Tropical Grasses and Legumes:

Optimizing Genetic Diversity for Multipurpose Use

Courtesy of Chaisang Phaikaew

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Project IP-5

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

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

Project Manager: Carlos E. Lascano
Fax: (57-2) 4450073
Email: c.lascano@cgiar.org

Cover photo: Commercial seed harvest of hybrid *Brachiaria* cv. Mulato in Thailand. This initiative of Semillas Papalotla will benefit approximately 1,800 Thai farmers, each with an average of less than 1 hectare of Mulato. Photo courtesy of Chaisang Phaikaew.

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1.0 Project Overview: IP5: Tropical Grasses and Legumes: Optimizing Genetic Diversity for Multipurpose use

Objective: To develop and utilize superior gene pools of grasses and legumes for sustainable agricultural systems in subhumid and humid tropics.

Outputs
Optimized genetic diversity for quality attributes, for host-parasite-symbiont interactions, and for adaptation to edaphic and climatic constraints, for legumes and selected grasses.
Selected grasses and a range of herbaceous and woody legumes evaluated with partners, and made available to farmers for livestock 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 supply to children, cash flow for small livestock and non- livestock farmers, while conserving and enhancing the natural resource base.

Milestones
2004 Defined utility of *Flemingia*, and *Lablab* hay as feed resources for dairy cows.
Opportunities identified in Africa to promote the utilization of forages developed by CIAT.
2005 Methods and tools available to enhance targeting and adoption of multipurpose forage germplasm in smallholder production systems in Central America.
A new *Brachiaria* hybrid with better adaptation to dry season and with higher seed yield available for release in the dry tropics.
2006 Widespread adoption of improved forage technologies in the subhumid and humid tropics (e.g. Central America and SE Asia).
A *Brachiaria* hybrid with resistance to some species of spittlebug, with high forage quality and with higher seed production than the available commercial hybrid (Mulato) is available to farmers in the tropics.

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; AROs (Universities of Hohenheim and Göttingen, CSIRO, JIRCAS, ETHZ); private sector (e.g. Papalotla).

CGIAR system linkages: Enhancement & Breeding (30%); Livestock Production Systems (15%); Protecting the Environment (5%); Saving Biodiversity (40%); Strengthening NARS (10%). Participates in the Systemwide Livestock Program (ILRI) through the Tropileche Consortium.

CIAT project linkages: Genetic resources conserved in the Genetic Resources Unit will be used to develop superior gene pools, using where necessary molecular techniques (SB-2). Selected grasses and legumes will be evaluated in different production systems of LAC, Asia and Africa using participatory methods (SN-3) to target forages (PE-4, SN-2) and to assess their impact (BP-1) for improving rural livelihoods and conserving natural resources (PE-2, PE-3, PE-4).

2.0 Research Strategy and Activities

Strategy: To accomplish its goal and the main objective, the Forage Project relies on CIAT's forage germplasm collection [housed in the Genetic Resources Unit (GRU)], which, with more than 20,000 accessions, is the largest forage germplasm collection in the CGIAR and on collaborative research with a range of public and private sector partnerships. From past multilocational evaluation through networking (RIEPT), a number of key genera of grasses (*Brachiaria, Paspalum, Panicum*) and legumes (*Arachis, Stylosanthes*, *Desmodium*, *Cratylia*) with high potential for animal feed and natural resource conservation have been identified. Within the key species in these genera, elite germplasm accessions are identified and characterized in the target area to develop cultivars with high feed quality and broad adaptation to biotic and abiotic stress factors. The improved genotypes are tested with partners in production systems using farmer participatory methods and NARS and private seed companies in the region release those selected cultivars.

It follows, that our main strategy is to exploit genetic diversity from collections of natural germplasm of selected key forage species based on strict prioritization of potential impact. Artificial hybridization to create novel genetic combinations is used in cases where clear constraints have been identified and where evaluation of large germplasm collections has failed to identify the required character combinations (e.g. spittlebug resistance and edaphic adaptation in *Brachiaria* or anthracnose resistance and seed yield in S*tylosanthes*).

Activities: To implement the R&D strategy a multidisciplinary team together with partners carries out the following main activities:

a) Define quality and antiquality factors in grasses and legumes to develop better screening procedures and identify nutritional synergism among species
b) Define mechanisms of adaptation of grasses and legumes to low fertility soils and drought to develop better screening protocols, e.g., for resistance to spittlebug and tolerance to edaphic and climatic constraints
c) Improve grasses and legumes with well-defined constraints of economical importance through selection in core collections and through artificial hybridization
d) Evaluate selected grasses and legumes for multipurpose use in smallholder livestock/ cropping systems
e) Link forage and socio economic databases to GIS to facilitate targeting of germplasm to different agro-ecosystems.
f) Create partnerships with other CIAT Projects and with NARS, NGO's, and private sector organizations to deliver superior grasses and legumes to farmers

3.0 Target Areas

Tropical grasses and legumes being developed at CIAT are targeted to three main agroecological zones in the tropics: Savannas, Forest Margins and Hillsides. These agroecosystems are characterized by low fertility soils and variable rainfall, ranging from sub-humid (600- 1500 mm/year rainfall and 4-8 months dry season) to humid (2,000 to 4,500 mm/year rainfall and limited or no dry season stress) areas.

Traditional land use in some savannas regions of Colombia, Venezuela and Brazil is characterized by extensive cow-calf operations with low management input and almost no purchased inputs, with corresponding low productivity. However, the area planted to improved grass pasture has expanded but the proportion of degraded pastures has also increased alarmingly. Intensive tillage for annual crop production has also resulted in soil degradation leading to severe compaction in the profile and increased runoff and erosion.

Variable topography and drainage and high weed potential generally characterize the Forest Margins in Central America, the Amazon, Africa and SE Asia. Many regions are far removed from markets and suffer from lack of infrastructure. Land is used predominantly for subsistence agriculture following slash and burn of the forest by smallholders and for dual-purpose cattle in cut and carry systems or in grass-based pastures in different stages of degradation.

Many soils that support crop and livestock systems managed by smallholders in subhumid areas of Central America, the Andean zone, Africa, and SE Asia are in different stages of degradation, which leads to deforestation. In addition, farmers in these regions have shortage of labor to collect feed from forests or wastelands and as a consequence livestock intensification is severely limited.

It follows, that a common constraint across the three agroecosystems being targeted by CIAT’s Forage Team is low quantity and quality of forage biomass available to feed livestock in pasture or cut and carry systems and as a result animal production is low and environmental degradation is high. Thus improved forage options can improve livelihoods of smallholders while contributing to reduce deforestation and restore degraded lands in the tropics.

4.0 Beneficiaries

Large, medium and small livestock producers are capturing the benefits of the improved grasses and legumes being developed by CIAT. Increasing incomes and urbanization in developing countries is creating a higher demand for livestock products (meat, eggs and dairy products) than staple crops. Because the poor derive a greater proportion of their income from livestock than do the wealthy, and because of the huge expected growth in demand for livestock products, the livestock revolution could become a key means of alleviating poverty in the next 20 years. To make this a reality, livestock production in developing countries will need to double by 2020 and to meet this goal improved forage-based feeding systems need to be developed and adopted by farmers.

5.0 PROJECT WORK BREAKDOWN STRUCTURE
2004

PROJECT PURPOSE

To identify and deliver to farmers superior gene pools of grasses and legumes for sustainable agriculture systems in subhumid and humid tropics

OUTPUTS	**Grass and legume genotypes with high forage quality attributes are developed**	**Grass and legume genotypes with known reaction to pests and diseases, and to interaction with symbiont organisms are developed**	**Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed**	**Superior and diverse grasses and legumes delivered to NARS partners are evaluated and released**
ACTIVITIES	• Screening of *Brachiaria* hybrids for high digestibility and protein • Animal production potential with selected grasses and legumes • Assessment of the potential of tanniferous legumes to improve ruminant nutrition	• *Brachiaria* genotypes resistant to biotic and abiotic stresses • Screening *Brachiaria* genotypes for spittlebug resistance • Identify host mechanisms for spittlebug resistance in *Brachiaria* • Selection of *Brachiaria* hybrids for resistance to *Rhizoctonia* foliar blight disease • Elucidate the role of endophytes in tropical grasses • Association of bacteria with *Brachiaria* genotypes • Antifungal proteins in tropical forages	• Genotypes of Brachiaria and *Arachis* with adaptation to edaphic factors • Genotypes of *Brachiaria* with dry season tolerance • Grasses with adaptation to poorly drained soils • Nitrification inhibition in tropical grasses • Legumes (herbaceous and woody) with adaptation to acid soils and drought • Annual legumes for multipurpose use in different agroecosystems and production systems	• Partnerships in LAC to undertake evaluation and diffusion of new forage alternatives • Partnerships in Asia to undertake evaluation and diffusion of new forages alternatives • Partnerships in Africa to undertake evaluation and diffusion of new forage alternatives • Forage Seeds: Multiplication and deleivery of experimental and basic forage seeds • Enhancing livestock productivity in Central America • Impact of forage research in LAC • Expert systems for targeting forages and extension materials for promoting adoption of forages • Facilitate communication through journals, workshops, and the Internet

6.0 Project Log-Frame

2004-2006

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 and case studies on socio-economic benefits and natural resource conservation in smallholder livestock farms in the subhumid and humid tropics	Policies are put in place by governments to favor sustainable livestock and forage development in marginal areas occupied by small farmers
Purpose To identify and deliver to farmers superior gene pools of grasses and legumes for sustainable agriculture systems in subhumid and humid tropics.	• Demonstrated economical and ecological benefits of multipurpose grasses and legumes to livestock and crop farmers in tropical regions of Latin America, Africa and South East Asia	• Range of genetic variation in desirable plant traits • Performance of forage components in systems	• Support from traditional and nontraditional donors • Effective collaboration: • CIAT's Projects • ARO's, partners and farmers, NGOs
Outputs 1. Grass and legume genotypes with high forage quality attributes are developed.	• Defined utility of *Flemingia*, and *Lablab* hay as a feed resource for dairy cows by 2004. • Determined utility of legume mixtures for increasing protein supply in ruminants while reducing methane emissions by 2005 • New *Brachiaria* genotypes with superior forage quality for improved animal performance characterized by 2006	• Demonstrated differences under field conditions • Scientific publications • Annual Reports • Theses	• Effective collaboration with CIAT Projects (PE-2), AROs, partners and farmer groups
2. Grass and legume genotypes with known reaction to pests and diseases and to interaction with symbiont organisms are developed.	• Efficient screening method to assess *Rhizoctonia* resistance in *Brachiaria* developed by 2004. • Role of endophytes on drought tolerance determined under field conditions by 2004. • QTL's for resistance to spittlebug and high aluminum in the soil in *Brachiaria* are available for marker-assisted selection by 2005. • *Brachiaria* genetic recombinants with combined resistance to different species of spittlebug are available by 2006.	• Demonstrated differences under field conditions • Scientific publications • Annual Reports • Theses	• Effective collaboration with CIAT Projects (SB-1, SB-2), AROs, partners and farmer groups
3. Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed.	• Improved accessions of *Vigna* and *Lablab* with adaptation and known value to farmers in hillsides of Central America are available to partners by 2004. • Defined variability for nitrification inhibition in *Brachiaria* genotypes by 2005. • *Brachiaria* genetic recombinants with resistance to low P and high aluminum in the soil and with drought tolerance are available by 2006.	• Demonstrated differences under field conditions • Scientific publications • Annual Reports • Theses	• Effective collaboration with CIAT Projects (SB-1, PE-2, PE-4), AROs, partners, NGOs and farmer groups
4. Superior and diverse grasses and legumes delivered to NARS partners are evaluated and released	• Scaling process of *Vigna*, *Lablab* and *Cratylia* and improved *Brachiaria* are in place in Central America by 2004. • New market opportunities in Central America for processed forages assessed by 2006. • A Decision Support Tool for targeting forages to different environments and production systems in Central America is available by 2005 • Opportunities identified in Africa to promote the utilization of forages developed by CIAT by 2004 • An information network on forages and effective forage multiplication systems are established in benchmark sites in SE Asia by 2004. • Improved multipurpose grasses and legumes result in increased on-farm milk, meat, and crop production, and reduced labor requirements in benchmark sites in SE Asia by 2005. • Widespread adoption of forage technologies in the subhumid and humid tropics by 2006. Improved processes for scaling-out the impacts of forage technologies on farms in SE Asia.	• Promotional publication – Newsletters – Journal – Extension booklets • Surveys on adoption impact of new grasses and legumes: – Seed sold – Area planted – Production parameters – Environmental/socioeconomic indicators	• Effective collaboration with CIAT Projects (PE-2, SN-1, SN-2, SN-3, BP-1 and Ecoregional Program), partners, NGOs and farmer groups

2005-2007

Area: Genetic Resources Research
Project: 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 and case studies on socio-economic benefits and natural resource conservation in smallholder livestock farms in the subhumid and humid tropics	Policies are put in place by governments to favor sustainable livestock and forage development in marginal areas occupied by small farmers

Purpose
To identify and deliver to farmers superior gene pools of grasses and legumes for sustainable crops-livestock systems in subhumid and humid tropics.
	• Demonstrated economical and ecological benefits of multipurpose grasses and legumes to livestock and crop farmers in tropical regions of Latin America, Africa and South East Asia	• Range of genetic variation in desirable plant traits
		• Performance of forage components in systems
			• Support from traditional and nontraditional donors
			• Effective collaboration:
			  • CIAT's Projects
			  • ARO's, partners and farmers, NGOs

Outputs
1. Grass and legume genotypes with high forage quality attributes are developed.
	• Determined the utility of legume mixtures for increasing protein supply in ruminants while reducing methane emissions 20% by 2005
	• Selected at least 10 Brach aria hybrids (sexuals) with high digestibility (>60%) and crude protein (> 10%) by 2006
	• The little bag silage technology with selected forage species adopted by at least 100 small farmers in Honduras and Nicaragua, results in 20-30%% milk yield increase in the dry season by 2007
		• Demonstrated differences under field conditions
		  • Scientific publications
		  • Annual Reports
		  • Theses
			• Effective collaboration with CIAT Projects (PE-2), AROs, partners and farmer groups

2. Grass and legume genotypes with known reaction to pests and diseases and interaction with symbiont organisms are developed.	• Validated a rapid screening method, with a capacity to evaluate 1000 genotypes in five days, to assess *Rhizoctonia* resistance in *Brachiaria* by 2005
	• At least 10 *Brachiaria* genetic recombinants with combined resistance to at least three species of spittlebug in Colombia are available for regional testing in Central/South America by 2006
	• At least three *Brachiaria* genetic recombinants with resistance to *Rhizoctonia* are available for regional testing in Central/South America by 2007
		• Demonstrated differences under field conditions
		  • Scientific publications
		  • Annual Reports
		  • Theses
			• Effective collaboration with CIAT Projects (SB-1, SB-2), AROs, partners and farmer groups

3. Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed.	• Two improved accessions of Vigna and one of Lablab multiplied (500 or 100 kg of seed produced, respectively) and distributed to two national partners (DICTA, INTA), one NGO (SERTEDESO), one farmer organization (Campos Verdes) one development project (GTZ), in Honduras and Nicaragua by 2005
	• A new *Brachiaria* hybrid (CIAT 36087, cv. Mulato-II) with better adaptation to acid soils and tolerance to dry season (50% higher dry season forage yield on acid soils than the current hybrid cultivar), and resistance/tolerance to at least three Colombian species of spittlebugs, and with 2-3 times higher seed yield available for release (50 tons of commercial seed available) by 2006
	• Defined the genetic variability for nitrification inhibition in at least 500 *Brachiaria* hybrids by 2007
		• Demonstrated differences under field conditions
		  • Scientific publications
		  • Annual Reports
		  • Theses
			• Effective collaboration with CIAT Projects (SB-1, PE-2, PE-4), AROs, partners, NGOs and farmer groups

4. In partnership with NARS, superior and diverse grasses and legumes are evaluated and disseminated through participatory research.	• New market opportunities for processed forages assessed trough surveys to at least 100 farmers with and without livestock in Honduras and Nicaragua by 2005
	• *Brachiaria brizantha* cv. Toledo seed produced (500 kg to 1 t) by one farmer enterprise (PRASEFOR). in Honduras by 2006
	• A forage production systems established with >5000 farmers in 4 countries of SE Asia supported by >50 experienced staff and key technical information about forage technologies and their development by 2006
	• At least 5,000 ha of Brachiaria hybrid (Mulato II) planted in Colombia, Honduras, Nicaragua and Mexico by 2007
	• Improved multipurpose grasses and legumes result in 20% more on-milk, and in 30% reduced labor requirements in benchmark sites in SE Asia by 2007.
		• Promotional publication
		  • Newsletters
		  • Journal
		  • Extension booklets
		• Surveys on adoption impact of new grasses and legumes:
		  • Seed sold
		  • Area planted
		  • Production parameters
		  • Environmental/socioeconomic indicators
			• Effective collaboration with CIAT Projects (PE-2, SN-1, SN-2, SN-3, BP-1 and Ecoregional Program), partners, NGOs and farmer groups

7.0 Research Highlights

Improvement of *Brachiaria* for biotic and abiotic stresses

The apomictic *Brachiaria* hybrid (CIAT 36087--Mulato 2) in the pre-release stage combines resistance to at least two species of spittlebug, adaptation to acid infertile soils and tolerance to drought. Significant progress has also been made in developing sexual *Brachiaria* hybrids that combine spittlebug resistance with Al resistance. Selected sexual genotypes are being used in recurrent selection to generate superior apomicitic hybrids of *Brachiaria* for release as cultivars.

- **Selected sexual *Brachiaria* hybrids with high levels of resistance to several species of spittlebug in the greenhouse and field:** In 2004, intensive screening of selected hybrids was conducted under greenhouse and field conditions with three species of spittlebug. A set of 731 pre-selected sexual (SX03) hybrids was simultaneously screened for resistance to *A. varia*, *A. reducta*, and *Z. carbonaria.* We found that 40% of the sexual hybrids combined low damage levels and high levels of antibiosis resistance to all three species of spittlebug. Sexual hybrids (22) previously selected in Palmira under greenhouse conditions were included in a field test with artificial infestation of spittlebug. All of the sexual hybrids showed adequate levels of field resistance to four species (*Aeneolamia varia*, *Zulia carbonaria*, *Z. pubescens*, and *Mahanarva trifissa*) of spittlebug.

- **Selected sexual *Brachiaria* hybrids with resistance to Rhizoctonia foliar blight**: A rapid detached leaf inoculation method coupled with a new rating scale (0- 5) was used to screen 745 sexual *Brachiaria* genotypes for resistance to Rhizoctonia foliar blight. Ten percent (73 genotypes) of the plant materials that showed average disease ratings below 3.0 in the screen with detached leaves were then evaluated in the greenhouse by inoculating complete plants under high humidity. Among the 73 selected *Brachiaria* genotypes evaluated in the greenhouse, 26% had high level of resistance to Rhizoctonia foliar blight.

- **Selected sexual and apomictic *Brachiaria* hybrids with high levels of resistance to Al under low nutrient conditions**: As reported previously, high values of total root length and low values of mean root diameter after exposure of plants to toxic level of Al in solution are indicative of resistance to Al. Using this selection criteria, 3 sexual hybrids (SX03NO/0846, SX03NO/2367, SX03NO/0881) were identified with greater level of Al resistance than that of the original sexual parent (*B. ruziziensis* 44-02). One apomictic hybrid (BR02NO/1372) was outstanding in its level of Al resistance. This hybrid is also resistant to spittlebug and has greater fine root (which give plants the ability to acquire nutrients from low fertility soils) development than *B. decumbens* CIAT 606 in the absence of Al in solution.

- ***Brachiaria* hybrid cultivar Mulato 2 (pre-release stage) combines good adaptation to acid infertile soils, drought tolerance and resistance to spittlebug**. Results from a field trial that included 4 *Brachiaria* hybrids (BR98NO/1251; BR99NO/4015; BR99NO/4132; Mulato 2) showed that the spittlebug resistant *Brachiaria* hybrid (Mulato 2) performed better than the other hybrids into the third year after establishment in the acid infertile soils in the Llanos. The superior performance of Mulato 2 was associated with its ability to acquire great amounts of nutrients, particularly calcium and magnesium from low fertility soils. Leaf and stem N content and shoot N uptake values indicated that Mulato 2 can also use N efficiently to produce green forage in the dry season.

- **Genetic variability exists for nitrification inhibition (NI) in *Brachiaria humidicola*.** Significant differences were found among accessions (10) of *B. humidicola* in total and specific NI activity of root exudates. Several accessions of *B. humidicol*a with NI activity that is two to three times higher than the standard cultivar CIAT 679 used for nitrification inhibition experiments at JIRCAS and CIAT were identified. The accession CIAT 26159 was repeatedly tested along with the standard cultivar CIAT 679, and the high NI activity nature of confirmed by JIRCAS. The existence of substantial differences among accessions of *B. humidicola* in NI activity of root exudates demonstrates the genetic nature of this plant attribute and the possibility of improving further the NI ability in *B. humidicola* through systematic evaluation of germplasm and breeding.

Development of multipurpose legumes for smallholder systems

Progress was made in defining the effect of planting site on quality of shrub legumes and in selecting legumes with drought tolerance as green manures for the dry hillsides of Central America where traditional legumes such as Mucuna used by farmers in the more humid areas do not perform well.

- **Selecting appropriate planting sites improves the feeding value of *Calliandra calothyrsus*.** While the tanniniferous shrub legume *Calliandra calothyrsus* is widely used by smallholders to supplement dairy cattle and goats in hillsides of Kenya, farmers in Colombia have hardly adopted this supplementation strategy. We hypothesized that this was mainly due to differences in the forage quality of this legume in the two countries. To test this hypothesis, an *in vitro* experiment was performed to compare the nutritional value and the ruminal fermentation characteristic of *C. calothyrsus* var. Patulul cultivated in sites in Colombia (Santander de Quilichao—acid-infertile soils—1000 masl) and in Kenya (Embu--fertile soils--1480 masl). Although the foliage of the two provenances of *C. calothyrsus* tested had similar contents of OM, CP and NDF, they differed in nearly all fermentation properties and the material from Kenya showed a higher apparent nutrient degradability. These differences in nutritive value were mainly explained by lower levels of condensed tannins in the foliage from Kenya. Work is in progress to define environmental factors (e.g. soil type and fertility) responsible for differences in forage quality and type and concentration of condensed tannins of a range of tanniniferous shrub legumes.

- **The drought tolerant *Canavalia brasiliensis* is an excellent green manure option for dry hillsides of Central America**. In farmers fields in hillsides of Central America soil fertility is declining and weeds are becoming a major problem. In order to overcome these limitations we have been working with local farmer organizations to introduce, evaluate and promote the use of legumes as green manures. At the SOL Wibuse site in San Dionisio, Nicaragua, four crop rotation treatments (maize/beans, maize/natural fallow, maize/*Canavalia brasiliensis* and maize + cowpea/*C. brasiliensis*) were evaluated. Results indicated that after two years of rotation the highest yields were observed in the maize + cowpea / *C. brasiliensis* and maize / *C. brasiliensis* rotation plots. The higher maize yields with *C. brasiliensis* as green manure were associated with the high biomass production and permanent soil cover with green foliage provided by this legume during the entire dry season.

Constraints for adoption and Impact of Forage R&D

This year we documented constraints associated with adoption of added value forage technologies by smallholders and the impact of improved *Brachiaria* in Central America. We are also documenting the success story of seed production of Mulato by smallholders in Thailand.

- **Constraints for animal feed production as an objective of poor farmers in Central-America were identified.** Given that animal feed related activities (production of dry season feed for sale to cattle owners) have been identified as promising income generating options for poor farmers in the hillsides of Central-America, an analysis was carried out to identify (mainly household related) factors inducing or inhibiting farmers to opt for production of animal feed. Results indicate that farmers owning land and cattle are more likely to include animal feed as a research and production objective than the poorer farmers, except for those who are not self-sufficient in maize. Farmers without full decisive power over their land are reluctant to engage in animal feed production for the market. Whereas research and development work on added value forage technologies to link farmers to markets can continue to be directed at all farmer categories in Central-American hillsides, special attention is justified for farmers without full land ownership and those who depend on outside jobs for acquired basic grains for their food security.

- ***Brachiaria* grasses have had a major impact in tropical milk production systems in Mexico and Central America.** A study was carried out to estimate the adoption and impact of *Brachiaria* grasses released through the forage network (RIEPT) operated by CIAT in the 80's and 90's, using as a basis seed sold during the period 1990-2003. Results indicated that during this period the annual increase rate (32 to 62%) in seed sales was high in all countries and that total area planted with *Brachiaria* cultivars during this period represented 6.5% of the total area of permanent pastures in Mexico, 12.5% in Honduras, and 18.7% in Costa Rica. The main beneficiaries from the adoption of *Brachiaria* cultivars have been small and medium size producers oriented toward dairy and to a lesser extent to beef production. For example, in Costa Rica more than 55% of the national milk production but only 18% of the beef produced in 2003 was due to the marginal increase in the productivity of *Brachiaria* pastures compared to the traditional technology from degraded or naturalized pastures.

- **Smallholder farmers producing seed of Mulato in Thailand have an assured market and earn more income.** *Brachiaria* Mulato hybrid was by CIAT to Southeast Asia in 1996 as part of a large *Brachiaria* variety trial. In Thailand, The Thai Department of Livestock Development (DLD) selected Mulato and seed production trials were initiated in 2003 with 7 small farmers. This year 1793 small farmers planted 700 ha of Mulato to produce 143 Tons of seed thanks to a guaranteed market by Papalotla. Earnings with Mulato seed production are 25% higher / ha than with the traditional seed production of Ruzi grass.

8.0 Proposed plans for the next 5 years

On- going Brachiaria Improvement Program

Our focus will continue to be on developing apomictic *Brachiaria* hybrids with adaptation to biotic (spittlebug and rhizoctonia foliar blight resistance) and abiotic (acid soils, drought and poor drainage) stresses, and with high forage quality and seed yield. To reach this objective we will implement recently developed screening methods, initiate work on mechanisms affecting adaptation to drought and seek funding to work on tolerance to poorly drained soils.

Spittlebug

- **Initiate studies to assess the resistance of genotypes to adult feeding of different species.** Screening for spittlebug has been limited to selecting genotypes with antibiotic resistance to nymphs. In the field pastures of *Brachiaria* are subject to attacks of both nymphs and adults and the damage in the plant caused by adults can be as severe as the damage caused by nymphs.

- **Identify partners in ARO and donor support to study the biochemical resistance of *Brachiaria* to spittlebug.** Identification of the biochemical mechanism of antibiotic resistance in *Brachiaria* will facilitate the development of biochemical and/or molecular markers for the screening process.

Rhizoctonia foliar blight

- **Implement screening procedure to handle large number of genotypes**
  - First screen- Laboratory: Inoculated detached leaves in petri dishes
  - Second screen- Greenhouse: Genotypes selected with detached leaf method will be grown in pots in the greenhouse and inoculated under high humidity conditions.

Edaphic constraints

- **Study mechanisms associated with phosphorus (P) uptake and Plant use efficiency.** The focus on screening *Brachiaria* hybrids for edaphic constraints will continue to be in Al resistance under low nutrients. However, studies are needed to define the mechanisms responsible for the superior adaptation of *Brachiaria decumbens* under low P conditions in order to help develop an additional screening procedure.

Drought

- **Study mechanism associated with drought tolerance.** Currently genotypes selected for spittlebug, and Al resistance are evaluated under field conditions to determine performance under acid- low fertility soils and dry season conditions. To increase the selection pressure for drought tolerance we need to understand the different plant mechanisms associated with this trait. Thus studies in the greenhouse will be undertaken with contrasting genotypes exposed to two levels of moisture to define shoot and root traits that contribute to superior adaptation to drought.

Poor soil drainage

- **Seek extra funding to initiate studies on adaptation to poorly drained soils.** There are large areas in tropical regions (i.e. the Atlantic region in Central America, piedmont of the llanos of Colombia) where poor soil drainage is a major constraint for commercial Brachiaria cultivars. To undertake this task we need to find additional human and financial resources to support the Plant Nutritionist working on abiotic constraints in forages.

Forage quality

- **Implement the screening procedure for digestibility and protein in a selected sexual population.** We have now standardized a protocol to estimate in vitro digestibility and crude protein using near-infrared reflectance spectrometer (NIRS). Thus what we need to do now is implement the screening procedure using replicated plants in pots.

Genetic improvement of *Brachiaria humidicola*

Among the different *Brachiaria* species *B. humidicola* has a number of important commercial attributes such as high adaptation to acid infertile soils, tolerance to poorly drained soils and to heavy grazing and capacity to inhibit nitrification (conversion of ammonium to nitrate) in soil. Some negative attributes of the commercial *B. humidicola* cultivar are susceptibility to spittlebug, low forage quality and low seed yield.

Recent results in collaboration with JIRCAS indicate that there are accessions of *B. humidicola* with greater capacity to inhibit nitrification than the commercial cultivar CIAT 679. In addition, the germplasm collection held at CIAT contains accessions that are putatively sexual. This opens up the option of initiating a breeding program by making crosses.

With the current level of funding we can only undertake some limited work on the improvement of *B. humidicola*. What we propose to do in the following years is:

- Screen the collection for capacity to inhibit nitrification in collaboration with JIRCAS
- Confirm sexuality of accessions (11) in the collection by carrying out progeny tests in the field
- Perform crosses and determine quality of seed produced.

Legume germplasm development

Our future efforts will continue to focus on the development of forages for mixed crop-livestock systems. In these more intensive systems the need for legumes is great particularly for dry season feeding and to contribute to soil conservation and improvement. We have also seen the potential utility of high quality legumes grown by farmers to feed monogastrics.

Shrub legumes are a good alternative to overcome feed shortages in critical dry periods in livestock systems. However, there are few high quality forage shrub legumes available adapted to acid soils. In addition, many farmers in subhumid areas are demanding herbaceous legumes with dry season tolerance to be used as green manure and to supplement crop residues.

To address these demands we will evaluate new core collections of woody and herbaceous legumes for adaptation to abiotic and biotic constraints within a systems perspective as follows:

- ***Desmodium velutinum*.** Within the collection of shrub legumes in the GRU, *D. velutinum* is one that has high forage quality. We want to examine the collection in terms of adaptation to soils of variable pH and fertility, persistence under cutting and grazing, variation in forage quality and seed yield.

- ***Canavalia brasiliensis* as dry season feed and green manure.** Work in Central America has shown that *C. brasiliensis* is very vigorous and drought tolerant. Thus we need to explore the genetic diversity for key agronomic traits within the collection held at CIAT.

- ***Leucaena diversifolia***. There are few options of shrub legumes for higher altitudes. Among the shrub legume species available, *Leucaena diversifolia* appears to be one that is well adapted to acid soils and mid-high altitudes. Accessions include material available in the GRU and collections obtained from CSIRO, ICRAF and ILRI. We will attempt to get accessions from the U of Hawaii.

- **Legumes for monogastrics (swine, fish and poultry) in LAC and Asia.** Legumes (grain and foliage) for feeding monogastric animals should be highly productive and of high quality. Thus the challenges ahead are to a) select of forage legumes with high quality and low antinutritional factors, b) define post harvest processing of grain and foliage for leaf meal production to reduce antinutritional factors, c) determine animal responses and d) link farmers to markets.

Development and delivery of improved forages in regions

Forage R&D in Africa: We will continue to place high priority in identifying opportunities for R&D work in forages in Africa through the development of proposals for funding. Specifically we will seek collaboration with the TSBFI for developing proposals that aim to improve soil condition in mixed crop-livestock systems through grasses and legumes selected by the Forage Project. Finally, we will continue to respond to forage germplasm requests from initiatives led by CIAT and NARS in different regions.

Forage R&D in Asia: The regional AusAID project (the Forages for Smallholders Project- FSP) was a *"proof of concept"* project. Working in 7 countries, a small suite broadly adapted of varieties was identified and the potential for considerable impacts confirmed. AusAID agreed to fund a bi-lateral project to take these results one-step further and demonstrate that impacts could be delivered on a larger scale. This resulted in the Forages and Livestock Systems Project (FLSP) in Laos; a *"proof of delivery"* project that ends in 2005.

At the same time, the Asian Development Bank (ADB) agreed to continue to fund the regional project FSP (now called Livelihood and Livestock Systems Project – LLSP) to develop participatory methodologies for dissemination of forage technologies and to address other production and marketing constraints in smallholder livestock systems. This project will also end in 2005.

The Asia Forage Team is exploring ways to continue the work to scale-out the impact-yielding forage/livestock systems that have been developed. What is needed is a fully-fledged development project to build on the experienced teams, the technologies, the methodologies and the impacts that have emerged from the FSP, LLSP and FLSP.

A Capacity Building project between the Laos National Agriculture and Forestry Research Institute (NAFRI) and CIAT (US$500,000) is expected to commence in January 2005. This project is designed to build a bridge between the end of the FLSP and the start of the PLDP (Participatory Livestock Development Project), which is a $10 million investment project in Laos for the Asian Development Bank. CIAT and ILRI are participating in the preparation of the project through a PPTA **(**Project Preparation and Technical Assistance) grant.

Forages for monogastrics is a new area of research identified by the Asia Team as having high priority and payoff. During this year, ACIAR invited CIAT to develop the proposal: "Forage legume supplementation of village pigs in Laos" with an indicative budget of Aust $ 400k. The proposed project will a) define criteria for selecting forage legumes for pig feeding, b) generate information on the nutritional value for pigs of selected forage legumes and c) integrate forage legumes in existing pig production systems as part of diets composed of other locally available feed resources (primarily new varieties of cassava, sweet potato and maize)

Forage R&D in LAC: Results from past work in Central America indicated the need to further develop forage alternatives for the dry season such as silage and hay. Both livestock or non-livestock owners can produce these silages and hays for self consumption or for sale provided they are exposed to simple technologies. Thus research is needed to define the usefulness and acceptability by farmers of such forage conservation technologies through on-station and on-farm studies.

Further research is also needed on the adaptation of new forage-based products to smallholder farm constraints to facilitate a market linkage between producers and end users. Success in this area would have an impact on income generation and livelihoods of smallholder farmers.

The efficiency of approaches facilitating innovation versus more traditional extension approaches of forage technologies needs to be evaluated (i.e. promotion of innovation versus promotion of adoption).

The mentioned research questions are the core of the recently funded BMZ proposal 'Demand-Driven Use of Forages in Fragile, Long Dry Season Environments of Central America to Improve Livelihoods of Smallholders".

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

1.1 Screening of *Brachiaria* hybrids for high digestibility and protein

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

Highlight

- Found acceptable correlations in quality parameters measured in different sampling periods in *Brachiaria* hybrids planted as spaced plants in the field

Rationale

We had previously reported a low correlation between IVDMD values obtained in samples of the same *Brachiaria* population cut at different times. We had postulated that this lack of correlation between samplings had to do with the sampling procedure and to a lesser extent with the processing of the harvested material. Thus we modified the sampling procedure in such a way that only leaves from individual plants were harvested. Results from three successive samplings in unreplicated plots of the same *Brachiaria* population using the modified sampling scheme indicated a higher correlation (r = 0.5) in IVDMD between sampling periods than had been previously found (r = 0.1).

Last year we sequentially sampled leaves from replicated (3) plants of 50 *Brachiaria* hybrids derived from a population *B. ruziziensis* x *B. brizantha* cv. Marandu and planted in pots in the greenhouse. The only difference from one sampling period to another was the age of the leaves harvested for quality analysis, but still results indicated that the ranking of genotypes of *Brachiaria* was significantly affected by sampling period and a result their were low correlations between periods (CP: r = 0.30; IVDMD: r = 0.01).

Thus we concluded that in order to select *Brachiaria* hybrids for quality parameters such as CP and IVDMD we still needed to develop a standard sampling procedure, which included fertilizer management and a uniform chronological or physiological age for harvesting the forage.

Materials and Methods

In 2003 we transplanted 50 *Brachiaria* hybrids (*B. ruziziensis* x *B. brizantha* cv. Marandu) in the field in Quilichao in replicated (3) plots (spaced plants). Initially the plants were cut at a uniform height and after 6 weeks of growth, leaves were harvested in three successive samplings (September 25, November 18 and December 18, 2003). After each harvest 2 g of urea were applied per plant. All samples (leaves) were analyzed in CIAT's Forage Quality Laboratory for CP and IVDMD using NIRS. Results were subject to an analysis of variance and to correlation analysis.

Results and Discussion

Results showed that mean CP and IVDMD values differed between sampling periods. While CP increased from sampling period 1 to 3, the reverse occurred with IVDMD (Table 1). In the three sampling periods the mean CP and IVDMD observed were high for a tropical grass probably related to both genetic factors as well as management factors (i.e. N fertilization and age of leaf at harvest).

The ANOVA showed that for both CP and IVDMD there was a significant sampling period ($P < 0.0001$) and genotype ($P< 0.002$ –0.006)

Table 1. Variation in crude protein (CP) and in vitro digestibility (IVDMD) in *Brachiaria* hybrids

Sampling Period (No of Samples)	CP (%)		IVDMD (%)	
	Mean	Range	Mean	Range
1 (143)	14.9	12.1-17.2	70.2	65.4-74.1
2 (141)	16.1	12.6-19.4	68.3	64.2-72.4
3 (150)	17.7	13.5-21.8	66.7	63.5-71.2
<u>Significance (P)</u>				
Sampling Period	0.0001		0.0001	
Rep (sampling period)	NS		0.0025	
Genotype				
Genotype x Sampling Period	0.0026		0.0064	
	NS		NS	

effects (Table 1). However, it was interesting to observe that the sampling x genotype interaction was not significant for both quality variable measured. With the 50 *Brachiaria* hybrids evaluated the variance associated with CP and IVDMD was 5.1 and 3.7 times greater, respectively than the variance associated with the interaction of genotype x sampling period.

The correlations between sampling periods for CP ranged from 0.58 to 0.73 (Figure 1), while in the case of IVDMD the correlation between periods ranged from 0.51 to 0.55 (Figure 2).

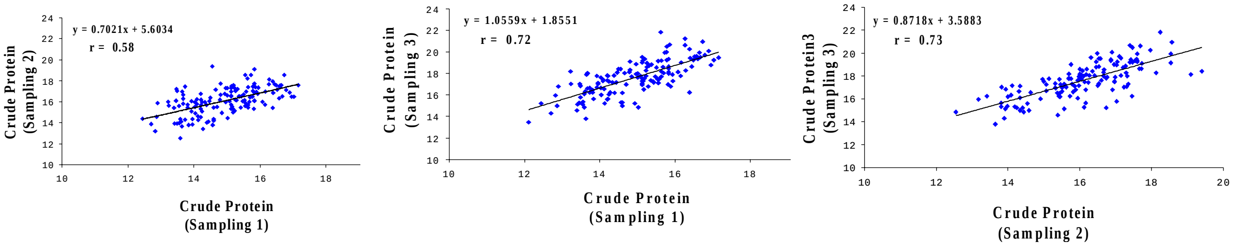


Figure 1. Relationship between crude protein (CP) values measured with NIRS in *Brachiaria* hybrids harvested in three sampling periods

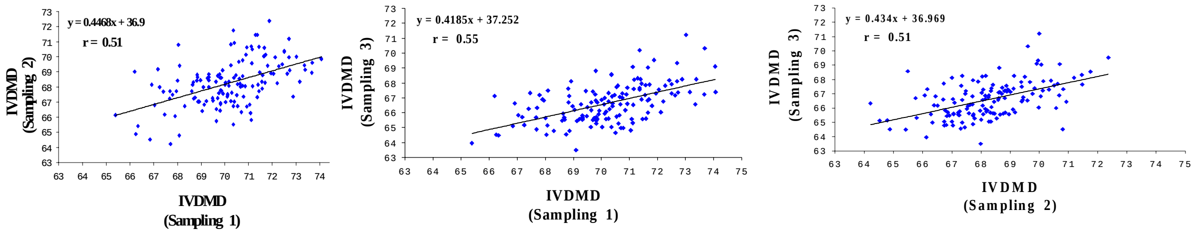


Figure 2. Relationship between in vitro digestibility (IVDMD) values measured with NIRS in *Brachiaria* hybrids harvested in three sampling periods.

From these results we conclude that with the methodology used it is possible to detect with NIRS differences in CP and IVDMD between *Brachiaria* hybrids planted as spaced plants in the field, even though the variability in these quality attributes seemed to be lower than expected. One of the advantages of *Brachiaria* hybrid cv Mulato is its high crude protein (CP) content relative to commercial cultivars when growing in good soils. Thus we also believe that hybrids should be screened for both CP and IVDMD, recognizing that selection for improved forage quality is justified if genetic variance for quality traits 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 IVDMD caused by genotype was four times greater than the variance from G x E. This may not be the case for CP since this quality parameter is significantly affected by soil N content.

To implement screening for IVDMD and for CP in the *Brachiaria* improvement program we still need to adjust a protocol for plants grown in pots in the greenhouse. From the results of this year with plants grown in the field, the greenhouse methodology to be implemented will include:

a) Transplanting vigorous cuttings in replicated pots with water and nutrients not being limiting.
b) Plants well established in the pots will be subject to a uniformity cut and allowed to growth for 6 weeks to harvest leaves for quality analysis

This protocol will be implemented and results will be reported next year.

1.2 Animal production potential with selected grasses and legumes

Highlights

- Sorghum silage with or without molasses had better fermentation (lactic acid process) than Cratylia silage (acetic process).
- Adding molasses improved the intake of Cratylia silage by goats.

1.2.1 Milk production in Brachiaria pastures supplemented with LabLab hay

Collaborators: P. Avila (CIAT), J. Miles (CIAT) and C. Lascano (CIAT)

Rationale:

The forage Project has been evaluating different annual legumes such as cowpea (from IITA) and Lablab (from CSIRO) as green manures in crop –livestock systems. In addition, we have been interested in looking at the feed value in the dry season of cowpea and Lablab hay harvested at pre-flowering and after grain harvest.

There is abundant literature on production and utilization of silage and hay, but in most cases the technology available is not useful to small farmers given that it relies on machinery (i.e. tractors and mechanical forage harvesters) out of the reach of these farmers. Thus we have been investigating alternative technologies such as the "bag silage" and hay packed in bags that could be more appropriate for smallholders livestock systems. Results from last year indicated that intake of cowpea hay was higher than the intake of Cratylia silage, but that this difference did not translate into a significantly higher milk yield. The supplementation of Cowpea hay resulted in18% more milk yield of cows grazing *B. decumbens* pastures when compared with production of cows receiving no supplement.

In this section we report the results of feeding LabLab hay to milking cows grazing different Brachiaria pastures.

Material and Methods

A grazing trial was carried out in a rainy –dry transition period (August 25 to October 7, 2003) in the Quilichao research station using 8 crossbred (Holstein x Zebu) arranged in a 3 x 3 Latin Square design. Four cows received Lablab hay (0.5% of BW) and the other for cows were left as control. All cows grazed pastures of *Brachiaria brizantha* cv Toledo, *Brachiaria* hybrid cv Mulato and *Brachiaria* hybrid CIAT 36087. 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. The hay of Lablab used in the experiment was harvested after 12 weeks of regrowth. The whole plant (leaves, stems) were sun dried (3 days) and packed and stored in a well-ventilated room prior to being fed to the cows.

Results and Discussion

Milk yield was not affected by the supplementation of Lablab, but was affected by pasture type, being higher (P< 0.05) in the *Brachiaria* hybrid CIAT 36087 pasture than in Mulato and Toledo (Table 2). In the case of milk composition only the milk non- fat solids were higher in cows receiving the Lablab hay as supplement. The higher milk yield in CIAT 36087 was related to higher CP (14.4 %) in the forage (leaves) on offer relative to the other grasses (9.0 % and 12.9 % for Toledo and Mulato, respectively).

In general, our results indicate that the lack of response to the supplementation of the high quality Lablab hay may have been associated with the low level of supplementation (0.5% of BW) of the hay and/or to the fact that the grasses had relatively high leaf protein contents.

The average consumption of Lablab hay was 4.2 kg DM per day/ cow (79% of the forage offered), which is lower than expected and maybe the reflection of the high quality of the grass in the pastures grazed by the cows.

Table 2. Milk yield and composition of cows grazing *Brachiaria* pastures with and without supplementation of Lablab hay

Treatments	Milk Yield (l/cow/d)	Milk Fat (%)	Milk Non-Fat Solids (%
Supplementation Effect			
- Lablab	8.0	4.3	9.1 a
+ Lablab	7.7	4.0	8.7 b
Pasture Effect			
B. brizantha CIAT 26110	7.9 b	3.9	8.8
Brachiaria Hybrid Mulato	7.5 c	4.2	8.9
Brachiaria Hybrid CIAT 36087	8.3 a	4.3	8.9

a, b means different (P < 0.05)

1.2.2 Quality and goat intake of *Cratylia argentea* and *Sorghum* sp. silage mixed in different proportions in plastic bags

Contributors: Carlos A. Chaves Quiroz and Carlos Jiménez (U. of Costa Rica); Pedro J. Argel and Guillermo Pérez (CIAT)

Rationale

The legume *C. argentea* cv. Veraniega (Cratylia) is an important forage legume mainly used by farmers either fresh or as silage to supplement the diet of milking cows during the dry period in Costa Rica and elsewhere. Silage making is almost always associated with large cattle feeding systems, which implies the use of sophisticated machinery and abundant resources. However, it has been shown that forages can be conserved using simple technology such as plastic bag silage. This forage conservation method is less costly and available to small farmers with no machinery, particularly for those who live along the dry or sub-humid tropics that experience a significant lack of forage during prolonged dry periods, with subsequent losses in animal production.

Finally, little attention has been paid to the benefits of mixing cereals and legumes for the production of high quality silage for livestock feeding.

Materials and Methods

An 111 days old re-growths of Cratylia and 88 days old re-growths of sweet forage *Sorghum* sp. were harvested and mechanically chopped into 2-5 cm pieces, and mixed in different proportions, with or without molasses to form eight silage treatments. An unrestricted randomized block design was used and treatments arranged in a 4 x 2 factorial for a total of 8 treatments. Molasses was used at 5% (on forage fresh weight basis), and three plastic bags (30-kg capacity) were used for each treatment. Plastic bags were hand packed and a vacuum machine was used to extract the air before sealing the bags with plastic bands as shown in Photo 1.

Photo 1. A30-kg plastic bag silage of *Sorghum* sp.

Plastic bags were stored in a protected room and opened 2 months later. Silage pH, color, smell, DM and OM contents, CP and NDF were measured.

Goat intake was measured only with Cratylia silage with and without molasses, and with the mixture of 66% Cratylia 33% sorghum plus molasses, namely treatments 1, 5 and 6. For this test, eight -50 kg additional bags were prepared for each treatment.

The different proportions of forage and molasses components for each treatment used were as follows:

Treatment	Cratylia (10%)	*Sorghum* (%)	Molasses
1	100	0	–
2	66	33	–
3	33	66	–
4	0	100	–
5	100	0	+
6	66	33	+
7	33	66	+
8	0	10	+

Silage coming from treatments 1, 5 and 6 was offered *ad libitum* to 4 goats per treatment in a complete randomize block design. The observation lasted 10 days and the daily silage offered and rejected was measured during the last 3 days to estimate animal intake and in vivo digestibility.

Results and Discussion

With the exception of maize and sorghum species, it is admitted that tropical grasses and legumes are not ideal material to ensile because at harvesting they have low contents of water-soluble carbohydrates that are essential for successful fermentation. However, mixing legumes with cereal crops such as sorghum, is a practice that contributes to improve the levels of fermentable carbohydrates reduces buffering and prevents proteolysis. In this case the silage color and smell averaged a value of 2.7, which indicated a silage of acceptable quality (Table 3).

Silage with pH 4 or less indicates a proper fermentation process since epiphytic lactic acid bacteria ferments the water-soluble carbohydrates. The bag silage made of pure sorghum, either with or without molasses (treatments 4 and 8) had the lowest pH content (3.5 and 3.9 respectively) (Table 3); however pure Cratylia silage had a pH of 5.0 (treatment 1) that was slightly reduced to pH 4.8 by adding molasses (treatment 5), indicating a strong buffering capacity and a more acetic than a lactic acid fermentation process.

In general, the fermentation process was acceptable for the different proportions of forage mixtures ensiled. Adding molasses at 5% (fresh weight basis) did not have a significant effect in this process, and as has been suggested in other experiments a proportion of at least 10% is needed to overcome the strong buffering effect of Cratylia silage.

It is commonly accepted that a crude protein (CP) content over 7% is required for animal production, and in this case only the silage made of pure sorghum (treatment 4) could be considered as limiting in protein. As expected, as the proportion of legume decreased in the mixture so did the CP content, particularly when no molasses was added.

The residues remaining after neutral detergent extraction (NDF) represents the proportion of plant dry matter made up of cellulose, hemicellulose, lignin, lignified N, and insoluble ash. This component is inversely correlated with intake and digestibility. In our study NDF ranged from 60.3-67.2% for silages without molasses and from 51.4-56.7% for silages with molasses, indicating that this additive may increase both digestibility and intake of the silage.

Silage intake with goats ranged from 0.09 kg/day/animal for the Cratylia pure silage to 0.52 kg/day/animal for the Cratylia plus molasses silage. The intake of the 66% Cratylia, 33% sorghum silage plus molasses was intermediate (0.20 kg/day/animal).

An intake value of 0.52 kg/day of silage represented only 1.3 dry matter (DM) intake per 100 kg body weight (BW), which is low and may be associated with the high cell wall contents recorded in the silages mainly due to plant age and shown by both the Cratylia and sorghum silage. Associated to this were the low organic matter digestibility values observed that range from 52.1% for Cratylia silage, to 48.7% when molasses was added, and a slight improvement in the mixed silage with sorghum (53.2%).

This demonstrated that small bag silage is a viable practice for forage conservation and that as expected, sorghum silage with or without molasses had better fermentation (lactic acid process) than Cratylia silage (more acetic

Table 3. Smell, color and some quality characteristics of *C. argentea* cv. Veraniega and *Sorghum* sp. silage mixed in different proportions with and without molasses in plastic bags.

Treatment	Smell*	Color*	pH	CP (%)	NDF (%)	DM (%)
1	2	2	5.0 a **	11.8 ab	66.8 ab	30.2
2	3	3	4.2 b	10.7 abc	67.2 a	27.1
3	2	3	3.8 c	9.3 bc	60.3 bc	27.1
4	2	2	3.5 c	6.8 d	66.3 a	24.1
5	3	3	4.8 a	12.0 a	55.7 dc	29.1
6	3	3	4.1 b	11.1 ab	56.7 c	29.7
7	3	3	4.0 bc	9.7 bc	51.4 d	29.6
8	3	3	3.9 c	7.7 d	56.5 c	22.4

* Related to smell and color: 1, is poor; 2, medium and 3, good (silage with lactic acid smell and greenish color)

**Within columns means followed by the same letter are not statistically significant ($P < 0.05$)

process). On the other hand, intake of Cratylia silage by goats was low and this may be associated with the type of fermentation produced; however, adding molasses to Cratylia improved the intake of Cratylia silage.

1.3 Assessment of the potential of tanniniferous legumes to improve ruminant nutrition

Highlights

- Selecting appropriate planting sites may improve the feeding value of *Calliandra calothyrsus* cv Patulul
- High quality legumes may be partially replaced by tanniniferous legumes without any negative effect on ruminal fermentation characteristics of the complete diet

Relations between the plant nutritional status and forage quality defined

While the tanniniferous shrub legume *Calliandra calothyrsus* is widely used by smallholders to supplement dairy cattle and goats in Kenya, farmers in Colombia have hardly adopted this supplementation strategy. We hypothesized that this was due to contrasting environmental conditions, which may affect the forage quality of this legume species. Results from in vitro studies carried out this year showed that foliage from *C. calothyrsus* var. Patulul cultivated in Kenya had a higher nutritional value than foliage from the same variety cultivated in Colombia, which was mainly due to a much lower concentration of condensed tannins in the foliage produced in Kenya.

Future work will concentrate in defining the environmental factors (e.g. soil type and fertility) responsible for differences in forage quality and type and concentration of condensed tannins of a range of tanniniferous shrub legumes. For this purpose experimental plots have been established at two contrasting sites in Colombia.

Suitable combinations of tanniniferous legumes with forages free of tannins for improved protein supply and efficiency of rumen fermentation available

Results from in vitro and in vivo experiments carried out during the last two years showed that condensed tannins in tropical legumes contribute to lower methane emission from ruminal fermentation. However, this methane suppressing effect was generally associated with a decrease in the ruminal degradation of nutrients. This year, our work was therefore focused on the identification of types and proportions of tannin rich legumes in mixtures with legumes free of tannins, which improve protein supply without negatively effecting ruminal nutrient degradation.

The results indicate that the tanniniferous legumes *C. calothyrsus* and *Flemingia macrophylla*, when supplemented in a mixture with *Vigna unguiculata* (a legume free of tannins), may be included in the diet at a proportion of up to 13% of total DM without negative effects on rumen fermentation. For *Leucaena leucocephala*, another tanniniferous legume, this maximal proportion seems to be around 22% of the total diet. When higher proportions of tanniniferous legumes were included, the apparent degradation of crude protein was drastically suppressed.

It is likely that this decrease would result in a greater flow of non-ammonia nitrogen to the lower digestive tract of ruminants fed such mixtures which in turn could result in improved animal performance. This hypothesis will be tested in future feeding trials, using sheep fitted with ruminal and duodenal cannulas.

1.3.1 Assessment of the effects of plant nutritional status on forage quality and concentration and chemical properties of condensed tannins of selected legume species

1.3.1.1 Effect of the cultivation site (Kenya or Colombia) on ruminal fermentation characteristics of *Calliandra calothyrsus* var. Patulul

Contributors: F. Noto (ETH Zurich), H.D. Hess (ETH Zurich), T.T. Tiemann (ETH Zurich), M. Kreuzer (ETH Zurich), S. Franzel (ICRAF), and C.E. Lascano (CIAT)

Rationale

Insufficient quality of ruminant diets, as is prevalent in tropical agriculture, in conjunction with low feed conversion efficiency, leads to low levels of animal production. Protein deficiency is by far the most important cause of low performance of ruminants maintained on low-quality forages, and ensuring adequate ammonia levels in the rumen for microbial growth is the first priority in optimizing fermentative digestion of forage. In this respect, shrub legumes are adequate supplements as small-scale farmers can grow them with additional advantage that they supply both rumen-degradable and undegradable protein in higher amounts than most grasses and crop residues.

Calliandra calothyrsus is a shrub legume with excellent agronomic characteristics and high crude protein content. However its feeding value may be limited by low palatability and high concentrations of condensed tannins. Although *C. calothyrsus* is widely used by smallholders to supplement dairy cattle and goats in Kenya, this supplementation strategy has hardly been adopted by farmers in Colombia. We hypothesized that this was mainly due to differences in the forage quality of this legume in the two countries.

To test this hypothesis, an in vitro experiment was performed to compare the nutritional value and the ruminal fermentation characteristic of *C. calothyrsus* var. Patulul cultivated in Colombia, Quilichao (site with acid soils) and Kenya, Embu (site with fertile soils) when used as supplements to a low-quality grass hay.

Material and Methods

In this experiment a grass-alone diet (negative control), a urea-supplemented diet (positive control) and five legume supplemented diets were evaluated. The legume supplements (33% of dietary dry matter) consisted of *Cratylia argentea* (100%), *Calliandra* Colombia (100%), *Calliandra* Kenya (100%), or mixtures (1:1) of *C. argentea* with *Calliandra* Colombia or *Calliandra* Kenya (Table 4). Foliage from *C. calothyrsus* var. Patulul was harvested from mature plants grown in Kenya and Colombia and oven dried at 50°C. Plant material from *Brachiaria humidicola* CIAT 6133 (grass) and *Cratylia argentea* was harvested in Colombia and sun dried. The different diets were tested using a rumen simulation technique (Rusitec) during 4 × 10 day periods (n=4) with four days for adaptation and 6 days for data collection. The daily dry matter supply to the fermenter was maintained constant at 15 g (Table 4).

Results and Discussion

Ammonia concentration in the fermenter fluid was increased ($P<0.05$) by supplementation with urea or *C. argentea* alone but was not affected ($P>0.05$) by supplementation with *Calliandra* alone. While supplementation with the legume mixture containing *Calliandra* Kenya increased ($P<0.05$) ammonia concentration, the mixture containing *Calliandra* Colombia had no effect on ammonia concentration. Independent of the growing site, ammonia concentration decreased linearly ($P<0.05$) with increasing *Calliandra* proportion in the diet. Overall, supplementation with *Calliandra* Kenya resulted in higher ($P<0.01$) ammonia concentrations than

Table 4. Composition (% of dry matter) of experimental diets.

	Diet						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Diet ingredients							
B. humidicola CIAT 6133	100	100	67	67	67	67	67
C. argentea			33			16.5	16.5
C. calothyrsus Colombia				33		16.5	
C. calothyrsus Kenya					33		16.5
Urea		1					
Analyzed composition							
OM	88.9	88.9	89.8	90.2	90.5	90.2	89.9
CP	3.5	5.9	9.7	9.1	9.6	9.4	9.7
NDF	72.1	70.4	63.8	55.5	57.6	60.1	60.7
ADF	39.2	38.8	37.7	33.1	34.7	35.5	36.8

supplementation with *Calliandra* Colombia. Counts of ciliate protozoa and bacteria were not affected (P>0.05) by supplementation.

The apparent degradation of organic matter (OM) increased (P<0.05) with any type of supplementation, except when the supplement consisted of pure *Calliandra* Colombia. Apparent crude protein (CP) degradation was increased (P<0.05) by supplementation with urea or *C. argentea*, but was not affected (P>0.05) by supplementation with the legume mixtures, and was decreased (P<0.05) by supplementation with pure *Calliandra* from Colombia or Kenya. Supplementation with urea, *C. argentea* or the legume mixtures increased (P<0.05) apparent degradation of neutral detergent fiber (NDF), but supplementation with pure *Calliandra* had no effect (P>0.05) on NDF degradation. Independent of growing site, the apparent degradation of OM, CP and NDF decreased linearly (P<0.05) with increasing *Calliandra* proportion in the diet. However, apparent nutrient degradation was higher (P<0.05) in diets containing *Calliandra* Kenya than in those containing *Calliandra* Colombia.

Daily methane release as well as methane release relative to OM degraded increased (P<0.05) with any type of supplementation, except when the supplement consisted of pure *Calliandra* Colombia. Diets containing *Calliandra* Colombia resulted in a 10-20% lower methane release (P<0.01) than those containing *Calliandra* Kenya. With increasing proportions of *Calliandra*, methane release decreased linearly (P<0.05), independent of the site where the legumes were harvested.

Nitrogen supply to the fermenters increased with any type of supplementation. However, depending on the type of supplement, there were significant shifts between N fractions. The proportion of N recovered as ammonia was increased (P<0.05) by supplementation with urea or *C. argentea*, but supplementation with *Calliandra* alone or in mixture with *C. argentea* had no effect on this N fraction. The fraction of apparently not degraded N was increased (P<0.05) by supplementation with *Calliandra* alone, but was not affected (P>0.05) by supplementation with the mixtures, and decreased (P<0.05) when urea or *C. argentea* alone were supplemented. Compared to *Calliandra* Colombia, supplementation with *Calliandra* Kenya resulted in higher proportions of N degraded, of N recovered as ammonia and of N apparently incorporated in microbial protein, but in a lower proportion of apparently not degraded N (P<0.001).

Although the two provenances of *C. calothyrsus* tested in this experiment had similar contents of OM, CP and NDF, they differed in nearly all fermentation properties and the material from Kenya showed a higher apparent nutrient degradability. Thus we conclude from these results that foliage from *C. calothyrsus* var. Patulul cultivated in Kenya had a higher nutritional value than foliage from the same variety cultivated in Colombia, which is mainly due to a much lower concentration of condensed tannins in the foliage produced in Kenya (20 vs. 38% in DM). Both materials had a much lower nutritional value than foliage of *C. argentea*. The effects of urea supplementation on ruminal fermentation were almost identical to the effects caused by supplementation with *C. argentea*. This indicates that the *C. argentea*-related effects on ruminal fermentation were mainly the result of an increased availability of fermentable nitrogen.

1.3.1.2 Establishment of experimental plots in Palmira and Matazul

Contributors: T.T. Tiemann (ETH Zurich), L.H. Franco (CIAT), C. Plazas (CIAT), M. Peters (CIAT), C.E. Lascano (CIAT), H.D. Hess (ETH Zurich), E. Frossard (ETH Zurich), and M. Kreuzer (ETH Zurich)

Rationale

Type and concentration of secondary metabolites in plants may be affected by environmental factors. Results resported in Activity 1.3.1.1 indicate that the forage quality of tanniniferous shrub legumes could be improved by the selection of appropriate planting sites or by improving the growing conditions (e.g. by fertilization). Therefore we are carrying out research to definine the environmental factors (e.g. soil type and fertility) responsible for differences in forage quality and type and concentration of condensed tannins of a range of tanniniferous shrub legumes. For this purpose, experimental plots were established at two contrasting sites in Colombia. The aim of this activity is to identify growing conditions, which could contribute to an improved forage quality of tanniniferous legumes.

