of this tropical grass on nitrification of the soil will be about 24 days.

The Genetic Resources Unit of CIAT has a germplasm collection of about 62 accessions of *Brachiaria humidicola*. We plan to evaluate these germplasm accessions of *B. humidicola* in order to identify genotypic differences in their ability to suppress nitrification. This work is expected to contribute toward identification of germplasm accessions with greater ability to inhibit nitrification process in soil. The selected accessions could then be used in breeding programs for developing genetic stocks that combine greater forage production potential with high levels of nitrification inhibition capacity.

Currently we are also working on identification of the organic compound/s that are responsible for this unique property of nitrification inhibition in the root exudates of *Brachiaria humidicola*. Also, efforts are underway to understand the mechanisms of nitrification inhibition in these root exudates of *B. humidicola.*

3.1.3 Differences in phosphorus acquisition from less available phosphorus forms in an oxisol as determined by isotope exchange kinetics

Contributors: S. Buehler, A. Oberson, E. Frossard (ETH, Zurich, Switzerland), D. Friesen (IFDC-CIMMYT, Nairobi, Kenya) and I. M. Rao (CIAT)

Rationale

The ability to grow on low P soils differs widely between plant species and even among varieties. These differences have been attributed to several strategies for optimizing P uptake or P use efficiency. The strategies for P uptake improvement include root system morphology, root hair density, symbiosis with mycorrhizal fungi and modification of the rhizosphere by root exudates or phosphatases to access P forms of low availability. Previous research showed that *Arachis pintoi* accesses sparingly soluble P sources by high response to fertilization with Al-phosphate, Ca-phosphate or organic phosphate (phytic acid). *Brachiaria* species are reported to be well adapted to low P acid soils with the variety *Brachiaria decumbens* CIAT 606 being planted on over 40 million ha of low P acid soils in Latin America.

Adaptation of *Brachiaria* species to low-P soils is mainly attributed to the extensive fine root system, mycorrhizal association, and lower internal requirement of P for plant growth together with enhanced secretion of phytase under low P conditions (e.g., *Brachiaria decumbens*). Infertility of acid tropical soils is caused by multiple stress factors, among others Al-toxicity. *Brachiaria decumbens* also tolerates high Al-concentrations in the soil solution, possibly due to intracellular complexation of Al-ions with organic acids. Plants with special P uptake mechanisms may contribute to more efficient soil P use or could increase the recovery of applied fertilizer if they take up P that is normally not available to other plants. This might reduce the need of high P fertilizer inputs, although on the long-term the use of germplasm with special P uptake mechanisms would lead to soil P mining. A strategy to contribute to agricultural sustainability would be to minimize the P fertilizer requirement needed to produce an economic return through the use of more efficient crop and forage germplasm.

Differences between plants in the accessed P forms can be detected by growing plants on soils labeled with radioactive P isotopes and comparison of their isotopic composition. The **L** value is derived from the isotopic composition of the P taken up by the plant and expresses the total amount of soil P, which is potentially available to the plant. This amount can be compared to the E value of the same soil, which is based on the isotopic composition, measured or extrapolated, in the solution of a labeled soil suspension after a defined time of isotopic exchange. It was shown for a large range of soils, L values determined with common bentgrass (*Agrostis capillaris*) and E values calculated for the same time of isotopic exchange are not significantly different, and therefore *Agrostis capillaries* takes up only isotopically exchangeable P. In contrast, significant differences between E values and L values on the same soil for the same time of isotopic exchange are interpreted by plant P uptake from non-exchangeable P pools. Different L values of different plants on the same soil indicate that these plants do access P pools with different isotopic exchangeability. Higher L values were attributed to special P uptake mechanisms, especially exudation of organic acids, as citric acid in the case of lupine or piscidic, malonic or oxalic acid in the case of pigeon pea.

Seed P is an important factor affecting the isotopic composition of the P taken up by the plant and with this L values, especially under P-limitation and with little plant P uptake. Adding carrier-P, i.e. a simultaneous ^{31}P addition with the radioactive label, could increase plant growth and P uptake. It is assumed that L values are independent of the amount of carrier added to the soil. Besides the effect of enhancing plant growth, the application of carrier with the ^{33}P label is recommended to avoid the fixation of the label.

As part of a Ph.D. thesis study funded by a special project from SDC, Switzerland, two key forage species (*Arachis pintoi* and *Brachiaria decumbens*) were compared with three low-P adapted crop cultivars (maize, bean and upland rice) in terms of their ability for P uptake from sparingly available P pools based on their L values. A soil was chosen with very low available P (determined with Bray II) in order to guarantee P limitation for plant growth. The L value of *Agrostis capillaris* was determined as reference assuming that this plant does not access any non isotopically exchangeable P. E values were determined in a batch experiment without carrier application to determine the isotopically exchangeable soil P. L values were determined in two experiments, one without and one with a P carrier application of 10 mg P kg^{-1} soil. This amount was chosen as, with the application of the same amount to a similar soil, *Bachiaria* species and *Arachis pintoi* increased biomass production and P uptake but remained P limited.

Materials and Methods

The soil chosen (well-drained oxisol) for this study was cultivated as improved grass legume pasture starting with rice in 1993, with under sown pasture. The procedure to determine E values is based on the measurement of the specific activity ($^{33}PO_4$/ $^{31}PO_4$) of phosphate ions in the soil solution after an addition of carrier free $^{33}PO_4$ in a soil-solution system at steady state. The isotopically exchangeable P (E_t) was calculated assuming that, at any given exchange time, the specific activity of phosphate in solution is equal to the specific activity of the exchangeable phosphate on the solid phase:

$$\frac{r_t}{10 * C_p} = \frac{R}{E_t} \qquad \text{[Eq. 1]}$$

or:

$$E_t = R \times \frac{10 C_p}{r_t} \qquad \text{[Eq. 2]}$$

and r_t/R is extrapolated as:

$$\frac{r_t}{R} = \frac{r_1}{R}\left[t + \frac{r_1}{R}^{(\frac{1}{n})} \right]^{-n} + \frac{r_\infty}{R} \qquad \text{[Eq. 3]}$$

where R is the introduced radioactivity in MBq $ml^{-1}$and r_t is the radioactivity remaining in the solution after t minutes. The other parameters can be determined experimentally: n is a parameter calculated as the slope of the linear regression between $\ln(r_t/R)$ and $\ln(t)$ for $t \leq 100$ minutes of the measured values, r_1/R is the interception of the regression when t=1, and r_∞/R is calculated as:

$$\frac{r_\infty}{R} = 10 * \frac{C_p}{P_i} \qquad \text{[Eq. 4]}$$

Where P_i is total inorganic P and the ratio r_∞/R represents the radioactivity remaining in the soil solution at infinite time.

L value determination. The experimental conditions of the two pot experiments carried out to determine L values are summarized in Table 2. The cultivars used were *Brachiaria decumbens* (CIAT 606), *Arachis*

pintoi (CIAT 18744) and rice (*Oryza sativa* var Savanna-6) in experiment one. Additionally we used beans (*Phaseolus vulgaris* AFR 475) and maize (*Zea mays* NST 90201(s) co-422-2-3-1-7-2-1), an inbred line derived from a triple hybrid developed by the Thai Department of Agriculture, selected as tolerant to low-P conditions. In both cases common bentgrass (*Agrostis capillaris*) was grown as control plant without adaptation to low P conditions. The soil was labeled by adding the quantities of $^{32}PO_4$ or $^{33}PO_4$ ions in 10 mL water to portions of 1.5 kg incubated soil and were thoroughly mixed to ascertain an even distribution of the isotope (details given in Table 65).

Table 65. Soil (0-15 cm) properties determined on air-dried samples (except bulk density).

Total P (mg kg^{-1})	242
Total P_i (mg kg^{-1})	86.4
Resin P (mg kg^{-1})	1.5
Bray-II P (mg kg^{-1})	3.1
pH (in H_2O)	4.3
Total C (g kg^{-1})	23.7
Total N (g kg^{-1})	1.6
Aluminum-saturation (%)	68
Bulk density (g cm^{-3})	1.3

Agrostis was grown from 100 mg of seeds (corresponding to about 800 seeds) in both experiments, which were sown directly into each pot. All other plants were pregerminated on filter paper before planting into the pot at numbers indicated in Table 66. The pots with beans and *Arachis pintoi* were inoculated with a suspension of the *Rhizobium* strains CIAT 899 and CIAT 3101, respectively. During the experiment soil moisture was controlled by weighing and kept at 50 % of the water holding capacity.

Table 66. Experimental conditions in pot experiment 1 and 2 for determination of L values.

	Experiment 1	Experiment 2
Plant species, quantities of soil and plants per pot	*Arachis pintoi*, 2 kg soil, 2 plants *Brachiaria decumbens*, 2 kg soil, 2 plants Rice, 2 kg soil, 2 plants	*Arachis pintoi*, 0.9 kg soil, 1 plant *Brachiaria decumbens*, 0.9 kg soil, 2 plants Rice, 0.9 kg soil, 2 plants Beans, 0.9 kg soil, 1 plant Maize, 3.4 kg soil, 1 plant
	Agrostis capillaris, 500 g, 100 mg seeds	*Agrostis capillaris*, 400 g soil, 100 mg seeds
Labeling	$^{32}PO_4$, 5.2 MBq kg^{-1} soil	$^{33}PO_4$, 3.7 MBq kg^{-1} soil
Carrier	none	10.26 mg P as KH_2PO_4 kg^{-1} soil, applied with labeling solution
Replicates	4	5
Location	greenhouse, CIAT, Colombia	Biotron, ETH, Switzerland
Experimental conditions	Maximum light intensity ~ 1100 µ mol m^{-2} s^{-1}	16 h daylight, light intensity ~ 300 µ mol m^{-2} s^{-1}
Temperature	38/20 °C (max/min d/n, over whole growth period)	24/20 °C (constant)
Humidity	90/40 % (max/min)	65 % (constant)
Duration of plant growth:	2 months	11 weeks

After two months or eleven weeks, respectively, plant shoots were harvested and dry matter was weighed after 48 h drying at 80° C. About 200 mg of a homogenized sample of the whole shoot biomass in the first or half of the total shoot in the second experiment, cut in pieces <2mm, was calcinated at 550° for 4 hours. Plant P content (p) was determined after solubilization of the ash in 1-5 mL of 11.3 *M* HCl. The same method was used for the determination of the seed P content, measuring ball-milled samples of 100 mg (*Agrostis capillaris*), two seeds (*Arachis pintoi*, rice, beans and maize) or five seeds (*Brachiaria decumbens*), with five replicates each.

The plant ^{33}P (r) content was measured by scintillation counting of diluted (to avoid quench effect) samples using a liquid scintillation analyzer (Packard 2500 TR) and Packard Ultima Gold scintillation liquid. The measured radioactivity was decay corrected back to the day of soil labeling. The L values, expressed as mg P kg^{-1} soil, were calculated with the P-concentrations and activities measured in the total shoot.

Experiment 1:
Without carrier:

$$L = \frac{R * p}{r} \qquad \text{[Eq.5]}$$

Experiment 2
With carrier:

$$L = Q\left(\frac{R * p}{Q * r} - 1\right) \qquad \text{[Eq.6]}$$

The source of P taken up by the plant in the experiment with carrier addition can be calculated as:

$$P_{\text{soil}} = p - P_{\text{carrier}} \qquad \text{[Eq.7]}$$

$$P_{\text{carrier}} = \frac{Q * r}{R} \qquad \text{[Eq.8]}$$

where R is the quantity of $^{33}PO_4$ or $^{32}PO_4$ used to label exchangeable soil P (MBq kg^{-1} soil) and Q the quantity of carrier added (mg P kg^{-1} soil), r is the quantity of $^{33}PO_4$ or $^{32}PO_4$ (MBq kg^{-1} soil) and p is the quantity of $^{31}PO_4$ (mg kg^{-1} soil) in the plant shoots. $P_{carrier}$ and P_{soil} are the total amount of P derived from the carrier solution or from soil respectively. However, the P content of the seed is a third P source and uptake from this source could not be distinguished from the P taken up from soil. Therefore, P_{soil} is actually the sum of the P taken up from soil and from the seed, and the specific activity of the P taken up from soil is diluted. This results in an overestimation of the L value in both experiments. To increase the accuracy of the L value, the following correction was applied:

$$L_{\text{th}} = L\frac{p}{\left(p + P_{seeds}\right)} \qquad \text{[Eq.9]}$$

where L_{th} is the corrected value, L the value calculated with Eq. 5 or 6 and P_{seeds} the P content of the sown seeds per pot. Another possibility to correct for the seed P influence is to subtract the total seed P content from plant P uptake for the calculation of the L value: This correction assumes that 100 % of seed P was taken up by the plant and allocated to the shoot. Therefore, it corrects for the highest possible influence of seed P.

$$L = \frac{R\,(p - P_{seed})}{r} \qquad \text{[Eq. 10]}$$

Acid phosphatase activity determination: Three bulk soil samples were taken at random after harvesting plants in all pots, air dried and roots were removed carefully by sieving soil at 2 mm. Acid phosphatase activity at pH 6.5 of soil samples derived from the planted pots and soil incubated without plants at the same conditions was measured using 1 g air-dried soil.

Statistical Analysis: The effect of plants in the pot experiment and the effect of the experimental conditions on parameters of isotopic exchange in the E value determination were tested by analysis of variance (ANOVA). If the F-test was significant ($P<0.05$), the means were compared using Tukey's multiple range test.

Results and Discussion

L values determined without carrier and correction for seed P influence: The biomass production of all plants in the first experiment was very low and the total P uptake was hardly higher than the P content of the seeds (Tables 68 and 69). Correction for the contribution of seed-P according to Eq. 9 resulted in a marked reduction of the uncorrected L values (Table 68). It is, however, doubtful whether this correction, which was established for L value determination with *Agrostis capillaris* and *Lolium perenne* as model plants on sand culture, is also valid for other test plants and for all soil types.

The correction with Eq. 10 was only applicable in the case of *Brachiaria decumbens* as for all other test plants $P_{seed}>p$. Consequently, the corrected L values may rather give an impression of the order of magnitude of the seed P influence than represent exact values. Most studies comparing L values of different plants on low P soils, may have underestimated the problem of seed P influence. As the P reserves in the seed are in most cases relatively high in comparison to the P taken up from soil, the L value can not be calculated without correction for seed P uptake.

Due to the uncertain influence of seed-P, the interpretation of the L values remains limited. Additionally, the L value of *Agrostis capillaris* could not be used as reference as the ratio of plant P uptake to seed P was very low, too. However, in the case of *Brachiaria decumbens* with the smallest influence from seed P (Table 68 and 69), the corrected L_{th}-value remains much higher (131 mg kg^{-1} with Eq.9 or 127 mg kg^{-1} with Eq.10, respectively) than the extrapolated E_{8weeks}-value of 64 mg kg^{-1} determined for the same soil (Table 67). As Eq. 10, with the subtraction of total seed P from P export in the plant shoot, corrects for the highest theoretically possible influence of seed P, the L value of *Brachiaria decumbens* indicates that P additional to the isotopically exchangeable P was taken up. However, it should be mentioned that the extrapolation of E values on such very low P soils is difficult and the precision of the calculated E_{8weeks} is therefore limited. On the other hand, the L value of *Brachiaria decumbens* is also higher than the total soil P_i extracted with the sequential P fractionation (Table 65). This fact reinforces the assumption that organic P or very recalcitrant P forms contributed to the P uptake of *Brachiaria decumbens*.

Table 67. Parameters of isotopic exchange of the used soil.

r_1/R†1	0.03
c_p ‡	0.003 mg l^{-1}
n¶	0.43
E_1 #	1.1 mg kg^{-1}
E_{8weeks}#	64 mg kg^{-1}

† ratio of radioactivity remaining in soil solution to radioactivity added at time 0 after 1minute of isotopic exchange
‡ P concentration in the soil solution measured at soil:water ratio 1:10
¶ Parameter of isotopic exchange describing the decrease of radioactivity in the soil solution
#Quantity of P exchangeable within 1 minute or within 8 weeks (calculated with Eq. 3)

Table 68. Biomass production, P uptake and L values of the compared plants in experiments 1 and 2.

Plant Material	Shoot dry weight		P uptake		Shoot P concentration		L†		L_{th}‡	
	(g per pot)		(mg per pot)		µg g^{-1} dry weight		(mg P kg^{-1} soil)			
	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
A. pintoi	1.6a	2.4b	0.9a	2.1b	561ab	877b	185a	4.0	46b	3.1
B. decumbens	0.3bc	1.9bc	0.22b	1.1b	729a	581c	153ab	0.9	131a/ 127§	0.9
Rice	0.6b	2.3b	0.25b	1.1b	417b	478cd	125b	1.4	39b	1.1
Maize	-	6.3a	-	3.9a	-	622c	-	4.7	-	3.8
Beans	-	1.0c	-	1.5b	-	1350a	-	1.7	-	1.1
Agrostis capillaris	0.2c	1.0c	0.14b	0.4b	697ab	392d	128ab	3.3	6.7b	1.6
ANOVA	***	***	***	***	*	***	*	n.s.¶	***	n.s.

*,*** Significant at the 0.05 or 0.001 probability level, respectively. Values within columns followed by the same letter do not differ significantly (P=0.05) according to Tukey's test.
†L value without seed-P correction
‡L value with the seed -P correction, Eq. 9
§ second value: corrected with seed P correction, Eq. 10
¶ not significant

Table 69. Average seed weight and seed P content of the used varieties

Plant material	Weight per seed	Total P in sown seeds
	(mg)	(µg)
Arachis pintoi	158	1200/ 2 seeds
Brachiaria decumbens	4.6	26.7/ 2 seeds
Rice	44	282/ 2 seeds
Maize	306	900/ 1 seed
Beans	174	540/ 1 seed
Agrostis capillaris	0.125	480/100 mg seeds

The adaptation of *Brachiaria* species to low P soils is mainly attributed to soil exploration by an abundant fine root system and mycorrhizal association. In addition, it was shown in a pot experiment with different

added P sources, that *Brachiaria dictyoneura* cv. Llanero can acquire P from less available inorganic (aluminum phosphate, as $AlPO_4$) and organic (phytic acid) forms. Acid phosphatase activity in roots of *Brachiaria dictyoneura* was increased with decreasing soil P supply, and *Brachiaria decumbens* grown under low P condition in nutrient solution was shown to secrete the highest amount of phytase in comparison to 15 other plant species.

In our study acid phosphatase activity measured in the pot soil samples, and in turn the potential to mineralize available phosphomonoesters, was only significantly increased ($p<0.001$) for *Arachis pintoi* in the first experiment and was increased significantly ($p<0.001$) for all plants but *Agrostis capillaris* in comparison to the control soil without plant in the second experiment (Table 70). However, as the measurements were not restricted to rhizosphere soil, local effects in that zone would not have been detected.

Table 70. Phosphatase activity in soil samples derived from pots after plant harvest.

Plant species	Phosphatase activity	
	Exp. 1	Exp. 2
	μg nitrophenol g^{-1} h^{-1}	
Arachis pintoi	426a	322a
Brachiaria decumbens	285b	342a
Rice	295b	295ab
Maize	-	332a
Beans	-	286b
Agrostis capillaris	236b	242c
Control	219b	225c
ANOVA	***	***

***Significant at the 0.001 probability level, values within a column followed by the same letter do not differ significantly (P=0.05) according to Tukey's test.

The influence of carrier application: As the correction for seed P influence was difficult, the L value determination without carrier application was unsatisfying for the tested plants, with exception of *Brachiaria decumbens*. To overcome the difficulties of small total P uptake and biomass production, the second experiment was carried out with the application of KH_2PO_4 (10.3 mg P kg^{-1} soil) as a carrier with the labeling solution and the duration of plant growth was extended from two month to eleven weeks and smaller pots were used to reach higher biomass production and a higher soil exploration by the roots. The application of a P carrier resulted in much smaller L values (mean of all plants 2.7 mg P kg^{-1} soil) than without carrier (mean 148 mg kg^{-1}) and there were no significant differences between plants.

One possible explanation of the difference found between L values determined with or without carrier application is that an application of 10 mg P kg^{-1} to a soil with a very low P concentration in the solution (in this case approximately 3 μg l^{-1}) could have a high impact on the processes in this system. Instead of isotopic exchange, a net diffusion process may dominate and sizes of pools are changed. High influences of carrier application on E values, especially for high P sorbing soils, were found before and were explained by the influence of carrier P on the process of isotopic exchange as well as by the fact of $^{32}PO_4$ fixation.

Additionally to the carrier application also the different experimental conditions, especially the smaller pot size, might have influenced the L-value. However, a smaller soil volume and therefore higher root exploration and biomass production per kg soil should, if at all, lead to an increase of L values by higher

root activity and P mobilization and not to a decrease.

In our study, the nearly identical values of the specific activities of the plants and the applied carrier indicate that the carrier P was the main source for the P taken up by the plant. Separation of the P sources using Eq. 7 and 8 shows that on average 81% of P taken up by the plant derived from the carrier (Table 71). Of the 19 % of the total plant P uptake derived from another source a part is actually seed P. Therefore it can be assumed that almost no soil P was taken up and that the application of carrier is not valid for the determination of L values on low-P highly P sorbing soils.

Table 71. Amount of P derived from applied carrier and percentage of P derived from other sources in Experiment 2 (calculated with Eq. 7 + 8).

Plant species	Total P uptake in plant shoot	P derived from carrier	P derived from other sources (soil and seed)
	(mg per pot)		(%)
Arachis pintoi	2.1b	1.5b	27a
Brachiaria decumbens	1.1b	1.0b	9b
Rice	1.1b	0.9b	16b
Maize	3.9a	2.6a	26a
Beans	1.5b	1.0b	13b
Agrostis capillaris	0.4b	0.3C	24a
(average)			19
ANOVA	***	***	***

*** Significant at the 0.001 probability level, values within columns followed by the same letter do not differ significantly (*P*=0.05) according to Tukey's test.

Conclusions

A higher L value than E value for *Brachiaria decumbens* suggested P uptake from less available P forms from a low P soil. For all other plants, the contribution of the seed P to plant P uptake did not allow the calculation of exact L values. Therefore, drawing conclusions about the access of different P pools by different plants was not possible. L values determined with or without carrier P differed widely and suggested that a carrier application is not recommendable for using soils with very low P supply.

Results from this study indicate that it is possible to use L value determination as a screening method to identify the most promising *Brachiaria* hybrids with adaptation to low P supply in soil. Further research work is needed to identify specific physiological and biochemical mechanisms contributing to the ability of *B. decumbens* to acquire P from less aavailable forms from low P oxisol.

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

Contributors: N. Castañeda, N. Claassen (University of Goettingen, Germany); A. Betancourt, R. Garcia, J. Ricaurte and I. M. Rao (CIAT)

Rationale

For the last two years, 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.

Materials and 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). 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 32 months after establishment.

Results and Discussion

Last year, we reported that CIAT 22159 was outstanding in terms of persistence during second year as determined by leaf area index, shoot biomass and shoot P and Ca uptake. But this accession was relatively slow in establishment.

At 32 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. Initial application of PR and TSP at establishment had no residual effect on the performance of 10 accessions. (Table 72). Live forage yield was greater for CIAT 22159 and CIAT 18744 with no P application treatment. This was not due to better leaf production but due to a large biomass of stolons (Table 73). With no P application treatment, 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 this accession with no P application.

Table 72. Influence of P fertilizer source on genotypic differences in leaf area index, live forage biomass and dead forage biomass of *Arachis pintoi* grown a low P soil at Montañita, Caquetá. Measurements were made at 20 months after planting.

Plant	P source	Accession of *A. pintoi*										$LSD_{0.05}$
Attributes		17434	18744	18745	18747	18748	18751	22155	22159	22160	22172	
Leaf area	NP	1.34	0.78	0.58	0.68	0.99	0.65	0.76	1.29	0.71	0.71	0.57
index	RP	0.89	0.75	0.72	0.44	1.10	0.81	0.69	0.78	0.63	0.77	0.48
(m^2/m^2)	TSP	1.19	0.78	0.76	0.56	1.06	0.93	0.58	0.71	0.87	0.93	0.49
Live forage	NP	2.22	3.08	1.39	2.32	1.93	2.15	3.07	3.54	1.74	2.84	1.14
yield	RP	2.31	2.38	1.60	1.86	2.83	2.33	2.19	3.39	2.15	2.40	0.91
(t/ha)	TSP	2.04	3.14	1.41	2.18	2.35	2.12	1.41	2.44	1.79	2.84	1.14
Dead forage	NP	1.03	1.55	0.96	1.05	1.11	1.52	1.71	1.80	1.22	0.99	0.65
yield	RP	1.39	1.92	1.16	1.23	1.40	1.34	0.84	1.22	1.12	0.76	0.64
(t/ha)	TSP	1.47	1.23	1.60	1.31	1.18	1.19	0.55	1.16	2.24	1.12	0.77

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

The differences among the 10 genotypes in terms of leaf N and P contents were small when compared with leaf biomass production (Table 73). The content of N and P in green leaves was greater with the accession CIAT 18751.

Nutrient uptake, particularly P and N by green leaves during the dry spell was not significantly different among 10 acessions for no P treatment. Among the ten accessions, CIAT 17434, the commercial cultivar was outstanding in its ability to acquire Ca particularly from no P treatment (Table 74).

Table 73. Influence of P fertilizer source on genotypic differences in leaf biomass, leaf N content and leaf P content of *Arachis pintoi* grown a low P soil at Montañita, Caquetá. Measurements were made at 20 months after planting.

Plant	P source	Accession of *A. pintoi*										$LSD_{0.05}$
Attributes		17434	18744	18745	18747	18748	18751	22155	22159	22160	22172	
Leaf	NP	629	501	327	417	578	406	498	541	423	423	235
biomass	RP	518	431	388	258	666	576	417	477	389	438	267
(kg/ha)	TSP	562	505	409	329	568	548	361	431	522	564	NS
Leaf N	NP	24.9	32.6	27.8	32.8	26.8	32.6	30.1	29.5	30.5	28.5	NS
content	RP	30.2	36.2	36.4	38.1	35.1	34.9	29.5	31.5	33.9	30.9	4.4
(g/kg)	TSP	32.5	34.8	40.0	35.0	32.0	36.3	30.0	33.2	33.6	28.3	6.9
Leaf P	NP	2.1	2.2	2.5	2.4	2.1	2.4	2.4	2.1	2.1	2.0	NS
content	RP	2.1	2.7	2.8	2.8	2.4	2.6	1.9	2.3	2.3	2.2	0.4
(g/kg)	TSP	2.4	2.5	2.7	2.8	2.1	2.7	2.0	2.3	2.7	2.0	NS

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

Table 74. Influence of P fertilizer source on genotypic differences in P, N and Ca uptake by green leaves of *Arachis pintoi* grown a low P soil at Montañita, Caquetá. Measurements were made at 20 months after planting.

Plant	P source	Accession of *A. pintoi*										$LSD_{0.05}$
Attribute		17434	18744	18745	18747	18748	18751	22155	22159	22160	22172	
	NP	1.30	1.10	0.84	0.98	1.22	1.02	1.05	1.16	0.90	0.85	NS
P uptake	RP	1.11	1.15	1.06	0.69	1.57	1.47	0.82	1.09	0.86	0.95	0.59
(kg/ha)	TSP	1.37	1.24	1.08	0.92	1.19	1.47	0.66	1.00	1.37	1.13	0.60
	NP	15.6	16.58	9.23	13.99	15.32	13.65	15.66	15.99	12.92	12.12	NS
N uptake	RP	15.77	15.50	13.98	9.72	23.37	20.10	12.52	15.11	12.50	13.47	9.2
(kg/ha)	TSP	18.77	17.54	15.98	11.77	18.43	19.85	10.63	14.39	17.09	15.97	NS
	NP	8.25	4.17	2.55	3.50	3.95	3.73	3.67	5.33	3.74	5.00	3.73
Ca uptake	RP	7.07	4.05	4.07	2.92	5.34	4.66	3.70	6.51	2.91	5.31	3.32
(kg/ha)	TSP	5.72	3.23	4.34	3.17	4.32	3.71	3.53	3.87	3.71	5.83	NS

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

Conclusions

It appears from this field study that CIAT 22159 may be a better accession in terms of persistence with no P fertilizer input.

3.1.4.1 Field evaluation of most promising accessions of *Arachis pintoi* in the Llanos of Colombia

Contributors: I. M. Rao, M. Peters, C. Plazas and J. Ricaurte (CIAT)

Rationale

Last year, we reported the progress from the field studies carried out at Caqueta that were aimed to determine genotypic differences among ten accessions of *Arachis pintoi* in persistence with low P supply in soil. Based on these data and the data collected from multilocational evaluation, we have assembled a set of 8 genotypes for further testing at two sites (Piedmont and Altillanura) in the Llanos of Colombia. The site in Piedmont is close to La Libertad (CORPOICA Experimental Station) and the soils in this region are relatively more fertile than in the Altillanura. The site in Altillanura is at Matazul farm where the soils are relatively infertile (sandy loam).

Materials and Methods

Two field studies were established during May this year. The trial in Piedmont was planted as monoculture while the trial in Altillanura was planted in association with a grass. This is based on the expected end use of the legume. We expect multiple use for this legume in the Piedmont area (e.g., cover legume in plantations). The trial in the Piedmont included 8 accessions of *Arachis pintoi* (CIAT 17434; 18744; 18747; 18748; 18751; 22159; 22160 and 22172). The trial in the Altillanura included 4 accessions (CIAT 17434; CIAT 18744; CIAT 18748 and CIAT 22159) planted in association with *Brachiaria decumbens*. Both trials were planted as 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 with 3 replications.

Results

At 140 days after establishment of the trial in Piedmont, CIAT 18751 was found to be outstanding in its ability to establish rapidly. Two accessions (CIAT 17434 and 18751) were responsive to high level of fertilization while one of those two accessions (CIAT 18751) together with CIAT 18747 were among better performers with low fertilizer application as determined by soil cover.

A number of plant attributes including forage yield, dry matter distribution and nutrient uptake are being monitored for both trials.

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 moderate dry season in the llanos of Colombia, was associated with lower levels of K and N content in green leaves.
- Field screening of 16 genotypes of Brachiaria and 13 accessions of *Arachis pintoi* with long dry season in Costa Rica resulted in identification of *Brachiaria* hybrid CIAT 36061 as not only adapted to drought but also superior in maintaining greater level of nitrogen (crude protein) in green leaves.

Progress towards achieving milestones

- ***Brachiaria* accessions and hybrids with superior tolerance to drought relative to commercial cultivars identified.**
  One hybrid of *Brachiaria* (FM9503-S046-024) was identified as promising material for areas with moderate drought stress in the acid soil regions. Recently released *Brachiaria* hybrid cv. Mulato (CIAT 36061) was identified as not only adapted to long dry season stress but also superior in its nutritional (protein) quality of green leaf forage to animals.

- ***Arachis* accessions with superior tolerance to drought identified.**
  Field testing of 13 accessions of *A. pintoi* did not result in identification of specific shoot attributes that are related to superior drought adaptation. Further research work is needed to evaluate shoot and root attributes under controlled conditions in the glasshouse.

- **Advanced in the development of an improved screening method to evaluate drought tolerance in *Brachiaria*.**
  Field screening of *Brachiaria* accessions at Atenas, Costa Rica were not very successful in identifying specific shoot attributes that contribute to superior adaptation to drought. Further research work is needed to evaluate shoot and root attributes under controlled conditions in the glasshouse to develop improved screening methods to evaluate drought adaptation of *Brachiaria* accessions and hybrids.

3.2.1 Determination of the genotypic variation in dry season tolerance in *Brachiaria* accessions and genetic recombinants in the Llanos of Colombia

Contributors: I.M. Rao, J. W. Miles, C. Plazas, J. Ricaurte and R. García (CIAT)

Rationale

Quantity and quality of dry season feed is a major limitation to livestock productivity in subhumid regions of tropical America. A field study is in progress at Matazul Farm in the Llanos of Colombia. The main objective was to evaluate genotypic differences in dry season (4 months of moderate drought stress) tolerance of most promising genetic recombinants of *Brachiaria*. Last year, we showed that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024, which maintained greater proportion of green leaves during dry season during the first year of establishment, was associated with lower levels of K and N content in green leaves. This year, we continued our efforts to monitor the dry season performance into second year after establishment.

Materials and 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*. Among the germplasm accessions, *B. brizantha* (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 (20 months after establishment; March 2001).

Results and Discussion

Initial application of high amounts of fertilizer (at the time of establishment) did not improve forage yield of most of the genotypes compared with low fertilizer application (Table 1). This indicates very little residual effects of initial application into the second year. At 20 months after establishment (4 months after dry sseason), live forage yield with low fertilizer application ranged from 0.14 to 1.48 t/ha and the highest value of forage yield was observed with one spittlebug resistant genetic recombinant, FM9503-S046-024 (Table 75).

Table 75. 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 20 months after establishment (at the end of the dry season - March 2001). 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
	(kg/ha)					
Recombinants:						
BR97NO-0082	475	407	204	286	679	693
BR97NO-0383	502	449	223	310	725	759
BR97NO-0405	478	690	287	380	765	1070
cv. Mulato (CIAT 36061)	985	702	611	377	1596	1079
CIAT 36062	440	609	639	476	809	1085
FM9503-5046-024	1485	1106	626	510	2111	1616
Parents:						
CIAT 606	548	541	560	590	1108	1131
CIAT 6294	785	727	385	382	1170	1109
BRUZ/44-02	141	196	85	64	226	260
CIAT 26646	835	1077	792	1115	1627	2192
Accessions:						
CIAT 26110	1008	1266	407	732	1415	1998
CIAT 26318	1074	806	754	702	1828	1508
Mean	730	715	442	494	1172	1208
LSD *(P=0.05)*	552	452	379	438	821	786

As expected, the performance of one of the parents, BRUZ/44-02 was very poor compared with other parents and genetic recombinants. Among the four parents, CIAT 26646 performed better but it had greater amounts of dead biomass than the other test materials.The superior performance of the hybrid, FM9503-S046-024 was mainly attributed to its ability to produce green leaf biomass during dry season (Table 76). But this hybrid produced less stem biomass than another hybrid, FM 9201-1873. Among the parents, CIAT 26646 showed greater leaf and stem biomass (Table 76).

Results on leaf and stem N content indicated that BRUZ/44-02 had greater amount of N per unit leaf dry weight but its ability to acquire N (shoot N uptake) with low fertilizer application was lowest compared with other parents and genetic recombinants (Table 77).

Shoot N uptake with low fertilizer application was greater for two accessions (CIAT 26110 and 26318), one parent (CIAT 26646) and one genetic recombinant (FM9503-S046-024). This genetic recombinant was also outstanding in its ability to acquire greater amounts of P, K, Ca and Mg from low fertilizer

application when compared with parents, accessions and other genetic recombinants (Tables 4 and 5). Among the parents, CIAT 26646 and CIAT 6294 were superior in P, K, Ca and Mg acquisition from low fertilizer application.

Table 76. 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 20 months after establishment (at the end of the dry season - March 2001). LSD values are at the 0.05 probability level.

Genotype	Leaf biomass		Stem biomass	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	Kg/ha			
Recombinants:				
BR97NO-0082	436	383	39	24
BR97NO-0383	431	381	71	68
BR97NO-0405	389	561	89	129
cv. Mulato (CIAT 36061)	493	605	492	97
CIAT 36062	402	563	38	46
FM9503-5046-024	1320	1044	165	62
Parents:				
CIAT 606	366	375	182	166
CIAT 6294	672	648	113	79
BRUZ/44-02	115	176	26	20
CIAT 26646	595	648	240	429
Accessions:				
CIAT 26110	827	985	181	281
CIAT 26318	640	581	434	225
Mean	557	579	173	136
LSD *(P=0.05)*	400	342	308	154

Table 77. 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 20 months after establishment (at the end of the dry season - March 2001). 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	1.360	1.140	ND	ND	ND	ND
BR97NO-0383	1.230	1.190	ND	ND	ND	ND
BR97NO-0405	0.910	0.970	1.11	0.83	3.72	6.31
cv. Mulato (CIAT 36061)	1.260	1.040	0.48	1.03	6.39	6.85
CIAT 36062	1.200	1.120	ND	ND	ND	ND
FM9503-5046-024	1.070	0.910	0.99	0.59	15.35	10.01
Parents:						
CIAT 606	1.360	1.180	0.84	0.91	6.21	5.10
CIAT 6294	0.990	0.900	1.05	0.67	6.99	6.06
BRUZ/44-02	2.240	1.900	ND	ND	ND	ND
CIAT 26646	1.060	0.960	0.68	0.67	8.05	8.72
Accessions:						
CIAT 26110	1.040	0.870	0.90	0.84	10.12	10.53
CIAT 26318	0.970	1.110	0.65	0.81	9.08	7.41
Mean	1.160	1.070	0.8	0.8	7.21	6.78
LSD *(P=0.05)*	0.451	0.481	NS	0.36	6.11	4.58

ND = not determined due to small size of the sample; NS = not significant.

Table 78. 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 20 months after establishment (at the end of the dry season.

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	0.116	0.126	ND	ND	ND	ND
BR97NO-0383	0.088	0.108	ND	ND	ND	ND
BR97NO-0405	0.101	0.116	0.124	0.130	0.42	0.78
cv. Mulato (CIAT 36061)	0.108	0.109	0.090	0.103	0.62	0.71
CIAT 36062	0.132	0.163	ND	0.152	ND	0.97
FM9503-5046-024	0.116	0.119	0.136	0.184	1.75	1.33
Parents:						
CIAT 606	0.118	0.137	0.117	0.172	0.63	0.76
CIAT 6294	0.113	0.109	0.165	0.099	0.87	0.74
BRUZ/44-02	0.092	0.143	ND	ND	ND	ND
CIAT 26646	0.104	0.127	0.084	0.106	0.82	1.24
Accessions:						
CIAT 26110	0.106	0.106	0.128	0.165	1.06	1.47
CIAT 26318	0.095	0.114	0.088	0.107	0.97	0.89
Mean	0.108	0.123	0.112	0.130	0.76	0.85
LSD *(P=0.05)*	NS	0.039	0.040	0.053	0.68	0.63

ND = not determined due to small size of the sample; NS = not significant.

Table 79. 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 20 months after establishment (at the end of the dry season - March 2001). 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	7.62	7.24	0.90	1.23	0.83	1.15
BR97NO-0383	7.59	6.17	1.01	0.95	1.17	0.94
BR97NO-0405	7.00	11.86	1.08	1.93	1.08	1.99
cv. Mulato (CIAT 36061)	6.94	9.38	1.26	1.59	1.41	1.81
CIAT 36062	7.96	9.35	0.91	1.53	0.87	2.09
FM9503-5046-024	23.58	13.32	3.70	3.55	3.69	3.63
Parents:						
CIAT 606	9.55	8.16	1.01	1.04	1.30	1.62
CIAT 6294	10.48	9.53	1.47	1.49	1.75	1.82
BRUZ/44-02	2.49	4.20	0.30	0.75	0.31	0.85
CIAT 26646	9.50	13.95	1.28	1.84	1.62	2.88
Accessions:						
CIAT 26110	14.10	15.54	1.73	2.51	1.94	3.60
CIAT 26318	12.05	8.95	1.67	1.40	2.10	2.12
Mean	10.40	9.92	1.43	1.67	1.59	2.07
LSD *(P=0.05)*	8.25	6.14	1.70	1.22	1.73	1.60

Correlation analysis between green leaf biomass produced in the dry season and other shoot attributes indicated that superior performance with low fertilizer application was associated with lower level of N in green leaves (Table 80).

Significant negative association was also observed between green leaf biomass and lower level of K and N in green leaves with high fertilizer application. This observation indicates that genotypes that are efficient in uitilization of N for the production of green forage is an important mechanism for superior performance with low fertilizer application in the dry season.

Conclusions

Results from this field study indicated that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024 which maintained greater proportion of green leaves during moderate dry season in the llanos of Colombia, was associated with lower levels of K and N content in green leaves.

Table 80. 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.

Shoot traits	Low fertilizer	High fertilizer
Live forage yield (t/ha)	0.87***	0.72***
Total forage yield (t/ha)	0.81***	0.81***
Dead biomass (t/ha)	0.54***	0.51***
Stem biomass (t/ha)	0.20	0.45**
Leaf N content (%)	-0.33*	-0.45**
Leaf P content (%)	0.05	-0.10
Leaf K content (%)	-0.15	-0.54***
Leaf Ca content (%)	-0.16	0.06
Stem N content (%)	0.20	-0.11

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

3.2.2 Determination of the genotypic variation in dry season tolerance in *Brachiaria* and *Arachis* in Costa Rica

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Rationale

Field studies were continued at Atenas, Costa Rica. The main objective was to evaluate genotypic differences in dry season (6 months) tolerance among 16 accessions of *Brachiaria* species and 13 accessions of *Arachis* species. We continued our work to test further the hypothesis that tolerance to dry season is greater in genotypes that accumulate greater amounts of total nonstructural carbohydrates (TNC) combined with less amounts of minerals (ash content) per unit dry weight of leaves and stems. The use of shoot attributes such as ash content, Ca content and nonstructural carbohydrate levels as selection criteria for dry season tolerance in *Brachiaria* is being tested further using green leaves developed during dry season compared to dry leaf and stem tissue.

Materials and Methods

Trial 1 included 16 genotypes (15 accessions and 1 hybrid) of *Brachiaria* species and trial 2 included 13 accessions of *Arachis* species selected from agronomic evaluation of the germplasm. Atenas site provided excellent field conditions to evaluate the impact of long dry season (5 months) while keeping nutrient supply in soil adequate for growth. Forage yield, nutrient composition, and nonstructural carbohydrates and ash content in green leaves, dry leaves and stem tissue were measured.

Results and Discussion

Trial 1 - Forage yield among *Brachiaria* species during dry season ranged from 2988 to 9988 kg/ha and the greatest forage yield was observed with *B. brizantha* CIAT 26646 (Table 81). The superior performance of this accession at Atenas site is consistent with its outstanding performance at Matazul site in the Llanos of Colombia (see above Activity 3.2.1). *B. brizantha* CIAT 26110, which maintained greater proportion of green leaves during dry season (visual observation) maintained greater amountn of N (crude protein) and TNC in green leaves while its ash (mineral) content was markedly lower than most accessions. This observation indicates that this accession combines drought adaptation with greater nutritional value of the green forage. One of the Brachiaria hybrids tested (CIAT 36061) was particularly outstanding in its N status of the green leaves. It apperas that this hybrid also combined adaptation to drought with greater nutritional value of the green forage. Among the 16 genotypes *B. brizantha* CIAT 667 was outstanding in maintaining greater amounts of nonstructural carbohydrates in green leaves.

Table 81. Genotypic variation in forage yield, green leaf nutrient composition, ash content and total nonstructural carbohydrates (TNC) of 16 genotypes of *Brachiaria* species grown during dry season at Atenas, Costa Rica.

Genotype (CIAT number)	Forage yield (kg/ha)	Green leaf composition							
		C	N	P	K	Ca	Mg	Ash	TNC
		(%)							(mg/kg)
B. brizantha (26646)	9988	25.3	0.45	0.074	1.345	0.546	0.648	9.0	156
B. brizantha (16305)	8828	27.8	0.67	0.057	1.468	0.418	0.513	8.3	141
B. brizantha (16322)	8132	26.5	0.56	0.050	1.150	0.566	0.721	11.4	174
B. brizantha (16319)	8068	27.0	0.39	0.038	1.379	0.337	0.517	6.8	180
B. brizantha (26110)	7028	27.1	0.71	0.049	1.472	0.409	0.295	7.5	178
cv. Mulato (CIAT 36061)	6692	28.0	0.81	0.062	1.334	0.701	0.755	11.4	161
B. brizantha (16300)	6640	26.9	0.47	0.045	1.139	0.495	0.623	7.1	158
B. brizantha (16467)	6628	27.2	0.57	0.076	1.860	0.606	0.519	9.6	146
B. brizantha (667)	6492	25.5	0.69	0.079	1.586	0.506	0.437	8.4	263
B. brizantha (16168)	5548	26.9	0.49	0.038	1.445	0.656	0.622	10.3	93
B. brizantha (16549)	5372	26.5	0.50	0.050	1.151	0.569	0.525	7.8	164
B. brizantha (16289)	5308	27.1	0.51	0.050	1.494	0.564	0.477	9.7	199
B. briznahta (16488)	5080	26.4	0.50	0.069	1.161	0.564	0.587	10.8	121
B. brizantha (16135)	4548	26.4	0.73	0.077	1.054	0.651	0.828	8.8	154
B. decumbens (16497)	3492	26.2	0.68	0.063	1.205	0.546	0.587	7.7	125
B. brizantha (6387)	2988	25.7	0.76	0.101	1.126	0.881	0.742	10.7	184
Mean	6302	26.7	0.59	0.061	1.336	0.563	0.587	9.1	162

Results on composition of dry leaves in terms of nutrients and TNC indicated that *B. brizantha* CIAT 667 is also outstanding in maintaining greater levels of TNC and nutrients (Table 82). Among the 16 genotypes tested. *B. brizantha* CIAT 16300 showed the lowest amount of ash content of both green leaves and dry leaves indicating that this genotype had greater nutrient use efficiency to produce forage

during dry season. Genotypic variation in nutrient and TNC composition of stems indicated that the hybrid CIAT 36061 is outstanding in maintaining greater composition of N and TNC than other genotypes. These results indicate that the hybrid is not only productive during dry season but also nutritive to the animals (Table 82).

Table 82. Genotypic variation in dry leaf nutrient composition, ash content and total nonstructural carbohydrates (TNC) of 16 genotypes of *Brachiaria* species grown during dry season at Atenas, Costa Rica.

Genotype (CIAT number)	Dry leaf composition							
	C	N	P	K	Ca	Mg	Ash	TNC
				(%)				(mg/g)
B. brizantha (26646)	25.4	0.32	0.073	0.873	0.537	0.629	9.5	140
B. brizantha (16305)	27.0	0.42	0.028	0.769	0.265	0.302	8.9	129
B. brizantha (16322)	26.5	0.36	0.048	0.868	0.561	0.565	11.0	134
B. brizantha (16319)	26.5	0.18	0.027	1.099	0.379	0.677	8.1	137
B. brizantha (26110)	26.5	0.51	0.041	1.211	0.460	0.361	8.9	154
cv. Mulato (CIAT 36061)	26.7	0.41	0.041	0.630	0.624	0.609	11.8	152
B. brizantha (16300)	28.6	0.33	0.054	0.780	0.568	0.631	7.7	247
B. brizantha (16467)	26.9	0.23	0.059	1.146	0.479	0.438	9.7	159
B. brizantha (667)	26.4	0.34	0.055	0.972	0.545	0.468	10.6	258
B. brizantha (16168)	28.7	0.43	0.044	1.098	0.547	0.533	10.1	201
B. brizantha (16549)	25.2	0.35	0.036	0.809	0.570	0.548	8.9	146
B. brizantha (16289)	27.9	0.48	0.054	1.027	0.479	0.398	9.0	139
B. brizanhta (16488)	27.0	0.38	0.065	1.012	0.435	0.540	10.9	143
B. brizantha (16135)	26.8	0.68	0.096	1.025	0.514	0.717	10.3	134
B. decumbens (16497)	26.5	0.58	0.074	0.870	0.573	0.560	8.6	90
B. brizantha (6387)	28.1	0.70	0.095	0.823	0.699	0.533	10.6	151
Mean	26.9	0.42	0.056	0.938	0.515	0.532	9.7	157

Trial 2- Although forage yield data were not available, among the 13 accessions of *Arachis pintoi* tested, three accessions, CIAT 17434, 22159 and 22161 maintained greater concentration of N in green leaf tissue (Table 83). One of the accessions, *A. pintoi* CIAT 22160, which was selected as dry season tolerant accession from field evaluation in cerrados of Brazil showed level of K in green leaf tissue. It also showed greater levels of Ca, Mg and TNC in green leaf tissue.

Results on genotypic variation in dry leaf nutrient composition, ash content and TNC content showed that CIAT 22160 had lower levels of TNC indicating that it may have greater ability to mobilize photosynthates (TNC) from older dry leaves to young green leaves (Table 84). This may be an important mechanism in the shoots in addition to its better rooting ability to avoid drought stress.

Further research is needed to test this accession compared with the commercial check, CIAT 17434 under glasshouse conditions to compare root and shoot attributes with drought stress.

Conclusions

Results from the above 2 trials did not provide any clear evidence that using green leaf nutrient status, ash content or TNC one good indicator of drought adaptation of *Brachiaria* and *Arachis* gentoypes.

Table 83. Genotypic variation in stem nutrient composition, ash content and total nonstructural carbohydrates (TNC) of 16 genotypes of *Brachiaria* species grown during dry season at Atenas, Costa Rica.

Genotype (CIAT number)	Stem composition							
	C	N	P	K	Ca	Mg	Ash	TNC
	(%)							(mg/g)
B. brizantha (26646)	27.3	0.11	0.040	0.374	0.169	0.334	3.9	144
B. brizantha (16305)	24.9	0.19	0.047	0.752	0.117	0.233	4.8	165
B. brizantha (16322)	26.1	0.19	0.042	0.582	0.148	0.202	5.8	116
B. brizantha (16319)	26.6	0.21	0.040	0.721	0.144	0.362	4.4	174
B. brizantha (26110)	25.4	0.30	0.046	0.723	0.151	0.157	4.4	99
cv. Mulato (CIAT 36061)	26.4	0.50	0.039	0.528	0.208	0.292	4.9	267
B. brizantha (16300)	27.4	0.25	0.050	0.561	0.164	0.374	4.6	153
B. brizantha (16467)	27.5	0.13	0.044	0.973	0.161	0.276	5.0	142
B. brizantha (667)	26.4	0.14	0.056	0.999	0.170	0.208	6.5	153
B. brizantha (16168)	27.6	0.07	0.025	0.513	0.110	0.179	4.4	152
B. brizantha (16549)	27.2	0.19	0.035	0.417	0.185	0.218	3.6	152
B. brizantha (16289)	24.8	0.11	0.024	0.863	0.141	0.193	5.6	150
B. brizanhta (16488)	26.3	0.11	0.042	0.600	0.151	0.320	6.4	133
B. brizantha (16135)	26.7	0.19	0.044	0.618	0.133	0.341	5.6	95
B. decumbens (16497)	26.0	0.26	0.057	0.626	0.145	0.200	4.7	116
B. brizantha (6387)	26.5	0.20	0.071	0.608	0.149	0.186	5.6	214
Mean	26.4	0.20	0.044	0.654	0.153	0.255	5.0	152

Table 84. Genotypic variation in green leaf nutrient composition, ash content and total non-structural carbohydrates (TNC) of 13 accessions of A*rachis pintoi* grown during dry season at Atenas, Costa Rica.

Genotype (CIAT number)	Green leaf composition							
	C	N	P	K	Ca	Mg	Ash	TNC
	(%)							(mg/kg)
A.pintoi (17434)	28.8	2.67	0.119	0.959	2.470	0.861	8.9	104
A.pintoi (18744)	26.2	2.62	0.103	0.785	2.423	0.981	9.1	69
A. pintoi (22148)	25.4	2.21	0.069	0.834	2.363	0.534	8.3	139
A. pintoi (22149)	26.3	2.48	0.091	0.929	2.371	0.501	8.8	127
A. pintoi (22150)	26.3	2.16	0.071	1.017	2.307	0.579	8.7	143
A. pintoi (22151)	26.3	2.31	0.076	0.571	2.590	0.710	8.9	64
A. pintoi (22155)	26.0	2.26	0.076	1.284	2.280	0.502	8.8	138
A. pintoi (22156)	-	-	-	-	-	-	-	-
A. pintoi (22157)	26.5	2.62	0.105	1.175	2.193	0.612	9.0	122
A. pintoi (22158)	26.7	2.47	0.086	0.956	2.403	0.765	9.5	143
A. pintoi (22159)	26.6	2.67	0.094	0.811	2.398	0.857	9.4	73
A. pintoi (22160)	27.2	2.35	0.077	0.490	2.511	1.004	9.5	117
A. pintoi (22161)	29.9	2.67	0.112	0.952	2.030	0.933	8.5	95
Mean	26.9	2.46	0.090	0.897	2.362	0.680	9.0	111

Table 85. Genotypic variation in dry leaf nutrient composition, ash content and total nonstructural carbohydrates (TNC) of 13 accessions of *Arachis pintoi* grown during dry season at Atenas, Costa Rica.

Genotype	Dry leaf composition							TNC
(CIAT number)	C	N	P	K	Ca	Mg	Ash	(mg/g)
A. pintoi (17434)	26.2	2.03	0.089	0.681	2.487	0.861	9.2	63
A. pintoi (18744)	28.6	2.15	0.092	0.654	2.886	0.981	10.3	42
A. pintoi (22148)	26.7	1.87	0.060	0.607	2.304	0.534	8.3	90
A. pintoi (22149)	26.3	2.21	0.081	0.544	3.096	0.501	8.2	80
A. pintoi (22150)	27.3	1.79	0.057	0.793	2.337	0.579	9.0	64
A. pintoi (22151)	26.7	1.59	0.045	0.377	3.017	0.710	10.3	61
A. pintoi (22155)	27.1	1.82	0.056	0.968	2.508	0.502	9.2	91
A. pintoi (22156)	26.7	1.90	0.055	0.789	2.524	-	9.1	53
A. pintoi (22157)	24.8	1.97	0.076	0.747	2.760	0.612	9.4	109
A. pintoi (22158)	27.1	2.11	0.088	0.685	2.798	0.765	9.8	82
A. pintoi (22159)	27.3	2.01	0.066	0.585	2.527	0.857	9.5	36
A. pintoi (22160)	28.5	1.99	0.069	0.355	2.598	1.004	9.3	52
A. pintoi (22161)	26.0	2.05	0.074	0.970	2.341	0.933	9.0	134
Mean	26.9	1.96	0.070	0.673	2.629	0.737	9.3	73

Activity 3.3 Shrub legumes with adaptation to drought and cool temperatures

Highlights

- Found significant differences within and between a collection of *Cratylia argentea* accessions and *Leucaena* spp. in quality attributes.
- Accesssions of *Cratylia argentea* with superior performance than *C. argentea* cv. Veraniega identified
- Developed map of potential distribution of *Flemingia macrophylla* in tropical Asia and found genetic variability for forage quality parameters in this legume species.

Progress towards achieving milestones

- **List of new accessions of *Cratylia argentea* and *Leucaena* species with known forage value**
  Our results show considerable variability in growth habit, DM yields, and quality parameters in accessions of *C. argentea*, which open the opportunity for the selection of new cultivars in the near future. Accessions CIAT 18674, 22375, 22406, 22408 and 22409 had higher dry matter yields than CIAT 18516/18668 (cv. Veraniega) in dry and wet seasons. The new accessions have also showed dry tolerance and good re-growth during prolonged dry seasons, which is one of the outstanding characteristics that makes *C. argentea* a valuable forage for dual purpose cattle farms.

  The legume *Leucaena leucocephala* is very well known for its high forage value. However, a great diversity exists within this genus that has not been fully characterized. For instance, our results showed that *L. macrophylla* susp. *nelsonii* OFI 47/85, species commonly found along the coasts of Oxaca and Guerrero in Mexico, showed high CP content (28.3%), and acceptable IVDMD (62.2%), indicating that it deserves to be evaluated with animals in futures studies.

- **List of *Flemingia macrophylla* accessions characterized for yield and quality**
  Results indicate that several accessions have superior dry matter yield and better digestibility than the control CIAT 17403. The most promising accession (CIAT 21090) will be multiplied for further

testing. Studies to better understand the difference in digestibility among *Flemingia* accessions were initiated and these studies will be complemented with palatability trials.

3.3.1 Characterization of a core collection of *Cratylia argentea* and *Leucaena* sp. in a subhumid environment of Costa Rica

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Rationale

One of the limitations of *Leucaena leucocephala* is its limited adaptation to acid soils and to pest such as psyllid. On the other hand, *C. argentea*, a shrub native to the South American tropics, has shown good adaptation to acid soils, and excellent tolerance to prolonged dry periods. Thus, we were interested in characterizing core collections of *C. argentea* and *Leucaena* for yield and quality attributes in a site characterized by having a long dry season and acid soils of medium fertility.

Materials and Methods

Leaves and young stems (edible forage) were harvested from 18 lines of *Leucaena* spp. and 30 lines of *Cratylia argentea* planted in Atenas, Costa Rica, that had been under cutting evaluation for 2 years. The site is located in a subhumid environment with a total annual rainfall of 1600 mm, and 5 to 6 months dry from December to May. The soils are Inceptisol of medium fertility with pH 5.0, and low P and low aluminum content. The samples were dried in an air forced oven set at 60 °C for 72 hours, and then ground to less than 5-mm particles. Dry matter (DM) and protein content (CP) were determined using standard AOAC procedures. Neutral-detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest and Roberston (1979), while invitro dry matter digestibility (IVDMD) was determined as described by Tilley and Terry (1963).

Results and Discussion

Results on quality of *C. argentea* and *Leucaena* spp. are presented in Table 86 and Table 87, respectively. The mean CP was higher in *Leucaena* sp. (mean of 23.3%) compared to *Cratylia* (mean of 17.7%). *L. macrophylla* subsp. *nelsonii* OFI 47/85 had the highest value of CP (28.3%), while *L. pulverulenta* OFI 83/87 had the lowest (17.2%).

In general, variation of CP content within *Cratylia argentea* accessions was less than in the *Leucaena* species, which indicates more genetic variability within the latter group. On the other hand, *C. argentea* CIAT 22382 had the highest CP content (19.4%), and *C. argentea* CIAT 22389 the lowest CP value (15.1%).

The NDF contents for both *Cratylia* and *Leucaena* accessions are within the range reported in the literature for tropical tree legumes. The NDF fraction varied from 43-80% for the *Leucaena* species, and from 62-77% for *C. argentea*. Considerable interspecific variation in NDF content existed within the species of *Leucaena*, evaluated with *L. macrophylla* subsp. *nelsonii* OFI 47/85 showing the highest content of NDF (80%) while *L. diversifolia* subsp. *diversifolia* OFI 83/92 the lowest (43%). There was little variability of NDF values within the *Cratylia* group, indicating a good degree of genetic uniformity for the 30 accessions evaluated.

Table 86. Quality components of *Cratylia argentea* accesions established in the subhumid enviroment of Atenas, Costa Rica.