Material and Methods

Based on the current knowledge, five multipurpose legume species with contrasting forage quality and types and contents of tannins were selected from the germplasm collection held at CIAT. Namely these were *Cratylia argentea* (CIAT 18516), *Calliandra calothyrsus* (CIAT 22310), *Flemingia macrophylla* (CIAT 17403), *Leucaena leucocephala* (CIAT 17502) and *Desmodium velutinum* (CIAT 33443). In experiment 1, one legume plot was established on a Mollisol at CIAT's headquarters in Palmira and one on an Oxisol in Matazul in the Eastern Plains of Colombia in order to assess the effect of soil type on forage quality. At both sites two fertilization levels are tested. Experiment 2 was established on an Oxisol in Matazul and three contrasting fertilization treatments awere applied to assess the effect of sulfur and phosphorus fertilization on forage quality of shrub legumes. The five experimental species and the fertilization treatments were arranged in randomized complete block designs with three replicates each. The two plots in experiment 1 consist of 30 subplots each (5 species × 2 fertilization levels × 3 replicates) and the plot in experiment 2 consists of 45 subplots (5 species × 3 fertilization treatments × 3 replicates). Subplots are of 6 × 5 m each with 1 m between subplots. In each subplot 30 individual plants have been established.

Seven to nine months after the establishment, the experimental plants will be cut for the first time. This will be done at a height of 75cm for *L. leucocephala* and of 50 cm for the remaining species. Thereafter dry matter production will be evaluated every 8 weeks and twice year edible forage will be harvested to determine forage quality. Plant tissues will be analyzed for C, P, N, K, S, Ca, Mg, condensed tannin, astringency, monomer composition and molecular weight. At the end of the experiment the total dry matter accumulated in the different organs of the plant will be evaluated and the total amount of C, N and P in the soil profile will be measured.

1.3.2 Assessment of in vitro ruminal fermentation dynamics of tanniniferous legumes using the gas transducer technique

Contributors: C.D. Stürm (ETH Zurich), H.D. Hess (ETH Zurich), T.T. Tiemann (ETH Zurich), P. Avila (CIAT), G. Ramirez (CIAT), C.E. Lascano (CIAT), and M. Kreuzer (ETH Zurich)

Rationale

Ruminants play an important role as assets and sources of high quality food and income for rural populations in the developing world. Ruminant productivity is usually low due to inadequate and insufficient nutrition, frequently based on roughages, which are deficient in fermentable protein. Promising forage legume species have been identified to overcome these limitations. Many of these legumes contain tannins that could be either advantageous or disadvantageous in terms of feed efficiency and metabolizable protein supply to the animal. Four multipurpose legume species, *Flemingia macrophylla*, *Cratylia argentea*, *Calliandra calothyrsus* and *Leucaena leucocephala*, have shown great agronomic potential and could serve as forage supplements for ruminants fed tropical grass-based diets.

Prior in vitro studies showed that the incorporation of the tannin-rich legumes *C. calothyrsus* and *F. macrophylla* did not improve the feeding value of low-quality grass diets when used alone. However, in combination with legumes free of tannins (e.g. *Vigna unguiculata*), these tanniniferous legumes could contribute to improve animal nutrition. Very little information is available on the fermentation dynamics and the tannin-related effects of legume mixtures when supplemented to tropical low-quality grass diets. Furthermore it is completely unknown which combinations and proportions of individual legumes species would be most efficient in improving animal nutrition. Therefore several in vitro experiments were performed, to evaluate the effects of contrasting mixtures of tanniniferous and tannin-free legumes as supplements to the tropical grass *Brachiaria dictyoneura* on ruminal fermentation. Additionally, the tannin related effects were assessed by incubating different diets with and without the addition of PEG (a tannin-binding compound which inactivates soluble condensed tannins).

Material and Methods

The plant material of the four shrub legumes, *C. calothyrsus*, *C. argentea*, *F. macrophylla* and *L. leucocephala*, was harvested manually eight weeks after the last cut. The youngest three fully developed leaves of actively growing branches were used for these experiments. This material was immediately stored at 20°C. Subsequently plant samples were freeze-dried. The material of *B. humidicola* CIAT 6133 was cut after a growing period of 12 weeks and sun- dried for two days. In the case of *V. unguiculata*, eight-week-old herbage (the whole plant, before flowering) was harvested and sun dried for two days. The dried plant material of all species was ground in a laboratory mill using a 1 mm screen.

In experiment 1, *B. humidicola* CIAT 6133, *V. unguiculata* and *C. calothyrsus* were tested either alone or in combination with each other. The legume supplemented diets contained either 1/3 or 2/3 of legume and the remaining part was *B. humidicola* CIAT 6133. The legume supplements consisted either of *V. unguiculata*, *C. calothyrsus* or mixtures of the two legumes (4:1, 3:2, 2:3 or 1:4).

In experiment 2, *B. humidicola* CIAT 6133 was evaluated either alone or supplemented with legumes (1/3 of dietary DM). The legume supplements consisted either of a single legume (*V. unguiculata*, *C. calothyrsus*, *F. macrophylla*, *C. argentea* or *L. leucocephala*) or of a mixture of the herbaceous legume *V. unguiculata* with one of the four shrub legumes in ratios of 1:2 or 2:1. Overall, a total of 15 diets were evaluated in experiment 1, and 14 diets in experiment 2. In both experiments all diets were evaluated with and without the addition of PEG (3.5% of DM).

Results and Discussion

Experiment 1: The highest ($P<0.05$) in vitro DM degradability (IVDMD) was observed in the pure *B. humidicola* CIAT 6133 and *V. unguiculata* diets and in grass-legume mixtures with high proportions of *V. unguiculata*, whereas the mixtures with high *C. calothyrsus* proportions and the pure *C. calothyrsus* diet showed the lowest IVDMD ($P<0.05$). The IVDMD decreased linearly ($P<0.001$) with increasing *C. calothyrsus* proportion in the legume mixture. This decrease was much lower when PEG was added to the diets than without PEG (interaction: $P<0.05$). The maximal gas production after 144 h was highest in the pure *B. humidicola* CIAT 6133 diet and lowest in the pure *C. calothyrsus* diet ($P<0.05$). The pure *V. unguiculata* and the mixed diets showed intermediate values.

The maximal gas production decreased linearly ($P<0.001$) with increasing *C. calothyrsus* proportion in the mixture, regardless of the legume proportion This decrease was greater in diets with high legume proportion than in the diets with low legume proportion. The interaction between diet and addition of PEG was highly significant ($P<0.001$) and the PEG-related increase in total gas production was larger in the diets containing high proportions of *C. calothyrsus* than in the remaining diets. The gas production rate was highest with the pure *V. unguiculata* diet and lowest with the pure *C. calothyrsus* diet and mixtures containing high proportions of this legume ($P<0.05$). In the remaining grass-legume mixtures and the pure *B. humidicola* CIAT 6133 diet intermediate values were observed. The interaction between diet and PEG addition was highly significant ($P<0.001$) and the PEG-induced increase in the gas production rate was much larger in the diets containing large proportions of *C. calothyrsus* than in the other diets.

The highest amount of protein was degraded in the pure *V. unguiculata* diet and the lowest amount in the pure *B. humidicola* CIAT 6133 diet ($P<0.05$). The interaction between diet and PEG addition was highly significant ($P<0.001$). The addition of PEG increased ($P<0.05$) the amount of apparently degraded crude protein in the pure *C. calothyrsus* diet and in all diets containing more than 13% of *C. calothyrsus* but had no effect in the remaining diets. Additionally, there was a quadratic effect ($P<0.001$) of the *C. calothyrsus* proportion on the amount of crude protein degraded when no PEG was added to the diets. With PEG, this effect was not apparent in the diets with low legume proportion, and in the diets with high legume proportion the negative effect of *C. calothyrsus* was much less pronounced than without PEG.

The inclusion of *C. calothyrsus* in the legume mixture had a quadratic effect on crude protein degradation. As a result, crude protein degraded was not affected by *C. calothyrsus* when a small proportion of the legume was used in the diet. In contrast, with higher proportions of legume (>13%) the amount of crude protein degraded decreased very sharply. The effects of diet and PEG on the apparent degradability of crude protein were very similar to the effects on the amount of crude protein apparently degraded described above. Crude protein degradability was high in the pure *V. unguiculata* diet and low in the pure *C. calothyrsus* diet and there was a highly significant interaction ($P<0.001$) between diet and PEG addition. In all *C. calothyrsus* -containing diets, the addition of PEG significantly increased protein degradability. With increasing *C. calothyrsus* proportion in the legume mixtures, protein degradability decreased quadratically ($P<0.001$).

Experiment 2. The highest IVDMD was observed the pure *B. humidicola* CIAT 6133 diet and the mixtures of *B. humidicola* CIAT 6133 with *V. unguiculata* and *C. argentea* ($P<0.05$). The partial or complete replacement of *V. unguiculata* by *C. argentea* did not affect IVDMD, but with other shrub legumes the IVDMD decreased with increasing proportion of the legume in the diet ($P<0.05$). The lowest ($P<0.05$) IVDMD was found in the mixture that contained 1/3 of *F. macrophylla*.

There was a highly significant interaction ($P<0.001$) between diet and PEG addition which was mainly due to the fact, that the addition of PEG increased IVDMD in the diets with high proportion of tanniniferous legumes, but had no effect in the other diets. The maximal gas production after 144 h was highest ($P<0.05$) with the pure *B. humidicola* CIAT 6133 diet and lowest with the diets containing the tanniniferous legumes at a proportion of 1/3. The replacement of *V. unguiculata* by *C. argentea* had no effect on the maximal gas production, but the inclusion of the other shrub legumes suppressed the gas production.

The addition of PEG increased the maximal gas production in the diets with high proportion of tanniniferous legumes but not in the remaining diets (interaction: $P<0.001$). The highest gas production rates were observed in the mixtures of *B. humidicola* CIAT 6133 with *V. unguiculata* and *C. argentea*, whereas the lowest values were found in the pure *B. humidicola* CIAT 6133 diet and the diets which consisted of 1/3 of *F. macrophylla* or *C. calothyrsus* ($P<0.05$). The partial or complete replacement of *V. unguiculata* by *C. argentea* did not affect gas production rate. With other shrub legumes the gas production rate decreased with increasing proportion of the legume in the diet ($P<0.05$). The interaction between diet and PEG addition was highly significant ($P<0.001$). The gas production rate increased when PEG was added to the diets consisting of 1/3 of *C. calothyrsus* or *F. macrophylla*, but not when PEG was added to the other diets.

The highest amount of crude protein was degraded in the diets containing *C. argentea* and the lowest amount in the pure *B. dictyoneura* diet ($P<0.05$). The diets containing *L. leucocephala* and the diet containing *V. unguiculata* to 1/3 showed intermediate values. In the diets containing *C. calothyrsus* or *F. macrophylla*, the amount of protein degraded was lower than in any other legume supplemented diet, but still higher than in the pure *B. dictyoneura* diet ($P<0.05$). The amount of crude protein degraded decreased ($P<0.05$) with increasing proportion of tanniniferous legumes in the diet when no PEG was added, but with PEG this effect was not observed (interaction: $P<0.001$). The apparent degradability of crude protein was highest ($P<0.05$) in the diets consisting of 1/3 of *C. argentea* or *V. unguiculata* or mixtures of these two legumes. The diets containing *L. leucocephala* or low proportions of *C. calothyrsus* or *F. macrophylla* as well as the pure *B. dictyoneura* diet showed intermediate values. The lowest ($P<0.05$) apparent crude protein degradability was observed in the diets consisting of 1/3 of *C. calothyrsus* or *F. macrophylla*.

There was a highly significant interaction ($P<0.001$) between diet type and PEG addition. In all diets containing *C. calothyrsus* or *F. macrophylla* as well as in the diets containing medium or high proportions of *L. leucocephala*, apparent degradability of crude protein was significantly higher ($P<0.05$) when PEG was added. With the other legume diets the addition of PEG had no effect ($P>0.05$). Additionally, the apparent crude protein degradability decreased ($P<0.05$) with increasing proportion of tanniniferous legumes in the diet when no PEG was added. In the treatments with PEG this effect was not observed or much less pronounced. Among the three tanniniferous legumes, *Leucaena* showed the lowest negative effects on crude protein degradability.

In summary, these results indicate that *B. humidicola* CIAT 6133 has a high potential in vitro dry matter degradability (>65%) but an extremely low crude protein content (<5%). Therefore it may be a good source of fermentable energy, provided that it is adequately supplemented with a source of fermentable protein. The herbaceous legume *V. unguiculata* has a great potential as protein supplement for ruminants fed low quality grasses, because it improves protein supply without negatively affecting IVDMD.

The effects of partial or complete substitution of *V. unguiculata* in the legume supplement for foliage of shrub legumes strongly depends on the tannin content of the shrub species. The supplementation with the tannin-free *C. argentea* results in similar or even slightly better fermentation characteristic of the mixed diet than the supplementation with *V. unguiculata*. Thus, *C. argentea* represents a valuable alternative for smallholders to improve protein supply to ruminant livestock, particularly because, compared to *V. unguiculata,* it has several agronomic advantages like being drough tolerant, and being perenial. In contrast to the tannin-free legumes, supplementation with *C. calothyrsus* or *F. macrophylla* does not increase the amount of degraded protein and also suppresses IVDMD compared to the pure *B. humidicola* CIAT 6133 diet. However, when these high tannin legumes are supplemented in a mixture with *V. unguiculata*, both may be included in the diet at a proportion of up to 13% of total DM without negative effects on rumen fermentation. For *L. leucocephala* this maximal proportion seems to be around 22% of the total diet. When higher proportions of tanniniferous legumes are included in the diet, the apparent degradation of crude protein is drastically decreased. It is likely that such a decrease would result in a greater flow of non-ammonia N to the lower digestive tract of ruminants fed such mixtures. However, as in this study only ruminal fermentation processes were considered, effects on N digestion and utilization in the lower digestive tract have to be assessed in animal feeding trials. If a certain amount of the protein-tannin complexes gets dissolved in the lower digestive tract and is available for digestion and absorption, a high proportion of these tanniniferous legumes in mixed diets could result in an increased animal performance.

1.3.3 Assessment of the effects of legume mixtures on rumen fermentation parameters using the Rumen Simulation Technique (Rusitec)

Contributors: M. L. Mera Álvarez (CIAT), H.D. Hess (ETH Zurich), T.T. Tiemann (ETH Zurich), G. Ramirez (CIAT), P. Avila (CIAT), C.E. Lascano (CIAT), and M. Kreuzer (ETH Zurich)

Material and Methods

Two experiments were carried out to evaluate the effects of the partial replacement of *Vigna unguiculata* by tanniniferous shrub legumes of contrasting quality in a basal diet of *Brachiaria humidicola* CIAT 6133.

In experiment 1, all experimental diets consisted of 50% *B. humidicola* CIAT 6133 and 50% legume supplement. The legume supplements consisted either of *V. unguiculata* alone or a mixture (3:2) of this legume with *Calliandra calothyrsus*, *Flemingia macrophyllus* or *Leucaena leucocephala*. The four diets were evaluated with and without the addition of PEG (1% of DM).

In experiment 2, a grass alone diet (negative control), a urea supplemented diet (positive control) and six legume -based diets (50% of DM) were evaluated. The legume supplements consisted either of *V. unguiculata* alone or in mixtures (3:2) with *C. calothyrsus*, *F. macrophyllus* or *L. leucocephala*. In the case of *C. calothyrsus* and *F. macrophylla* two accessions were tested per species, one with low in vitro dry matter digestibility (L-IVDMD) and one with high IVDMD (H-IVDMD). The experimental diets were tested in a Ruistec apparatus during 4 × 10 day periods (n=4) and daily dry matter supply to the fermenters was maintained constant at 15 g.

Results and Discussion

Experiment 1. The partial replacement of *V. unguiculata* by any tanniniferous shrub legumes used decreased ($P<0.05$) ammonia concentration in the fermenter fluid, but the decrease was more pronounced with *C. calothyrsus* than with *F. macrophylla* or *L. leucocephala*. On average, *L. leucocephala*. On average, PEG addition increased (P<0.05) ammonia concentration from 3.3 to 3.7 mmol/l and no interaction (P>0.05) between PEG addition and diet was observed. Counts of ciliate protozoa and bacteria were not affected (P>0.05) by diet but bacteria counts increased (P<0.05) with PEG addition.

Apparent degradability of organic matter (OM) and crude protein (CP) decreased (P<0.05) when *V. unguiculata* was partially replaced by *C. calothyrsus* or *F. macrophylla*, but not when *L. leucocephala* was included in the diet (P>0.05). The inclusion of any shrub legume decreased (P<0.05) the apparent degradability of fiber (NDF), but the decrease was more pronounced with *C. calothyrsus* and *F. macrophylla* than with *L. leucocephala*. Addition of PEG had no effect (P>0.05) on the apparent degradability of OM and CP but increased (P<0.05) degradability of NDF. No interaction (P>0.05) between PEG addition and diet on apparent nutrient degradability was observed. Daily methane emission as well as methane emission relative to OM degraded were similar with all diets and were not affected by PEG addition (P>0.05). Taken as such, the lack of an interaction between PEG addition and diet would suggest, that tannins present in the shrub legumes had no effect on ruminal fermentation. However, the changes caused by the inclusion of these tanniniferous legumes agreed well with those observed in previous experiments (see also Activity 1.3.2) and indicate that microbial activity was suppressed in the same way, as it would be expected from tannin addition. We therefore assume that the level of PEG added to the diets (1% of DM) was not sufficient to inactivate all tannins and to avoid negative effects on ruminal fermentation.

Experiment 2. The supplementation with urea or legumes clearly increased (P<0.05) ammonia concentration in the fermenter fluid. The largest increase was observed with urea supplementation, the smallest with the legume mixture containing *C. calothyrsus* L-IVDMD. Ciliate protozoa and bacteria counts were not affected (P>0.05) by supplementation. Legume supplementation increased (P<0.05) the apparent OM degradability regardless of species or mixture. The largest increase was achieved with the supplement consisting of pure *V. unguiculata*, the smallest with the legume mixtures containing any of the *C. calothyrsus* accessions or *F. macrophylla* L-IVDMD.

Apparent CP degradability was increased (P<0.05) by supplementation with urea, *V. unguiculata* alone or in mixture with *F. macrophylla* H-IVDMD, but was not affected (P>0.05) by the other legume supplements. Apparent NDF degradability was increased (P>0.05) by supplementation with urea, *V. unguiculata* alone and the mixtures containing *F. macrophylla* H-IVDMD or *L. leucocephala*. Daily methane release was not affected (P>0.05) by supplementation with mixtures containing any *C. calothyrsus* accession, *F. macrophylla* L-IVDMD or *L. leucocephala*, but increased (P<0.05) when urea, *V. unguiculata* alone or the mixture containing *F. macrophylla* H-IVDMD were supplemented. Methane release relative to OM degraded was not affected (P>0.05) by dietary treatments.

In summary, these results confirm the high potential of *V. unguiculata* as a supplement to low-quality diets and suggest that the effects of supplementing this legume on ruminal fermentation are mainly the result of an increased supply of fermentable nitrogen. The partial replacement of *V. unguiculata* by *F. macrophylla* H-IVDMD resulted only in small changes in the fermentation characteristics of the complete diet. Therefore, this shrub legume seems to have a high potential as a supplement for ruminants in combination with high-quality legumes. Based on the present results, the evaluated shrub legumes can be ranked according to their feeding value as follows: *F. macrophylla* H-IVDMD > *L. leucocephala* > *F. macrophylla* L-IVDMD = *C. calothyrsus* H-IVDMD > *C. calothyrsus* L-IVDMD.

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

2.1 *Brachiaria* genotypes resistant to biotic and abiotic stresses

Highlights

- Sexual clones with resistance to spittlebug and Rhizoctonia foliar blight and high levels of Aluminum were selected.
- Modified breeding scheme: Assessment of merit of sexual clones will be assessed not only on their phenotypes but also of their hybrid progeny.

2.1.1 Selection of sexual clones

Contributors: C. Cardona, S. Kelemu, I.M. Rao, C.E. Lascano and J.W. Miles (CIAT)

Rationale

A broad-based, synthetic tetraploid sexual *Brachiaria* population is the core of the breeding program. This population has been cyclically selected over eight years (four cycles), mainly on spittlebug resistance and general agronomic performance. Stunning progress in spittlebug resistance has been achieved (see Section 2.2).

Recently, selection pressure on Al tolerance, Rhizoctonia resistance, and nutritional quality is being incorporated in the selection process as screening protocols for these additional traits improve in capacity and speed. As the sexual population is upgraded, genetic gain is captured in apomictic genotypes by crossing selected sexual clones with elite apomicts.

Materials and Methods

Nearly 3,000 clones of the most recent cycle of the sexual population were established, as individual spaced plants, in two unreplicated field trials (CIAT-Quilichao and Matazul Farm, Puerto López) in May, 2003. Each clone was included in each of the two locations. Between May and October, the initial population was culled to 746 "pre-selections" on the basis of periodic visual inspection at the two field sites.

Between January and July 2004, These "pre-selections" were screened for spittlebug resistance in a two-stage process, including an unreplicated evaluation in which each clone was separately infested with each of the three species followed by a replicated evaluation of the best (apparently most resistant) 120 clones with the same three species.

Information on reaction to Al and Rhizoctonia were obtained simultaneously.

Results and Discussion

In July 2004, an isolated crossing block was set up by vegetative propagation of 42 selected clones established in 25-cm pots in three randomized complete blocks.

Given the large number of highly spittlebug-resistant sexual clones identified, in addition to establishing the sexual population crossing block, it was decided to cross the same 42 clones to *B. decumbens* cv. Basilisk.

2.1.2 Crossing elite sexual selections to *B. decumbens* CIAT 606

Contributors: J.W. Miles, A. Betancourt, and J. Muñoz (CIAT)

Rationale

The overall objective of the *Brachiaria* breeding project is to produce useful new, apomictic cultivars. *B. decumbens* CIAT 606 possesses many highly desirable characters, including edaphic adaptation and strongly stoloniferous growth habit. It is susceptible to spittlebug. Given a planned change in the breeding scheme no large field planting was scheduled for 2005. This 1-yr lull in field trials plus the high levels of multiple resistance found in many sexual clones motivated a decision to attempt to generate a large hybrid population to evaluate next year.

Given the large number of highly spittlebug-resistant sexual clones identified, in addition to establishing the sexual population crossing block, it was decided opportunistically to cross the same 42 clones to *B. decumbens* CIAT 606 to generate a large hybrid population segregating for apomixis.

Materials and Methods

An area of *B. decumbens* of approx. 1.25 ha was in the process of being prepared (mown, replanted, fenced) to serve as a "top-cross nursery" for activities in 2005. We simply accelerated this preparation and, in July 2004, were able to space plant (very approx. at 5 x 5m) four vegetative replicates of each of 120 pre-selected sexual plants in this plot of *B. decumbens*. In mid-August, when final results of the replicated, reconfirmation spittlebug screening were available, the plants of 78 clones were physically culled and the four replicates of each of the 42 most resistant clones left to cross with the surrounding *B. decumbens*.

Results and Discussion

Four hundred eighty (120 clones * 4 replicates) SX03 transplants were successfully established. Poor rainfall following planting has retarded growth of both the sexual plants and the surrounding *B. decumbens* until recent rains in September. We anticipate harvesting several thousand viable hybrid seeds from the 168 (42 clones * 4 replicates) sexual plants that remain following culling. The resulting hybrid population will be planted in 2005 in space-planted field trials in two locations.

2.1.3 Establishment and selection in the field of new hybrid clones (3000 SX x AP— Series BR03/04) clones

Contributors: J.W. Miles, A. Betancourt, J. Muñoz, C. Plazas and D. Vergara (CIAT)

Rationale

The *Brachiaria* breeding project seeks to create, identify and propagate useful apomictic hybrids. Each cycle of selection in the synthetic sexual population identifies a small number of elite sexual clones. These are recombined and also are crossed to selected apomictic accessions to generate novel apomictic hybrids.

Materials and Methods

In the sexual population planted in 2001, two clones with exceptional spittlebug resistance were identified. These two clones were each crossed during 2003, to four apomictic accessions to produce eight hybrid families. A small population of these hybrids (~420) was produced in 2003 (series BR03) and a very preliminary spittlebug screening (3 spp., 2 reps) was done late that year. A much larger seedling population was established in 2004 (series BR04). A total of thirty-six BR03 seedlings with promising initial reaction to spittlebugs and 2,131 BR04 seedlings (and appropriate checks) were propagated to establish two unreplicated, space-planted field trials trials at CIAT Quilichao and at the Matazul Farm (Puerto López). Hybrids are from eight families, formed by crossing two spittlebug-resistant sexual selections (series SX01) by four apomictic *Brachiaria*.

Results and Discussion

The hybrid population has been culled down to 492 clones based on several visual assessments. Seed will be harvested (for progeny tests) and the "pre-selected" plants propagated from the field at CIAT-Quilichao to CIAT-Palmira during October for detailed screenings for spittlebug, aluminum, Rhizoctonia, and quality.

2.1.4 Recombination of sexual hybrids (7) with CIAT 16320 (Series BR02): Reconfirmation of reproductive mode and screening for reaction to Rhizoctonia

Contributors: J.W. Miles; S. Kelemu; J. Muñoz (CIAT)

Rationale

Our mainstream tetraploid sexual breeding population is generally very susceptible to Rhizoctonia foliar blight. The *B. brizantha* accession CIAT 16320 shows exceptional resistance to Rhizoctonia foliar blight. In 2001, several selected clones from the sexual population were crossed with CIAT 16320. Several hundred of these hybrids were assessed for Rhizoctonia resistance by the detached-leaf assay. Selected hybrids were progeny tested. Seven apparently sexual hybrids with an intermediate level of resistance were identified. By recombining these few genotypes to create a large segregating population, we expect to identify tetraploid, sexual clones with higher levels of resistance so that this resistance (originally derived from CIAT 16320) can be introgressed into the mainstream tetraploid sexual breeding population.

Materials and Methods

Seven apparently sexual (on progeny test), apparently more or less resistant to Rhizoctonia foliar blight (on detached-leaf assay) hybrids with the highly Rhizoctonia-resistant *B. brizantha* accession CIAT 16320 were identified in 2003, from crosses made in 2001. These seven clones were propagated vegetatively and a small isolated recombination block established early in 2004. Open-pollinated seed is being hand harvested as it matures.

Results and Discussion

Open-pollinated seed is being harvested currently (Sept. 2004), to establish a large population in 2005 with the expectation of being able to isolate tetraploid sexual clones with high levels of resistance.

2.1.5 Seed multiplication from apparently apomictic hybrids (36- Series BR02) for evaluation in Colombia, Mexico, and Thailand

Contributors: J.W. Miles, A. Betancourt, J. Muñoz, P. Horne, and I.M. Rao (CIAT)

Rationale

With each selection cycle a new cohort of sexual-by-apomictic hybrids is formed. As these are assessed for a series of attributes, most are culled and a small group of promising apomictic pre-selections identified for distribution for wider evaluation.

Materials and Methods

A new cohort of pre-selected, experimental apomictic hybrids is generated each selection cycle (generally every two years). From several thousand new sexual-by-apomictic hybrids first planted in Colombia in 2002 (series BR02), 36 hybrids were selected on vigor and apomictic reproduction. After assessing seed fill, 21 of these selections were culled, so that 15 lines still are under consideration. The main selection criteria have been vigor and seed fill, as well as spittlebug reaction. Given the absence of pest spittlebug species in tropical Asia, even susceptible genotypes merit testing there.

Seed of 15 selections, harvested from progeny tests conducted at CIAT in 2003 was mostly used in an unsuccessful attempt to produce sufficient plants for an agronomic trial to be planted in the Colombian Llanos. Small amounts of remnant seed of 9 of the 15 selections was sent to Peter Horne (CIAT-Laos) for initial observation trials in Laos and Thailand. All 15 selections were vegetatively propagated during 2004 and seed multiplication plots established at CIAT-Popayán at mid-year. We anticipate having sufficient seed of these lines for wider distribution (Asia, Mexico) during 2005.

Results and Discussion

Small seed multiplication plots (50 spaced plants) of 15 BR02 selections have been established at Popayán.

2.1.6 Establishment of *B. decumbens* CIAT 606 at CIAT-Popayán for formation of testcrosses in 2005

Contributors: A. Betancourt and J.W. Miles (CIAT)

Rationale

The *Brachiaria* breeding program has relied to date on cyclic intra-population selection in a broad-based, synthetic, tetraploid sexual population. Since the objective of the program is to develop superior hybrids, and given the improvements achieved to date in the sexual breeding population (e.g., greatly improved spittlebug resistance), a decision has been taken to modify the population improvement scheme such that assessment of the merit of sexual clones will be based not only on their own phenotype, but also on that of their testcross (hybrid) progeny. A single apomictic ("male") tester will be crossed to candidate sexual ("female") clones in the field to generate the testcross progenies.

Materials and Methods

An area of approx. 1.25 ha of *B. decumbens* was identified at CIAT-Popayán (where *B. decumbens* flowers continuously throughout the year). The area was fenced and mown. We are already using this area in an (opportunistic) attempt to produce a large hybrid population with the 42 SX03 clones selected for resistance to three spittlebug species.

These sexual plants will be eliminated after seed harvest (early 2005), and 500 SX05 plants (resulting from recombination of SX03 selections) spaced planted (at 5 x 5 m) to be pollinated by *B. decumbens*, thus forming 500 testcross families for testing during 2006 and 2007.

Results and Discussion

The "testcross crossing block" is established and functioning. There should be no obstacle to producing the desired testcross families next year, particularly with opportune establishment of the spaced sexual plants with first semester rainy season.

2.1.7 Agronomic evaluation in Mexico apomictic hybrids from material evaluated in 2002 and 2003: Selection of candidate cultivars

Contributors: E. Guzmán (Papalotla), and J.W. Miles (CIAT)

Rationale

Approx. 125 "pre-selections" from nearly 4,000 hybrids (series MX02) established at Semilla Papalotla's Santa Elena Farm were progeny-tested during 2003 (in Mexico). Approx 60 of these appear to be highly apomictic. A number of selections were culled on poor seed fill, and 34 selected for further evaluation. Seed of these 34 apomictic selections was received at CIAT in January this year.

Evaluations of their reaction to spittlebugs, both Mexican species (in Mexico) and Colombian species (at CIAT), as well as initial seed increase have begun.

Materials and Methods

Seed was harvested from apparently apomictic progeny rows at Sta. Elena Farm (Mexico) during 2003. From seed samples of 34 progenies received at CIAT, seedlings were established and propagated for assessment of spittlebug reaction. Following quarantine clearance by Colombian authorities, 20-plant initial seed multiplication field plots were established at CIAT in September.

Results and Discussion

We anticipate having sufficient seed of these promising experimental lines for further field assessment in Colombia and SE Asia during 2005.

2.2 Screening *Brachiaria* genotypes for spittlebug resistance

Highlights

- Mass rearing of *Zulia carbonaria* and *Prosapia simulans* were successfully established.
- New sexual hybrids with high levels of antibiosis resistance to *Aeneolamia varia*, *A. reducta* and *Zulia carbonaria* were identified.
- 11 apomictic hybrids "pre-selected" for Mexican conditions showed high levels of antibiosis resistance to *Prosapia simulans*, one of the most important spittlebug species affecting *Brachiaria* in Mexico and Central America.
- The resistance of 22 sexual hybrids to *A. varia*, *Z. carbonaria*, *Z. pubescens* and *Mahanarva trifissa* was confirmed in replicated field trials conducted in Caquetá.

2.2.1 Continuous mass rearing of spittlebug species in Palmira and Macagual

Contributors: G. Sotelo and C. Cardona (CIAT)

A permanent supply of insects is essential in the process of evaluating genotypes for resistance to spittlebug. At present, the progress made in mass rearing of nymphs and in obtaining eggs from adults collected in the field allows us to conduct simultaneous screening of large number of *Brachiaria* genotypes for resistance to all major spittlebug species present in Colombia. Insects produced in our mass rearing facilities are used for greenhouse evaluations in Palmira and field evaluations in Caquetá.

2.2.2 Selection of *Brachiaria* genotypes resistant to spittlebug

Contributors: C. Cardona, G. Sotelo, J. W. Miles, P. Sotelo, U. Castro, and A. Pabón (CIAT)

2.2.2.1 Greenhouse screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species

Rationale

Assessment of resistance to spittlebugs is an essential step in the process of breeding superior *Brachiaria* cultivars at CIAT. In 2004, intensive screening of selected hybrids was conducted under greenhouse and field conditions.

Materials and Methods

Screenings for resistance were conducted with *Aeneolamia varia, A. reducta, Zulia carbonaria,* and *Prosapia simulans*. Test materials were usually compared with five checks fully characterized for resistance or susceptibility to *A. varia*. Plants were infested with six eggs per plant of the respective spittlebug species and the infestation was allowed to proceed without interference until all nymphs were mature (fifth instar stage) or adult emergence occurred. Plants (usually 5-10 per genotype) were scored for symptoms using a damage score scale (1, no visible damage; 5, plant dead) developed in previous years. 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 susceptible hybrids were discarded.

Results and Discussion

A set of 731 pre-selected sexual (SX03) hybrids was simultaneously screened for resistance to *A. varia*, *A. reducta*, and *Z. carbonaria*. We used one rep per hybrid per insect species. For comparison, we used 16 well-known checks replicated 10 times per insect species. In terms of damage scores, 78.3%, 84.3%, and 74.9% of the hybrids were rated as resistant to *A. varia*, *A. reducta*, and *Z. carbonaria*, respectively (Table 5). After percentage survival was recorded, 120 hybrids combining low damage levels and high levels of antibiosis resistance were selected for reconfirmation tests. These were conducted using five replications per genotype per insect species. Results (Table 6) clearly indicated that a very significant progress has been made in incorporating antibiosis resistance to all of the three test species in a relatively short period of time.

Table 5. Frequency distribution (percentages) of resistance reactions in a set of 731sexual *Brachiaria* hybrids screened for resistance to three spittlebug species.

Category	*Aeneolamia varia*	*Aeneolamia reducta*	*Zulia carbonaria*	All three species
Resistant	64.2	75.2	59.1	39.5
Intermediate	14.1	9.1	15.8	33.9
Susceptible	21.7	15.7	25.1	26.6

The rapid progress made in incorporating resistance to spittlebug is also illustrated in Figure 3. There has been a steady increase in the frequency of resistant genotypes as a result of recurrent selection through cycles.

As part of on-going studies on mechanisms of resistance to spittlebug species of economic importance in Mexico, we screened 34 hybrids for resistance to *Prosapia simulans*. These hybrids had been pre-selected in Mexico for good adaptation and desirable agronomic characteristics. Using a level of infestation of six nymphs per plant and 10 replications, the hybrids were compared with four accessions, and two susceptible and two resistant checks.

Results shown in Table 7 indicate that 11 hybrids have antibiosis resistance to *P. simulans*. This

Table 6. Levels of resistance to three spittlebug species in selected sexual *Brachiaria* hybrids

Genotype	Damage scores			Percentage nymph survival		
	Aeneolamia varia	*Aeneolamia reducta*	*Zulia carbonaria*	*Aeneolamia varia*	*Aeneolamia reducta*	*Zulia carbonaria*
			Elite hybrids			
SX03/2483	1.0	1.0	2.4	8.0	0.0	26.7
SX03/2226	1.0	1.0	1.7	3.3	6.7	16.7
SX03/2061	1.3	1.0	1.3	16.7	0.0	16.7
SX03/4043	1.3	1.0	1.2	10.0	10.0	6.7
SX03/3744	1.0	1.4	1.6	13.3	6.7	3.3
SX03/4351	1.1	1.4	1.4	20.0	13.3	10.0
SX03/3882	1.0	1.3	1.2	13.3	20.0	10.0
SX03/2053	1.0	1.0	2.4	20.0	20.0	33.3
SX03/1100	1.0	1.5	2.7	25.0	21.7	13.3
SX03/4224	1.4	1.2	1.2	20.0	23.2	4.2
SX03/0282	1.0	1.0	1.5	30.0	6.7	6.7
SX03/0770	1.3	1.3	1.2	30.0	10.0	3.3
SX03/1090	1.3	1.7	1.7	30.0	17.3	13.3
SX03/1408	1.0	1.2	1.2	26.7	23.3	13.3
SX03/2784	1.5	1.1	2.0	26.7	16.7	0.0
			Checks			
CIAT 36062[a]	1.0	1.4	2.2	25.0	21.7	60.0
SX01NO/0102[a]	1.6	1.0	2.2	26.7	10.0	20.0
CIAT 0606[b]	4.6	3.8	4.0	91.7	75.0	53.3
BRX-44-02[b]	4.8	4.6	3.8	83.3	80.0	68.3

[a] Resistant check; [b] Susceptible check.

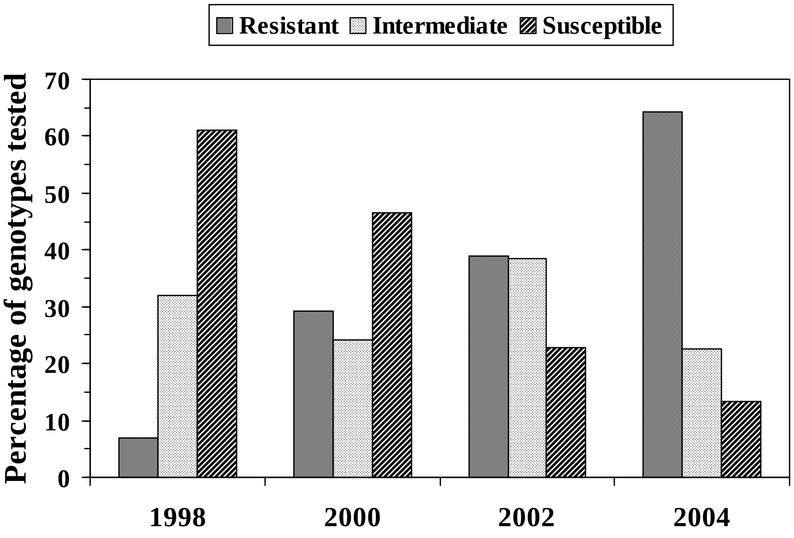


Figure 3. Progress in the incorporation of resistance to *Aeneolamia varia* in *Brachiaria*; note the steady increase in the frequency distribution of resistance genotypes and the decline in the frequency of susceptible genotypes as a result of continuous cycles of selection.

information will be crossed with that obtained in Mexico with the species *Aeneolamia albofasciata* and *A. postica* (part of Ulises Castro's M. Sc. thesis on mechanisms of resistance to Mexican species). In progress is the evaluation for resistance to *A. varia*, *A. reducta* and *Z. carbonaria* of 422 apomictic hybrids derived from crosses between the highly resistant sexual hybrid SX01NO/0102 and *B. decumbens* 'Basilisk' and other susceptible genotypes. The main purpose of this study is to identify patterns of segregation of resistance for each of the spittlebug species involved. Results will be reported in 2005.

Table 7. Levels of resistance to *Prosapia simulans* in *Brachiaria* hybrids pre-selected for Mexican conditions.

Genotype	Damage scores	Percentage nymph survival	Rating	Genotype	Damage scores	Percentage nymph survival	Rating
		Hybrids				Hybrids	
MX 1905	1.1	3.3	Resistant	MX 1660	2.9	47.9	Susceptible
MX 1561	1.3	5.6	Resistant	MX 1769	3.0	3.3	Resistant
MX 3056	1.6	1.7	Resistant	MX 1548	3.1	50.0	Susceptible
MX 1423	1.8	1.7	Resistant	MX 1565	3.1	31.7	Susceptible
MX 1880	1.8	29.6	Intermediate	MX 2775	3.1	40.7	Susceptible
MX 3641	2.0	25.0	Intermediate	MX 1638	3.2	56.7	Susceptible
MX 2295	2.2	10.0	Resistant	MX 3861	3.2	66.7	Susceptible
MX 1809	2.2	16.7	Resistant	MX 2273	3.2	6.2	Resistant
MX 1388	2.2	16.7	Resistant	MX 2090	3.2	23.3	Susceptible
MX 3567	2.2	26.7	Intermediate	MX 1614	3.3	46.7	Susceptible
MX 2552	2.2	33.3	Intermediate	MX 3626	3.4	71.7	Susceptible
MX 1788	2.3	31.5	Susceptible	MX 3582	3.7	75.9	Susceptible
MX 3426	2.3	26.7	Intermediate			Checks	
MX 1263	2.4	42.6	Susceptible	CIAT 16827	1.2	5.0	Resistant
MX 3731	2.4	18.5	Resistant	CIAT 26110	1.8	21.7	Resistant
MX 2135	2.5	48.3	Susceptible	CIAT 36087	1.8	1.7	Resistant
MX 2531	2.6	50.0	Susceptible	CIAT 36061	3.1	27.1	Intermediate
MX 1942	2.6	41.7	Susceptible	CIAT 36062	1.8	0.0	Resistant check
MX 3850	2.7	50.0	Susceptible	CIAT 06294	1.6	6.7	Resistant check
MX 2783	2.7	60.0	Susceptible	CIAT 0606	3.6	50.0	Susceptible check
MX 3213	2.8	9.2	Resistant	BRX-44-02	4.0	68.3	Susceptible check
				LSD 5%	1.97	12.5	

2.2.2.2 Field screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species

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

Rationale

Assessment of spittlebug resistance under natural levels of infestation in the field is very difficult due to the focal, unpredictable occurrence of the insect. This problem has been overcome since 1998 when we developed an artificial infestation technique that allows us to properly identify resistance under field conditions. The purpose of field evaluations is to reconfirm levels of resistance identified under greenhouse conditions.

Materials and Methods

Using the experimental unit described in our 1998 Annual Report, the genotypes (usually 10 replicates) are initially infested in the greenhouse with an average of 10 eggs per stem. Once the infestation is well established, with all nymphs feeding on the roots, the units are transferred to the field and transplanted 10-15 days after infestation. The infestation is then allowed to proceed without interference until all nymphs have developed and adults emerge some 30-35 days thereafter. The plants are then scored for damage by means of the 1-5 visual scale utilized in greenhouse screenings. The number of stems per clump is counted before and after infestation and a tiller ratio (tillers per plant at the end of the infestation process/tillers per plant at the beginning of the infestation process) is then calculated. Using this methodology, 12 major screening trials (four with *A. varia*, four with *Zulia carbonaria*, two with *Z. pubescens*, and one with *Mahanarva trifissa*) were conducted in Caquetá in 2004.

The main purpose of these trials was to reconfirm resistance in 22 sexual hybrids (SX01) previously selected in Palmira under greenhouse conditions.

Results and Discussion

As shown in Table 8, virtually all of the sexual hybrids showed adequate levels of field resistance to all four species tested. Consistently, average damage scores were significantly lower than those obtained with the susceptible checks, CIAT 0606 and BRX-44-02. Tiller ratios for the sexual hybrids were significantly higher than those of susceptible checks, suggesting that antibiosis resistance present in the hybrids protects the plants from intense insect damage, allowing the plant to grow and produce new tillers. On the contrary, susceptible plants lose tillers As in previous occasions there were significant ($P < 0.01$) negative correlations between damage scores and tiller ratios (r = -0.844 for *A. varia*, -0.887 for *Z. carbonaria*, -0.785 for *Z. pubescens*, and -0.697 for *M. trifissa*). This means that damage scores are useful in predicting tiller losses resulting from intense insect damage.

One of the commercial checks (CIAT 36087, 'Mulato 2') was resistant. Surprisingly, the commercial check CIAT 36061 ('Mulato'), which

Table 8. Damage scores and tiller ratios obtained with 22 selected sexual *Brachiaria* hybrids and checks tested for resistance to *Aeneolamia varia* (Av), *Zulia carbonaria* (Zc), *Z. pubescens* (Zp), and *Mahanarva trifissa* (Mt) under field conditions.

Genotype	Damage scores				Tiller ratios[a]			
	Av	Zc	Zp	Mt	Av	Zc	Zp	Mt
SX01/NO/0067	1.8	2.1	1.6	1.3	1.09	1.21	1.29	1.62
SX01/NO/0102	2.0	1.7	1.6	1.4	1.29	1.76	1.92	1.60
SX01/NO/0159	1.6	1.8	1.5	1.3	1.37	1.66	1.53	1.82
SX01/NO/0233	2.7	1.9	1.8	1.9	0.94	1.29	1.43	1.33
SX01NO/0263	2.0	1.9	1.6	1.2	1.34	1.48	1.55	1.76
SX01/NO/0446	1.8	1.8	1.6	1.1	1.09	1.26	1.38	1.47
SX01/NO/0878	1.9	1.8	1.8	1.7	1.38	1.44	1.65	1.71
SX01/NO/1090	1.9	1.9	1.8	1.2	1.06	1.61	1.26	1.24
SX01/NO/1175	1.8	2.0	1.8	1.3	1.12	1.26	1.35	1.43
SX01NO/1186	2.1	2.1	1.9	1.4	1.22	1.46	1.34	1.18
SX01NO/1710	1.7	2.0	1.6	1.5	1.33	1.52	1.46	2.72
SX01/NO/2017	1.9	2.0	1.6	1.3	1.48	1.39	1.72	1.83
SX01/NO/2420	1.9	1.8	1.6	1.7	1.35	1.57	1.46	1.18
SX01/NO/2619	1.7	1.7	1.7	1.2	1.11	1.46	1.69	1.49
SX01/NO/3168	1.8	1.8	1.8	1.4	1.10	1.33	1.52	1.38
SX01/NO/3178	1.9	1.8	1.8	1.3	1.12	1.41	1.34	1.47
SX01/NO/3390	2.1	2.3	1.8	1.9	0.92	1.14	1.30	1.14
SX01/NO/3439	1.9	1.9	1.6	1.0	1.25	1.61	1.58	2.22
SX01/NO/3615	1.7	1.8	1.7	1.4	1.22	1.54	1.32	1.47
SX01/NO/4506	2.1	2.1	1.7	1.6	0.92	1.17	1.29	1.44
SX01/NO/4785	1.9	2.0	1.6	1.1	1.22	1.27	1.57	1.83
SX01/NO/4861	1.7	1.7	1.7	1.6	1.28	1.64	1.62	1.74
Mean 22 hybrids	1.9b	1.9b	1.7b	1.4b	1.19b	1.43b	1.48a	1.59a
CIAT 36087	2.0	1.6	1.6	1.4	1.31	1.60	1.50	1.50
CIAT 36061	1.7	1.5	1.3	1.3	1.44	2.00	1.59	1.71
Mean commercial checks	1.8b	1.6bc	1.4c	1.3b	1.37a	1.80a	1.54	1.60a
CIAT 36062	1.6	1.3	1.4	1.1	1.64	1.92	1.69a	1.89
CIAT 6294	1.1	1.4	1.2	1.1	1.22	1.58	1.33	1.46
Mean resistant checks	1.3c	1.3c	1.3c	1.1b	1.43a	1.75a	1.51a	1.67a
CIAT 0606	4.0	3.1	2.9	3.7	0.37	0.62	0.59	0.46
BRX-44-02	4.5	3.5	3.4	4.0	0.30	0.64	0.70	0.54
Mean susceptible checks	4.2a	3.3a	3.1a	3.8a	0.33c	0.63c	0.64b	0.50b

a. Tillers per plant at the end of the infestation process/Tillers per plant at the beginning of the infestation process
Means of 10 reps per genotype per species, 4 trials in the case of *A. varia* and *Z. carbonaria*, two trials with *Z. pubescens* and one trail with *M. trifissa*. Means within a column followed by the same letter are not significantly different at the 5% level according to Scheffe's multiple range test for arbitrary comparisons. Each species analyzed separately.

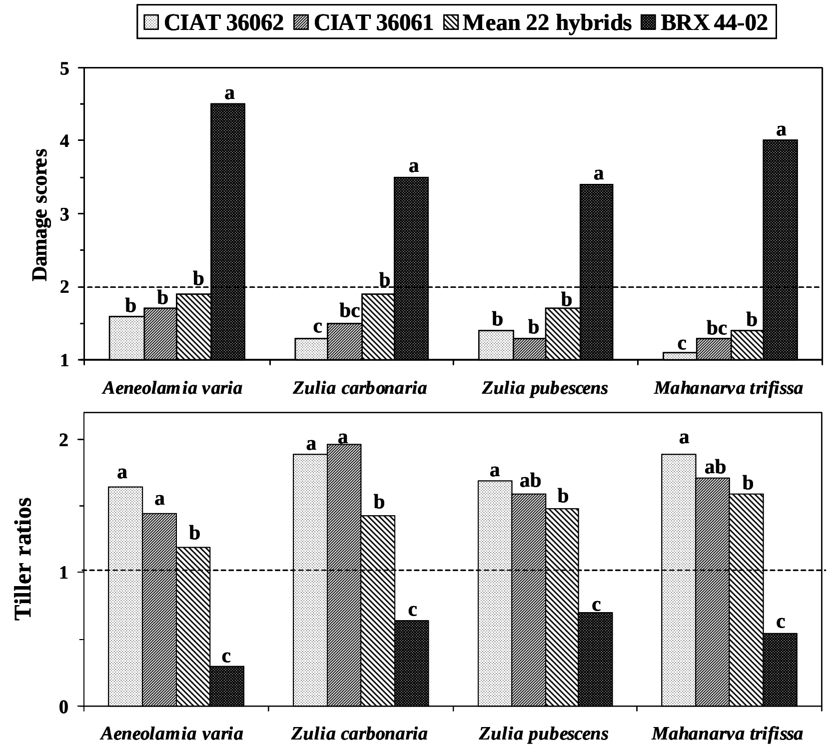


Figure 4. Resistance to four spittlebug species in selected *Brachiaria* genotypes tested under field conditions. Dotted lines represent cut-off points for resistance rating and selection. Within a given spittlebug species, bars with the same letter are not significantly different at the 5% by LSD. Each species analyzed separately.

is not antibiotic to any spittlebug species, showed a very interesting level of field tolerance both in terms of damage scores and tiller ratios (Figure 4). Damage scores obtained with the 22 sexual hybrids and assorted *Brachiaria* accessions in the greenhouse correlated very well (r = 0.76; $P < 0.01$) with damage scores recorded in the field (Figure 5). This is further proof that the technique we are using to screen for resistance in the field is a reliable one to reconfirm resistance detected under greenhouse conditions.

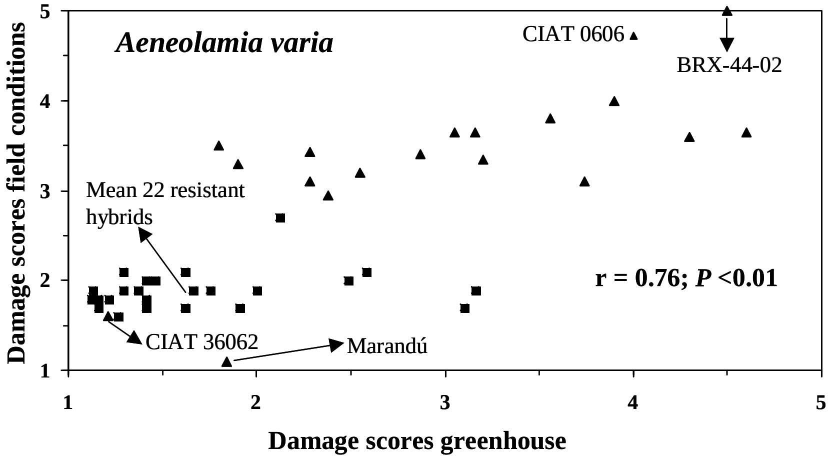


Figure 5. Damage scores obtained with selected sexual *Brachiaria* hybrids (%) and accessions (%) tested for resistance to *Aeneolamia varia* under greenhouse and field conditions.

2.3 Identify host mechanisms for spittlebug resistance in *Brachiaria*

Highlights

- The antibiosis to nymphs in the resistant *Brachiaria* hybrid CIAT 36062 causes significant sub-lethal effects on the reproductive biology of resulting adults.

- The high immature mortality and sub-lethal effects of antibiosis on resulting adults caused by the resistant *Brachiaria* hybrid CIAT 36062 have a major impact on the demography of *A. varia*.

2.3.1 Effect of host plant resistance on the demography of *Aeneolamia varia*

Contributors: P. Sotelo, G. Sotelo and C. Cardona (CIAT)

Rationale

Varying levels of antibiosis resistance to nymphs of several spittlebug species have been well characterized in a number of resistant *Brachiaria* genotypes. The effects of antibiosis on the biology of nymphs have also been studied. Not much was known about possible direct effects of antibiotic genotypes on the biology of adults. Even less was known about sub-lethal effects (i. e., reduced oviposition rates, reduced longevity, prolonged generation times, reduced rates of growth, etc.) on adults resulting from nymphs feeding on antibiotic genotypes. We initiated a series of studies aimed at measuring how antibiotic genotypes may directly or indirectly (through sub-lethal effects) affect the biology of adults of *A. varia*. We used the life-table technique, which is widely recognized as one of the most effective means of teasing apart the subtle, interrelated aspects of changes in population density. Longevity, age-specific fecundity, sex ratio and generation time can be examined and compared among treatments as they relate to the most important demographic parameter, the intrinsic rate of natural increase.

Materials and Methods

A comprehensive series of experiments aimed at determining whether antibiosis to nymphs has an adverse effect on the demography of *A. varia* were conducted. For this, 18 life tables (nine fecundity, nine complete) were constructed. Treatment combinations are shown in Table 9.

Table 9. Treatment combinations to study possible sub-lethal effects of intermediate and high levels of nymphal antibiosis on adults of *Aeneolamia varia*.

Nymphs reared on:	Resulting adults feeding on:	Null hypothesis
BRX 44-02[a]	BRX 44-02	Absolute check
BRX 44-02	CIAT 06294	A genotype that is moderately antibiotic to nymphs does not affect adults
BRX 44-02	CIAT 36062	A genotype that is highly antibiotic to nymphs does not affect adults
CIAT 06294	BRX 44-02	Intermediate antibiosis to nymphs does not affect resulting adults
CIAT 06294	CIAT 06294	Intermediate antibiosis to nymphs does not affect resulting adults even when these are feeding on a moderately antibiotic genotype
CIAT 06294	CIAT 36062	Intermediate antibiosis to nymphs does not affect resulting adults even when these are feeding on a highly antibiotic genotype
CIAT 36062	BRX 44-02	High antibiosis to nymphs does not affect resulting adults
CIAT 36062	CIAT 06294	High antibiosis to nymphs does not affect resulting adults even when these are feeding on a moderately antibiotic genotype
CIAT 36062	CIAT 36062	High antibiosis to nymphs does not affect resulting adults even when these are feeding on a highly antibiotic genotype

[a] BRX44-02 is a highly susceptible accession; CIAT 6294 (an accession) and CIAT 36062 (a resistant hybrid) possess intermediate and high levels of antibiosis resistance to nymphs of *A. varia*, respectively.

For each of these treatments we established cohorts of 105 pairs of spittlebug and the fate and reproductive rate of individuals were recorded until death occurred.

From these data the following life-table statistics were derived: net reproductive rate (R_o) [net contribution per female to the next generation]; mean generation time (T) [mean time span between the birth of individuals of a generation and that of the next generation]; doubling time (D) [time span necessary to double the initial population]; finite rate of population increase (ë) [multiplication factor of the original population at each time period]; and intrinsic rate of natural increase (r_m) [innate capacity of the population to increase in numbers]. Life-table statistics were analyzed using the SAS program based on jackknife estimates of demographic parameters. Other variables recorded were sex ratios, percentage egg fertility and adult dry weights. These data were submitted to analysis of variance and when the *F* test was significant, we performed mean separation by LSD.

Results and Discussion

A. Sub-lethal effects of resistance on adults of *Aeneolamia varia*: The impact of antibiosis to nymphs on the reproductive biology of resulting adults

Both resistant genotypes caused significant effects on the demography of *A. varia*. For simplicity, we will limit the discussion to the results obtained with the most resistant genotype, CIAT 36062. In general, rearing of nymphs of *A. varia* on the resistant genotype had a deleterious effect on the weight of resulting males and on the number and fertility of eggs laid per female (Table 10). Females feeding on the susceptible genotype BRX-44-02 weighted significantly more than those feeding on the resistant genotype. Age-specific survival and age-specific fecundity curves for *A. varia* adults are presented in Figure 6. Mean survival times for the four treatment combinations did not differ at the 5% level, meaning that there was not a major impact of nymphal antibiosis on the survival of resulting males or females. On the contrary, rearing of the insect on the resistant genotype CIAT 36062 did have a pronounced effect on the ability of resulting females to lay eggs. Independently of the food substrate used to feed the adults, females obtained from rearing the nymphs on the resistant genotype laid fewer eggs for a slightly shorter period of time, than those obtained from rearing the insect on the susceptible genotype. This can be interpreted as a sub-lethal effect of nymphal antibiosis on the reproductive capacity of the insect.

All demographic parameters of *A. varia* adults were significantly affected by the antibiotic effect of CIAT 36062 on the nymphs (Table 11).

Females originating from nymphs reared on the resistant genotype had lower net reproductive rates, lower intrinsic rates of natural increase, lower finite rates of increase and longer generation times than those reared on the susceptible genotype. We conclude that antibiosis to nymphs in the resistant *Brachiaria* hybrid CIAT 36062 causes significant sub-lethal effects on the reproductive biology of resulting adults.

Table 10. Life history parameters of *Aeneolamia varia* as affected by all possible combinations of rearing immature stages and feeding resulting adults on susceptible (BRX 44-02) or resistant (CIAT 36062) *Brachiaria* genotypes.

Treatment[a]		Adult dry weight (g x 10^{-3})		Eggs per female	Percentage egg fertility
Nymphs reared on:	Resulting adults feeding on:	Females	Males		
BRX 44-02 (S)	BRX 44-02 (S)	5.73a	3.79a	130.4ab	93.0a
BRX 44-02 (S)	CIAT 36062 (R)	4.90b	3.66ab	147.8a	92.6a
CIAT 36062 (R)	BRX 44-02 (S)	5.46ab	3.28b	108.0bc	80.6b
CIAT 36062 (R)	CIAT 36062 (R)	4.37c	3.10c	86.1c	80.4b

[a] S, susceptible; R, resistant.

Within a column, means followed by the same letter are not significantly different at the 5% level by LSD.

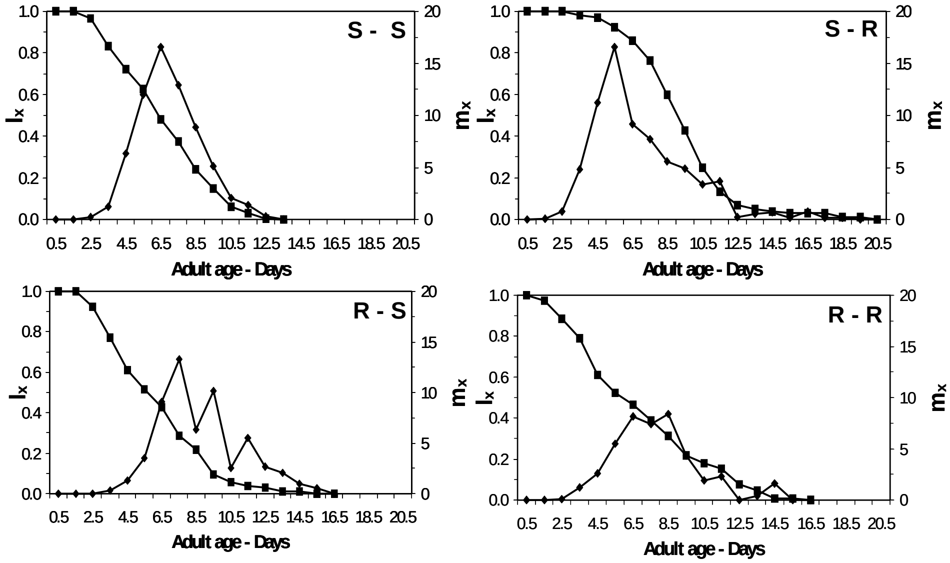


Figure 6. Age-specific survival (l_x) (%) and age-specific fecundity (m_x) (f&) curves for adults of *Aeneolamia varia* as affected by all possible combinations of food substrate for adults and nymphs. First initial in letter combinations indicates the food substrate for nymphs followed by the initial for the food substrate for resulting adults. S, susceptible genotype (BRX 44-02); R, resistant genotype (CIAT 36062).

Table 11. Fecundity life-table statistics for *Aeneolamia varia* adults as affected by all possible combinations of rearing immature stages and feeding resulting adults on susceptible (BRX 44-02) or resistant (CIAT 36062) *Brachiaria* genotypes.

Treatment[a]		Demographic parameters			
Nymphs reared on:	Resulting adults feeding on:	Net reproductive rate (R_o)	Intrinsic rate of natural increase (r_m)	Mean generation time (T)	Finite rate of increase (λ)
BRX 44-02 (S)	BRX 44-02 (S)	65.8a	0.724a	5.8b	2.06a
BRX 44-02 (S)	CIAT 36062 (R)	69.5a	0.747a	5.7b	2.11a
CIAT 36062 (R)	BRX 44-02 (S)	52.5b	0.576b	6.9a	1.77b
CIAT 36062 (R)	CIAT 36062 (R)	42.2b	0.574b	6.3a	1.80b

[a] S, susceptible; R, resistant.

Within a column, means followed by the same letter are not significantly different at the 5% level by LSD Jackknife estimates of the intrinsic rate of increase (per capita rate of population growth).