CIAT No.	DM (%)	CP (%)	NDF (%)	ADF (%)	IVDMD* (%)
22374	34.2	15.8	68.6	52.7	54.0
22386	30.8	16.8	69.9	50.2	60.9
22379	31.6	16.6	69.5	50.9	50.5
22382	33.0	19.4	67.3	46.4	64.9
22381	31.0	18.8	67.7	51.5	56.5
22375	32.8	15.7	66.5	49.9	54.1
22389	34.0	15.1	68.7	48.6	55.7
(BRA 000621)	42.1	18.2	71.1	57.0	-
22391	33.3	16.6	61.6	44.9	51.3
(BRA 000876)	31.6	19.3	62.3	45.3	61.6
22393	31.9	18.4	63.6	44.2	65.0
22378	32.3	18.5	68.1	46.4	58.8
(Yacapani)	32.9	18.6	64.4	43.2	55.8
22380	34.3	17.5	68.9	50.6	53.1
22385	37.2	17.1	65.4	45.6	54.8
22383	32.2	16.2	69.7	47.8	53.7
22384	34.5	16.1	65.8	48.2	57.2
22395	33.8	19.0	67.7	48.4	55.1
22373	32.1	18.6	67.6	52.2	57.1
22390	32.2	18.7	66.6	47.7	50.3
22392	31.2	19.0	64.2	44.8	57.3
22387	32.0	17.5	66.4	49.7	-
22394	34.2	18.4	67.9	48.7	60.3
(BRA 000884)	32.8	19.3	65.9	42.8	62.5
22388	31.2	18.2	68.4	48.4	58.9
(BRA 000604)	37.3	17.8	65.3	46.5	55.4
(BRA 000841)	34.5	18.4	65.6	47.1	55.9
22396	32.5	19.0	64.6	47.1	47.8
22377	33.8	17.9	67.3	54.4	48.1
22376	32.0	17.3	76.7	48.6	57.4
Mean	33.2	17.7	69.1	48.1	55.9
Sd	2.3	1.2	2.9	3.2	4.4

*Quality components of eatable forage (leaves and young stems) 8 weeks old

The mean level of ADF was slightly higher in *Cratylia* than in the *Leucaena* accessions, but within the range expected for tropical legumes. On the other hand, DM digestibility was higher, and more variable in *Leucaena* (range from 50-84%), compared to *Cratylia* (range from 48-65%).

Forage quality parameters of *C. argentea* cv. Veraniega (CIAT 18516/18668) at 90 days of re-growth, are within the range observed in the core collection of *C. argentea* accessions evaluated. On the other hand, *L. leucocephala* subsp. g*labrata* cv. Taramba en Australia (OFI 34/92), showed high CP (33%) and IVDMD (65%), indicating a high potential feed value; however, this line is susceptible to the psyllid, which may limit its commercial use in sites with high incidence of the insect.

Table 87. Quality components of *Leucaena* species established in the subhumid environment of Atenas, Costa Rica.

Species	ID No. (OFI)	DM (%)	CP (%)	NDF (%)	ADF (%)	IVDMD* (%)
L. trichanda	53/88	29.2	24.9	50.6	46.6	51.8
L collinsii	52/88	29.5	26.6	45.1	35.5	84.0
L. leucocephala subsp. *glabrata*	34/92	32.6	23.8	65.7	40.3	64.6
L. pallida	14.96	30.6	22.9	70.6	53.5	53.4
L. hybrid	1/95	31.8	21.3	63.3	42.3	-
L. macrophylla subsp. *nelsonii*	47/85	43.4	28.3	80.2	45.4	62.2
L. leucocephala CIAT	17263	31.4	24.3	46.9	41.6	71.5
Leucaena hybrid	52/87	32.3	25.0	52.3	41.3	-
L. salvadorensis	17/86	35.9	22.4	49.7	38.5	69.9
L. lanceolata	43/85	33.8	24.2	46.4	41.1	73.3
L. diversifolia subsp. *diversifolia*	83/92	30.1	26.1	43.4	38.0	55.7
L. pallida	79/92	31.4	24.4	55.2	49.6	50.1
L. esculenta subsp. *esculenta*	47/87	33.6	20.7	68.0	40.1	60.6
L. pulverulenta	83/87	38.0	17.2	62.7	47.4	73.1
L. collinsii subsp. *zacapana*	56/88	30.5	21.7	52.3	46.3	70.9
L. lempirana	6/91	31.3	24.5	47.6	36.0	80.7
L. shannonii subsp. *magnifica*	19/84	31.2	23.5	51.0	39.9	75.7
L. trichodes	61/88	32.3	22.7	55.9	45.2	66.6
Mean		32.4	23.3	54.3	42.2	64.9
Sd		3.4	2.2	10.2	4.8	10.2

*Quality components of eatable forage (leaves and young stems) 8 weeks old

3.3.2 Genetic diversity in the multipurpose shrub legumes *Flemingia macrophylla* and *Cratylia argentea*

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CIAT projects: SB-2

Rationale

Work on shrub legumes in CIAT, emphasizes the development of species to be utilized as feed supplement during extended dry periods. Tropical shrub legumes of high quality for better soils are readily available, but germplasm with similar characteristics adapted to acid, infertile soils is scarce. Shrub legume species, such as *Flemingia macrophylla* and *Cratylia argentea* are well adapted to low fertility soils and to prolonged drought, respectively. Thus, work on these genera is of high priority in CIAT's Forage Project.

In order to define the extent of genetic diversity within ex-situ collections of *F. macrophylla* and *C. argentea* we initiated a project with three main objectives. (a) to identify new, superior forage genotypes based on conventional germplasm characterization/evaluation procedures (morphological and agronomic traits, forage quality parameters, including IVDMD and tannin contents), and (b) to optimize the use and management, including conservation, of the collections. To accomplish these objectives, different approaches are being used with a core collection: (a) genetic diversity assessment by a germplasm origin information; and (b) molecular markers (AFLPs). This information should also be useful to define future collection needs in terms of geographical focus.

Materials and Methods

Agronomic characterization and evaluation: Spaced-plants of *Cratylia argentea* (39 accessions) and *Flemingia macrophylla* (73 accessions) were established in Quilichao in March 1999 (Photo 13) and March 2000 (Photo 14), respectively. Additionally two replications were sown for morphological characterization and for seed production. The following parameters are being measured: vigor, height and diameter, regrowth, seasonal dry matter yield during incidence of diseases, pests and mineral deficiencies.

Photo 13. *Cratylia argentea* at Quilichao

Photo 14. *Flemingia macrophylla* accession at Quilichao

For the morphological evaluation, qualitative and quantitative parameters are recorded, such as days to first flower, days to first seed, flower color, flowers per inflorescence, flowering intensity, pod pubescence, seeds per pod, seed color, branching capacity, leaf length and width, peduncle length, etc. To assess nutritive value, we are measuring crude protein (CP) and in vitro dry-matter digestibility (IVDMD) in leaf samples of the two collections.

For *F. macrophylla*, a more detailed chemical analysis will be conducted on a representative subset which will include accessions with high intermediate and low CP and IVDMD. Other chemical analysis in selected accessions of *F. macrophylla* will include NDF, ADF, condensed tannins calcium, and phosphorus.

Analysis of available origin information: Based on geographical information on the site of origin of accessions, a core collection will be created under the assumption, that geographic distances and environmental differences are related to genetic diversity. The analysis will be conducted with FloraMap^{TM}, a GIS tool developed by CIAT

Genetic analysis by molecular markers (AFLPs): Genetic variability will also be assessed through AFLP molecular markers. Based on the results of molecular markers group of accessions will be formed, using multivariate statistic tools.

Data analysis and synthesis: Individual and combined analyses of all data generated will be performed, including the use of GIS tools and multivariate statistics. In the analysis of each of the different approaches (agronomic characterization, origin information, molecular marker analysis), Principal Component Analysis and Cluster Analysis will be utilized to assist in the formation of core collections. Correlation between the different approaches and clusters obtained will also be determined.

These results are expected to help in deciding which of the three methods or which combination is most appropriate (time and cost efficiency) to create a core collections. For example, if an agronomic evaluation of the entire collection is not feasible because of time constraints, a core collection may be created using origin information and/or molecular markers. In addition, based on similarity of molecular marker and GIS analysis, we hope to provide information that will be useful for defining future collections on areas with particularly high diversity. Accession duplicates in the world collections will also be identified.

Results and Discussion

Based on the origin of existing germplasm accessions (73), the potential natural distribution of *F. macrophylla* extends throughout vast areas of tropical Asia (Photo 15). This map, however, is not to be considered as a 'prediction' of the probable natural distribution of *F. macrophylla* but merely as an indication of conditions based on climate and latitude/longitude matching those of the germplasm collection sites.

Agronomic characterization and evaluation: Results from one evaluation in the dry season and one in the rainy season show that there is considerable phenotypic and agronomic variation in the collection of *Cratylia argentea* evaluated (Table 88) and *Flemingia macrophylla* (Table 89). In the case of *C. argentea* mean dry matter production was 45 g/plant in the wet and 60 g/plant in the dry season, whereas IVDMD varied between 61 and 67% and CP content between 18 and 21%.

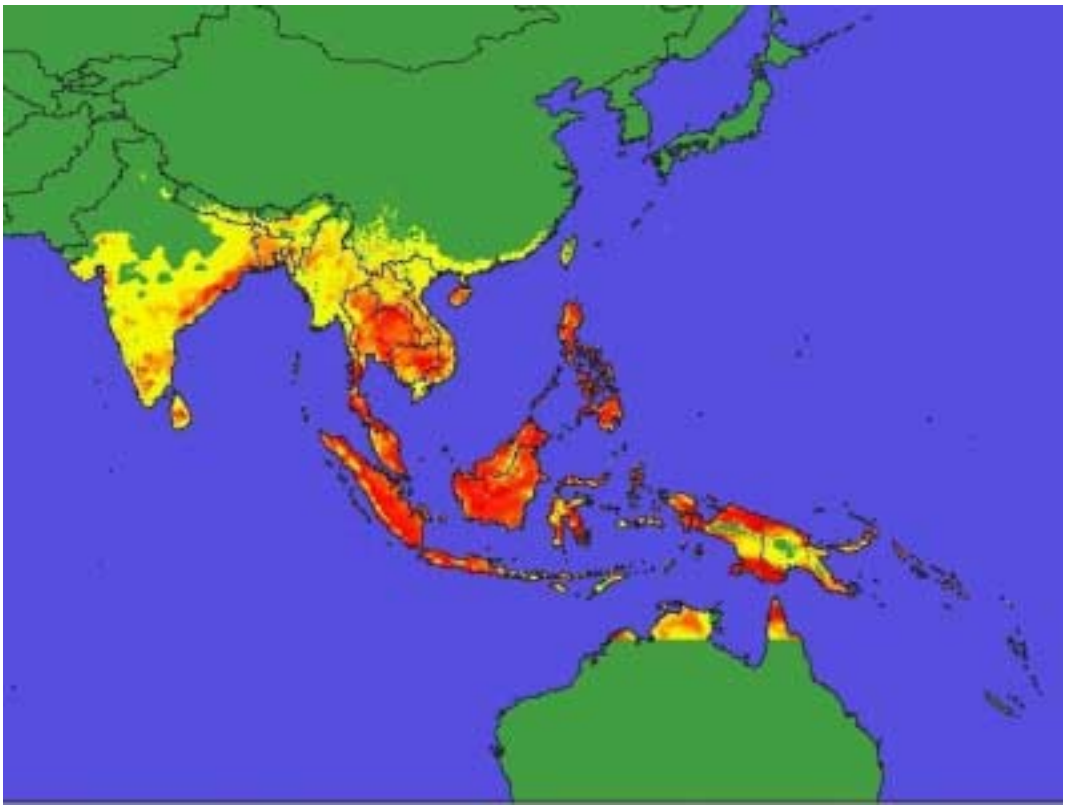

Photo 15. FLORAMAP analysis of potential natural distribution of *Flemingia macrophylla* in tropical Asia, based on passport data of the world germplasm collection maintained at CIAT.

Principal component analysis performed with the agronomic data of 39 accessions of *C. argentea* revealed high correlations between total dry matter production, diameter, regrowth points and vigour. Cluster analysis resulted in 9 groups and 5 of the clusters contained only one accession, among them three of the most productive accessions (CIAT 18674, 22406 and 22408).

Based to these initial results, we have pre-selected *C. argentea* accessions CIAT 18674, 22375, 22406, 22408 and 22409 given that productivity of these accessions is higher than the genotypes released in Costa Rica (an accession mix of CIAT 18516/18668) as cv. Veraniega.

In the case of *F. macrophylla* the average dry matter production was 60 g/plant in the wet and 42 g/plant in the dry season. The most productive accessions were CPI 104890, CIAT 21090, 21241, 21529 and 21580 with a total dry matter production >100 g/plant. We also found high variability in IVDMD (31 to 51%) and CP (16 to 24%), which is an interesting results since one limitation of *F. macrophylla* is low feed value.

Principal component analysis performed with the agronomic data of 73 accessions of *F. macrophylla* revealed high correlations between total dry matter production, plant height and diameter and vigour (>70%). Cluster analysis (UPGMA) resulted in 7 clusters and 2 of the clusters contained only one accession, among them one of the most productive accessions (CIAT 21090). Based on these results we selected the *F. macrophylla* accession CIAT 21090 (semi-erect type, high forage yield and quality for seed multiplication and evaluation with animals.

Table 88. Agronomic evaluation of a collection of *Cratylia argentea* in Quilichao. Preliminary data of four cuts (two in the dry season and two in the wet season).

Treatment No. CIAT	Height (cm)	Diameter (cm)	Regrowing points (No.)	Mean dry matter yields (g/pl)			IVDMD (%)	Crude protein (%)
				Wet	Dry	Mean		
18516	112	105	19	55	78	66	65.0	20.7
18667	112	101	18	45	68	56	64.6	20.4
18668	106	110	17	48	68	58	65.2	19.9
18671	111	106	20	54	55	54	64.3	18.3
18672	96	83	13	34	39	37	62.1	20.1
18674	118	122	23	91	109	100	63.9	20.0
18675	112	97	15	47	63	55	63.3	19.0
18676	105	93	14	46	50	48	61.2	19.7
18957	111	102	16	50	76	63	62.5	20.1
22373	109	93	15	38	57	48	64.4	20.2
22374	116	102	17	55	71	63	66.4	19.6
22375	125	98	16	59	76	68	67.0	21.2
22376	95	70	11	23	36	29	64.1	19.6
22378	103	81	12	34	39	36	61.7	19.8
22379	111	89	16	47	65	56	63.5	19.6
22380	107	90	11	31	43	37	61.3	20.4
22381	105	85	11	34	46	40	64.0	19.1
22382	110	92	12	41	62	52	64.2	20.4
22383	99	90	13	34	43	39	62.6	18.6
22384	113	91	9	43	47	45	64.5	18.9
22386	111	86	12	39	47	43	64.7	18.6
22387	111	90	12	41	57	49	62.5	19.1
22390	99	92	13	45	47	46	64.8	18.5
22391	108	96	15	44	62	53	63.4	18.9
22392	114	83	13	33	53	43	63.2	21.0
22393	110	92	17	41	58	49	63.5	20.6
22394	112	88	13	33	46	40	64.0	20.5
22396	101	79	10	30	43	36	63.8	21.3
22399	102	86	13	35	42	39	66.1	19.8
22400	119	104	16	52	74	63	61.7	20.7
22404	110	97	13	42	68	55	67.0	20.9
22405	111	96	16	41	61	51	62.9	19.9
22406	113	112	20	63	86	74	62.6	21.0
22407	111	99	16	46	59	53	65.3	20.8
22408	120	109	18	69	88	79	67.2	20.1
22409	113	115	17	57	81	69	66.5	21.2
22410	116	96	14	42	60	51	64.3	19.8
22411	103	88	14	37	58	47	64.5	20.2
22412	116	90	11	42	63	52	64.9	18.7
Mean	110	95	15	45	60	52	64.1	19.9
Range	95-125	70-122	9-23	23-91	36-109	29-100	61-67	18-21

Table 89. Agronomic evaluation of a collection of *Flemingia macrophylla* in Quilichao. Preliminary data of two cuts (one in each season). Growth habit: =erect, s=semierect, p=prostrate.

Treatment No. CIAT	Height (cm)	Diameter (cm)	Regrowing Points (No)	Mean dry matter yields (g/pl)			IVDMD (%)	Crude Protein (%)
				Wet	Dry	Mean		
J 001 (e)	125	85	30	102	58	80	40.1	22.3
801 (e)	125	90	29	103	62	82	36.3	22.9
7184 (e)	124	95	34	101	82	92	34.0	21.4
C 10489 (e)	108	99	34	121	79	100	33.6	22.7
I 15146 (e)	98	70	24	103	58	80	39.9	22.9
17400(s)	63	98	33	55	52	53	33.2	21.5
17403 (s)	67	96	32	68	57	62	35.8	22.2
17404 (s)	58	79	32	46	45	45	32.9	22.5
17405 (s)	65	94	36	71	67	69	36.1	21.9
17407 (s)	78	106	39	87	62	74	32.8	21.9
17409 (s)	56	109	35	87	66	77	33.0	20.2
17411 (s)	55	86	33	56	54	55	35.5	22.4
17412 (s)	73	96	39	61	63	62	38.6	20.2
17413 (s)	58	93	35	51	39	45	35.2	20.1
18048 (s)	32	43	19	12	8	10	42.8	20.4
18437 (s)	54	101	37	57	55	56	47.8	22.5
18438 (s)	58	71	31	36	22	29	51.5	23.5
18440 (s)	59	87	38	65	44	55	33.4	21.4
19453 (e)	105	78	20	65	33	49	36.0	21.6
19454 (e)	115	82	24	73	52	63	39.1	19.7
19457 (e)	116	85	25	52	64	58	33.1	21.3
19797 (s)	57	90	22	58	46	52	38.5	21.0
19798 (s)	55	95	27	61	55	58	38.3	20.9
19799 (s)	50	69	19	28	39	33	37.3	21.7
19800 (s)	65	85	29	34	48	41	32.0	20.7
19801 (s)	82	91	40	68	57	63	35.7	21.7
19824 (e)	62	93	35	54	61	58	35.6	21.3
20065 (p)	15	21	4	0	1	1	32.1	18.9
20616 (s)	67	108	34	86	54	70	32.1	22.0
20617 (s)	72	92	27	51	44	48	30.6	20.1
20618 (s)	74	95	31	57	60	58	33.2	21.6
20621 (e)	84	88	32	58	54	56	31.6	21.6
20622 (e)	146	88	30	105	76	91	42.8	22.9
20624 (s)	74	122	39	101	91	96	34.5	19.8
20625 (e)	128	86	26	105	69	87	42.5	22.8
20626 (e)	115	92	28	88	70	79	39.5	22.3
20631 (e)	121	90	25	97	75	86	41.5	20.9
20744 (e)	125	87	27	102	65	84	42.9	23.1
20972 (p)	24	56	31	12	14	13	39.6	23.4
20973 (p)	24	45	17	4	10	7	34.2	19.6
20975 (s)	52	83	45	44	24	34	45.3	20.3
20976 (s)	45	57	27	17	11	14	40.8	20.0
20977 (s)	33	35	9	5	4	4	46.1	18.5
20978 (s)	52	56	24	21	11	16	46.6	22.1

Continues…..

Table 89. Agronomic evaluation of a collection of *Flemingia macrophylla* in Quilichao. Preliminary data of two cuts (one in each season). Growth habit: =erect, s=semierect, p=prostrate.

Treatment No. CIAT	Height (cm)	Diameter (cm)	Regrowing Points (No)	Mean dry matter yields (g/pl)			IVDMD (%)	Crude Protein (%)
				Wet	Dry	Mean		
20979 (s)	48	76	38	27	25	26	38.9	21.2
20980 (s)	43	55	26	27	18	22	41.8	21.0
20982 (s)	49	61	28	26	23	25	41.0	19.9
21079 (s)	47	78	44	51	25	38	37.9	20.2
21080 (s)	41	58	13	32	11	21	39.2	15.5
21083 (e)	93	79	36	71	43	57	45.8	21.4
21086 (s)	27	29	4	NA	3	3	-	-
21087 (s)	64	66	46	47	32	39	42.3	20.4
21090 (s)	88	106	48	135	66	100	50.0	21.3
21092 (s)	72	81	23	57	39	48	49.2	18.0
21241 (e)	133	93	27	134	66	100	36.2	20.2
21248 (e)	127	92	30	106	77	91	33.5	23.6
21249 (e)	129	104	34	167	85	126	40.9	22.0
21519 (e)	127	101	28	109	67	88	39.5	22.3
21529 (e)	132	102	31	145	71	108	42.0	23.1
21580 (e)	131	101	32	184	86	135	39.1	19.8
21982 (p)	19	62	38	26	11	19	42.1	20.9
21990 (p)	35	66	43	27	19	23	31.9	19.1
21991 (p)	29	52	24	13	10	11	37.5	22.6
21992 (p)	29	50	24	12	9	11	48.5	20.2
21993 (s)	42	77	45	34	24	29	43.9	19.9
21994 (p)	24	42	9	8	7	8	34.5	16.4
21995 (p)	29	50	26	11	8	9	40.8	19.6
21996 (p)	23	44	14	7	6	6	42.5	21.8
22058 (e)	84	58	13	41	29	35	37.2	18.5
22082 (s)	79	82	58	69	37	53	48.4	20.0
22087 (p)	27	51	17	15	4	10	40.3	17.8
22090 (s)	44	47	10	10	5	7	41.1	17.5
22285 (s)	43	75	42	32	21	27	38.7	20.4
22327 (s)	41	62	33	20	21	21	48.4	19.3
Mean	70	78	29	60	42	51	39.0	20.9
Range	15-146	21-122	4-58	0-184	1-91	1-135	31-51	16-24

Genetic analysis by molecular markers (AFLPs): Samples of 5 g of young leaves were taken of all *C. argentea* and *F. macrophylla* accessions and DNA was been extracted and quantified. To identify efficient primers for the AFLP analysis, 2 supposedly genetically contrasting accessions of each *F. macrophylla* and *C. argentea* (CIAT 21990 and 21529, and CIAT 18672 and 18516 respectively) were tested with different primer combinations and the resulting polymorphic bands were counted (Table 90).

Table 90. Polymorphic bands of different primer combinations for *Flemingia macrophylla* (accessions CIAT 21990 and 21529) and *Cratylia argentea* (accessions CIAT 18672 and 18516).

Primer combination	Polymorphic bands		
	F. macrophylla	*C. argentea*	Total
E-AAC / M-CAA	n.a.	n.a.	n.a.
E-AAG / M-CAA	n.a.	n.a.	n.a.
E-AAG / M-CAT	28 / 24	2 / 4	58
E-ACA / M-CAT	18 / 20	7 / 9	54
E-ACA / M-CTG	15 / 8	4 / 5	32
E-ACT / M-CTG	13 / 8	4 / 5	30
E-ACC / M-CAG	20 / 15	9 / 8	52
E-ACG / M-CAG	16 / 24	2 / 21	62
E-ACG / M-CAC	26 / 24	19 / 12	81
E-AGC / M-CTA	11 / 18	3 / 3	35
E-AGG / M-CTC	24 / 21	9 / 12	66
E-AAC / M-CTT	45 / 20	18 / 3	86

3.3.3 Agronomic characterization of a collection of *Rhynchosia schomburgkii*

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Rationale

From the evaluation of a range shrub legumes with tolerance to cool temperatures *Rhynchosia schomburgkii* emerged as one of the most promising species for higher altitude hillsides. Thus, we were interested in characterizing its potential feed value (Photo 16).

Photo 16. *Rhynchosia schomburgkii* at Quilichao

Materials and Methods

A total of 13 accessions of *Rhynchosia schomburgkii*, mostly originating from Colombia, were planted at Quilichao. Plants were transplanted into single-row plots, with 4 replications. Dry matter yield, drought tolerance and forage quality are the main parameters being measured.

Results and discussion

Results from last year had indicated that of the 13 accessions evaluated, CIAT 17918, 22134, 918 and 19235 showed the highest yields. This year we were interested in measuring quality parameters in the collection of *R. schomburgkii* as affected by seasonal variation.

Results indicated that during the season with maximum rainfall, there were differences among accessions for IVDMD but not for CP (Table 91). In the drier period no significant (P>0.05) differences among accessions in terms of quality were recorded. However, season had a large effect on digestibility, but the effect was not the same for all accessions.

Table 91. Fodder quality of accessions in a collection of *Rhynchosia schomburgkii* grown in Quilichao in Minimum and Maximum precipitation.

Accession	Season					
	Minimum		Maximum			
	IVDMD	CP	IVDMD	CP	Tannins	
					Soluble	Bound
8582	42	22	42	20	4.81	0.78
19235	39	22	40	22	3.68	0.40
20800	38	21	38	21	2.49	0.84
17918	38	19	49	22	3.06	0.73
20456	38	19	52	23	3.31	0.68
22134	37	20	42	22	3.69	4.55
7389	36	22	44	23	2.95	0.67
7810	36	20	41	22	5.23	0.72
18490	36	21	40	22	3.44	0.92
918	31	19	38	22	5.85	0.72
LSD	NS	NS	5.7 (P <0.001)	NS		

The concentration of condensed tannins measured in the wet season was relatively low (2.5 to 5.8%) and not as variable as IVDMD and CP.

In general, our results show that, in the small collection of *R. schomburgkii* evaluated there is limited variability in CP and IVDMD which limits the scope for selecting genotypes based on quality.

Activity 3.4 Selection of legumes for multipurpose use in different agroecosystems

Highlights

- Accessions of *Vigna unguiculata* with specific adaptation to acid or neutral soil and more broadly adapted accessions identified

- Research on *Vigna unguiculata* carried to Honduras and Nicaragua, Participatory evaluations in preparation
- *Lablab purpureus* accessions with outstanding performance on neutral soils identified

Progress towards achieving milestones

- **Suitability of *Vigna unguiculata* for acid and neutral soils defined**
  Accessions were identified with specific and broad adaptation to variable soil pH and fertility conditions.

- **List of accessions of *Vigna unguiculata* for use as feed and/or green manure in Central America**
  A core collection of *Vigna unguiculata* from IITA is now in Honduras and Nicaragua, for participatory evaluation. Seed multiplication of promising accessions is underway in Costa Rica.

- **Results on characterization of a core collection of *Lablab purpureus* in acid and neutral soils**
  The *Lablab purpureus* accessions evaluated were well adapted to acid low fertility soils, eventhough productivity was much lower than on neutral higher fertility soils. However, there is intra-specific variation in adaptation to soil and climate conditions. Some accession have more specific adaptation while other accessions showed a more broad adaptation. The next step is to carry out more detailed studies with a limited number of accessions, focusing on small farmers in Colombia and Central America. For comparison, we are trying to obtain seed of available commercial cultivars (cv. Endurance Rongai, Highworth and Koala) from Australia.

3.4.1 Evaluation of a core collection of *Vigna unguiculata* for multipurpose uses in Colombia, Nicaragua and Honduras

Contributors: M. Peters, Luis H. Franco, A. Schmidt, H. Cruz Flores, P. Avila, G. Ramírez, B. Hincapié, (CIAT), and B.B. Singh (IITA, Nigeria)

CIAT projects: PE-2, PE-3

Quilichao and Palmira

Rationale

Cowpea (*Vigna unguiculata*) is utilized in the subhumid/semi-arid tropics of West Africa and India as a source of food and feed for livestock. Work of CIAT with a limited number of accessions had indicated potential of cowpea for soil improvement, but the utilization of cowpea in Latin America is so far limited. We visualize that, cowpea could be an alternative crop for the second planting season in the central hillsides region of Nicaragua and Honduras where the legume could provide not only higher grain yields as compared to common beans, but could also allow for a third crop in November/December in order to provide hay as animal feed in the dry season or contribute to soil fertility enhancement for the following maize crop.

Adaptation to climatic and edaphic conditions, especially to water stress, are prerequisites for a successful development of a cowpea option for the traditional maize-bean cropping systems in Central America. It remains to be seen if cultural traditions allow for the inclusion of cowpeas in the daily menu of people in Central America.

A) Evaluation of cowpea in Quilichao and Palmira, Colombia

Materials and Methods

A core collection of 15 cowpea accessions was obtained from Dr. B.B. Singh, cowpea breeder of IITA and complemented with two local accessions from Colombia (cultivar Sinu) and Brazil (cultivar Verde Brasil). After initial experiments on acid soils (Annual report 2000), these accessions were again planted at CIAT's Quilichao Research Station. Accessions were evaluated for grain and forage yield and their value as green manure for a succeeding maize crop (Photo 17).

Photo 17. *Vigna unguiculata* in grass production phase at Quilichao

Results and Discussion

In the Quilichao site, all accessions established rapidly, reaching soil covers of >80%, 8 weeks after planting. At the time of incorporation into the soil (9 weeks after planting) all accessions were well established and vigorous. No significant differences ($P>0.05$) were found among accessions for DM yields (Table 92).

However, significant differences ($P<0.05$) were found in maize dry matter production and in grain yield following the incorporation of cowpea accessions. Highest maize yields were recorded after green manuring with IT93K-573/5, with yields being 3.6 t/ha grain and almost 9 t of dry matter. In contrast, with no N grain and dry matter yields were 1.5 t and 4.1 t, respectively. Fertilizations higher than 80 kg N had a negative effect on maize grain and dry matter yields.

Results confirm data obtained in the initial experiments (AR 2000). All green manure treatments except IT96D-759 led to higher maize yields than obtained with any level of nitrogen fertilizer applied.

Table 92. 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 in Quilichao, 2nd phase.

Accessions	Cowpea	Maize	
	Herbage	Grain	DM Total
	(kg/ha)		
IT93K-573/5	3180	3619	8882
IT90K-284/2	2187	3558	8442
IT89KD-391	2387	3382	7576
IT95K-1088/4	2033	3350	7765
IT86D-716	2293	3290	8433
IT95K-1088/2	2213	3255	7779
IT86D-715	3313	3280	8308
IT6D-733	2867	3192	8993
IT96D-740	1940	3104	7321
IT90K-277/2	1913	3067	7520
IT93K-503/1	2047	2868	7219
IT86D-719	2393	2803	8238
IT93K-637/1	2613	2636	6338
IT89KD-288	3513	2558	6728
IT96D-759	1047	2331	5194
80N	-	2405	5213
160N	-	2104	4337
200N	-	2094	4360
0N	-	1487	4105
40N	-	1478	3577
120 N	-	1330	3785
LSD (P<0.05)	NS	1862	3804

Forage quality of cowpea accessions in terms of CP, lignin, digestibility, P and Ca concentrations varied among accessions (Table 93). Nevertheless, with CP concentrations of 14-21 % and a digestibility of dry matter of 80% or more cowpea is also an excellent fodder for livestock (Table 93).

Table 93. Fodder quality in accessions of *Vigna unguiculata* (cowpea) grown in Quilichao.

Accessions	Forage					Grain		
	Protein	IVDMD	Lignin	P	Ca	N	P	K
				%				
IT86D-715	21	80	4.5	0.14	2.1	4.19	0.36	1.22
IT90K-277/2	19	82	2.5	0.12	2.1	3.00	0.28	1.00
IT93K-573/5	19	82	2.7	0.13	1.5	3.71	0.30	1.13
IT96D-740	18	83	5.9	0.13	1.6	3.20	0.33	1.20
IT90K-284/2	18	81	2.4	0.13	1.6	3.41	0.36	1.28
IT96D-733	17	84	4.4	0.12	1.5	3.37	0.33	1.22
IT86D-719	17	83	4.2	0.11	1.8	3.58	0.35	1.25
IT93K-673/1	17	85	2.7	0.13	1.5	3.47	0.34	1.25
IT93K-503/1	17	83	2.1	0.12	1.3	3.16	0.31	1.17
IT95K-1088/2	16	85	4.5	0.11	1.4	3.39	0.35	1.18
IT89KD-391	16	82	1.7	0.10	2.1	3.28	0.33	1.27
IT95K-1088/4	16	84	3.4	0.14	1.6	3.42	0.36	1.27
IT89KD-288	15	85	2.6	0.09	1.4	3.47	0.30	1.16
IT86D-716	14	86	5.6	0.10	1.3	3.78	0.35	1.3
LSD (P<0.05)	3.1	2.66	1.2	0.02	0.61			

In a 3rd phase, cowpea accessions were sown in the same season in Quilichao and Palmira to compare the effect of climate and soil on performance and possibly identify accessions with broad adaptation, which is key for Central American Hillsisdes with highly variable soil and climatic conditions.

The establishment of the accessions of cowpea included in the trial was slower in Palmira than in Quilichao, due to higher incidence of insects and weeds. In Quilichao, the incidence of pest and diseases was minimal, with the exception of a localized incidence of ants.

Results showed that no differences in DM yields among accessions in the two sites (Table 94). However, mean dry matter yield in Quilichao (2229 kg/ha) was 30% higher than in Palmira (1752 kg/ha). In addition, we observed a G x E interaction in performance of accessions tested.

For example, accessions IT86D-715 and IT89KD-391 had high DM yields on the acid soils in Quilichao, but were among the lowest yielding accessions on the more neutral fertile soils in Palmira. However, other accessions such a IT95K-1088/4 had high dry matter yields in the two sites.

Table 94. Dry matter yield (kg/ha) of cowpea green manure herbage and grain before soil incorporation en Quilichao and Palmira, 2001.

Accessions	Quilichao	Palmira
	DM Herbage (kg/ha)	
IT86D-715	3147	1280
IT89KD-288	2653	1873
IT6D-733	2567	1627
IT89KD-391	2413	1187
IT95K-1088/4	2373	2480
IT93K-503	2353	1627
IT96D-740	2230	1947
IT90K-277/2	2220	1307
IT86D-716	2187	1807
IT90K-284/2	2080	1900
IT93K-573/5	1993	1493
IT95K-1088/2	1827	2040
IT93K-637/1	1813	1927
IT86D-719	1773	2326
LSD (P<0.05)	1,130	1,252

B) Evaluation of cowpea in Nicaragua

Materials and Methods

A core collection of 19 accessions of *Vigna unguiculata* (Table 95) was established at the SOL SECO site (the Spanish acronym for Supermarket of technologies for hillsides – dry) in San Dionisio, Matagalpa, Nicaragua. The accessions were replicated three times in a randomized block design. Plots measured 5 x 2.5 m and seeds were sown at a distance of 0.25 m within a row and 0.5 m between rows.

Table 95. Accessions of *Vigna unguiculata* sown in San Dionisio/Nicaragua and Yorito/Honduras and Nicaragua as green manures for maize-based systems; *Lablab purpureus* DICTA was sown in Honduras only as a local check.

Accessions	Accessions
Vigna unguiculata IT86D-277/2	*Vigna unguiculata* IT86D-715
Vigna unguiculata IT90K-284/2	*Vigna unguiculata* IT96D-740
Vigna unguiculata IT89KD-391	*Vigna unguiculata* IT95K-1088/4
Vigna unguiculata IT86D-716	*Vigna unguiculata* IT89KD-288
Vigna unguiculata IT93K-503/1	*Vigna unguiculata* IT93K-573/5
Vigna unguiculata IT93K-637/1	*Vigna unguiculata* CIDDICO1
Vigna unguiculata IT86D-719	*Vigna unguiculata* CIDDICO2
Vigna unguiculata IT95K-1088/2	*Vigna unguiculata* CIDDICO3
Vigna unguiculata IT6D-733	*Lablab purpureus* DICTA

Evaluations will include seed emergence, ground cover, plant height, plant vigour, biomass/grain production flowering patterns, and incidence of pest and disease. Local farmers will be invited to participate in the evaluation of the core collections and soil fertility enhancement effects will be measured through the planting of a maize crop at the onset of the next wet season and comparing maize yields with N-fertilized plots.

Expected results

The selection of superior accessions based on agronomic performance on a farmer criteria will provide a clear indication on the potential of *Vigna unguiculata* in farming systems found in Hillsides of Central America. Further evaluations with the selected accessions will be necessary in order to optimize management techniques.

C) Evaluation of cowpea in Honduras

In 2001, an experiment was established in the SOL Yorito to evaluate a core collection of cowpea (Table 96). In this case the experiment was complemented with the addition of *Lablab purpureus* DICTA as a control.

In Yorito, the focus is again on selecting cowpeas for green manures in maize-based systems for soils with neutral to alkaline pH. Significant ($P<0.0001$) differences among accessions were found for DM yield. The highest biomass production was recorded with CIDICCO3 (6.2 t of DM/ha) and IT90K-284/2 (6.1 t of DM/ha).

In general, the ranking of accessions compares favourably with results obtained on neutral soils in Palmira though yields are much higher in Honduras.

Table 96. Dry matter yields of *Vigna unguiculata* (cowpea) genotypes before soil incorporation before a maize crop in Yorito, Honduras.

	DM yield	
	Soil cover (%)	kg/ha
CIDICCO3	93	6212
IT90K-284/2	90	6123
CIDICCO1	83	5282
Lablab purpureus DICTA	85	5230
IT96D-740	72	5112
IT93K-637/1	72	5101
IT86D-716	67	4944
IT95K-1088/2	72	4042
IT95K-1088/4	68	3923
IT6D-733	50	3827
IT93K-503/1	60	3672
CIDICCO2	78	3521
IT93K-573/5	50	2926
IT89KD-391	43	2867
IT89KD-288	53	2734
IT86D-719	38	2381
IT86D-715	37	2175
IT90K-277/2	33	1754
LSD (P<0.05)		2014

3.4.2 Evaluation of core collection of *Lablab purpureus* for multipurpose uses (Quilichao and Palmira)

Contributors: M. Peters, L. H. Franco, B. Hincapié, and G. Ramírez (CIAT)

Rationale

Lablab purpureus is a free seeding, fast growing or short-term perennial legume, with widespread use through the tropics as a fodder plant. In Africa the use of Lablab for human consumption is also common. The origin of the Lablab germplasm currently utilized is mainly Eastern/Southern Africa and Asia. In addition, it is well documented that *Lablab purpureus* is best adapted to lower altitudes and to areas with rainfall regimes of 750–2000 mm/year. This species grows in a variety of soils, but the ideal pH for growing Lablab is reported to be between 5.0 and 7.5.

In order to evaluate the potential of Lablab in tropical America, we obtained a collection available at ILRI/CSIRO. Our main objective with the collection is to select accessions with broad adaptation to different soils and climate conditions in tropical America. However, of immediate interest is to evaluate the Lablab collection in acid and neutral soils to define niches of Lablab for green manure and fodder (especially for hay and silage or deferred feed), with emphasis on Central America where soils are highly variable in pH.

Materials and Methods

A total of 44 accessions of *Lablab purpureus* were initially sown on an acid soil (pH 4.0) in the Quilichao Research Station for seed multiplication. In 2001, 42 and 25 accessions were planted for agronomic evaluation in neutral (Palmira), and acid soil (Quilichao), respectively (Photo 18).

Photo 18. Seed of *Lablab purpureus* at Quilichao

Results and Discussion

Results of the agronomic evaluations are shown in Tables 97 and 98. In Quilichao (Table 97), accessions 14442, I 14411, I 14437, 76996, 21603 and 99985 were the fastest to establish, with soil cover of >95 % and vigour ratings of 4 to 5, 12 weeks after sowing. Of the 25 accessions sown 15 (60%) had a soil cover above the mean of the experiment (87% soil cover). As expected, early flowering accessions were less productive than late flowering accessions.

The highest yields were recorded with accessions CIAT 34777, 52535 and 21603, and the lowest yields were recorded for T 52508, 17192, I-6536 and I-11613. However, it is interesting to note that accession I-6536, which had very low yields at 12 weeks, became one of the most productive accessions, 4 weeks later.

Plant vigour and yields of Lablab in Palmira (Table 98) were higher than in Quilichao. In this site significant ($P<0.05$) differences among accessions were found for DM yield and soil cover. The accessions with fastest soil cover and ability to compete with weeds measured 8 weeks after planting were 14442, I-11630, I-14437, 29398, 76996, 34777, I-6533, I-14411, L-987 and 106494.

In general, the accessions with the best adaptation across different soil and climate conditions were 34777, 96924, 21603, I-11630, I-14411, I-14441, 67639 and 52535.

Table 97. Dry matter yield (kg/ha) and soil cover (%) of *Lablab purpureus* herbage in Quilichao, 2001.

Accessions	Vigour	Cover (%)		DM (kg/Ha)	
	1 a 5	12 Weeks	16 Weeks	12 Weeks	16 Weeks
34777	4	83	50	2447	2153
52535	4	77	57	2440	1973
21603	5	97	90	2327	2367
I-11632	3	73	50	2067	1453
76998	4	93	77	2060	1607
99985	5	95	97	2027	1927
106494	5	87	93	2013	1153
36903	3	73	53	1920	1453
I-14411	5	100	93	1913	1573
100602	4	80	87	1887	1627
I-14437	4	98	83	1853	2447
I-11630	5	70	100	1840	2093
14442	5	100	97	1840	2667
67639	4	93	80	1820	2313
CQ-2975	4	93	87	1807	2046
106548	4	90	93	1793	1833
81626	3	80	60	1707	2073
106500	4	93	90	1640	1560
76996	5	98	90	1560	1873
I-14441	4	90	73	1493	2067
I-6533	3	77	70	1426	1273
I-11613	4	92	93	1280	1387
I-6536	5	93	97	1213	1980
17192	2	80	77	1160	1060
52508	3	67	50	1147	1053
22183				.	1453
LSD (P<0.05)		22.5	16.4	878	NS

Table 98. Dry matter yield (kg/ha) and soil cover (%) of *Lablab purpureus* herbage in Palmira, 2001.

Treatment	Vigour	cover(%)		DM kg/ha	
	1 a 5	8 weeks	13 Weeks	8 Weeks	13 Weeks
35894	2	93	70	3493	9067
I-14437	4	97	97	3280	7760
I-11615	2	87	60	3293	7340
34777	4	97	77	3380	6933
96924	2	83	77	2840	6927
21603	4	93	100	3760	6847
I-11630	5	100	100	4807	6740
2160	3	87	77	2707	6707
I-14411	4	97	93	3080	6587
I-14441	4	87	95	2420	6547
67639	4	87	97	2780	6533
29398	4	97	93	3447	6527
L-987	5	77	100	2367	6407
52535	3	93	73	3360	6340
I-6533	4	97	87	3193	6300
52544	3	87	83	2413	6187
106494	4	97	100	2440	6120
100602	3	87	90	2113	5660
L-1683	5	77	100	2367	5500
76998	4	93	93	3433	5480
76996	4	97	93	3213	5440
81626	2	83	53	2487	5433
CQ-2975	4	80	90	2387	5407
17197	3	57	80	1560	5193
I-6536	3	73	90	2000	5173
I-11613	4	87	90	2780	5027
I-11632	2	83	63	2793	5007
36903	3	93	60	2660	4900
106548	4	77	90	2067	4747
14442	5	100	97	2367	4640
99985	3	90	63	3113	4587
I-6930	2	83	57	2247	4527
52508	2	77	47	2473	3927
22183	4	83	93	2100	3853
106500	3	73	100	1373	3827
69498	2	63	73	1387	3747
17196	2	50	77	867	2987
51564	2	40	73	500	2880
17193	1	43	60	680	2020
17192	1	23	43	393	1147
17195	1	17	73	187	1140
17189	1	20	20	247	853
LSD (P<0.05)		35.2	47.3	2514	5557

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

Activity 4.1 Development of 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

- Confirmed that *Desmodium ovalifolium* CIAT 13651 is an excellent legume cover for rubber and oil palm plantations in the Llanos.
- Observed fast establishment, excellent ground cover and high DM yields with some genotypes of *Vigna* evaluated as green manures in the llanos piedmont.
- Using farmer criteria pre-selected *B. brizantha* 26124 as a suitable alternative for pasture systems in the Llanos of Colombia.
- Selected and multiplied seed of multipurpose forages for degraded Hillsides of Haiti.
- Completed a Technical Bulletin for the release in early 2002 in Costa Rica of *Cratylia* as cv. Veraniega.
- Defined that deficient technical assistance and lack of information by farmers are the major constraints to increase adoption of *Cratylia argentea* and *Arachis pintoi* in Costa Rica.
- Made progress in the development of collaborative forage-based initiatives for Africa.

Progress towards achieving milestones

- ***Cratylia* released and available to farmers in Costa Rica**
  As part of the release process a Technical Bulletin was prepared for distribution to technicians and farmers. The official release by MAG of *Cratylia argentea* cv. Veraniega will now take place in February 2002 in a farm using the forage to supplement dairy cows in the dry season.

- **Suitable legume covers for plantations in the Llanos of Colombia defined**
  We have confirmed that *Desmodium heterocarpon* var. *ovalifolium* CIAT 13651 is a excellent option to recover degraded pastures and to use as cover in rubber and oil palm plantations in the Llanos of Colombia. The task ahead is to promote the use of this legume by graziers and plantation growers through multiplication of seed, and diffusion activities (workshops, field days and technical bulletins).

- **Selected grass and legume species for on-farm testing in Hillsides of Haiti**
  Through agronomic trials carried out in two sites in Haite we selected a range of grasses and legumes (herbaceous and shrubs) for multipurpose in diverse farming systems. To accelerate the diffusion of improved forages selected materials were multiplied and the seed sent to Haiti.

4.1.1 On-going collaboration in forage evaluation with partners

4.1.1.1 Use of Forages for recuperation of degraded areas in hillsides of Colombia

Contributors: FIDAR (NGO lead partner), UMATA, Comite Cafeteros, REVERDECER, CVC, Alcaldia de Restrepo, Universidad de Valle, Comite de Cafeteros, University of Hohenheim

Rationale

The study area located in the north of the 'Valle de Cauca' is 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 fauna and floral biodiversity, lack of vegetative cover and resulting high erosion, and reduced crop yields. Communities at the lower end of the watershed 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 carried out 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 wide adoption and maintenance by the community.

The present collaborative project aims to develop a concerted effort with different actors in the region including the community to reverse the degradation problem in the watershed. 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

Simulation models

In 2000/2001, the calibration of the SWAT (Soil and Water Assessment Tool) was refined, to serve in the future as tool for diagnosis, planning and NRM. Maps for land use were elaborated. The community is familiarized with the tools used

Monitoring and evaluation

Changes in the administration of the municipality have led to changes in key contacts in collaborating institutions. Labor and purchased inputs are being monitored together with the identification of market opportunities with active participation of the community.

Open evaluation with farmers is strengthening the interaction with farmer groups and a process of dissemination of project results was initiated

Cover crops and mechanical erosion barriers established

On an area of 9 ha exposed to severe erosion and 14 ha exposed to moderate erosion, cover crops and mechanical barriers were established in collaboration with farmers. For materials selected by farmers seed banks were set up, one with farmers and one with students in their last year of education; the latter contributes to the environmental education. These activities are accompanied by training activities and facilitation of organization processes. A CIAL is supported with technical assistance.

4.1.1.2 Evaluation of legumes as covers for plantations in the Llanos of Colombia

Contributors: C. Plazas, M. Peters, L.H. Franco, B. Hincapie (CIAT) and Oil Palm and Rubber Growers of the Colombian Llanos

Rationale

There is a need in plantations of the Llanos of Colombia to find sustainable ways to reduce weed infestation, to maintain and improve soil fertility, to control erosion and increase soil 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.

In 1999 a range of legume accessions of the species *Arachis pintoi, Desmodium heterocarpon* subsp. *ovalifolium* and *Pueraria phaseoloides* were sown under shade and no-shade conditions in the Meta department of Colombia. Based on initial results, this work was expanded to include the evaluation of different establishment procedures for the most promising cover (*Desmodium heterocarpon* subsp. *ovalifolium* CIAT 13651) in comparison with the most commonly used cover *Pueraria phaseoloides*.

Materials and Methods

In plots of 80 m^2 we established legumes covers in commercial young and old rubber and oil palm plantations in the savannas and piedemonte areas of the Llanos.

The following legumes were sown in a Randomized Block Design with three replications: *Arachis pintoi*: 17434, 18744, 18748, 22159, 22160 (seed rate 10 kg/ha); *Desmodium heterocarpon* subsp. *ovalifolium* (*D. 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. Measurements carried out include: % cover, DM yield and weeds.

Results and Discussion

In Table 99 we show the effects of different establishment procedures for *Desmodium ovalifolium*. Planting was in August 2000, and measurements were taken 6 months (dry season) and 15 months (wet season) after planting.

Table 99. Soil cover of *Desmodium heterocarpon* subsp. *ovalifolium* (*D. ovalifolium*) in rubber and oil palm (palma) plantations under different establishment procedures in two sites in the Llanos of Colombia.

Treatments	Savanna (rubber)		Piedemont (oil palm)	
	Season			
	Dry	Wet	Dry	Wet
	Cover (%)			
Soil preparation + *D. ovalifolium* CIAT 13651 (1 kg/ha)	26	85	10	2
Soil preparation + *D. ovalifolium* CIAT 13651 + Fusilade	24	84	13	3
Soil preparation + Roundup + *D. ovalifolium* CIAT 13651	24	84	4	0
Soil preparation + Kudzu. (3 kg/ha).	54	55	16	5

Establishment and development of *Desmodium* under rubber in the Altillanura has been very good as indicated by more than 80% cover in the wet season, which is better than the cover obtained with the traditional kudzu. However, during the early establishment phase (dry period) the kudzu treatment had a higher cover. In denser oil palm plantations, establishment of the legume covers was not good as a result of shading caused by the trees.

In Table 100 we present results on soil cover with different legumes in plantations two years after establishment.

Table 100. Soil cover of different forage legumes in plantations two years after sowing in two sites in the Llanos of Colombia.

Treatments	Savannas - Rubber				Piedmont-Oil Palm	
	Dry		Wet		Dry	Wet
	Old Plantation				Young Plantation	
	Shade	Open	Shade	Open		
	Cover (%)					
A.p 17434	20	15	50	47		35
A.p 18744	65	37	83	70		63
A.p 18748	32	23	75	68		47
A.p 22159	27	18	63	62		62
A.p 22160	27	23	62	63		67
D.h 350	90	93	30	83		68
D.h 13105	93	88	17	75		68
D.h 13110	92	93	12	60		77
D.h 13651	87	90	43	95		87
D.h 23762	92	92	27	82		53
P.p 8042	17	33	30	40		37
P.p 9900	25	55	30	57		37
Asoc. *Ap/Dh*	83	87	63	90		87

Under rubber in savannas *Desmodium* and the *Desmodium/Arachis* mixture maintained soil covers >80% between rubber rows (open). Cover with *A. pintoi and P. phaseoloides* (Kudzú) was much lower, oscillating between 15 and 23 % for *Arachis* and between 17 and 55% for Kudzú.

Performance of the different legume covers under palm trees in the Piedemont was not recorded in the dry season as management of palm trees includes slashing down the vegetation. In the wet season best covers were achieved with *D. ovalifolium* CIAT 13651 and the *Arachis/Desmodium* mixture.

The results from this year confirm those from last year (Annual Report 2000), but also indicate that in old rubber plantations soil cover by legumes improved substantially form one year to another. While soil

covers of *D. ovalifolium* and *A. pintoi* increased in the second year, soil covers achieved with *P. phaseoloides* increased only in old rubber plantations. Under oil palm tree, *P. phaseoloides* declined from one year to another.

In this study we have identified *D. ovalifolium* CIAT 13651 as an excellent legume cover for plantations in the Colombian Llanos. The low establishment cost and superior soil cover obtained with *Desmodium* as compared to the traditionally used kudzu (*Pueraria phaseoloides*) makes it an interesting option to plantation owners. Of interest is also the association of *Arachis pintoi* CIAT 18744/*D. ovalifolium* CIAT 13651, since combining these two species led a to a more stable soil covers across seasons

Based on our results, we will now start promoting the use of *D. ovalifolium* CIAT 13651 as a legume cover in plantations in the llanos. Actions needed include seed multiplication by selected farmers, demonstration field days, technical brochures and training events for farmers and technicians.

4.1.1.3 Evaluation of green manures in the Llanos of Colombia

Contributors: C. Plazas, M. Peters, and B. Hincapie, CIAT

Rationale

One of the aims of the Forage Project is to develop green manures for rice and maize based systems in the Llanos of Colombia. It is expected that suitable legumes will reduce the need for external inputs and make thus make the crops more competitive.

Materials and Methods

Based on prior experience, several l ”best bet” legume accessions were selected to evaluate as green manures in the llanos:

1. *Mucuna pruriens* CIAT 9349,
2. *Canavalia ensiformis* CIAT 715,
3. *C. brasiliensis* CIAT 17009,
4. *Pueraria phaseoloides* CIAT 9900, 8042, 7182
5. *Stylosanthes guianensis* CIAT 11844, 184,
6. *Chamaecrista rotundifolia* CIAT 8990,
7. *Centrosema pubescens* CIAT 15160,
8. *C. rotundifolium* CIAT 5260,
9. *Arachis pintoi* CIAT 17434,18744,
10. *Desmodium heterocarpon* var. *ovalifolium* (*D. ovalifolium*) CIAT 13651, 13105,

The legumes were established in 25 m^2 plots in a Randomized Complete Block Design with three replicates at the Santa Rosa Rice station. A subset of legumes (*Vigna unguiculata* 288,716 and 733; *S. guianensis* 11844, 11833; and *Mucuna pruriens* 9394) were also established at La Libertad Station of CORPOICA and subsequently incorporated prior to planting rice.

To determine the effect of the legume green manures we compared rice yields in: a) green manure + 0 N, b) green manure + 40N c) fallow and d) fallow+ 6 increasing levels of N.

Results and Discussion

In Santa Rosa station, we found significant differences among legume species and accessions in soil cover and biomass yield at time of incorporation of the plant material (2 to 3 months after planting). The highest soil covers (> 60%)and ability to compete with weeds was observed with *S. guianensis* CIAT 11844 and 184, *Canavalia brasiliensis* CIAT 17009, *C. ensiformis* CIAT 715, *Pueraria phaseoloides* CIAT 8042. Other legumes, such as *D. ovalifolium* CIAT 13105, 13651, *Arachis pintoi* CIAT18744, 17434 and *Centrosema rotundifolia* CIAT 5260 had soil covers below 40%. On the other hand, the highest above ground DM yields were obtained with *S. guianensis* CIAT 11844 (6.7 t DM/ha), followed by *S. guianensis* CIAT 184 and *Ch. rotundifolia* CIAT 8990 with yields above the mean (2.1 T DM/ha) of treatments (Table 102).

Given that rice yields were not affected by green manure + 0 N or by green manure + 40 N, data were pooled for comparing yields after green manures with yields obtained with different levels of N fertilizer (Table 101). The incorporation of different legume species into the soil did not result in a significant increase (P>0.05) in rice yield relative to the fallow control (weeds). On the other hand, it was evident that yields of rice responded linearly to N from 0 to 120 kg/ha, but declined at higher levels (160 and 240 kg of N/ha).

Table 101. Dry matter yield of green manure and grain before soil incorporation and grain and dry matter yield of subsequent rice crops en Santa Rosa, Villavicencio, Llanos de Colombia.

Treatment	Herbage (kg/ha)	Rice (kg/ha)	
		Yield (2000)*	Yield (2001)**
S. guianensis CIAT 11844	6757	3905	3187
S. guianensis CIAT 184	4627	4171	4335
C. rotundifolia CIAT 8990	3303	2887	4297
Fallow Control	3129	3401	3933
C. pubescens CIAT 15160	2085	2512	3401
P. phaseoloides CIAT 8042	2081	3099	3914
P. phaseoloides CIAT 9900	2079	3417	3407
C. brasiliensis CIAT 17009	1967	2848	3184
P. phaseoloides CIAT 7182	1695	3609	3802
C. ensiformis CIAT 715	1543	3802	4029
D. heterocarpon subs. *ovalifolium* CIAT 13651	1501	3440	3393
D. heterocarpon subs. *ovalifolium* CIAT 13105	1411	3052	3064
M. pruriens CIAT 9349	591	3420	3614
A. pintoi CIAT 18744	585	3308	3238
A. pintoi CIAT 17434	293	3030	4312
C. rotundifolium CIAT 5260	227	2405	3465
120 N		5797	3848
80 N		4928	4435
40 N		4431	5506
240 N		4316	2550
160 N		4000	3317
0 N		3286	3159
LSD (P<0.05)	2226	1478	1115

*After incorporation of legumes

**Residual effect of legume green manures incorporated the previous year

The highest rice grain yields in the first harvest (2000) were obtained after using *S. guianensis* CIAT 184, 11844, *C. ensiformis* CIAT 715 and *P. phaseoloides* CIAT 7182 as green manures, but yields were not

different from those recorded in the natural fallow. The highest rice yield obtained with a green manure (*S. guianensis* 184) was comparable to the yield obtained with 40N.

The 2nd rice yields (2001) were not significantly affected by the residual effect of legume green manures when compared with yields recorded in the natural fallow. However, it was interesting to observe that with some green manures (i.e. *Ch. rotundifolia* CIAT 8990, *A. pintoi* CIAT 17434, *C. rotundifolium* CIAT 5260 and *C. pubescens* CIAT 15160) rice yields were increased by 35% or more in the second crop as compared to the first crop, but still yields were below those recorded with 40 or 80 kg/N. In the legume green manure experiment established in 2001 in La Libertad station, the cowpea (*Vigna unguiculata)* accessions were the quickest to cover the soil when compared with S. *guianensis* accessions. Highest DM production were also achieved with cowpea, with yields above 4 t/ha DM in 80 days, confirming results obtained at other sites. (Table 102) In this experiment rice yields were not affected by green manure treatments (data not shown). In general, our results indicate that we have some excellent legume options for use as short-term green manures in annual cropping systems in the llanos.

Table 102. Dry matter yield (kg/ha) of green manure herbage before soil incorporation for subsequent rice crop at La Libertad, Villavicencio, Llanos of Colombia.

Treatment	Herbage	
	Cover (%)	kg/ha
Vigna unguiculata (IT86D-716)*	100	4917
Vigna unguiculata (IT6D-733)*	100	4132
Vigna unguiculata (IT89KD-288)*	100	4065
Vigna unguiculata cv CN	99	3971
M. pruriens CIAT 9349	100	2422
Fallow control	95	2218
S. guianensis CIAT 11844	78	2071
S. guianensis CIAT 11833	73	1680
S. guianensis CIAT 184	53	1205
S. guianensis pobl 3	74	966
LSD (P<0.05)	11.9	1275

* IITA numbers

Among the species tested, we are particularly interested in Cowpeas developed in IITA, given their fast growth and high biomass production. However, it is evident from our results that the use of short-term green manures to increase soil N in the piedemont rice production systems will not pay off. Thus, in 2002 we plan to test selected legumes as green manure in maize grown in savannas soils, since it may be more responsive to green manures than rice. It is import to indicate that maize is gaining importance as a crop in the llanos and that a legume green manure technology may be an option attractive to farmers.