B. Total effects of resistance on the demography of *Aeneolamia varia*

To measure the total impact of antibiosis resistance on the demography of *A. varia,* we took into account the rates of immature mortality caused by both the resistant and the susceptible genotypes. Age-specific survival curves for nymphs and adults, as well as age-specific fecundity curves for *A. varia* adults are presented in Figure 7. The antibiosis to nymphs present in the resistant genotype CIAT 36062 had a significant deleterious effect on the biology of the insect, which reflected in very high levels of immature mortality. As a result, survival curves were very low as compared to those obtained with the susceptible genotype. Rearing of the insect on the resistant genotype caused a delay of about 15 days in the emergence of adults. Antibiosis also had a significant effect on the ability of resulting females to lay eggs. Independently of the food substrate used to feed the adults, females obtained from rearing the nymphs on the resistant genotype laid less eggs than those obtained from rearing the insect on the susceptible genotype.

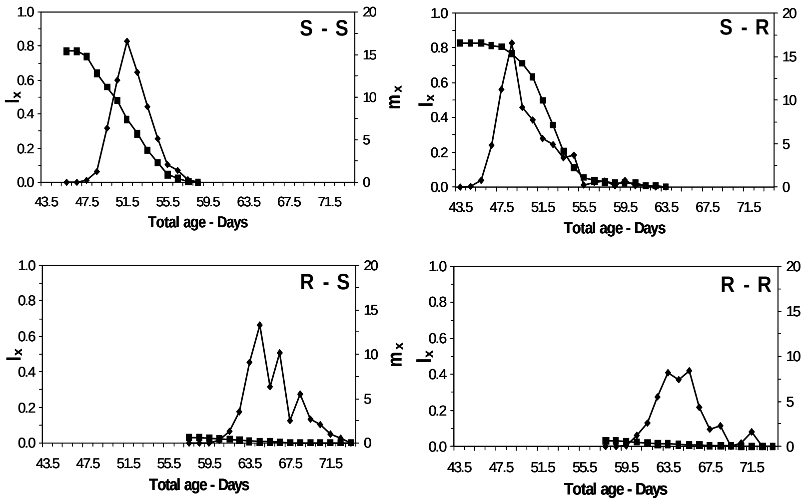


Figure 7. Age-specific survival (l_x) (%) and age-specific fecundity (m_x) (f&) curves for *Aeneolamia varia* as affected by all possible combinations of food substrate for adults and nymphs. First initial in letter combinations indicates the food substrate for nymphs followed by the initial for the food substrate for resulting adults. S, susceptible genotype (BRX 44-02); R, resistant genotype (CIAT 36062).

As a result of high immature mortality and sub-lethal effects on resulting adults, all demographic statistics of the *A. varia* population tested were significantly affected by the antibiosis present in CIAT 36062 (Table 12).

Populations derived from the resistant genotype had lower net reproductive rates, lower intrinsic rates of natural increase, lower finite rates of increase and longer generation times than those obtained from rearing the insect on the susceptible genotype. The finite rate of increase is a parameter that describes deleterious effects on a given population. It is defined as a multiplication factor of the original population at each time period.

The decimal part of the finite rate of increase corresponds to the daily rate of increase expressed as a percentage. This means that populations reared on the susceptible genotype would grow by 9.5 to 10.3% whereas those on the resistant genotype would grow by 0.4-0.8% (Table 12). We conclude that high immature mortality and sub-lethal effects of antibiosis on resulting adults caused by the resistant *Brachiaria* hybrid CIAT 36062 have a major impact on the demography of *A. varia*.

Table 12. Life-table statistics for *Aeneolamia varia* as affected by all possible combinations of rearing immature stages and feeding resulting adults on susceptible (BRX 44-02) or resistant (CIAT 36062) *Brachiaria* genotypes.

Treatment[a]		Demographic parameters			
Nymphs reared on:	Resulting adults feeding on:	Net reproductive rate (R_o)	Intrinsic rate of natural increase (r_m)	Mean generation time (T)	Finite rate of increase (λ)
BRX 44-02 (S)	BRX 44-02 (S)	50.7a	0.090b	43.3b	1.095b
BRX 44-02 (S)	CIAT 36062 (R)	57.7a	0.098a	41.5c	1.103a
CIAT 36062 (R)	BRX 44-02 (S)	1.6b	0.008c	54.4a	1.008c
CIAT 36062 (R)	CIAT 36062 (R)	1.3b	0.004c	53.8a	1.004c

[a] S, susceptible; R, resistant.

Within a column, means followed by the same letter are not significantly different at the 5% level by LSD Jackknife estimates of the intrinsic rate of increase (per capita rate of population growth).

2.4 Selection of *Brachiaria* hybrids for resistance to *Rhizoctonia* foliar blight disease

Highlights

- Developed a visual rating system for screening for resistance to Rhizoctonia foliar blight disease.
- Identified *Brachiaria* genotypes with resistance to *Rhizoctonia solani*.

2.4.1 Developing a rating scale for rhizoctonia foliar blight disease of *Brachiaria*

Contributors: Carolina Zuleta, Gustavo Segura, John Miles and Segenet Kelemu (CIAT)

Rationale

Disease management through the use of host resistance, when available, remains to be the most preferred, practical and environmentally friendly method. Differences in reaction to *Rhizoctonia solani* exist in genotypes of *Brachiaria.* The assessment of such differences requires not only a reproducible inoculation method but also a reliable rating scale. The ability to uniformly induce disease and measure resistance accurately is essential in a breeding program for developing resistant cultivars. To meet this need, we developed a simple 0-5 disease rating scale.

Materials and Methods

Brachiaria materials*:* Eighty-seven *Brachiaria* genotypes that showed varying degrees of disease reaction were used. Fully developed leaves of same maturity were detached from each of the 87 genotypes of the BR-02 series (codes given by the IP-5 forage breeding program). In addition, control resistant (CIAT 16320) and susceptible (CIAT 36061) controls were included in all the tests.

Fungal inoculum and inoculation: Isolate *R. solani* originally collected from CIAT 36061 was used as inoculum. Sclerotia of R. solani isolates originally isolated from species of Brachiaria were germinated on potato dextrose agar (PDA) at 28 °C. Mycelial discs (6 mm in diameter) were cut out of the actively growing 2 days old PDA culture for inoculation. Fully developed young leaves collected from each of the 87 *Brachiaria* genotypes were trimmed to about 12 centimeters in length. Two leaves were placed in a Petri dish of 15 cm in diameter containing Whatman #3 filter paper soaked with 5.5 ml sterile water. A mycelial disc was placed on the center of each leaf and the Petri dish was sealed with parafilm and incubated at room temperature (approximately 25 °C) on a large table in the lab. Tests were conducted in a completely randomized design with three replications.

Evaluation: The leaf samples were photographed 96 hours after inoculations. The disease areas were defined, as shown in Photo 2, using Photo Editor and Paint Brush of Microsoft. Once the images of disease areas were defined (white areas diseased, black areas healthy; see Photo 2), they were analyzed using WinRHIZO software (Regent Instruments Inc.) to determine percentage of leaf area infected.

Photo 2. Defining precise percentage of rhizoctonia foliar blight diseased leaf area in species of *Brachiaria* (shown also in AR2003).

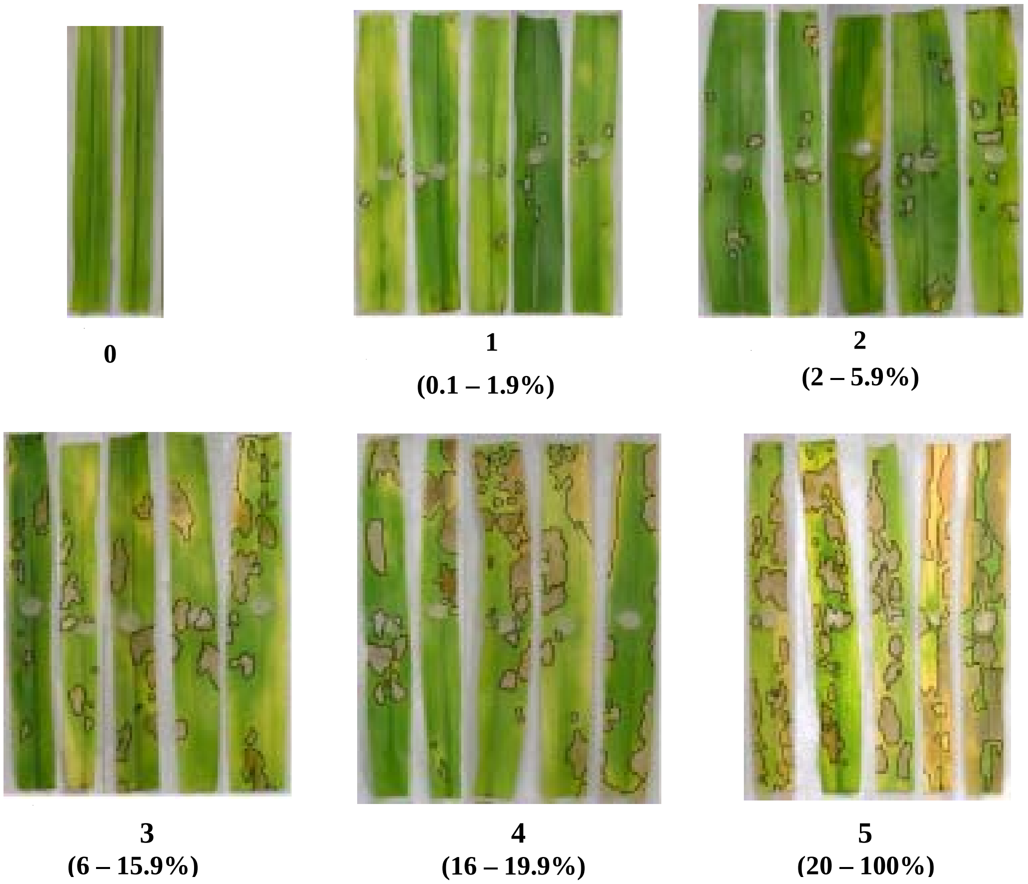


Photo 3. A visual rating system for rhizoctonia foliar blight disease in species of Brachiaria, corresponding to ranges of lesion areas, where 0 = 0%, 1 = 0.1-1.9%, 2 = 2-5.9%, 3 = 6-15.9%, 4 = 16-19.9%, and 5 = 20-100%.

Results and Discussion

After 96 hours of incubation and disease symptom development, pictures of leaves demonstrating various levels of disease reaction were taken and lesion areas calculated using the WinRHIZO software (Regent Instruments Inc.) as described under *materials and methods* section. A visual rating system corresponding to ranges of lesion areas was developed, where 0 = 0%, 1 = 0.1-1.9%, 2 = 2-5.9%, 3 = 6-15.9%, 4 = 16-19.9%, and 5 = 20-100% (Photo 3).

Because results of visual disease rating systems can vary from person to person (what seems a rating of 2 by one person may be rated as 1 by another), we further verified the accuracy of the rating scale. Two people (one who intimately worked to develop the scale, but not the second one) used the scale to evaluate the same materials independently. The two evaluators came up with similar results (with a correlation of 84%). In addition, the data independently generated by the two evaluators had correlations of 83% and 87%, respectively, with the actual precise disease lesion areas.

Using this newly developed rating scale of 0-5, 87 *Brachiaria* genotypes of the codes BR02- series and resistant control CIAT 16320 and susceptible control CIAT 36061 were evaluated. Ten of these (BR02-1995, -1811, -1919, -1667, -1917, -1717, -1805, -1973, - 465, -1968) and CIAT 16320 scored less than 3 (with BR02-1995 with the lowest score i.e. high level of resistance). The new rating scale coupled with the detached leaf inoculation method have enhanced our screening efficiency enabling us to evaluate hundred of plant materials in a relatively short period of time.

2.4.2 Screening *Brachiaria* genotypes for resistance to *Rhizoctonia* foliar blight disease

Contributors: Carolina Zuleta, Gustavo Segura, Ximena Bonilla, John Miles and Segenet Kelemu

Rationale

Rhizoctonia foliar blight, caused by *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk), is a disease of increasing importance on a number of crops. The disease is rapid and destructive when environmental conditions are particularly conducive (high relative humidity, dense foliar growth, high nitrogen fertilization, and extended wet periods).

Rhizoctonia solani complex consists of an economically important group of soil-borne pathogens that infect various plant species worldwide. The fungus is a basidiomycete and does not produce any asexual spores (conidia). Occasionally the fungus produces sexual basidiospores. The pathogen survives in soil in the form of thick-walled mycelia commonly called sclerotia, associated with organic debris. These sclerotia can germinate and produce hyphae that can infect host plants.

In *Brachiaria*, initial symptoms appear water-soaked, then darken, and finally turn to a light brown color. Lesions may coalesce quickly during periods of prolonged leaf wetness and temperatures between 21 and 32 °C resulting in entire leaf or plant death. As the plant cells die due to infection, the fungal hyphae continue to grow and colonize dead tissue, eventually forming sclerotia for another cycle of infection. The fungus infecting *Brachiaria* belongs to anastomosis group AG-1 (Kelemu et al. 1995. Tropical Grasslands 29:257-262). (There are about 12 anastomosis groups described in *R. solani*). The emergence of *R. solani* as an important *Brachiaria* pathogen is perhaps attributed to the development of high quality, high tillering Brachiarias and an increase in fertilization.

Host resistance, when available, is the most practical and cheapest means of disease control. The ability to uniformly induce disease and measure resistance accurately is crucial in a breeding program for developing resistant cultivars. The objective of this work is to identify resistant materials among *Brachiaria* genotypes

Materials and Methods

Storage of isolates: Fungal sclerotia produced either in PSY broth (20 g peptone, 20 g sucrose, 5 g yeast extract , 1 L deionized water) or on potato dextrose agar (PDA) were air-dried on sterile Whatman filter paper in a laminar flow hood. Dry sclerotia were placed in sterile glass tubes and stored at 4 °C.

Inoculum production and inoculation on detached leaves in Petri dishes*:* Sclerotia of *R. solani* isolates originally isolated from species of *Brachiaria* were germinated on potato dextrose agar (PDA) at 28 °C. Mycelial discs (6 mm in diameter) were cut out of the actively growing 2 days old PDA culture for inoculation. Fully developed young leaves were collected from each of the 745 *Brachiaria* genotypes evaluated, and trimmed to about 12 centimeters in length. Two leaves were placed in a Petri dish of 15 cm in diameter containing Whatman #3 filter paper soaked with 5.5 ml sterile water. A mycelial disc was placed on the center of each leaf and the Petri dish was sealed with parafilm and incubated at room temperature (approximately 25 °C) on a large table in the lab (Photo 4).

Inoculation on plants in greenhouse tests: Mycelial discs were cut out of actively grown PDA cultures as described above. The 73 selected materials from the detached leaf tests plus resistant and susceptible controls were used. A disc was placed in contact with each plant stem at the base and wrapped with parafilm (Photo 5). Inoculated plants were kept in the greenhouse at about 28 ^{o}C, high humidity (≥ 90 %), using a randomized complete block design and 4 replications over time. Plants were evaluated 15 days after inoculation.

Photo 4. Large -scale artificial inoculation of detached *Brachiaria* leaves placed in Petri dishes of 15 cm in diameter containing wet Whatman #3 filter paper. A mycelial disc was placed on the center of each leaf and the Petri dish was sealed with parafilm and incubated at room temperature (approximately 25 °C) on a large table in the laboratory.

Evaluation of resistance: Detached leaves were collected from 745 *Brachiaria* genotypes of SX03-series. CIAT 16320 and CIAT 36061 were included as resistant and susceptible controls, respectively, in all the experiments. The samples were randomized and experiments repeated three times over time. The plant materials were evaluated for disease reaction 96 hours after inoculation, using a 0-5 rating scale we developed, where 0 = 0%, 1 = 0.1-1.9%, 2 = 2-5.9%, 3 = 6-15.9%, 4 = 16-19.9%, 5 = 20-100% (Photo 3).

Results and Discussion

The 745 genotypes evaluated differed in their reactions to *R. solani*. Ninety percent of these (672 genotypes) exhibited severe foliar blight symptoms. Ten percent of the plant materials (73) showed average disease ratings below 3.0. Of the materials with average ratings above 3.0, 323 had average rating values between 3.0 and 3.7. The remaining 349 hybrids scored with ratings above 3.7.

To further verify the results, the 73 genotypes with ratings of less than 3.0 along with the susceptible check (CIAT 36061) and resistant control (CIAT 16320) were re-evaluated with 10 repetitions in a completely randomized design. The repetitions were increased to 10 in order to reduce the variation further. All the 73 selected materials (SX03-8, -11, -130, -203, -227, -252, -257, -258, -261, -282, -290, -293, -325, -520, -846, -864, -869, -872, -881, -946, -979, -1085, -1100, -1195, -1225, -1367, -1384, -1444, -1450, -1479, -1489, -1500, -1504, -1536, -1873, -1922, -1982, -1997, -2011, -2053, -2068, -2145, -2166, -2226, -2391, -2424, -2425, -2431, -2483, -2494, -2545, -2617, -2626, -2640, -2716, -2763, -2873, -2892, -2909, -3017, -3282, -3551, -3566, -3604, -3709, -3736, -3744, -3753, -3799, -3884, -4194, -4235, -4312), [Note: These codes of the SX03-series were assigned by the *Brachiaria* breeder (J. Miles)] consistently showed disease ratings below 3.0, indicating that the method of inoculation and evaluation as well as the plant tissue reactions to the pathogen are reproducible and consistent.

Photo 5. Reaction of *Brachiaria* plants (left, susceptible; right, resistant) to *Rhizoctonia solani*. A mycelial disc was placed in contact with each plant stem at the base and wrapped with parafilm Inoculated plants were kept in the greenhouse at about 28 ^{o}C, high relative humidity (≥ 90 %).

Inoculations conducted on complete plants under high humidity conditions in the greenhouse generally resulted in higher levels of disease reactions along with more variability than those observed on detached leaves. Among the 73 selected *Brachiaria* genotypes that were tested in the greenhouse, 19 genotypes (SX03- 2166, -1479, -252, -1982, -214, -3551, -3753, -4194, -3884, -3799, -3604, -2425, -2494, -257, -3017, -2716, -3282, -2431, -4312) had high level of resistance. Some of the genotypes that were evaluated as intermediate reaction to the disease using the detached leaf inoculation method developed high levels of disease reaction in glasshouse tests.

In conclusion, the detached leaf inoculation and evaluation method is very useful in screening large numbers of genotypes very quickly at a lower labor and material cost. The technique enabled us to quickly discard large number of susceptible materials and select a few materials for greenhouse tests using complete plants. Therefore, a combination of the two methods allowed us to screen large number of materials for resistance to the disease rapidly and reliably.

2.5 Elucidate the role of endophytes in tropical grasses

Highlights

- The presence of the fungal endophyte *Acremonium implicatum* in some hybrids of *Brachiaria* confirmed.
- *Acremonium implicatum* transformed with green fluorescent protein gene for the first time.
- A methanol extract compound from endophyte-infected *Brachiaria* plants showed strong fungal inhibition activity.
- Found that endophyte infection had no significant effect on dry season performance after 2 years of establishment of two accessions of *Brachiaria brizantha*.

2.5.1 PCR analysis and screening of *Brachiaria* genotypes for endophytes

Contributors: Tomoko Sakai (JICA), Martin Rodriguez and Segenet Kelemu (CIAT)

Rationale

The fungus *Acremonium implicatum* can develop an endophytic association with *Brachiaria* species that is asymptomatic. Endophyte-plant associations are widespread in nature. Grasses harboring nonpathogenic and intercellular endophytes benefit in various ways such as having enhanced drought tolerance and vigor, and increased resistance to attacks from insect pests and pathogens. Systemic infections of grasses in the *Festuca* and *Lolium* genera with *Neotyphodium* species and the corresponding teleomorph *Epichlöe* species have been studied extensively. These fungi are often transmitted by seed to the next host generation.

Many *Brachiaria* species are apomictic and reproduce asexually through seed. Apomictic reproduction permits plant genotypes to breed true through seed. This type of reproduction also offers advantages to research on endophyte-host associations and use. If the specific endophyte in question were seed-transmitted, almost all seeds of an endophyte-infected apomictic plant would contain the endophyte, as well as being genetically identical to each other. We had demonstrated for the first time that *A. implicatum* is transmitted through seeds of *Brachiaria* grasses (Dongyi and Kelemu, 2004, Plant Disease, *in press*). A previously developed polymerase chain reaction (PCR)-based method used a pair of endophyte-specific primers to amplify a diagnostic, 500-bp, DNA fragment for a rapid and reliable detection of *A. implicatum* in tissues of *Brachiaria* grasses (Kelemu et al., 2003, Mol. Plant Pathol. 4:115-118). We used this method to examine tissues of *Brachiaria* hybrids for the presence or absence of *A. implicatum*.

Materials and Methods

Plant materials: Forty-one genotypes of *Brachiaria* (CIAT 16320, SX99/0711, SX99/0574, SX99/0275, BR99NO/4015, FM9503/S046/024, BR99NO/4132, SX99/3564, SX99/2514, SX99/2857, SX99/1370, SX99/2606, SX99/1260, CIAT 606, SX99/2173, SX99/3690, SX99/2621, SX99/2280, SX99/0835, SX99/0823, SX99/0246, SX99/2663, SX99/3770, SX99/1630, SX99/2030, SX99/1513, FM9201/1873, BRUZ4X/4402, SX99/0731, SX99/0029, CIAT 6294, SX99/1616, SX99/3488, SX99/2115, SX99/1145, SX99/2162, SX99/0236, SX99/2341, SX99/2927, SX99/1833, SX99/1622) provided by the forage-breeding program of CIAT were tested. These were maintained either in the greenhouse or planted in field plots.

DNA isolation: Leaf blades were collected from *Brachiaria* hybrids and known endophyte-infected or endophyte-free plants and macerated separately in liquid nitrogen for genomic DNA isolation. DNA was extracted using an improved CTAB (Hexadecyltrimethylammonium bromide) method. Extraction buffer [2% CTAB, 100mM Tris-HCl (pH8.0), 20mM EDTA (pH8.0), 1.4mM NaCl and 1% PVP40) and 1/50 volume of Rnase A (10 mg / ml)] was added to macerated plant tissue, and incubated at 65 ^{o}C for 30 min. An equal volume of Chloroform: Isoamylalcohol (24:1) was added and mixed well by vortexing. The mixture was then centrifuged at 13,200 rpm for 10 min. The supernatant was transferred to a new tube. About 0.8 ~ 1 volume of ice-cold isopropanol was added to the supernatant and kept at room temperature for 15 minutes to precipitate the DNA. DNA pellet was generated after centrifugation at 13,200 rpm for 20 min. The pellet was washed with 70 % ethanol and re-suspended in 50-ìl Tris-EDTA (TE) buffer (10 mM Tris-HCl (pH7.5), 1 mM EDTA (pH8.0)).

PCR analysis: PCR was carried out using the specific primer pairs P1 (5'-TTCGAATGATAAGGCAGATC-3 and P4 (5'-ACGCATCCACTGTATGCTAC-3'). The PCR reaction volume was 20-ìl, and composition was as follow: 1x PCR buffer (QIAGEN); 3mM $MgCl_2$; 0.26mM each deoxynucleotide triphosphate (dNTPs); 1.25ìM each olygonucleotide primer; 1 units Taq DNA polymerase (Invitrogen) and 30ng template DNA. Amplification cycles were programmed in a Programmable Thermal Controller (MJ Research, Inc.) as follows: step 1, 94°C 3min; step 2, 94°C 30 sec; step 3, 53°C 40 seconds; step 4, 72°C for 45 seconds; step 5, go to step 2 for 35 cycles; then 72°C 4 min. The amplification products were separated by electrophoresis in a 1.2% agarose gel (Invitrogen), stained with ethidium bromide and photographed under UV lighting.

Results and Discussion

The presence of *A. implicatum* in *Brachiaria* leaf tissues was determined by the presence of a diagnostic 500-bp amplification product (eg. Photo 6). Plants belonging to the genotypes SX99/1833, SX99/0236, SX99/2162, SX99/2663, SX99/0823, SX99/1260 tested negative for the presence of the 500-bp amplified DNA fragment and thus, for the endophyte *A. implicatum*, whereas all remaining genotypes examined tested positive. Genotypes CIAT 16320, CIAT 606, SX99/0275, SX99/2857, SX99/2173, SX99/2621, and SX99/2280 showed strong associations with the endophyte under field and greenhouse conditions. These results indicate that the *A. implicatum-Brachiaria* associations are widespread naturally. Many of the genotypes were maintained both in the greenhouse and in the field. Some genotypes tested either positive or

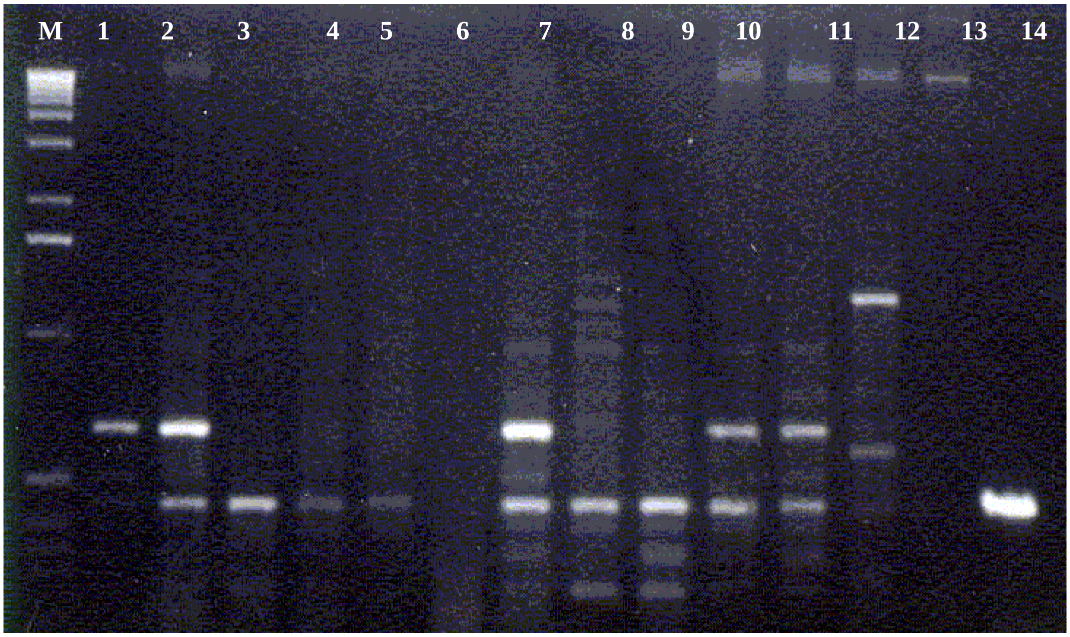


Photo 6. Specific detection of *Acremonium implicatum* in hybrids of *Brachiaria*.
Genomic DNA isolated from leaves of *Brachiaria* plants maintained in the greenhouse. Lanes 1-12, CIAT 06294 (05), BR99NO/4132 (06), SX99/0574(09), SX99/2030 (20), SX99/1513 (23), SX99/0236 (27), SX99/3564 (28), CIAT 16320 (32), BR99NO/0415 (37), FM9503/S046/024 (45), SX99/3770 (51) and SX99/2162 (17), respectively. Lane 13 is template DNA from *Rhizoctonia solani* as negative control. Lane 14 is positive control, genomic DNA from endophytic fungus *Acremonium implicatum*. Lane M = 1Kb-ladder.

negative depending on whether they were maintained in the field or in the greenhouse. Although we don't know the exact reasons for some of these inconsistencies, we suspect that environmental conditions may play a role in the amount and distribution of endophyte mycelia within the plant tissues.

2.5.2 Analysis of alkaloid profile results (collaboration with University of Kentucky, USA)

Contributors: Christopher Schardl (Department of Plant Pathology, University of Kentucky), Gustavo Segura, Ximena Bonilla and S. Kelemu (CIAT)

Rationale

Endophytic fungi associated with temperate grasses are known to produce a variety of alkaloids. The endophytes *Neotyphodium coenophialum* (previously known as *Acremonium coenophialum*), from tall fescue (*Festuca arundinacea* Schreb.), and *N. lolii* (*A. lolii*), from perennial ryegrass (*Lolium perenne* L.), have been a major focus of research associated with toxicity in livestock grazing on endophyte-containing pastures. In most cases, the toxic syndromes have been attributed to alkaloids in endophyte-containing tall fescue and perennial ryegrass.

Although several livestock disorders have been associated with cattle grazing some *Brachiaria* pastures, the exact cause or causes of these syndromes are unknown. These syndromes include conditions called "fallen cow" which affects cows in late gestation or early lactation and grazing *B. decumbens* cv. Basilisk; and "swollen face", which occurs in horses grazing

B. humidicola pastures in the Brazilian *Cerrados*. Deaths have been reported in Brazil, Colombia and Venezuela. In Brazil, livestock grazing *B. decumbens* suffer a hepatic disorder that affects young animals, causing weight lose and even death. In the State of Mato Grosso, Brazil, sheep grazing *B. decumbens* have suffered poisoning with symptoms such as swelling and dermatitis of the face, ears and eyelids, and blindness (de Lemos et al. 1996. Cienc. Rur. Santa Maria 24:109-113.). Although the causes of these disorders are still unknown, endophytic fungi may possibly play a role.

A collaboration has been set up with Dr. Christopher Schardl, Department of Plant Pathology, University of Kentucky, to determine the alkaloid profile in *Acremonium*/*Brachiaria* associations, with funds made available by USAID-University linkage programs. Some preliminary results are presented here below.

Materials and Methods

Plant samples: Freeze-dried plant samples were prepared from endophyte-infected and endophyte-free *Brachiaria* grasses in our laboratory (CIAT) and were sent to Kentucky. The endophyte-containing grasses were infected with the fungal endophyte *Acremonium implicatum*.

Fungal isolates: In addition eleven isolates of the endophyte *A. implicatum* were sent to Kentucky in pure cultures. Test fungi to be used and described in a paper by Abou-Jawdaw et al. (2002, Journal of Agricultural and Food Chemistry, 50, 3208-3213) are *Botrytis cinerea, Alternaria solani, Penicillium* sp., *Cladosporium* sp., *Fusarium oxysporum* f. sp. *melonis*, *Verticillium dahlia*, *Phytoptora infestans, Colletotrichum* , and *Rhizoctonia solani*. Since the *Dreschleria* complex was shown to be affected by the endophyte *A. implicatum* (Kelemu et al.. 2001. An endophyte of the tropical forage grass *Brachiaria brizantha*: isolating, identifying, and characterizing the fungus, and determining its antimycotic properties. Canadian Journal of Microbiology 47:55-62), this would need to be included in our analysis as well as some of the listed ones from above.

Sample analysis: Samples of freeze-dried plant materials were extracted by two different methods; soxhlet, which is basically a warm methanol reflux, and by shaking in methanol at room temperature. In pilot experiments, other solvents were also used on the soxhlet. Solvents are of analytical grade from Aldrich.

Assay for fungicidal activity: Extracts from freeze-dried plant samples were applied on to Petri dishes containing potato dextrose agar (PDA) (extract from endophyte-free (E-) plant samples to half of a plate and extract from endophyte-containing (E+) sample to the other half). The plates were then sprayed with spores of *Colletotrichum graminicola* and incubated for fungal growth. The dark color indicates abundant growth of *C. graminicola* (Photo 7).

Chemical compound analysis: Extracts exhibiting fungal growth inhibition will further be looked at with thin layer chromatography (TLC) autobiography tests. For the TLC autobiography (Hamburger and Cordell, 1987, Journal of Natural Products, 1, 19-22), aliquots of these extracts will

Photo 7. Growth inhibition of *Colletotrichum graminicola* by methanol extract compounds from freeze-dried leaf tissues of *Brachiaria* containing the endophyte *Acremonium implicatum*. The dark color indicates abundant growth of *C. graminicola*, whereas the clear transparent zone shows growth inhibition.

be spotted onto a silica gel plate in duplicate and run using different solvent systems, initially chloroform-methanol (9:1) as reported by Abou-Jawdaw et al. (2002, Journal of Agricultural and Food Chemistry, 50, 3208-3213). The duplicate plate is for later isolation of compounds from the corresponding areas on test plates that exhibited inhibition of growth.

These plates will be dried at 37°C for one hour to remove solvent residue before a suspension of spores of the different test organisms in potato dextrose broth is sprayed onto the plates and control plates (fresh silica gel plates and silica gel plates run in solvent). These plates are grown in a humidity chamber for several days to observe zones of inhibition. The corresponding areas on the unsprayed plates are then scraped and analyzed via GC-MS or LC-MC to identify compounds of interest.

Results and Discussion

In pilot experiments, other solvents were used on the soxhlet, but only the methanol extraction method gave compounds that resulted in growth inhibition of test fungi. The difference in growth on the plates between inhibited and uninhibited is quite dramatic (Photo 7).

The main problem is that some of the samples that were sent as endophyte-free also showed inhibition. One explanation is that there was a possible error in labels being switched (particularly E- and E+) either in the labs or at the APHIS offices that received the samples for examinations before forwarding them to the University of Kentucky. Alternatively, perhaps we are just seeing differences in plant genotypes, with the endophyte playing no role.

However, the results from similarly numbered plants are not always consistent either when we disregard the presence or absence of endophytes, but only consider the plant genotype. Thus, the most plausible explanation is that some sample labels had been switched somewhere along the line.

We plan to resend samples and repeat the tests.

2.5.3 Endophytes as gene delivery system

Contributors: Javier Abello and Segenet Kelemu (CIAT)

Rationale

Ever since D. C. Prasher cloned a cDNA for the green fluorescent protein (GFP) gene from the jellyfish *Aequorea victoria* in 1992 (Gene 111:229-233), this gene or its derivatives have been expressed in a wide array of organisms including plants and microbes. The protein (27 kDa) absorbs light at maxima of 395 and 475 nm and emits at a maximum of 508 nm. The protein is a success as a reporter because it requires only UV or blue light and oxygen, but requires no cofactors or substrates as many other reporters do for visualization. GFP-expressing transgenic fungal isolates have been used for analysis of *in planta* fungal development and interaction.

Enhanced color variants [ECFP (cyan), EGFP (green), EYFP (yellow)] have been generated through mutagenesis and these are some of the most widely used reporters in biological research. They can be used as tags to track proteins in living cells, as reporters to monitor promoter activity, and as labels to visualize specific tissues, whole cells or subcellular organelles. They are useful for monitoring gene expression and protein localization.

This work describes the transformation and expression of the GFP gene in an isolate of *Acremonium implicatum*, and endophyte in species of *Brachiaria*, in order to study endophyte-*Brachiaria* interactions as well as to examine the potential use of endophytes as gene delivery systems. The practical implication of seed transmission of endophytes in *Brachiaria* is significant: once associated with the plant, the fungus can perpetuate itself through seed, especially in apomictic genotypes of *Brachiaria*, for as long as seed storage conditions do not diminish the survival of the fungus. Several *Brachiaria* hybrids obtained from CIAT's forage breeding program were shown to harbor *A. implicatum*. We may be able to exploit this association and its high seed transmission [Dongyi, H. and Kelemu, S. 2004. *Acremonium implicatum*, a seed-transmitted endophytic fungus in *Brachiaria* grasses. *Plant Disease* (in press)] by using a transgenic *A. implicatum* as a vehicle for production and delivery of gene products of agronomic interest into the host plant to enhance protective benefits and other traits, and thus improve livestock production. In addition, we want to exploit the qualities of GFP as a reporter and study the interactions between *A. implicatum* and its host *Brachiaria*.

In this study, we used two GFP expression vectors, pWGFP20 and pC74, to transform *A. implicatum*. In this initial phase of the work our immediate objective is to develop an efficient transformation protocol for *A. implicatum*.

Materials and Methods

GFP expression vectors: Vectors pWGFP20 and pCT74 were kindly provided by Dr. Jin-Rong Xu of Purdue University, USA, and Dr. Lynda Ciuffetti of Oregon State University, respectively. Vector pWGFP-20 has successfully been used to transform *Magnaporthe grisea*. Vector pCT74 is a GFP expression vector for filamentous fungi that expresses the protein from a *Tox*A promoter of *P. tritici-repentis*. It has successfully been used in *Fusarium sambucinum*, *Botrytis cinerea*, *Pyrenophora tritici-repentis*, *Alternaria alternata*, *Cochliobolus sativus*, *Sclerotinia sclerotiorum*, *Colletotrichum magna*, and *Verticillium dahliae*.

Bacterial transformation and plasmid extraction: Vector DNA was sent either on Whatman 3 MM paper (pCT74) or in a tube (pWGFP20). To recover the plasmid from the filter paper we cut out a marked circle (where the plasmid DNA was placed), placed it in an eppendorf tube, added 50 µl of 10 mM Tris, pH 7.6, vortexed and let it rehydrate for about 5 minutes. After brief centrifugation, the supernatant liquid was used to transform competent bacterial cells. Plasmid DNA was isolated using standard alkaline lysis procedure (Sambrook et al., 1989).

Electroporation- mediated transformation of Agrobacterium tumefaciens LBA 4404: Bacterial cells were cultured in Luria agar supplemented with 0.1% glucose and incubated for 2 days at 28 °C. Cells from a single colony were transferred to Luria broth medium (100 ml) supplemented with 0.1 % glucose and grown at 28 °C in a shaker at 250 rpm till it reached an optical density of $OD_{600}=1.2$. Once it reached this growth density, the culture was kept in ice. Bacterial cells were collected by centrifugation at 4000 rpm for 10 minutes. The cells were resuspended in 750 µl of 10 % glycerol to generate a concentration of about 7×10^9 bacterial colony forming units/ ml. Aliquots of this suspension were distributed in eppendorf tubes containing 45 µl and stored at -80 °C. For electroporation, a mixture of bacterial cells (40 µl) and 1 µl of plasmid DNA (concentration 200-500 ng/µl) were placed in 0-2 cm electrode gap Gene Pulser® cuvettes (Bio-Rad), and pulsed at various voltages. Electroporated cells were added to SOC medium [Tryptone (Difco) 20 g, Yeast-extract (Difco) 5 g, NaCl 0.5 g. This was autoclaved and when the medium cooled, filter sterilized solution of 1 M $MgCl_2$ (10 ml), 1 M Mg SO_4 (10 ml), 1 M glucose 20 ml was added] and incubated for 1.5 hr at 28 °C in a shaker at 250 rpm. The cells were then plated on agar medium containing ampicillin (50 µg/ml).

Agrobacterium tumefaciens–mediated transformation of Acremonium implicatum: Monoconidial cultures of *A. implicatum* were cultured on fresh potato dextrose agar at 28 °C for conidial production. Conidia were collected by adding sterile water containing 0.15 M NaCl to the plates and gently rubbing with a sterile glass rod. The conidial suspension was filtered through sterile Whatman # 2 to remove large particles (mycelia).

A. tumefaciens LBA4404 containing either pWGFP20 or pCT74 was cultured in minimal medium [2.05g K_2HPO_4, 1.45 g KH_2PO_4, 0.15 g NaCl, 0.50 g $MgSO_4.7H_2O$, 0.1g $CaCl_2$. $6H_2O$, 0.0025g $FeSO_4.7H_2O$, 0.5g $(NH_4)_2SO_4$, 2.0 g glucose, 1 L water] supplemented with ampicillin (50 µg/ml) at 28°C for 2 days. The *A. tumefaciens* were diluted to optical density (OD_{600}) of 0.45, 0.54 and 0.5 in induction medium [40 mM 2-(N-Morpholino) ethanesulfonic acid (MES) pH 5.3, 10 mM glucose, 0.5 % (v/v) glycerol] with or without 200µM of acetosyringone. The cells were grown for 6 hours. The various concentrations of cells were mixed with an equal volume of each of the three conidial concentrations ($3.25x10^6$, $12.5x10^6$ y $18.1x10^6$). A sample of each mix (200 µl per plate) was placed on nitrocellulose filter (0.45-µm pore size) and plated on cocultivation medium (which is the same as the induction medium, but it contains half of the glucose) with or without 200 µM of acetosyringone. After incubation for 2 days at 25°C, the filter was transferred to minimal medium containing hygromycin B (50 µg/ml) as a selection agent for transformants and cefotaxime (200µM) to eliminate *A. tumefaciens* cells. Individual transformants were transferred to small centrifuge tubes of 2 ml volume containing 1.5 ml of YMG agar medium (4 g glucose, 10 g malt extract, 4 g yeast extract, 10 g agar, 1L) supplemented with hygromycin B (50 µg/ml) and incubated until conidia formation. Conidia of individual transformants were suspended in sterile distilled water and plated on potato dextrose agar containing hygromycin B (50 µg/ml). Monoconidial cultures of each transformant were created. Conidia from these cultures were stored in 20% glycerol at –80 °C for further analysis.

Results and Discussion

The transformation of *A .tumefaciens* through electroporation generated few successful transformants on the selection medium. The vectors were isolated from the transformants to further confirm their presence. *A. implicatum* is a slow growing fungus. Although a recommended concentration of cefotaxime (200 µM) was used to inhibit the growth of *A. tumefaciens*, there was still enough bacterial growth to further impede the growth of *A. implicatum*. However, we managed to have fungal transformants free of *Agrobacterium* although the green fluorescence emitting appears to be weak with those containing the vector pWGFP20 (Photo 8). Work is in progress to get a more pronounced emission with those containing pCT74.

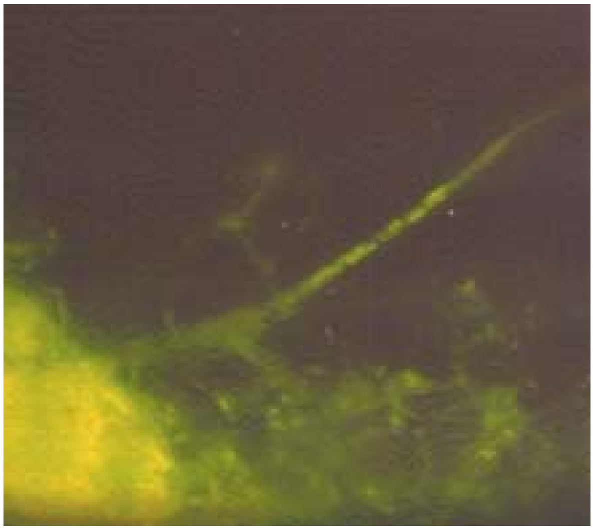


Photo 8. *Acremonium implicatum* transformed with vector pWGFP20 containing green fluorescent protein.

2.5.4 Drought tolerance in endophyte-infected plants under field conditions

Contributors: S. Kelemu, X. Bonilla, Carolina Zuleta, C. Plazas, J. Ricaurte, R. García and I. M. Rao (CIAT)

Rationale

We showed before with soil-grown plants in the greenhouse that endophyte-infected plants under severe drought stress conditions could maintain better leaf expansion and produce significantly greater leaf biomass (IP-5 Annual Report, 1999;

2000). In 2002, to validate the findings from the greenhouse study, we initiated a field study in the Llanos of Colombia to quantify the impact of endophytes in improving drought tolerance and persistence in *Brachiaria.* Last year, we reported preliminary results from this field trial that indicated that endophyte infection could improve dry season performance by improving the uptake of nutrients by two accessions of *Brachiaria brizantha.* This year, we conducted further field evaluation to confirm the role of endophytes in improving dry season tolerance of *Brachiaria* grasses.

Materials and Methods

A field trial was established at Matazul farm in May of 2002. The trial included 2 accessions of *Brachiaria brizantha* (CIAT 6780 and CIAT 26110). Plantlets were propagated from the original mother plant containing the endophyte *Acremonium implicatum* (J. Gilman and E. V. Abbott) W. Gams. Half of these plants were treated with the fungicide (Folicur) to eliminate the endophyte (method described in Kelemu et al. 2001. Canadian Journal of Microbiology 47:55-62) while the remaining half was left untreated. The trial was planted as a randomized block in split-plot arrangement with the presence or absence of endophytes as main plots and two accessions as subplots with 3 replications. Each plot included 3 rows with 8 plants per row (24 plants/plot). The plot size was 5 x 1.5 m. The trial was established with low levels of initial fertilizer application (kg/ha: 20 P, 20 K, 33 Ca, 14 Mg, 10 S) that are recommended for establishment of grass alone pastures. A number of plant attributes including forage yield, green leaf production, dry matter distribution and green forage nutrient composition, leaf and stem total nonstructural carbohydrate (TNC) content, leaf and stem ash (mineral) content, and shoot nutrient uptake were measured at the end of wet season (November 2003) and dry season (March 2004).

Results and Discussion

At 18 months after establishment, i.e., at the end of rainy season, the endophyte-infected plants (E+) showed greater values of stem biomass (Figure 8a) in both accessions of *Brachiaria brizantha* (CIAT 6780 and CIAT 26110). Between these two accessions, CIAT 6780 was more productive. Endophyte infection had no significant effects on leaf and stem nutrient composition at the end of rainy season (Table 13). Leaf TNC content was not affected by endophyte infection but stem TNC content was slightly higher in CIAT 26110 than in CIAT 6780 but endophyte infection had no effect. Leaf and stem ash contents were also not affected by endophyte infection.

Results on shoot nutrient uptake showed that uptake of N, P, K, Ca and Mg was greater with endophyte-infected (E+) plants than that of uninfected (E-) plants (Figure 8b).

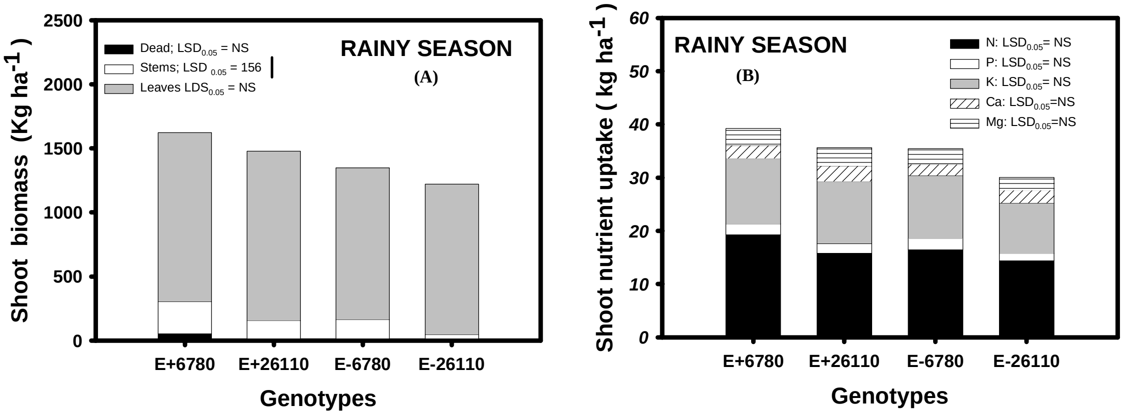


Figure 8. Influence of endophyte infection on (a) shoot (green leaves + stem) biomass production and (b) nutrient uptake of two accessions of *Brachiaria brizantha* CIAT 6780 and CIAT 26110 at 18 months after establishment (at the end of rainy season). E+ are endophyte-infected plants while E- are endophyte-free plants.

Table 13. Influence of endophyte infection on stem biomass and leaf and stem nutrient content of two accessions of *Brachiaria brizantha* CIAT 6780 and CIAT 26110 at 18 months after establishment (at the end of rainy season). E+ are endophyte-infected plants while E- are endophyte-free plants.

Genotype	CIAT 6780		CIAT 26110		
Endophyte infection	E +	E -	E +	E -	$LSD_{0.05}$
Stem biomass (t/ha)	248	164	156	48	156
Leaf N content (%)	1.33	1.32	1.14	1.18	NS
Leaf P content (%)	0.13	0.15	0.13	0.11	NS
Leaf K content (%)	0.74	0.86	0.79	0.77	NS
Leaf Ca content (%)	0.16	0.16	0.21	0.20	NS
Leaf Mg content (%)	0.20	0.21	0.25	0.20	NS
Leaf TNC content (mg g^{-1})	164	160	141	164	NS
Leaf ash content (%)	5.77	5.61	6.58	5.45	1.05
Stem N content (%)	0.84	0.81	0.71	0.69	NS
Stem P content (%)	0.11	0.12	0.10	0.09	NS
Stem K content (%)	0.99	1.23	1.23	0.99	NS
Stem Ca content (%)	0.11	0.12	0.11	0.12	NS
Stem Mg content (%)	0.18	0.22	0.21	0.21	NS
Stem TNC content (mg g^{-1})	117	125	123.1	136	16.8
Stem ash content (%)	5.42	6.03	6.36	5.47	0.52

Table 14. Influence of endophyte infection on (a) shoot biomass production and (b) nutrient uptake of two accessions of *Brachiaria brizantha* CIAT 6780 and CIAT 26110 at 22 months after establishment (at the end of dry season). E+ are endophyte-infected plants while E- are endophyte-free plants.

Genotype	CIAT 6780		CIAT 26110		
Endophyte infection	E +	E -	E +	E -	$LSD_{0.05}$
Leaf N content (%)	1.60	1.55	1.29	1.39	NS
Leaf P content (%)	0.15	0.14	0.11	0.10	0.05
Leaf K content (%)	1.18	1.02	0.95	0.85	0.14
Leaf Ca content (%)	0.31	0.25	0.19	0.26	0.04
Leaf Mg content (%)	0.27	0.30	0.16	0.21	NS
Leaf TNC content (mg g^{-1})	121	123	134	108	NS
Leaf ash content (%)	7.47	7.07	5.49	5.73	NS
Stem N content (%)	1.15	1.24	0.97	0.93	NS
Stem P content (%)	0.13	0.11	0.09	0.08	NS
Stem K content (%)	1.08	1.00	1.06	1.02	NS
Stem Ca content (%)	0.21	0.18	0.11	0.14	NS
Stem Mg content (%)	0.28	0.29	0.18	0.22	NS
Stem TNC content (mg g^{-1})	109	115	112	88.3	NS
Stem ash content (%)	7	6.78	6.12	6.2	0.86

In contrast to the results at the end of rainy season, at 22 months after establishment, i.e., at the end of dry season, the endophyte infected plants showed no significant increase in either green leaf or stem biomass (Figure 9a) in both accessions of *Brachiaria brizantha* (CIAT 6780 and CIAT 26110). This observation is not consistent with the results from the greenhouse study where the benefits of endophyte infection were noted under severe drought stress. But endophyte free plants showed the tendency of greater dead biomass. Leaf nutrient composition indicated that leaf K was significantly greater in endophyte infected CIAT 6780 than the endophyte free plants (Table 14). Leaf and stem TNC content were significantly greater in the endophyte infected plants of CIAT 26110 than the endophyte free plants (Table 14). Results on nutrient uptake at the end of dry season also

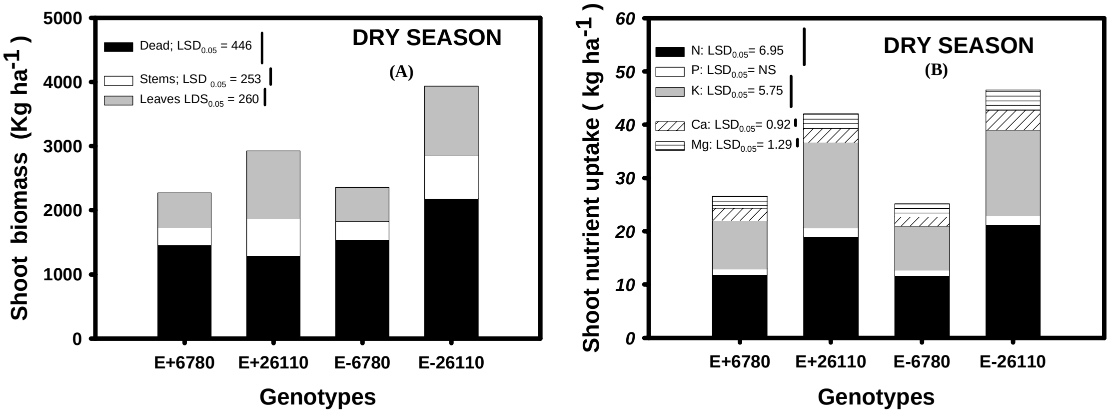


Figure 9. Influence of endophyte infection on (a) shoot biomass production and (b) nutrient uptake of two accessions of *Brachiaria brizantha* CIAT 6780 and CIAT 26110 at 22 months after establishment (at the end of dry season). E+ are endophyte-infected plants while E- are endophyte-free plants.

showed that the uptake of N, P and K was not significantly different between the endophyte-infected plants and the endophyte free plants (Figure 9b). This on-going field study indicated beneficial effects of endophyte infection on drought tolerance in the first year and almost no effect in the second year. This might be due to the regrowth of the endophyte in the endophyte free plants. There is evidence for this possibility in greenhouse grown plants. We are testing the persistence of the endophytes in these plants in order to see if the lack of effects is due to the disappearance of the endophytes in the infected plants.

2.6 Association of bacteria with *Brachiaria* genotypes

Highlight

- A mutant strain was marked for resistance to rifampicin from the original bacterial strain isolated from *Brachiaria* CIAT 36062 and that tested positive for *nif*H gene sequences that was introduced into *Brachiaria* CIAT 36061.

2.6.1 Characterization of endophytic bacteria isolated from *Brachiaria*

Contributors: Raul Sedano, Carolina Zuleta and Segenet Kelemu (CIAT)

Rationale

Endophytic bacteria that reside in plant tissues without causing any visible harm to the plant have been isolated from surface-sterilized *Brachiaria* tissues. The primary point of entry for many of these bacteria is the root zone, although aerial plant parts like flowers and stems may also be entries. Once inside a plant, they may either be localized at the point of entry or spread throughout. Bacterial endophytes have been reported to live within cells, in the intercellular spaces or in the vascular system of various plants.

Many plant-growth-promoting bacteria (PGPB) that include a diverse group of soil bacteria are thought to stimulate plant growth by various mechanisms such as plant protection against pathogens, providing plants with fixed nitrogen, plant hormones, or solubilized iron from the soil. Three bacterial isolates 01-36062-R2, 02-36062-H4, and 03-36062-V2 were isolated from *Brachiaria* CIAT 36062 in roots, leaves and stems, respectively, that tested positive for sequences of the nifH gene (the gene that encodes nitrogenase reductase) [IP-5 Annual Report 2003]. As stated in the 20003 Annual Report, the fatty acid analysis matched the bacterium coded 03-36062-V2 with *Flavimonas oryzihabitans* at 0.887 similarity index. *F. oryzihabitans* has been described as a plant growth promoting rhizobacterium in graminicolous plants. The analysis matched isolate 02-36062-H4 with *Agrobacterium rubi* at 0.845 similarity index. The name *A. rubi* is synonymous to *Rhizobium rubi*. The match using fatty acid data of the isolate 01-36062-R2, however, was not conclusive, matching it with *Leclercia adecarboxylata*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*, at 0.879, 0.841, and 0.820 similarity index, respectively. Of these, *E. cloacae* has been described as one of the dominant endophytic bacteria isolated from citrus plants (Araújo et al., 2002. Applied and Environmental Microbiology 68:4906-4914). A nitrogen-fixing endophytic strain of *Klebsiella pneumoniae* (Kp342) has been ioslted from a nitrogen-efficent line of maize (Chelius and Triplett, 2000. Applied and Environmental Microbiology 66:783-787). This strain has been described to have a very broad host range and is capable of colonizing the interior of many plants with fewer than 10 cells in the inoculum (Dong et al., 2003. Plant Soil 257:49-59). More recently, endophytic colonization and nitrogen fixation in wheat were demonstrated upon inoculation with *Klebsiella pneumoniae* strain Kp342 (Iniguez et al., 2004. Molecular Plant Microbe Interaction 17:1078-1085).

The objective of this study is to isolate and characterize bacterial strains with potential plant growth promoting properties.

Materials and methods

Marking bacterial cells for antibiotic resistance: An overnight culture of bacterial cells (strain 01-36062-R2) were plated on nutrient agar medium containing rifampicin (50 μg/ml) and incubated at 28 C for 48 h. Individual colonies which appeared on the medium were transferred on to freshly prepared medium containing the same concentration of rifampicin. The growing colonies were transferred on to freshly prepared medium containing 50μg/ml rifampicin. The same process was repeated until a mutant bacterium was obtained which grew the same on rifampicin-containing medium at a concentration of 50 μg/ml as well as on medium containing no rifampicin. Dilution series of the mutant bacterium were plated on nutrient agar medium with and without rifampicin to determine that the mutant grew equally on both media. Growth curves of the mutant bacterium were also conducted in nutrient broth media with and without rifampicin. The growth of the rifampicin-resistant mutant strain was determined in comparison with that of the original isolate from which the mutant was derived. In addition, nested PCR amplifications were conducted on both the marked mutant and the original bacterial isolate to make sure that the *nifH* gene sequences can be detected in both.

Nested PCR Amplification: Three primers were used, which were originally designed by Zehr and McReynolds (1989. Appl. Environ. Microbiol. 55: 2522-2526) and Ueda, et al. (1995. J. Bacteriol. 177: 1414-1417) to amplify fragments of *nifH* genes. Amplification steps described by Widmer et al (1999. Applied and Environmental Microbiology 65:374-380) were adopted.

Inoculation of Brachiaria: Rifampicin–resistant bacterial cells were used to inoculate *Brachiaria* CIAT 36061 (Mulato) plants. Plants were inoculated with the rifampicin-resistant mutant either by injection or immersing the roots in bacterial suspension for 48 hours. In the root immersion inoculation method, roots of 19 plants were washed with sterile distilled water. The plants were then transferred to a suspension of bacterial cells (rifampicin resistant mutant derived from strain 01-36062-R2 at a concentration of optical density (OD_{600} = 0.1). The plants were removed from the suspension two days later and rinsed with sterile distilled water. They were then planted in sterile soil. Mutant bacterial cell suspensions (200 µL of OD_{600} = 0.1) were injected into stems and leaves of each plant (a total of 19 plants). Control plants were treated with sterile distilled water.

Evaluation of inoculated plants: root tissues or above ground tissues were macerated in 200 µL sterile distilled water, 3, 7, 12, 21, 26 and 75 days after inoculations. A dilution series was made and plated on nutrient agar containing rifampicin at a concentration of 50 µg/mL. The colonies were counted after 48 hours incubation at 28 C. The values were used to calculate the approximate number of colony forming units per tissue sample. The isolated bacteria were also tested with nested PCR to determine whether they were positive for *nif*H gene sequences.

Results and discussion

Bacterial cells were re-isolated from inoculated plants (hybrid *Brachiaria* CIAT 36061; cv. Mulato) on nutrient agar medium containing rifampicin (50 µg/mL) as late as 75 days after inoculation. No bacterial cells that can grow on the rifampicin-containing media were isolated from control plants. These rifampicin-resistant bacterial colonies also tested positive for sequences of *nif*H (Photo 9). In summary, we took a bacterial strain isolated from what appeared to be a nitrogen-efficient *Brachiaria* CIAT 36062 and that tested positive for *nif*H sequences, marked it for resistance to the antibiotic rifampicin at 50 µg/mL, introduced it to

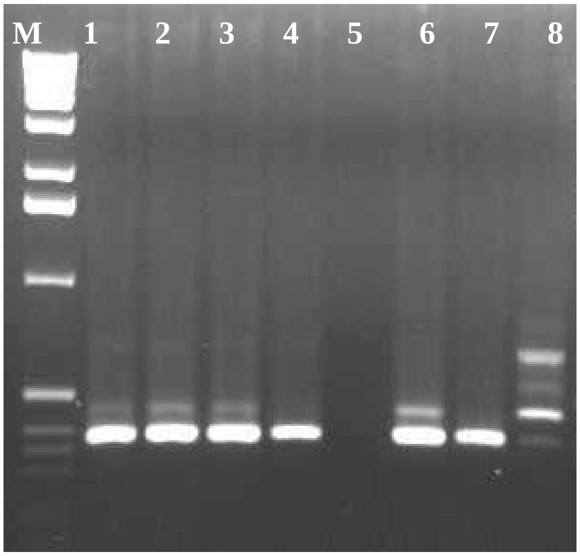


Photo 9. Nested PCR analysis of rifampicin-resistant bacterial colonies reisolated from artificially inoculated CIAT 36061 (cv. Mulato) plants, for *nif*H gene sequences. Lanes 1-4 are rifampicin-resistant independent bacterial colonies re-isolated from Mulato plants 23 days after inoculations (lanes 1 and 2 DNA of bacteria isolated from leaves; lanes 3 and 4 isolated from roots). Lane 5 is negative control. Lanes 6 and 7 are positive control and DNA from original positive bacterium from which rifampicin resistant mutants were derived, respectively. Lane 8 is a randomly picked bacterium. Lane M is size marker.

cv. Mulato and re-isolated it 75 days after inoculations, indicating that the bacterium was established in artificially inoculated plants of cv. Mulato.

The rifampicin-resistant bacterial cells were isolated both from leaves and roots of inoculated plants, although the bacterial population is not evenly distributed in all the leaves. The bacterium was consistently re-isolated from root tissues. Although both inoculation methods (plant injections or root immersions) gave successful results, more bacterial cells were recovered following root immersion inoculations and thus, root immersion method is a better inoculation method. Not surprisingly, the bacterial cell population in *Brachiaria* was much lower than that observed for a plant pathogenic bacterium such as *Xanthomonas campestris* pv. *graminis*.

By introducing this strain into cv. Mulato, we can now study the effect of the bacterial strain on the growth of Mulato plants in comparison with genetically identical plants without the bacterial strain.

2.6.2 Creation of plants with and without endophytic bacteria

Contributors: Raul Sedano and Segenet Kelemu (CIAT)

Rationale

An important component of the rhizosphere (the soil portion that forms the complex habitat of plant roots) is the actively growing microbial community that flourishes due to organic nutrients in root exudates. The microbes that colonize the rhizosphere affect plant and rot biology in relation to nutrition, development and general health. Microorganisms that colonize the rhizosphere can have various effects on plants.