4.1.1.4 On-farm evaluation of new grasses and legumes options for livestock systems in the Llanos of Colombia

Contributors: C. Plazas, J. Miles and C. Lascano, CIAT

Rationale

One major limitation for beef and milk production in Neotropical savannas is the degradation of introduced grasses, as a result of nitrogen deficiencies and overgrazing. Thus CIAT's Forage Project (IP5) 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.

Introduction of legumes to reclaim degraded pastures

The introduction of *Arachis* to reclaim degraded *Brachiaria* pastures in the piedmont of the llanos has been successful. Results from two farms in the llanos piedmont indicate that after 2- 3 years the legume content in the pastures range from 22% when in association with *B. humidicola* (Figure) to 40% when in association with *B. decumbens* (Figure 34).

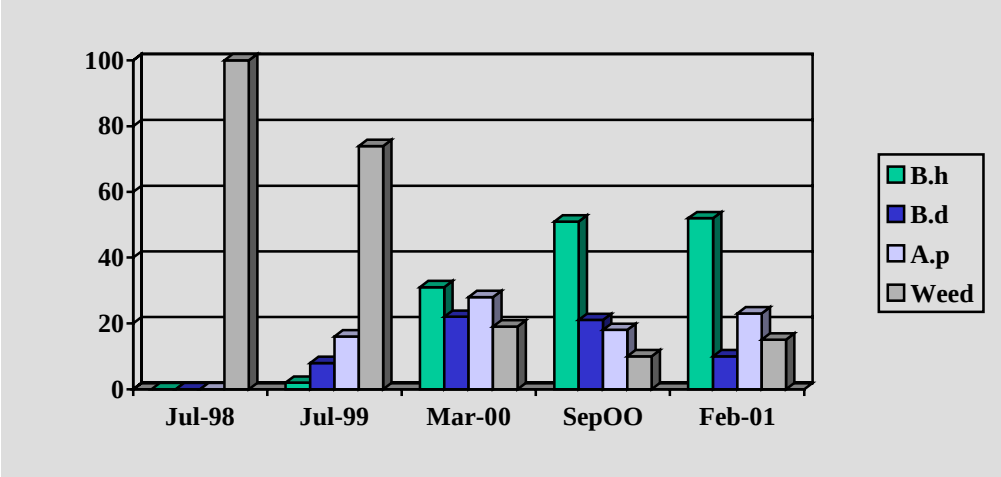

Figure 34. Botanical composition of *B. humidicola* pasture reclaimed with *Arachis pintoi* in the llanos piedmont (Farm 1)

In Farm 1, the Arachis–based pasture has been managed with high stocking rates because of heavy spittlebug attack on *B. decumbens* pastures in the property. This heavy grazing of the *Arachis*-based pasture has favored the legume and has allowed the farmer to release pressure on pastures damaged by spittlebug. The alternative was to sell animals to reduce stocking rate or to graze the spittlebug damaged pastures, with negative consequences on animal performance and on productivity of the pastures. In Farm 2, the pasture reclaimed with *Arachis* in April 1999 is now very productive and with a high legume content as shown in Figure 35.

The CP content in *Brachiaria* has increased form 5 % to 10%, which has had a significant impact on productivity of the pasture. Last year we reported that 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.

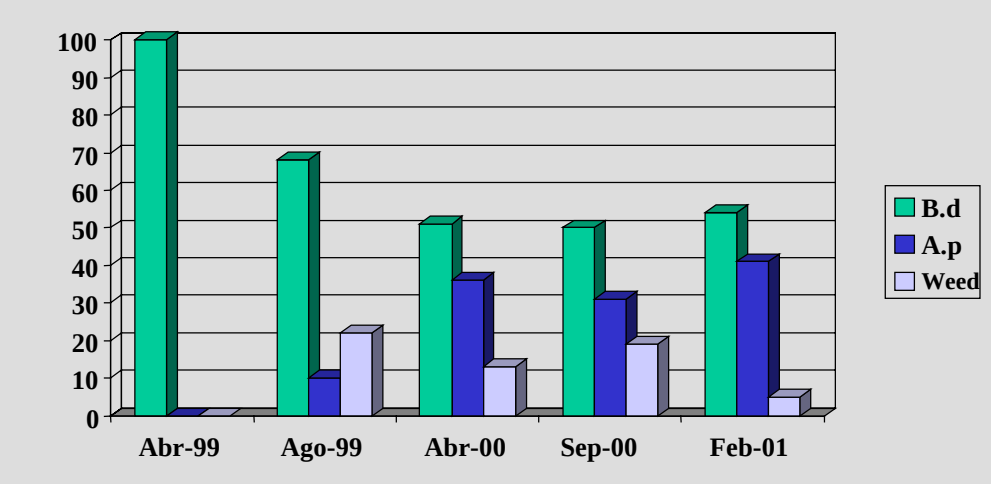

Figure 35. Botanical composition of *B. decumbens* pasture reclaimed with *Arachis pintoi* in a farm in llanos piedmont (Farm 2)

We have now established the use of *Arachis* to reclaim degraded pastures in well drained sites in the llanos will require high use of management and fertilizer inputs, but it is unlikely that farmers would be willing to pay the extra cost. The alternative is the use of *Desmodium* heterocarpon subsp. *ovalifolium* (*D. ovalifolium*) which is better adapted to acid-low fertility soils.

The value of *D. ovalifolium* CIAT 13651 to reclaim degraded pastures was evaluated in one farm located in well-drained site in the llanos. The pasture reclaimed had low availability of *B. decumbens* and high proportion of weeds. To reclaim the pasture, we plowed the land and introduced *Desmodium* (250 g of seed /ha) in May 2000. One year after the pasture has 20% legume and it is being successfully used to fatten steers (Figure 36).

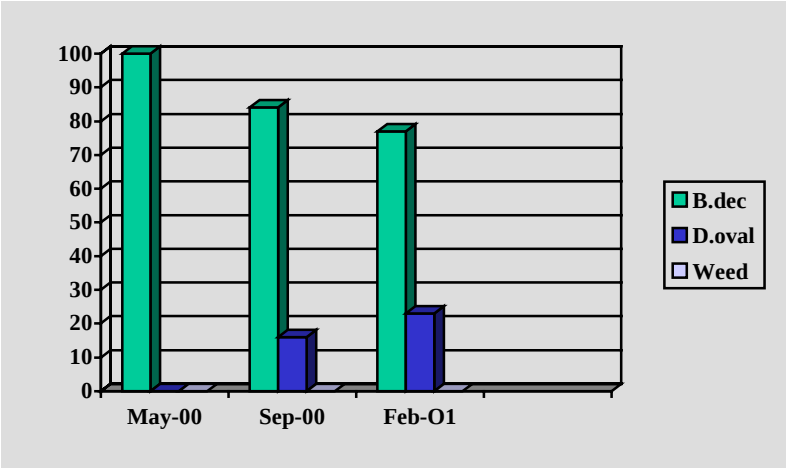

Figure 36. Botanical composition of a pasture reclaimed *with Desmodium ovalifolium* in a farm in the llanos

Given the successful introduction of *D. ovalifolium* CIAT 13651, farmers have shown interest in its use to reclaim degraded pastures. A total of 140 ha of degraded pasture in well- drained savannas have been recuperated by the initiative of farmers. Farmers in the piedmont have also reclaimed more pastures (180 ha) using *D. ovalifolium* rather than *Arachis* due to lower seed cost. We have estimated that the cost of

reclaiming degraded pastures with *D. ovalifolium* CIAT 13651 is ½ of what it is with *Arachis* ($US70 vs. 152/ha) and this cost differential is a driving force in the adoption of legumes in the llanos.

Introduction of new grasses to reclaim degraded pastures

Last year we reported that in one farm located in a well-drained savanna site with acid-low fertility soils we introduced in 1999 two new *B. brizantha* accessions (CIAT 26110 and 26318). In 2000 we introduced in the same farm *B. brizantha* 26556 G and *B. brizantha* 26124. In addition, we introduced *B. brizantha* 26110 in one farm in the piedmont. Results on performance of these pastures are shown in Figure. In the dry season (February 2001), *B. brizantha* 26610 produced more biomass than the other accessions being evaluated in a well- drained savanna site. However, in the wet season (May 2001) *B. brizantha* 26556 G and 26124 have been more productive than the other grasses being tested in savannas. We have also observed that *B. brizantha* 26110 is more productive in the piedmont (P) than in a well-drained savanna (WDS) site (Figure 37).

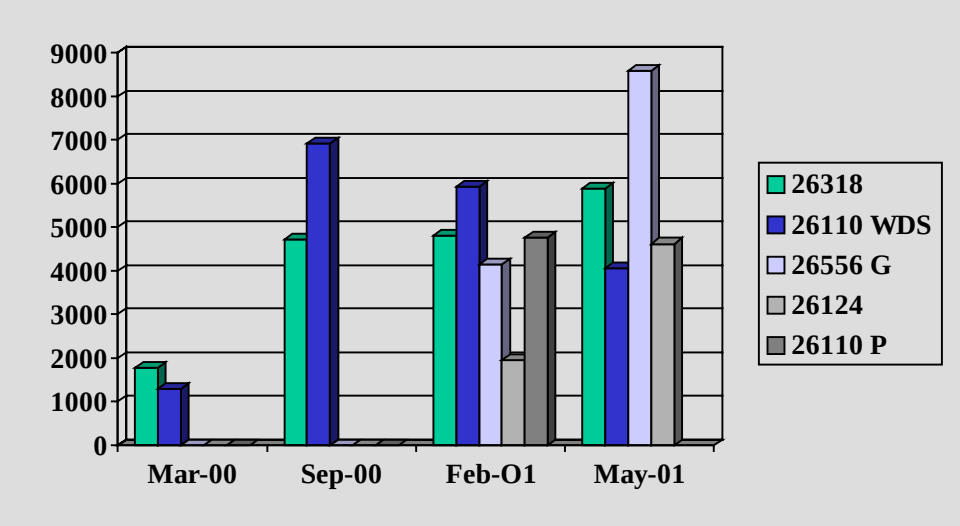

Figure 37. Forage on offer of different *Brachiaria brizantha* accessions under grazing in the llanos

The following feedback was obtained from a participatory evaluation of the new grasses being tested in the llanos:

1. *B. brizantha* 26318: It recovers fast after grazing but does not seem to be very palatable to cattle given it high stem content. Poor overall rating.
2. *B. brizantha* 26110: High forage production and this can result in forage loosing quality if not properly managed. Most farmers like CIAT 22610 but concur that it needs to be well managed (high stocking rates, short rest periods) to obtain good animal performance.
3. *B. brizantha* 26556 G: This grass was ranked No 2 by producers due to green intense color, high leaf content, large leafs and soft leaves. Unfortunately this accession was heavily attacked by spittlebug and recovery following grazing is slow. In spite of the susceptibility of this accession to spittlebug farmers expressed an interest in this genotype.
4. *B. brizantha* 26124: This accession was given the highest rating by producers given its high leaf content, large and soft leaves. One advantage farmers see with his accession is that it recovers fast after grazing and that it produces stolons that root.

Thus from the results on evaluation of new *B. brizantha* accessions, it would seem that CIAT 226110 (cv Toledo) is better adapted to the piedmont with better soils than to the more acid-low fertility soils found in well- drained savannas. In addition, it is clear that for farmers in the llanos important selection criteria for grasses include plant factors associated with quality and palatability such as leaf: stem ratio and leaf softness. Recovery of the grass after grazing is also an important selection criterion of farmers. Finally, there is an urgent need to multiply seed of selected grasses in order to expand the on-farm evaluation of the new grasses. An alternative is to make seed available to private seed companies that have interest in establishing a multilocational-testing program with new grass alternatives.

4.1.1.5 Ex-ante analysis of the utility of *Cratylia argentea* in dual-purpose production systems of the Llanos piedmont of Colombia

Contributors: F. Holmann, C. Plazas and C. Lascano (CIAT)

Rationale

About 90% of the milk produced in the Llanos comes from dual-purpose herds where pastures are the main source of feed. However, the quality of existing forages is low. On the other hand, shrub legumes have the potential to improve the feed quality of offered forages. Shrub species produce more biomass than herbaceous legumes, have a higher regrowth capacity, and produce a good quality forage in areas with prolonged dry season. Among the shrub legumes that have been evaluated and selected by CIAT is *Cratylia argentea*.

This study forms part of larger project in which we will evaluate with farmer participation the utility of *Cratylia argentea* to substitute the use of feed supplements in dual-purpose cattle production systems found in the Llanos piedmont of Colombia. The specific objective of the ex- ante economical study is to define if by using *Cratylia* farmers can reduce feed costs. Different uses of *Cratylia* are being analyzed: a) offered alone in cut-and-carry systems at milking time; (b) mixed with molasses and offered at milking time; and (c) under direct grazing by milking cows.

Materials and Methods

In order to understand the current production systems, technologies used, use of resources, and input and output prices we collected data through direct interviews of 32 farmers that operate dual-purpose production systems located in the target area. We also used data from secondary sources from the region where farms are located. To meet the objectives of the study a linear programming (LP) model was utilized as the main tool of analysis. This LP model was initially developed by the Tropical Agronomical Center for Teaching and Research (CATIE) and the International Network of Animal Production Systems of Latin America (RISPAL), which was later expanded by CIAT. The LP model was developed in an electronic spreadsheet with the objective to evaluate ex-ante the costs and benefits of the current and potential land use and their interactions between the technological components and the biological productivity.

Results and Discussion

In Table 103 we present descriptive statistics of the current dual-purpose production systems found in the Llanos piedmont. As can be observed, about 85% of total farm area is under pastures with very little area allocated to crops (1.5 ha/farm), consisting mainly of fruit trees and annual crops for self-consumption. Mean herd size is about 63 animals/farm of which 27 are mature cows.

Table 103. Means for land use, livestock inventory, stocking rate, milk production, and feed supplements for 32 farms representative of production systems in the Llanos piedmont.

Parameters	Quantity
Total farm area (ha/farm)	59.6
Area under pastures (ha/farm)	50.7
Total number of cattle (heads/farm)	63.1
Number of cows (#/farm)	26.9
Stocking rate (AU/ha)	0.92
Milk production (kg/farm/day)	112
Milking cows (#/farm)	19
Milk productivity (kg/cow/day)	5.9
Feed supplements used (kg/milking cow/day)	
* Oilpalm cake	0.43
* Feed concentrates	0.42
* Molasses	0.41
* Mineralized salt	0.12

Milk yield is about 5.9 kg/cow/day, which is significantly higher than the average for the whole of the region, which is 3.4 kg/cow/day. The amount of feed supplements offered to milking cows is low, which suggests that producers used them strategically. In Table 104 we show the forage quality parameters used in the linear programming model to do the ex-ante analysis for the scenarios considered in this study.

Table 104. Forage parameters utilized to run the linear programming model to do the ex-ante analysis in dual-purpose farms in Villavicencio.

Parameter	*Brachiaria decumbens*	*Brachiaria humidicola*	Napier Grass	*Cratylia argentea*	
				Cut and carry	Direct grazing
Crop duration (years)	10	10	10	20	3, 6, 9
High rainfall season					
* Available biomass (tm DM/ha)	4	4	8	16	4
* Crude protein (%)	6	4.5	8	15	23
* Degradability of CP (%)	60	50	60	70	70
* IVDMD (%)	55	50	55	50	60
Low rainfall season					
* Available biomass (tm DM/ha)	2	2	2	8	2
* Crude protein (%)	5	3.5	7	15	23
* Degradability of CP (%)	55	45	55	65	65
* IVDMD (%)	50	45	55	50	60
Lost forage to trampling (%)					
* High rainfall season	30	30	0	0	10
* Low rainfall season	25	25	0	0	10
Biomass transfer from high to low rainfall season (tm DM/ha)[1]	1	1	2	4	1

[1]Equivalent to 25% of biomass production during high rainfall season for grasses and Cratylia under direct grazing system and 100% for Cratylia in cut-and-carry systems.

These parameters were determined based on field data collected from several years in similar regions. It is expected that the simulation runs for the ex-ante analysis and the discussion of results will be completed by December of this year.

4.1.1.6 Analysis of intensification of milk production systems in Colombia

Contributors: Juan Carulla (U. Nacional, Bogotá), Silvio Guzmán (Fundación San Martín, Barranquilla), Manuel Martínez (U. de los Llanos, Villavicencio), Luis A. Giraldo (U. Nacional, Medellin), B. Rivera (U. de Caldas, Manizales), F. Holmann y Andrew Farrow (CIAT)

Rationale

From 1992 to 1999, milk production in Colombia grew at an annual rate of 4.3% and dairy imports during the same period represented 2.6% of domestic production. Thus, Colombia is practically self-sufficient in milk production. On the other hand, Colombia has always been a net exporter of beef, but with a clear loss in relative importance throughout this decade. In 1991 Colombia exported about 5% of its domestic production. Since then, the reduction in exports has been noticeable, dropping to less than 1% in 1999.

Nonetheless, Colombia has a significant potential to increase livestock production given the high proportion of land under pastures and abundant feed resources, good public infrastructure, genetic potential of its livestock inventory, human resources, and availability of technologies and livestock services. However, internal discusion exists within Colombia as to whether its farmers will be able to survive and compete in a scenario of free trade without tariff barriers.

The objectives of this study were to (1) identify the technologies that have a positive effect in the productivity and profitability of milk in five contrasting regions of Colombia; (2) quantify the effect of these technologies on the productivity and profitability of milk; (3) quantify the investment needed to adopt these technologies at the farm level; (4) geo-reference farms in Colombia to identify patterns of adoption with regards to level of infrastructure, human population, and market access; and (5) analyze the comparative advantages in each region to increase the future supply of milk.

This study is a collaborative project between CIAT and five institutions in Colombia: Universidad Nacional (Bogota and Medellin sites), Universidad de Caldas, Universidad de los Llanos, and Fundacion San Martin. In addition, this study is an integral part of a transregional analysis led by ILRI.

Materials and Methods

Data for this study came from direct surveys to 545 producers done between February and November of 2000 in five contrasting regions of Colombia distributed as follows: (1) 145 farms in the Eastern plains (Arauca, Casanare, and Meta); (2) 116 farms in the Caribbean region (Atlantico, Guajira, Magdalena, Cesar, Bolivar, and Cordoba); (3) 105 farms in the coffee region (Quindio, Valle, Caldas, and Risaralda); (4) 97 farms in Antioquia; and (5) 82 farms in the highlands of Cundinamarca and Boyaca.

The survey information was then developed into a database using Access and completed in April of 2001. The statistical analysis of the database started in May of 2001 and it is expected to be finished in May of 2002. For the geo-reference analysis new software developed at CIAT (i.e., accessibility wizard) will be used to identify patterns of intensification in order to complement farm-level data and to better understand the drivers of adoption of technologies.

Expected Outputs

The final product of this study will be a publication which includes (a) an analysis of the milk production systems found in each of the five regions studied (i.e., 5 chapters); (b) an additional chapter which integrates the five regions as a country; (c) a chapter using GIS which analyzes market access and identify patterns of intensification; and (d) a final chapter analyzing the comparative advantages and disadvantages of different milk production systems to meet the future supply of milk.

4.1.1.7 Participatory evaluation of forages for multipurpose use in Haiti

Contributors: Levael Eugene (CIAT), Ellisssaint Magloire (ORE), Joseph Andrefoine (ORE), P. Argel, C. Lascano, M. Peters, Luis A. Hernández, CIAT

Rationale

This year we completed a collaborative project in Haiti through the HGRP (Hurricane George Recovery Program), which financed by USAID and administrated by the Pan American Development Foundation (PDF), an ONG based in USA, and which in turn subcontracted with other ONG's 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.

The short-term objective of CIAT's work on forages in Haiti through the HGRP was to select 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 were to:

1. Select 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. Train Professionals on Forage Agronomy and Seed Multiplication.
3. Define a 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 the following activities:

1. Reviewed available rainfall and soils data in target areas and previous experiences with introduction of grasses and legumes.
2. Reviewed CIAT Forage database and selected grass and legume species that could be adapted to target areas.
3. Delivered small quantities of forage seed to Haiti through ORE to establish selected grasses and legumes species in small plots in replicated trials in two sites (Camp Perrin, and Deron) to assess seed multiplication potential.
4. Prepared research protocols to ORE's staff for the evaluation of forage germplasm for multipurpose use (feed resource and covers**).**
5. Evaluated a range of forage species and selected best bet options for seed multiplication.
6. Provided short-term training to a staff from ORE on Forage Agronomy in CIAT- Palmira and Costa Rica.

7. Provided training in forage agronomy and in participatory evaluation methods to professionals of different organizations in Haiti.

Two contrasting sites were chosen for the evaluation activity: 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 2000 with the participation of Agronomist from ORE and CIAT. ORE personnel established the trial in Debron in September of the same year. 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.

Camp Perrin is located close to sea level, while Deron is placed at mid altitude (around 600 masl)). At both sites the soils are of calcareous origin; however at Deron the soils give the appearance of being acid soils because of the intense red color they have, but this occurs because most of the soil basis are washed away with the rains except the iron compounds that produce the reddish color observed, however the pH is over 6. Both soils have low contents of organic matter (around 2.0%) and phosphorus (around 1.4 ppm), meanwhile the calcium content is high (over 2.5%) as well as the exchange capacity (over 31 meq/100 g).

Results and Discussion

At both sites the forage germplasm planted consisted of shrub legumes, herbaceous grasses and legumes, and legumes as green manure/cover crops. Plant emergence was limited by heavy rains following planting, particularly of small seeded legumes such us *Desmodium ovalifolium* and *Stylosanthes guianensis.*

At Camp Perrin the plots had a uniform cut during April and a first evaluation of dry matter yields was carried out in August. At Deron the plots were cut two weeks before the evaluation in June of this year. A visual rating of adaptation of the species allowed us to rank species based on adaptation and production potential in the two sites (Table 105).

In Camp Perrin and Deron, the shrub legumes *Leucaena leucocephala* subsp. *glabrata* (OFI 34/92) and *L. leucocephala* CIAT 17263 were outstanding given the calcareous nature of the soils in the two sites. However, it was interesting to note that *L. collinsi* OFI 52/ 88 and *L. macrophylla* subsp. *nelsonii* OFI 57/85 performed well in Camp Perin but failed in Deron, which could be related to differences in rainfall distribution in the two sites. Other shrub legume species such as *Cratylia argentea* were slow to establish but it is expected to produce good biomass once established, particularly in the dry season.

In terms of grasses it was evident that several accessions of *Panicum maximum* and *Brachiaria* sp performed well in both sites. Of the herbaceous legumes tested, *Centrosema macrocarpum* and *C. pusbescens* performed well in both sites and could be excellent supplement to animals on low quality feed such as crop residues used in Haiti to feed cattle. *Arachis pintoi* established well in both sites, but growth was retarded possibly as a result of micronutrients. Other legumes such as *Mucuna* sp and Cana*valia ensiformis* are very well adapted in the two sites and are obvious choices for green manures.

Based on these initial results we selected a number of grasses and legumes for seed multiplication and subsequent delivery to Haiti. A total of 340 kg of seed of grasses and legumes were sent to Haiti this year as part of HGRP. This seed was stored in ORE and will be used for regional and on-farm testing in the new HAP project in which CIAT will participate.

Table 105. Evaluation of multipurpose forage established in Deron and Cam Perrin during 2000 in Haiti.

Species	Sites	
	Camp Perrin	Deron
Shrub legumes		
L. leucocephala subsp. *Glabrata* OFI 34/92	Excellent	Excellent
L. leucocephala CIAT 17263	Excellent	Excellent
L. collinsii OFI 52/88	Excellent	Failed
L. macrophylla subsp. *Nelsonii* OFI 47/85	Excellent	Failed
C. callothyrsus CIAT 22310	Regular	Good
C. callothyrsus CIAT 22316	Good	Good
F. macrophylla CIAT 17403	Good	Failed
C. argentea CIAT 18515/668	Good	Poor
Herbaceous Grasses and Legumes		
P. maximum CIAT 16051	Good	Excellent
P. maximum CIAT 16028	Good	Excellent
P. maximum CIAT 16031 (cv. Tanzania)	Good	Good
B. brizantha CIAT 26110 (cv. Toledo)	Good	Excellent
B. decumbens CIAT 606 (cv. Basilisk)	Good	Excellent
B. dictyoneura CIAT 6133 (cv. Llanero)	Regular	Poor
B. humidicola CIAT 679 (cv. Humidicola)	Regular	Poor
B. humidicola CIAT 26427	Regular	Poor
S. guianensis CIAT 1844	Failed	Good
N. wightii CIAT 204	Poor	Failed
C. ternatea cv. Tejuana	Poor	Poor
A. pintoi CIAT 18744 (cv. Porvenir)	Regular	Regular
A. pintoi CIAT 22160	Regular	Regular
C. macrocarpum CIAT 25522 (cv. Ucayali)	Good	Good
C. pubescens CIAT 15160	Good	Regular
D. ovalifolium CIAT 33058	Failed	Failed
Green manure/cover Legumes		
Mucuna sp.	Excellent	Regular
Canavalia ensiformis CIAT 715	Excellent	Good
Pueraria phaseoloides CIAT 7182 (Kudzú)	Good	Regular

4.1.2 Releases and adoption by farmers of new forage species

4.1.2.1 Release of *Cratylia argentea* as cv. Veraniega by MAG in Costa Rica

Contributors: P. J. Argel (CIAT), Carlos Hidalgo, Marco Lobo, Vidal Acuña (MAG), Jesús Gonzalez (ECAG) and Carlos Jiménez (UCR)

Rationale

The shrub legume *Cratylia argentea* was introduced in 1988 for evaluation in Costa Rica at the experimental station Los Diamantes (Guápiles), within the cooperation agreement between MAG-CATIE-ECAG and the Tropical Forage Program of CIAT. The legume is a shrub that reaches between 1.5 and 3.0 m high, and adapts well to a wide range of sites in Costa Rica located between 0 and 900 m.a.s.l., above this altitude plant growth slows down. The plant grows well in well-drained Ultisol soils and in Inceptisols of good to moderate fertility located in subhumid ecosystems with 5 to 6 months dry. The plant does not grow well in calcareous soils or in heavy soils with tendency to high moisture saturation.

The cultivar Veraniega is a physical blend of the accessions *C. argentea* CIAT 18516 and *C. argentea* CIAT 18668, both collected in Brazil respectively at the localities of Sao Domingos (Goiás) and Cuibá (Mato Grosso). These accessions have identical growth habit, similar adaptation to climate and soil and similar contents of minerals, crude protein (around 20% in 3 months old plants), and in vitro dry matter digestibility (around 54%). Cultivar Veraniega flowers and sets good quality seeds in humid and subhumid tropical conditions; the seed has low physical and physiological dormancy.

Fodder banks of cv. Veraniega can be established by direct seeding following a conventional disking soil preparation or after minimum soil tillage. Forage yields depend upon re-growth age, cutting height and planting distance. For instance, dry matter yields per cut have been reported of 2.6 t/ha and 5.1 t/ha, and 1.9 t/ha and 5.3 t/ha, after increasing respectively the cutting height from 30 to 90 cm and the cutting frequency from 60 to 90 days, in a one year old forage banks planted at 1.0 m x 1.0 m between plants and rows. The plant tolerates prolonged dry seasons and up to 30% of the DM yield has been reported during this period of the year.

In dairy farms of cv Veraniega can substitute concentrate or chicken manure when offered fresh or as silage to milking cows during the dry season, together with high energy sources such as sugar cane or king grass. Because the good adaptation and productivity of this shrub in Costa Rica and elsewhere, the Ministry of Agriculture and Livestock (MAG) of this country decided to release it as cv. Veraniega, in a joint activity with the University of Costa Rica, the ECAG and CATIE, all of them members of the Tropileche Consortium.

Actions for the release of Cratylia

A Technical Bulletin entitled “Cultivar Veraniega (*Cratylia argentea* (Desv) O. Kuntze). Una Leguminosa Arbustiva para la Ganadería de América Latina Tropical”, is presently under printing. Four thousand units will be published, which contain information related to the species origin, botanical description, soil and climate adaptation, establishment, pest and disease tolerance, seed quality and production, nutritive value, utilization and management, and response of milking cows to feeds based on Cratylia.

The Technical Bulletin will back up the official release of the cultivar programmed in a field day in February 2002 in one dual purpose cattle farm that has been utilizing successfully *C. argentea* cv. Veraniega for the last 4 years.

The main use of cv. Veraniega in Costa Rica is as protein supplement for lactating dual purpose cows during the dry period (5 to 6 months dry), offered either fresh or as silage. Lately the legume is being used in partially confined cattle feeding systems, as a substitute of highly costly concentrates. These systems are being promoted in Costa Rica in pronounced hilly country where small and medium size farmers fat between 5 to 20 bulls per year; thus the confined or partially confined fattening system is a practice that reduces the animal pressure on steep highly eroding soils.