Some are pathogens that have deleterious effects, whereas others have beneficial ones. Among the beneficial effects, nitrogen fixation is conducted by phylogenetically diverse groups of prokaryotes. Evidence on nitrogen fixation by rhizospheric bacteria associated with grass roots was first presented in the tropics (Döbereiner and Day, 1976. Associated symbioses in tropical grasses: characterization of microorganisms and nirogen-fixing sites. In: W. E. Newton and C. J. Nyman ed. Proc. of the 1st International Symposium on nitrogen fixation, Washington State Univ. Press, Pullman, pp. 518-538). Plant growth promoting beneficial microbes have various mechanisms of actions including production of secondary metabolites such as antibiotics, phosphate solubilization, and siderophore production.

Brachiaria CIAT 36062 stays green under both greenhouse and field conditions even under limited nitrogen conditions and it appears to be nitrogen-efficient. A bacterium was consistently isolated from *Brachiaria* CIAT 36062. The bacterial strain tested positive for *nif*H gene (encodes nitrogenase reductase) sequences in a nested PCR test.

In this study, we attempted to create genetically identical clones of CIAT 36062 with and without the bacterium in order to study the effect of the bacterium on plant growth and development.

Materials and Methods

Treatment with antibiotics*:* In an attempt to eradicate bacteria associated with *Brachiaria* CIAT 36062, plant tillers were treated with 5 different antibiotics; ampicillin (30 µg/mL), kanamycin (15 µg/mL), streptomycin (15 µg/mL), nalidixic acid (20 µg/mL), and chloromphenico (20 µg/mL) either sequentially or in combinations by immersing the roots in antibiotic solutions in beakers.

Tillers were soaked in antibiotic solutions for a total of 3 days, i.e. either sequentially treating the tillers in individual antibiotic solutions approximately every 14 hours or for 3 days in solution mix of all three antibiotics. Tillers were then rinsed with sterile distilled water and planted in sterile sand subsequently receiving nutrient solutions.

Heat treatment: Roots of young *Brachiaria* tillers were soaked in hot water at 75 °C for 1.5, 3, 6,10, 20 minutes in an attempt to remove bacterial association from plants and create genetically identical clones with and without bacteria. The tillers were subsequently transferred to sterile sand receiving nutrient solutions.

DNA isolations: DNA extraction was done using a modified protocol based on combinations of standard methods, which includes growing bacterial cells in liquid media LB (tryptone 10g, yeast extract 5g, NaCl 10g, 10 ml of 20% glucose in 1 L of distilled water), treatment of cells with a mixture of lysozyme (10 mg.ml in 25 mM Tris-Hcl, ph 8.0) and RNase A solution, and extraction of DNA with STEP (0.5% SDS, 50 mM Tris-HCl 7.5, 40 mM EDTA, proteinase K to a final concentration of 2mg/ml added just before use. The method involves cleaning with phenol-chloroform and chloroform/isoamyl alcohol and precipitation with ethanol. Wizard Genomic DNA purification Kit (Promega, Madison, WI) was used to extract DNA from *Brachiaria* tissues. The quality of DNA was checked on 1 % agarose gel.

PCR amplifications: Three primers were used, which were originally designed by Zehr and McReynolds (1989. Use of degenerate oligonucleotides for amplification of the *nifH* gene from the marine cyanobacterium *Trichodesmium thiebautii*. Appl. Environ. Microbiol. 55: 2522-2526) and Ueda, et al. (1995. Remarkable N2- fixing bacterial diversity detected in rice roots by molecular evolutionary análysis of *nifH* gene sequences. J. Bacteriol. 177: 1414-1417), to amplify fragments of *nifH* genes.

Amplification steps described by Widmer et al (1999. Analysis of *nifH* gene pool complexity in soil and litter at a douglas fir forest site in the Oregon cascade mountain range. Applied and Environmental Microbiology 65:374-380) were adopted.

Staining roots method: In order to sample new root tissues for presence or absence of bacteria after antibiotic treatment, roots were stained with safranine (0.5%) dissolved in water. Right after antibiotic treatments were completed, the roots were immersed in the stain for an hour, rinsed with sterile distilled water and transferred to pots containing sterile sand. Plants received nutrient solution.

Results and Discussion

None of the antibiotic treatments nor the heat treatment procedures successfully eliminated bacteria that tested positive for *nif*H gene sequences. Samples were collected 4 weeks after treatments. Template DNA isolated from root tissues grown after antibiotic treatment (white colored ones, see Photo 10) as well as from those treated with antibiotics (red colored ones, Photo 10) all produced the diagnostic 370

Photo 10. Roots of *Brachiaria* stained with 0.5% safranine solution dissolved in water. Roots stained red were treated with antibiotics; new root growth after treatment is seen as whitish.

bp amplification nested PCR product (data not shown).

New root growth did not seem to be affected by safranine staining procedure. High plant mortality resulted after the heat treatment procedure.

We are currently studying the possibility of eliminating endophytic bacteria associated with *Brachiaria* CIAT 36062 through tissue culture and regeneration procedures. If successful, we will be able to generate genetically identical clones with or without bacteria and study subsequent plant development.

2.7 Antifungal proteins in tropical forages

Highlight

- Fungal pathogens in beans and *Brachiaria* controlled in direct spray applications of an antifungal protein isolated from *Clitoria ternatea* seeds

2.7.1 An antifungal protein from *Clitoria* and its direct application in disease control

Contributors: Gustavo Segura, George Mahuku and Segenet Kelemu (CIAT)

Rationale

When wounded, or attacked by harmful microorganisms, plants can trigger an array of potent defense mechanisms, one of which is to synthesize proteins, peptides and low-molecular-weight compounds that have antimicrobial effects. Antimicrobial proteins and peptides are widely distributed in nature and are synthesized not only by plants but also by bacteria, insects, fungi and mammals.

Seeds use strategies to germinate and survive in soils that are inhabited by a wide range of microfauna and microflora. Various antifungal and/or antibacterial proteins such as chitinases, â-glucanases, thionins, ribosome-inactivating proteins and permatins have been detected in seeds.

Antimicrobial proteins and peptides have been isolated and characterized from seeds of maize (*Zea mays* L.), radish (*Raphanus sativus* L.) and various other plants. They are believed to play a role in plant defense because of their strong antimicrobial activity *in vitro*. This belief is further supported by their ability to confer resistance (to pathogens) in transgenic plants containing genes that encode them. The list of antifungal proteins from various organisms is long, with new ones continuously being discovered.

Other plant-derived proteins have insecticidal properties that can, for example, protect seeds from attack by larvae of various bruchids and inhibit the growth and development of *Helicoverpa punctigera* (Wallengren) larvae.

Of particular interest are plant-derived proteins called cyclotides (circular proteins in which the N and C termini are linked via a peptide bond), which have antimicrobial and insecticidal properties. Ocatin, a protein isolated from the Andean tuber crop oca (*Oxalis tuberosa* Mol.), is reported to have antibacterial and antifungal effects.

We reported the isolation, purification and characterization of a protein with an antifungal, antibacterial and insecticidal properties from seeds of *Clitoria ternatea* (L.) [*IP-5 AR 2003*; Kelemu, S., Cardona, C., and Segura, G. 2004. *Plant Physiology and Biochemistry* (in press); Kelemu, S., Cardona, C., and Segura, G. 2004. *Phytopathology* 94:S50). In this study, we examined the direct applications of the crude preparations of the protein in disease control on various plants.

Materials and Methods

Protein extractions: Large quantities of seeds *Clitoria ternatea* CIAT 20692 were produced on field plots at CIAT headquarters in Palmira, Colombia, for protein extractions. Seeds (100 g) of *C. ternatea* CIAT 20692 were surface-sterilized in 3.25% NaOCl solution for 10 min, then in 70% ethanol (3 min), and rinsed 6 times with sterile distilled water. The seeds were left in sterile distilled water overnight to facilitate maceration. The imbibed seeds were then macerated in 1,000 mL of sterile distilled water with a sterilized mortar and pestle. The macerated solution was filtered through several layers of cheesecloth to get rid of the seed debris. The filtrate was then centrifuged at 4 °C in tubes (50 mL) at 13 000 × g for an hour. To remove any potential microbe associated with the filtrate, the supernatant was filtered through 0.22-µm-pore-size cellulose acetate membranes. Aliquots (7 mL) of the filtrate were distributed in 15-mL tubes and lyophilized for 7 hours.
The lyophilized samples were stored at - 20 °C for further use.

This lyophilized crude protein extracts were re-suspended in sterile distilled water (10 % of the original volume) to conduct the antifungal activity bioassay on plants.

Inoculum: A highly virulent isolate (PG8 HND) of the pathogen *Phaeoisariopsis griseola*, causal agent of angular leaf spot, was grown on V8 agar at 24°C for 12 days. Conidia were collected and suspended in sterile distilled water at a concentration of 2 x 10^4 conidia per mL. This inoculum was used on *Phaseolus vulgaris* variety Sprite bean plants. This variety is one of the most susceptible varieties to *P. griseola*.

Rhizoctonia solani, causal agent of foliar blight disease of *Brachiaria* was used as inoculum. Inoculum production and inoculation methods were as described in section 2.4.2.

Plant inoculations and treatment applications: Seeds of a highly susceptible bean variety (Sprite) were planted in pots in the greenhouse at CIAT headquarters. Seventeen-day old bean plants (15 plants per treatment) were first sprayed with, either the fungicide benlate (500 ìg/ml), crude antifungal protein preparation, or sterile water. Two hours later all the plants were inoculated with *P. griseola* conidia at a concentration of 2 x 10^4 conidia per mL. The inoculated plants were placed in a humidity chamber for 4 days. They were then transferred to the greenhouse for development and symptom expression. Treatments with crude antifungal protein, benlate or sterile water continued every 2 days.

Disease evaluations were conducted 7, 10, 12, 14 and 17 days after inoculation.

Brachiaria CIAT 36061, which is highly susceptible to *R. solani*, was used in this test. Fully developed detached leaves were used for inoculations as described in Materials and Methods under section 2.4.2.

Treatment of *P. griseola* conidia with the protein Finotin: A conidial suspension of 2 x 10^4 conidia per mL was diluted 10^{-2} - 10^{-5} and examined under a microscope in order to determine the right concentration with evenly distributed and separated conidia. The dilution 10^{-4} was chosen for treatment with the antifungal protein Finotin and further examination. Twenty-µl of this conidial suspension was placed on a slide and subsequently covered with a thin layer of potato dextrose agar medium. A 200-µl crude protein preparation (the same concentration that was used to spray onto bean plants) was applied on the agar. Control slides had water instead of the antifungal protein. These were placed in Petri dishes containing wet filter paper and incubated at room temperature. Pictures of conidia were taken under the microscope at 0, 2, 7, 24, 32 and 96 hours to observe the development of individual conidia.

Results and Discussion

Effect of antifungal protein Finotin on bean angular leaf spot: The crude protein extract from seeds of *C. ternatea* CIAT 20692 showed antifungal activity in vitro on the pathogen *P. griseola* (data not shown). Plants treated with the crude antifungal protein preparation consistently developed fewer angular leaf spot disease lesions than the control plants that were treated with sterile distilled water (Photo 11; Figure 10). Had a purified protein been used to control the disease on bean plants, the level of disease control would perhaps have been even higher.

It is interesting to note that even a crude protein extract sprayed directly onto plants provided protection against the disease. Experiments are currently in progress to control tomato diseases under field conditions and natural infections using crude protein preparations. Tomatoes are generally susceptible to a number of diseases.

The purpose of these experiments is to develop a simple disease control strategy for small producers using this antifungal protein.

Effect of antifungal protein on conidia of *P. griseola* in vitro: Conidia treated with crude protein or sterile water along with a layer of potato dextrose agar as described earlier reacted differently. Conidia failed to germinate in the presence of the antifungal protein Finotin 96 hours after treatment, whereas those treated with sterile water germinated and converted into mycelia (Photo 12).

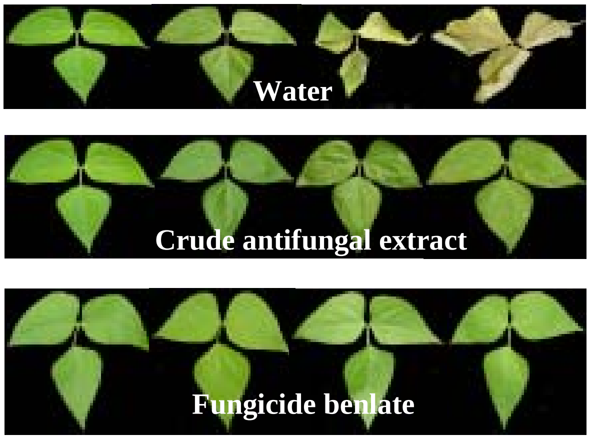


Photo 11. Treatment of bean plants with crude protein extract from seeds of *C. ternatea* CIAT 20692 against the fungal pathogen *P. griseola*, causal agent of angular leaf spot disease. Plants treated with the crude antifungal protein preparation consistently developed fewer angular leaf spot disease lesions than the control plants that were treated with sterile distilled water.

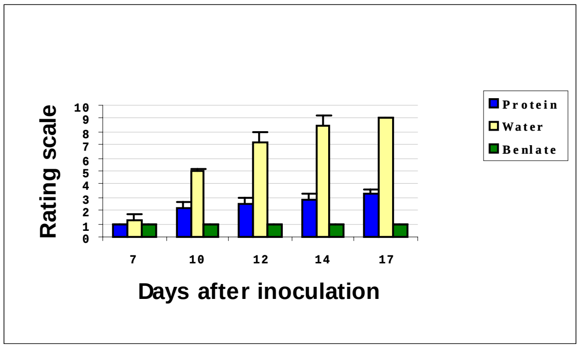


Figure 10. Angular leaf spot disease development in artificially inoculated bean plants following treatment with crude antifungal protein preparations isolated from *C. ternatea* CIAT 20692, the fungicide benlate, or water control.

Thus, one of the mechanisms of pathogen control by the protein may be by preventing fungal spore germination. However, a more detailed work on plant tissue as well as on culture is needed to fully establish the mechanism of disease control by this protein.

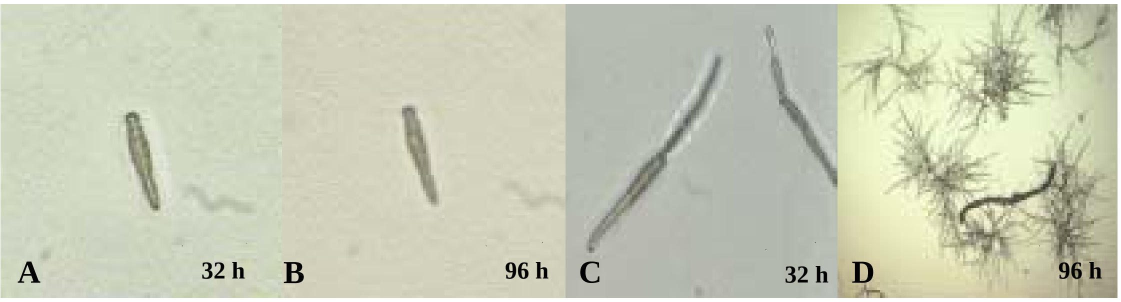


Photo 12. Treatment of *Phaeoisariopsis griseola* conidia with the antifungal protein Finotin. Conidia failed to germinate in the presence of the antifungal protein Finotin 32 and 96 hours (A and B) after treatment, whereas those treated with sterile water germinated and grew into mycelia (C and D) .

Effect of antifungal protein Finotin on Brachiaria foliar blight: Detached *Brachiaria* CIAT 36061 leaves sprayed with crude protein extract and subsequently inoculated with *R. solani* mycelial discs developed very limited or no foliar blight lesions, whereas control leaves developed severe lesions (Photo13) when evaluated 72 hours after inoculation. Although we don't intend to use the antifungal protein for direct applications in the control of foliar blight disease in *Brachiaria* (it will be impractical to do so), we are exploring the possibilities of transforming some of the endophytic microbes associated with *Brachiaria* with the gene encoding the protein.

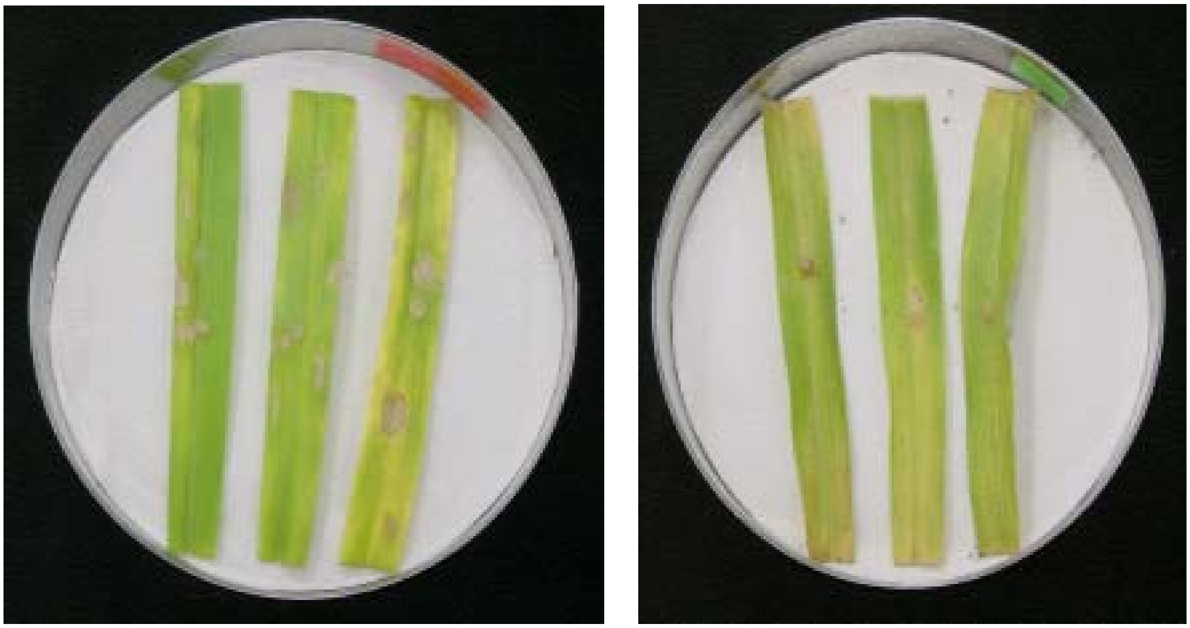


Photo 13. Detached *Brachiaria* CIAT 36061 leaves sprayed with crude antifungal protein isolated from the forage legume *Clitoria ternatea* (L.), and subsequently inoculated with *Rhizoctonia. solani* mycelial discs (right) and control leaves (left).

Output 3: Grass and legumes genotypes with superior adaptation to edaphic and climatic constrains are developed

3.1 Genotypes of *Brachiaria* and *Arachis* with adaptation to edaphic factors

Highlights

- Collaborative research conducted with Hokkaido University in Japan generated the first evidence based on ^{27}Al NMR analysis that organic acids within root tissue help detoxify aluminum in non-accumulator species such as in the *Brachiaria* hybrid cv Mulato.

- Showed that the high level of aluminum resistance in signal grass (*Brachiaria decumbens*) is part of a generic resistance mechanism that is effective against trivalent cations in general.

- Showed that hematoxylin staining could be employed as a quick selection criterion to discard Al-sensitive genotypes in the *Brachiaria* breeding program, because most of the Al accumulates in the external layer of root meristems and should be readily stainable with hematoxylin in intact root apices.

- Constructed a genetic linkage map for aluminum resistance in *Brachiaria*, evaluated 50% of the polymorphic SSRs and AFLPs in the F1 cross of *B. decumbens* x *B. ruziziensis* and found preliminary associations between markers SSRs and AFLPs and three phenotype root traits of aluminum resistance.

- Using microarray technology, the 3'-UTR sequences of candidate genes associated with aluminum (Al) resistance in *Brachiaria decumbens* were identified by comparing expression levels between genotypes and treatments.

- Using screening procedure to evaluate aluminum resistance, 3 sexual hybrids (SX03NO/0846, SX03NO/2367, SX03NO/0881) were identified with greater level of Al resistance than that of the sexual parent. One of the apomictic hybrids (BR02NO1372) was outstanding in its level of Al resistance and this hybrid is also resistant to spittlebug

- Showed that the *Brachiaria* hybrid, FM9503-S046-024 (Mulato 2) performed well into the third year after establishment in the Llanos and its superior performance at 30 months after establishment was associated with its ability to acquire greater amounts of nutrients, particularly Ca and Mg from low fertility soil

- Results from a 4-year field study in the Piedmont showed that the *Arachis pintoi* accessions CIAT 18744, 18751 and 22159 were superior to the commercial cultivar (CIAT 17434) in terms of persistence with low amounts of initial fertilizer application.

- Collaborative research conducted in Goettingen, Germany, under controlled environmental conditions in a growth chamber, showed that the *Arachis pintoi* accessions CIAT 18744 was more efficient acquiring P from less available P-pools in a low-P oxisol than the commercial cultivar, CIAT 17434. This high P efficiency and the increase of P uptake were found to be due to a high P influx. The activity of acid phosphatase on root surface and exudation of organic acids (lactic and acetic) did not contribute to this increase in P influx.

3.1.1 Edaphic adaptation of *Brachiaria*

Contributors: I. M. Rao, P. Wenzl, J. W. Miles, J. Tohme, M. Ishitani, J. Ricaurte, R. García, A. L. Chaves, M. E. Recio, A. Arango, D. F. Cortes, G. Gallego, E. Gaitán, and C. Plazas (CIAT), T. Watanabe, H. Yano, M. Osaki (Hokkaido University, Japan)

Previous research on mechanisms of adaptation of *Brachiaria* species to acid soil stress factors indicated that *Brachiaria decumbens* cv. Basilisk is highly resistant to toxic levels of Al and low supply of P. Based on this knowledge, rapid and reliable screening procedure to evaluate Al resistance was developed to improve the efficiency of the on-going *Brachiaria* breeding program. The use of improved screening methods and identification of QTLs and candidate genes responsible for Al resistance and adaptation to low P supply will contribute toward development of superior genotypes that combine several desirable traits to improve pasture productivity on acid, infertile soils and to combat pasture degradation.

3.1.1.1 Investigating physiological and genetic aspects of aluminum resistance in *Brachiaria*

As part of the restricted core project funded by BMZ-GTZ of Germany, we continued our efforts to investigate physiological and genetic aspects of aluminum resistance in *Brachiaria*.

A) Aluminum resistance coincides with differential resistance to trivalent lanthanide cations in *Brachiaria*

Contributors: **P. Wenzl (CAMBIA, Australia), A.L. Chaves, M.E. Recio, J. Tohme and I.M. Rao (CIAT)**

Rationale

Signalgrass (*Brachiaria decumbens*) has evolved a highly effective Al-resistance mechanism that does not appear to rely on chelation of Al ions with organic-acid anions. Electrical charges at the external surface of root cells generate an electrostatic potential that modulates cell-surface ion activities and hence ion uptake and intoxication. We hypothesized that the superior Al resistance of signalgrass compared to closely related ruzigrass (*B. ruziziensis*) could be due to a less negative surface potential at root cells that are critical to root growth and elongation. We tested this hypothesis by investigating whether Al resistance of signalgrass was associated with superior resistance to other cations toxicants and greater susceptibility to anionic toxicants.

Materials and Methods

Seeds were germinated in continuously aerated 200 µM $CaCl_2$ (pH 4.20) for 3-4 days. Homogeneous seedlings were selected and their root lengths were recorded. The seedlings were then transferred to continuously aerated treatments solutions in the greenhouse (toxicant + 200 µM $CaCl_2$, pH 4.20; see left panel below). After three days root length were measured again. Each experiment comprised six toxicant levels plus the toxicant-free basal solution. Three independent experiments were performed for each toxicant. The concentration of a toxicant that inhibited relative root elongation (RRE) by 50 % (C_{50}) was determined for each of the two species after fitting a Weibull function to the pooled data from the three replicate experiments by using the Marquardt-Levenberg algorithm (right panel above). The SE of C_{50} was computed based on error propagation rules. The C_{50} values of the two species for a particular toxicant were considered to be different if their 95 % confidence intervals did not overlap.

Results and Discussion

The superior Al resistance of signalgrass compared to ruzigrass was associated with greater resistance to all the trivalent lanthanide cations tested (Figure 11). If a lower root cell surface negativity was the cause for the greater lanthanide resistance of signalgrass, signalgrass should be more resistant to other cationic toxicants and more sensitive to anionic toxicants. The two species, however, were equally sensitive to the majority of divalent and monovalent cations (Figure 10b) and most anions (Figure 10c). Apart from lanthanides and Cd^{2+}, signalgrass was more resistant than ruzigrass only for those inorganic toxicants that are in redox equilibrium with a trivalent cationic form: $Fe^{2+} \leftrightarrow Fe^{3+}$, $Co^{2+} \leftrightarrow Co^{3+}$, $Cr_2O_7^{2"} \leftrightarrow Cr^{3+}$. An organic trivalent cation (spermidine^{3+}), by contrast, was equally toxic to both species.

These results suggest that Al resistance in signalgrass is part of a more generic resistance mechanism that is effective against trivalent cations in general, a finding that confirms the unique physiological basis of Al resistance in signalgrass.

The pattern of resistance to cationic and anionic toxicants, however, is not consistent with the idea that a less negative root cell surface potential confers resistance to cationic toxicants as a result of electrostatic interactions, that is, solely based on the charge but not the structural properties of a toxicant. The cross-resistance of signalgrass to Al and other, mostly trivalent inorganic cations may instead be based on interspecific differences in critical cellular sites to which trivalent cations such as Al^{3+} and lanthanides bind. More work is required to elucidate the nature of these sites and to develop biochemically-based strategies to isolate the underlying genes. It may be possible to use lanthanide cations as proxies for Al to circumvent some of the problems and ambiguities caused by the difficulties to predict Al speciation.

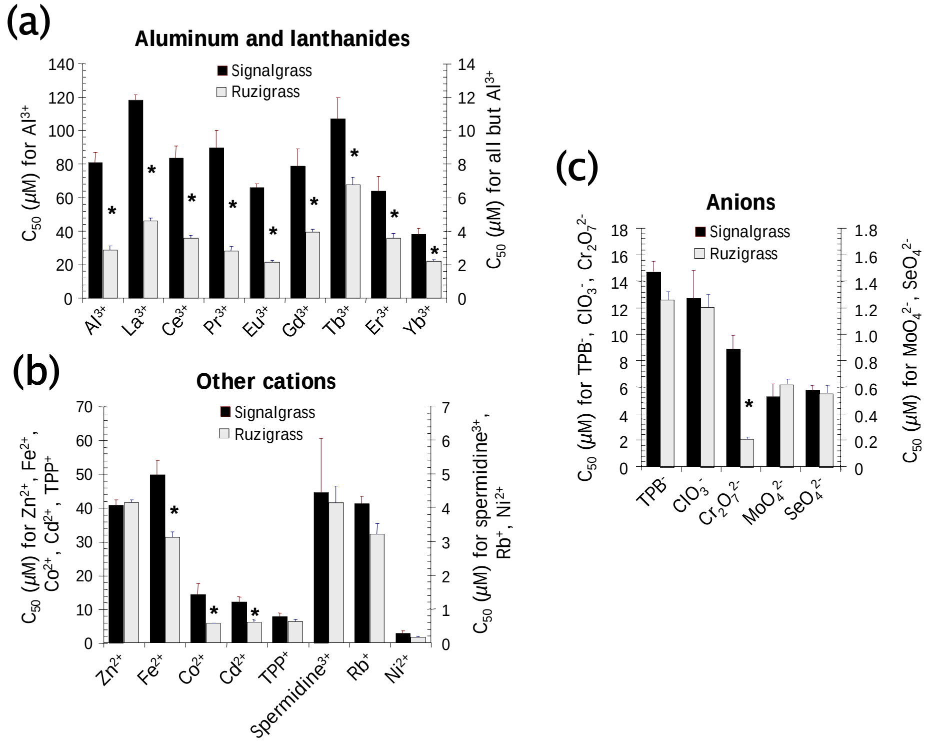


Figure 11. Concentrations of cationic and anionic toxicants required for inhibiting root elongation of signalgrass and ruzigrass by 50 % (C_{50}).

B) Accumulation of callose and aluminum in root tips of *Brachiaria* spp.

Contributors: A. Arango, P. Wenzl, I.M. Rao, and J. Tohme (CIAT)

Rationale

The effects of aluminum (Al) toxicity on callose accumulation were evaluated in root tips of *Brachiaria* populations previously evaluated for physiological and genetics response (IP-5 Annual report 2002, 2003). *Brachiaria decumbens* (Al resistant), *B. ruziziensis* (Al sensitive) and two contrasting *B. ruziziensis* x *B. decumbens* hybrids were evaluated after 3, 12 and 21 days of Al treatment (200 µM $AlCl_3$).

Materials and Methods

Rooted stem cuttings of *B. decumbens*, *B. ruziziensis* and two contrasting hybrids were cultivated as described previously (IP-5 Annual Report, 2003). Root apices (5 mm), collected after different times of exposure to Al, were fixed during 48 hours in 2.5 % glutaraldehyde to detect callose with aniline blue, or in a 1:1 mixture of 3.7 % phormol (pH 7.4) and 2.5 % glutaraldehyde to detect Al accumulation with hematoxylin.

Samples were cut (7 µm) and processed and aluminum was visualized by staining with 0.1% (w/v) hematoxylin and 0.01% (w/v) KIO_3 for 20 min. Callose was visualized by staining with 0.1% (w/v) aniline blue and 1M glycine NaOH (pH 9.5).

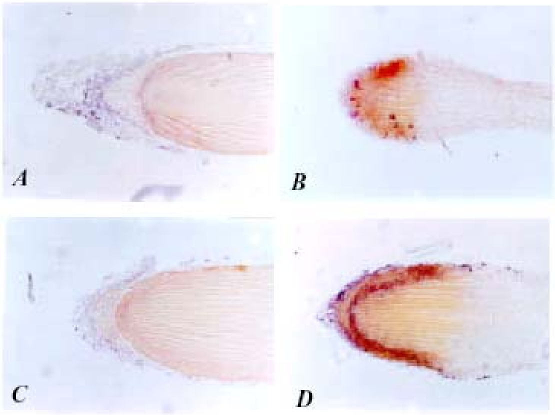


Figure 12. Hematoxylin staining of *Brachiaria*.root apices. **A**. Al-tolerant parent (*B. decumbens*); **B**. Al-sensitive parent (*B. ruziziensis*); **C**. Al-tolerant hybrid; **D**. Al-sensitive hybrid (12 days of Al treatment).

Results and Discussion

Differential hematoxylin staining was observed between sensitive and tolerant genotypes. Root apices of *B. decumbens* (Figure 12A) and an Al-resistant hybrid (Figure 12C) did not accumulate much Al. By contrast, root apices of *B. ruziziensis* (Figure 12B) and an Al-sensitive hybrid (Figure 12D) accumulated Al in the outer layer of root meristems. Aniline-blue staining of histological sections revealed a higher content of callose for Al-sensitive genotypes.

The pattern of callose accumulation was only partially correlated with that of Al (visualized by hematoxylin). Al-tolerant *B. decumbens* accumulated callose exclusively in the root cap and at the surface of the root meristem. Al-sensitive *B. ruziziensis* accumulated a large amount of callose in cortical and vascular tissues, an area where little Al was detected in the hematoxylin stain (Figure 13).

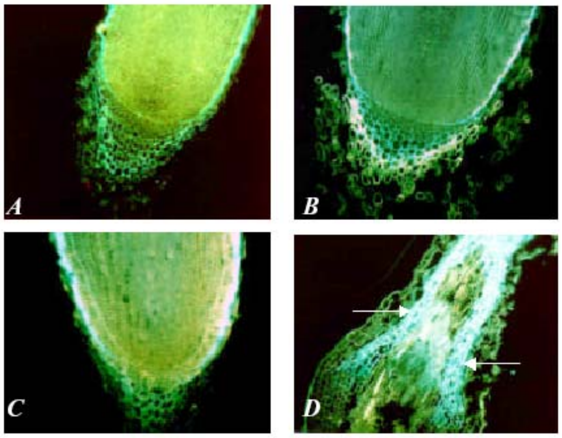


Figure 13. Callose detection by aniline-blue staining in histological sections of *Brachiaria* root apices. **A, B**. *B. decumbens* (without and with Al). **C, D**. *B. ruziziensis* (without and with Al). Arrows designate callose deposition (3 days of Al treatment).

Hematoxylin staining could be employed as a quick selection criterion to discard Al-sensitive geotypes in the Brachiaria breeding program, because most of the Al accumulates in the external layer of root meristems and should be readily stainable with hematoxylin in intact apices.

C) Identification of molecular markers and QTLs associated with gene(s) conferring aluminum resistance in a *Brachiaria ruziziensis* × *Brachiaria decumbens* cross (F1)

Contributors: J. Vargas, C. Quintero, C. Rosero, G. Gallego, M.C. Duque, M.E. Buitrago, A.L. Chaves, J.W. Miles, P. Wenzl, I.M. Rao and J. Tohme

Rationale

Acid soils have been estimated to occur on about 40% of the arable land (3.95 billions of ha). Plant growth on these soils is constrained mainly by aluminum (Al) toxicity and deficiencies of nutrients such as phosphorus (P), nitrogen (N), and calcium (Ca). There is considerable variation within and between plant species in their ability to resist Al, and this variation within some species has allowed breeders to develop genotypes that are able to grow on acid soils. Within the *Brachiaria* genus, Al resistance of signal grass (*B. decumbens* Stapf cv Basilisk), a widely sown tropical forage grass, is outstanding compared with the closely related ruzigrass (*B. ruziziensis* Germain and Evrard cv Common). The main objective of this work is to identify microsatellites, AFLPs and QTLs associated with the gene(s) conferring aluminum resistance in a *Brachiaria ruziziensis* × *Brachiaria decumbens* cross.

Materials and Methods

An F_1 hybrid population of 263 individuals (*B. ruziziensis* x *B. decumbens*) was used for this study. Young leaves were cut and placed in paper bags in an incubator previously set at 45-50°C. Samples were allowed to dry for at least 20h, or until the leaves were dry enough that they break easily. Samples were stored at –80°C until grinding. Dried leaf tissue was grinded with stainless steel spheres with vigorous shaking. Genomic DNA was extracted using a CTAB-Chloroform protocol with some modifications for small amounts of tissue. DNA was quantified on a DyNA Quant 200ä Flourometer (Hoefer Scientific Instruments) and diluted at 4ng/ul for SSRs amplification and 25ng/ul for AFLPs amplification. Methods for the isolation of microsatellites, and the methodology for PCR amplification and evaluation of polymorphism, were as described previously (SB-2 Annual Report, 2000; 2001) with some modifications. The AFLP Analysis System I kit, and AFLP Analysis System II Small Genome, from Invitrogenâ were used for AFLP amplification, following the instructions, with some modifications. Silver staining (Promega Inc., USA), was used to visualize allele segregation of the markers on 6% denaturing polyacrylamide gels with 5M Urea and 0.5X TBE.

Results and discussion

Microsatellites: 73 SSRs were evaluates in the parental genotypes of which 40 were found to be polymorphic. When run in the progeny, three sets of primers did not amplify in 30% of the progeny, so they were discarded together with three more monomorphic microsatellites. Ninety-seven polymorphic alleles were scored in the population, out of which 63 were found with the *B. decumbens*, Al-tolerant, 606 genotype, while 34 carried the *B. ruziziensis*, Al-sensitive, 44-2 genotype (Figure 14).

AFLPs: 64 combinations of primers were assayed with the two parental genotypes. Among them, 12 having high number of polymorphic bands, were chosen. To date 3 combinations (E-ACC/M-CAC; E-ACT/M-CTA and E-ACG/M-CAG) were run in the progeny and yielded 63 polymorphic bands distributed as follows: 50 were found in the Al-tolerant parental and 13 in the Al-sensitive genotype. (Figure 15).

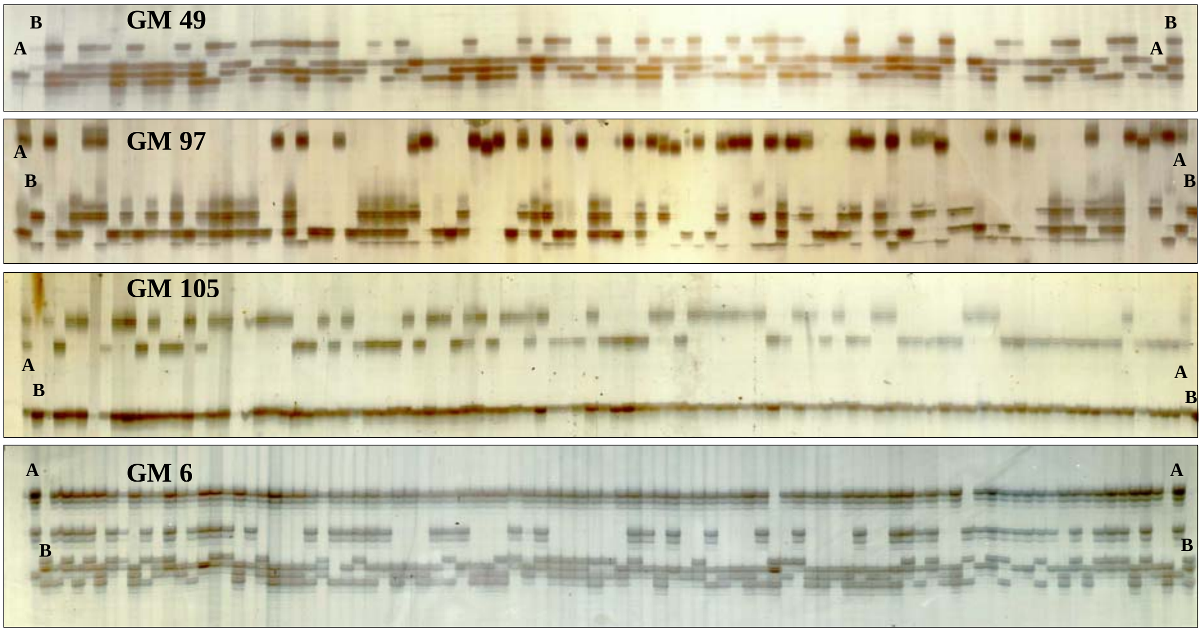


Figure 14. Segregation of four SSR alleles in some individuals of the F_1 *B. ruziziensis* x *B. decumbens* progeny. **A**, Parental genotype 606 (Al-resistant) and **B**, parental genotype 44-2 (Al-sensitive).

Linkage analysis: Segregation of markers in a 1:1 ratio, as single dose restriction fragments (SDRF), will be determined by a Chi-square test. The data matrixes for presence or absence of bands were analyzed with MAPMAKER v3.0b for PC, and MAPMAKER v2 for Macintosh. Using 56 SSR and 50 AFLPs molecular markers, we constructed a putative linkage map with 18 linkage groups. These linkage groups span 445.3 cM, and the average marker density is one per every 6.1 cM. The position of 78 markers is shown in Figure 15, on the framework molecular genetic map of *Brachiaria* (LOD = 25 and tetha (θ) = 25). Map distances are shown in Kosambi map units.

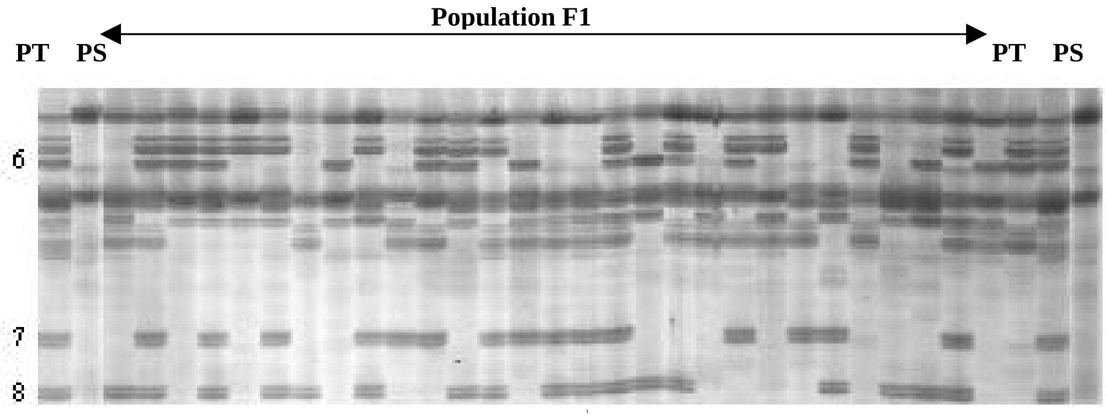


Figure 15. Segregation of AFLPs bands in individuals of the F_1 *B. ruziziensis* x *B. decumbens* progeny. **PT**, Parental genotype 606 (Al-resistant) and **PS**, parental genotype 44-2 (Al-sensitive).

Association between Molecular Markers and Al resistance: To find association with molecular markers, a preliminary analysis of 106 markers at the 10% level was done using SAS. Putative associations were found between 78 SSRs and AFLPs markers and the phenotypic characterization under greenhouse conditions. The three phenotypic variables for Al resistance (root length, abundance of root tips, mean root diameter) were analyzed for association with molecular markers. We found 13 molecular markers with R^2 between 0.0124-0.0267 that explain the variance for these traits; molecular markers associated with phenotypic characterization are in blue (Table 15 and Figure 16).

Further work is in progress: (i) to saturate linkage map of *B. decumbens* CIAT 606 parent with SSR and AFLPS; (ii) to analyze data for mapping for Al resistance; (iii) to conduct QTLs analysis for Al resistance; and (iv) to design of SCARs for marker assisted selection.

Table 15. Association analysis between molecular markers and phenotipic variable for Al resistance in a *Brachiaria decumbens* x *Brachiaria ruziziense*s hybrid population.

Marker	**R^2**	**Linkage Group**	**Phenotipic Variable**
GM 44d2	0.0267	B	Rl[a]
GM 44d2	0.0218	B	Tips[b]
C1b4	0.0181	R	Tips
GM 109c	0.0177	N	Rd[c]
C1b17	0.166	D	Rd
C2b17	0.0161	R	Tips
C2b15	0.016	I	Rd
GM 109d	0.0156	N	Rd
GM 79c	0.0156	A	Rl
GM 58a	0.0151	D	Rd
GM 44d1	0.0149	B	Tips
C2b12	0.0145	B	Rd
C1b12	0.014	R	Tips
GM 79d	0.013	D	Rd
GM 44d2	0.0124	B	Rd

a Root Length
b Abundance of root Tips
c Root Diameter

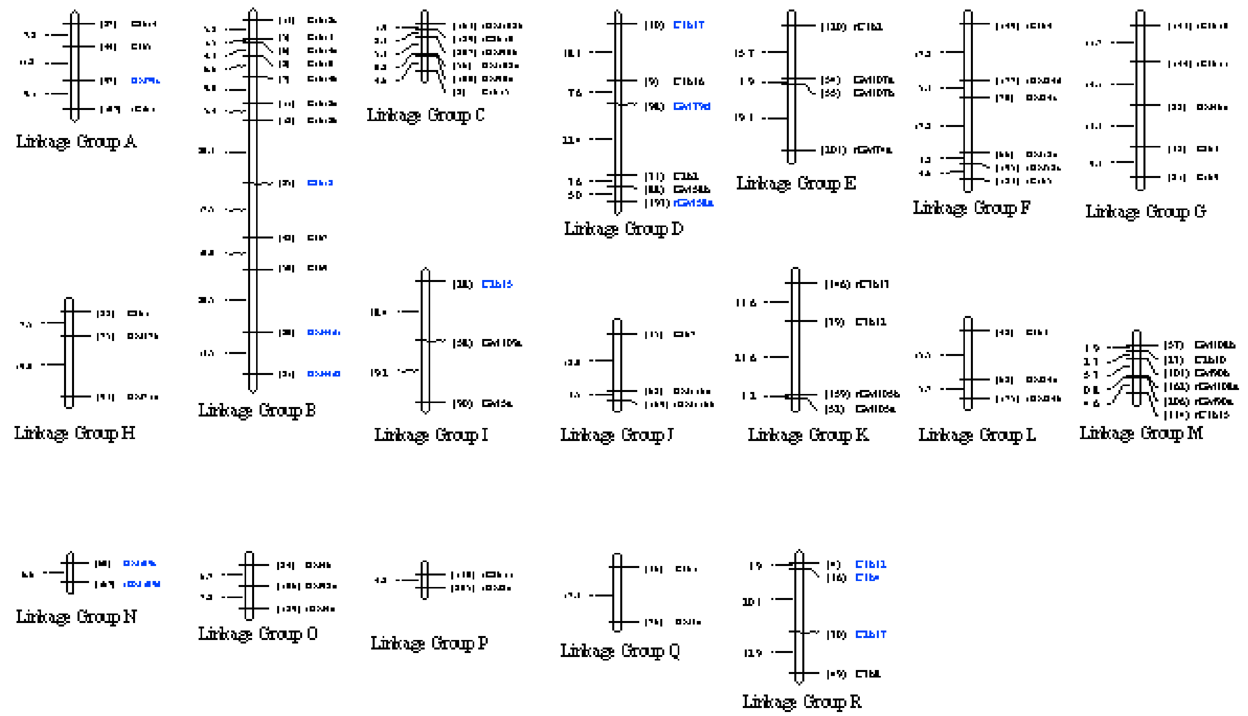


Figure 16. Preliminary *Brachiaria* linkage map of a *B. ruziziensis* × *B. decumbens* F1. Markers in blue indicate putative association with Al resistance.

D) Identification of candidate genes associated with aluminum resistance in *Brachiaria decumbens*.

Contributors: A. Arango, G. Gallego, D. Bernal, P. Wenzl, I.M. Rao, M. Ishitani and J. Tohme (CIAT)

Rationale

The 3'-UTR sequences of candidate genes associated with aluminum (Al) resistance were identified by comparing expression levels between genotypes and treatments. Subtractive libraries were prepared from root apices of contrasting genotypes grown in the presence and absence of Al: *Brachiaria decumbens* (Al-resistant parent), three Al-resistant *B. ruzizie*nsis x *B. decumbens* hybrids, *B. ruziziensis* (Al-sensitive parent), and seven Al-sensitive hybrids (Annual Report 2003). Microarray technology was then used to catalogue clones derived from genes that were differentially expressed between samples.

Materials and Methods

Subtractive libraries of 3'UTR fragments were prepared with the differential subtraction chain (DSC) method (IP-5 Annual Report, 2003). Inserts were amplified and arrayed in duplicate on glass slides. Pairs of contrasting RNA populations (control, target) were hybridized to microarrays (Table 16). Two pairs of dye-swap hybridizations were performed per combination of control and target.

Microarray sample pools (MSPs) were synthesized from 2.4 to 240 ng of cDNA to cover a 100-fold range of signal intensities. They were arrayed together with other controls, such as negative controls (spotting buffer, polylinker of the vector used for library preparation, unrelated genes such as insulin, Sp1 β-cell, HPH) and positive controls (GADPH of *Brachiaria*, α-tubulin, Spy genes). A total of 768 controls were spotted onto the array. The logarithms of the crude ratios between the two channels were first normalized by using the lowess algorithm and then analyzed with the Significance Analysis of Microarrays (SAM) software.

Differentially expressed clones were amplified, purified (Qiagen kit) and sequenced (ABI BigDye terminator kit). The sequences obtained were compared against those in the UTR database at http://bighost.area.ba.cn.it/BIG/UTRHome.

Results and Discussion

The microarray hybridizations identified a total of 35 3'-UTR fragments of candidate genes that were expressed differentially in the four comparisons between target and control pools (Figure 17, Table 16).

Seven clones contained the post-transcriptional control sequence 15LOX-Dice (15-Lypoxigenase Differentiation Control Element), seven clones contained the ribosomal regulatory element IRES (Internal Ribosome Entry Site), and one clone was homologous to the 3'-UTR of a *Arabidopsis thaliana* gene coding for the germination protein

Table 16. Populations evaluated in conditions of Al toxicity by Microarrays.

Combination	Control	Treatment		Target	Treatment
1	Al-sensitive parent + hybrids	$AlCl_3$ (200 µM)	*vs*	Al-resistant parent + hybrids	$AlCl_3$ (200 µM)
2	Al-resistant parent + hybrids	Al (0 µM)	*vs*	Al-resistant parent + hybrids	$AlCl_3$ (200 µM)
3	*B. ruziziensis*	$AlCl_3$ (200 µM)	*vs*	*B. decumbens*	$AlCl_3$ (200 µM)
4	*B. decumbens*	Al (0 µM)	*vs*	*B. decumbens*	$AlCl_3$ (200 µM)

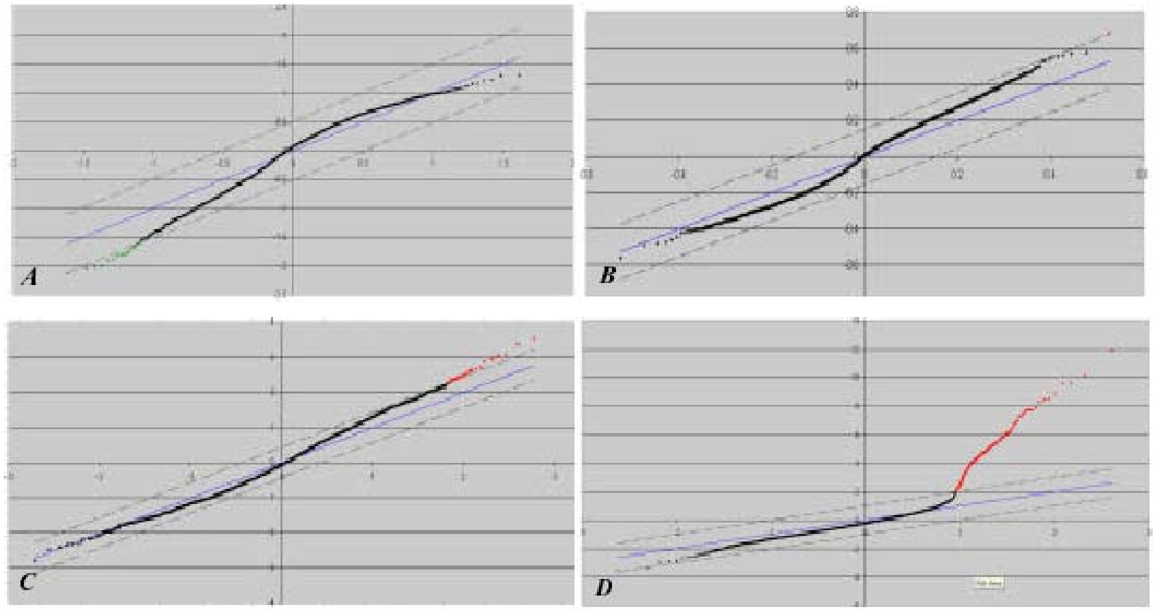


Figure 17. Differentially expressed genes associated with Al resistance (red points). **A**. Al-sensitive parent + hybrids in Al treatment *vs* Al-resistant parent + hybrids in Al treatment; **B**. Al-resistant parent + hybrids in control treatment *vs* Al-resistant parent + hybrids in Al treatment; **C**. Al-sensitive parent (*B. ruziziensis*) in Al treatment *vs.* Al-resistant parent (*B. decumbens*) in Al treatment; **D**. Al-resistant parent (*B. decumbens*) in control treatment *vs.* Al-resistant parent (*B. decumbens*) in Al treatment.

GLP2 (EMBL: BT002170). Five clones had no match in the data base. The 3'-UTR clones of differentially expressed genes identified in these experiments will be used as probes to isolate the corresponding full-length genes in a cDNA library previously prepared from root apices of the Al-resistant parent (*B. decumbens*).

E) Internal mechanisms of plant adaptation to aluminum toxicity and phosphorus starvation in three tropical forages

Contributors: T. Watanabe, M. Osaki, H. Yano (Hokkaido University, Japan) and I. M. Rao (CIAT)

Rationale

Soil acidity inhibits plant growth, principally because of toxicity from excess aluminum (Al) and lack of nutrients, especially phosphorus (P). Plant mechanisms for tolerating toxic levels of Al are usually grouped into two: external mechanisms to prevent Al invading root cells, or internal mechanisms that provide tolerance to excess Al. One significant external mechanism is for roots to exude organic acids into the rhizosphere, where they then make stable complexes with Al. Internal mechanisms are often found in Al-accumulator species, which prevent Al toxicity within the plant by creating Al complexes with organic acids or silicon.

Phosphorus is a major nutrient that plays a role in forming phospholipids, nucleic acids, nucleosides, coenzymes, and phosphate esters in plants. Under P starvation, adapted plants produce more fine roots or root hairs and/or increase root mass to acquire more P from soils. The roots release chelating compounds (e.g. organic acids) to mobilize and use insoluble phosphate compounds (e.g. those with Al, Fe, and Ca). Roots also release enzymes (e.g. acid phosphatase or APase) to use organic P. In addition, under P deficiency, adapted plants have the strategy of efficiently using P in cells. Phosphohydrolases may function as a P-recycling mechanism in plants. Inorganic P, liberated by APase or ribonuclease (RNase) in old tissues are retranslocated to young tissues. Induction of APase and RNase in plants under P deficiency has been intensively studied. Another strategy—mycorrhizal symbiosis—is also important for many plant species.

Brachiaria species are adapted to low-fertility acid soils in the tropics, tolerating both excess Al and P starvation very well. However, their mechanisms of high level of adaptation have yet to be defined. In *B. decumbens*, for example, Al-exclusion mechanisms, such as exudation of organic acids and rhizosphere alkalinization, are not involved in its high level of resistance to Al. Many other tropical forage grasses and legumes also grow well in acid soils, adapting to excess Al and P starvation stresses by using mechanisms that are still unclear. We therefore studied the mechanisms of adaptation to Al toxicity and P starvation in three tropical forages: two grasses (a *Brachiaria* hybrid and *Andropogon gayanus*) and one legume (*Arachis pintoi*).

Materials and Methods

For both experiments, we used seeds from three tropical forages: the grass *Brachiaria* hybrid (*B. ruziziensis* Ger. & Ev. clone 44-06 × *B. brizantha* (A. Rich.) Stapf CIAT 36061, also known as cv. Mulato); the grass *Andropogon gayanus* Kunth (CIAT 621); and the legume *Arachis pintoi* Krap. & Greg. (CIAT 17434). The seeds were sterilized with sodium hypochlorite for 10 min, washed with deionized water, and sown on a moderately moist perlite-vermiculite mixture (1:1, v/v) in a greenhouse at Hokkaido University, Sapporo, Japan. Uniform seedlings (shoot height = 10 cm) were transplanted to containers containing a standard nutrient solution with 0.06 mM P at pH 4.0, and grown for one week to adapt to hydroponic conditions.

Experiment 1: Tolerance of Al stress and P starvation: After precultivation, seedlings were transferred to 36-litre containers containing treatment solutions, which comprised: the standard nutrient solution with 0.06 mM P (control); the same solution but no P (-P, i.e. 0 mM P); and the same solution but with Al added (+Al, i.e. 0.37 mM Al and 0.06 mM P). Al and P were added as $Al_2 (SO_4)_3$ and NaH_2PO_4, respectively. At the end of 10 days of treatment, the roots of seedlings from each treatment were first washed with tap water, then with deionized water, and transferred to polyethylene bottles containing 0.1 mM $CaCl_2$ (pH 4.0) for collecting root exudates. After being left overnight from 17:00 to 8:00 (i.e. 15 h) in a greenhouse, the exudates were collected, the roots washed with deionized water, and the seedlings cut to separate roots from shoots. The fresh weight of each set of organs was determined. Half of the fresh samples were dried in a forced-air oven at 80°C for 72 h, then weighed and digested with H_2SO_4-H_2O_2 for mineral analysis. The other half of the samples was used to determine organic acids. Acid phosphatase activity in root exudates was determined. One unit of acid phosphatase activity was defined as the amount of enzyme that hydrolyzed 1 µmol of *p*NPP per minute. Soluble organic acids in fresh samples were determined and the concentration of organic acids was measured by capillary electrophoresis. The levels of Al, K, Ca, and Mg in leaves and roots were determined. Concentrations in leaves and roots were determined by the semi-micro Kjeldahl method for N and the vanado-molybdate yellow method for P.

Experiment 2: ^{27}Al NMR study: *Brachiaria* seedlings were prepared as described above, and transferred to 36-litre containers carrying the standard nutrient solution, but with 2.8 mM Al at pH 3.7 added, and left to grow for 1 month. The much higher Al concentration was used to ensure clear peaks in the ^{27}Al NMR spectrum. Even so, the *Brachiaria* seedlings grew well (data not shown). After treatment, roots were removed from the seedlings and washed, first with tap water, then with deionized water. The roots were grouped into three: Fraction (a), roots given the water washings only, and used to determine total amounts of Al and organic acids; Fraction (b), roots were also washed with 0.1 N HCl for 5 min to remove apoplastic, soluble or loosely bound, components; and Fraction (c), roots were also washed with 0.1 N HCl for 5 min, frozen at -50°C for 1 h to rupture cell membranes, thawed, and washed again with 0.1 N HCl for 5 min to remove symplastic, soluble or loosely bound components. Al and organic acid concentrations in each fraction were determined as described for Experiment 1. Each fraction of *Brachiaria* roots was placed in a 10-mm-diameter NMR tube. $AlCl_3$ (0.1 M) solution was used as an external reference to calibrate the chemical shift (0 ppm). ^{27}Al NMR spectra were recorded, using a Bruker MSL400 spectrometer at 104.262 MHz. The spectra were obtained by using a frequency range of 62.5 kHz, a pulse width of 12 µs, a delay time of 0.16 ms, a cycle time of 0.5 s, and 4000 scans.

Results and Discussion

Experiment 1: Tolerance of Al stress and P starvation. The effect of each treatment on plant growth was expressed as dry matter accumulation in each treatment relative to that of the control treatment (Figure 18). The +Al treatment (+0.37 mM Al) did not affect growth in any of the species used in the study. The -P treatment did not inhibit growth in *Brachiaria* and *A. pintoi*, but did in *A. gayanus*. Relative growth rate (RGR) in the control treatment was much higher in the two grasses than in the legume (Figure 18). The root-to-shoot ratio in *Brachiaria* and *A. pintoi* increased remarkably with -P treatment, whereas it remained unchanged in *A. gayanus* (Figure 18). The treatments hardly affected acid phosphatase activity in root exudates of seedlings, except in *A. gayanus* where it increased with +Al treatment (Table 17).

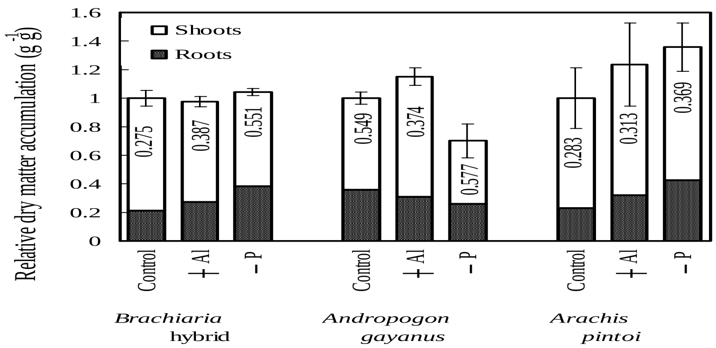


Figure 18. Effects of P starvation and Al toxicity on plant growth in Experiment 1. Plant growth was expressed as the relative dry matter accumulation (i.e. [dry weight after treatment – initial dry weight in each treatment]/[dry weight after treatment – initial dry weight in control treatment]). Bar values indicate root-to-shoot ratios, and the range for each bar indicates the ± SE value. Relative growth rate (dry weight after treatment – initial dry weight)/initial dry weight) in control treatment was 10.4, 8.5, and 1.1 g g^{-1}, in a *Brachiaria* hybrid, *Andropogon gayanus*, and *Arachis pintoi*, respectively.

Table 17. Acid phosphatase activity in root exudates of forage seedlings grown under three treatments

	Acid phosphatase (mU g^{-1} f. wt 15 h^{-1})		
Species	Control	+Al	-P
Brachiaria hybrid	170.9 ± 72.2	100.7 ± 42.1	202.9 ± 24.9
Andropogon gayanus	182.3 ± 3.2	213.6 ± 24.5	166.6 ± 21.4
Arachis pintoi	76.1 ± 2.9	76.6 ± 7.0	54.6 ± 1.0

Mineral concentrations in leaves and roots, and total organic acid concentrations in roots are shown in Table 18. Nitrogen concentrations were hardly affected by the treatments. Phosphorus concentrations in leaves and roots of all species declined drastically in the -P treatment, especially in the *Brachiaria* hybrid and *A. gayanus*. Phosphorus concentrations tended to decrease as K concentrations decreased. Aluminum concentrations in *Brachiaria* leaves with the +Al treatment were less than 100 mg kg^{-1}, whereas in *A. gayanus* and *A. pintoi*, they were about 600 and 340 mg kg^{-1}, respectively. Organic acid concentrations in roots increased under the +Al treatment in the *Brachiaria* hybrid and *A. gayanus*.

Experiment 2: ^{27}Al NMR study. Aluminum, oxalate, malate, and citrate concentrations as determined by the ^{27}Al NMR spectrum are shown for each fraction in Figure 19. Aluminum concentration in fraction (b) (roots after removing apoplastic soluble components) did not differ significantly from that in fraction (a) (intact

Table 18. Concentrations of N, P, K, Ca, Mg (mg g^{-1}d. wt), Al (mg kg^{-1}d. wt) and total organic acid (μmol g^{-1}f. wt) in organs of *Brachiaria* hybrid, *Andropogon gayanus* and *Arachis pintoi*. Values are the means of three replicates±SE. SEs were not shown in organic acid concentrations because of two replicates. ND = not determined.