4.1.2.2 Opportunities and constraints to adoption of *Cratylia argentea* in Costa Rica

Contributors: Ewout van den Ouwelant (U. of Wageningen, Holland), P. J. Argel and F. Holmann (CIAT)

Rationale

Although many factors influence animal production, the availability of feed is undoubtedly one of the most important. Scientists and farmers are jointly seeking alternative sources of animal feed for the dry season. The legume *Cratylia argentea* was introduced to Costa Rica as an alternative source of feed for

cattle during the 6-month dry season. Since 1995, an increasing number of farmers are now using this legume, but the process of incorporating this new feeding alternative into production systems and changing farmers' attitudes will take some time. Since then, information about this legume has spread and its use has become more common. This study was carried out to learn more on the knowledge, experience, and benefits that early users in Costa Rica have of Cratylia.

Materials and Methods

Data was obtained by direct interviews with farmers located at three sites in Central-Pacific Costa Rica during March-April 2001. Two groups of farmers were interviewed; one group consisted of 39 Cratylia users and the other, of 25 non-users. Users were asked about their experiences in the initial years of establishment and non-users provided information on potential uses of this legume.

Results and Discussion

More than 50% of farmers who had planted Cratylia were still not using it as feed because of its recent establishment. Of the farmers surveyed, 80% indicated that the most important reason for planting Cratylia was its availability as feed during the dry season and 65% indicated that it was mostly offered fresh, mixed with other feed sources.

Cratylia was well accepted by cattle and mostly fed to the most productive animals, according to 80% of the participants. Leaf retention, regrowth after cutting, and seed production were considered, attributes of Cratylia as good by 70% of the respondents; whereas 60% thought Cratylia helped prevent soil erosion.

Although no major problems were reported, some disadvantages of planting Cratylia were indicated. The legume's slow initial growth and sometimes difficult establishment are seen as negative aspects, as well as the high labor costs involved in cutting the forage and in initial land preparation. Cratylia's biggest advantages are its excellent resistance to drought and its potential to reduce production costs.

With proper management, Cratylia can completely replace chicken manure, thereby substantially reducing feeding costs during the dry season. Figure 38 shows the different opinions about Cratylia by early adopters.

Over the last five years, the average area sown to Cratylia per farm has been maintained because of the failed attempts of several producers who lacked know-how and technical assistance to plant more area. Perspectives for the future are, however, optimistic.

Of the farmers currently using Cratylia, 85% are planning to increase the area planted over the next five years, with an average of 0.7 ha/farm. Most of the farmers (88%) not using Cratylia have no objections about changing their farming practices and adopting the legume.

The lack of information and deficient technical assistance are the major constraints to increasing the rate of adoption of *Cratylia* in Costa Rica. The communication between producers and researchers is mainly through the offices of the Ministry of Agriculture (MAG). The future of *Cratylia* could prove promising with improved services and communications toward farmers.

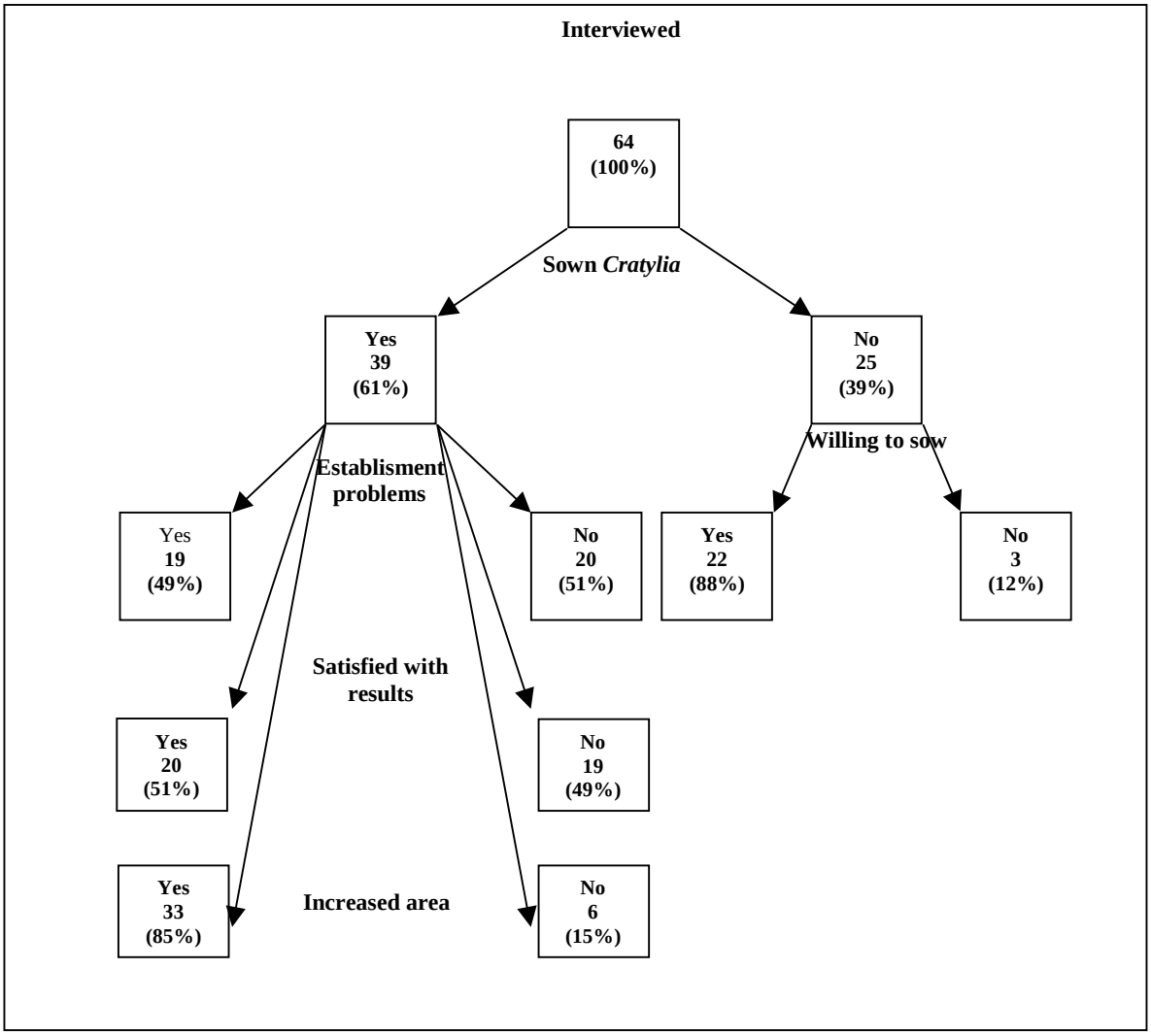

Figure 38. Overview of number of farmers with different adoption characteristics of *Cratylia argentea* In Costa Rica

4.1.2.3 Adoption of *Arachis pintoi* in Costa Rica

Contributors: Tobías Wuenscher (U. of Hohenheim, Germany), R. Schultze-Kraft (U. Hohenheim), P. J. Argel, L. Rivas y F. Holmann, M. Peters (CIAT)

Rationale

Degradation of pure grass pastures is a frequent problem in the tropics. The legume *Arachis pintoi* was found to show a number of characteristics, which can contribute to the development of sustainable and productive pastures in the tropics. The legume was introduced in Costa Rica in 1987 and is used as a pasture plant, as a cover crop in plantations, as a ground cover on roadsides and steep slopes, and as an ornamental plant. It can be grown pure to form protein banks or it can be grown in association with grass species. *A. pintoi* can be fed to various animals including horses, donkeys, sheep, goats, pigs and chicken. The forage has a high protein content and good digestibility. With relatively little area of pure *A. pintoi*

good extra weight gains can be achieved and grass/ legume pasture associations are more profitable than improved, grass-alone pasture system.

The objective of this study was to learn about the adoption process by livestock farmers at an early stage in order to accelerate the diffusion process using as a case study the region of Huetar Norte of Costa Rica.

Materials and Methods

The zone known as Huetar Norte was chosen to be the study region because both milk and beef production are predominant farming activities. It was decided to gain relevant information by means of thoroughly structured interviews. The farmers were visited on their properties to be directly and personally interviewed.

The target population was livestock holders and owners of pastures. The frame population was a list of 7131 livestock holders. A simple random sampling was applied to the frame population. 115 interviews were conducted within the simple random sample. We also interviewed 34 farmers in a directed sample. Two questionnaires were used in the interviews: a) short version for the random sample, and b) long version to be used with farmers, which had already worked with *A. pintoi*. MAG extension workers (21) and the author of this study conducted interviews. All interviews were conducted between January and March 2000.

Results and Discussion

The average farm size in the random sample was 69.8 ha. The average farm was made up of 52.5 ha of pasture, 3.3 ha of perennial plantations, 1.9 ha of annual crops and 12.1 ha of land with other uses like trees for wood. The average herd size was 86.4 head of cattle per farmer. The most frequent pasture species was Ratana (*Ischaemum indicum*). The most frequent improved pasture species was Estrella (*cynodon nlemfuensis*). A total of 104 farmers (90.4%) had already heard about *A. pintoi* and 29 farmers (25.2%) had already sown the legume on their land, mostly for ornamental purposes. Only 8 farmers said to have sown it to improve livestock nutrition.

Comparison of adopters and non-adopters: Adopters seek technical information about new forage-based technologies more intensely than non-adopters. For example, 66.6% of adopters but only 34.3% of non-adopters had attended at least one workshop or field day in 1999. In addition, adopters have an average farm area of 128.2 ha and an average herd size of 196 animals compared non-adopters with an average farm area of 69.9 ha and an average herd size of 86 animals. Adopters have herds with an average of 49.8% being dairy cattle, whereas non-adopters have herds with only 19.8% of dairy cattle. Of the adopters, 42.4% belong to the highest gross income category whereas only 15.0% of non- adopters belong to this category. Similarly, only 9.1% of adopters but 17.8% of non-adopters belong to the lowest income category. Thus, adopters of *A. pintoi* tend to be larger producers obtaining higher incomes with more emphasis on milk production and who are more interested in obtaining new information about forage technologies.

Adopters and their experiences with *A. pintoi* as a forage legume: The majority of adopters received information about *A. pintoi* through the extension service of MAG (40.6%) and the ITCR (15.6%) and a 59.4% of the adopters acquired the planting material from one of the three "*Arachis pintoi* centers". The majority of plots were sown with stolons (82.2%).

The main reason for planting *A. pintoi* was to improve pasture (50 % of the answers) quality. Of all adopters 51.5% planted *A. pintoi* in association with grasses and 48.5% planted it in pure stands. The

majority of farmers feed it to all types of cattle, some only to milk cows, calves or sick cattle. Within the group of all interviewed adopters (33 farmers) the total area planted with the legume was 73.87 ha.

Of all adopters 87.9% (29 farmers) said to be satisfied with the results they have so far obtained with the use of the legume. However, 3 farmers (9.1%) said to be more or less satisfied and 1 farmer (3.0%) said to be not satisfied.

Advantages of the legume were described as; a) good quality feed (36.6%), b) increases cattle production (15.9%), c) persistence and d) ability to improve soil fertility. Most frequent disadvantages mentioned by adopters were that it a) attracts slugs (12.8%) and b) difficulty to control broad leaf weeds.

The majority of the adopters found that the establishment of *A. pintoi* was slow and 73.1% said that their cattle did not need time to get used to the legume. Whereas 37.5% o said *A. pintoi* could disappear when mixed with improved grasses. Nearly a third of the adopters found it performed better than other improved grass species in the dry season and 81.8% of adopters wanted to increase the area planted with *A. pintoi* in the next five years.

Establishment and maintenance costs differed substantially within the group of adopters. The establishment costs ranged from US$ 57.60 to US$ 348.67 per ha. The maintenance costs ranged from US$ 6.40 US$ to US$ 91.39 per year. The maintenance costs for other pastures ranged from US$ 34.67 to US$ 156.67 per year. Thus in the opinion of farmers the maintenance of *A. pintoi* – based pastures was less than the maintenance of another pasture. Not a single farmer who had planted A. pintoi had taken credit to pay for the investment.

Non-adopters and their perceptions of *A. pintoi*: Of 109 interviewed non-adopters, 98 (89.9%) had already heard about *A. pintoi* and of these 22 said to have already sown it on their farm, mostly for ornamental purposes. The majority of non-adopters (51.6%) has obtained information about the legume from colleagues, neighbours or friends.

Of the non-adopters, 62.4% knew that *A. pintoi* could be used as a feed for cattle and 55 (58.5%) of the farmers though that the legume could serve a useful role on their farm. The majority of these farmers would use it in association with grasses for grazing (51.7%). Most frequent reasons for not using A. pintoi was a) lack of information (27.9%) and b) seed not readily available (13.4%).

In summary our results show that the estimated adoption rate of *Arachis pintoi* as a forage legume is very low in “Huetar Norte” region of Costa Rica. An estimated 3.5%, or 248 farmers had planted an estimated 0.0006% (252 ha) of Arachis-based pastures. Thus the diffusion process is at a very early stage.

The most negative experiences with *A. pintoi* are related to establishment of the legume and to persistence under grazing when in association with a grass. The establishment was seen by a large number of the farmers interviewed as a very time consuming, and costly matter. Persistence was often seen to be a problem, particularly when the legume was in association with a grass. However, after having the legume well established farmers generally had less expenditure in maintenance of the pasture as compared to other pastures and thus experienced an economical benefit from the legume.

In order to accelerate the adoption process it is recommended to develop technologies that ensure a relatively low cost of establishment (i.e. reduced price of seed) as well as management practices that ensure its persistence under grazing. Moreover, it is recommended that training courses be given to extension workers and to ensure the on-farm establishment of Arachis-based pastures for demonstration purposes.

4.1.3 Development of new collaborative research proposals with NARS, NGO's IARC and ARIS

4.1.3.1 New initiatives for the evaluation and promotion of multipurpose forages, with focus in Africa

Contributors: Michael Peters, R. Kirkby, and Thomas Oberthuer, Douglas White, Arturo Franco

In line with the advances made by CIAT's Bean Program in Central, Eastern and Southern Africa, CIAT is expanding its efforts with work on multipurpose forages. Such work is intended to have impact not only on feed improvement for livestock but also on Natural Resource Management (NRM).

Currently, there are three initiatives under way, which include Africa. Common to all initiatives is the collaboration with partners in and outside the region, farmers, NGOs, NARS, ARIS and other International Research Centers.

Improvement of smallholder dairy systems in Southern and Eastern Africa: This initiative with more regional focus is in collaboration with the International Livestock Research Institute (ILRI) and cuts across many of the on-going work of ILRI and CIAT in Southern and Eastern Africa. CIAT and ILRI have done a reconnaissance survey and consulted with partners in the region. Based on this survey and experiences we aim to concentrate on the dairy sector in the region. Criteria for site selection would be based on: a) complementing existing activities, b) representatives, c) systems that are evolving and driven by market forces, and d) potential impact. Tanzania, Uganda and Malawi are currently seen as the most likely countries for reference sites.

The focus is on dairy production, as dairy is seen to both increase food security and income**.** The dairy systems in Eastern and Southern Africa are also particular appealing given that in current intensive farming systems, NRM and poverty alleviation needs are strongly related. In many cases NRM and Poverty alleviation have linked entry points, i.e. the use soil conservation strips as potentials for growing improved feed or, vice versa, growing improved feed to promote soil conservation.

To implement the work ILRI/CIAT will use a participatory approach but will attempt to compare the efficiency of Participatory and Non-Participatory approaches at different points in the Research Development continuum. In addition, CIAT/ILRI will define strategic research issues based on demand and perceived problems and opportunities.

Some basic problems in the target periurban areas are land tenure, land scarcity and HIV/AIDS. In dairy, production often responsibilities for particular processes are shared between women and men. However, in view of the HIV/AIDS problem – i.e. the insecurity who in the household would be there in a decade - we believe that we need to involve both genders and all age groups in the technology development process and concentrate on labor conserving technologies. As forage seed systems are less well developed than in other Regions and the development of the milk-marketing sector is highly variable, Agroenterprise Development will be an important part of the Research and Development Process.The two other initiatives relating to forages incorporate Africa as part of a global or transregional initiatives.

Linking forage databases to Expert Systems to define entry points of forages: This initiative, which involves CSIRO, Australia, ILRI and CIAT, seeks to develop a Forage Database linked to an Expert System, which will aim to define potential entry points for forages in systems. This effort should result in enhanced adoption of improved forages and in a more efficient use of past and future Research and Development experiences. This activity will also complement and strengthen existing database and targeting efforts based on spatial analysis carried out by CIAT and its partners contributing to the project proposal.

Development of DST for targeting forages: This initiative developed by ILRI, IITA, CIAT and Universities, intends to include local knowledge and demand into a decision making tool. Though a great number of research results could be relevant across regions, the final decision on uptake of multipurpose forages will depend on the clients, the small and medium sized farmers. Their decisions will depend on a number of economic, cultural and environmental considerations. Therefore the idea is to define at the community level the most important criteria for decision-making and incorporate this as an interactive component in a Forage Targeting Tool, which links to existing or evolving databases. Intended users of such a tool would be primarily NGOs, Development projects and NARS.

Activity 4.2 Evaluation with farmer participation of multipurpose forages in crop and livestock systems

Highlights

- Adapted participatory methods for the evaluation of forages and identified farmer criteria for selecting forages for hillsides of Honduras.
- Advanced farmer led-testing of forages in Honduras and observed higher preference to evaluate grasses followed by shrub legumes.
- Established experiments in 8 communities of San Dionisio, Nicaragua to evaluate the effect of legume green manures in crop production
- Found significant increases in daily milk production of dual purpose cows grazing a Brachiaria-Leucaena paddock as compared to only paddock.
- Found significant increases of liveweight gains of young female calves during the dry period that had access to a protein bank of *Leucaena leucocephala* for 3 hours daily in contrast with those that had no access to the protein bank.

Progress towards achieving milestones

- **Model for participatory selection of forages developed**
  We have made progress in the development of a glossary of local terminology used by livestock farmers in hillsides of Central America to facilitate communication with technicians. In addition we have defined criteria used by farmer to select forages and have determined preference ranking of forages by farmers. Finally, we have initiated activities at the Honduras bench mark site to design and carry out forage-based experiments with crop and livestock farmers.

- **Known value of different legume green manures for crop production in hillsides of Central America**
  We established experiments in eight communities of San Dionisio, Nicaragua to evaluate with farmers the effect of three legumes on maize yield. Production economical and acceptability data will be analyzed to determine adoption potential of green manure technology by farmers in hillsides of Central America.

- **Known value of *Leucaena* to supplement milking cows and pre-weaved**
  We demonstrated that with small areas of *Leucaena leucocephala* CIAT 17263 farmers can produce 20% more milk and improve by 67% the liveweight gain of calves during the dry season in dual-purpose cattle farms.

4.2.1 Development of Participatory Methods to enhance adoption of forages as feed resources and for INRM

Contributors: R.Van der Hoek (University of Hohenheim**),** M. Peters, V. Hoffmann (University of Hohenheim)**,** J. Ashby, M. Ayarza

Rationale

Many regions in developing countries, including the hillsides of Central America, are characterized by deteriorating biophysical conditions such as erosion, loss of soil fertility and deforestation, resulting into diminishing resources, like depletion of nutrients, and in lower crop and livestock production. In addition, the growing population in these countries demands a higher food production. Maintaining and improving soil fertility and soil conservation are crucial. For this, a broad range of inputs are available, such as organic materials like animal manure, green manure and composted crop residues.

It is well recognized that forage-based technologies can play an important role in improving the environmental and socio-economic sustainability of smallholder production systems in the tropics, especially in situations with a fragile balance between the availability of natural and economic resources and their utilization. Forages can serve multiple objectives, such as provision of animal feed, enhancing soil conservation and maintaining and improving soil fertility.

Most of this research is being carried out in CIAT's reference site in hillsides of Honduras with the financial support of BMZ, Germany.

Expected outputs include a manual on participatory methods for forage-based technologies, scientific publications and a Ph.D. thesis (Hohenheim University).

The main activities being carried out are:

1. Systematization of farmers' perception on their own land use and the influence of new technologies
2. Development and adaptation of participatory approaches for the identification, testing, evaluating of forage-based technologies in complex systems.

During the last 10-20 years, many participatory methods and tools have already been developed. Some of these existing methods will be selected during this research process and their suitability will be tested in the process of identifying, testing and evaluating of forage based technologies as well as systematizing of farmers' perceptions on their own land use and the influence of new technologies. Consequently, existing methods will be adapted, aggregated in new combinations, or new methods will be developed if necessary.

Results and Discussion

Up to present, a detailed research proposal has been elaborated and a first analysis of the farming systems in the research area has been carried out.

Some results and conclusions are:

Farmer Categories. From approximately 40 interviews conducted with farmers in different zones in CIAT's site in Honduras we now have categorized farmers and their current production systems as follows:

a) Farmers in the lower lying areas (<500 m) have around 15 ha of arable land, whereas farmers in the medium (500-1000 m) and higher (>1000 m) zones have access to 5 and 3 ha, respectively.
b) Maize is the main crop at lower altitudes, beans are mostly grown at the medium and higher altitudes, whereas coffee is mostly found at the higher altitudes. Many farmers from the lower regions have bean and coffee fields at medium and higher altitudes.
c) Livestock (and more specifically cattle) ownership shows the same tendency. Many farmers in the lower areas posses more than 10 heads, whereas in the medium altitude zones farmers have 5 animals or less. In the higher areas almost all farmers have no cattle at all. Other types of livestock, such as pigs and poultry, are found in all zones. In some higher situated villages considerable amounts of sheep and goats are found.

Current activities with participatory methods in the region. In the region many Research and Development organizations are active with farmer groups. With respect to the use of participatory methods CIAT and IPCA (Investigación Participativa en Centro America) should be mentioned. The latter organization works with groups of farmers that conduct research in their communities. SERTEDESO is another important organization that offers a wide range of technologies focused on product diversification and soil fertility and conservation. All organizations offer important entry points with regard to the research theme.

Local experiences with forage-based technologies. Most cattle owners have experiences with local forages and CIAT and SERTEDESO are carrying out research activities to introduce new species. However, up to present, the activities have been focused mainly on cattle owners who represent only a small part (15%) of all farmers.

Future research activities. The next steps in the research process will address the following topics:

- Identification of research priorities and definition of objectives of experiments with forage based-technologies and development of forage ideotypes for different uses within the system. Participatory methods on priority setting, formulation of objectives and development of ideotypes will be used and adapted

- Design of experiments and protocols to test with farmer participation forage ideotypes. Extensive use will be made of participatory methods on formulation and design of experiments.

- Execution of several types of experiments with forage-based technologies (i.e. testing of forage germplasm, experiments on management and utilization of forages and crop residues, use of manure, compost). During and after the experiments monitoring and technical (e.g. treatment effects), economical (e.g. assessments of profitability and risks) and social (e.g. taking into account gender aspects, age, consumption patterns) evaluations will take place. The application and testing of participatory monitoring and evaluation tools will be a key element in this work.

The objective of this research is to work not only with the “conventional” users of forage- based technologies, (i.e. cattle keepers). Given the diverse production systems in the region there would seem to be ample scope (enough entry points) for the development of participatory methodologies for the utilization of forage -based technologies for farmers with cropping systems on steep slopes (forage crops for soil conservation and fertility) or farmers keeping small animals (forages for poultry, pigs, sheep, goats).

4.2.2 Evaluation of forages for multipurpose use with farmer participation in Hillsides of Central America

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CIAT Projects: SN-3, PE-2, PE-3

Costa Rica: The project in collaboration with the Ministerio de Agricultura y Ganadería (MAG) works in two sites: Bajo de Jorco in Acosta and Bocana in Puriscal. Both sites are located in the Central/Southern Regions of the San Jose Province.

In the first site, we are evaluating pasture grasses and herbaceous and shrub legumes, as well as live barriers of *Cratylia argentea* in degraded hillside areas. In the second site the work so far has been restricted to the use of *C. argentea* as live barriers.

In Acosta, MAG is managing a project called 'Reconversión Productiva', in which about 30 livestock producers (all men between 16 and 60 years) participate. Though women occupy a very important space in the production system through their work, but up to now there is no direct participation of women.

Capitalizing on this interest and existing linkages with MAG the project identified initially 17 farmers for the Participatory Evaluation of Forages, which was later reduced to 10. To maintain group motivated, objectives were clarified and the importance of active participation of all involved for the project was stressed.

In Puriscal, the project via MAG is working with a producers association, some of which are involved in livestock production. Currently the group is composed of 25 farmers between 19 and 65 years, with both women and men involved.

All farmers concur that the scarcity of forage in the dry season is their main problem, followed by steep slopes and low soil fertility. These problems limit the intensification of production, which is one of their main goals. A glossary of terms utilized by farmers with technical translations was elaborated to integrate terminology used by farmers and technicians.

Honduras: Participatory diagnoses with 59 farmers of which 45% were women (age 18-75 years) was carried out in three hillside sites (Victoria, Sulaco, Yorito) of Honduras. Scarcity of feed for the dry season and lack of seed availability were identified as the major limitations to raise livestock in these sites.

Based on the participatory diagnoses carried out initially, forage nurseries were established with farmers participating in land preparation, planting and in selection of forage options. Each group is composed of 15 to 20 farmers, and participation in individual events is from 65 to 75%, which is an indication that farmers have maintained interest in the evaluations of new forage options. However, only 10-15% of farmers participating regularly are women, particularly in Jicaro. Age groups are much better represented, varying between 25 and 76 years.

Particular strengths of these groups are high interest, unconditional participation and high stability. Initial weaknesses include lack of knowledge on forage germplasm, fear to communicate their assessment on forage options, lack of knowledge of what constitutes participatory evaluations and low level of organization.

To maintain the groups interested, the project facilitates activities that will result in stronger organizations such as initiatives for artesanal seed production and technical information on dairy products such as cheese. As a result of this process farmers now have less fear in communication and have obtained a better understanding of the utility of different forage materials.

Nicaragua: The project is working in three sites in the municipality of San Dionisio: Wibuse, El Corozo y Piedras Largas.

In 2000, the institutions involved (PRODESSA, INTA and CIAT) in the Project identified farmer leaders, who then invited small to medium crop-livestock owners for a meeting to select interest groups, present the project and establish farmer committees. A total of 56 farmers (15, 18 and 23, in El Corozo, Piedras Largas and Wibuse, respectively) got involved (only 3% women).

During the same meeting information was obtained on feeding strategies employed by farmers throughout the year. Inmost cases, during the wet season cattle are maintained on native pastures, on Jaragua (*Hyparrhenia rufa*.) and Estrella (*Cynodon* spp.). In the dry season crop residues (maize, bean and sorghum stover) becomes an important feed alternative. However, most producers stress the problem of quantity and quality of available feed resources during the dry season and several indicated the need to buy supplements (i.e. rice bran) at very high prices. Poorer livestock owners that cannot purchase supplements in the dry season face a reduction in production, lower reproduction and even death of their animals.

During 2000 and 2001, a Rapid Participatory Diagnosis on livestock production was carried out in the El Corozo and Wibuse sites to further characterize farmer groups. About 90-95% of the farmers indicated the low productivity of jaragua, estrella and native pastures as the principal problem for raising livestock. Hence farmers suggested the introduction of a variety of forages to be compared to existing grasses. Of the 56 farmers initially participating in the 3 committees, 60% maintain active participation, while the participation of the other 40% is more irregular or have been replaced by new-interested farmers.

Farmers are now conscious about the need to look for new forage alternatives to improve production and conserve Natural Resources and as a result, there is an increasing demand for seeds of selected grasses and legumes. However, we should indicate that there is still a lack of understanding of the participatory process by some farmers and their role in it. Further progress has also been limited by conservative attitudes of some livestock owners and lack of trust in religious and political processes sometimes has limited participation in collective activities. Moreover some producers had high expectation to obtain benefits from the project without any participation.

To maintain the interest of the groups the projects continuously clarifies concepts and relies on farmers with leadership to motivate other farmers. Also there is an active process of feed- back to local organizations, with the aim that farmers and local organization obtain ownership of the project. The project also participates in local events showing results and facilitates an active interaction between the different sites of the project. Events are scheduled to correspond to times of lower activity of farmers.

Adaptation of Participatory Methods for forage evaluation

One outcome of working with farmers in CA has been the adaptation of participatory procedures for forage germplasm development and deployment. The procedure is based on open evaluations using preference-ranking techniques. The first product obtained through open evaluations has been a glossary of local terminology, which is developed with farmers in response to the technology farmers are exposed to, in this case forages. Based on this, local terminology has been developed to facilitate communication

between farmers and technicians/researchers. At the same time an interactive process of communication and verification has been initiated. A second product has been the identification of criteria for selecting forage species from a farmers perspective. An example is shown in Table 106 for herbaceous legumes evaluated in Wibuse, Nicaragua.

The first column corresponds to technical criteria, which then are described according to farmers reasons in the second column. In this case, yield is related to the quantity and development of leaves, to drought tolerance and retention of leaves to green color, cover is important for soil conservation and adaptation is a positive response of the forage to biophysical conditions. Frequency indicates the number of responses for each criteria, indicating its relative importance. For better interpretation is important to record the number of farmers participating and their composition according to age and gender. The last column shows the qualitative scale used by farmers for each criteria. As for other technologies, farmers in Latin America seem to use expressions like good, regular and bad.