Species		Leaf			Root		
		Control	+Al	- P	Control	+ Al	- P
Brachiaria hybrid	N	47.3 ±1.7	48.0 ±0.6	55.8 ±0.8	27.0 ±0.9	27.6 ±1.3	25.4 ±1.0
	P	8.8 ±0.7	14.3 ±0.3	0.8 ±0.0	5.6 ±0.2	8.8 ±0.5	1.1 ±0.0
	K	24.9 ±0.5	25.3 ±0.8	9.7 ±0.0	33.7 ±0.4	33.1 ±2.2	16.1 ±3.5
	Ca	4.5 ±0.6	2.8 ±0.3	2.3 ±0.2	1.3 ±0.1	1.3 ±0.0	0.7 ±0.1
	Mg	3.7 ±0.3	3.7 ±0.3	3.3 ±0.1	3.5 ±0.1	4.5 ±0.3	2.3 ±0.1
	Al	49 ±6	75 ±11	56 ±7	357 ±40	3285 ±76	687 ±24
	Organic acid	ND	ND	ND	0.79	4.71	0.23
Andropogon gayanus	N	21.7 ±0.2	27.0 ±0.3	20.5 ±0.2	18.7 ±1.3	20.6 ±0.2	26.0 ±0.7
	P	6.5 ±0.0	5.5 ±0.2	0.6 ±0.0	10.6 ±0.9	7.0 ±0.0	0.2 ±0.3
	K	18.8 ±0.2	20.7 ±0.5	14.6 ±0.7	24.9 ±1.7	28.8 ±0.2	10.8 ±1.4
	Ca	6.5 ±0.0	2.9 ±0.0	4.4 ±0.1	2.1 ±0.1	2.0 ±0.0	1.7 ±0.1
	Mg	3.7 ±0.1	3.0 ±0.0	3.1 ±0.0	3.5 ±0.2	4.5 ±0.0	2.8 ±0.1
	Al	39 ±3	594 ±22	23 ±4	219 ±15	5822 ±4	117 ±139
	Organic acid	ND	ND	ND	3.44	10.70	3.71
Arachis pintoi	N	49.5 ±1.8	51.4 ±1.4	56.0 ±1.3	31.4 ±0.5	35.1 ±0.4	37.2 ±1.0
	P	17.1 ±0.5	10.1 ±0.7	2.4 ±0.1	31.1 ±0.5	22.9 ±1.3	2.3 ±0.1
	K	27.5 ±0.1	25.0 ±1.6	22.6 ±0.6	30.2 ±0.7	28.8 ±1.6	33.5 ±1.8
	Ca	15.6 ±0.2	19.5 ±1.5	13.8 ±0.8	5.1 ±0.0	3.0 ±0.6	3.5 ±0.1
	Mg	8.8 ±0.0	8.8 ±1.6	6.5 ±0.3	15.3 ±0.4	5.0 ±0.3	3.5 ±0.2
	Al	83 ±6	339 ±15	58 ±4	605 ±1	4402 ±143	356 ±6
	Organic acid	ND	ND	ND	0.47	0.53	1.19

roots). The differences in organic acid concentrations between fractions A and B were also small. ^{27}Al NMR spectra obtained from fractions A and B were very similar, and showed several peaks downfield and a small peak at 0 ppm. In contrast, in fraction (c) (roots after removing apoplastic soluble components, followed by removing symplastic soluble components), Al concentration was much lower than in fractions A and B. The ^{27}Al NMR spectrum was also different, with the resonance peaks downfield decreasing and a peak at 0 ppm becoming higher and sharper. Of the three organic acids, malate decreased drastically in fraction (c) (Figure 19).

All three tropical forages used in the present study were highly tolerant of Al and no growth reduction was observed with the +Al treatment (+0.37 mM Al) (Figure 18). Concentrations of Ca and Mg, the uptake of which appeared inhibited by Al, decreased in the +Al treatment (Table 18). However, concentrations of other major nutrients were not affected by Al application (Table 18), suggesting that Al did not affect root function for these nutrients.

To prevent or evade Al toxicity, plants growing in acid soils have developed mechanisms to exclude Al from roots or to tolerate high Al concentrations in tissues. Although organic acid exudation from roots is a major mechanism for excluding Al from roots, this mechanism was not evident in *Brachiaria* species. Previous research showed that Al application increases organic acid concentrations in roots of *Brachiaria* species. In our study, organic acid concentrations increased with Al application, but not with P starvation in *Brachiaria* and *A. gayanus* (Table 18).

We speculated before that the increase in organic acids in roots suggested that they play a role in the internal detoxification of roots from Al by acting as ligands for Al. In many Al-accumulator species, leaves with high concentrations of Al are detoxified by organic ligands, such as Al-oxalate, Al-catechin, and Al-citrate. The same mechanisms are considered possible in roots.

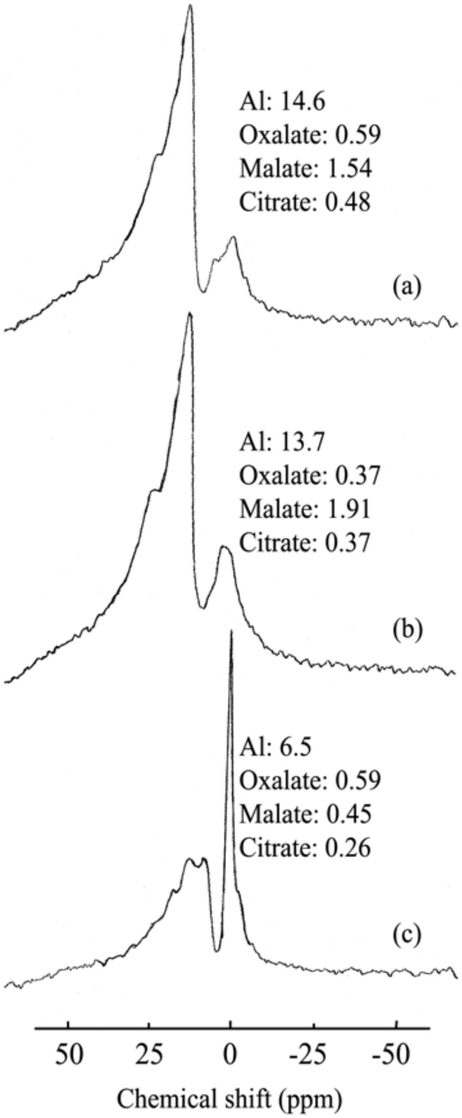


Figure 19. ^{27}Al NMR spectra and concentrations of Al (mg g^{-1} d. wt) and organic acids (µmol g^{-1} f. wt) in three root fractions of a *Brachiaria* hybrid. Fraction (a) = intact roots; fraction (b) = roots after removing soluble or loosely bound apoplastic components; fraction (c) = roots after removing soluble or loosely bound apoplastic and symplastic components. $AlCl_3$ (0.1 mM) was used as an external reference to calibrate the chemical shift (0 ppm).

An important result of our study was to demonstrate that Al-ligand complexes occur inside the root cells of a *Brachiaria* hybrid, an Al non-accumulator genotype. We applied simple fractionation of Al and ^{27}Al NMR to roots of the *Brachiaria* hybrid to evaluate the occurrence of Al-ligand complexes in root tissues. The ^{27}Al

NMR spectrum obtained from intact roots (fraction a) showed several peaks downfield at 10-20 ppm, suggesting that most of the soluble Al in roots makes octahedral complexes, presumably with organic acids. Both the ^{27}Al NMR spectrum and Al concentrations in fraction (b), in which soluble or loosely bound apoplastic Al was removed, were almost the same as those of intact roots (fraction a), indicating that root apoplast is not a primary site for Al accumulation in *Brachiaria* hybrid (Figure 19). In contrast, in fraction (c), the peaks downfield in the ^{27}Al NMR spectrum, Al concentrations, and malate concentrations all drastically decreased after removing symplastic components (Figure 19), indicating that most of the chelated Al occurs in root symplast, and that malate may participate in formation of Al complexes.

In Al-accumulator species, Al that penetrates the plasma membrane of root cells is immediately transferred to shoots. In *Brachiaria*, however, Al accumulated only in roots and Al concentrations in shoots were kept very low (Table 18). In shoots of buckwheat, an Al-accumulator species, Al-ligand complexes are mostly isolated in vacuoles. Al in the *Brachiaria* hybrid may also compartmentalize in vacuoles and, thus, not be translocated from roots to shoots.

Of the three species tested in this study, the *Brachiaria* hybrid and *A. pintoi* were extremely tolerant of low P stress (Figure 18). The *Brachiaria* hybrid grows well under P starvation, despite very low P concentrations in its leaves (Table 18). This suggests that *Brachiaria* can reuse P more efficiently. In *A. gayanus*, growth under the -P treatment was inferior to that of the control treatment, probably because of a higher relative growth rate (RGR). Based on values of P-use efficiency (the reciprocal of P concentration in plant), both *A. gayanus* and the *Brachiaria* hybrid appear to be tolerant of P starvation (Table 18). Higher P-use efficiency was observed before in *Brachiaria humidicola (dictyneura)* than in forage legumes, including *A. pintoi*, in acid Oxisols.

Although APase activity in root exudates, which contributes to the use of organic P in the rhizosphere, was hardly affected by P deficiency in any of the species (Table 17), it was higher than that of other forage species. While the ability to use organic P in the rhizosphere did not affect plant growth in the solution culture experiment, high APase activity in root exudates may significantly affect P acquisition in soils. In addition, the higher root-to-shoot ratio under P starvation in *Brachiaria* hybrid and *A. pintoi* would increase P uptake in soils (Figure 18).

This study showed that the *Brachiaria* hybrid, *Andropogon gayanus*, and *Arachis pintoi* adapt well to acid soils with low P and excess Al. The results indicate that the *Brachiaria* hybrid makes chelating complexes of Al (possibly with organic acids) inside root cells (symplast) to detoxify the cells. Until now, Al detoxification with organic acids in plant tissue has been observed mainly in Al-accumulator species.

This study provides direct evidence for the first time that organic acids inside root tissues help detoxify Al in non-accumulator species of Al. In Al non-accumulator species, Al is assumed to be detoxified outside the root cells, in the rhizosphere and/or root apoplast, by exuded organic acids. However, the *Brachiaria* hybrid, an Al non-accumulator, accumulates large amounts of Al in the root symplast (Figure 19), probably in the vacuoles. Thus, in some cases, Al accumulation and detoxification in roots involve the same mechanism in Al-accumulators and non-accumulators. Differences in characteristics of Al accumulation in shoots between Al-accumulators and non-accumulators may be caused by the existence of ligands for controlling translocation from roots to shoots.

Conclusions

We tested how Al toxicity and P starvation affect growth, concentrations of minerals and organic acids, and acid phosphatase activity in root exudates of *Brachiaria* hybrid, *Andropogon gayanus* and *Arachis pintoi*. The two tropical grasses tolerated high levels of Al toxicity and P starvation, with the *Brachiaria* hybrid maintaining very low levels of Al concentration in shoots. ^{27}Al NMR analysis revealed that, in *Brachiaria* hybrid, Al makes complexes with some ligands in root symplast. The three forages are probably adapted to P starvation through high P-use efficiency. These experiments provide the first evidence we know of that organic acids within root tissue help detoxify Al in non-accumulator species such as the *Brachiaria* hybrid.

3.1.1.2 Screening of *Brachiaria* hybrids for resistance to aluminum

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

Rationale

For the last three years, we have implemented screening procedure to identify Al-resistant *Brachiaria* hybrids that were preselected for spittlebug resistance. In 2002, we have identified 2 sexual hybrids (SX 01NO3178 and SX01NO7249) and one apomictic hybrid (BR99NO/4132) with greater level of Al resistance than that of the sexual parent, BRUZ/44-02. Last year, we have identified 2 hybrids ((BR02NO1372 and BR02NO1621) with greater level of Al resistance than that of the most hybrids generated from the *Brachiaria* breeding program. With the partial support of BMZ-GTZ of Germany and Papalotla (seed company) of Mexico to the *Brachiaria* improvement project, this year we evaluated Al resistance of a sexual population of 745 hybrids along with 14 checks. The increase in Al resistance of the sexual hybrids has been very marked compared with the sexual population of 2001.

Materials and Methods

A total of 745 sexual hybrids generated from 2003 population together with 14 checks including 3 parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6294 and *B. ruziziensis* 44-02) were included for evaluation of Al resistance. All the new sexual hybrids were screened for spittlebug resistance (C. Cardona, personal communication; also see Output 2 and Activity 2.2 of this report). All the hybrids that rooted well were screened initially using 200 µM Al treatment to eliminate the Al sensitive hybrids. From this initial screening, a total of 124 sexual hybrids were selected to test under both treatments of with Al (6 experiments) and without Al (3 experiments) in solution. Out of these 124 sexual hybrids, data were obtained for 86 sexuals for both treatments. Mean values from all the experiments (ranging from 3 to 20 observations) are reported. Stem cuttings of hybrids and checks were rooted in a low ionic strength nutrient solution in the glasshouse for 9 days. Equal numbers of stem cuttings with about 5 cm long roots were transferred into a solution containing 200 µM $CaCl_2$ pH 4.2 (reference treatment) and a solution containing 200 µM $CaCl_2$ and 200 µM $AlCl_3$ pH 4.2 (Al treatment). The solutions were changed every second day to minimize pH drifts. At harvest on day 21 after transfer, the dry weight of stems was measured. Roots were stained and scanned on a flatbed scanner. Image analysis software (WinRHIZO) was used to determine root length and average root diameter.

Results and Discussion

As reported for the past 3 years, higher values of total root length per plant and lower values of mean root diameter after exposure to 21 days with or without toxic level of Al in solution indicate that the parent *B. decumbens* CIAT 606 is outstanding in its level of Al resistance (Figures 20 and 21).

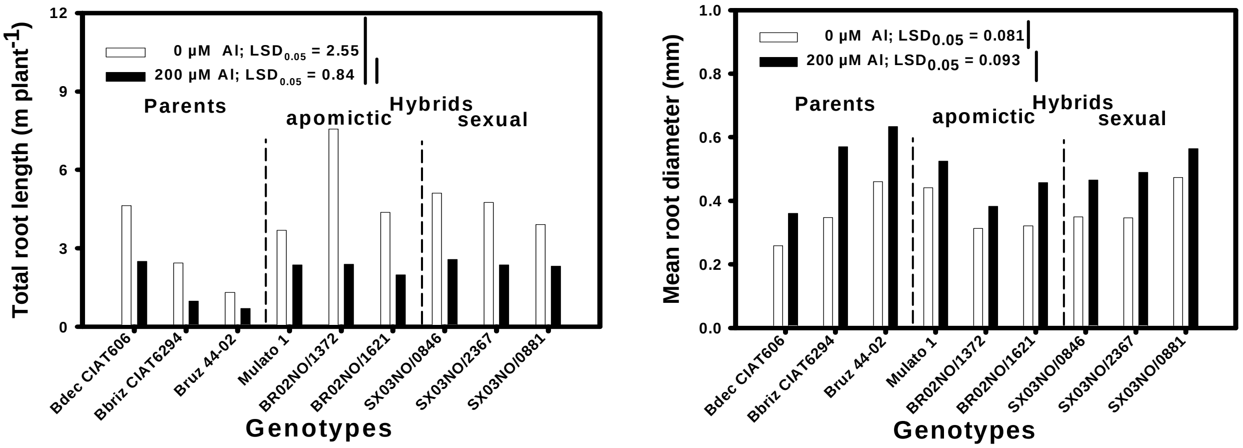


Figure 20. Al resistant apomictic and sexual hybrids of *Brachiaria* based on total root length and mean root diameter. Total root length and mean root diameter were measured after exposure to 0 or 200 ìM $AlCl_3$ with 200 ìM $CaCl_2$ (pH 4.2) for 21 days.

Among the 745 sexual hybrids and checks tested, 3 sexual hybrids (SX03NO/0846, SX03NO/2367, SX03NO/0881) and 3 apomictic hybrids (Mulato, BR02NO1372 and BR02NO1621) showed greater level of Al resistance based on total root length per plant (Figure 20; Table 19). Among these promising hybrids, BR02NO1372 showed greater fine root development than CIAT 606 in the absence of Al in solution (Figure 20). Total root length of the three sexual hybrids, both in the presence or absence of Al, was markedly superior to the sexual parent, *B. ruziziensis* (Figure 20). Among the hybrids and the checks tested, *B. humidicola* (*dictyoneura*) CIAT 6133 showed the lowest values of mean root diameter in the presence or absence of Al in solution (Table 19; Figure 22). This could be a desirable attribute for persistence under infertile acid soil conditions.

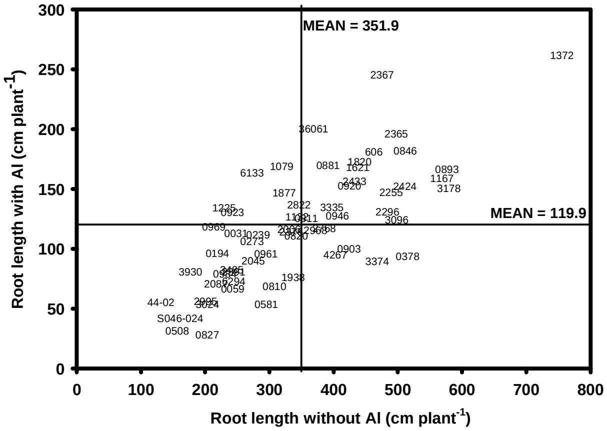


Figure 21. Relationship between total root length and mean root diameter of 78 genotypes of *Brachiaria* with presence or absence of aluminum in solution. Gentoypes that develop finer root system were identified in the upper box of the left hand side.

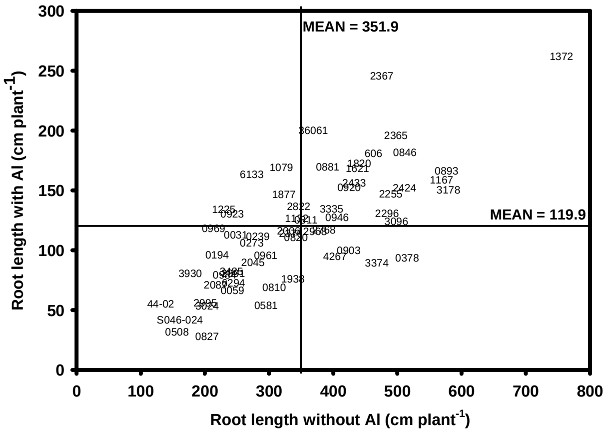


Figure 22. Relationship between total root length with Al and total root length without Al of 78 genotypes of *Brachiaria* with presence or absence of aluminum in solution. Genotypes that develop greater root length were identified in the upper box of the right hand side.

Table 19. Root length and mean root diameter of *Brachiaria* sexual hybrids evaluated with (200 µM Al) and without Al (0 µM Al) in solution in comparison with their parents and other checks.

Genotypes	Root length (m plant^{-1})		Root diameter (mm)	
	0 µM Al	200 µM Al	0 µM Al	200 µM Al
Sexual hybrids				
SX03NO/0846	5.115	2.575	0.349	0.465
SX03NO/2367	4.756	2.365	0.346	0.490
SX03NO/0881	3.912	2.314	0.474	0.564
SX03NO/2433	4.323	2.170	0.314	0.448
SX03NO/2365	4.976	2.170	0.332	0.456
SX03NO/1167	5.685	2.069	0.349	0.505
SX03NO/1079	3.192	1.992	0.351	0.479
SX03NO/0311	3.573	1.969	0.350	0.483
SX03NO/2968	3.718	1.966	0.397	0.578
SX03NO/2424	5.109	1.855	0.389	0.521
SX03NO/0893	5.765	1.804	0.373	0.555
SX03NO/2255	4.896	1.797	0.390	0.584
SX03NO/1820	4.404	1.734	0.341	0.419
SX03NO/0273	2.731	1.726	0.394	0.502
SX03NO/0903	4.241	1.703	0.473	0.457
SX03NO/2296	4.839	1.634	0.317	0.532
SX03NO/1132	3.429	1.634	0.378	0.494
SX03NO/0961	2.946	1.593	0.386	0.554
SX03NO/2995	2.005	1.578	0.426	0.636
SX03NO/3096	4.983	1.535	0.320	0.475
SX03NO/2391	2.441	1.509	0.454	0.580
SX03NO/2006	3.305	1.486	0.375	0.532
SX03NO/2768	3.855	1.480	0.404	0.562
SX03NO/0946	4.060	1.454	0.405	0.549
SX03NO/0059	2.428	1.450	0.357	0.528
SX03NO/2045	2.749	1.449	0.360	0.539
SX03NO/0378	5.153	1.440	0.396	0.702
SX03NO/0920	4.249	1.400	0.357	0.554
SX03NO/3930	1.770	1.399	0.555	0.724
SX03NO/0810	3.079	1.398	0.371	0.610
SX03NO/0923	2.426	1.356	0.436	0.579
SX03NO/3335	3.973	1.338	0.426	0.625
Sexual hybrids				
SX03NO/2087	2.168	1.337	0.505	0.658
SX03NO/0969	2.135	1.294	0.399	0.561
SX03NO/0194	2.191	1.292	0.420	0.555
SX03NO/1225	2.294	1.292	0.430	0.476
SX03NO/2376	3.337	1.286	0.397	0.590
SX03NO/3374	4.679	1.263	0.367	0.609
SX03NO/1877	3.231	1.249	0.480	0.692
SX03NO/0031	2.481	1.237	0.463	0.535
SX03NO/0239	2.825	1.185	0.443	0.664
SX03NO/2822	3.459	1.181	0.393	0.576
SX03NO/4267	4.026	1.180	0.404	0.543
SX03NO/0820	3.418	1.108	0.393	0.516
SX03NO/0508	1.565	1.096	0.454	0.550
SX03NO/1938	3.371	1.065	0.417	0.649
SX03NO/3485	2.415	1.025	0.478	0.698
SX03NO/3024	2.034	1.009	0.400	0.575
SX03NO/0581	2.953	0.977	0.390	0.636
SX03NO/0984	2.308	0.944	0.419	0.548
SX03NO/0827	2.033	0.764	0.446	0.656
Parents				
B.decumbens CIAT606	4.625	2.496	0.259	0.360
B. brizantha CIAT6294	2.444	0.979	0.348	0.570
B.ruziziensis 44-02	1.310	0.705	0.461	0.634
Checks				
BR02NO/1372	7.557	2.395	0.313	0.383
Mulato	3.688	2.361	0.441	0.525
BR02NO/1621	4.377	1.980	0.321	0.458
B.humidicola (*dictyoneura*) CIAT6133	2.732	1.922	0.221	0.287
SX01NO/3178	5.794	1.341	0.394	0.653
FM9503-S046-024 (CIAT 36087-Mulato 2)	1.609	0.934	0.512	0.765
Means	3.519	1.537	0.395	0.553
LSD (P<0.05)	2.548	1.077	0.081	0.093

attribute for persistence under infertile acid soil conditions.

Relationship between root length and mean root diameter with Al in solution showed that several sexual hybrids were superior to the sexual parent, *B.ruziziensis* 44-02 (Figure 21). Exposure to Al decreased the mean value of total root length of the 78 genotypes from 352 to 120 cm plant^{-1} (Figure 22). Relationship between total root length without Al and with Al in solution showed that several apomictic and sexual hybrids were superior to the Al resistant parent, *B. decumbens* CIAT 606 in the absence of Al in solution (Figure 22). The greater root vigor of these hybrids could

The sexual hybrids SX03NO/846 and SX03NO/0881 were found to be spittlebug resistant while SX03NO/0311 was resistant to Rhizoctonia foliar blight. Two sexual hybrids that were found to be spittlebug resistant, SX03/2483 and SX03/1820, were also found to be moderately resistant to Al stress. Another spittlebug resistant sexual hybrid, SX03/2694 was more sensitive to Al than the sexual parent, *B. ruziziensis* 44-02. The promising sexual hybrids that combine spittlebug resistance with Al resistance and other desirable attributes are being used in recurrent selection to generate superior hybrids of *Brachiaria*.

3.1.1.3 Field evaluation of most promising hybrids of *Brachiaria* in the Llanos of Colombia

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

Rationale

Evaluation of a large number of *Brachiaria* hybrids for their resistance to spittlebug and adaptation to infertile acid soils resulted in identification of a few promising *Brachiaria* hybrids. We selected 4 of these hybrids for further field-testing in comparison with their parents. The main objective was to evaluate growth and 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 of 2001. The trial included 4 *Brachiaria* hybrids (BR98NO/1251; BR99NO/4015; BR99NO/4132; FM9503-S046-024) 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. A number of plant attributes including forage yield, dry matter distribution, nutrient (N, P, K, Ca and Mg) uptake, leaf and stem total nonstructural carbohydrate (TNC) content and leaf and stem ash (mineral) content were measured at 30 months after establishment (November 2003).

Results and Discussion

After 30 months of establishment, forage yield with low fertilizer application was greater with one spittlebug resistant genetic recombinant, FM 9503-S046-024 (CIAT 36087—Mulato 2) and one parent (CIAT 6294) (Table 20). With high initial fertilizer application also these two genotypes were outstanding in live forage yield (Figure 23). Among the 4 hybrids tested, Mulato 2 was outstanding in its adaptation to low initial fertilizer application. It is important to note that CIAT 6294 had greater amount of dead biomass and stem biomass under low fertilizer application (Figure 23).

Table 20. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low or high initial fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer	High fertilizer
Total (live + dead) shoot biomass (t/ha)	0.84***	0.91***
Dead shoot biomass (t/ha)	0.54	0.76***
Leaf biomass (t/ha)	0.94***	0.94***
Stem biomass (t/ha)	0.69***	0.68***
Leaf N content (%)	-0.24	-0.35
Leaf P content (%)	0.21	0.06
Leaf TNC content (mg g^{-1})	-0.25	-0.31
Leaf ash content (%)	0.05	0.01
Stem N content (%)	-0.45	0.79***
Stem P content (%)	-0.43	-0.31
Stem TNC content (mg g^{-1})	0.45	0.12
Stem ash content (%)	-0.12	0.05
Shoot N uptake (kg/ha)	0.93***	0.85***
Shoot P uptake (kg/ha)	0.91***	0.93***
Shoot K uptake (kg/ha)	0.76***	0.78***
Shoot Ca uptake (kg/ha)	0.85***	0.81***
Shoot Mg uptake (kg/ha)	0.84***	0.89***

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

As observed last year, results on shoot nutrient uptake, particularly Ca and Mg, indicated that the hybrid, Mulato 2 was superior to CIAT 606 under low fertilizer application (Figure 24). Nutrient acquisition by Mulato 2 was also greater than the rest of the hybrids with high initial fertilization.

These results are consistent with the results reported last year from the same experiment. Correlation coefficients between live forage yield and other plant attributes indicated that greater nutrient acquisition with low initial fertilizer application contributed to superior performance (Table 20). No significant correlations were found between live forage yield and leaf and stem TNC or ash contents.

The performance of the 4 hybrids in comparison with two parents with maintenance fertilizer application will be monitored for next year in terms of forage yield and nutrient acquisition.

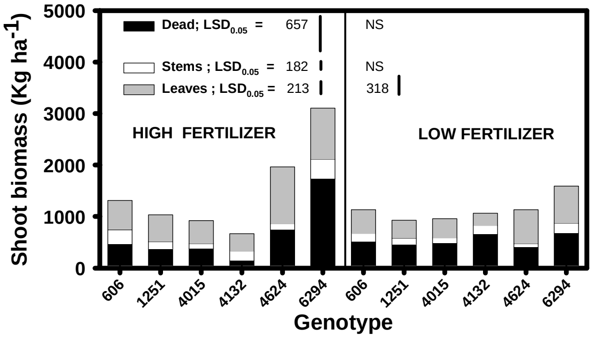


Figure 23. Genotypic variation as influenced by fertilizer application in shoot biomass production (forage yield) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 30 months after establishment (November 2003). LSD values are at the 0.05 probability level. NS = not significant.

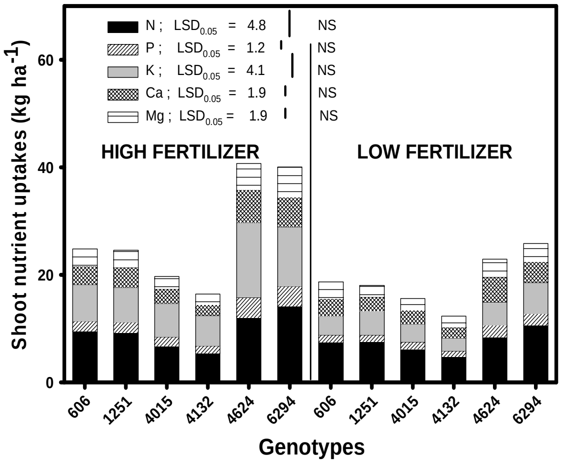


Figure 24. Genotypic variation as influenced by fertilizer application in nutrient uptake (N, P, K, Ca and Mg) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 30 months after establishment (November 2003). LSD values are at the 0.05 probability level. NS = not significant.

3.1.2 Participatory evaluation of *Brachiaria* accesions/hybrids in comparison with commercial cultivars in Nicaragua

Contributors: A. Schmidt, C. Davis, M. Peters, J. Miles and I. M. Rao (CIAT)

Rationale

As part of the BMZ-GTZ project on developing aluminum resistant *Brachiaria* hybrids, in 2002 we initiated field studies in Nicaragua for evaluation of new hybrids of *Brachiaria* along with commercial *Brachiaria* cultivars with farmer participation. The opinion of farmers is very important in the process of identifying and selecting promising forage germplasm, because their selection criteria are not necessarily the same as those of researchers. Thus the main objective of participatory evaluation was to expose the promising hybrids to farmers and generate information on farmer selection criteria. This information is highly useful to *Brachiaria* improvement program to incorporate farmer perspectives on *Brachiaria* ideotypes for multiple uses in crop-livestock systems.

Materials and Methods

The experiment site was chosen in Ubú Norte (12° 58’ 44" N, 84° 54’ 23" E, 261 masl) in the Region Autónoma del Atlántico Sur (RAAS) where acid soils are predominant. Soil characteristics and rainfall distribution from January to December are presented in Table 21 and Figure 24, respectively. A total of 14 *Brachiaria* accessions and hybrids (CIAT No. 606, 654, 679, 6133, 6780, 16322, 26110, 26124, 26318, 26646, 26990, 36061, 36062, and “Mixe” (no CIAT No.) were sown in three replicates in a split-plot design with fertility levels as main plots and genotypes as subplots. Site preparation was initiated in September and plots (5x4m) were sown early October 2002 upon the beginning of the second rainy season.

Table 21. Soil characteristics of experimental site Ubú Norte.

SOM (%)	pH	P-Bray (ppm)	K (meq)	Ca (meq)	Mg (meq)	Na (meq)
6.61	5.66	3.22	0.55	5.50	2.42	0.09

The establishment of grasses in the plots was heavily affected by unusual high precipitation, leaving Ubú with 1/3 more precipitation as the average of the last 20 years and causing floods in the area. Most of the plots established did not germinate and thus plots were replanted in May 2003 (Photo 14). Two fertilization treatments (high/low) were applied upon plot establishment. Fertilization levels were adjusted to soil analysis results. Agronomic and participatory evaluations with farmers were conducted in August 2003 (max. rainfall.), March 2004 (min. rainfall) and July 2004 (max. precip) in each case 6 weeks after standardization cut (Figure 25).

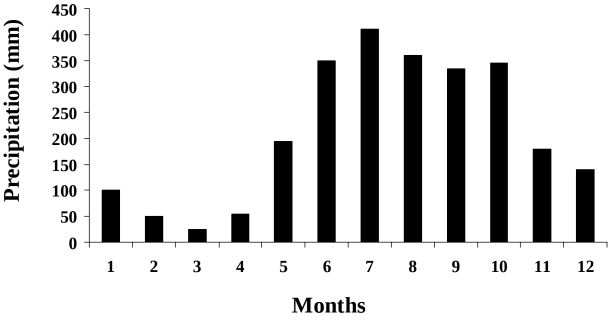


Figure 25. Rainfall distribution at the experimental site of Ubú Norte, Nicaragua.

Photo 14. *Brachiaria* accessions and hybrids tested on acid soils in Ubú Norte, Nicaragua.

Results and Discussion

Evaluation 2003: Results from agronomic evaluations showed no significant fertilizer effect on plant height, soil cover and dry matter yield (Table 22). Significant differences were found among accessions/ hybrids, but no fertilizer x accession/hybrid interactions were detected.

Data obtained in 2003 showed *Brachiaria brizantha* cv. Toledo (CIAT 26110) and *Brachiaria* hybrid cv. Mulato (CIAT 36061) as the top ranking accessions with regard to dry matter yield, followed by *B. brizantha* CIAT 26124 and *B. brizantha* cv. Marandú (CIAT 6780). The lowest yields were obtained with *B. humidicola* CIAT 679 and *B. humidicola (dictyoneura)* CIAT 6133 due to their slow establishment. Best soil cover was observed in plots with *B. hybrid* cv. Mulato (CIAT 36061), *B. brizantha* “Mixe”, *B. brizantha* CIAT 26124, and *B. brizantha* cv. Marandú (CIAT 6780). Plant height was greater with *B. brizantha* cv. Toledo (CIAT 26110) and *B. brizantha* cv. Marandú (CIAT 6780). *B. humidicola* CIAT 679 did not recover well from the standardization cut and was discarded from the experiment in 2004.

Table 22. Plant height, soil cover and DM yield of 14 *Brachiaria* accessions and hybrids in Ubú Norte, Nicaragua, 2003-2004.

Fertility Levels/ Accessions	2003						2004		
	Max			Min			Max		
	Plant height (cm)	Soil Cover (%)	DM yield (g/m^2)	Plant height (cm)	Soil Cover (%)	DM yield (g/m^2)	Plant height (cm)	Soil Cover (%)	DM yield (g/m^2)
Fert. Level									
- High *	88	64	802	56	73	310	76	75	338
- Low	82	60	747	54	69	283	71	67	303
No. CIAT									
606	90	76	578	56	73	244	73	82	329
654	71	48	538	39	52	239	55	37	188
679	32	17	-	-	-	-	-	-	-
6133	55	30	339	37	46	234	55	65	347
6780	126	89	1000	40	78	171	65	91	244
16322	98	88	646	72	84	252	94	88	348
26110	108	65	1682	72	70	375	76	64	363
26124	99	89	1239	64	67	264	75	66	320
26318	90	34	854	62	47	359	61	17	342
26646	75	48	505	65	70	305	76	76	275
26990	87	60	695	64	53	357	73	48	277
36061	99	93	1546	52	74	305	69	81	308
36062	70	32	440	52	76	322	54	49	253
“Mixe”	89	90	775	56	81	331	77	86	355
Mean Acc.	85	61	834	56	67	289	69	65	304
LSD (0.05)	31	30	645	13	24	190	21	30	263

(*Differences at fertilization levels were not significant at $p \leq 0.05$)

Results obtained during the period of low rainfall showed no fertilizer effect on agronomic parameters. Best performing accessions were *B. brizantha* cv. Toledo (CIAT 26110), *B. brizantha* CIAT 26318, *B. brizantha* CIAT 26990, and *B. brizantha* “Mixe”. In early 2004, Rhizoctonia foliar blight detected in *Brachiaria* hybrid cv. Mulato, had a negative effect on dry matter production. Plots of *B. brizantha* CIAT 16322 developed very well in the period of low rainfall and outperformed other accessions in plant height and soil cover, indicating good adaptation to prevailing environmental conditions. Dry matter yield, however, was below average because of the very fine leaf structure of this accession.

Evaluation 2004: Due to severe reduction in rainfall during the first semester of 2004 in Nicaragua, dry matter yields were significantly lower than in 2003. The highest yields were recorded for *B. brizantha* cv. Toledo (CIAT 26110), *B. brizantha* “Mixe”, *B. brizantha* CIAT 16322, and *B. brizantha* CIAT 26318. *Brachiaria* hybrid cv. Mulato showed good soil cover and above average dry matter yield. *B. brizantha* cv. Marandú (CIAT 6780), widely planted in the area of Ubú Norte, established well and showed good adaptation, soil cover and height, especially in the first year, but in subsequent evaluations dry matter yield was below average. Apart from the Rhizoctonia foliar blight attacks no significant or limiting pest and disease incidents were recorded throughout the experiment.

Participatory evaluations with farmers: Prior to agronomic data collection, farmer groups evaluated the plots in accordance with their own criteria. Their preference rankings are summarized in Table 23.

The main criteria applied by farmers throughout the experiment were: plant height, soil cover, foliage production, and ease of establishment, leaf size and color. While the high ranking of

Table 23. Preference ranking of *Brachiaria* accessions and hybrids at Ubú Norte, Nicaragua (2003-2004) (Max = Maximun rainfall; Min = Minimum rainfall).

Max 2003	Min 2003	Max 2004
B. brizantha cv Marandú	*B. brizantha* cv. Toledo	*B. brizantha* cv. Toledo
B. brizantha cv. Toledo	*B. brizantha* cv Marandú	*B. hybrid* cv. Mulato
B. hybrid cv. Mulato	*B. brizantha* “Mixe”	*B. brizantha* cv Marandú
B. brizantha CIAT 26990	*B. hybrid* cv. Mulato	*B. brizantha* “Mixe”
B. brizantha CIAT 26124	*B. brizantha* CIAT 16322	*B. brizantha* CIAT 16322
B. brizantha CIAT 16322	*B. brizantha* CIAT 26646	*B. brizantha* CIAT 26318

B. brizantha cv. Marandú (CIAT 6780) was somewhat expected, the cultivar is known in the area for years and well-adapted to the prevailing conditions, *B. brizantha* cv. Toledo (CIAT 26110), *Brachiaria* hybrid cv. Mulato (CIAT 36061) were preferred because of their abundant foliage with bright green leaves. The fact that both materials were sold on the seed market could have influenced the ranking. Accessions such as *B. brizantha* CIAT 26990, 26124, 16322 were often classified as less productive because of their leaf size. Most other materials were rated low due to low soil cover or plant height.

As mentioned earlier, farmer rankings could have been influenced to some extent by the active presence of a livestock project in the area promoting *B. brizantha* cv. Toledo and *B. hybrid* cv. Mulato. The main difficulty during the participatory evaluation was the large number of accessions to be ranked. Some farmers had difficulties to keep track of all plots. In the future, smaller number of materials should be presented to farmers in order to avoid confusion, especially with farmers who do not have experiences with ranking of forage germplasm.

Although the chosen experimental site represented an acid soil region of Nicaragua, soil pH and aluminum contents were not limiting factors for the tested accessions/hybrids. Since real drought conditions did not prevail throughout the experiment, hybrids such as cv. Mulato could not express their full potential, but did show susceptibility to Rhizoctonia foliar blight. Nevertheless farmers seem to appreciate the Mulato hybrid, since seed is increasingly available to them. *Brachiaria brizantha* accession CIAT 16322 and *Brachiaria brizantha* “Mixe” are high-potential materials due to their excellent adaptation and growth. Their small leafs might be a decisive factor for their adoption by farmers.

3.1.3 Edaphic adaptation of *Arachis pintoi*

3.1.3.1 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

Based on field studies conducted in Caqueta, Colombia and the data collected from multilocational evaluation, we have assembled a set of 8 genotypes for further testing at Piedmont 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 main objective of this work was to identify plant attributes related to superior adaptation of the most promising accessions for the llanos of Colombia.

Materials and Methods

A field trial was established in Piedmont in May, 2001 as monoculture. The trial included 8 accessions of *Arachis pintoi* (CIAT 17434; 18744; 18747; 18748; 18751; 22159; 22160 and 22172). The trial was 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. Genotypic differences in agronomic performance were determined at 42 months after establishment (December 2004) at low and high initial fertilizer application. Maintenance fertilizer at half the level of initial applications was applied at 26 months after establishment (August 2003).

Results

At 42 months after establishment of the trial, the response in terms of shoot biomass production with fertilizer application was greater with CIAT 22160 (Figure 26). Overall the performance of CIAT 18744 and CIAT 18751 and CIAT 22159 was better than the other accessions. These results are consistent with the observations made last year. Shoot TNC content was markedly greater in CIAT 18751 under both low and high initial fertilizer application (Tables 24 and 25).

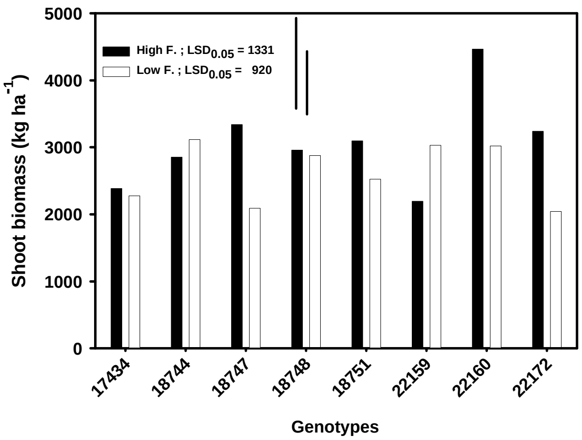


Figure 26. Genotypic variation in forage yield of 8 accessions of *Arachis pintoi* at 42 months after establishment (December, 2003) in forage yield (kg/ha) as influenced by initial level of fertilizer application to a clay loam oxisol at La Libertad, Piedmont.

Shoot ash (mineral) content was higher in CIAT 22172 under both low and high initial fertilizer application (Tables 24 and 25).

The superior performance of CIAT 18744 was associated with greater TNC (Total Non-structural Carbohydrates) and lower ash content in the shoot biomass under low initial fertilization indicating greater nutrient use efficiency for producing green forage. Measurements of nutrient uptake indicated that CIAT 22160 was outstanding with high initial fertilizer application

Table 24. Shoot biomass, cover, vigor and shoot total nonstructural carbohydrates (TNC) content and shoot ash content in 8 *Arachis pintoi* accessions evaluated with high initial fertilizer application to low fertility acid soils at La Libertad (December 2003).

Accession	Shoot biomass (kg ha^{-1})	Cover (%)	Vigor	Shoot TNC (mg g^{-1})	Shoot ash (%)
17434	2384	98	3.0	79.3	8.073
18744	2853	100	4.3	79.3	7.867
18747	3339	100	4.3	56.0	8.367
18748	2959	100	4.0	81.3	7.693
18751	3095	100	4.0	102.1	7.527
22159	2193	100	4.0	63.1	7.967
22160	4464	100	4.3	73.2	7.600
22172	3240	98	4.0	67.1	8.400
Mean	3066	99.5	4.0	75.2	7.937
LSD(P>0.05)	1331	NS	0.66	NS	NS

Table 25. Shoot biomass, cover, vigor and total nonstructural carbohydrates and ash content in 8 *Arachis pintoi* accessions evaluated with low initial fertilizer application to low fertilizer on low fertility acid soils at La Libertad (December 2003).

Accession	Shoot biomass (kg ha^{-1})	Cover (%)	Vigor	Shoot TNC (mg g^{-1})	Shoot ash (%)
17434	2276	100	3.3	90.7	8.033
18744	3114	100	4.0	135.9	7.240
18747	2088	98	3.7	64.2	7.673
18748	2878	97	3.3	84.3	7.927
18751	2525	100	4.0	125.1	7.040
22159	3028	100	4.0	69.7	7.560
22160	3021	100	4.3	100.8	7.373
22172	2043	97	3.0	65.1	8.187
Mean	2622	99	3.7	92.0	7.629
LSD (P>0.05)	920	NS	0.71	NS	0.611

while CIAT 22159 was superior with low initial fertilizer application (Tables 26 and 27).

Correlation coefficients between green forage yield and other shoot attributes indicated that the superior performance with low initial fertilizer application was associated with lower level of Ca content in the shoot tissue (Table 28).

Results from this field study indicated that after 4 years, the *Arachis pintoi* accessions CIAT 18744, 18751 and 22159 are superior to the commercial cultivar (CIAT 17434) in terms of persistence with low amounts of initial fertilizer application.

Table 26. Shoot N, P, K, Ca and Mg uptake of 8 *Arachis pintoi* accessions evaluated with high initial fertilizer application to low fertility acid soils at La Libertad (December 2003).

Accession	Shoot nutrient uptake (kg ha^{-1})				
	N	P	K	Ca	Mg
17434	65.5	7.5	32.5	51.0	23.3
18744	76.6	8.5	35.8	55.3	27.9
18747	88.6	10.1	46.9	84.8	34.2
18748	75.2	7.9	34.2	68.3	28.5
18751	80.0	8.0	31.0	70.7	28.0
22159	63.2	7.9	29.4	47.8	21.4
22160	113.6	12.0	59.6	90.2	43.9
22172	90.6	10.0	47.0	75.5	31.5
Mean	81.7	9.0	39.5	67.9	29.8
LSD(P>0.05)	28.8	3.1	23.5	NS	13.6

Table 27. Shoot N, P, K, Ca and Mg uptake of 8 *Arachis pintoi* accessions evaluated with low initial fertilizer application on low fertility acid soils at La Libertad (December 2003).

Accession	Shoot nutrient uptake (kg ha^{-1})				
	N	P	K	Ca	Mg
17434	60.0	7.3	26.7	52.6	23.9
18744	79.8	9.7	31.5	55.8	36.8
18747	61.4	7.1	20.2	48.3	19.8
18748	78.0	8.4	19.9	70.0	32.1
18751	64.3	8.2	27.5	47.2	21.8
22159	91.1	11.7	35.6	61.7	29.9
22160	75.2	9.0	21.2	62.3	35.2
22172	56.1	5.4	15.7	55.5	23.1
Mean	70.7	8.3	24.8	56.7	27.8
LSD (P>0.05)	24.7	3.0	14.0	NS	12.4

Table 28. Correlation coefficients (r) between green forage yield (t/ha) and other shoot traits of *Arachis pintoi* genotypes grown with low or high initial fertilizer application in a low fertility Oxisol in La Libertad, Colombia.

Shoot traits	Low fertilizer	High fertilizer
Soil cover (%)	0.20	-0.05
Vigor (visual scale)	0.44*	0.55**
Shoot N content (%)	-0.13	-0.49**
Shoot P content (%)	-0.06	-0.58**
Shoot K content (%)	0.11	0.28
Shoot Ca content (%)	-0.45*	0.04
Shoot Mg content (%)	0.13	-0.17
Shoot TNC content (mg g^{-1})	0.05	-0.01
Shoot ash content (%)	-0.29	-0.02

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

3.1.3.2 Genotypic variation in *Arachis pintoi* for tolerance to low P supply

Contributors: N. Castañeda, N. Claassen (University of Goettingen, Germany) and I. M. Rao (CIAT)

Rationale

Several studies conducted in Colombia as well as in Goettingen confirmed that *Arachis pintoi* genotypes showed a different growth potential when grown in Ultisols or Osixols with extremely low P availability. A growth chamber study conducted last year in Goettingen indicated significant genotypic difference in P acquisition among the accessions CIAT 17434, CIAT 18744 and CIAT 22172. Therefore this P acquisition was related to an increase of P soil solution concentration with CIAT 18744 i.e. to P mobilization in the rhizosphere. These results indicated that the rapid establishment as well as the sustained yield of CIAT 18744 and 22172 was due to their great ability to acquire P from P-deficient soil per unit root length. There was no significant difference among the genotypes in their ability to utilize acquired P. This year, we continued our efforts to determine the physiological basis of differences in P influx between the 2 accessions, CIAT 17434 and 18744.

Material and Methods

Plant cultivation - Germinated seedlings in sand of *Arachis pintoi* CIAT 17434 and 18744 were pre-cultured in –P nutrient solution for 5 days and then the taproot was excised to develop lateral roots for additional 15 days till the lateral roots had reached about 5 cm long. The 20-day-old seedlings were transplanted to pots divided in three compartments (Figure 27) and placed on the dividing walls and their roots split so that 50% grew in soil and 50% in sand.

Each one of the outer compartments was filled with 2 kg of air dry clay loam fossil Oxisol (clay 50%, organic carbon 0.35%, pH_{CaCl2} 5.1, pH_{H2O} 5.2, P-CAL 0.4 mg /100 g soil and P-Bray II 1.4 mg/100 g soil, Fe/Al-P 788 mg kg^{-1} and Ca-P 330 mg kg^{-1}; in soil solution pH 4.9 and 0.1 µM P) from Lich in the Vogelsberg area (Hessen – Germany). The middle compartment was filled with 3 kg air dry sand (size 3-5 mm) in which P were removed using 5% HCl solution and then washing the sand till getting the same pH of distilled water. The sand compartment was watered three times per day with nutrient solution without P. Its composition (M) was: $Ca (NO_3)_2$ 5.0×10^{-3}, KCl 5.0×10^{-4}, K_2SO_4 3.5×10^{-3}, $MgSO_4$ 2.5×10^{-3}, H_3BO_3 5.0×10^{-5}, $MnSO_4$ 5.0×10^{-6}, $ZnSO_4$ 2.5×10^{-6}, $CuSO_4$ 1.0×10^{-6}, $(NH_4)_{6Mo7O24}$ $5.0\times10_{-8}$, and FeEDTA 1.0×10^{-4}. This compartment was allowed to percolate water through a hole in the bottom which were later leached the root exudates. Two levels of P supply (0, and 1000 mg kg^{-1}) as $Ca (H_2PO_4)_{2.H2O}$ were given only in the soil compartments. Basal nutrients were applied (mg kg^{-1}) every 30 days to each soil compartment: 50 K as K_2SO_4, 40 Mg as $MgSO_4$, 0.2 B as H_3BO_3, 0.1 Mo as $(NH_4)_{6Mo7O24}$ and 100 N as $Ca (NO_3)_2$. The soil surface in each soil compartment was covered with a layer of quartz sand (1 to 2 cm) to avoid the formation of a superficial crust due to the watering. Two weeks before transplanting, water was added to get moisture content of 25% w/w. One pot for each P treatment was left unplanted to measure soil moisture evaporation losses and as control of P dynamic under unplanted conditions. The pots were watered daily and water was added to

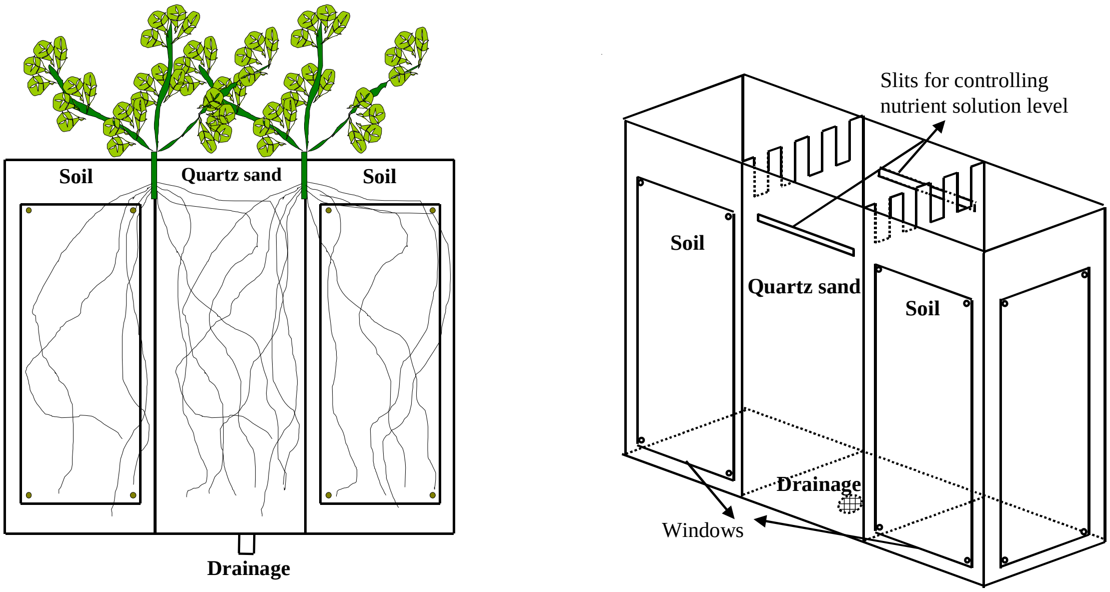


Figure 27. Schematic representation of plant growing technique with split root system.

maintain the soil with 60% of its water holding capacity by weight. The plants were grown in a growth chamber, maintained at 25°C, with a photon flux density of 300 µmol m^{-2} s^{-1} and 80% relative humidity during 16 h day and at 20 °C and 70% relative humidity during 8 h night.

There were four replicates for each treatment. The pots were completely randomized and re-positioned weekly to minimize any effect of uneven environmental factors.

Collection of root exudates using a percolation system - Prior to collection of root exudates, the sand compartment was thoroughly washed with distilled water until removing nutrient ions, specially NO_3, which affects accurate determination of organic acids by HPLC. The sand compartment drainage's was closed and then filled with bi-distilled water for 1 hour. After that the leached root exudates were collected to avoid O_2 stress (deficiency) in the roots and immediately the sand compartment was again filled with the collected root exudates for one hour. Finally, leaving the drainage open, the collected root exudates were once more added to the sand by a pressure bottle in order to leach all as possible root exudates from the sand. The collected root exudates were lyophilized to concentrate them and after that the dry root exudates were diluted in 1 mL bi-distilled water and put into 1.5 mL Eppendorf reaction vials. Thereafter, they were subjected to shaking for three-times (30 seconds each) for extraction of carboxylates. The aliquot was centrifuged at 2000g for 10 min. The supernatant was collected by a micropipette and stored at –20ºC for analysis of organic acid anions by HPLC.

Analysis of carboxylates - The organic acid anions in root exudate samples were analyzed by reversed phase HPLC in the ion suppression mode. Separation was conducted on a 250 × 4 mm reversed phase column (LiChrospher 100 RP-18, 5 µm particle size) equipped with a 4 × 4 mm LiChrospher 100 RP-18 guard column (Merck, Darmstadt, Germany). Sample solutions (100 µL) were injected onto the column, and 18 mM KH_2PO_4 adjusted to pH 2.2 with H_3PO_4 was used for isocratic elution, with a flow rate of 0.25 mL min-1 at 24°C and UV detection at 210 nm. Identification of organic acids was performed by comparing retention times and absorption spectra with those of known standards.

Determination of root surface APase activity - Excised segments (2-3 root tips at 1.5-2 cm) of root tips were harvested in the soil compartments and transferred to 1.5 mL Eppendorf reaction vials. The root segments were washed 2 times with distilled water for 5 min to remove contents of wounded cells, followed by adding 0.5 mL water, 0.4-mL Na-Ac buffer and 0.1 mL pNPP substrate. After a reaction time of 10 min at 25-30°C, an aliquot of 0.8 mL of the reaction medium was taken out and mixed with 0.4 mL of 0.5 M NaOH to terminate the reaction. The absorption of reaction solution was measured at 405 nm. The fresh weight of excised segments of root tips was recorded after determination of APase activity.

Quantitative determination of the pH values at the root surface with the antimony electrode In the soil compartments, the pH values at the root surface of young and old root were measured potentiometrically with an antimony electrode.

Plant and soil harvests - The plants were harvested as separate roots and shoots at 30, 60 and 100 days after transplanting (DAT) into split-root pots. The dry weight was determined by drying the shoots and roots in an oven at 65% for 1 day and then at 105°C till constant weight. After grinding, the plant material was used for determination of nutrient composition. To determine P concentration in plant tissue, shoot and root samples were wet digested in HNO_3 and P was determined with Molybdate-Vanadium method.

Shoot P uptake, shoot growth rate, P acquisition efficiency (mg of P uptake in shoot biomass per unit root length), P use efficiency (g of forage production per g of total P acquisition), and P-Influx (the amount P taken up per unit of root and time) were determined. Soil solution pH value was measured directly. Soil pH was determined in 0.01 M $CaCl_2$ in soil to solution ratio of 1:2.5. Data were subjected to an analysis of variance using the SAS computer program. Least-significant differences were calculated by an tuckey-test. A probability level of 0.05 was considered statistically significant.

Results and Discussion

At high P supply (P-1000) the shoot yield (Figure 28) and the shoot P concentration of both genotypes was similar. i.e. the growth potential of both genotypes is the same. But under low P availability, the shoot dry matter yield and shoot P concentration were different between the genotypes. Since under sufficient P condition the genotypes show the same growth, the difference at P-0 is because they have different P efficiency (Figure 29). At 30 DAT, the two genotypes had different shoot P contents (Figure 30) under low P availability and at 100 DAT the P content in the shoot of CIAT 18744 is 5 times higher than that of CIAT 17434. This shows, that the high shoot dry matter yield by CIAT 18744 was due to its high P uptake.

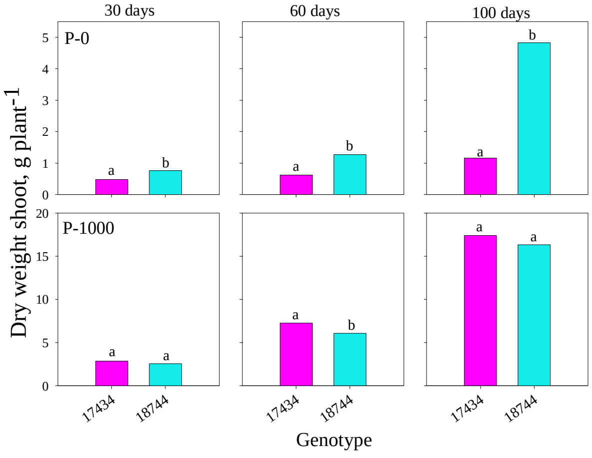


Figure 28. Influence of phosphorus supply (P-0 and P-1000) on genotypic differences in shoot biomass (forage) production of 2 accessions of *Arachis pintoi* grown for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different ($P<0.05$) if letters above bars are different within P supply level at a given age. DAP = days after planting.

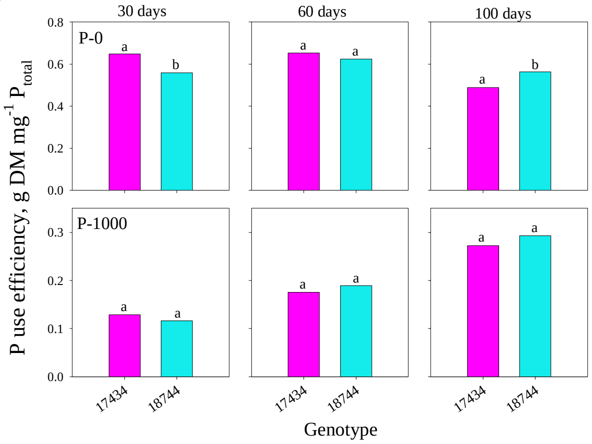


Figure 29. Influence of phosphorus supply (P-0 and P-1000) on genotypic differences in P use efficiency of 2 accessions of *Arachis pintoi* grown for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different ($P<0.05$) if letters above bars are different within P supply level at a given age.

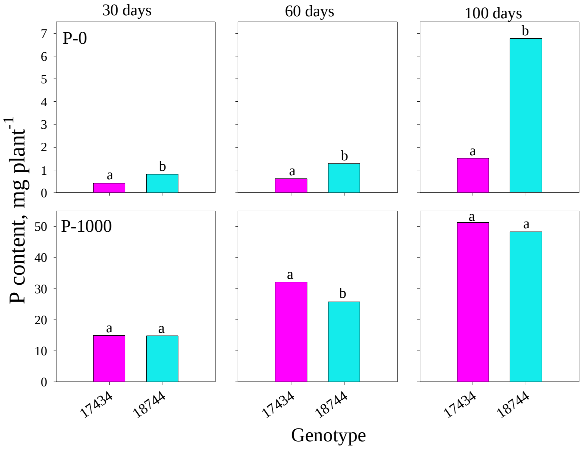


Figure 30. Influence of phosphorus supply (P-0, P-50 and P-1000) on genotypic differences in shoot P content of 2 accessions of *Arachis pintoi* grown for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different ($P<0.05$) if letters above bars are different within P supply level at a given age.

Differences in P uptake by a plant could be due to differences in the size of the root system and/or differences in P influx i.e. the amount taken up per unit of root and time. As a measure of the size of the root system, we used the root length-shoot biomass ratio (Figure 31). CIAT 18744 showed the lower ratio at each harvest. Thus the high shoot P content, of the efficient genotype, was not associated with more roots but to a greater P influx. The P influx (ability of plant P uptake per unit length of the root per unit time) was determined for the period between 30 and 60 days and 60 and 100 days. The P influx by the CIAT 18744 was markedly greater than that of CIAT 17434 (ca. 2 times and 3 times for the first and second harvest, respectively).

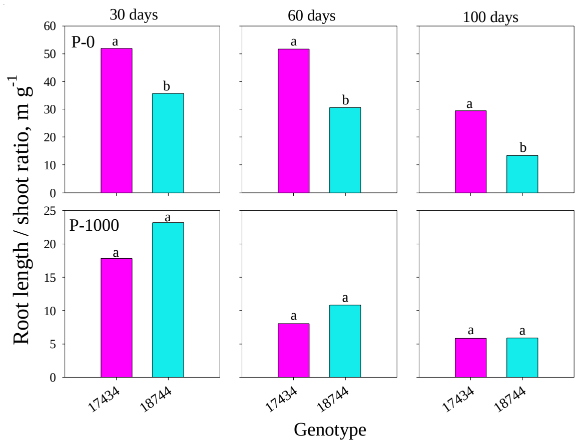


Figure 31. Influence of phosphorus supply (P-0 and P-1000) on genotypic differences in root length/shoot biomass ratio of 2 accessions of *Arachis pintoi* grown for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different ($P<0.05$) if letters above bars are different within P supply level at a given age.

With age, the P influx increases 4 times higher for CIAT 17434 and 6 times higher for CIAT 18744 (Figure 32). One possible reason for high P influx could be due to increase in P absorbing surface area per cm root that was infected by hyphae of native arbuscular-mycorrhizae (AM). Only at 100 DAT and -under low P availability, CIAT 18744 had a higher colonization of native AM than CIAT 17434 (Table 29), which may be the reason for the higher P influx of CIAT 18744, during the second growth period. However, the infection found after 30 and 60 DAT cannot explain the P influx differences between the genotypes, at 30 DAT.

To explore further possible reasons for differences in P influx between the two accessions, we investigated the acid phosphatase (APase) acitivity on the root surface and exudation of organic acids from roots. At each growth stage, CIAT 18744 had a lower APase activity at the root surface than the CIAT 17434 (Figure 33).

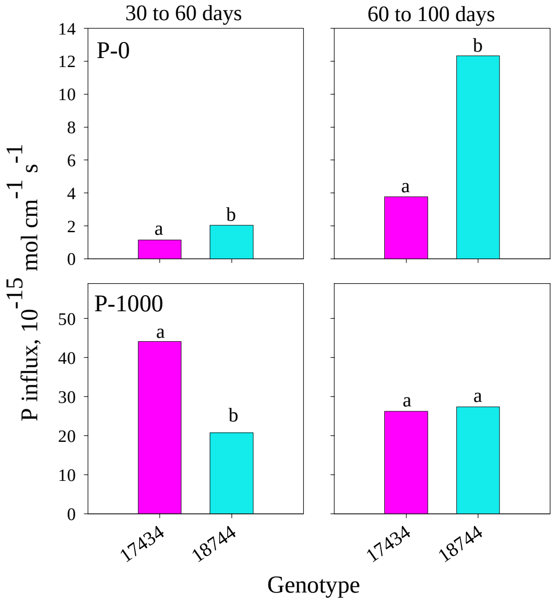


Figure 32. Influence of phosphorus supply (P-0 and P-1000) on genotypic differences in P influx of 2 accessions of *Arachis pintoi* grown for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different ($P<0.05$) if letters above bars are different within P supply level at a given age.

Table 29. Influence of P supply on colonization of roots by mycorrhizae.

P-level	Genotype	Colonization %		
		30 d	60 d	100 d
P-0	17434	< 1	< 5	25 a
	18744	< 1	< 5	56 b
P-1000	17434	< 1	< 1	5 a
	18744	< 1	< 1	12 a

Thus the APase activity may not have contributed to either the high P influx of the efficient genotype or the increase of P influx with age. The P absorbing surface area and the hydrolysis of organic P cannot explain the higher P influx by CIAT 18744.

Another possibility could be that the plants mobilise P, i.e. increase P concentration in soil solution. P concentration in soil solution of unplanted and planted soil was also determined (Figure 34).

After 30 days of planting, the P concentrations for all were approximately 0.1 µM, which is

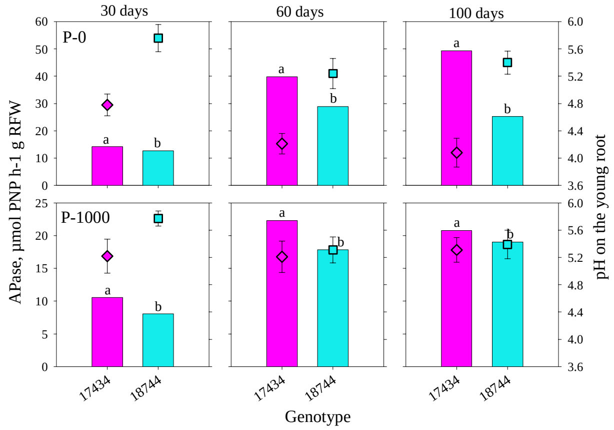


Figure 33. Influence of phosphorus supply (P-0 and P-1000) on genotypic differences on APase activity at the root surface and pH on the young root of 2 accessions of *Arachis pintoi* grown for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different ($P<0.05$) if letters above bars are different within P supply level at a given age.

below CLmin found for *Arachis hypogea*. At 60 DAT, especially at 100 DAT, the P concentration of the planted soil increased markedly and the efficient genotype showed the highest P concentration. It was 6 times higher than that of the unplanted soil

P mobilization has very often been related to organic acids exuded by roots. Twelve different organic acids were analyzed from the collected root exudates in which only lactic acid and acetic acid were detected in significant amounts with the HPLC. Organic acids, like citric acid or malic acid, that are often shown to be associated with P mobilization, were only found in trace amounts (Figure 35).

The observed organic acids were related to the P soil solution concentration. The highest exudation rate was associated with the lowest P concentration in soil solution with CIAT 17434, and the increase of the P concentration with age was associated with a decrease of organic acid exudation. Thus the organic acid exudation by the genotypes could not explain the increase of P concentration in soil solution. Moreover, monocarboxylic acids such as lactic or acetic acid have not been associated with P mobilization.

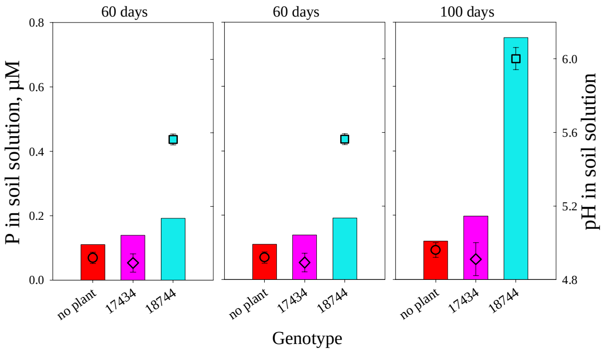


Figure 34. Influence of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* on P concentration in soil solution and pH in soil solution when grown with no phosphorus supply (P-0) for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different ($P<0.05$) if letters above bars are different within P supply level at a given age.

Further research work is necessary to identify the physiological mechanisms responsible for the high P uptake observed with the *Arachis pintoi* CIAT 18744.

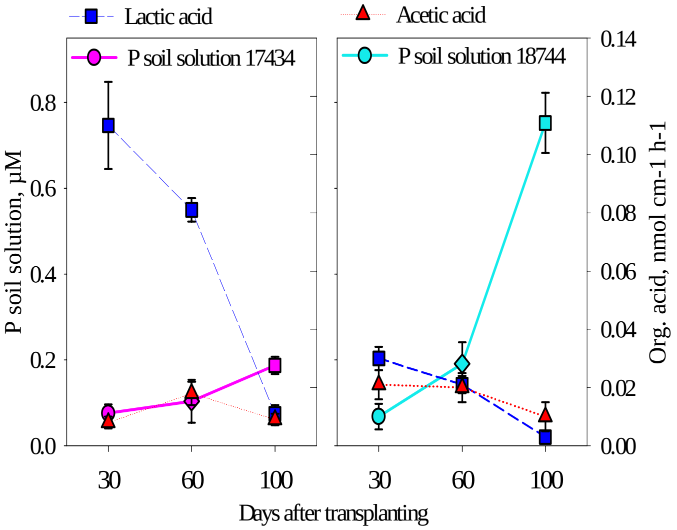


Figure 35. Influence of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* on P concentration in soil solution and organic acid exudation from roots when grown with no phosphorus supply (P-0) for 30, 60 and 100 days in a clay loam oxisol in a growth chamber. Means are different ($P<0.05$) if letters above bars are different within P supply level at a given age.

3.2 Genotypes of *Brachiaria* with dry season tolerance

Highlights

- Found that total nonstructural carbohydrate (TNC) content in stems of *Brachiaria* genotypes increased with increasing drought stress in large plastic soil cylinders under greenhouse conditions.

- Found that *Brachiaria* hybrid Mulato 2 is superior to cv. Mulato in terms of dry season tolerance under low fertility acid soil conditions in the Llanos of Colombia. This was associated with a greater proportion of fine roots in the top 5 cm of the soil profile.

3.2.1 Genotypic differences in root distribution and drought tolerance of 2 hybrids and 3 parents

Contributors: J. Rincón, J. Polania, F. Feijoo, R. García, J. Ricaurte, J. W. Miles and I. M. Rao (CIAT)

Rationale

Identification of shoot and root attributes that are associated with superior drought adaptation will help to develop rapid and reliable screening methods. These methods are needed to develop *Brachiaria* hybrids that combine drought adaptation with other desirable attributes. Field studies conducted for the past few years in the Llanos of Colombia indicated that one of the *Brachiaria* hybrids, CIAT 36087 (FM9503-S046-024 or Mulato 2) is superior to its parents and other hybrids. This hybrid maintained greater proportion of green leaves during dry season under field conditions. A greenhouse study was conducted to characterize shoot and root responses of this hybrid in comparison to its parents and another hybrid, cv. Mulato when subjected to moderate and severe drought stress conditions.

Materials and Methods

A greenhouse study was conducted using a sandy loam oxisol from Matazul farm in the Llanos of Colombia. The trial comprises 5 entries, including three parents (*B. decumbens* CIAT 606, *B. brizantha* CIAT 6780, *B. ruziziensis* 44-02) and two hybrids (cv. Mulato and CIAT 36087). Plants were grown in large plastic cylinders (100 cm long and 15 cm diameter) covered with PVC tubes (Photo 15).

The trial was planted as a randomized block in split-plot arrangement with three levels of water supply: 100% field capacity (well-watered), 60% field capacity (moderate drought stress) and 30% field capacity (severe drought stress) as main plots and genotypes as sub-plots. Soil was fertilized with adequate level of nutrients (kg/ha of 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S and micronutrients).

Treatments of water stress were imposed after three weeks of initial growth of plants established with seed. Water stress was maintained by weighing each cylinder every week and applying water to the soil at the top of the cylider. After 2 months of stress treatment (at 85 days after germination), shoot biomass distribution, root

Photo 15. A large plastic cylinder showing root distribution across soil depth.

biomass and root length distribution in different soil depths, and leaf and stem nutrient composition, ash content and TNC (total nonstructural carbohydrates) contents were determined.

Results and Discussion

Reducing the water supply to 30% of field capacity markedly decreased the leaf, stem and root biomass of the three parents and the two hybrids (Figure 36). Leaf biomass of CIAT 606 was lower than the other genotypes tested at all three levels of water supply. Differences between the two hybrids (CIAT 36061 and CIAT 36087) in leaf biomass were not significant within each level of water supply.

Results on root length distribution showed that the hybrid Mulato 2 had a greater proportion of fine roots in the top 5 cm of soil profile at all 3 levels of water supply (Figure 37). Higher values of root length observed for 50 to 100 cm soil depth in some genotypes at 60% and 100% of field capacity indicates the growth of roots on the surface of the plastic tube and reaching to the bottom of the cylinder. We also noted problems of compaction in some cylinders. Therefore we conducted some additional studies to overcome these problems and found that use of 2:1 of soil and sand in smaller plastic tubes (50 cm long and 5 cm diameter) could overcome some of the problems encountered with large cylinders. Use of small cylinders will also facilitate evaluation of a larger number of genotypes.

Results on the determination of N, P, ash (mineral) and TNC contents in leaves and stem tissue indicated that water stress could markedly increase stem TNC content (Figure 38). Severe water stress also increased stem N, P and ash contents. But leaf N, P, ash and TNC contents were not markedy influenced by water stress conditions.

Work is in progress to evaluate the usefulness of fine root production in top soil, root penetration in subsoil and % increase of stem TNC as indicators of drought tolerance and green leaf production in *Brachiaria*.

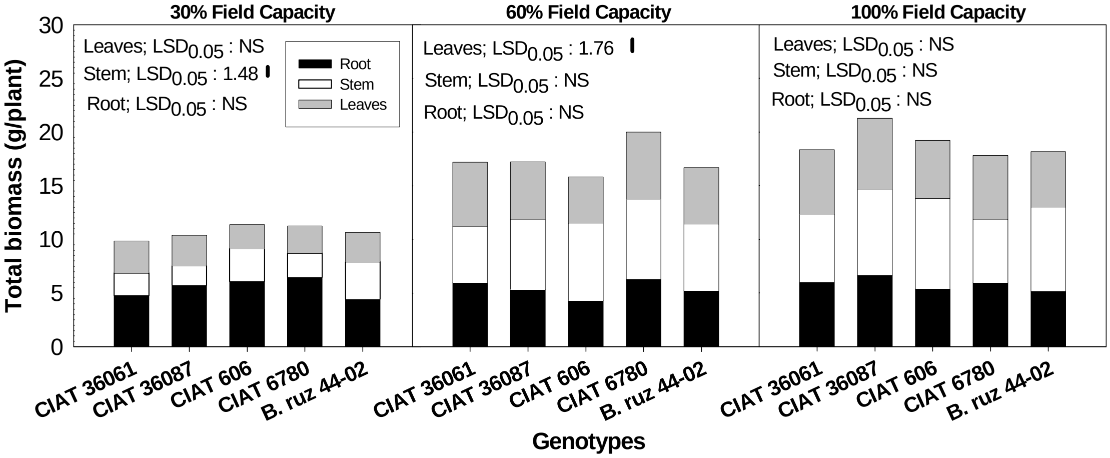


Figure 36. Influence of three levels of water supply (100%, 60% and 30% of field capacity) on dry matter distribution among leaves, stem and roots of five *Brachiaria* genotypes.

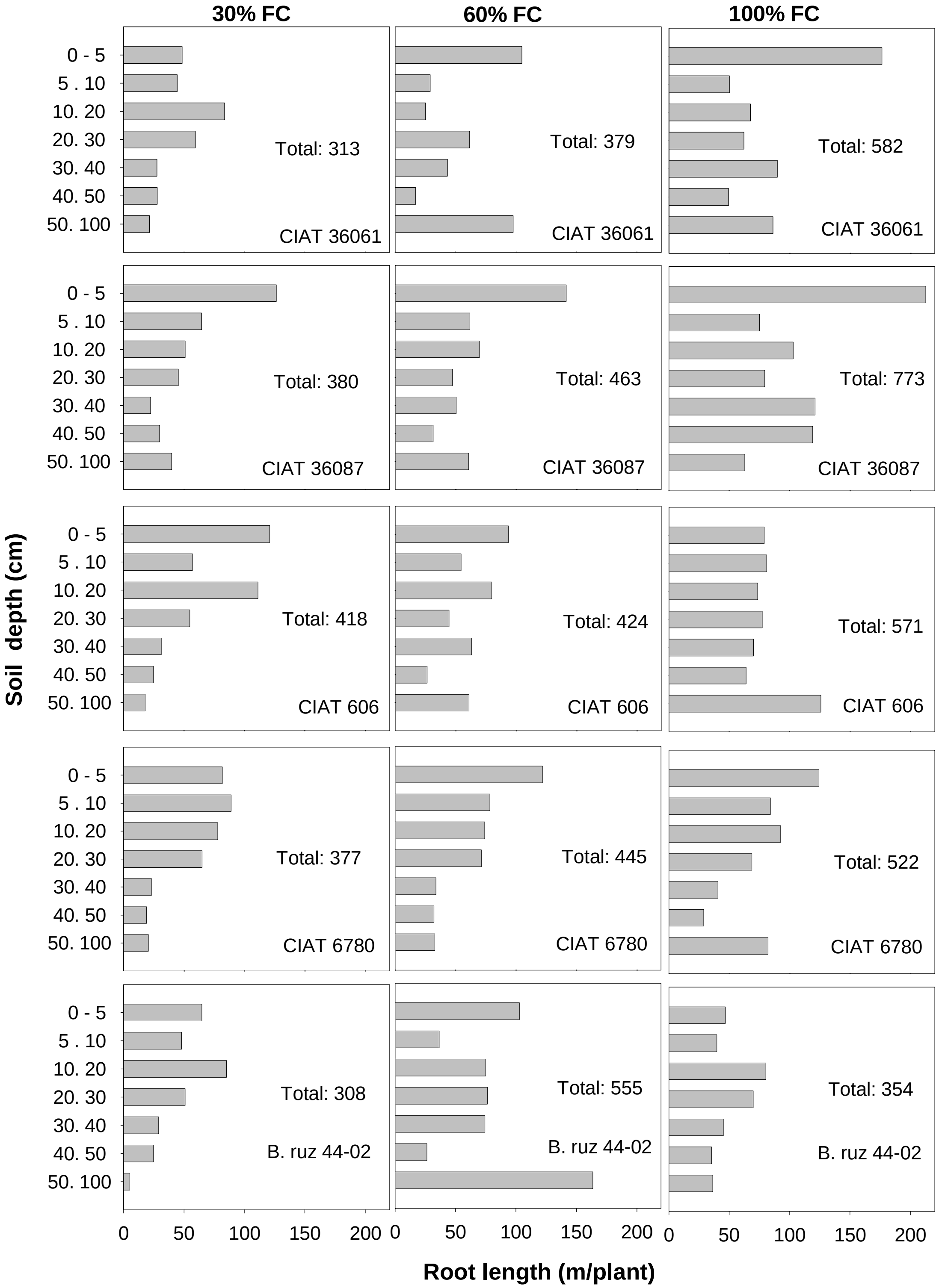


Figure 37. Influence of three levels of water supply (100%, 60% and 30% of field capacity) on root length distribution across soil depth in five *Brachiaria* genotypes.

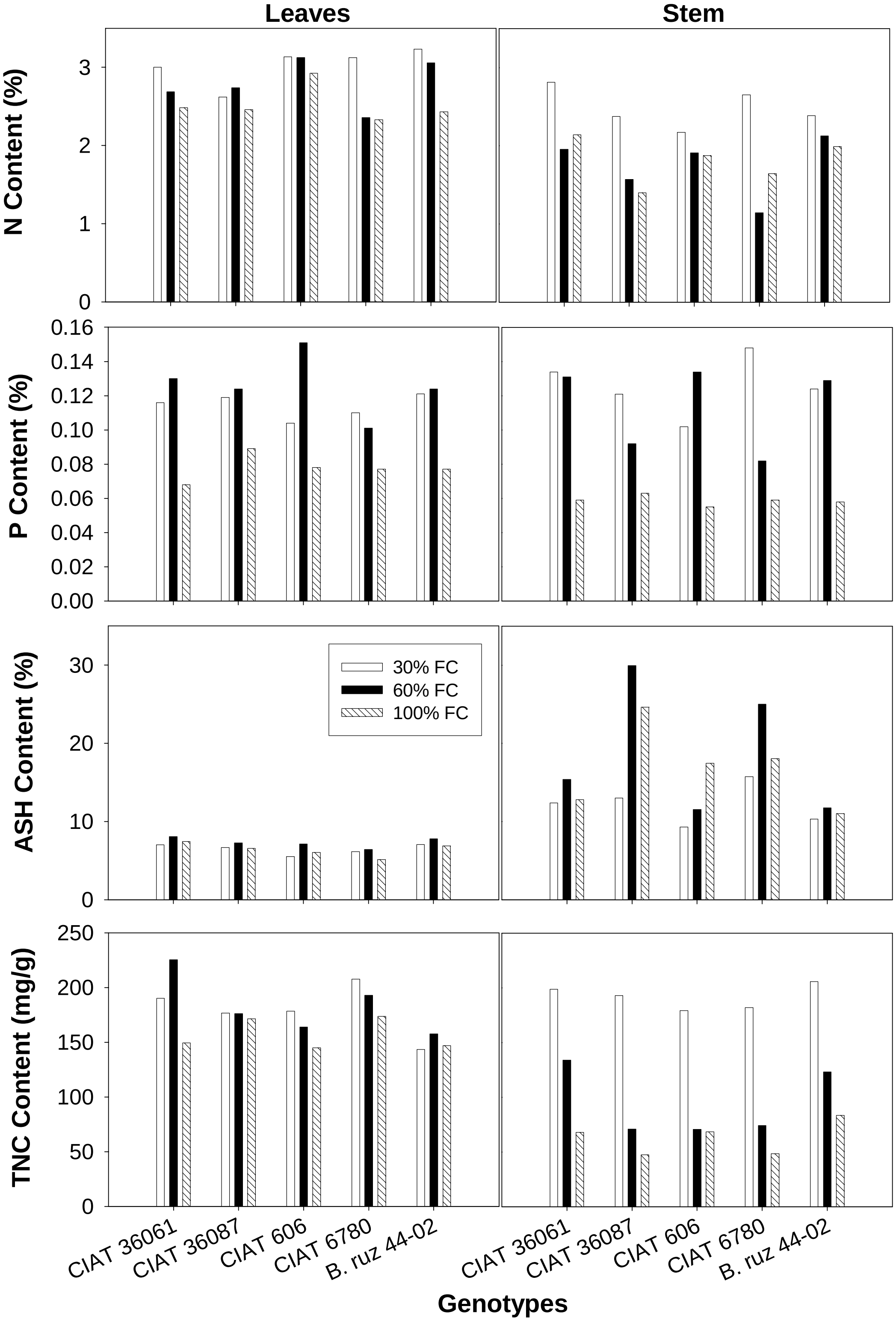


Figure 38. Influence of three levels of water supply (100%, 60% and 30% of field capacity) on leaf and stem N, P, ash and TNC contents of five Brachiaria genotypes.

3.2.2 Genotypic variation in dry season tolerance in *Brachiaria* accessions and hybrids in the Llanos of Colombia

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

Rationale

A major limitation to livestock productivity in subhumid regions of tropical America is quantity and quality of dry season feed. A field study is completed this year 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*. Results from this field study for the past 2 years indicated that the superior performance of the germplasm accession CIAT 26110 and the *Brachiaria* hybrid Mulato 2 (FM9503-S046-024), which maintained greater proportion of green leaves during moderate dry season in the llanos of Colombia, was associated with greater acquisition of nutrients under water deficit conditions. This year, we report results from the dry season performance into fifth 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, 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 20 P, 20 K, 33 Ca, 14 Mg, 10 S; and high: 80 N, 50 P, 100 K, 66 Ca, 28 Mg, 20 S and micronutrients) as main plots and genotypes as sub-plots. Live and dead forage yield, shoot nutrient composition, shoot nutrient uptake and leaf and stem TNC (total nonstructural carbohydrates) in six entries (2 hybrids, 3 parents and 1 accession) were measured at the end of the dry season (56 months after establishment; March 10, 2004). Maintenance fertlizer (half the levels of initial application) was applied at the beginning of the wet seasons of 2001 and 2003.

Results and Discussion

Because of the application of maintenance fertilizer, forage yields with high fertilizer treatment were greater than those with low fertilizer treatment (Figure 39; Table 30). At 56 months after establishment (4 months after dry season – March 10, 2004), live forage yield with low

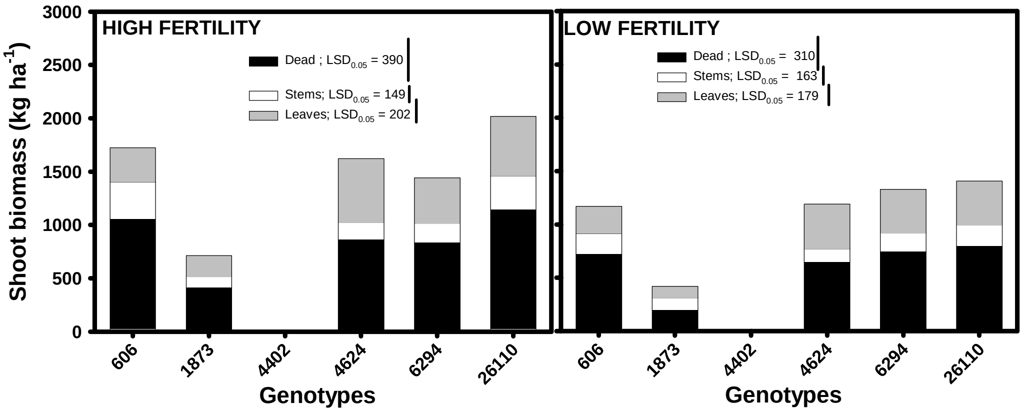


Figure 39. Genotypic variation as influenced by fertilizer application in dry matter distribution among green leaves, stems and dead biomass of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 56 months after establishment (at the end of the dry season – March 2004). LSD values are at the 0.05 probability level.

fertilizer application ranged from 0 to 609 kg/ha and the highest value of forage yield was observed with a germplasm accession CIAT 26110. This accession was released in Costa Rica as cultivar Toledo and is known for its dry season tolerance. Among the 3 parents, CIAT 6294 was outstanding in green live forage and dead biomass production with low fertilizer application. A spittlebug resistant genetic recombinant, Mulato 2 was superior among the genetic recombinants in terms of greater live shoot biomass, both with low and high fertilizer application. As expected, the performance of one of the parents, BRUZ/44-02 was very poor compared with other parents and genetic recombinants as it produced almost no live forage after dry season. The values of leaf to stem ratio of the genetic recombinant, FM9503-S046-024, were markedly superior to other genotypes under low and high levels of initial fertilizer application (Table 31).

Table 30. Genotypic variation as influenced by fertilizer application in live shoot biomass, leaf to stem ratio 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 56 months after establishment (at the end of the dry season – March 10 2004). LSD values are at the 0.05 probability level.

Genotype	Live shoot biomass (kg ha^{-1})		Leaf to stem ratio (%)		Total forage yield (kg ha^{-1})	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
Recombinants:						
FM9201-1873	223	301	0.9	2.0	420	714
FM9503-5046-024	542	760	3.4	3.7	1190	1622
Parents:						
CIAT 606	447	665	1.3	0.9	1169	1723
CIAT 6294	582	605	2.4	2.4	1329	1442
BRUZ/44-02	0	0	.	.	0	0
Accessions:						
CIAT 26110	609	872	2.1	1.8	1408	2019
Mean	401	534			919	1253
LSD (P>0.05)	327	318			582	667

Table 31. 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 56 months after establishment (at the end of the dry season – March 10 2004). LSD values are at the 0.05 probability level.

Genotype	Leaf N content (%)		Stem N content (%)		Shoot N uptake (kg ha^{-1})	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
Recombinants:						
FM9201-1873	1.05	1.11	0.72	0.89	4.04	3.12
FM9503-5046-024	0.99	0.86	0.67	0.48	4.94	5.94
Parents:						
CIAT 606	1.28	1.34	0.62	0.63	4.45	6.57
CIAT 6294	1.24	0.94	0.72	0.58	6.13	4.99
BRUZ/44-02	.	.	.	.	.	.
Accessions:						
CIAT 26110	1.21	0.97	0.61	0.49	6.12	6.61
Mean	1.15	1.04	0.67	0.61	5.14	5.45
LSD (P>0.05)	0.26	0.17	NS	0.35	NS	2.90

Results on leaf N content indicated significant differences among genetic recombinants, parents and accessions with both levels of fertilizer application (Table 31). Shoot N uptake with both low and high fertilizer application was markedly greater for the hybrid Mulato 2 than the cv. Mulato (Table 31; Figure 39). Leaf and stem N content and shoot N uptake values indicated that the genetic recombinant Mulato 2 could use N more efficiently to produce green forage in the dry season (Table 31).

3.2.3 Dry season tolerance of most promising hybrids of *Brachiaria* in the Llanos of Colombia

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

Rationale

Evaluation of a large number of *Brachiaria* hybrids for their resistance to spittlebug and adaptation to infertile acid soils resulted in identification of a few promising *Brachiaria* hybrids. We selected 4 of these hybrids for further field-testing in comparison with their parents. The main objective was to evaluate growth and persistence in dry season 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 of 2001. The trial included 4 *Brachiaria* hybrids (BR98NO/1251; BR99NO/4015; BR99NO/4132; Mulato 2) 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. A number of plant attributes including forage yield, dry matter distribution, nutrient (N, P, K, Ca and Mg) uptake, leaf and stem total nonstructural carbohydrate (TNC) content and leaf and stem ash (mineral) content were measured at 34 months after establishment (March 2004).

Results and Discussion

At 34 months after establishment, live forage yield with low fertilizer application was greater with one spittlebug resistant genetic recombinant Mulato 2 and one parent (CIAT 6294) (Table 32). With high initial fertilizer application also these two genotypes were outstanding in live forage yield (Figure 40).

Table 32. 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)	0.81***	0.87***
Dead shoot biomass (t/ha)	0.66**	0.70***
Leaf biomass (t/ha)	0.94***	0.89***
Stem biomass (t/ha)	0.77***	0.83***
Leaf N content (%)	-0.63**	-0.71***
Leaf P content (%)	0.05	-0.07
Leaf TNC content (mg g-1)	-0.08	-0.22
Leaf ash content (%)	0.15	-0.11
Stem N content (%)	-0.37	-0.58**
Stem P content (%)	-0.61	-0.19
Stem TNC content (mg g-1)	0.21	-0.2
Stem ash content (%)	0.40	0.53
Shoot N uptake (kg/ha)	0.95***	0.88***
Shoot P uptake (kg/ha)	0.93***	0.89***
Shoot K uptake (kg/ha)	0.61**	0.91***
Shoot Ca uptake (kg/ha)	0.86***	0.85***
Shoot Mg uptake (kg/ha)	0.86***	0.89***

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

Among the 4 hybrids tested, 4624 was outstanding in its adaptation to low initial fertilizer application. It is important to note that both the hybrid 4624 and CIAT 6294 had greater amount of dead biomass and stem biomass under low fertilizer application (Figure 40).

As observed last year, results on shoot nutrient uptake, particularly Ca and Mg, indicated that the hybrid, 4624 was superior to CIAT 606 under low fertilizer application (Figure 41). Nutrient acquisition by the hybrid 4624 was also greater than the rest of the hybrids with high initial fertilization. These results are consistent with the results reported last year from the same experiment. Correlation coefficients between live forage yield and other plant attributes indicated that greater nutrient acquisition with low initial fertilizer application contributed to superior performance (Table 32). No significant correlations were found between live forage yield and leaf and stem TNC or ash contents.

The performance of the 4 hybrids in comparison with two parents with maintenance fertilizer application will be monitored for next year in terms of green forage yield and nutrient acquisition.

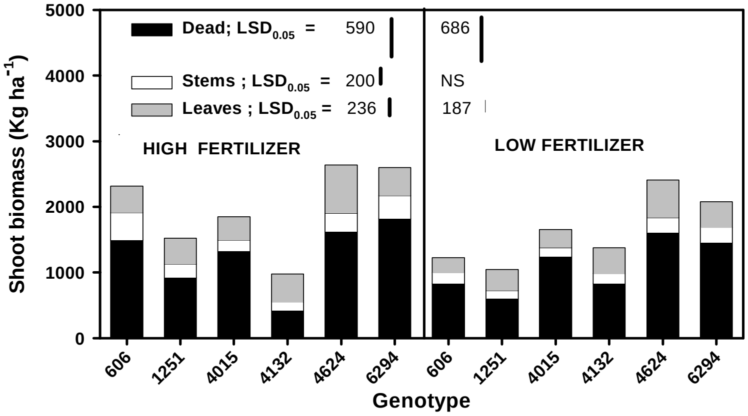


Figure 40. Genotypic variation as influenced by fertilizer application in shoot biomass production (forage yield) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 30 months after establishment (November 2003). LSD values are at the 0.05 probability level. NS = not significant.

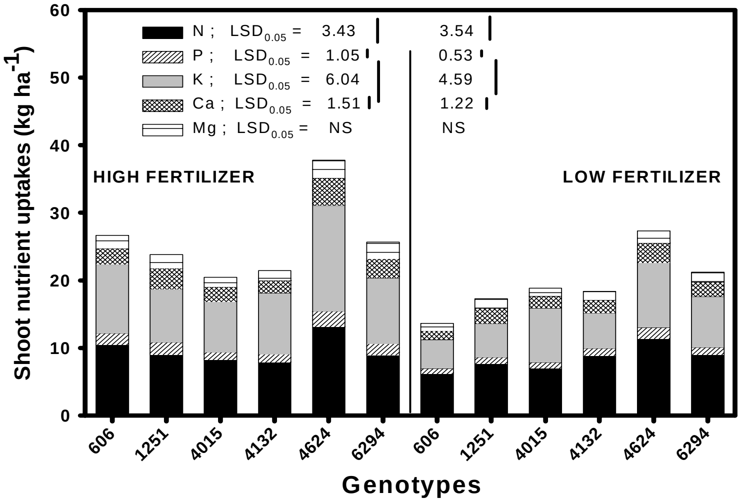


Figure 41. Genotypic variation as influenced by fertilizer application in nutrient uptake (N, P, K, Ca and Mg) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 30 months after establishment (November 2003). LSD values are at the 0.05 probability level. NS = not significant.

3.3 Grasses with adaptation to poorly drained soils

Highlights

- Plant survival and yield of *Brachiaria* hybrids and accessions were affected by waterlogged conditions. However, *Brachiaria* hybrid CIAT 36087 (Mulato 2) showed less plant mortality than other hybrids under these conditions.

- *Paspalum atratum* cv. Pojuca showed no plant mortality and increased yield under waterlogged soil condition.

3.3.1 Field evaluation of *Brachiaria* and *Paspalum* genotypes in poorly and well drained sites in Costa Rica

Contributors: Pedro J. Argel and Guillermo Pérez (CIAT)

Rationale

Poorly drained sites are frequently found in many areas of the low land tropics were cattle is an important economic activity. However, improved forage options are limited for permanent or periodically waterlogged conditions, and as a result native or naturalized grasses of medium to poor feeding qualities predominate in these areas. For this reason field tests are necessary to characterize the adaptation of promising forage germplasm to poorly drained soil conditions.

Material and Methods

As described in the IP-5 Annual Report 2003, seedlings of the *Paspalum atratum* cv. Pojuca (CIAT 26986), *B. brizantha* CIAT 26124, CIAT 26318, CIAT 26990, a line of this species called Mixe, and the *Brachiaria* hybrids CIAT 36061 (cv. Mulato), CIAT 36087, CIAT 4015 and CIAT 36062, were transplanted for evaluation in a site with variable slope, sufficient to create three different moisture conditions. The soil is a heavy clay (45-55% clay) with the following characteristics: pH 5.6, 0.4 meq/100 ml of Al content, high content of Ca (26.9), Mg (10.4) and medium content of K (0.44). Phosphorous (4 ppm) and Zinc (2.5 ppm) contents are low, whereas Mn (27.5), Cu (16.3) and Fe (39.7) contents are medium.

Twelve plants were established in each strata as described in 2003, and in September of this year three dikes were built along the lower part of the plots to create variable gradients of soil humidity: (a) waterlogged, (b) moderately waterlogged and, (c) well drained condition. Plant mortality, vigor and plant yield were measured during the wet period of 2004 along the three humidity strata that were created.

Results and Discussion

The dikes built created the expected waterlogged conditions. At the lower part of the plots a permanent water table of 5 to 10 cm depth remained and covered partially the grass plants during the evaluation period. At the middle of the plots, moderately waterlogged conditions were also created, and the water table remained around 20 cm. The well drained conditions had a water table that ranged from 30 to 50 cm.

Plant vigor and plant mortality of all *Brachiaria* species were affected during the evaluation period by the soil moisture conditions created as shown in Table 33. With the exception of *P. atratum* cv. Pojuca, the *Brachiaria* species lost vigor as the soil humidity increased, indicating poor adaptation to waterlogged soils. At the waterlogged site, plant mortality was relatively high (3 plants out of 12 plants) with *Brachiaria*

Table 33. Plant vigor and plant mortality of grass species established along a humidity gradient formed by (1) well drained conditions, (2) moderately drained, and (3) poorly drained (waterlogged) conditions in a heavy soil of Atenas, Costa Rica.

Species	CIAT No.	Vigor*			Plant mortality (No.)		
		(1)	(2)	(3)	(1)	(2)	(3)
Brachiaria hybrid (cv. Mulato)	36061	3.8	4.0	2.0	0	0	3
B. hybrid (Mulato 2)	36087	4.2	4.3	3.5	0	0	1
B. hybrid	36062	4.8	2.8	2.3	0	1	2
B. hybrid	4015	3.2	3.3	1.8	0	0	3
B. brizantha	26124	4.3	2.8	1.7	0	0	3
B. brizantha	26318	2.5	2.2	1.5	0	0	2
B. brizantha	26990	2.5	2.0	1.2	1	2	2
B. brizantha	(Mixe)	3.7	3.2	1.8	0	0	3
Paspalum atratum cv. Pojuca	26986	3.5	4.0	5.0	0	0	0

* Vigor rated: 1.0 = poor vigor; 5.0 = highly vigorous plant.

hybrids cv. Mulato and CIAT 4015, for *B.brizantha* CIAT 26124 and Mixe, and moderately (2 plants dead out of 12 plants) for *Brachiaria* hybrid CIAT 36062, *B. brizantha* CIAT 26318 and CIAT 26990. The *Brachiaria* hybrid CIAT 36087 (Mulato 2) had only 1 dead plant at the flooded site, while *P. atratum* cv. Pojuca did not show any sign of plant mortality under these conditions, indicating the good adaptation of this species to waterlogged soils.

As the soil moisture increased, there was a tendency for reduced plant yields in all *Brachiaria* species. In contrast with *P. atratum* cv. Pojuca there was higher yields under water-logged conditions (Table 34).

It was interesting to observe that the yields recorded for the *Brachiaria* hybrid cv. Mulato and other *Brachiaria* species, were similar at the three humidity treatments, indicating that for the degree of plant mortality recorded, the survival plants compensated in growth for the lost ones, with the exception of *B.* hybrid CIAT 36087 (Mulato 2) and Mixe that produced significant more plant yield at well drained sites.

Table 34. Dry matter yields (DMY) of grass species established along a humidity gradient formed by (1) well drained conditions, (2) moderately drained, and (3) poorly drained (waterlogged) conditions in a heavy soil of Atenas, Costa Rica (means of 4 cuts of 5 weeks re-growths during the wet period 2004).

Species	CIAT No.	DMY (g/plant) Well drained	Moderately drained	Poorly drained	Probability
Brachiaria hybrid (cv. Mulato)	36061	86.7 a*	76.1 a	72.5 a	ns
Brachiaria hybrid (Mulato 2)	36087	99.4 a	75.0 b	75.2 b	p=0.02
Brachiaria hybrid	36062	71.3 a	59.3 a	56.7 a	ns
Brachiaria hybrid	4015	55.4 a	51.7 a	43.8 a	ns
B. brizantha	26124	67.7 a	62.3 a	61.2 a	ns
B. brizantha	26318	56.3 a	48.8 a	44.6 a	ns
B. brizantha	26990	37.0 a	30.6 a	30.0 a	ns
B. brizantha	(Mixe)	64.8 a	57.3 ab	47.2 b	p=0.10
Paspalum atratum cv. Pojuca	26986	101.5 a	104.3 ab	237.4 c	p=0.0001

*****Within the same line means followed by the same letter are not statistically significant

3.4 Nitrification inhibition in tropical grasses

Highlights

- Root exudates from *B. humidicola* are effective, persistent and stable at inhibiting nitrification up to at least 75 days.

- Presence of NH_4-N stimulates the synthesis and release of NI activity in exudates produced by the roots of *B. humidicola*.

- Genetic variability in capacity to inhibit nitrification was found among accessions of *B. humidicola* held by CIAT, which opens up the possibility for breeding for this trait

3.4.1 Bioassay – Further improvements and refinements to the methodology: Expression of NI activity in AT (equivalent to allylthiourea inhibition) units

Contributors: G.V. Subbarao, O. Ito, T. Ishikawa, and K. Nakahara (JIRCAS, Japan)

Rationale

We have further improved the bioassay methodology and developed ways to express inhibitory effect (on nitrification from root exudates) in equivalent standard inhibitor, allylthiourea (AT) units. The transgenic *Nitrosomonas* -responds linearly to the AT concentration in the bioassay medium (Figure 42). Using this relationship, the inhibitory effect from root exudates (that is determined using bioassay) is expressed in AT units, which can be subjected to statistical analysis. One AT unit of NI is defined as the inhibitory activity caused by the presence of 0.22 μM of AT in the bioassay medium. These improvements in the bioassay methodology will make it now possible to characterize the nitrification inhibition phenomenon in root exudates of plants. Also, the bioassay methodology will make it possible for the evaluation and comparative analysis of crop and forage germplasm accessions and breeding lines for the NI (nitrification inhibitory) activity in root exudates.

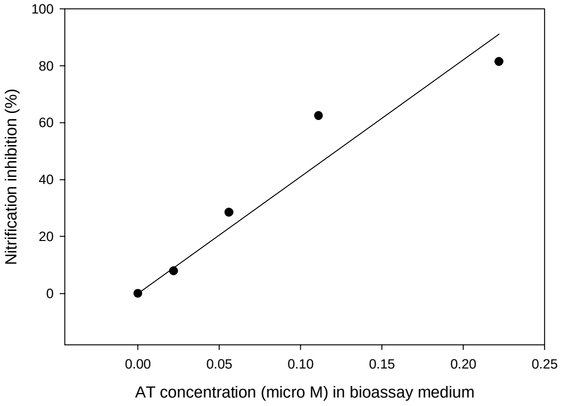


Figure 42. Transgenic *Nitrosomonas europaea* response to synthetic nitrification inhibitor, allylthiourea in the bioassay medium.

3.4.2 Stability, persistence and effectiveness of *Brachiaria humidicola* root exudates in inhibiting nitrification in soil

Contributors: G.V. Subbarao, H. Wong, T. Ishikawa and O. Ito (JIRCAS, Japan); M. Rondon and I.M. Rao (CIAT)

Rationale

This year, we have improved further the protocols in processing and testing of root exudates to determine the inhibitory effect on nitrification in soil (IP-5 Annual Report, 2003). We have tested the stability, persistence and effectiveness of the inhibitory effect from root exudates of *B. humidicola* on nitrification in soil. NI activity of 10 AT units g^{-1} soil (Soil from Tsukuba, Japan) was added to the soil with 182 ppm of N as $(NH_4)_2SO_4$ and incubated at 20 °C and 95% RH. The experiment was replicated three times. Sequential sampling was done at 25 d interval~~s~~ and the incubation was continued for 100 days. NI activity of 10 AT units g^{-1} soil was very effective in inhibiting nitrate formation in soil (about 70% inhibition) and remained effective in inhibiting nitrification (about 50%) until 75 days. A substantial portion of the inhibitory effect from NI activity was lost between 75 and 100 period of incubation in soil.

The synthetic nitrification inhibitor, ©Nitrapyrin did not inhibit nitrification effectively (only about 20% inhibition on nitrate formation) at 4.5 ppm under these conditions and lost its effectiveness after 30 days of incubation (Figure 43).

Our results demonstrate that root exudates from *B.humidicola* are effective, persistent and stable in inhibiting nitrification in soil (up to 75 days at least). Our results indicate that two *B. humidicola* plants of 60 to 70 d old can release up to 100 AT units of NI activity (in 24 h period) under optimum conditions.

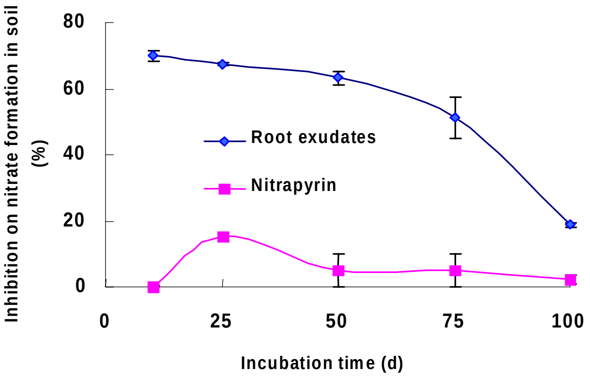


Figure 43. Inhibitory effect from root exudates (10 AT units NI activity g-1 soil) and nitrapyrin (4.5 ppm) on nitrate formation in soil during 100 d incubation period (Note: In control, nearly 90% of the added NH_4-N was nitrified by 75 days).

Our results also indicate that the NI activity release rates mentioned above can be maintained for long periods of time (we have tested up to 15 days and that the release rates were maintained).

This is the first time that we have demonstrated the effectiveness, stability and persistence of root exudates (from *B. humidicola*) inhibitory effect on nitrification in soil.

3.4.3 Influence of NH_4-N on expression/regulation and release of NI activity in root exudates of *B. humidicola*

Contributors: G.V. Subbarao, H. Wong, T. Ishikawa, O. Ito and K. Nakahara (JIRCAS, Japan); M Rondon and I.M. Rao (CIAT)

Rationale

We have tested the hypothesis that nitrogen forms (NH_4-N *vs* NO_3-N) can influence the release of NI activity from roots in *B.humidicola*. Plants of *B. humidicola* were grown hydroponically with two sources of nitrogen – 1 mM N as $(NH_4)_2SO_4$ or KNO_3 for 70 days. The experiment was replicated three times. Root exudates were collected by keeping intact plant roots in distilled water, 1 mM NH_4Cl or 1 mM KNO_3 for 24 h. NI activity of root exudates was determined with the NI bioassay. Root exudates of NH_4-N grown plants showed NI activity, whereas NI activity was completely absent in the root exudates of NO_3-N grown plants (data not shown for NO_3-N grown plants as there was no NI activity detected in root exudates).

Presence of NH_4-N in the root exudates collection solutions further stimulated the release of NI activity in NH_4-N grown plants (Figure 44).

The NI activity released in the presence of NH_4-N was several-fold higher than in the absence of NH_4-N (i.e. when root exudates are collected using distilled water).

Our results support the hypothesis that presence of NH_4-N stimulates the synthesis and release of NI activity from root exudates (data not presented on the root tissue NI levels). The release of NI activity from roots appears to be a highly regulated phenomenon and NH_4-N in the rhizosphere is certainly one of the important regulating factors for the release of NI activity. Also, regulatory role of NH_4-N in the rhizosphere for the release of NI activity from roots further indicates the functional significance of NI activity in protecting NH_4-N in soil from nitrification.

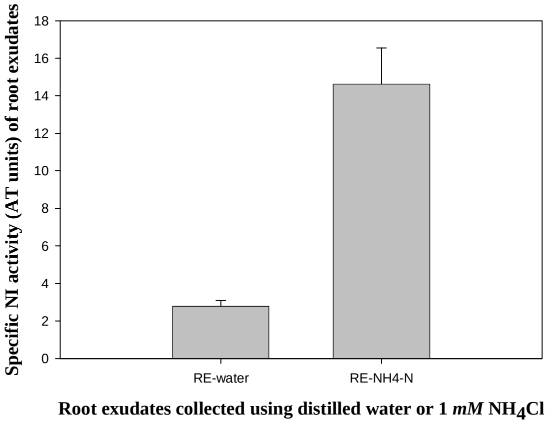


Figure 44. Influence of NH4-N in the root exudates collection medium on the release of NI activity into root exudates from *B. humidicola* roots (Specific NI activity = NI activity g^{-1} root dry weight).

3.4.4 Screening for genetic variability in the ability to inhibit nitrification in accessions of *B. humidicola*

Contributors: M. Rondón, I.M. Rao, C.E. Lascano, J.A. Ramírez, M.P. Hurtado, J. Ricaurte (CIAT); G.V. Subbarao, T. Ishikawa, K. Nakahara, and O. Ito (JIRCAS, Japan)

Rationale

Ongoing collaborative research with JIRCAS, Japan, has shown that *B. humidicola* CIAT 679 inhibits nitrification of ammonium and reduces the emission of nitrous oxide into the atmosphere. Given these findings with the commercial cultivar of *B. humidicola* CIAT 679, and the fact that a range of inhibition of nitrification was observed among different tropical grasses, there is a need to determine the extent of genetic variation among the 69 accessions of *B. humidicola* that are part of CIAT germplasm bank. This information will be extremely useful to develop screening methods to select genetic recombinants of *Brachiaria* grasses that not only are resistant to major biotic and abiotic stress factors but also can protect the environment. Given the vast areas under *B. humidicola* in the tropics, reductions in net emissions of N_2O could also have important environmental implications.

The main objective was to quantify differences among 10 accessions of *B. humidicola* regarding the nitrification inhibition activity of root exudates collected from plants grown under greenhouse conditions using infertile acid soil. Also we intend to test the relationship between nitrification inhibition and root production in terms of biomass and length.

Materials and Methods

A sandy loam Oxisol from the Llanos (Matazul) of Colombia was used to grow the plants (4 kg of soil/pot) under greenhouse conditions. A basal level of nutrients were applied before planting (kg/ha): 40 N, 50 P, 100 K, 66 Ca, 28.5 Mg, 20 S and micronutrients at 2 Zn, 2 Cu, 0.1 B and 0.1 Mo. A total of ten accessions were used (accessions CIAT 679, 6133, 6369, 6707, 16866, 16867, 16886, 16888, 26149, 26159). A control without plants was also included. The experiment was arranged as a completely randomized block design with four replications. Each pot contained four plants. After sowing, plants were allowed to grow for 15 weeks and were cut to 10 cm height to simulate grazing effects under field conditions. Plant tissue was dried and saved.

Plants were allowed to re-grow during 5 weeks more to promote a well developed root system and then ammonium sulfate was applied in solution at a rate of 38.5 mg N-NH$_4$/kg soil (equivalent to 100 kg N-NH$_4$ per hectare). Five weeks later plants were harvested (at 25 weeks after sowing). At the end of the experiment, plants were carefully removed from soil minimizing mechanical damage to the roots. Soil adhered to the fine toots was removed and the roots were rinsed with deionized water. Once clean, the roots were fully immersed in 1 liter of deionized water and were allowed to produce root exudates during 24 hours. Collected root exudates were kept in the refrigerator and were reduced in volume to approximately 100 ml using a freeze drier.

Harvested plants were separated into shoot and roots. Root length was measured using a root length scanner. Dry matter content and N status of both shoot and root biomass was determined. At harvest time, soil samples were extracted with KCL and analyzed for nitrate and ammonium levels. The concentrated root exudates were further concentrated using a rotovapor using protocols that were developed for this purpose. The final concentrate was tested for its nitrification inhibitory activity using a specific bioassay developed at JIRCAS.

Results and Discussion

Results on dry matter partitioning among shoot and root biomass from the comparative evaluation of the ten accessions are presented in Table 35. No significant differences were found in total biomass production among most of the CIAT accessions except for the accessions of 16866 and 16867, which were lower than the rest of the accessions. However, significant differences among accessions were found in root biomass production. The commercial cultivar, CIAT 679, which has been used in most of the previous work, seems to have root biomass around the average value for the group tested. The accession 6707 produced the highest root biomass among the tested accessions. Values of root biomass of this accession were more than two fold greater than the value for the lowest in the group, the accession 26149.

Table 35. Dry matter partitioning differences among ten accessions of *B. humidicola* grown in pots under greenhouse conditions. Plants were harvested at six months after planting.

CIAT Accession Number	Dry matter (g/pot)		
	Root biomass	Shoot biomass	Total biomass
CIAT 679	4.29 (1.19) a	14.76 (3.76) d	19.05 (3.68) f
CIAT 6133	4.14 (1.65) a	15.06 (1.90) d	19.20 (3.49) f
CIAT 6369	4.77 (1.58) b	14.35 (1.59) d	19.12 (2.52) f
CIAT 6707	4.92 (0.72) a	17.84 (2.75) d	22.75 (2.61) f
CIAT 16866	3.52 (0.89) a	13.45 (0.96) e	16.97 (0.95) g
CIAT 16867	3.50 (0.38) a	14.70 (1.65) e	18.20 (1.56) g
CIAT 16886	4.48 (1.09) b	15.53 (4.56) d	20.01 (5.12) f
CIAT 16888	3.26 (0.72) a	16.97 (1.40) d	20.22 (1.17) f
CIAT 26149	2.39 (0.30) c	17.31 (3.20) d	19.70 (3.09) f
CIAT 26159	2.96 (1.43) c	16.15 (2.09) d	19.10 (2.20) f

Numbers in parenthesis indicate standard deviation. In a given column, data followed by the same letter indicate non- significant differences (LSD, p<0.05).

Results from the bioassay indicated substantial level of NI (nitrification inhibitory) activity in the root exudates of most of the accessions tested (Table 36). However a range in NI activity was found among the tested accessions. Accessions could be grouped in 3 classes in relation to their specific NI activity. Group 1 with the accession CIAT 16867 showed no NI effects, behaving

Table 36. Nitrification inhibitory activity (total NI activity pot^{-1} and specific activity .g root dry weight^{-1}) of the root exudates from ten accessions of *B. humidicola* grown under glasshouse conditions. Plants were grown for six months before the collection of root exudates.

CIAT Accession Number	NI activity (in AT units pot^{-1})	Specific NI activity (in AT units g root dwt^{-1})
CIAT 679	68.84 (24.1) cd	17.48 (8.4) c
CIAT 6133	51.58 (16.9) cd	12.24 (2.83) c
CIAT 6369	86.94 (14.3) c	20.72 (4.2) c
CIAT 6707	69.68 (5.5) cd	14.86 (1.2) c
CIAT 16866	41.48 (6.9) d	11.26 (2.9) c
CIAT 16867	-48.55 (18.1) e*	-13.42 (3.35) d
CIAT 16886	128.05 (15.3) ab	27.95 (5.8) bc
CIAT 16888	160.95 (6.08) a	53.76 (17.45) a
CIAT 26149	33.5 (39.8) d	15.22 (18.15) c
CIAT 26159	126.17 (19.9) b	46.33 (19.0) ab

Note: Numbers in parenthesis indicate standard deviation. In a given column, data followed by the same letter indicate non-significant differences (LSD, p<0.05). NI activity is expressed as AT units; One AT unit is defined as the inhibitory activity caused by the addition of 0.22 µM of allylthiourea (AT) in the bioassay medium. Thus, the inhibitory activity of the test samples of root exudates is converted into AT units for the ease of expression in numerical form.

[*] Negative activity indicates that nitrification was stimulated by the root exudates.

similarly to other grasses such as *Panicum maximum,* which also lack the NI activity. Group 2 that included accessions CIAT 6133, 6707, 16866, 26149, 6369, and 6707 showed similar levels of NI that was observed with the commercial cultivar CIAT 679. Group 3 that included the accessions 16886, 16888, and 26159 showed significantly higher levels of NI than the accession 679. The accession 16888 was outstanding in its NI activity with a value of more than three times to that of the value of CIAT 679.

Results on NI activity indicate that wide genetic variability exists among accessions of *B. humidicola* in relation to the effectiveness of root exudates to inhibit nitrification in soils. This genetic variability for NI activity could be exploited in a breeding program to select for genotypes with different levels of NI activity. Once all the accessions in the gene bank are tested, accessions with superior NI activity could be used as parents to regulate NI activity in the genetic recombinants together with other desirable agronomic traits.

The presence of substantially higher levels of NI activity in the root exudates of the two CIAT accessions (16888 and 26159) draws attention to the need to study these accessions in more detail. The immediate task is to continue the screening of other accessions of *B. humidicola* from the gene bank and to initiate screening of other commercially important grasses and crops for their ability to inhibit nitrification. As a continuation of this work, this year we have initiated the screening of another 11 accessions of *B. humidicola* including all materials that are classified as putatively sexual. An additional experiment will be conducted to obtain and test NI activity of root exudates from maize, rice, sorghum, soybean, cowpea and common bean. Results from this study will be reported next year. Further research work is needed to determine the relative importance of total NI activity *vs*. specific NI activity in influencing the nitrification process (i.e. inhibition) in a soil environment.

3.4.5 Field validation of the phenomenon of nitrification inhibition from *Brachiaria humidicola*

Contributors: M. Rondón, I.M. Rao, C.E. Lascano, M. P. Hurtado, J. Ricaurte (CIAT); G.V. Subbarao, T. Ishikawa and O. Ito (JIRCAS, Japan)

Rationale

Research conducted at JIRCAS and CIAT for the past three years using *B. humidicola* has shown that root exudates from this tropical grass have the capability to inhibit/suppress the nitrifying populations in the soil. Factors such as presence of NH_4-N in the soil seem to have a stimulating effect on the expression of nitrification inhibition (NI) activity in the root exudates of *B. humidicola*. Differences have been found among accessions of *B. humidicola* with regard to their NI activity. Also, our recent studies involving soils incubated with root exudates of *B. humidicola* and soybean have shown that root exudates from *B. humidicola* have suppressed the N_2O emissions and inhibited the nitrification process, while those of soybean seem to stimulate the nitrification process in soils. Soybean (usually in rotation with maize) is becoming increasingly important as a crop not only in Latin America but also in many tropical and temperate regions. Other grasses such as *Panicum maximum* lack the NI activity, while the *Brachiaria* hybrid cv. Mulato was found to have a moderate level of NI activity. The use of this hybrid is expanding rapidly in Latin America due to its high productivity and forage quality.

All these above studies were conducted either using hydroponic systems or soil in pots under greenhouse conditions to test and verify the concept of the biological phenomenon of nitrification inhibition. There is a clear need to validate some of these findings under field conditions. This year a collaborative (CIAT-JIRCAS) long-term experiment was initiated to validate the phenomenon of NI under field conditions and to monitor whether the NI activity is a cumulative process in the soil

Given the vast areas currently grown in the tropics on tropical grasses, an understanding of the NI process and the possibility of managing it to improve fertilizer N use efficiency, reduce nitrate pollution of surface and ground waters as well as reduce net impact on the atmosphere through reduced emissions of nitrous oxide, could have potential global implications for sustainable agricultural development and environmental protection.

Materials and Methods

The field experiment was established on 31 August 2004 at CIAT-Palmira on a Mollisol (Typic Pellustert) as a randomized complete block (RCB) design with six treatments and 3 replications. Annual rainfall at this site is about 1000 mm with a mean temperature of 25 °C. Soil is fertile with a pH of 6.9. Two accessions of *B. humidicola* were included: the reference material (CIAT 679) that has been used for most of our previous studies, and the high NI activity germplasm accession (CIAT 16888). The Hybrid Mulato was included as a moderate NI and *Panicum maximum* var. common was included as a negative non-inhibiting control. A crop rotation (maize-soybean) was included to assess under field condition the recent finding that Soybean lacks NI ability (indeed accelerate nitrification), while maize shows some degree of inhibitory capability. As first crop of the rotation we used maize variety (ICA V109). A plot without plants where emerging weeds are removed manually is used as an absolute control.

Plot size for each treatment was 10m x 10m. Irrigation will be provided if necessary. Maize was planted from seeds and the tropical forage grasses were propagated from vegetative cuttings. Fertilizer will be applied (broadcast) for every crop cycle, consisting of (kg/ha) 96 N (as urea), 48 K, 16 P, 0.4 Zn, 0.4 B and 8 S. The fertilizer is split into two equal applications: one at 20 days after sowing of each crop (either maize or soybean) and the other at flowering time at approximately 60 days after sowing.

A number of soil and plant parameters will be measured at every four months. These include nitrate and ammonium availability in the soil, dynamics of nitrifier organisms in soil, plant nitrogen uptake and nitrous oxide (N_2O) emissions. The NI activity of soil water extracts will be measured using the bioassay. Soils samples will be periodically collected and sent to JIRCAS to assess changes in inhibitory compounds in the soil. Gas samples for measuring N_2O fluxes will be collected every month. Once a year, soil incubation studies will be conducted using rhizosphere soil, to monitor nitrogen dynamics and fluxes of N_2O. Currently plants are growing well and the initial sampling is expected in January 2005. Results from this field study will be reported next year.

3.5 Legumes (herbaceous and woody) with adaptation to acid soils and drought

Highlights

- Selected accessions of *Desmodium velutinum* with superior productivity, forage quality and that have persisted over 2 years under cutting.

- Selected accessions of *Canavalia brasiliensis* with superior productivity and drought tolerance for seed multiplication.

3.5.1 Evaluation of new collections of the multipurpose shrub legumes *Flemingia macrophylla, Flemingia* spp. and *Cratylia argentea*

Contributors: M. Peters, L.H. Franco (CIAT), R. Schultze-Kraft (University of Hohenheim), B. Hincapié, P. Ávila and G. Ramírez (CIAT)

Rationale

The work of CIAT on shrub legumes emphasizes the development of materials to be utilized as feed supplement during extended dry seasons. Tropical shrub legumes of high quality for better soils are readily available, but germplasm with similar characteristics adapted to acid, infertile soils is scarce. *Flemingia macrophylla* and *Cratylia argentea* have shown promising results in such environments and hence work on these genera is part of the overall germplasm development strategy of the CIAT Forages team.

C. argentea is increasingly adopted and utilized, particularly in the seasonally dry hillsides of Central America, and more recently, the Llanos Orientales de Colombia. However, most research and development is based on only few accessions and hence activities to acquire and test novel germplasm of *C. argentea* are of high priority.

F. macrophylla also is a highly promising shrub legume with excellent adaptation to infertile soils. In contrast to *C. argentea*, whose adaptation is limited to an altitude below 1200 masl, *F. macrophylla* can successfully be grown up to altitudes of 2000 masl. However, the potential utilization of *F. macrophylla* is so far limited by the poor quality and acceptability of the few evaluated accessions.

The project aims to investigate the genetic diversity within collections of *F. macrophylla*, *Flemingia* spp. and *C. argentea* with two main objectives:

1) 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).
2) To optimize the use and management, including conservation, of the collections. For this, different approaches to identify core collections for each species were tested and compared based on: (a) genetic diversity assessment by agronomic characterization/ evaluation and (b) germplasm origin information.

Material and Methods

In the Annual Report of 2003 we presented results on the evaluations of large collections of both *Flemingia macrophylla* and *Cratylia argentea*. To complement this work, we acquired additional germplasm of *C. argentea* and *F. macrophylla*. In view of the interest of *Flemingia* as a genus; we are also evaluating a new collection of different *Flemingia* species.