Table 106. Criteria and reasons for farmers selecting legumes in Wibuse, San Dioniosio Nicaragua.

Criteria	Reason given by farmers	Frequency of responses	Farmer's Qualitative Scoring
1. Yield	Quantity of leaves	10	Good
2. Drought tolerance/Leaf retention	Green Color in the dry season	5	Good
3. Soil conservation	Cover	4	Good
4. Adaptation	Adapted to climate and resistant to pest and disease	1	Regular

The third product is Preference Ranking of Forage Technologies, using regression procedures. In Figure 43 we show an example for cover legumes in one of the sites in Nicaragua. It is clear that Caballero (a local *Lablab purpureus*) has high acceptance while mungo (*Vigna mungo*) is the least accepted by the farmers.

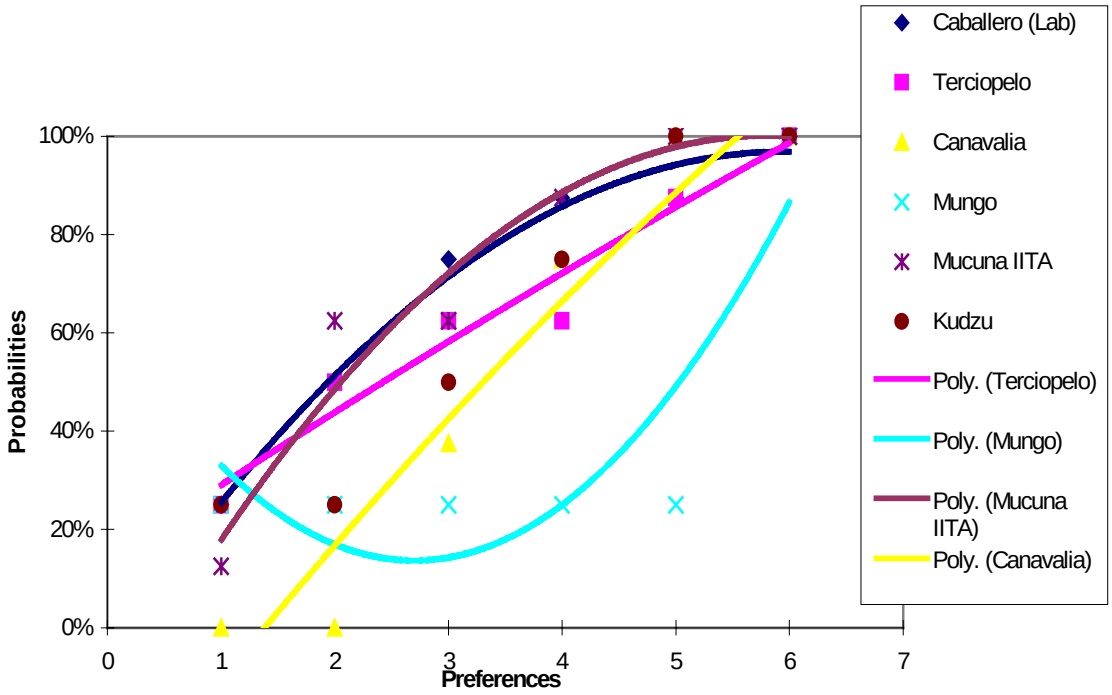

Figure 39. Preference ranking of six cover legumes, Piedras Largas, Nicaragua

These results then are related to the criteria obtained in the same evaluation, giving indication for potentially successful technologies. The participatory methods being developed are disseminated through training courses, workshops and field practices. At the end two main achievements are expected:

1. That people participating in the training become trainees themselves and
2. Development of a Training Module to guide technicians through the process.

Agronomic Evaluation of Forages in Hillsides of Central America

In the following section agronomic results from Honduras are shown as an example of the data being recorded in the three hillside sites of Central America.

Green manures: Soil cover 7 months after establishment was good with most species tested, the exception being Pueraria phaseoloides CIAT 7182 in Las Cañas (Table 107). Significant (P<0.05) differences among accessions were observed in yields in the two sites. However, in both sites Mucuna deerengianum and Lablab exhibited high yields.

Table 107. Soil cover and Dry Matter yield of herbaceous legumes in two sites of Yoro, Honduras.

Species	Sites			
	Las Cañas		Luquigue	
	Cover (%)	Yield (kg/ha)	Cover (%)	Yield (Kg/ha)
Mucuna pruriens Negra	95	8355	-	-
Mucuna pruriens IITA BENIN	95	8315	90	11193
Lablab purpureus DICTA	43	6980	93	12017
Mucuna deerengianum	95	6471	95	12321
Canavalia brasiliensis CIAT 17009	77	3859	80	8681
Pueraria phaseoloides CIAT 7182	3	450	52	2313
LSD (P<0.05)		3466		7236

Productivity of grasses in the dry season is shown in Table 108. Yields were slightly higher in Luquique than in Las Cañas. However, due to severe drought, yields were relatively low in both sites. However, it was interesting to observe that in spite the prolonged dry season the cut and carry grass 'Camerún' performed well in the two sites.

Table 108. Dry Matter yield (kg/ha) of 8-week regrowth of shrub legumes in the dry season, in two sites in Yoro, Honduras.

Species	Site			
	Las Cañas		Luquigue	
	Cover (%)	Yield kg/ha	Cover (%)	Yield kg/a
Pennisetum purpureum cv Camerun	37	1430	25	1022
Panicum maximum CIAT 16031	25	624	18	413
Brachiaria brizantha CIAT 26646	42	521	30	936
Andropogon gayanus CIAT 621	26	510	30	638
Brachiaria brizantha CIAT 26110	25	464	23	668
Brachiaria brizantha CIAT 16322	33	439	20	1088
Brachiaria hybrid CIAT 36061	25	385	17	640
Brachiaria humidicola CIAT 6133	60	133	28	137
LSD (P<0.05)		525		462

With shrub legumes we only found differences in DM yield among accession in Luquigue (Table 109). The highest yields were recorded for *L. leucocephala* CIAT 17263 in Las Cañas and for *C. calothyrsus* CIAT 22310 in Luquigue. *Cratylia argentea* had low yields in the two sites due to its slow establishment. The distribution of forage materials to farmers around the Yorito reference site are shown in (Table 111).

Table 109. Dry matter yield (g/plant) of 9-week regrowth of shrub legumes in the wet season, in two sites in Yoro, Honduras.

Treatment	Sites			
	Las Cañas		Luquigue	
	Leaf and fine stem	Total Dry Matter	Leaf and fine stem	Total Dry Matter
	(gDM/plant			
Leucaena leucocephala CIAT 17263	219	293	210	256
Calliandra calothyrsus CIAT 22316	183	230	230	312
Leucaena macrophylla 47/85	155	208	0	0
Calliandra calothyrsus CIAT 22310	135	154	437	607
Cratylia argentea CIAT 18668	48	50	95	107
Local control	0	0	0	0
LSD (P<0.05)	NS	NS	258	354

Table 110. Distribution of forage materials in the Yorito Reference Site, Honduras (2001).

Material	Area	Quantity			Farmers	Institution	CIAL/ Farmer Association
		Seed	Vegetative Material				
			Stacks	Bags (50 kg approx)			
	(m^2)	(g)	(No.)	(No.)	(No.)	(No.)	(No.)
A. gayanus 621	2400	2400			5	1	
B. dictyoneura 6133	1200	4800		4	8	4	
B. brizantha 26110	5600	4875			17	6	1
P. maximum 16031	6400	3400			11	4	2
P. purpureum cv. Camerún	6400		3200		16		
A.pintoi 22160	400			1	1		
C. argentea 18668	5000	5080			12	2	1
C. pubescens 15160	400	100			2		
L. leucocephala 17263	5500	495			9	2	
L. purpureus	1000	1000			1		
S. guianensis 184	1200	900			5	1	
M. pruriens IITA BENIN	1000	1000			5	1	
Total	36500	24050	3200	5	88	20	4

Most of the forage species that have been distributed in the Yorito area has been through sexual seed, the exception being *P. purpureum* that does not produce seed. In addition more area has been planted with grasses (60%) than with legumes and farmers have preferred to evaluate shrub legumes rather than herbaceous legumes. An interesting development is that farmers harvested 188 kg of pure seed of *Brachiaria brizantha* 26110 for further testing in 2002.

Small fund scheme

The project is carrying out a competitive funding scheme to facilitate the diffusion of results and methods being developed. The main problem for approving proposals has been lack of knowledge on participatory

methods, which need to be part of the work. Most proposals therefore were assigned to partners already involved in the project.

The following projects were approved and will start in the 2nd half of 2001

- Asociación de Campos Verdes (a farmer grassroot organization), San Dionisio, Nicaragua: Improving our soils with green manures – a participative project of communities of San Dionisio
- Instituto de Tecnología Agropecuaria, INTA, Nicaragua: Participatory evaluation on the use of *Cratylia argentea* as feed supplement to milking animals in San Dionisio
- Ministerio de Agricultura y Ganadería de Costa Rica, MAG: Participatory evaluation of *Cratylia argentea* cv. Veraniega as live barrier and feed resource in degraded areas of Puriscal hillsides
- Fundación Ecotrópica, Puriscal, Costa Rica: Participatory evaluation of establishment methods for *Brachiaria brizantha* 26110 (Toledo) in association with *Desmodium ovalifolium* 33058 and *Arachis pintoi* 18744 in the Puriscal hillsides
- Servicios Técnicos para el Desarrollo Sostenible, SERTEDESO, Yorito, Honduras: Artesanal seed production of multipurpose forage grasses in Yorito, Sulaco and Victoria

4.2.3 On-farm evaluation of selected forages as feed resources in dual cattle systems of Central America through the Tropileche Consortium

4.2.3.1 Grazing of *Leucaena leucocephala* by lactating cows and young calves in a dual purpose cattle system located in a sub-humid area of Costa Rica

Contributors: Beatriz Sandoval, Marco Lobo, Carlos Hidalgo and Vidal Acuña (MAG), P. J. Argel (CIAT)

Rationale

The low animal production figures associated with cattle farms in the Latin American tropics are mainly related to poor quality and availability of the pastures used, which consist mainly of introduced African grasses. It is also well known that tropical legumes, particularly shrubs and trees, can improve the quality of the diet offered to the animals due to high levels of protein and mineral contents. This is particularly true during the dry period, when grasses become mature and loose quality. Tropileche is a project aimed at the improvement of animal feed supplies with emphasis on forage legumes with farmer participation, and one alternative promoted has been the use of the tree legume *L. leucocephala* to feed lactating cows and young calves in dual purpose cattle systems.

Materials and Methods

Two paddocks were planted with *L. leucocephala* subsp. *leucocephala* CIAT 17263 in one farm located in the district of Miramar (Costa Rica). The soils are moderately acid sandy loams (pH 4.8 to 5.2), with low aluminum and phosphorus content. A mean temperature of 27°C and an annual rainfall of 2040 mm, distributed from May to November, had been recorded. One paddock consisted of 4.0 ha of Leucaena planted on strips and associated with the grass *Brachiaria decumbens* cv. Basilisk to be grazed by lactating cows (see IP-5 Annual Report 2000). The other paddock consisted of a Leucaena protein bank of 0.8 ha planted by direct seeding at a distance of 1.0 m between rows and 0.75 m between plants (13,300 plants/ha), to be grazed by young (around three months old) female calves.

Forage availability in Leucaena was measured using the forage reference pattern method known as "Adelaida technique", while forage availability of the grass was measured by the well known rank dry weight method. Milk production was monitored in the associated Brachiaria – Leucaena pastures, and in a

pure Brachiaria stand with dual purpose lactating cows using a simple cross over design. Twenty cows were assigned randomly to each treatment (paddock), and the cycles consisted of 10 days grazing (7 days adaptation and 3 days of measurements).

Animal liveweight of 3-4 months old female cebu x swiss brown calves was monitored in the protein bank of Leucaena. Following the milking of the cows, 20 female calves were allowed to graze Leucaena for 3 hours daily, and later moved to a *Brachiaria* spp. pasture, for a period of 31 days. A similar group of young female calves was maintained only in the Brachiara pastures, and the liveweight of both groups was measured at the end of the observation period. A t test was used to compare liveweight means of both groups.

Results and Discussion

Milk production of cows which had access to the Brachiaria – Leucaena pastures produced significantly ($p<0.05$) more milk (6.4 kg milk/cow/day) in contrast with cows grazing only Brachiaria (5.6 kg milk/cow/day), as a response to improvements of quality in associated pastures. In addition, DM availability was higher (1.6 t more DM) in the associated grass-legume pasture shown in Table 111. This is consistent with results observed elsewhere in pastures that have a legume component, which gives the opportunity to increase the stocking rate or to prolong the days of ocupation of the paddocks.

Table 111. Dry matter availability and protein content of *B. decumbens* cv. Basilisk in monoculture and associated with *L. leucocephala* CIAT 17263 after 45 days of rest in a farm located in Miramar, Costa Rica.

	Pastures		
	B. decumbens (Monoculture)	*B. decumbens + L. leucocephala*	
DM availability (t/ha)	2.5	3.4	0.7
Crude protein (%)	8.8	10.0	23.4

Liveweight gains of young female calves with access or not to the Leucaena protein bank is shown in Table 112. It is clear that the larger effect of the legume on weight gain was experienced during the dry period, that last for about five months in this site. During the wet period there was no effect of the legume or gain when the animals had access to grasses like *B. brizantha* cv. Marandú and La Libertad. These grasses maintain acceptable levels of crude protein and digestibility during the wet period and thus the effect of the legume is less noticeable.

Table 112. Liveweight gains of young female calves with or without access to a protein bank of *L. leucocephala* CIAT 17263 of 45 days of re-growth in a cattle dual purpose farm located in Miramar, Costa Rica.

	Liveweight gain (g/day)	
Calves group	Dry	Wet
With access to Leucaena	400 a*	525 a
Without access to Leucaena	240 b	520 a

* t test ($p<0.04$)

4.2.4 Alternative strategies for on-farm forage seed production

Contributors: M. Peters, A Schmidt, SERTEDESO, Guillermo Giraldo

CIAT projects: PE-2, PE-3

Rationale

Lack of seed often limits adoption of forage- based technologies as in most cases commercial seed production is not well developed, with notable exceptions of the seed sector for tropical grasses in LAC. Even where a commercial seed sector exists, it may be limited to a few forage options and quantities of seed demanded by small farmers may at least initially not be an attractive market for profit oriented private companies. Hence any forage germplasm development program directed towards farmers needs to address this problem. At the same time forage seed production may be an opportunity for farmers to increase income.

Key in the success of functional seed systems directed towards smallholders is the matching of demand and supply of seed and planting materials and cost. As smallholders are living often in marginal or remote areas, this is particular important as these groups often have limited access to information market, and cash to purchase seed.

Activities

Both in Honduras – through a small fund scheme – and in Nicaragua activities are underway to enhance forage seed production by smallholders. Approaches are different according to sites. In Honduras farmers have started seed production but need help in organization, while in Nicaragua there is a need to start from scratch. Beyond facilitating and learning from these seed production efforts in Central America, a strategy for forage seed production is being elaborated.

4.2.5 On-farm evaluation of green manures in hillsides of Nicaragua

Contributors: Campos Verdes (San Dionisio, Nicaragua), Axel Schmidt, Michael Peters, Edmundo Barrios, Luis Alfredo *Hernández*

CIAT projects: PE-2, PE-3, SN-3

Rationale

Farmers are increasingly concerned about the lower prices they ar getting for their harvest products and increasing input prices on the market. At the same time soil fertility on farmer fields is decreasing. Weeds become a larger problem over time. In order to overcome these limitations and backed up by CIAT, the local farmer organization “Campos Verdes” initiated a project to introduce, evaluate and promote the use of cover crops and green manures in the communities of San Dionisio.

Cover crop and green manure legumes may significantly contribute to enhanced soil fertility, water and soil conservation and weed suppression. Some of these green manure crops show high drought tolerance and can be used as forage or even for human consumption. It was also taken into account that growing green manures may result in a smaller amount of applied agrochemicals, which are already contaminating the scarce water resources of the people in San Dionisio.

While plant adaptation/management and technical feasibility are important factors, economic viability is

considered decisive for adoption of cover crops and green manures. Therefore, cost-benefit analyses are one of the main objectives of the project in order to compare the current management including N-fertilizer and agrochemical application with the use of cover crops and green manures. Other objective are objectives are the demonstration are selection of legumes for green manure that have drought tolerance, management of cover crops and green manures with active participation of the local community and identification of key indicators of soil organic matter status.

Materials and Methods

A workshop was held in San Dionisio in April 2001 to which all members of Campos Verdes had been invited. A total of 27 farmers attended the event and the proposed project was presented and discussed.

Sites with different soil and climate conditions throughout San Dionisio were identified and legume cover and green manure options were discussed. Farmers chose *Mucuna pruriens*, *Canavalia ensiformis* and *Lablab purpureus* as legumes for the experiment. At the end of September 2001 the experiments were established on 8 farms in different communities of San Dioniso (Table 113).

The experiments consist of seven treatments, which were arranged in a randomized block design with 3 replicates at each site. The treatments are summarized in Table 114.

Table 113. Location and site description of on-farm cover crop/green manure experiments in San Dionisio, Nicaragua.

Farmer	Community	Latitude	Longitude	Altitude	Observations
D. Salgado	Piedra Colorada	12° 49’ 47.2 N	85° 51’ 51.1” W	504	River valley
A. Castro	Susuli central	12° 48’ 29.2” N	85° 50’ 24.5” W	564	Slope
J. Hernández	Susuli arriba	12° 47’ 48.0” N	85° 50’ 05.2” W	565	Steep slope
V. Cebilla	Corozo	12° 47’ 02.2” N	85° 52’ 17.6” W	484	Slope
J. Orozco	Carizal	12° 47’ 08.2” N	85° 54’ 15.0” W	715	Moderate slope
J. Jarquín	Piedras Largas	12° 43’ 32.6” N	85° 49’ 43.1” W	474	Slope
J. Hernández	Jícaro	12° 46’ 19.2” N	85° 50’ 15.6” W	530	Very steep slope
E. Ochoa	Ocote arriba	12° 45’ 23.2” N	85° 53’ 17.3” W	735	Slope

Table 114. Treatments included in on-farm cover crop/green manure experiments in San Dionisio, Nicaragua.

Treatment	Year 200*	Year 2002
1	Maize	Maize without N-fertilizer (Control)
2	Maize	Maize with low N-fertilizer level
3	Maize	Maize with high N-fertilizer level
4	Maize	Maize with very high N-fertilizer level
5	Maize with *Mucuna*	Maize without N-fertilizer
6	Maize with *Canavalia*	Maize without N-fertilizer
7	Maize with *Lablab*	Maize without N-fertilizer

*Cover crops/green manures were sown into existing maize plots in September when the maize was entering its mature stage.

Legumes were sown in maize plots (4 x 4 m) at the traditional bean sowing distance (0.4 x 0.4 m). Legume evaluation will be carried out on a monthly basis recording field emergence, plant height, ground

cover, incidents of pests and diseases, weed presence, biomass production, drought tolerance, flowering patterns and seed production.

Soil samples will also be taken prior to the establishment of the experiments, prior to the maize planting and at the end of the experiments and analyzed. The cover crops and green manures will be kept in the maize plots throughout the dry season and maize will be planted in the wet season. Fertilizer treatments will be applied and maize yields recorded. Pest, disease and weed incidents will be evaluated throughout the project. Cost-benefit analyses will be conducted and presented in a final workshop in November 2002. Field days will be held at strategic points of the project (legume establishment, legume drought tolerance, mulching and maize planting, maize harvest) in order to demonstrate and discuss practical management issues with the communities. By discussing soil fertility issues key informants for local soil organic matter management techniques and indicators will be identified.

Expected outputs

Local on-farm evaluation data and economic analyses will be available for further promotion and dissemination of legumes species for use as cover crops and green manure. It is expected that a growing number of farmers in San Dionisio will adopt green manure technologies for growing maize in the future and will show further interest in other legume species. Local indicators for soil organic matter will be identified and the local knowledge on the management of soil organic matter documented.

Activity 4.3 Forage seeds: reproductive biology, quality, multiplication and delivery of experimental and basic seed

Highlights

- Continued to multiply and deliver to partners in the region, experimental and basic seed of a wide range of promising forage species.
- Found that main seed dormancy mechanism in *B. brizantha* cv. Toledo is related to physical factors in the seeds

Progress towards achieving milestones

- **Seed of selected forages delivered to partners and seed companies**
  Seed multiplication and delivery from our two seed units continue to be an important mechanism to promote adoption of new forages. This year we delivered basic seed of different forage species to Haiti for on-farm testing and of a new *Brachiaria* hybrid to a seed company for regional testing.

4.3.1 Multiplication and delivery of selected grasses and legumes in the Seed Units of Atenas and Palmira

4.3.1.1 Seed Unit of Atenas

Contributors: P. J. Argel and Guillermo Pérez (CIAT)

Seed multiplication activities continued in the Atenas Seed Unit (Costa Rica) in collaboration with the Escuela Centroamericana de Ganadería (ECAG). The seed produced is destined to support advanced evaluations of promising forage germplasm both by CIAT's projects and regional research/development institutions.

From August 2000 through August 2001 a total of 262.48 kg of experimental and basic seed was either produced at Atenas or procured from collaborator farmers. The bulk of the seed was formed by *Cratylia argentea* (125.04 kg), *Brachiaria* spp. (99.73 kg), *Arachis pintoi* (9.50 kg), *Leucaena* spp. (3.57 kg), *Centrosema* spp. (6.49 kg), *Desmodium heterocarpum* spp. *ovalifolium* (3.80 kg) and *Paspalum* spp.(4.92 kg). In addition, small quantities of experimental seed of *Panimum maximum, Desmodium velutinum, Chamaechrista rotundifolia* spp. *grandiflora, Clitoria ternatea*, *Calliandra* spp. and *Stylosanthes guianensis* was multiplied.

During the period August 2000-August 2001 a total of 614.01 kg of experimental and basic seed were delivered by the Seed Unit of Atenas (Costa Rica). Table 115 shows that eighty eight seed requests were received from 10 countries, where more than half of the requests came from Costa Rica, the host country of the forage project. However, a significant amount of seed was delivery to Haiti (282.59 kg), a country recently involved in forage projects with the assistance of CIAT. *C. argentea* has been the species with more demand regionally, which indicates the strong expectation that this shrub legume has generated both among researchers and cattle farmers.

Table 115. Countries, number of requests and amount of experimental/basic forage seed delivered by the Seed Unit of Atenas (Costa Rica) during the period August 2000-August 2001.

	No. of	Forage species (kg)			
Country	Requests	*Brachiaria* sp.	*A. pintoi*	*C. argentea*	Other species
Belize	1		25.0		29.0
Brazil	2		0.52		1.6
Colombia	6	6.47	0.5	22.0	2.0
Costa Rica	62	52.0	21.0	107.3	30.5
Philippines	1				0.3
Guatemala	1			2.0	
Haiti	3	61.22	1.9	1.7	217.77
Honduras	1			3.0	
Mexico	3	5.0	7.0		
Nicaragua	8	2.9	0.8	0.2	7.3
Total	88	127.57	63.72	136.2	286.52

4.3.1.2 Seed Unit of Palmira

Contributors: A. Ortega, A. Betancourt, B. Hincapie y J. W. Miles (CIAT)

The Project operates a small seed multiplication unit which is intended mainly to service the seed requirements of the Project. Where possible, seed produced in excess of Project needs is donated (small quantities) or sold (larger volumes) to bona fide researchers or private producers. In May 2001, this Unit suffered the irreplaceable loss of the technician who handled with exceptional competence all field and laboratory operations -- Mr. Alcibiades Ortega -- who was seriously injured in a highway accident returning from the CIAT-Popayán multiplication site. We are still adjusting to this terrible tragedy.

A total of nearly 2 t of seed was harvested during the previous 12 mo (July 2000 to June 2001). Half of this total is represented by seed of three accessions of *A. pintoi*. Twenty-one *Brachiaria* accessions were multiplied, mostly for on-farm evaluation under grazing, including promising new hybrid selections. Thirty-four accessions of *Cratylia caliandra* were multiplied. Several additional legume genera [*Calliandra* (4 accessions), *Centrosema* (3 accessions), *Clitoria* (1 accession), *Desmodium* (5 accessions), *Leucaena* (4 accessions), *Mucuna* (5 accessions), *Rhynchosia* (1 accession), *Stylosanthes* (7 accessions), and *Vigna* (16 accessions)] complete the list.

During the first 6 mo of 2001, 134 individual samples of seed of 16 forage genera were dispatched from the Seed Multiplication Unit, accounting for a total of nearly 400 kg of seed (Table 117). This seed was distributed to recipients in 14 different countries, as detailed in Table 116.

Table 116. Genus distributed, weight (kg) and number of sample send during the first semester of 2001.

Genus	Kilograms	Number of Sample
Aeschynomene	0.010	1
Andropogon	10.000	1
Arachis	308.45	20
Brachiaria	6.426	20
Calopogonium	0.070	2
Canavalia	0.150	1
Centrosema	8.260	7
Cratylia	18.155	15
Desmodium	28.403	30
Flemingia	0.040	1
Leucaena	2.250	4
Mucuna	1.000	1
Panicum	0.120	1
Pueraria	3.105	24
Stylosanthes	0.195	5
Zornia	0.010	1

This sample were send to 14 countries: Germany (15); Brazil (2); Costa Rica (11); Haiti (13); Honduras (37); Japan (1); Mexico (1); Nicaragua (52); Senegal (2); Tanzania (4); Uganda (1); Uruguay (6); USA (2), and Colombia (320) distributed: CIAT (190), CORPOICA (18), Particular (36), Universities (11), Others-GNO (65).

4.3.1.3 The effect of storage conditions on viability and germination of acid-scarified and non-scarified seeds of *Brachiaria brizantha* cv. Toledo (CIAT 26110)

Contributors: P. J. Argel and Guillermo Pérez (CIAT)

Rationale

Commercial cultivars of several species of the genus *Bachiaria* grass are widely used in the Latin American tropics. However, given the variability of the livestock ecosystems in terms of soils and climate, as well as management, new Brachiaria cultivars are developed by research institutions aiming at more productive plants with good agronomic attributes and of high forage quality. Planting by seed is the common practice to establish Brachiaria fields, and the seed industry has grown considerable during the last 20 years to cope with an increasing demand for Brachiaria cultivars. Seed yield and quality are variable among the Brachiaria species, and one of the key components in the development of new cultivars deals with seed yield, viability and dormancy. Highly dormant seeds require seed storage and treatment to breakdown this conditions and that may limit a wide and opportune use of the cultivar. Thus the studies carried out to understand the degree of dormancy and methods to break it down are justified, particularly with new cultivars such as *B. brizantha* cv. Toledo (CIAT 26110).

Materials and Methods

Seeds of cv. Toledo were hand-harvested in November 1999 in Atenas. The seed was cleaned, processed and stored at ordinary ambient conditions, namely mean temperature of 24°C and Relative Humidity (RH)

of 60-80%. Three months after harvesting (15 February 2000) half of the seed was scarified with concentrated sulfuric acid for 12 minutes and washed with abundant water. Then two lots of scarified and non-scarified seed were formed; half of the seed was stored at the ambient conditions described, and the other part was stored in a cool room kept a constant 20° C of temperature and 50% RH. Thus 4 different lots of seeds (treatments) were formed: 1) Cool room non-scarified seeds (CNS); 2) Cool room scarified seeds (CS); 3) Ambient non-scarified seeds (ANS), and ambient scarified seeds (AS).

One month latter (namely 4 months after harvest) a germination test was carried out on plastic trays filled with a mixture of 60% sand and 40% soil. Four replicates each of 100 seeds per tray were evaluated. Watering of the trays was done daily with sterile water and count on germinated seeds was recorded 3, 7, 10, 14 and 21 days after planting. These tests were repeated every two months for a total of 8 tests during approximately two years. Germination percentages were determined and statistically compared as well as the Rates of Speed Germination (RSG). The latter measures how quick the seeds germinate once they are put in adequate germination conditions; a higher value of RSG indicates that a high proportion of the seed germinated during the first days of the test. This has practical implications since a rapid germination may conduce to quicker pasture establishment and better competence with weeds.

Results and Discussion

It is very common to find high levels of seed dormancy in forage grass in process of domestication; the nature of the dormancy can be physical (e.g., glume and lemma tightly closed) or physiological (e.g., immature embryo), or the combination of both. This is a natural event of ecological importance that secures the survival of the species, but may have negative practical implications in commercial plantings, and on the reliability of germination tests. Figure 40 shows that four months after harvest scarified seed of *B. brizantha* cv. Toledo had high levels of germination independent of seed storage (85.5% and 78.0% germination respectively for scarified seed stored at ambient conditions and in the cool room).

Non-scarified seed germinated less indicating that the dominant dormancy factor present in Toledo seed is caused by physical factors. The controlled cool environment seems to increase the expression of this type of dormancy since germination was only 28.2% for the CNS treatment in contrast with 69.0% for the ANS. This could be associated with less seed moisture content that the seed may have reached in the cool room (50% RH), non-measured in this experiment, compared to the seed moisture content reached at ambient humidity (60-80% RH), since it is well known that as seed loses moisture, the physical dormancy increases.