In 2002, 8 accessions of *Cratylia argentea* - CIAT 22377, 22395, 22388, 22389, 22395, 22401, 22402, 22403 and Yacapani previously evaluated in Costa Rica were sown at CIATs research station in Santander de Quilichao (Photo 16). In contrast to other accessions of *C. argentea*, Yacapani is of prostrate growth habit. Accessions CIAT 18516 and 18668 were included as standards.

Photo 16. *Cratylia argentea* at Quilichao, 2002.

Plants were sown in the greenhouse in jiffys and transplanted to the field 6 weeks later. We employed the same evaluation methodology as for the larger collections of *C. argentea* and *F. macrophylla* described in the Annual Report 2003 (for more details on the methodology please refer to the Annual Report 2003).

Results and Discussion

Agronomic evaluation: Three cuts were carried out in each the dry and wet season and results on dry matter yield are presented for *Cratylia argentea* and *Flemingia macrophylla* in Tables 37 and 39.

***Cratylia argentea* (2002):** Except for the distinct growth habit of Yacapani, no morphological differences between accessions were observed. Though Yacapani has normally a prostrate growth habit, erect plants were also encountered; it is not clear if this variation is to be attributed to contamination of the seed, outcrossing or diversity within the accession. Additional plants of the accession were sown to study the growth habit of this particular accession in more detail. No disease and pest problems were observed.

In general, yields were much higher than previously obtained in Costa Rica for the same accessions. DM yields of the accessions CIAT 18516, 22377, 22395, 22389, 22403 and 18668 were higher than reported for the larger collection in the Annual Report 2003, most likely as a result of a more favorable growing conditions in 2003/2004. No significant (P>0.05) yield differences among accessions were recorded in either the wet or dry season. Mean dry season yields were superior to those recorded in the wet season, and better regrowth was also found under dry conditions.

Highest DM yields were obtained for CIAT 18516, which had a higher yield and regrowth ability than accession CIAT 18668. The mixture of these accessions was released in Costa Rica as cv. Veraniega and in Colombia as cv Veranera. Lowest DM yields in both seasons were recorded for accession CIAT 22401 (Table 37). In line with results obtained for DM yield, in-vitro dry matter digestibility (IVDMD) was higher in the dry than in the wet period. The accessions 18516 and 18668 (mixture of these two accessions formed cv. Veranera) had lower IVDMD and CP as compared to accessions CIAT 22402, 22389, 22041 and 22388. However, with the exception of CIAT 22389, accessions of higher quality had relatively low DM yields (Table 38).

***Flemingia macrophylla* (2002):** In contrast to the large diversity in growth types encountered in the larger collection of *F. macrophylla* (see

Table 37. Agronomic evaluation of a collection (2002) of *Cratylia argentea* in Quilichao. Data of six evaluation cuts (three in the dry season and three in the wet season). Underlaid in grey: Accessions CIAT 18516/18668.

Accession No. CIAT	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
18516	169	149	28	580	151	126	31	683
22395	142	137	20	436	145	123	25	542
22389	152	121	17	389	156	111	22	515
22377	145	110	18	370	144	111	21	437
22403	148	124	18	351	144	113	23	437
18668	140	120	19	325	136	106	23	425
22388	146	118	14	321	150	101	18	411
22402	144	106	14	310	150	87	17	378
Yacapani	135	131	17	284	122	111	27	400
22401	121	107	17	219	129	93	17	247
Mean	144	122	18	351	143	108	22	439
LSD (P<0.05)				246.9				344.6

Table 38. Forage quality of accessions of *Cratylia argentea* evaluated in Quilichao, 2004. Grey underlaid: Accessions CIAT 18516/18668.

Accession No. CIAT	Wet IVDMD	 C P	 ADF	Dry IVDMD	 C P	 ADF
			(%)			
22402	60.2	20.2	24.1	70.6	22.8	22.8
22389	65.0	20.1	22.4	70.6	22.1	20.7
22401	60.1	21.7	24.9	68.4	22.4	24.9
22388	63.2	21.8	23.5	67.6	22.0	22.1
22395	60.6	21.1	25.1	65.7	23.5	25.1
22403	57.6	20.5	25.9	65.5	20.6	27.8
18668	58.6	19.3	25.2	65.1	20.8	25.4
18516	60.6	20.1	23.3	65.0	23.2	22.3
22377	55.9	21.2	27.0	64.3	22.8	26.4
Yacapani	56.8	21.5	23.5	64.1	22.0	22.7
Mean	59.8	20.8	24.5	66.7	22.2	24.1
LSD (P< 0.05)	6.9	2.8	3.7	3.7	2.1	2.9

Annual Report 2003), materials evaluated this year were more homogenous and all of erect growth habit (Photo 17).

Differences in yield between the wet and dry seasons were not significant. However, significant (P<0.05) differences in DM yield were found among accessions when averaged across seasons. The highest DM yields and the best regrowth were recorded for accessions CIAT 659 and 906 (Table 39).

Photo 17. Collection 2002 of *Flemingia macrophylla* in Quilichao.

Table 39. Agronomic evaluation of a collection (2002) of *Flemingia macrophylla* in Quilichao. Data of six evaluation cuts (tree in the dry season and tree in the wet season). Grey underlaid: Accessions with digestibility >44% and dry matter yield >250 g/plant.

Accession Number	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
659	127	117	37	416	119	116	46	419
906	134	108	30	409	134	102	40	409
870	126	107	37	378	124	115	45	350
591	135	114	34	374	129	102	38	315
914	134	105	31	370	137	114	40	395
816	119	108	31	347	119	109	41	304
595	133	108	19	335	128	101	26	300
615	123	107	28	328	104	97	34	280
780	128	108	30	321	130	110	35	298
857	126	99	25	314	118	97	32	324
632	124	97	29	310	120	101	39	273
753	109	93	25	297	109	94	31	246
601	126	108	26	297	134	108	34	353
629	110	94	25	291	105	101	42	274
821	110	104	33	274	112	99	31	266
804	117	103	25	274	119	101	30	313
576	130	96	20	267	129	93	25	303
542	122	96	18	206	123	80	22	186
Mean	124	104	28	323	122	102	35	312
LSD(P< 0.05)				166.62				180.62

In terms of quality parameters significant (P<0.01) differences among accessions were measured for IVDMD, ADF and soluble tannin content (Table 40). While some accessions had a higher digestibility than CIAT 17403 (control), values were lower than for the accessions with highest quality selected from the larger collection of Flemingia macrophylla previously evaluated (see Annual report 2003).

Table 40. Forage quality of accessions of *Flemingia macrophylla* evaluated in Quilichao, 2004.*

Accession Number	Wet				Dry			
	IVDMD	C P	ADF	Tan Sol	IVDMD	C P	ADF	Tan Sol
				(%)				
870	45.4	21.7	23.0	4.8	44.5	20.4	21.4	1.6
816	41.3	21.1	23.4	7.4	41.1	21.1	21.6	5.5
601	41.4	20.6	22.1	3.3	40.1	20.4	21.8	5.9
780	44.6	21.5	22.2	6.0	40.1	21.4	22.2	3.9
595	41.6	21.7	21.4	2.4	39.3	20.6	22.0	6.2
857	44.8	20.0	22.3	6.5	38.6	19.8	22.7	3.4
804	37.0	22.0	22.3	6.9	38.4	21.2	23.3	6.2
542	40.4	21.3	22.8	5.3	38.2	20.6	22.7	5.3
914	38.5	19.0	23.5	5.8	37.8	19.8	24.4	1.8
576	40.1	20.9	20.5	5.9	36.9	19.9	21.3	7.4
906	42.7	20.4	22.3	5.0	36.8	20.5	23.0	2.4
591	39.6	21.7	20.9	2.1	36.7	21.1	20.9	8.0
659	38.2	20.6	20.5	7.6	35.7	20.6	21.2	4.1
753	37.8	21.9	23.8	6.8	35.4	20.4	23.6	6.5
615	39.3	21.1	24.6	3.9	35.4	20.0	24.6	8.1
821	38.0	20.1	21.7	4.7	34.8	20.3	21.1	3.7
629	32.6	20.2	23.4	3.0	32.9	19.3	20.3	8.0
632	37.1	20.1	23.2	4.7	30.9	18.7	21.4	7.3
Mean	40.1	20.9	22.3	5.1	37.4	20.3	22.2	5.3
LSD(P< 0.05)	5.8	2.8	2.3	3.9	7.0	2.5	1.93	2.7

* CIAT 17403 (IVDMD = 43.68 - 41.83; CP = 20.52 - 20.06; ADF = 20.36 - 24.09; T Sol= 4.32 - 9.62)

3.5.2 Genetic diversity in the multipurpose shrub legume *Desmodium velutinum*

Contributors: R. Schultze-Kraft (University of Hohenheim), F. Parra (Corpoica), N.Vivas (Universidad Nacional de Colombia, Palmira), M. Peters, L.H. Franco, B. Hincapié, and G. Ramírez (CIAT)

Rationale

For acid, low-fertility soils in drought-prone environments there are few options in terms of shrub legumes. Species such as Desmodium velutinum may offer an option in such environments (where they would complement Cratylia argentea). There are very few studies on D. velutinum and the ones available concentrates on one or two accessions. However, the available information indicates that this legume produces forage of high quality and has the potential to adapt to drought and (acid) low-fertility soils.

We are currently exploring the genetic diversity in a collection of D. velutinum held by CIAT in terms of morphology, yield and quality parameters. From this work we expect to derive a core collection based on agronomic and morphological parameters, origin information, (using GIS tools), and characterization with molecular markers, will be identified for more detailed regional evaluation.

Materials and Methods

A total of 137 accessions of *Desmodium velutinum*, mostly originating from Asia and to a lesser extent from Africa, were planted at Quilichao (Photo 18). Plants were sown in jiffy pots and transplanted 6 weeks later into the field in single-row plots, with 4 replications. Dry matter yield, drought tolerance and forage quality are the main parameters being measured.

Photo 18. Regrowth at 8 weeks of *Desmodium velutinum* at Quilichao

Results and Discussion

Five months after transplanting plants were well established, had a good vigor and were free of pests and diseases. Accessions were classified into three groups according to their growth habit: e = erect (54 accessions), se = semi-erect (66), r = prostrate (17 accessions).

DM yields, averaged over two cuts each in the wet and dry periods are presented in Tables 41, 42 and 43 for the 3 groups of *D. velutinum* that were formed based on growth habit. Each growth type was analyzed separately as these are likely to occupy different niches. Significant ($P < 0.01$) differences among accessions were recorded in each group.

In general, results indicate that for the group classified as erect, DM yields were slightly higher in the wet than in the dry season, with accessions CIAT 33443, 13953, 23985, 23994 and 33352 producing more than 200g DM/plant for an 8 week regrowth, in both seasons (Table 41). A higher number of regrowing points was recorded in the dry season, indicating that *D. velutinum* does not only survive dry periods but that it remains productive (Table 41).

Among the semi-erect groups DM yield differences among accessions were significant ($P < 0.05$), with slightly higher yields in the wet than the dry season. In this group only 2 accessions, CIAT 13218 and 23983 had DM yields above 200 g DM/plant (Table 42).

As observed in the erect group, there were more regrowing points in the dry than in the wet season. Quality of the semi-erect types was similar to the erect types, with a range of 59 to 76% and 17 to 25 % IVDMD and CP, respectively. A larger number of accessions in this group had digestibilities above 70%, with accessions CIAT 23992, 23923, 23922, 33387, 23986, 23995 and 23975 having values above 73% though at a low yield level.

In the prostrate group significant ($P<0.01$) yield differences among accessions were measured (Table 43). However yields were lower than for the other groups, with only CIAT 13212 having DM yields above 200g/plant (Table 43). As digestibilities were also relatively low, this group is probably of the least agronomic interest.

Forage quality parameters of some promising *Desmodiun velutinun* accessions in the Erect Group are presented in Table 44. The IVDMD and CP content ranged between 59% and 75%, and 19% and 26%, respectively.

Accessions CIAT 23988, 23079, 23272, 33138 and 13948 had digestibilities above 70%. Highest stability for digestibility and yield across seasons was measured for accessions CIAT 33443, 23985, 33352, 23994 and 33138 (Table 44).
The level of CP was high in all accessions evaluated.

Table 41. Erect Group: Agronomic evaluation of a collection of *Desmodium velutinum* in Quilichao. Data of four evaluation cuts (two in the dry season and two in the wet season). Grey underlaid: Accessions with digestibility > 67 % and dry matter yield >200 g/plant.

Accession No. CIAT	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
33443	102	178	54	300	85	163	73	237
23985	109	151	41	230	105	143	76	215
33352	97	143	51	195	88	132	78	207
13953	99	152	61	301	88	140	60	204
23994	92	152	65	201	84	144	64	204
33138	108	134	48	185	106	107	69	194
23081	105	142	68	244	84	130	74	177
23086	90	143	62	184	78	136	72	170
33247	89	153	51	177	83	131	74	170
23136	112	143	43	151	103	123	52	158
23079	104	127	45	172	95	117	57	155
23989	112	134	37	165	101	115	46	153
D2430	80	133	53	145	81	129	65	141
23083	93	124	53	136	85	118	78	140
23132	98	131	38	129	90	107	56	134
23988	95	157	50	168	80	138	71	133
D3456	93	138	33	145	80	134	71	126
13947	108	119	42	159	93	112	59	125
13391	93	124	48	131	80	111	62	120
23133	118	131	35	148	98	108	47	116
13948	90	120	35	119	85	115	56	113
D81995	85	141	44	185	73	119	57	105
13222	90	119	37	132	78	120	56	105
23929	92	122	40	113	79	112	53	103
33250	94	125	43	121	81	101	62	103
23135	102	129	34	109	82	100	56	101
23930	77	135	42	92	73	133	56	100
14314	102	121	42	138	83	96	52	98
33254	85	134	36	117	74	112	53	93
23084	82	112	42	85	79	110	61	92
23158	89	135	39	91	84	132	53	89
23667	94	122	36	86	87	103	42	81
23272	80	128	48	82	78	124	53	79
23987	93	125	48	100	81	112	65	77
13945	105	102	29	102	94	97	47	74
23320	82	125	45	75	86	113	48	69
23669	86	103	41	67	83	101	49	69
23157	103	114	37	102	94	100	42	68
13954	88	121	34	104	70	111	46	68
23160	85	126	36	84	78	119	48	68
23324	85	130	43	70	85	120	50	65
D6	75	132	33	95	71	87	33	64
23322	77	132	43	64	78	119	62	64
23274	76	124	48	91	71	111	52	59
23326	89	123	46	66	80	107	48	55
23248	74	133	48	81	67	107	63	53
13221	95	119	32	57	84	106	32	52
23134	107	103	25	71	85	75	30	49
23321	79	112	33	41	79	114	46	46
23319	63	103	37	51	55	95	46	37
33255	74	83	17	32	70	68	23	33
D7NAPRI	67	110	24	77	56	86	24	30
23323	68	100	26	27	73	104	34	20
23082	71	118	34	44	67	92	42	19
Mean	91	128	42	123	82	114	55	105
LSD(P< 0.05)				112.099				124.81

Table 42. Semi-Erect Group: Agronomic evaluation of a collection of *Desmodium velutinum* in Quilichao. Data of four evaluation cuts (two in the dry season and two in the wet season). Grey underlaid: Accessions with digestibility >66 % and dry matter yield >190 g/plant.

Accession No. CIAT	Height	Diameter	Regrowing point	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
13218	78	163	61	218	73	148	61	225
23983	81	179	37	223	71	169	76	200
23982	90	166	51	225	79	148	72	198
23981	86	173	59	281	77	155	69	194
23276	70	171	51	224	60	172	81	194
33463	79	164	63	241	74	149	73	193
33003	98	162	53	209	87	152	79	177
33396	70	179	52	207	62	146	65	177
23928	87	173	55	217	76	159	70	171
23996	90	173	51	325	72	153	71	169
33459	88	154	59	172	88	144	65	168
33451	69	177	41	230	64	167	70	164
23920	69	144	52	210	63	151	78	161
33428	82	155	48	184	79	151	81	159
13216	75	162	32	185	73	150	81	159
23991	68	166	46	181	62	155	71	155
23923	82	156	54	176	79	140	55	154
23977	82	169	45	195	77	156	68	151
23325	86	160	61	228	74	135	62	146
13691	84	165	54	227	73	139	68	146
23980	88	174	59	197	74	147	73	142
23922	76	150	45	133	66	138	69	129
13220	100	148	41	176	69	123	58	127
23279	96	164	59	181	97	160	63	123
33242	84	142	41	123	73	126	60	122
23993	68	163	51	154	55	151	68	121
13219	67	161	51	141	59	144	48	115
23973	56	154	41	153	59	144	62	112
23927	86	136	42	122	80	129	60	111
23275	73	138	48	176	64	120	61	110
23921	76	159	56	224	67	148	52	106
13417	78	150	38	116	74	142	48	105
13952	78	134	51	141	75	129	37	105
33356	79	146	47	125	65	139	63	103
23975	86	145	52	179	67	117	74	103
13692	53	158	54	154	56	130	55	102
23986	77	153	62	160	63	129	73	99
13227	78	152	44	121	66	133	63	98
33249	71	145	44	115	58	111	52	90
23979	87	162	53	145	78	128	67	88
23278	92	161	53	116	83	142	56	87
23080	69	149	40	122	68	137	55	86
23668	82	131	38	87	65	123	58	84
13526	69	149	34	126	66	133	46	83
23974	63	143	50	142	54	124	61	82
23995	68	151	46	96	62	133	64	79
13676	94	146	51	95	77	128	62	76
23282	73	125	47	76	72	119	59	72
33401	77	142	46	112	68	115	52	72
13204	64	148	52	179	56	118	74	68
33464	80	130	40	100	71	116	50	67
23271	80	141	48	80	70	117	48	66
23277	90	135	44	91	75	111	58	64
23280	59	95	26	48	48	87	35	59
23992	75	140	45	104	61	110	59	58
33387	71	149	52	89	58	116	53	57
23327	74	129	41	88	70	117	59	56
23976	75	143	46	92	52	120	48	54
13690	73	137	54	125	58	120	69	49
23915	61	113	50	71	54	97	53	44
23273	74	113	37	59	53	88	46	41
23926	61	111	49	70	56	113	60	41
23925	69	111	41	69	53	106	45	37
13207	64	107	33	46	56	94	44	29
23924	49	83	33	30	47	84	44	19
23281	51	76	29	14	47	73	35	15
Mean	76	147	47	147	67	131	61	109
LSD(P<0.05)				113.1				110.6

Table 43. Prostrate Group: Agronomic evaluation of a collection of *Desmodium velutinum* in Quilichao. Data of four evaluation cuts (two in the dry season and two in the wet season).

Accession No. CIAT	Height	Diameter	Regrowing points	Mean DM yields	Height	Diameter	Regrowing points	Mean DM yields
	Wet				Dry			
	(cm)		(No.)	(g/pl)	(cm)		(No.)	(g/pl)
33520	44	171	41	179	42	154	59	143
13694	50	162	49	124	56	156	68	132
33481	42	174	45	158	45	154	59	121
13213	51	152	45	142	58	134	61	118
13215	53	167	50	148	48	151	68	94
33484	48	161	42	119	47	152	62	92
13693	41	160	51	127	38	142	58	92
13697	36	173	52	199	52	150	64	92
13212	52	163	54	206	45	141	55	91
33471	61	169	56	164	57	151	59	91
23990	56	168	43	126	50	158	54	82
13214	28	152	48	110	32	141	58	77
13695	46	162	50	145	37	151	53	75
13687	38	147	37	115	41	132	49	74
13688	53	139	42	81	34	132	52	70
13211	44	155	39	82	42	129	51	68
13217	37	147	48	171	38	144	45	58
Mean	46	160	47	141	45	145	57	92
LSD(P<0.05)				92.3				64.5

Table 44. Forage quality of selected accessions (erect group) of *Desmodium velutinum* evaluated in Quilichao, 2003-2004.

Accession	IVDMD	CP	NDF	ADF
			%	
23988	73.2	22.5	29.8	21.4
33451	70.6	22.7	30.8	21.6
23986	70.1	24.2	32.2	20.6
23994	69.7	22.0	32.8	23.9
23982	69.7	23.3	31.5	21.9
23981	69.2	21.7	34.4	23.8
23985	68.6	21.8	33.0	23.1
33463	68.3	23.4	36.0	25.8
13953	68.0	19.9	31.5	23.3
23921	67.9	21.9	33.8	24.0
23996	66.8	20.9	38.7	26.5
33443	66.8	20.5	37.1	28.2
23325	66.5	22.9	32.9	23.9
13218	66.1	20.9	36.3	25.1
23275	66.1	21.9	33.2	23.2
13691	65.8	21.6	35.4	25.2
23928	65.8	21.7	34.6	24.8
23081	64.9	20.9	36.5	26.6
23086	64.1	20.7	38.2	26.8
13952	63.8	19.3	35.2	25.6
Mean	67.8	21.6	34.2	24.3

3.5.3 Evaluation of a core collection of *Canavalia brasiliensis* for multipurpose uses in Santander de Quilichao, Colombia, 2004

Contributors: M. Peters, R. Schultze-Kraft (University of Hohenheim), Luis H. Franco, B. Hincapié, and G. Ramírez (CIAT)

Rationale

Canavalia brasiliensis Mart. ex Benth. ("Brazilian jackbean") is a weakly perennial, prostrate to twining herbaceous legume with a wide natural distribution in the New World tropics and subtropics. In comparison with *C. ensiformis* ("jackbean"), research reports on *C. brasiliensis* are scattered and restricted to studies done in Latin America. The species develops a dense and extensive, deep-reaching root system and subsequently tolerates a 5 month dry period. Based on studies that generally were done with only one genotype, it is adapted to a wide range of soils, including very acid, low-fertility soils. Its main use is as green manure, for fallow improvement and erosion control. Due to medium biomass decomposition, nutrient release of *C. brasiliensis* green manure has the potential to synchronize well with the nutrient demand of the succeeding crop and leads to high N recovery rates.

In Central America, the legume is being used to improve the value of stubble grazing in the dry season. Antinutritive substances such as toxic amino acids (e.g., canavanin), lectins (e.g., concanavalin Br) and trypsin inhibitors have been reported in seeds of *C. brasiliensis*. However, there is little information on the nutritive value of the herbage of this species for ruminants.

Materials and Methods

A total of 53 accessions of *Canavalia brasiliensis*, mostly from Latin America, were sown into jiffy pots and transplanted 4 weeks later to the field at CIAT's research station in Santander de Quilichao. A total of 6 plants per plot were sown, in a Randomized Complete Block Design with 4 replications. DM yield, drought tolerance and forage quality parameters are measured (Photo 19).

Photo 19. *Canavalia brasiliensis* sown at Quilichao, 2004

Results and Discussion

Plants established quickly, and incidence of pest and diseases was low, in particular when compared to a collection of *Canavalia* sp. planted at the same time in Quilichao. 12 weeks alter transplanting the majority of accessions had soil covers above 70%, with accessions CIAT 808, 18515, 7319, 7648, 7970, 8557, 2009517008,17009 and 20096 showing the highest values (Table 45).

Forage yields 16 weeks after transplanting varied significantly (P< 0.01) among accessions, with yields above 4 t DM /ha recorded for accessions CIAT 808, 17009, 8557, 17012, 20098, 21824, 18515, 20303,17973, 7178, 20306, 7648 and 7319.

Table 45. Vigor, soil cover (%) and dry matter yields of *Canavalia brasiliensis* in Quilichao, 2004.

Accession	6 Weeks		12 Weeks		16 Weeks		
	Vigor	Soil cover	Vigor	Soil cover	Vigor	Soil cover	Mean dry matter yields
	1-5	%	1-5	%	1-5	%	kg/ha
808	4	50	5	92	5	100	6320
17009	4	43	4	85	5	100	5333
8557	4	47	5	87	5	97	4693
17012	3	35	3	78	4	90	4560
21824	3	40	3	73	3	78	4440
20098	3	33	3	75	4	80	4440
18515	3	45	4	88	5	100	4333
20303	3	35	3	77	4	80	4333
17973	3	32	3	68	4	75	4293
7178	4	40	3	73	4	82	4200
20306	3	30	3	72	4	85	4187
7648	4	52	4	87	5	100	4107
7319	4	52	4	87	4	90	4067
20095	3	47	4	87	4	93	3907
7969	3	35	4	80	5	93	3880
22132	3	40	3	73	4	77	3840
7321	3	35	3	78	4	87	3787
7973	3	40	4	77	4	80	3760
18501	3	38	3	70	4	75	3720
17008	4	42	4	85	4	80	3680
8770	3	38	3	75	5	100	3680
19034	3	43	4	83	4	85	3653
17462	3	40	4	80	5	97	3627
905	3	43	3	63	3	67	3613
7647	3	37	4	77	4	87	3507
7971	3	30	3	72	4	82	3400
7175	2	27	3	63	3	72	3373
19029	4	43	4	83	4	83	3320
20518	3	42	4	83	4	80	3147
20096	3	37	4	85	4	83	3120
20516	3	35	3	73	4	83	3053
21825	3	33	3	73	4	82	3040
17010	3	35	3	70	4	80	2933
19035	3	35	3	72	3	63	2893
20514	4	52	3	83	3	80	2840
19361	4	43	3	78	4	88	2813
17011	3	40	3	80	3	85	2813
7972	3	32	4	80	4	90	2787
20513	4	50	4	75	3	82	2773
20301	3	27	3	78	3	83	2720
8768	3	32	3	63	3	72	2653
7970	3	43	4	87	4	90	2640
20295	3	35	3	77	3	77	2627
19359	3	37	3	73	3	73	2600
9146	3	37	3	75	3	80	2560
20304	2	28	3	63	3	75	2560
19632	3	27	3	65	3	67	2440
20296	2	23	3	60	2	67	2360
20090	2	22	3	70	3	70	2333
5033	3	38	3	70	3	80	2227
7174	3	32	3	67	3	70	2227
17828	3	28	4	68	3	63	2160
7894	3	28	2	55	3	72	1813
Mean	3.1	37	3.4	76	3.8	82	3400
Range	2-5	20-70	2-5	30-100	1-5	30-100	1000-8600
LSD (P< 0.05)							2855.21

Accession CIAT 17009 is a line previously selected by farmers in Central America for use as green manure and to improve fallows and crop residues, due to its high yield and drought tolerance. Despite a severe dry period all accessions remained healthy, with soil covers above 80%; in a few materials some leaf loss under drought conditions was observed.

3.5.4 Evaluation of a core collection of *Canavalia* sp. for multipurpose uses in Santander de Quilichao, Colombia, 2004

Contributors: M. Peters, Luis H. Franco, R. Schultze-Kraft (University of Hohenheim), B. Hincapié, G. Ramírez, (CIAT)

Rationale

In view of the promising results obtained with *Canavalia brasiliensis*, there is an interest to define the potential of other species of *Canavalia* for use mainly as green manure and for fallow improvement in low fertility, drought prone environments.

Materials and Methods

A total of 47 accessions of *Canavalia* sp, originating from Latin America, China and Thailand were sown into jiffy pots and transplanted to the field in Santander de Quilichao (Photo 20).

The design and variables are the same as described for *C. brasiliensis*. Establishment of most accessions was slow, with soil covers below 53% after 12 weeks of transplanting. On the other hand, 16 weeks after transplanting, only 11 materials had soil covers above 80%. Several materials appear not to be well adapted to the acid soils in Quilichao and were severely affected by pests and diseases.

Accessions with the best adaptation during establishment phase were CIAT 21012, 21014, 19038, 21209, 7317, 7383, 8719, 21013, 21211 and 18587, all of which are showing good drought tolerance (Table 46).

Photo 20. *Canavalia* sp. sown at Quilichao, 2004.

Table 46. Vigor and soil cover (%) of *Canavalia* sp. in Quilichao, 2004.

Number Accession	6 Weeks		12 Weeks		16 Weeks	
	Vigor	Soil cover	Vigor	Soil cover	Vigor	Soil cover
	1-5	%	1-5	%	1-5	%
19038	5	55	5	90	4	100
21209	5	52	5	80	4	97
7317	5	50	4	83	4	93
21012	5	42	4	60	5	92
21014	4	40	4	57	5	90
8719	5	57	5	83	4	87
18271	5	53	4	73	3	87
7383	4	40	4	67	4	87
7318	5	58	4	80	3	83
21013	4	37	4	63	4	83
21211	4	33	4	63	4	82
7322	5	58	4	72	3	78
19033	5	45	4	70	3	78
20803	4	42	4	63	2	75
19031	4	42	4	60	3	73
20307	4	40	4	73	3	73
18587	4	33	4	50	4	73
18272	5	52	4	73	3	72
20145	4	37	3	47	3	72
18580	3	32	3	47	3	72
22031	4	35	3	50	3	70
20691	4	32	3	50	2	70
20748	3	28	4	57	2	70
8769	4	40	4	57	2	68
19032	4	37	4	63	3	68
20305	4	32	3	50	2	68
20298	4	42	4	70	3	67
19357	4	35	4	67	2	67
18270	4	45	4	60	3	65
21210	4	45	4	57	3	65
17929	4	45	4	70	2	63
17451	4	38	4	57	2	63
8771	3	30	3	43	2	63
21212	4	32	3	40	2	60
8185	4	45	3	43	2	57
20113	4	40	3	47	2	57
21487	3	28	3	47	2	57
19052	2	17	3	33	3	57
18268	2	11	2	20	2	37
19356	2	13	2	20	2	33
18258	2	18	2	13	2	28
18261	2	8	2	13	1	28
18263	2	8	2	13	1	25
20300	4	37	3	40	1	23
20093	3	32	2	20	1	10
20297	2	7	2	10	1	10
20299	2	6	2	10	1	5
Mean	3.7	36	3.5	53	2.6	64
Range	1-5	1-70	1-5	10-95	1-5	5-100

3.6 Annual legumes for multipurpose use in different agroecosystems and production systems

Highlights

- Selected an accession (IT95K-52-34) of cowpea with superior grain yield as compared to local checks in acid infertile and fertile soils.

- Showed that cowpea as green manure can substitute the N applied (80 kg) to maize by farmers in hillside of Nicaragua.

- Selected an accession (CPI-67639-early flowering) of *Lablab* in hillsides of Nicaragua based on rapid establishment, high cover and high seed yield.

3.6.1 Evaluation of new Vigna *unguiculata* accessions in Quilichao and Palmira, Colombia

Contributors: M. Peters, Luis H. Franco, B. Hincapié, G. Ramírez, (CIAT), R. Schultze-Kraft (University of Hohenheim) and B.B. Singh (IITA, Nigeria)

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, 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. Cowpea could also be used for hay, silage and feed meal production, which in turn could be an option for income generation by smallholder livestock and non-livestock owners.

Good adaptation to climatic and edaphic conditions, especially to water stress, are prerequisites for a successful development of cowpea as an 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.

Materials and Methods

A new collection of cowpea obtained from IITA was sown in Santander de Quilichao and Palmira in 2004 in order to select accession with both high forage and grain yields and good adaptation to contrasting soils. (Photo 21). Our previous selection criteria for cowpea had been mainly forage yield in alkaline and acid soils. The same evaluation methodology as presented in previous annual reports was utilized. The main variables measured are forage production and quality, grain yield and effect as green manure on a subsequent maize crop. A particular emphasis is given to material adapted to a wide range of soils.

Results and Discussion

The collection established was highly diverse in terms of flowering response, with very early to very late accessions present. The differential flowering pattern will be taken into account for the planning of subsequent trials. In Palmira it was necessary to replant one replication because of negative effects of waterlogging and consumption of seed by birds.

Soil cover was more rapid in Palmira than in Quilichao, with a mean of 80% and 64% covered respectively 10 weeks after planting. Pest and diseases were present in both sites but did not limit the development and productivity of plants. Grain was harvested 12

Photo 21. Cowpea (*Vigna unguiculata*) at Quilichao.

weeks after planting when pods where dry. Mean yields in Quilichao were above 2 t/ha and significant ($P \leq 0.05$) differences among accessions were measured.

The accessions IT97K-1069-6, IT95K-52-34 and IT98K-412-8, had yields above 3 t/ha. Mean grain yields in Palmira were double (4 t/ha) than those obtained in Quilichao. Differences among accessions were significant ($P \leq 0.01$) and the highest grains yields with more than 5 t/ha were achieved with local checks (CIDICCO3, CIDICCO4), and with the new accession, CIAT 9611, IT95K-52-34 (Table 47).

In terms of forage quality, significant ($P \leq 0.01$) differences among accessions were measured for IVDMD, P and K, but not for CP. In vitro dry matter digestibility was above 83% while CP contents ranged between 19.7% and 24.2 %, confirming the high quality of the forage from cowpea (Table 48).

Table 47. Vigor, soil cover (%) and yield (kg/ha) of *Vigna unguiculata* grain in Quilichao and Palmira, 2004.

	Quilichao (acid infertile soils)			Palmira (fertile soils)		
Accession	Vigor	Cover (%)	Grain (kg/ha)	Vigor	Cover (%)	Grain (kg/ha)
	1 - 5	10 weeks	12 weeks	1 - 5	10 weeks	12 weeks
IT97K-1069-6	5	85	3327	4	87	4360
IT95K-52-34	5	92	3313	5	100	5513
IT98K-412-8	4	72	3180	4	83	4900
IT98K-131-2	4	67	2873	3	77	4873
IT97K-819-118	4	68	2867	3	73	2460
IT98K-406-2	4	72	2607	3	77	3847
CIDICCO 3 (local check)	5	82	2560	5	100	6013
IT97K-1069-2	4	73	2533	4	90	4087
IT97K-818-35	4	72	2460	3	77	3020
CIDICCO 2 (local check)	5	85	2420	5	93	4653
IT99K-7-14	3	63	2360	3	63	3267
IT99K-409-8	4	77	2347	4	87	3440
IT99K-429-2	3	53	2327	3	67	3120
IT99K-1060	2	47	2280	3	62	3760
IT97K-825-3	4	75	2267	3	67	2853
IT98K-476-8	4	73	2260	3	73	3553
CIAT 9611 (local check)	4	60	2167	5	93	5433
IT98K-391-2	4	72	2153	5	93	4553
IT98D-1399	3	53	2087	3	60	3440
IT98K-412-13	4	67	1993	3	73	4533
IT96D-610	4	65	1987	4	83	4327
CIDICCO 1 (local check)	4	75	1980	5	93	4613
IT98K-428-3	4	70	1953	4	77	3333
CIDICCO 4 (local check)	5	70	1927	5	100	5533
IT97K-570-18	4	77	1820	5	93	5533
IT98K-506-1	3	55	1733	3	77	3913
IT97K-356-1	3	20	1673	3	70	3913
IT98K-205-8	3	53	1660	3	67	3800
IT97K-499-38	4	60	1627	4	78	2980
IT98K-390-2	4	63	1533	5	100	4233
IT99K-7-21-2-2	2	37	1520	3	67	2833
IT89KD-288[1]	2	43	1413	3	77	3480
IT97K-494-3	2	47	1380	3	70	2527
IT99K-216-24-2	2	53	1367	3	53	4153
IT99K-1122	2	50	1327	4	90	4233
IT97K-461-4	3	50	1260	5	87	4293
FHIA (local check)	2	43	1093	4	83	4487
IT98K-311-8-2	4	80	1027	4	90	3880
Mean	3.8	64	2070	3.8	80	4020
MSD ($P \leq 0.05$)			1810.2			3353.4

[1] Accession selected from the first core collection from IITA evaluated in Palmira and Quilichao.

Table 48. Quality of the forage in accessions of *Vigna unguiculata* grown in Quilichao, 2004.

Number Accession	Forage IVDMD	 Protein	 P	 K
		%		
IT98K-131-2	89.8	21	0.16	1.46
IT97K-825-3	89.2	20.6	0.16	1.85
IT96D-610	88.8	19.8	0.13	1.21
CIAT 9611 (local check)	88.8	20.2	0.16	1.44
FHIA (local check)	88.6	19.4	0.13	1.57
IT98K-311-8-2	88.4	19.4	0.15	1.44
IT98K-476-8	88.4	19.9	0.12	1.77
IT99K-7-14	88.3	20.5	0.14	1.29
IT89KD-288	87.9	20.4	0.12	1.45
IT99K-216-24-2	87.8	21.5	0.16	1.51
IT98K-205-8	87.5	20.4	0.14	1.44
IT95K-52-34	87.5	19.7	0.14	1.44
IT97K-819-118	86.8	20.8	0.16	1.64
IT97K-570-18	86.5	20.0	0.13	1.49
CIDICO 2 (local check)	86.8	20.7	0.14	1.59
IT97K-356-1	86.7	20.3	0.15	1.54
IT97K-818-35	86.7	20.9	0.15	1.44
IT97K-499-38	86.6	22.2	0.15	1.78
IT99K-429-2	86.6	20.3	0.14	1.43
IT99K-1122	86.5	23.1	0.20	1.95
IT98K-506-1	86.4	21.1	0.16	1.69
IT97K-1069-2	86.3	20.9	0.17	1.37
IT98K-412-13	86.2	22.1	0.16	1.77
IT97K-1069-6	85.7	23.5	0.18	1.73
IT97K-461-4	85.5	22.4	0.16	1.69
IT97K-494-3	85.3	22.7	0.17	1.86
CIDICO 4 (local check)	85.1	22.1	0.15	1.80
IT98K-412-8	85.1	22.6	0.16	1.93
IT98K-428-3	85.1	22.5	0.16	1.5
IT98D-1399	85.0	20.5	0.13	1.77
IT99K-7-21-2-2	84.7	22.6	0.16	1.95
CIDICO 1 (local check)	84.7	21.1	0.14	1.77
IT98K-390-2	84.6	21.7	0.15	1.69
IT98K-391-2	84.6	20.6	0.10	1.78
CIDICO 3 (local check)	84.5	23.6	0.14	1.81
IT99K-1060	83.7	21.6	0.15	1.5
IT99K-409-8	83.7	22.5	0.15	1.7
YT98K-406-2	83.0	24.2	0.21	1.9
Mean	86.4	21.3	0.15	1.64
LSD	4.79 ($P \leq 0.01$)	5.74 ($P \leq 0.07$)	0.08 ($P \leq 0.01$)	0.71 ($P \leq 0.01$)

3.6.2 Evaluation of *Vigna unguiculata* germplasm in Nicaragua

Collaborators: A. Schmidt, C. Davies, E. López, M. Peters, L.H. Franco, and G. Ramirez (CIAT)

Rationale

We visualize that cowpea could be an alternative crop for a) the first planting season (“primera”) as a soil improving starter crop for maize, and b) for the second planting season (“postrera”) in low fertility soils in hillsides of Nicaragua and Honduras. This legume could allow for a third crop in November/December the maize/bean systems in order to provide grain, hay for animal feeding in the dry season or contribute to soil fertility enhancement for the following maize crop. Cowpea could also be used for hay, silage and feed meal, which in turn could be an option for income generation by smallholder livestock and non-livestock owners. 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.

Materials and Methods

During 2002 a small core collection of *Vigna unguiculata* from IITA comprising 14 accessions, which were selected in Quilichao and Palmira for good adaptation to soils (acid and alkaline) was introduced into Nicaragua. In October 2002 the core collection, complemented with 5 Central American accessions, was planted out in small plots at the SOL seco site in San Dionisio, Matagalpa, Nicaragua (Lat N 12° 45' 05.8", Long. W 85° 53' 16.5", Alt. 537 masl, rainfall 990 mm/a, mean temp. 26°C). Standard evaluation procedures were applied as in previous years. At flowering 50% of each plot was cut and biomass kept on plot surface as mulch; from the remaining 50% grain was harvested in December 2002. Crop residues were equally kept on plot surface for the rest of the dry season. The respective results were reported in AR 2003, p.116). Upon the outset of the rainy season 2003 a maize crop was established in each plot. Total dry matter and grain production were recorded in November 2003 in order to detect possible residual fertility effects from the preceding *Vigna unguiculata* accessions. Additional plots were established where 45, 80, 140, 200 kg/ha N, respectively, were applied. A non-fertilized (N 0) treatment was also included.

Early 2003, due to increasing interest in *Vigna unguiculata* by farmers in San Dionisio (who demanded the re-establishment of our collection for further participatory evaluations and for seed increase for on-farm evaluation) the accessions were re-established in 6m x 6m plots without replicates. After 2 field events with participatory evaluations (n=21 persons) plots were harvested and seed distributed to 35 farmers.

Results and Discussion

In Table 49 maize plant height, DM and grain yields are presented. No significant differences ($P>0.05$) were detected among accessions or between treatments (mulch vs. grain harvested). Mean plant height, DM and grain yield are in-line with farmer maize crops in San Dionisio. Farmers, depending on their economic possibilities, apply up to 80 kg/ha N to their maize crops. Results of our experiment showed that a cowpea crop can easily replace the application of these amounts of nitrogen, even as a preceding dry season crop. This corroborates our findings from on-farm experiments, reported in AR 2003 (p. 145), where we argued that with a legume crop planted at the end of the rainy season, traditional nitrogen fertilizer applications for the following maize crop can be substituted in the dry hillsides of Nicaragua and Honduras.

Our results this year with cowpea indicate that farmers can not only reduce production cost of maize, but also have an additional legume grain harvest for human consumption or animal feeding. Since no significant differences between plots with mulched or grain-harvested cowpeas were found, farmers can choose their cowpea accession based on their utilization preference.

In Table 50 we present the results of participatory evaluations of the cowpea collection in Nicaragua. Selection criteria employed by farmers were: grain yield, leafiness and leaf size, plant vigor, pod size, and plant height. Of the accessions selected, the local accessions Rojo, INTA and Negro are leafy types for animal feeding, while the introduced IT90K-284/2 accession is a dual purpose type as shown before (see AR 2003, p. 117). This accession is widely adapted across different environments in Colombia and Central America.

Seed production began in the first planting season 2004 with the production of 50 kg of accession Rojo and IT90K-284/2. In the second planting season seed production efforts will be increased. Evaluation of new accessions, both local and introduced materials, will continue throughout

2004-2005. With regard to soil fertility effects resulting from cowpea as a green manure, we plan to intensify our collaborative work with the TSBF/Soils Group in Central America (see also 4.1.7 in this report).

Table 49. Plant height, DM and grain yields of maize planted after *Vigna unguiculata* (cowpea) at San Dionisio, Nicaragua, 2003.

Accession	Maize yields - Cowpea mulch			Maize yields - Cowpea grain harvested		
	Plant height (cm)	DM total (kg/ha)	Grain yield (kg/ha)	Plant height (cm)	DM total (kg/ha)	Grain yield (kg/ha)
IT95K-1088/2	194	7999	3385	198	6512	3255
IT95K-1088/4	191	8279	3149	184	6408	2041
IT90K-277/2	198	8278	3284	189	6873	2785
IT90K-284/2	188	5763	2844	172	4967	2213
IT89KD-288	202	10206	3528	190	8930	4209
IT89KD-391	200	6858	2699	201	8448	4048
IT93K-503/1	181	6408	3224	176	7910	3448
IT93K-573/5	191	6185	2792	199	7611	3006
IT93K-637/1	194	7029	3050	209	9095	4516
IT86D-715	204	7937	3416	191	5643	3008
IT86D-716	198	6772	2015	200	7534	3294
IT86D-719	201	6537	4014	192	7735	3422
IT6D-733	187	5607	2824	197	8367	3804
IT96D-740	197	6188	2683	193	7628	3094
Café	212	8163	4082	208	8399	3671
INTA	199	6955	2873	196	9115	3253
Negro	209	8129	3845	185	8124	3905
Rojo	190	8667	3432	210	8688	3770
SF Libre	201	6461	2777	189	7015	2886
Mean Acc.	197	7285	3153	194	7632	3349
LSD ($P \leq 0.05$)	46.9	6873.9	3044.9	42.1	4619.7	2349
Nitrogen treatments						
N 0	180	4324	2239			
N 45	179	5104	2621			
N 80	177	6668	3085			
N 140	164	7169	4060			
N 200	184	7612	4368			

Table 50. Farmers ranking of *Vigna unguiculata* accessions and their potential uses expressed by farmers in San Dionisio, Nicaragua, 2003.

Framers ranking		Potential use expressed by farmers	
Good	Regular	Animal feed	Grain production
Rojo	IT86D-715	Rojo	Rojo
Café	IT86D-719	INTA	IT90K-284/2
IT96D-740	IT93K-637/1	Negro	
IT89KD-391	IT6D-733		
INTA	IT95K-1088/2		
Negro	IT93K-573/5		
IT90K-284/2			

3.6.3 Evaluation of *Lablab purpureus* germplasm in Nicaragua

Contributors: A. Schmidt, C. Davies, E. Lopez, M. Peters, L.H. Franco, G. Ramirez (CIAT)

Rationale

A major problem facing livestock producers in Central America is inadequate animal nutrition during the dry season when pastures, sorghum and maize stover are limiting in quality. Problems such as sickness and weight loss due to a poor nutrition are frequent. One way for improving the utilisation of such crop residues is by adequate supplementation with leguminous forages of high quality.

The legume *Lablab purpureus* is recognized not only as drought resistant, but also for its adaptability to a wide range of environmental conditions. Though the legume is widely known in Central America under a number of names (e.g. dolichos, caballero) and has the capability of being an outstanding resource for crop-livestock systems in this region, (e.g. the legume can be used as cover crop, grazed in a pasture setting or as a companion crop to maize, cut as hay, or mixed with corn silage), it is not being used to its full potential. So far only two commercial lines have been available to farmers in Nicaragua, but seed availability remains a major limitation. Thus to select for more productive and better-adapted germplasm for the dry hillside regions of Nicaragua, a *Lablab purpureus* core collection from ILRI/CSIRO is currently under evaluation.

Material and Methods

During 2003 a core collection from ILRI/CSIRO comprising 12 accessions was introduced into Nicaragua. In October 2003, accessions were planted out in 2m x 2.5 m small plots in three replicates in a randomized complete block design at the SOL seco site in San Dionisio, Matagalpa, Nicaragua (Lat N 12° 45' 05.8", Long. W 85° 53' 16.5", Alt. 537 masl, rainfall 990 mm/a, mean temperature 26°C). After an initial evaluation of plant emergence, accessions were evaluated in a two-weeks interval for plant height, soil cover, plant vigour, flowering patterns, incidence of pests and diseases, and seed production. No fertilizer was applied throughout the experiment. Dry matter yields prior to flowering were not recorded due to the small initial amount of seed introduced. Priority was given to genotype characterization and seed increase.

Result and Discussion

Accessions of the core collection established well at the experimental site with an average of 79% of all seeds emerging (Table 51). Plants reached an average height of 49 cm and covered 12 weeks after planting on average 74% of the plots. Plants showed good vigour and no incidence of pests or diseases throughout the experiment, with the exception of accession CPI-36903.

Accession CPI-67639 (early flowering) performed outstanding in this experiment in terms of establishment, soil cover and seed yield. It remains to be seen if this seed high yield is correlated with low biomass production. Biomass production and on soil fertility effects on subsequent maize crops will be obtained on larger plots in the *postrera* 2004 and primera season 2005. Enough seeds was harvested in the present experiment to include an additional experimental site in 2004-2005, which will be under the responsibility of our national partner INTA.

We conclude from this first experiment with *Lablab purpureus* that the accessions evaluated adapted well to the dry conditions of the central region of Nicaragua where small farmer crop-livestock systems are predominant. Our results showed variability within the collection with regard to seed yield and flowering patterns. Nevertheless, biomass production data and the results from participatory evaluation by farmers will determine which accession will be multiplied for forage production, soil improvement or grain production. The selected *Lablab* accessions will be an additional annual legume alternative to farmers in the drier regions of Central America.

Table 51. Plant emergence, plant height, soil cover, plant vigour, flowering behaviour and seed yield of a *Lablab purpureus* core collection at San Dionisio, Nicaragua, 2003-2004

Accessions	CIAT No.	Plant Emergence (%)	Plant height (cm)	Soil cover (%)	Plant Vigour (1-5)	Flowering (Early/Late)	Seed Yield (g/m2)
CPI-67639	17197	100	41	97	4	E	127
CPI-34777	22598	67	59	62	4	L	113
21603	21603	77	44	70	5	L	92
I-14442	22768	88	50	87	5	L	83
CQ-2975	22735	90	47	83	5	E	82
L-987	22660	97	60	91	5	E	74
CPI-52535	22604	63	42	63	3	L	70
cv. Highworth	22660	85	56	83	4	E	70
I-11632	22764	90	50	72	4	L	43
I-6533	22770	85	51	85	5	L	37
CPI-36903	22653	33	32	30	3	L	34
CPI-106471	22663	77	56	62	4	E	28
Mean		79	49	74	4.3		71
LSD ($P \leq 0.05$)		78.8	43.5	72.4			81.9

3.6.4 Effect of *Lablab purpureus* accessions as a green manure in Quilichao and Palmira

Contributors: M. Peters, L. H. Franco, B. Hincapié, and G. Ramírez (CIAT)

Rationale

Lablab purpureus is a free seeding, fast growing, 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 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 the evaluation of the Lablab collection in acid and neutral soils to define niches for this species as 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 multilocational trial to evaluate of *Lablab purpureus* selected from previous work in Colombia was initiated in contrasting sites – soil, climate and altitude – in Colombia (6 sites), Costa Rica and Nicaragua. In this section we report results on effects of Lablab on a succeeding maize crop in Quilichao (acid low fertility soils) and Palmira (alkaline high fertility soils) (Photo 22).

Results and Discussion

Due to the short rainfall cycles in the bimodal rainfall system prevalent in Palmira and Quilichao, it is not possible to plant a crop directly after lablab as a green manure. Hence an alternative strategy was employed, with the lablab green manure being incorporated at the end of the dry season, followed by the crop sown at the beginning of the next wet season. Maize DM and grain yields after incorporating lablab as green manure are presented in Table 52.

Maize yields in Palmira were higher than in

Quilichao. In Palmira there was no positive effect neither of the green manure or N-fertilization, probably due to the inherent high fertility of soils. In contrast, in Quilichao, maize yields without fertilization were only 55% of the yields obtained with 120 kg/ha N. Following incorporation of lablab accessions CIAT 22663 and 21663, maize grain yields were similar to those recorded with the 3 levels of N applied.

Table 52. Effect of *Lablab purpureus* as green manure on biomass and grain yields of a succeeding maize crop (Palmira and Quilichao, 2004).

Accessions CIAT No.	Palmira (fertile soil)		Quilichao (acid infertile soil)	
	Yield (kg/ha)			
	DM Plant	Grain Maize	DM Plant	Grain Maize
22653	5648	6014	4548	4336
22768	3366	5319	4665	4554
22766	4411	5282	4381	4231
22762	4026	5162	4047	3268
22660	4385	4627	4242	3983
17197	4156	4870	4375	4080
22770	3591	4796	4175	3886
22652	5171	4657	3963	3886
N0	5824	4596	3240	2689
22598	4513	4545	5010	4426
22764	3738	4471	4431	3963
N120	5574	4439	4320	4826
21603	4012	4360	4821	4682
22663	5152	4179	5016	4826
22604	3897	4128	4336	4587
N80	4124	3929	4136	4292
N160	4694	3850	3674	4470
22735	2863	3627	4387	3607
N40	4138	3077	4119	3958
Fallow			3064	2906
Mean	4398	4558	4266	4087
LSD ($P \leq 0.05$)		3401		3134

Photo 22. (A) *Lablab purpureus* as green manure and **(B)** Maize after Lablab in Palmira.

3.6.5 Forage and green manure potential of a collection of *Mucuna* spp.

Contributors: M. Peters, L. H. Franco, B. Hincapié, and G. Ramírez (CIAT)

Rationale

Mucuna is a legume utilized by farmers in Central America (particularly in the humid tropics) as a green manure and rarely as forage. We were interested in determining the variability among Mucuna accessions when used as green manure.

Materials and Methods

Eight accessions of *Mucuna* sp., obtained from CIEPCA, and previously evaluated for L-Dopa content (see Annual report 2002) were planted in Quilichao (see Photo 23) to be used as a green manure for a succeeding maize crop. A Randomized Complete Block Design with 3 replications was employed.

Results and Discussion

The establishment of Mucuna was rapid, with the exception of *Mucuna* sp cv. Rayada-61. Mean soil cover was 90% 12 weeks after sowing. At 16 weeks soil cover declined to 81% due to some leaf loss. However, soil cover of *Mucuna*. sp cv. Preta-82 and *M. pruriens* CIAT 9349 remained stable with 100% soil cover even under drought conditions.

Biomass yields were above 5.8 t/ha both at 12 and 16 weeks, with *M. pruriens* cv. Jaspeada-106, *M. pruriens* CIAT 9349. These accessions had significantly (Pd$\leq$0.01) higher DM yields after 16 weeks, of growth that other accessions (Table 53).

However, highest maize grain yields (6.1 t/ha) were achieved after incorporation of *M. pruriens* cv. Utilis-109. Interestingly the lower biomass yielding accession (cv. Rayada-61) had a better effect as green manure as compared to the higher biomass accession (cv. Jaspeada –106), probably related to immobilization of nutrients in the soil.

Digestibilities of Mucuna accessions under evaluation ranged between 52% and 62%, and digestibility values were below the values obtained for lablab included as a control. The CP contents ranged between17% and 21% (Table 54).

Table 53. Soil cover (%) and biomass yield (kg/ha) of *Mucuna* sp and maize grain yield in Quilichao, 2004.

Accession	Biomass Mucuna				Maize	
	Soil cover (%)		DM yield (kg/ha)		DM yield (kg/ha)	
	12 Weeks	16 Weeks	12 Weeks	16 Weeks	Plant	Grain
M. pruriens cv. Cochinchinensis	100	85	7987	5720	11768	5773
M. pruriens cv. Jaspeada-106	100	80	6940	10033	11206	5099
M. sp cv. Ghana-4	70	32	6433	3020	9413	3991
M. sp cv. Rayada-61	53	17	6213	1687	12291	5728
M. pruriens CIAT 9349	100	100	5427	6293	11723	5160
M. pruriens cv. IRZ-99	100	85	5347	7867	11367	5422
L. purpureus CIAT I-14442 (control))	77	68	5053	5507	11846	5544
M. pruriens cv. Utilis-109	100	97	4420	6580	12748	6140
M. sp cv. Preta-82	100	100	4400	6007	10449	4787
Fallow				5720	8676	4259
Mean	90	81	5802	5857	11234	5222
LSD ($P \leq 0.05$)			3295.41	4788.27	4657.72	2355.44

Photo 23. *Mucuna* sp. at Quilichao

Table 54. Forage quality of a collection of *Mucuna* sp evaluated in Quilichao, 2003-2004.

Accession	PC %	DIVMS %
L. purpureus (control)	18.1	72.7
M. pruriens cv. Cochinchinensis	20.2	61.6
M. sp cv. Ghana-4	15.1	60.4
M. pruriens cv. IRZ-99	20.8	58.1
M. pruriens cv. Jaspeada-106	16.9	57.9
M. pruriens CIAT 9349	21.8	57.5
M. sp cv. Preta-82	20.0	57.2
M. pruriens cv. Utilis-109	21.4	55.3
M. sp cv. Rayada-61	17.0	51.9
Mean	19.0	59.2
LSD ($P \leq 0.05$)	6.1	6.6

3.6.6 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

In plantations of the Llanos of Colombia there is a need 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 species (*Arachis pintoi, Desmodium heterocarpon* subsp. *ovalifolium* and *Pueraria phaseoloides)* were sown under shade and no-shade conditions in the Meta department of Colombia.

Materials and Methods

In plots of 80 m^2 we established legumes covers in a commercial rubber (young and old) and oil palm plantations in the savannas and Piedmont 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.

These plots have been monitored through visual observation in regular intervals, to assess long-term persistence of legumes sown as plantation covers.

Results and Discussion

The legume covers were evaluated at the beginning of the wet season of 2004. Now 5 years after planting, several accessions sown under high shade conditions in established plantations disappeared. However, legume covers established in young plantations with moderate shade continue to be vigorous and cover the soil well. Several *A. pintoi* accessions have covers above 70%, while *D. heterocarpon* cv. Maquenque (CIAT 13651) covered 67% of the soil and *D. heterocarpon* (CIAT 23672) covered 80% of the soil. The control *P. phaseoloides* almost disappeared under low shade (Table 55).

Results confirm the utility of *D. heterocarpon* subsp. *ovalifolium* and *A. pintoi* as plantation covers, provided moderate light in available. These legume when used as covers should be established in the early phase of the plantation and/or utilized to cover more open spaces between rows of trees, where weeds are a major problem.

Table 55. Soil cover of different forage legumes under rubber 5 years after sowing, under high shade and low shade conditions, Llanos of Colombia.

Accession	High shade		Low shade	
CIAT No.	Soil cover (%)	Vigor	Soil cover (%)	Vigor
A. pintoi 17434	17	3	73	4
18744	5	3	73	4
18748	12	3	60	3
22159	5	2	72	4
22160	10	2	73	3
D. heterocarpon 350	0	0	57	3
13105	0	0	52	3
13110	2	1	52	3
13651 (cv. Maquenque)	2	1	67	3
23762	0	0	80	3
P. phaseoloides 8042 (control)	0	0	12	2
9900	2	1	2	1
Asoc. *A. pintoi – D. heterocarpon*	5	2	45	3

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

4.1 Partnerships in LAC to undertake evaluation and diffusion of new forage alternatives

Highlights

- High carrying capacity of Mulato pastures is a positive benefit to farmers since it results in greater animal production per unit area as compared to other grasses. However, the high biomass production potential of Mulato is associated with a high demand for N particularly in acid soils of low fertility.

- The *Brachiaria* hybrid (CIAT 36087 (Mulato II) showed good adaptation to both acid-low fertility soils and well drained fertile soil.

- Adoption of *Arachis pintoi* in the llanos piedmont is affected by (a) lack of commercial seed, (b) high cost of establishment with vegetative material and (c) limited information on management of legume-based pastures.

- *Brachiaria* cultivars (Toledo and Mulato) and *Paspalum atratum* were selected on the basis of adaptation in sites in Nicaragua with soils of low fertility, whereas *Paspalum atratum* and *Panicum maximum* cv. Tanzania were selected for sites with more fertile soils.

- The drought tolerant *Canavalia brasiliensis* when used as green manure resulted in higher yield of maize as compared to the traditional maize/bean system and maize-natural fallow system in dry hillsides of Nicaragua.

- The IITA accession IT90K-284/2 was selected for seed multiplication due to superior forage and grain yields across environments and acceptance by farmers.

- Participatory evaluation of forages in Central America revealed that farmers first selected grasses followed by shrub legumes and legumes for green manure and covers. Grass-legume pastures were viewed as a complex technology, which requires a great deal of demonstration and information before adoption is to take place.

- In hillsides of Central America efforts to link farmers to markets through added value forage technologies should be mainly addressed to small farmers without full land ownership and to those who depend on outside jobs to meet foods security.

- The farmer-led seed production enterprise (PRASEFOR) in Honduras continued to make progress. In two year PRASEFOR has produced over 2 tons of grass seed with a market value of over US$15,000.

4.1.1 On-farm evaluation of new *Brachiaria* hybrids in LAC

Contributors: Marisabel Caballero and Conrado Burgos from DICTA (Honduras), Heraldo Cruz (DICTA/CIAT, Honduras), Moisés Hernández and Marco Lobo from INTA (Costa Rica), Bolívar Pinzón from IDIAP (Panamá), and Hugo Cuadrado from CORPOICA (Colombia)

Rationale

On-farm evaluation of new promising forage species complements the results reported from on-station research sites. Usually new forage options, particularly grasses, have a wide range of adaptation and may be used successfully in different production systems, but this characterization needs to be quantified with farmers under their particular circumstances of climate, soil and individual management. Under this consideration the *Brachiaria* hybrid cv. Mulato has been monitored during the last two years in cattle dual-purpose farms of Central and South American tropics. The results up to know show that this grass offers a better alternative of production compared to traditional grasses, both for the high quality and high forage production reported.

Material and Methods

Agronomic evaluations of hybrids and accessions of *Brachiaria* are underway in Gualaca (Panamá) in collaboration with IDIAP, and in Guápiles in collaboration with INTA (Costa Rica). In Panama the site is located at 100 masl and within the very humid premontane ecosystem; the soils are clay loam acid inceptisols with pH 4.8, high in Al (1.1 meq/100 ml), medium in OM (4.0 %), low in P (1 ppm), medium in K content (59 meq/100 ml) and low in Ca and Mg (1.0 and 0.20 meq/100 ml respectively). Mean temperature is 26 ºC and the site has a record of 4000 mm total rainfall from May to November. Meanwhile that Guápiles is a very humid tropical forest located at 250 masl, 4260 mm of total rainfall and a mean temperature of 24.6 ºC. The soils are sandy loams of medium fertility with pH 5.7, low in Al (0.35 meq/100 ml), and high P content (37 ppm).

For the agronomic evaluations the grasses *Hemarthria altissima* and Braquipará were planted using cuttings in Costa Rica, while other species were direct planted with seeds in both countries. Plots size of 4 m x 5m replicated 3 times were used, and depending of the site, plant cover and cutting evaluations to measure dry matter yields were carried out every 4 to 12 weeks respectively for the dry and wet season.