Six months after harvest all the seed lots had high levels of germination, although again the sulfuric acid scarification improved the germination of the seeds. Germination tests carried out afterwards and for the following 18 months, have shown high levels of seed germination (close to 90%) independent of seed treatment, which indicates the high viability that the cv. Toledo seed has. The data also indicates that 6 months after harvest no scarification of the seed is needed to obtain high germination percentages, independent of seed storage conditions.

Under appropriated temperature and humidity conditions, the speed of seed germination varies according to the level of dormancy. Hard seeds for instance may take longer to become imbibed with water, thus delaying the initiation of the germination process. In this particular experiment, seed germination was monitored for 21 days and the rates of speed germination (RSG), a measure that indicates how fast the seeds germinate, varied among treatments as illustrated in Figure 41.

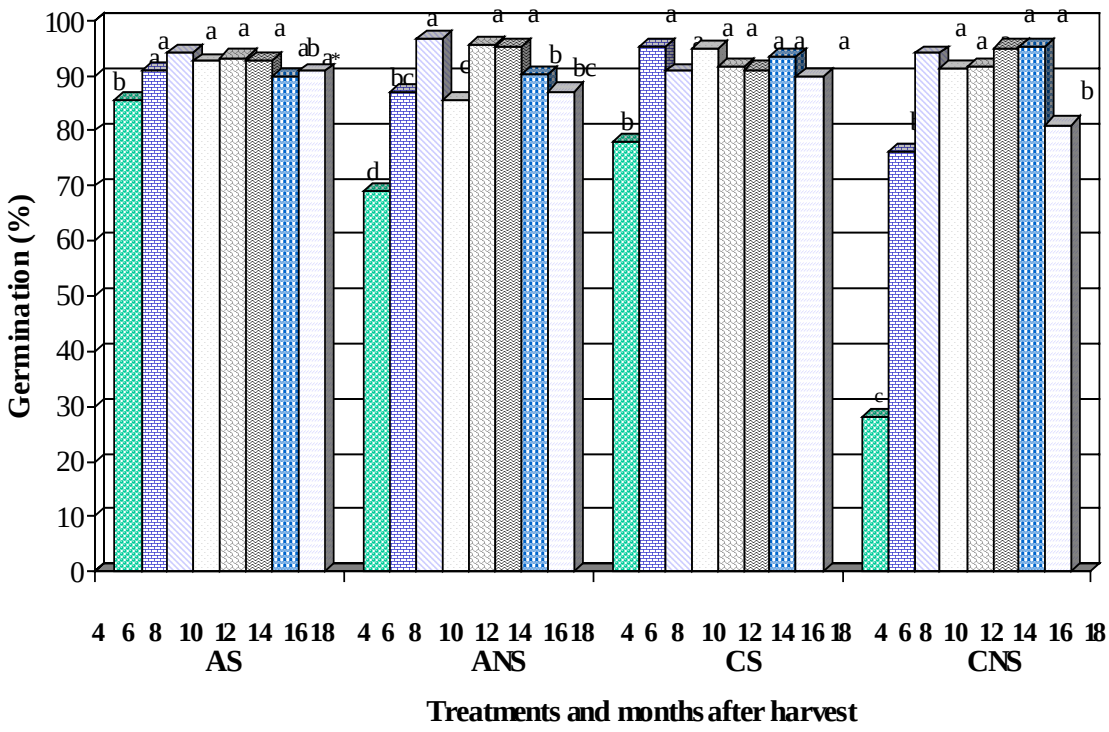

Figure 40. Germination percentages of *B. brizantha* cv. Toledo (CIAT 26110) seeds scarified and non-scarified with sulfuric acid and stored in ambient and controlled conditions (AS, ambient scarified seeds; ANS, ambien non-scarified; CS, controlled scarified and CNS, controlled non-scarified) (P<0.05).

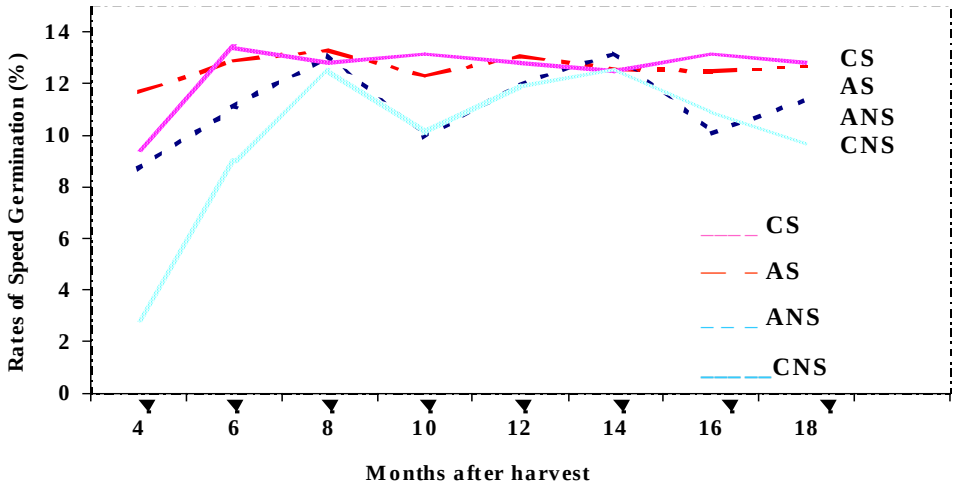

Figure 41. Rates of speed germination (RSG) of sulfuric scarified and non-scarified *B. brizantha* cv. Toledo (CIAT 26110) seeds stored at ambient and in controlled conditions for 18 months (CS=controlled scarified seeds; CNS=controlled non-scarified; AS=ambient scarified; ANS=ambient non-scarified).

For the first 8 months after harvest, the non-scarified seeds had lower RSG, particularly the seeds stored in controlled conditions, in contrast with the acid scarified seeds. This again indicates that dormancy was

mainly imposed by physical factors, which in turn had larger expression in cool storage conditions. Also, 4 months after harvest AS and ANS seeds had higher RSG in contrast with CS and CNS seeds. This may suggest a certain degree of physiological dormancy, which was short lived since the differences in RSG had practically disappeared 6 to 8 months after harvest for all the storage conditions. It is also clear that RSG are higher and more reliable for acid scarified seeds than for non-scarified seeds independent of the storage conditions.

Activity 4.4 Expert systems for forage biodiversity linking geographical information with biological data

Highlights

- CIAT's forage database, with a graphical interphase is now ready and will soon be able for distribution in a CD-ROM and in the Internet.
- Developed a conceptual model to target forages to different biophysical and socio-economic conditions.

Progress towards achieving milestones

- **Forage database published in a CD-ROM and distributed**
  The development of CIAT's forage database with a graphical interphase was completed and can now be accessed in Intranet. By the end of the year we expect to have the database in a CD-ROM and in CIAT's new Web page.

- **Developed a conceptual framework for a Decision Support System (DSS)**
  Progress was made in developing a conceptual model to target forages to differente environments and socio-economic niches. In addition, potential users and stakeholders of the DS Tool were identified, and an inventory of data, models and algorithms available was completed.

4.4.1 Development of a forage database with graphical interface

Contributors: A. Franco, F. Barco, B. Hincapié, L.H. Franco, G. Ramírez, C. Lascano and M. Peters (CIAT)

Rationale

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.

To access the forage data base an information system based on the fourth generation language ORACLE FORMS 3 was developed; this system is available via a CIAT's 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.

Materials and 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.

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.*

In 2001 new data was added, the layout improved and a help manual elaborated. Once the help manual is edited, the database will launch and CD-ROM version will be available to collaborators. Currently the database is available on the CIAT Intranet, and will be available on the Internet when the new CIAT web site is launched (Photo 19)

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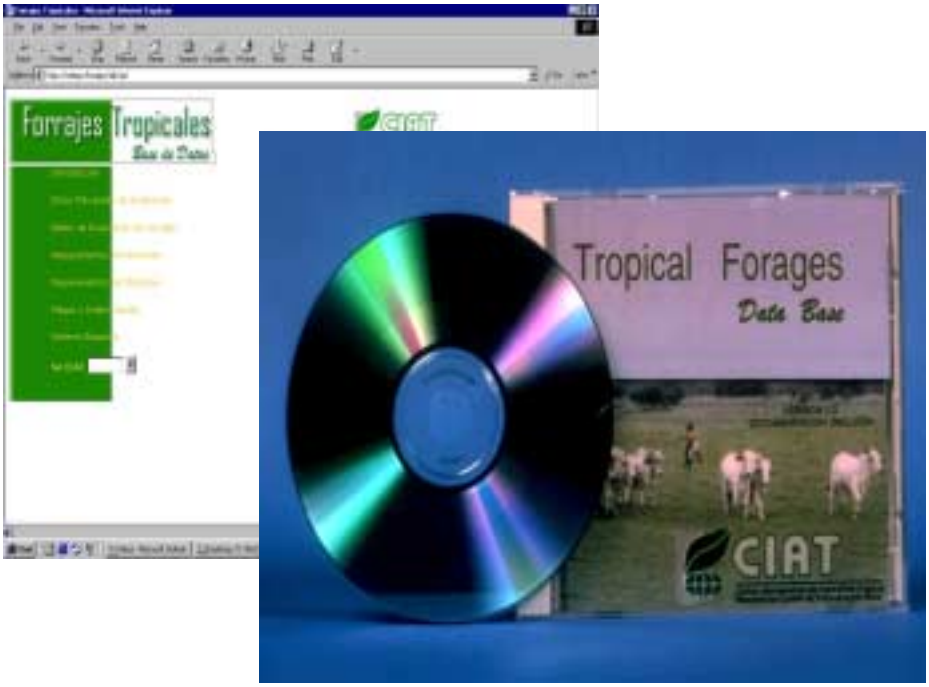

Photo 19. Tropical forages database-Internet and CD-ROM

4.4.2 Incorporating Socio-Economic Data and Expert Knowledge in representations of Complex Spatial Decision-Making

Contributors: R. O'Brien, R. Corner (both Curtin University), S. Cook, M. Peters, Th. Oberthür, G.G Hyman, L.H. Franco, A. Franco, B. Hincapie

CIAT projects: PE-4, SN-3

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 intends to enhance the utility of existing information and, in future, to integrate environmental and socio-economic adaptation of forage germplasm for multiple uses. It is anticipated, that this approach will allow a more accurate and client-oriented prediction of possible entry points for forage germplasm.

One product of this research will be a fully functional web-based or CD-ROM tool, primarily designed for targeting forage germplasm in Central America. The primary target users are, NGO's, development agencies, national research institutes and decision makers in government. In conjunction with farmers, these users will be able to more effectively target suitable locations for new forages, with the aid of the tool. This will result in more informed choices being made, thus allowing more effective use of public funds dedicated to agricultural development and natural resources conservation.

Tools to better target forages will also help improve the wellbeing of smallholders by assisting them to more effectively utilize their resources in sustainable ways. The addition of carefully selected forages to a farming system has a plethora of benefits both for the farmers and for the environment as well as the wider community. These benefits derive both from the direct influence of forage planting, and the indirect increase in cattle production and cropping system improvements, and include for example improved sustainable intensification, reduced erosion and alleviation of protein and micronutrient deficiencies in the community.

Materials and Methods

Literature and model review: Review of literature and of existing similar models and software will be ongoing. An effort will be made to identify current and as yet unpublished work on the topic, by contacting relevant university departments and research organizations, and by attending and presenting at conferences where possible. This will help identify possible approaches to representing spatial decision-making with particular emphasis on incorporating socio-economic data and expert knowledge. Existing tools will be evaluated to determine their effectiveness in representing expert spatial decision-making processes and in particular in targeting forage germplasm. Initial research suggests that that regression tree and Bayesian techniques will likely prove the most useful methods, as these techniques could cope with data uncertainties like gaps and errors in the data. A possible approach will be to use regression trees to determine rough climatic envelopes, followed by Bayesian modeling to estimate likelihood of adoption success within these envelopes.

Case study – design and develop a tool: As a case study, a decision support tool to target forage germplasm will be designed and developed, using geographical information system (GIS) technology.

This targeting consists of identifying which forages would be suitable or successful in a particular location, given data and/or knowledge about the forages and about the location in question.

The forages data will be in the form of spatial coordinates for a number of locations at which accessions have performed successfully. Success will be defined from data available in the CIAT forages database, other data sources and input from experts. It is recognized that definition of success may vary from species to species, and may also depend on the unique characteristics of the farmer. In particular, a risk-averse farmer may require a high level of certainty that the selected forage will be successful according to his or her criteria, whereas in other situations a lower level of certainty but a higher rating of success may be desirable.

The scope of the final tool will be decided in conjunction with end users. The majority of the research will be carried out at CIAT in Cali, Colombia. CIAT holds an extensive forage germplasm database, as well as various climate and other biophysical GIS databases. CIAT also will provide expertise on forage adoption and GIS technology. Data will be collected for one or more study areas in Central America, and the model will be developed to reflect the needs of the users in this area. The tool will then be provided to users in other areas for further testing and verification.

The aim is to produce a tool that can be adapted by the user to fit a particular purpose, using available data in their location, so portability is an important test. Some basic data may be built in to the tool, such as climate data, soil data and population density. Other data will be specifically collected to test the tool for targeting forage germplasm.

Consultation: Prior to and during the development of the tool, there will be consultation with potential users. These potential users will be representatives from CIAT, and development agencies, NGOs, national research institutions and ministries of agriculture and natural resources in Central America, with focus on Honduras, Nicaragua and Costa Rica.

Testing and validation: Once case study data has been collected, these will be applied to a number of algorithms including existing software packages. The appropriateness and accuracy of these methods will be evaluated using standard validation procedures, such as bootstrapping and jack-knifing, as well as expert opinion. From these trials, methods will be selected for development into a software tool for the case study. These methods will be verified and validated throughout the development period in collaboration with potential users.

The cooperation of targeted end users may be problematic, and this will be addressed by closely collaborating with selected users in the initial stages of the project, and by asking them to contribute to the testing and validation of the model.

Implementation: The tool will be implemented using existing GIS software and programming software, such as MapObjects with Delphi, or ArcView and Avenue. Ideally the final product will require no special GIS licenses and will run completely stand-alone to promote accessibility for poorer users in tropical developing countries.

Results and Discussion

Potential users and stakeholders: A large number of potential users and stakeholders in development agencies, NGOs, national research institutes and ministries of agriculture and natural resources, mainly in Central America, have been contacted via email with brief details on the project and a request for feedback. In addition a presentation on the project was given to Central American ministry of agriculture representatives during the 5th Global Spatial Data Infrastructure conference in Cartagena, Colombia, in

May 2001. Feedback so far has been limited, but positive. Next steps are to strengthen these linkages and ideally work alongside a potential user during the development of the model. Potential users will also be involved in the testing of early versions of the model.

Data: A review has been conducted of data available through CIAT as well as other readily available data for Central America (eg. FAO, USGS). Also data has been identified which has been used in similar projects, but may not currently be available for the region, as well as data which would be desirable in the model, according to forage and agriculture experts at CIAT. Next steps are to access available data in useful formats, and to identify methods for obtaining other data identified.

Models and algorithms: Literature on similar models and algorithms has been reviewed, as well as existing software packages. A number of these have been identified as potentially relevant and useful. In particular the Bayesian methods used in the Expector software have been applied to limited forages data, as a proof of concept. Next steps are to test this and other algorithms on a wider dataset, and start developing the actual tool.

Conceptual model: A conceptual model has been developed, taking into account the research into algorithms and data, and discussions with supervisors at CIAT. Several key concepts have been identified which will be addressed in the model. Next steps are to disseminate this research further for more input and feedback, and refine the conceptual model as research on data and algorithms continues.

Activity 4.5 Facilitate communication through Newsletters, Journals, Workshops and Internet

Highlights

- New information on the bioecology and management of spittlebugs shared in a 5-day workshop for sugar cane entomologists in Guatemala.
- Reference collection of 675 papers on the spittlebugs and the superfamily Cercopoidea strenghtened for conversion to an on-line bibliographic database.
- Advanced in the development of a Forage Page that is compatible with CIAT's format.

Progress towards achieving milestones

- Research information produced by CIAT's Forage team made available to partners.
- During 2001 we carried out workshops and training courses in Guatemala, Haiti and Colombia on bioecology of spittlebug, principles of forage evaluation and progress made in forage development in the Llanos, respectively.

4.5.1 Development of a Forage Web Page

Contributors: B. Hincapie, S. Staiger, and IP-5 staff

CIAT projects: Communication Unit

Rationale

It is becoming more and more important to quickly exchange information with partners and the community interested in research. CIAT is in the process of updating its Web Page and a as a result we initiated the development of a web page for the Tropical Grasses and Legumes Project in collaboration with CIAT’s Communication Unit (Photo 20).

Progress

During 2001 a graphic platform was developed. We are now in the process to compile information which should allow the user to obtain information on the 'What, How, and Who' of Project IP5. The layout is using the accepted CIAT format and templates.

Currently the following processes are underway

1) Development of a general framework established containing History, Personnel, Highlights and Products of the project. This part is near completion

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 areas for the web page include Annual reports, newsletters and publications in PDF format, a list of publications, indication of donors and collaborators and a list of released cultivars. This process is initiated and should be completed during 2001.

3) Establishing links to the newly developed Forage Database and linkages to sites of collaborators. Also to establish links to published information respective to the outputs of the project. This process is well advanced and contribution of all staff will amplify the number of linkages.

Completing these 3 steps is anticipated to yield a comprehensive web page which the will need to be regularly updated.

Photo 20. Screens showing of the Web Page of Forages

4.5.2 Information and technology transfer for spittlebug management in graminoids

4.5.2.1 Workshop in Guatemala on the Bioecology and Management of Spittlebugs in Graminoid Crops

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 (see section 4.6.2). To partially fill this gap, five workshops on the Bioecology and Management of Grassland Spittlebugs have been carried out from 1997 to 2001, three in CIAT, one in Ecuador and one in Guatemala.

The fifth workshop took place 13-17 August, 2001 at CENGICAÑA (Centro Guatemalteco de Investigación y Capacitación de la Caña de Azúcar), Santa Lucía Cotzumalguapa, Guatemala, sponsored by ATAGUA (Asociación de Técnicos Azucareros de Guatemala) and CAÑAMIP (Comité de Manejo Integrado de Plagas de la Caña de Azúcar). Unlike past events, the main interest of this group was information regarding spittlebugs pests in sugar cane. In Guatemala, spittlebugs are considered the most damaging pest in this crop. There are proposed IPM programs that achieve relatively good control largely through cultural techniques and biological control based in fungal entomopathogens.

The event was attended by 20 agronomists and entomologists representing the major sugar cane farms in southern Guatemala, the Ministry of Agriculture and Ranching, and CENICAÑA. 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. A 150-page manual with supporting information and notes was prepared for each participant as well as a compilation of 34 relevant articles.

4.5.2.2 Reference collection and on-line bibliography of the Cercopoidea

Contributors: Daniel Peck, Mariano Mejía, Edith Hesse, Carlos Saa

Rationale

A major limitation to advances in the management of spittlebugs in forage grasses and sugar cane is difficult access to information. First, there are no published reviews of the insect family Cercopidae or the superfamily Cercopoidea despite their economic significance in cultivated graminoids such as forage grasses and sugar cane. Such material exists for other groups of economically important Homoptera such as the leafhoppers, planthoppers, aphids, scales and whiteflies, but students of the spittlebugs and froghoppers must turn to articles and gray literature to acquire an understanding of this group of insects.

Second, reviews of the biology and management of spittlebugs are inadequate. The few that exist are not widely disseminated, are outdated, and contain overgeneralizations and erroneous information, particularly regarding taxonomy. Third, much of the available information is in gray literature sources that are difficult to access. The quality of research from small and isolated universities or research teams is challenged by not being able to acquire the information necessary to support studies on this pest group.

Materials and Methods

To start to overcome some of the limitations in information dissemination, we are strengthening our reference collection on the Cercopoidea. References have been gathered over the last 10 years. In 2001 we began working with CIAT Information Services to make this information source available on-line.

Results

At present, we have physical copies of 675 references related to the superfamily Cercopoidea. Of these, 468 are directly related to spittlebugs in graminoids, 320 related to forage grasses, 145 related to sugar cane and 23 related to other graminoids such as rice and turfgrass. At present, all references are housed alphabetically in filing cabinets of the Spittlebug Bioecology and IPM Research Group. All citations are entered into an electronic database (EndNote). This bibliography has been printed and deposited in the CIAT library. Key words were assigned to each citation to facilitate searching from within the program software (Table 117).

Table 117. Key words assigned to references in the Cercopoidea bibliography for key-word search in EndNote.

Region	Crop	Management	Habitat	Biology	Classification
Africa	Alfalfa	Ants	Biogeography	Aggregations	Aphrophoridae
Asia	Arachis	Biocontrol	Cover crops	Aposematism	Cercopidae
Australia	Beans	Burning	Dispersion	Bioacoustic behavior	Cercopoidea
India	Cacao	Cultural control	Distribution	Color polymorphism	Clastopteridae
Indonesia	Cassava	Cutting	Endophytes	Comparative phenology	Machaerotidae
New Zealand	Centrosema	Disease transmission	Habitat selection	Copulation	Pipunculidae
Canada	Citrus	Disturbance	Host plant selection	Defense	Procercopidae
Caribbean	Coffee	Economic impact	Host plants	Diapause	Taxonomy
Central America	Conifers	Economic threshold	Litter arthropods	Egg development	
Costa Rica	Cowpea	Entomopathogens	Original habitat	Fecundity	
Panama	Fruit trees	Fertilization	Pasture management	Feeding strategies	
Europe	Grasslands	Grazing	Plant architecture	Life table	
Mexico	Maize	Herbivore competition	Plant quality	Lights	
South America	Marijuana	IPM	Rainfall	Longevity	
Brazil	Millet	Marking	Vegetational diversity	Morphology	
Colombia	Oil palm	Mites		Movement	
Ecuador	Pecans	Natural enemies		Nymph development	
Peru	Rice	Nematodes		Oogenesis-flight syndrome	
Venezuela	Sorghum	Pasture assessment		Oviposition	
U.K.	Strawberry	Pasture pests		Preference-performance	
U.S.	Stylosanthes	Pesticides		Pheromones	
	Sugar cane	Phytotoxemia		Population dynamics	
	Turfgrass	Plant impact		Protandry	
		Plant resistance		Reflex bleeding	
		Rearing		Reproduction	
		Salpingogaster		Spittle mass	
		Sampling		Stadia	
		Spiders		Tenerals	
		Trampling			

Categorical labels were also assigned to facilitate subgrouping of references in the initial on-line database (Table 118).

For the on-line interface, references were converted from EndNote to ProCite. The initial version is a rigid (non-searchable) database divisible into categories with relevant references listed alphabetically. This version will probably be available on the CIAT web page by the end of the year.

Discussion: The reference collection and on-line bibliography will be further improved in the following steps: (1) continual acquisition of new references with a focus on neotropical spittlebugs in graminoids, (2) continual updating of the electronic database, (3) housing physical references in the CIAT library, (4) adding information to the on-line site on how to order copies of references from the CIAT library, and (5) making the on-line database completely searchable by author, category and key words.

Table 118. Codes assigned to references in the Cercopoidea bibliography for subdivision of references in rigid (non-searchable) on-line database.

A01 - Cercopids in graminoids
A02 - Other Cercopoidea
B00 - Bioecology
B01 - Behavior
B02 - Biology
B03 - Diapause
B04 - Ecology
B05 - Population dynamics
B06 - Taxonomy & Systematics
C00 - Management
C01 - Biological control & Natural enemies
C02 - Chemical control
C03 - Cultural control
C04 - Host plant resistance
C05 - Impact
C06 - Integrated pest management
C07 - Rearing
C08 - Sampling & Monitoring
C08 - Sampling & Monitoring
D00 - Host plants
D01 - Forage grasses
D02 - Other grasses
D03 - Sugar cane
D04 - Non-graminoid host

4.5.3 Training course activities

This year a training course on forages was organized as part of the activities of HGRP in Haiti. Technicians (30) from ORE, PADF, the Ministry of Agriculture and NGO's attended the training course. CIAT staff (Luis Horacio Franco, Luis Alfredo Hernández and P. Argel) presented topics related to the utility of tropical forages in mix-cropping systems, forage seed multiplication, methodologies for the evaluation of tropical forages, alternatives of grasses and legumes for Haiti and the use of participant methods for the evaluation of forages. A field visit was also organized to see the status of the forage plots established in Camp Perrin.

The topics presented were well received and generated a good deal of questions among the participants. It is obvious that the theme of tropical forages has not been a priority in Haiti, perhaps because the predominant production systems emphasize on grains and cash crops given the high population pressure on the land and the need to produce staple foods.

At the end of the presentations the participants group was divided in three sub-groups to discuss future needs and ways to promote the use of tropical forges in Haiti. The group also outlined the potential uses in agricultural systems of different forages species (Table 119).

Statements were also made of the possibility of using *B. brizantha* for direct grazing and of *Leucaena leucocephala* and *L. diversifolia* as firewood. But it is interesting to note that pastures for direct grazing are not considered a priority in Haiti and this is something to take into account when planning future pasture evaluation activities in the country.

Table 119. Potential uses of tropical forages in Haiti.

Species	Living fences	Cut and carry	Erosion control	Green manure	Contours
B. brizantha		XX*			X
P. maximum	X*	XX	X		X
P. purpureum	XX	XX	XX		X
T. laxum	X	XX	X		X
Mucuna			XX	XX	
L. diversifolia	X	XX			
G. sepium	X	XX			
Canavalia sp.			X	XX	

*Appropriate
**Highly appropriate

In general, there is consensus among technicians in Haiti that improved forages can have multiple uses in current agriculture systems such as:

- Barriers to reduce and control erosion
- Living fences with the option to cut and carry
- Green manure to recover or improve soil fertility
- Protein/energy banks established along fences or between crop borders
- Improve feeding quality of crop residues such as maize and sorghum

Therefore, in the selection of forage options for Haiti we need to keep in mind this possible utilization practices. We have already selected forage species for Camp Perrin and Deron, but more options need to be tested in other parts of the country. It is also important to initiate on-farm evaluation of the best choices and to advance on seed multiplication of the promising germplasm.

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List of Donors

	Project duration
Australia – **ACIAR**	1998-2001
Anthracnose disease in *Stylosanthes*	
CURTIN UNIVERSITY	2001-2003
Incorporating socio-economic data and expert knowledge in representations of complex spatial decision-making (stipend PhD Rachel O'Brien)	
Colombia – **COLOMBIAN GOVERNMENT**	1999-2003
Grasses and legumes with high nutritive quality Grasses and legumes adapted to low fertility acid soils Grasses and legumes with adaptation drought, poor soil drainage and resistant to pest and disease Integration of improved grasses and legumes in production systems in savannas	
PRONATTA	
Fungal entomopathogens as an alternative for spittlebug management	2000-2002
Intepretation and prediction of spittlebug phenology	2000-2002
Utilidad de la leguminosa semi-arbustiva *Cratylia argentea* en sistemas de ganado de doble propósito del piedemonte llanero: Validación y Difusión	2001-2003
COLCIENCIAS (through FIDAR)	2000-2001
Revegetalizacion de suelos degradados en zonas de ladera de la region andina del sur-occidente de Colombia	
ECOFONDO (through FIDAR)	2000-2002
Alternativas para la recuperacion de suelos degradados en zona ladera del departamento del Valle del Cauca	
COLCIENCIAS (through FIDAR)	2000-2003
Evaluación biofísica y económica de especies arbustivas y arbóreas del ciclo corto para el manejo de suelos erosionados en ladera	
Germany – **BMZ**	
Farmer Participatory Research in Action: Selection and Strategic Use of Multipurpose Forage Germplasm by Smallholders in Production Systems in Hillsides of Central America	2000-2003
Utilising multipurpose legume diversity to improve soil and feed quality including application in a watershed in the Central American hillsides – PostDoc program A. Schmidt	2000-2003
An integrated approach for genetic improvement of aluminium resistance of crops on low-fertility acid soils	2001-2003
Vater und Sohn Eiselen-Stiftung	2000-2001
Early adoption of *Arachis pintoi* as a forage legume by farmers in Huetar Norte, Costa Rica. Diplomarbeit WÜNSCHER, T.	
DAAD (German Academic Exchange Service, University of Hohenheim)	2001-2003
Genetic diversity and core collection approaches in the multipurpose shrub legumes *Flemingia macrophylla* and *Cratylia argentea*	

List of Donors

	Project duration
Haiti - **USAID-HGRP**- Haiti Hillsides Agricultural Project Germplasm Improvement Project	2001-2005
Italy - **Food and Agriculture Organization of the United Nations** (FAO) Update of Grassland Index	2000-2001
Japan – **The Ministry of Foreign Affairs**-JIRCAS The role of endophytes in tropical grasses	1995-2002
Mexico - **SEMILLAS PAPALOTLA, S.A. de C.V.** Mejoramiento de *Brachiaria*	2001-2005
Nigeria – **IITA/CIEPCA** Mucuna G*E trial L-Dopa	2001-2002
Zurich, Switzerland - ETHZ Assessing the impact of adapted germplasm on the phosphorus fertility of low phosphorus –supplying tropical soils	1998-2001
Strategies to increase feed utilization and to limit methane emission of tropical smallholder livestock using the potential of native plants	2000-2003

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