As resported in the IP-5 2002 Annual Report, a protocol for on-farm validation/promotion of *Brachiaria* hybrid cv. Mulato was developed and proposed to national institutions of Panamá, Guatemala, Nicaragua, Costa Rica, Honduras and Colombia. Using this protocol we have established on -farm trials with hybrids and other grasses in dual purpose and beef cattle farms the region of Yoro, Yorito, Victoria and Olancho (Honduras); León, Chinandega, Posoltega, Boaco, Chontales and San Dionisio (Nicaragua); Puriscal, Miramar, San Isidro, Nicoya, Guanacaste, San Jerónimo and Orotina (Costa Rica), Bugaba, Gualaca and Boquerón (Panamá); Nueva Conception, Coatepeque and Esquintla (Guatemala), and in the Llanos Orientales and the Caribbean Coast of Colombia.

Results and Discussion

Agronomic evaluations: Gualaca in Panamá is a site of infertile acid soils, while Guápiles in Costa Rica has soils of medium to good fertility. This difference in soil fertility accounts for the better forage yields observed in Guapiles. The *Brachiaria* hybrids have adapted well to both sites and the forage yields observed are within those recorded for other *Brachiaria* species such as *B. brizantha* cv. Toledo in Guápiles. Problems related with pests and diseases have not been reported up to now (Table 56).

Table 56. Dry matter yields of *Brachiaria* species and hybrids and other grasses evaluated under cutting in Panamá and in Costa Rica.

	CIAT No.	Gualaca (Panamá)	Guápiles (C. Rica)
Species	Yield (Kg DM /ha)		
Brachiaria hybrid	36062	1505*	2369**
Brachiaria hybrid	36087	1509	2527
Brachiaria hybrid	cv. Mulato (36061)	1603	2798
Brachiaria brizantha	26318	2241	na
B. brizantha	26124	1596	2642
B. brizantha	(Mixe)	1693	2559
B. brizantha	cv. Toledo (26110)	na	2670
Brachiaria sp.	(Braquipará)	na	2995
Other species			
Hemarthria altissima		na	3112
Paspalum atratum cv. Pojuca	26986	1505	na

* Means of 10 evaluation cuts every 4-5 weeks during the wet period

****** Means of 2 evaluation cuts every 6 weeks during the wet period

On-farm monitoring: Honduras is the country where *Brachiaria* hybrid cv. Mulato has been more closely monitored in dual -purpose cattle farms. Colleagues from the national institution (DICTA) have carried out important field -work in this regard along the Atlantic coast, the northwest and the central part of the country.

In Table 57 we show that from January to June 2004 (mostly the wet period), Brachiaria hybrid cv. Mulato did not affect individual animal production in six farms monitored ($P<0.05$); however, there is a consistent tendency of more milk produced per cow in these pastures.On the other hand, significant increases ($P<0.05$) in stocking rate in all farms were observed in pastures of cv. Mulato as compared with other naturalized or improved grasses such as Jaragua, Andropogon, Toledo and Swazi grass; this in turn lead to significantly ($P<0.001$) more milk production per unit area, which in most farms was more than twice in cv. Mulato relative to other grasses.

Similar results have been observed in other countries with milking cows, which confirms that one of the major advantages of *Brachiaria* hybrid cv. Mulato is the high production of high quality forage that traduces in more milk per unit area.

During the first semester of 2004 additional dual-purpose and beef cattle farms were selected for the establishment and monitoring of cv. Mulato in Costa Rica (18 farms), Nicaragua (17 farms), Honduras (9 farms) and Guatemala (12 farms), for a total of 56 farms. This activity forms part of the project Enhancing Beef Productivity, Quality, Safety and Trade in Central America, which is financed by CFC (Common Fund for Commodities of the European Union) and executed by ILRI, CIAT, IICA and SIDE.

Cultivar Mulato is in the process of establishment in all selected farms in areas that range from 1 to 7 ha. The grass will be monitored in terms of animal production related to other commercial *Brachiaria* species. Additionally, a grazing trial with beef cattle that compares animal performance of cv. Mulato and *Brachiaria* hybrid CIAT 36087 is in the process of

Table 57. Stocking rate and milk production per animal and per hectare of cows grazing *Brachiaria* hybrid cv. Mulato and other grass species in dual-purpose farms of Honduras during the period January-June 2004 (Information supplied by Conrado Burgos and Heraldo Cruz of DICTA).

Farm/ Pasture	Stocking rate (cow/ha)	Mean daily milk (kg/cow)	Mean daily milk (kg/ha)
Mulato	5.1 a**	7.1 c	37.5 d
Swazi*	1.6 b	6.8 c	8.6 f
Mulato	5.6 a	5.2 c	32.1 d
Swazi	2.7 b	4.8 c	13.5 f
Mulato	9.4 a	3.8 c	36.0 d
Toledo*	3.7 b	3.8 c	14.0 f
Mulato	5.0 a	13.1 c	64.5 d
Toledo	2.7 b	12.7 c	33.3 f
Mulato	6.1 a	10.7 c	65.3 d
Andropogon*	3.4 b	10.5 c	36.7 f
Mulato	4.7 a	6.3 c	29.9 d
Jaragua *	2.1 b	5.7 c	12.3 f

* Swazi (*Digitaria swazilandensis*), Toledo (*Brachiaria brizantha*), Andropogon (*Andropogon gayanus*) and Jaragua (*Hyparrhenia rufa*).

** Within each farm means followed by different letters are statistically significant ($P<0.05$).

establishment at Corpoica's Research Station Taluma in east acid savannas of Colombia.

Controlled grazing trial: Grazing trials to measure beef cattle animal performance were established during 2002 in Panama by IDIAP (Instituto Panameño de Investigación Agropecuaria) and in Colombia by Corpoica (Corporación Colombiana de Investigación Agropecuaria). In Panamá the site is located at 100 masl and within the very humid premontane ecosystem; the soils are clay loam acid inceptisols with pH 4.8, high in Al (1.1 meq/100 ml), medium in OM (4.0 %) and low in P (1 ppm). Mean temperature is 26 ºC and the site has a record of 4000 mm total rainfall from May to November. Meanwhile that the soils are of alluvial origin in Turipaná (Colombia) with pH 5.3, 5.9% OM, 25 ppm of P, 282 ppm of S and only Al traces.

In both sites cv. Mulato was established by direct seeding after controlling the existing vegetation with herbicides. In Panamá the 2 ha of the experiment were fertilized with 20 kg/ha of N and 10 kg/ha of P divided in 8 paddocks and established a grazing rotation of 3/21 days of grazing/rest. Meanwhile that in Colombia cv. Mulato is grazed and compared with *B. decumbens* cv. Basilisk in a rotational system of 2 days grazing, 22 rest during the wet period, and 3 days grazing, 33 rest during the dry period.

The experiments are still underway but preliminary data show a mean stocking rate of 3.2 AU/ha in Panamá and a liveweight gain of 0.630 kg/an/day during the wet season. Meanwhile in Colombia the stocking rates have been respectively 3.4 AU/ha and 2.7 AU/ha for cv. Mulato and cv. Basilisk, and similar liveweight gains of 0.530 kg/an/day for both grasses, indicating that the larger benefit of Mulato grass is to allow an increase in the number of animals under grazing per unit area. This is a result of significant more forage production of this cultivar as has been demonstrated elsewhere.

4.1.2 On-farm evaluation of new forage options for pastures rehabilitation in the llanos of Colombia

Contributors: Camilo Plazas, Daniel Vergara, J. Miles and C. Lascano (CIAT)

Rationale

Degradation of introduced pastures is one of the main constraints in livestock production systems of tropical America. This degradation results from poor pasture management and overgrazing. To address problems of pasture degradation in the llanos of Colombia in 1998- 1999 we introduced new grasses and legumes in degraded pastures in the well-drained savannas and in the piedmont. For a period of 5-6 years we have been monitoring the reclaimed pastures in commercial farms and under the management of the farmers.

Legumes

Legumes introduction in farms of the Piedmont: Results in one of the farms (La Esperanza) indicate that after 5 years of introduction of *A. pintoi* in a *B. decumbens* pasture, the legume content varied from 10% and 40%, depending on time of year. Thecrude protein (CP) of the grass on offer has varied between 9,4% and 10,2% in the legume-based pasture and between 4% and 8,7% in the grass only pasture.

In the pasture associated with Arachis LWG has been of 350 kg/ha per year, compared with 78 kg/ ha per year in the unimproved pasture. Milk production is equally higher in the associated pasture than in the contro (3000 vs. 900 kg/ha per year).

In another farm (San Pedro) a pasture of *A. pintoi* - *B. humidicola* was established in an area invaded by *Homolepis aturensis*, a low

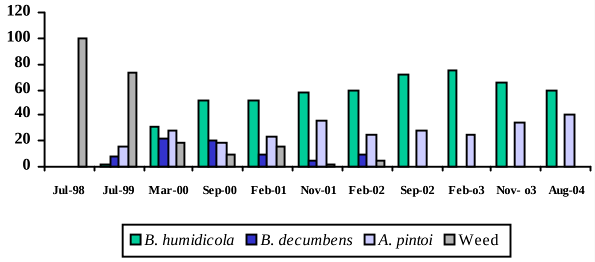


Figure 45. Legume proportion (%) in pastures of *Brachiaria humidicola* associated with *Arachis pintoi* after 6 years of establishment at the farm San Pedro in Piedmont of the Llanos of Colombia.

quality grass. After 6 years the legume has varied between 16% and 40%. The legume proportion averaged 25% during the dry period of 2003-04 and 40% in the rainy period of 2004. The weed *H. aturensis* has practically disappeared while the content of *B. humidicola* has increased (Figure 45). The carrying capacity of the pasture also increased over time. Currently between 20 and 20 steers are maintained in the pasture (10 ha) with an average weight of 280 kg under a continous grazing.

Legume introduction at farms of the well - drained savannas: The herbaceous legume *Desmodium heterocarpon* subsp. *ovalifolium* CIAT 13651 was established in 2000 a degraded *B.decumbens* pasture in a farm (Andremoni) in the well- drained savannas. The legume content increased up to 25% in the rainy season of 2001. The pasture was very productive (in fact one of the most productive in the farm). However, as a result of continous heavy grazing and no maintenance fertlizer thelegume proportion in the dry season of 2002 was reduced 5%. Its recovery in the rainy season of that year was very slow, and finally disappeared in the dry season of 2003. The alternative is to replant the seed (0.5kg/ha) and apply maintance fertilizer.

Grasses

Introduction of Toledo in the piedemmont and well-drained savannas: In 2000 at the San Pedro farm, located in llanos Piedmont, a pasture of 2,5 ha of *Brachiaria brizantha* cv. Toledo (CIAT 26110) was established in a degraded area. A group of 10 steers with average LW of 300 kg, are maintained in the pasture with occupation periods of 15 days and 30 days of rest. Forage availability in this pasture has varied between 3,5 and 5,8 t/ha of DM with a content of CP between 7,5% and 11% (Table 58). The control pastures namely *B. decumbens* cv. Basilisk in the same farm show very low contents of CP (4%, in average).

Results from the farm trial indicate that *Brachiaria brizantha* cv. Toledo is a commercial cultivar that has excellent productive potential in the Piedmont. The most important attributes of Toledo are its high carrying capacity and ability to whistand soils with poor drainage.

In 2000, at the farm El Porvenir in a well - drained savanna with acid infertile soils, a pasture of 3,5 ha was established with *B. brizantha* cv. Toledo. Results have indicated that forage availability varied between 2,6 and 6,8 t/ha of DM, with a CP content between 3,5% and 5% (Table 58). The pasture is managed with 12-15 steers of 280 kg of average LW, with 8 days of occupation and between 35 to 40 days of rest. Productivity of Toledo in the well-drained savannas has not been as good as in the piedmont. However, the grass has persisted under an intensive grazing regime and in the absence of maintenance fertilizer.

Table 58. Forage availability (t/ha of DM) and CP content (%) of *Brachiaria brizantha* CIAT 26110 cv. Toledo under grazing at farms of Piedmont and Altillanura of the Llanos of Colombia.

Season	Piedmont[a]		Altillanura[b]	
	DM (t/ha)	CP (%)	DM (t/ha)	CP (%)
March/2000	-	-	1.3	3.5
September/2000	-	-	6.8	4.2
February/01	4.8	11	5.9	4
November/01	4.4	10	3.4	5
February/02	3.5	8	2.6	2.5
September/02	3.5	7	3.9	5
February/03	5.8	8	5.5	4
November/03	4	6	4.2	4
August/04	3.5		4.9	

a. Farm San Pedro.
b. Farm El Porvenir.

On-farm evaluation of different accessions of *B. brizantha.* In 2000 at the farm El Porvenir, well drained savanna site, 5 ha of *B. brizantha* CIAT 26318 were established. In the following year, 3,5 ha of each of the accessions of *B. brizantha* CIAT 26990 and 26124 were also established in the same farm.

Forage on offer in these pastures has varied from 3,4 to 5,2 t DM/ha in *B. brizantha* CIAT 26318 and from 2,3 to 5,1 t DM/ha of DM in *B. brizantha* CIAT 26990. With *B. brizantha* CIAT 26124 production of DM has been low (between 1,98 and 0,378 t/ha of DM) due to its excellent palatability and heavy grazing system used (Table 59). The CP content of these accessions has been low in the dry season (3 to 4%) and average for a grass in the wet season (6 to 7%).

After 4 years of evaluation of the different *Brachiaria brizantha* accessions it is evident that each accession should be managed in a different form. For example, *Brachiaria brizantha* CIAT 26318 (the prefered accession of the farmer) is being used finish steers with 8 days of occupation and 35 of rest, while *B. brizantha* CIAT 26990 and 26124 are used with good results with cows with 5 days of occupation and 30 days of rest.

Grasses Evaluation at farms: *Brachiaria* Hybrid cv. Mulato (CIAT 36061). This hybrid that shows multiple attributes like its rapid establishment, excellent forage quality and drought resistance is being tested as pasture for milking cows in the piedmont and as a component in crop- pasture systems in the llanos of Colombia.

Piedmont of the Llanos: In a clay loam oxisol at the farm La Isla, Piedmont, in July 2001, 7,5 ha of this hybrid were established in a plot of *B. decumbens* in advanced satge of degradation.

At the beginning 3,2 steers /ha at the finishing stage were maintained in the pasture for one month. All animals received 1 kg daily of commercial concentrate as supplement and liveweight gains were in the order of 2 kg/d. In a second phase of utilization defined by the farmer, milking cows were introduced in a rotation grazing system in five plots of 0,75 ha and 3 days of occupation. With this system 12 cows milking were maintained (3,2 cows/ha) during a complete cycle of 15 days. These cows in pastures of *B. decumbens* produced on average, 5 kg of milk in morning milking, and some of them that were milked in the afternoon produced 3,8 kg more. In the new pastures of *Brachiaria* hybrid cv. Mulatto, the same cows produced daily 6,5 kg in morning milking and 4,7 kg in the afternoon milking, which meant a total increase of 23 kg of milk per day in Mulato.

At present the Mulato pasture in La Isla farm is managed under continuous grazing with 12 cows. With this management, forage offer is in the order of 2,9 t/ha of DM and milk production is of 5.0 l /cow/d. When these same cows grazing

Table 59. Forage availability (t/ha of DM) y CP content (%) of accessions of *Brachiaria brizantha* at the farm El Porvenir in the Altillanura of the Llanos of Colombia.

	DM (t DM /ha)			CP (%)		
	CIAT 26318	CIAT 26993	CIAT 26124	CIAT 26318	CIAT 26993	CIAT 26124
March /2000	1.4	-	-	2.6	-	-
September /2000	4.7	-	-	4.2	-	-
February /01	4.8	4.2	2.0	3.7	7.2	7.4
November /01	4.3	4.5	3.8	5.2	6.5	5.2
February /02	3.4	2.4	2.5	3.7	2.8	3.3
September /02	5.2	5.0	2.4	3.7	4.3	6.1
February /03	4.7	4.7	2.9	3.0	3.9	3.3
November /03	4.7	4.8	3.1	3.9	4.1	5.9
August /04	5.0	4.3	2.0			

other species of *Brachiaria* milk yield is 3.0 l/cow/day. One major problem of Mulato in La Isla farm has been plant mortalility due to poor soil drainage. The Mulato plants have been replaced by *Homolepis* sp in some areas of the pasture.

Well-drained savannas: Sowing commercial crops with grasses or crop-pasture rotations are alternatives to reduce establishment costs of pastures, to improve their productivity and quality due to high residual fertilizer and for sustainability of the crop phase over time.

With the support of Papalotla, of Mexico, and in the farm Costa Rica (located in a well drained savanna site, with a sandy loam Oxisol), in 2001 we established Mulato in association with maize in site (15 ha) with degraded *B. brizantha* cv. Marandu. Grain yield after 138 days of sowing the maize was 3,7 t/ha, while *Brachiaria* hybrid cv. Mulatto produced 4,2 t/ha with 8,7% of CP and 65% of DIVMS.

First grazing of the resulting Mulato pasture was carried with 39 old cows and heifers that were between 24 and 36 months old. These animals 36 days later weighed, in average, 506 kg, which means a daily gain of 1675 g and a total production of 2351 kg of LWG with a carrying capacity of 2,6 animal/ha. In the middle of the dry season (January 2003), after 31 days of rest, the Mulato pasture produced 3,5 t/ha of DM. In June 2004, at the beginning of the rainy seasons fertilizer was applied (67 kg/ha of N and 38 kg/ha of K) and the forage on offer increased from 2,9 to 5,1 t DM/ha. However, soon after the fertilization Mulato exhibited N deficiency (clorosis) indicating that Mulato requieres good soil fertility and frequent N fertilization (Photo 24). A small plot experiment was setup to study N fertilization management in Mulato under the llanos condition.

At present the Mulato pasture is been used with 37 heifers (2,5 animal/ha), with an average of initial living weight at wean of 250 kg, with occupation periods between 15 and 20 days and between 30 and 40 days of rests.

Other studies with Mulato and other hybrids in the Llanos are:

1. At the farm El Porvenir, Altillanura, we established 7,64 ha of *Brachiaria* hybrid cv. Mulato (CIAT 36061) in association with a rice crop, with an initial fertilization of 400 kg/ha of dolomite lime, 400 kg/ha of Calfos and 50 kg/h of smaller elements. At the same farm established 5,84 ha of *Brachiaria* hybrid cv. Mulato-2 (CIAT 36087) with the same fertilizer dose application used in Mulato.

Photo 24. Mulato pasture in the Llanos of Colombia: (A): Fertilized with N (67 kg/ha) and (B) 60 days after fertilization.

2. At the experimental station Taluma of Corpoica located in the Altillanura, Mulato 2 (CIAT 36087) was established in 1,8 ha with an initial fertilization of 500 kg/ha of dolomite lime, 300 kg/ha of phosphoric rock, 75 kg/ha of potassium chloride and 30 kg/ha of sulfur flower. This pasture will be used in alternate grazing and its productivity will be compared with *Brachiaria* cv. Mulato (CIAT 36061).
3. At the research center Corpoica La Libertad, Piedmont *Brachiaria* cv. Mulato (CIAT 36061), *Brachiaria* cv. Mulato-2 (CIAT 36087), *B. brizantha* cv. Toledo and *B. decumbens* cv. Basilisk were established in associated with *Pueraria phaseoloides* and *Arachis pintoi* in plots of 2 ha each, in order to evaluate productivity, and persistence.

4.1.3 Adoption of new soil conservation technologies in the llanos of Colombia: Arable layer building technology (Capa Arable)

Contributors: L. Rivas and D. L. Molina (CIAT)

Rationale

As a result of CIAT's collaborative research activities with Corpoica, Pronatta and Unillanos, with financial support from MADR and COLCIENCIAS, a series of soil improvement and conservation practices are available. These practices focus on arable layer building technologies —part of the soil profile that can be modified through a combination of biological and physical management— in soils of the savannas of the Llanos of Colombia. These practices include use of proper crop and pasture rotations in agropastoral systems.

In August 2004, a rapid survey was conducted in order to learn about the adoption of the arable layer building technology (Capa Arable) in farms located in the area of Puerto López - Puerto Gaitán in the llanos of Colombia.

In total, 18 farmers were interviewed, including those that were using and not using the technology. The survey was done using the methodology of semi-structured interviews to groups of producers, technicians and experts of the region, to identify and understand trends and main features of the adoption process.

Results and Discussion

Results of this survey make reference to technology adoption in early phases, since the first farmers adopted it 4 years ago and the majority of them started adopting it between 1 and 2 years ago. In Table 60 we summarize the main results obtained in the survey and we discuss the major findings. In general, producers showed great interest in maintaining and improving soils quality, since this practice has a high and rapid payoff in terms of crops and pastures yield. Farmers in the past attempted to establish crops without adequate soils management and used non-adapted pasture and crop germplasm, and consequently experienced large economical failures.

Table 60. Results of the survey on the use of the arable layer building technology by producers in the Llanos of Colombia.

Variable	Level
Number of producers interviewed	18
Land Use	
Total Area of farms (ha)	
Mean	883
Range	120-5000
Area under crops (ha)	
Mean	238
Range	0-1100
Area under pastures and other uses (ha)	
Mean	645
Range	0-3900
Area under crops/Total Area (%)	27
Proportion of producers with no crops (%)	28
Most common rotation	Maize-soybean
Most common land preparation method	Vertical tillage with chisel plow

In contrast to the previous experiences, utilization of soil conservation methodologies together with the use of improved germplasm have shown significant advantages in productivity and in economic returns to the investments made.

Practices for arable layer building include a vertical corrective tillage using rigid chisels, correction of nutrient deficiencies in soil and sowing of forages and acid soil adapted crops. In some cases, lack of key inputs such as machinery, fertilizers and seeds prevents establishment of crops or timely harvest of crops, which has negative implications for building arable layer and for the economics of the system. There is great interest from the Colombian government and also from the private sector (e.g., poultry) in emphasizing grain production in the savannas of the llanos of Colombia. Some field crops are being established in the farms of poultry farmers to improve feed production in the region. Thus the expansion of field crops in the region is beeing pushed by the poultry sector.

The most frequent rotation is maize in the first semester and soybean in the second. The new rice variety, Line 30, recommended in this technology and that has not been commercially released yet is creating great expectation among farmers due to its high productive potential.

Even though there is clear conscience among producers of the fact that continuous monoculture degrades the soils and provokes greater pest and disease pressure, price variations in crops seem to drive the rotation cycles. High price expectations for soybean in 2004 induced many producers to plant this crop in both semesters.

Many of the farmers that have introduced crops in their farms traditionally have been and continue to be livestock producers. The majority of these farmers interviewed said that they are working with crops to improve soils and to subsequently sow high productivity pastures. Thus it seems that there will be a high demand for new forage cultivars that are being developed by CIAT Tropical Forages Project, as is the case of the new Brachiaria hybrids such as Mulato already in the market.

Productivity gains constitute the principal benefit perceived by those who apply soil conservation practices in the well -drained savannas of the llanos. In general, yields in the first sowing are low, but increase subsequently as a result of soil improvement. With soybean, initial yields are between 1,5 and 1,9 t/ha, and in rare cases reaches 3t/ha. With maize, yields are between 4 to 5 t/ha. It is foreseen that over time crop yields will begin to decline and when this occurs it will be the time to introduce pastures in the rotation.

Since the introduction of crops is new in the Llanos, most farmers interviewed do not have enough clarity on the duration of the crop phase and pasture phase of the rotation. Several of the farmers interviewed consider that between 4 and 5 years of crop rotations is needed before reconverting the land to pastures; other farmers estimate that changing crops to pastures will occur when productivity of the crop starts to decline as a result of soil fertility loss.

Constraints for adopting the arable layer building technology are economic in nature and lack of infrastructure.

Some of the economical constraints have to do with:

1. Low availability of capital associated with inappropriate credit plans.
2. Market price fluctuations of products that generate large income variations.
3. Eventual oversupply of products such as rice, and
4. High cost of inputs and agricultural machinery.

In terms of infrastucture the following limitations were mentioned:

a) Poor roads that increase cost of transportation, and
b) Low quality of seeds sold in the market that results in poor yields and reduced income.

Those farmers who have not introduced crops in their farms mentioned various reasons for not having done so:

a) Land shortage, which is something critical in small properties that do not have sufficient areas to establish crops.
b) Lack of capital and machinery to start agricultural activities.
c) Lack of experience in agriculture.
d) Time shortage or little desire to invest additional time to crops, and
e) Farm topography that does not permit crop establishment.

In summary, our survey indicates that the most immediate impact area for the arable layer building technology developed by CIAT is the Puerto López – Puerto Gaitán region, approximately 180 thousand hectares. It is considered that for its rapid adoption, investment by the Colombian government in improving road infrastructure is critical. In addition, in the more remote areas of the llanos there are a number of other critical factors (i.e. lack of machinery, inputs, technical assistance, qualified hand labor and roads and communications) that prevent the introduction of crops to establish rotations with pastures in sustainable agropastoral systems.

4.1.4 Adoption of *Arachis pintoi* in in the Llanos of Colombia

Contributors: Camilo Plazas and D. Vergara (CIAT)

The adoption of *Arachis pintoi* cv. Mani Forrajero in the Piedmont of the Llanos of Colombia has been a slow process. In order to learn about the causes of this low adoption of this legume, a survey was conducted among 77 farmers located in Villavicencio, Restrepo and Guamal.

Results of the survey indicated the following:

1. 83% of farmers interviewed knew the legume and of these the majority learned about it in a field day, in a neighbor’s farm and as an ornamental plant. However, it was interesting to see that only half of the people surveyed had received information on the legume, and that a very low number (11%) had received information on establishment and grazing management of pastures with Arachis.

2. Of the total farmers surveyed, thirty six (47%) farmers had sown Arachis and of these, only eight had had problems with establishment due to an inadequate time of sowing. Only one farmer mentioned problems with seed germination. On the other hand, only 28 of farmers (36%) had between one and two plots established with this legume in their farms. Of those, 12 had less than 1 ha, 11 between 1 and 3 ha and five more than 3 ha. Of those using Arachis, 21 had used vegetative material for establishment.

3. Concerning utilization of Arachis, 32% of farmers that have Arachis use it to graze cows, 7% with fattening animals and the rest with different kinds of animals. Out of 41 farmers that use the legume, 19% had it in pastures associated with grasses, generally *Brachiaria* species and 49% had it as an ornamental legume. Other small uses of Arachis were as protein bank (15%), as cover (2%) and for rehabilitation of degraded pastures. On the other hand, only one farmer multiplied seed of Arachis for expanding the area the farm.

4. Concerning satisfaction with Arachis, the survey showed that out of 26 farmers that had used the legume in pastures, 23% were convinced that *A. pintoi* increased milk production, 15% that it increased LWG in animals and 11% that it improved soils characteristics.

5. By areas in the piedmont, in Villavicencio 89% of farmers surveyed knew *Arachis* for more than 10 years and 78% had received information on its establishment and management. In Restrepo, even though most farmers recognize the advantages of Arachis its adoption has been slow because of lack of information. In the Ariari, 88% of farmers in the survey knew the legume and 65% had information on how to utilize it as feed resource. In this zone the greatest use of Arachis was in protein banks and in association with grasses.

In summary, results indicate that 93% of farmers using Arachis were satisfied, among other reasons because of increased forage production, soil and moisture conservation, persistence, tolerance to pest and disease, good palatability for animals and because it is easy to propogate with vegetative material. Reasons of those not using Arachis for not having established the legume varied from not knowing it (38% of farmers surveyed), not knowing how to use it (16% of farmers surveyed) and to high cost of sexual seed (3% of farmers surveyed).

Results of this survey showed some features of the diffusion and adoption process of *A. pintoi* in the Llanos of Colombia:

(1) Lack of commercial seed at an accessible price for farmers in current cattle-raising situation in the region is, maybe, the main constraint for diffusion of *Arachis*.

(2) High costs of establishment with vegetative material.

(3) Limited information to farmers on establishment and management of Arachis based pastures

As a follow-up of the survey, between July and August 2004, a series of workshops were held with farmers on, establishment, fertilization establishment and maintenance and uses of Arachis. These workshops were held in San Martín (35 participants), Guamal (33), Villavicencio (60) and Restrepo (50) with cooperation of Comité de Ganaderos del Meta, the Farmers Training School of Restrepo, the Comité de Ganaderos of San Martín and the Asociación de Ganaderos of the Ariari (Aganar).

4.1.5 On-farm evaluation of forage options in hillsides in Colombia

Contributors: C.V. Duran (Universidad Nacional de Palmira), Luz Mary Ocampo (Secretaría de Agricultura del Valle), M. Valderrama (Instituto Técnico de Roldadillo, INTEP) and farmers from the Grupo de Productores de la Ondina, J.I. Roa (IPRA), L.H. Franco and M. Peters (CIAT)

Rationale

The Norte del Valle of Colombia is an important livestock area. However forage options available to livestock holders are limited and hence restrict productivity of livestock operations. Through a participatory approach it is aimed to define and adapt forage technologies hence improve livelihoods of farmers.

Material and Methods

Work was initiated in 2002 with a participatory diagnosis and several farmer visits to farms and research stations employing improved forage technologies. This was followed by planning meetings with farmers to establish a trial including several forage options, planted in contour lines at the ‘finca La Ondina’ in Roldanillo (800 mm annual rainfall, high temperatures), located in the piedmont of the Western Cordillera, in the Northern Valle. Farmers and researchers observe the adaptation of the various options through qualitative measurements (see Photo 25). Farmers then select and adapt forage technologies to their particular conditions, with the innovation process supported by close interaction with technicians and researchers.

Photo 25. Farmers at Roldanillo, Valle (trial at "Finca La Ondina"), 2004.

The following forages were established:
Brachiaria híbrido cv. Mulato
Brachiaria brizantha cv. Toledo
Panicum maximum cv. Mombasa
Brachiaria dictyoneura cv. Llanero
Cratylia argentea cv. Veranera
Leucaena leucocephala local.

Results and Discussion

Farmers defined the following criteria as important for selection of forages:

- Palatability
- Color
- Forage on offer
- Adaptation to low fertility soils
- Adaptation to drought
- Tolerance to ants
- Organic Matter production
- Cover (aggressiveness)
- Rooting capacity
- Persistence under grazing
- Adaptation to fertility gradients

The criteria selected are very similar to those selected in evaluations with farmers in Central America, confirming the validity of the participatory approach used. To support the farmer innovation process, parallel training events were organized on subjects such as forage utilization, pasture assessment (BOTANAL), pasture establishment, pasture management, ant control and silage and hay making. After two years, farmers are starting to adopt *Brachiaria* hybrid cv. Mulato, *B. brizantha* cv. Toledo, *Cratylia argentea* and *Leucaena leucocephala*. Farmers expressed the need to evaluate forage materials at different altitudes and rainfall regimes. Thus new trials were established at altitudes of 1200 to 1500m and 1500 to 1800 masl.

4.1.6 Participatory introduction of improved forages in smallholder dairy systems in hillsides of Nicaragua

Collaborators: A. Schmidt, C. Davies, E. López, M. Peters, L.H. Franco, and P. Argel (CIAT)

Rationale

It is recognized that many relevant agriculture technologies are not achieving their full potential impact due to low levels of adoption, which has led to more emphasis on the use of participatory research methods to enhance adoption of improved technologies. The BMZ funded forages project 'Farmer Participatory Research in Action: Selection and Strategic Use of Multipurpose Forage Germplasm by Smallholders in Production Systems in Hillsides of Central America' achieved the development of a technology package made up of different components: a) methods for participatory diagnosis and stakeholder analysis, b) characterized forage

germplasm acceptable to farmers,
c) demonstrated benefits of improved forages in animal production results including economic analysis, d) complementary training modules (participatory evaluation and selection, forage utilization/management, participatory monitoring and evaluation), and e) seed systems (informal and formal public, and private).

The package developed by the CIAT-led project attracted the attention of the ASDI-funded bilateral development project FONDEAGRO (Fondo de Desarrollo Agropecuario) based in Matagalpa as a key input to enhance milk production in the projects' target region Matiguás and Rio Blanco in Nicaragua. The target group consists of approx. 1,300 livestock holders in three zones. After adapting the technology package to the specific needs of FONDEAGRO, CIAT was asked to implement the package within their project.

Material and Methods

CIAT was contracted (1) to select and implement three forage nursery trials in different sites offering a wide basket of germplasm options to farmers, (2) implement on-farm animal production experiments to demonstrate pasture management and increased milk production based on improved grasses and grass-legume associations, (3) implement dry season feed opportunities based on the shrub legume *Cratylia argentea* in combination with cut&carry grasses, (4) conduct training modules on forage agronomy and pasture management, participatory methods for evaluation and extension, seed production and participatory monitoring and evaluation, and (5) develop a seed production system within the project in order to secure long term sustainability and adoption of the selected forage options. The project ended March 2004 after 18 months.

Results and Discussion

Adaptation of grasses

Despite the short project duration we could successfully introduce new germplasm into the project area. As shown in Table 61, the wide basket of forage germplasm options included grasses, and herbaceous and shrub legumes. Pasture establishment resulted difficult due to unusual high precipitation at the beginning of the project. Furthermore, increased sowing rates were required because of the high weed pressure in this region. A total of 367 plots were established at three sites and evaluated during the periods of maximum and minimum precipitation. Agronomic data was recorded as well as farmers' preferences in collaboration with project farmer groups. At the Ubú Norte site a fertilizer treatment was applied in order to demonstrate possible effects on yield of grass species.

Results from the agronomic evaluation of 18 grasses at Ubú Norte are presented in Table 62 No fertilizer effect could be observed throughout the experiment; however, it remains to be seen if pasture yields will decline in the area over time demanding at least regular maintenance fertilization. The objective of demonstrating to farmers the benefits of fertilizer application, who traditionally do not apply any fertilization to pastures, could not be achieved basically due to the short project duration.

At Ubú Norte *Panicum maximum* accession CIAT 16031 cv. Tanzania followed by *Paspalum plicatulum* accession CIAT 26989 produced the highest dry matter yields in combination with good soil cover indicating ease of establishment even under very high rainfall conditions.

Although the *Brachiaria* hybrid's cv. Mulato full potential is expressed under drier conditions, yields did not differ significantly between the periods of minimum and maximum precipitation. The robust *B. brizantha* accessions CIAT 26110 cv. Toledo and "Mixe" can also be considered promising and productive grasses for Ubú Norte. Plant diseases and pests were no limiting factor, except for one Rhizoctonia foliar blight attack on *B.* hybrid cv. Mulato.

The yield of grasses recorded at La Patriota site (Table 63) were lower than those observed at Ubú Norte. Again *Paspalum* accessions and

Table 61. Forage options established within the FONDEAGRO project.

Grasses	Herbaceous legumes	Shrub legumes
B. brizantha CIAT 6780	*A. pintoi* CIAT 18744	*C. argentea* CIAT 18668
B. brizantha CIAT 26110	*A. pintoi* Ciat 22160	*C. argentea* CIAT 18516
B. brizantha CIAT 16322	*D. ovalifolium* CIAT 33058	*C. calothyrsus* CIAT 22310
B. brizantha CIAT 26646	*C. pubescens* CIAT 15160	*C. calothyrsus* CIAT 22316
B. brizantha CIAT 26124	*C. brasiliensis* CIAT 17009	*L. leucocephala* CIAT 17263
B. brizantha CIAT 26318	*C. pascorum* cv. Cavalcade	*D. velutinum* CIAT 13953
B. brizantha CIAT 26990	*C. plumieri* DICTA	*Cha. rot. grandifolia* CIAT 18252
B. brizantha “Mixe“	*P. phaseloides* CIAT 7182	*Cajanus cajan* (local)
B. decumbens CIAT 606	*S. guianensis* CIAT 11844	*Gliricidia sepium* (local)
B. humidicola CIAT 679	*Lablab purpureus* (local)	
B. dictyoneura CIAT 6133	*Mucuna pruriens* (local)	
B. ruziziensis CIAT 654	*Vigna umbellata* (local)	
B. hybrid CIAT 36061		
B. hybrid CIAT 36062		
P. maximum CIAT 16051		
P. maximum CIAT 16031		
Pas. plicatulum CIAT 26989		
Pas atratum CIAT26868		

Table 62. Plant height, soil cover and DM yields of 18 grass accessions in the period of maximum and minimum precipitation in Ubú Norte, Nicaragua.

	Plant height (cm)		Soil cover (%)		DM yield (g/m^2)	
	Min	Max	Min	Max	Min	Max
Fert. Level						
- High	55	76	73	75	283	338
- Low	56	71	69	67	311	302
CIAT No.						
606	56	73	73	82	244	329
654	39	55	52	37	239	188
679	-	-	-	-	-	-
6133	37	55	46	65	234	347
6780	40	65	78	91	171	244
16322	72	94	84	88	252	348
26110	72	76	70	64	375	363
26124	64	75	67	66	264	320
26318	62	61	47	17	359	342
26646	65	76	70	76	305	275
26990	64	73	53	48	357	277
36061	52	69	74	81	305	308
36062	52	54	76	49	322	253
“Mixe”	56	77	81	86	331	355
16031	67	105	81	83	260	432
16051	51	82	68	83	256	332
26868	48	78	87	91	443	325
26989	42	71	95	97	328	400
Mean Acc.	55	73	71	71	297	320
LSD (0.05)	13.3	21.3	24.9	30.3	193.6	271.5

Panicum maximum cv. Tanzania were the most productive grasses. local check, *B. brizantha* cv. Marandú, resulted at all site less productive than the new grasses introduced by the project. *Brachiaria* cultivars Mulato and Toledo adapted well at the La Patriota site.

At the third project site Paiwítas, the *Paspalum* accessions performed well throughout the evaluation period, while *P. maximum* accessions were well below average, probably due to the lower pH and organic matter content of the soil at Paiwítas (Table 64). Soil conditions might also be the reason that grasses at this site showed the lowest dry matter production, height and soil cover of all three experimental sites. *Paspalum*, accessions *B. decumbens*, *B. brizantha* "Mixe", and *B. brizantha* cv. Toledo were the best forage options for Paiwítas.

Table 63. Plant height, soil cover and DM yields of 13 grass accessions in the period of maximum and minimum precipitation in La Patriota, Nicaragua.

CIAT No.	Plant height (cm)		Soil cover (%)		DM yield (g/m^2)	
	Min	Max	Min	Max	Min	Max
606	50	84	72	88	233	298
6133	51	62	68	65	311	154
6780	33	107	90	94	117	205
16322	55	90	87	93	164	239
26110	53	104	90	92	181	270
26646	53	96	70	90	167	269
36061	47	86	82	67	183	270
36062	53	73	80	67	215	212
"Mixe"	55	82	62	77	267	341
16031	76	108	93	73	425	192
16051	50	94	82	87	227	347
26868	57	94	95	93	453	259
26989	53	97	100	98	454	372
Mean Acc.	53	91	82	83	261	264
LSD (0.05)	24.8	22.6	33.2	39.6	333.6	320.6

Table 64. Plant height, soil cover and DM yields of 13 grass accessions in the period of maximum and minimum precipitation in Paiwítas, Nicaragua.

CIAT No.	Plant height (cm)		Soil cover (%)		DM yield (g/m^2)	
	Min	Max	Min	Max	Min	Max
606	57	81	90	92	256	233
6133	39	50	50	47	208	154
6780	48	69	60	67	141	111
16322	54	84	70	63	195	305
26110	67	93	67	72	287	191
26646	52	77	63	70	249	227
36061	45	71	70	73	220	120
36062	36	65	55	63	155	191
"Mixe"	52	63	68	72	224	276
16031	60	91	60	70	167	202
16051	42	67	83	87	159	126
26868	50	80	80	75	351	410
26989	52	84	88	93	316	636
Mean Acc.	50	75	70	73	225	245
LSD (0.05)	15.9	28.9	43.4	43.6	212.6	315.8

Adaptation of legumes

The accessions *Stylosanthes guianensis* CIAT 11844, *Arachis pintoi* CIAT 18744, *Desmodium ovalifolium* CIAT 33058 and *Canavalia brasiliensis* CIAT 17009 were the most promising herbaceous legume materials in the three sites (data not presented). *Pueraria phaseoloides* had establishment problems, which seemed to associated with low seed quality. The annual legume options *Lablab purpureus* and *Vigna umbellata* showed quick establishment and high dry matter production at all sites. The shrub legumes did not establish well at the three experimental sites. Due to its slow initial growth, *Cratylia argentea* only showed promising results at the end of the project period. *Cajanus cajan* and *Calliandra calothyrsus* seemed to be well adapted to the Ubú Norte and Paiwítas sites. At La Patriota site, however, high water table conditions throughout the year apparently affected the shrub legumes evaluated.

Participatory selection of grasses and legumes

Farmers' preference ranking (Photo 26) from all three project sites is presented in Table 65. While some of the expressed preferences by the farmers groups are coherent with recorded agronomic data, the high ranking of *B. brizantha* cv. Toledo and *B.* hybrid cv. Mulato seems to be triggered also by other factors such as seed availability and especially the promotion activities of other entities contracted by FONDEAGRO. Large quantities of seed of both cultivars were distributed among farmers clearly influencing their preferences. It has to be mentioned as well that a high percentage of the group members had not been exposed to forage germplasm before. Therefore, results should be regarded as highly biased and indicating limitations of participatory methods, which have to be taken into account in future projects.

Photo 26. Participatory evaluation of grasses in Paiwítas, Nicaragua.

Table 65. Farmer's dry and wet season preference ranking of grass and legume germplasm at three sites in Nicaragua.

Species	Ubú Norte		Paiwítas		La Patriota	
	Min	Max	Min	Max	Min	Max
Grasses	26110	26110	36061	26868	16031	26110
	6780	36061	26110	36061	26110	36061
	16031	6780	6780	26110	36061	6780
	36061	16031	36062	6780	16051	16031
	26989	26989	16051	36062	26989	26868
Herb. legumes	17009	17009	17009	11844	17009	18744
	7182	15160	15160	15160	Lablab	Mucuna
	15160	*V. umbellata*	18744	*C. plumieri*	11844	11844
	Lablab	71820	Lablab	Lablab	33058	33058
Shrub legumes	Cratylia	Cratylia	Cratylia	Cratylia	22310	Cratylia
	Cajanus	Cajanus	22310	Gliricidia	Cajanus	Cajanus
	17263	17263	Cajanus	Cajanus	Cratylia	17263
	22310	22310	22316	18252	17263	22316

Staff from CIAT facilitated the establishment of a series of plots with a smaller selection of materials in different locations across the target zones. These plots are managed directly by farmer groups under the supervision of Technoserve, which is an extension company (45 technicians) contracted by FONDEAGRO. The acquisition and import of 530 kg of grass and legume seed mainly from Honduras and Costa Rica was facilitated by CIAT. CIAT's Seed Unit in Atenas, Costa Rica, played a vital role in this effort.

Grazing experiments with milking cows

In order to demonstrate milk production potentials of the new forage options on offer, large grazing plots (1-2 ha) were established in each zone of the FONDEAGRO Project. The grass *B. brizantha* cv. Toledo (CIAT 26110) was chosen for this effort. Parts of the plots were established in association with *Arachis pintoi* CIAT 18744. On demand of FONDEAGRO, these plots are managed intensively with electric fences (Photo 27). Milk production is being recorded in comparison to the traditional pastures available in the area. First results indicate a milk increase of approximately 25% on the improved pastures over the control. This has to be confirmed through further grazing cycles in the future.

Photo 27. Intensive pasture rotations with electric fences in Ubú Norte, Nicaragua.

Forages for Cut and Carry

The establishment of *Cratylia argentea* and cut and carry grasses for dry season feeding resulted very difficult due to high rainfall and the inherent slow initial growth of the shrub legumes. While plots of *Pennisetum* spp. established well at all sites, *Cratylia argentea* only reached production stage at the Ubú Norte site (Photo 28). Further research is necessary on plant establishment of *Cratylia* in order to speed up initial growth and reduce the risk of failure under different environmental conditions. Nevertheless, farmers at Ubú Norte, convinced of the benefits of the shrub legume for the dry season, harvested the first *Cratylia* seed and doubled the area of *Cratylia* at this experimental site in 2004. Remaining seed was sold to other project areas.

Photo 28. *Cratylia argentea* for dry season feed in Ubú Norte, Nicaragua.

Training

Training of project technicians (45), and a small group of key farmers, was a major accomplishment of the project. Training courses were held on forage germplasm characteristics (Sept 2002/May, 2003), participatory monitoring and evaluation (Feb., 2003), forage seed production (Mar., 2003) and pasture management (Apr., 2003). Dry season feed strategies for all three project zones, taking into account available germplasm at the sites, were developed during a workshop in November, 2003 (Photo 29). Field days to observe seed harvest were conducted in

December, 2003.

In March 2004 a last training course was held on intensive pasture rotation utilizing electric fences in order to prepare technicians for the establishment and management of additional grazing plots in the target areas.

Photo 29. Grass silage for smallholders – training course for field technicians of FONDEAGRO, Nicaragua.

Development of Forage Seed Systems

The development of a forage seed production system within the project in order to secure long term sustainability and adoption of the selected forage options could only be initiated due to the short duration of the project. Efforts were made to organize farmers interested in seed production and initiate seed multiplication of *B. brizantha* cv. Toledo, *Arachis pintoi* and *Pueraria phaseoloides*. Since formal seed markets in Nicaragua are only beginning to evolve (only grass seed of few selected species available) with prices often beyond the economic ability of farmers, such a multiplication system is key for the FONDEAGRO project's success. CIAT facilitated the first steps of this process, but it remains to be seen if activities will continue after CIAT's project involvement ended.

Summary

During the project we successfully introduced new promising forage germplasm options in a region where natural pastures are the main source of feed for livestock and where farmers had only restricted access to information and seed of improved forage cultivars. Training technicians and key farmers allowed for the development of local capacities to ensure that the introduced forage germplasm will be managed and multiplied adequately. Benefits were shown and farmers, who formerly spent little time and effort on improving feed resources for their animals, establish now their own plots and buy seed on their own account. One important lesson learned in this project is the need to develop forage germplasm for water-logged areas.

Assisting development projects with forage germplasm options, methodologies and training is an effective way of scaling research results. However, more up-to-date training and information materials have to be developed for this purpose.

4.1.7 On-farm evaluation of green manures in hillsides of Nicaragua

Collaborators: A. Schmidt, P.P. Orozco (CIAT), Campos Verdes (San Dionisio, Nicaragua), M. Peters, L.H. Franco, G. Ramirez (CIAT), and E. Barrios (TSBF-Soils)

Rationale

Farmers are increasingly concerned by the high price of inputs used for agriculture production such as fertilizers. At the same time soil fertility on farmer fields is decreasing and weeds are becoming a larger problem. In order to overcome these limitations, the local farmer organization “Campos Verdes” initiated (backed up by CIAT) activities to introduce, evaluate and promote the use of cover crops and green manures (CCGM) in the communities of San Dionisio in 2001.

Drawing from former experiences, Campos Verdes hypothesized that CCGM legumes significantly contribute to soil fertility enhancement, water and soil conservation and weed suppression. Some of these green manure legumes may be even used as forage or for human consumption. It was also taken into account that growing CCGMs may result in a smaller amount of applied agrochemicals, which are already contaminating the scarce water resources of the people in San Dionisio. Further objectives were participatory learning about CCGM, and their management within the local community.

This initiative was a logical extension of our germplasm work with multipurpose legumes and scaling efforts within the Calico watershed in collaboration with TSBF. We therefore supported CCGM experiments at watershed level with traditionally known legumes (e.g. *Mucuna pruriens, Canavalia ensiformis*) and exposed farmers to newly introduced legume accessions of *Vigna unguiculata* and *Lablab purpureus*. Results (see AR 2003; p. 145) indicated the possibility to replace traditional nitrogen fertilizer application to following maize crops through the establishment of a CCGM crop in the outgoing preceding rainy season.

Since farmer-led experiments are always more susceptible to data loss, we established also a long-term crop rotation experiment at our Sol Wibuse site in order to monitor the effects of CCGM crops in relation to the traditional production system in San Dionisio. This experiment established in 2001 served as a mother-trial for an on-farm validation project funded by FUNICA (Fundación para el Desarrollo Tecnológico Agropecuario y Forestal de Nicaragua) in 2003. Within this project different legume options for soil fertility enhancement are currently being tested at 28 farms in the watershed. In this report we present the first results of these activities.

Materials and Methods

At the SOL Wibuse site in San Dionisio (Lat. N12°46’49.5"; Long. W 85°49’35.2", 560 masl, annual prec.1366 mm; 26°C) four crop rotation treatments (Table 66) were established on 5m x 5m plots with three replicates. In the first planting season each year, maize was established in the traditional way including basic, low level fertilization. For the maize + cowpea / *Canavalia brasiliensis* rotation, cowpea seed was sown in two lines between two maize rows; 15 days after maize establishment. Cowpea plants were cut 30 days later and left on the plot surface as mulch. In the second planting season, plots were either sown with beans, *Canavalia brasiliensis* or kept in fallow until the following year. *C. brasiliensis* was identified in a previous CCGM germplasm experiment as highly drought resistant, staying green during the entire dry season at the SOL site. Maize and other plant residues were left on the plots. Maize yields were recorded.

Results and Discussion

In Figure 46 Maize yields from four different crop rotation treatments are presented. The recorded yields did not differ in 2001 and 2002 as expected. Only in 2003 after two years of rotation significant differences between rotations were recorded. The maize-natural fallow rotation showed the lowest maize yields. The traditional

Table 66. Treatments of long-term CCGM crop rotation experiment at Sol Wibuse site, Nicaragua, 2001-2003.

Crop rotation/treatment	2001		2002		2003	
	Prim	Post	Prim	Post	Prim	Post
Trad. Maize / bean	M	B	M	B	M	B
Trad. Maize / natural fallow	M	NF	M	NF	M	NF
Maize / *C. brasiiensis*	M	CB	M	CB	M	CB
Maize+cowpea / *C. brasiliensis*	M+C	CB	M+C	CB	M+C	CB

Prim = “Primera” (first planting season); Post = “Postrera” (second planting season)

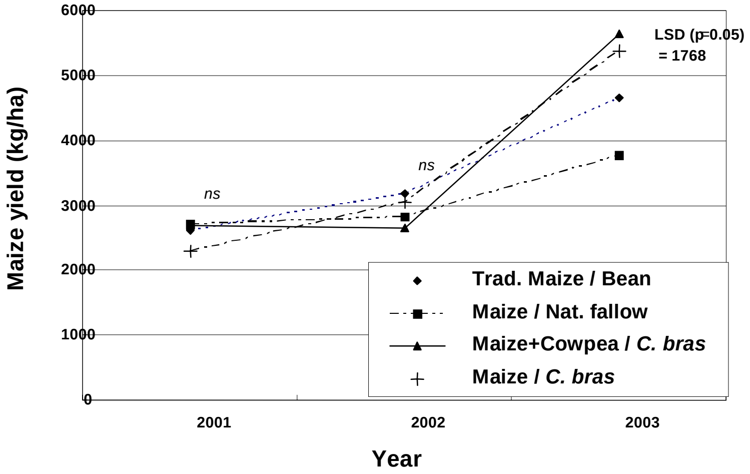


Figure 46. Maize yield of for different crop rotation systems at Sol Wibuse site, San Dionisio, Nicaragua, 2001-2003.

maize-bean rotation produced 4652 kg/ha of maize, which is above the average production level in San Dionisio. Highest yields were observed in the maize + cowpea/*C. brasiliensis* and the maize/*C. brasiliensis* rotation plots. Statistical analysis showed no significant differences between the two later ones.

Since the benefits of CCGM crops are related to soil organic matter accumulation at the topsoil we explain the observed differences between rotations with the amount of organic matter produced in each treatment. Plant species found in natural fallow plots showed only low biomass production (data not presented) and thus low organic matter accumulation in the topsoil. The relative high level of production in the traditional maize / bean rotation can be attributed to plant residue management applied, which differed from farmer fields. Both maize and bean residues were left on the plots and not burned as usually done by farmers, which leaves the rotation with a negative nutrient balance as shown by E. Sindhoj (TSBF, AR 2003).

The high yields observed in the maize + cowpea / *C. brasiliensis* and the maize/*C. brasiliensis* rotation are probably due to the massive biomass production of *C. brasiliensis* and the constant soil cover the plant specie provides during the entire dry season (Photos 30, 31).

Photo 30. *Canavalia brasiliensis* in the dry season in San Dionisio, Nicaragua

Photo 31. *Canavalia brasiliensis* sown in maize residues in the outgoing rainy season in San Dionisio, Nicaragua

The effect of cowpea on the maize yields is presumably low given the short time period of 30 days for establishment and biomass production as compared to *C. brasiliensis*. More data recorded including biomass data of cowpea, *C. brasiliensis*, and fallow species along with soil analysis and economic parameters will be recorded at the end of 2004 when the experiment ends. However, with the data presented we can conclude that legumes which drought resistance and produce enough biomass are able to enhance farmer production systems in the dry hillsides of Nicaragua and reverse the current tendency of these soils to degrade leaving farmers with higher yields and lower production costs.

The results presented were shown to farmers during participatory evaluations and field visits at the SOL site. In the FUNICA project similar crop rotations were established on 28 farms in 20m x 20 production plots under farmer management. First results indicate farmer preference for cowpea over *Mucuna pruriens* which was included as a rotation element on farmer's demand (Photo 32). Data analysis will be performed early 2005 and results will be presented in our next annual report. Further research on the mechanism behind the observed effects on maize yield in the hillside soils in Nicaragua is necessary and will be sought through our collaboration with TSBF/ Soils.

Photo 32. *Vigna unguiculata* as a cover-green manure crop prior to maize

4.1.8 Cafeteria type experiment with new grasses under dry hillsides conditions of Nicaragua

Collaborators: A. Schmidt, C. Davies, and E. López (CIAT)

Rationale

The importance of involving grazing animals at early stages of the germplasm selection process is widely recognized. Cafeteria type experiments are considered useful, efficient and rapid instruments to assist in the evaluation and selection of forage germplasm. However, we recognize that results from Cafeteria experiments only give relative indications of the acceptability of a given grass or legume accession. Therefore, we established an experiment to determine the relative acceptability of different accessions of *Brachiaria brizantha* and of *Brachiaria* hybrids developed by CIAT.

Materials and Methods

At the SOL Wibuse site in San Dionisio, Matagalpa, Nicaragua (Lat. N12°46'49.5"; Long. W 85°49'35.2", 560 masl, annual prec.1366 mm; 26°C; 6 months dry season from November to May) plots (5m x 5m) of *Brachiaria brizantha* cv. Toledo (CIAT 26110), *Brachiaria brizantha* accession CIAT 16322, *Brachiaria* hybrid cv. Mulato (CIAT 36061), and *Brachiaria* hybrid Mulato 2 (CIAT 36087) were established in three replicates on a pronounced slope in October 2003 (Photo 33). Plots were fertilized on establishment. First grazing (cycle 1, dry season) took place in March 2004, followed during the wet season by cycle 2 and 3 in June and July 2004, respectively.

Two fasted heifers were introduced to each block (replicate) (Photo 34) and during four days, the following animal activities were recorded at five-minute intervals for a total of nine hours (7 a.m. - 4 p.m.): grazing activity on each of the four grasses on offer, standing, walking, ruminating, or

Photo 33. Established plots of new grasses for the cafeteria type experiment at the SOL Wibuse site, San Dionisio, Nicaragua.

Photo 34. Heifers grazing a new grass cafeteria at the SOL Wibuse site, San Dionisio, Nicaragua.

dinking water. Based on the number of observations, animal activity profiles per site and relative acceptability indices for each grass (no. of observations of a given grass being grazed/ total no. of grazing observations expected in the respective block, if all grasses were of equal acceptability) were calculated. Accessions with low relative acceptability score indices less than 1 while those with higher acceptability had indices greater than 1. Plant height (cm), soil cover (%) and DM availability (g/m^2) were recorded before and after each grazing cycle.

Results and Discussion

In Table 67 relative acceptability indices of the four grasses are presented. In the dry season cycle no statistically significant differences were detected in relative acceptability among grasses since animals showed no preference towards one specific grass accession or hybrid. Similar results were recorded in the first wet season cycle. However in cycle 3, *B.* hybrid Mulato 2 (CIAT 36087) was less accepted by animals as compared with the other grasses of the experiment. Data on DM availability for the hybrid (not presented) confirm these findings.

It remains to be seen if the described differences continue to be observed in future cycles allowing for a better characterization of *B.* hybrid Mulato 2 (CIAT 36087) with regard to acceptability and persistence under intensive grazing. Grazing will be carried out every 4-6 weeks according to rainfall conditions. The experiment will conclude in December 2005.

Table 67. Relative acceptability indices (AI) of four Brachiaria accessions/hybrids in San Dionisio, Nicaragua.

Species	AI-Dry season	AI-Wet season	
	Cycle 1	Cycle 2	Cycle 3
B. brizantha CIAT 16322	0.98	0.89	1.14
B. brizantha CIAT 26110 (cv. Toledo)	1.15	1.12	1.13
B. hybrid CIAT 36061 (cv. Mulato)	0.94	1.08	0.98
B. hybrid CIAT 36087 (Mulato 2)	0.93	0.91	0.75*
	ns	ns	

* Species significantly different at $p<0.05$ (Ryan-Elliot-Gabriel-Welsh Range Test), ns = not significant.

4.1.9 Evaluation of milk production potential of different grass-legume associations in the hillsides of Nicaragua

Collaborators: A. Schmidt, C. Davies, E. López, M. Peters, L.H. Franco, and G. Ramirez (CIAT)

Rationale

Livestock farmers in Central America face severe forage shortages during the dry season preventing them from producing and earning an income in time periods with favourable market prices. But even in the wet season milk production levels are low due to insufficient protein intake and inadequate pasture management. Furthermore, improved animal breeds with genetic potential to produce milk are needed, but such improvements have to be based on equally improved feeding practices. As part of the BMZ/GTZ-funded project "Farmer Participatory Research in Action", where farmers evaluated and selected new forage technologies for the hillsides of Central America, the production potential of different forage technologies on offer under the environmental conditions prevailing in the area was assessed. Since farmer group members were particularly interested in grass-legume associations, our objectives were two-fold: a) to determine the milk production potential of selected grass-legume associations in both, the wet and the dry season, and b) to demonstrate how to manage grass-legume pastures.

Materials and Methods

At a farm close to the SOL Wibuse site in San Dionisio, Matagalpa, Nicaragua (Lat. N12°46'49.5"; Long. W 85°49'35.2", 560 masl, annual prec.1366 mm; 26°C; 6 months dry season from November to May) four different grass-legume associations were established. During the wet season 2001, the grasses *Brachiaria brizantha* cv. Toledo (CIAT 26110), *Brachiaria brizantha* cv. Marandú (CIAT 6780), *Brachiaria decumbens* (CIAT 606), and *Brachiaria humidicola (dictyoneura)* cv. LLanero (CIAT 6133) were associated with *Arachis pintoi* accessions CIAT 18744 and CIAT 22160 on 2 ha, respectively (Photos 35, 36). The associations were fertilized at establishment with 50 kg/ha N, P, K, respectively, and 20 ka/ha Mg. After two years a maintenance fertilization of 18 kg/ha N and 46 kg/ha P was applied. Standardization cuts were carried out at the end of each dry season.

Photo 35. Grass-legume association *B. brizantha* cv. Toledo (CIAT 26110) – *Arachis pintoi* CIAT 18744/22160 at Wibuse, San Dionisio, Nicaragua.

Photo 36. Grass-legume association *B. brizantha* cv. Marandú (CIAT 6780) – *Arachis pintoi* CIAT 18744/22160 on a steep slope at Wibuse, San Dionisio, Nicaragua.

A total of 16 cows of similar breed, age and stage of lactation grazed the four grass-legume association were arranged in a completely balanced crossover design during 5 cycles from the beginning of the wet season 2002 through the dry season 2003-2004 (Table 68).

Table 68. Completely balanced cross-over experimental design for milk production assessment of four grass-legume associations in San Dionisio, Matagalpa, Nicaragua, 2002-2004.

Animal No.	Group	Rotation period Days 1-14	 Days 15-28	 Days 29-42	 Days 43-56
1-4	1	D Bb26110	B Bd606	A Bhd6133	C Bb6780
5-8	2	B Bd606	C Bb6780	D Bb26110	A Bhd6133
9-12	3	A Bhd6133	D Bb26110	C Bb6780	B Bd606
13-16	4	C Bb6780	A Bhd6133	B Bd606	D Bb26110

Milk production of each cow was recorded throughout the experiment. The 14 days of each rotation were split in a 7-days adjustment period, in order to avoid residual effects, and a 7-days recording period. Animals were milked once a day leaving one quarter to the calf.

Results and Discussion

Average milk production/cycle for the grass-legume associations are presented in Table 69. Although the *B. brizantha* cv. Toledo (CIAT 26110) – *Arachis pintoi* CIAT 18744/22160 association showed the highest milk production, no significant differences could be established for the first cycle upon the onset of the wet season 2002. The cycle conducted in late 2002 resulted in increased production in the association with *B. brizantha* cv. Marandú, but again no significant differences between the four associations were recorded.

For the dry season cycle of 2003 a decline in production was observed in all pastires. However, in the wet season cycle in 2003 production levels were generally increased (up to 100%) probably due to the standardization cut and application of the maintenance fertilization. Associations with the grasses *Brachiaria brizantha* cv. Toledo (CIAT 26110), *Brachiaria brizantha* cv. Marandú (CIAT 6780), and *Brachiaria decumbens* (CIAT 606) outperformed significantly the association with *B. humidicola* (*dictyoneura*) which showed the lowest milk yields throughout all cycles. In the dry season cycle 2003/2004 increasing signs of weed infestation and thus degradation could be observed in the *B. humidicola* (*dictyoneura*) association.

Average daily milk production/animal (Table 70) followed the same general patterns as described in Table 68. Milk production with the locally available breeds can reach up to 6.17 L/day, which represents nearly 100% increase over what is obtained with local pastures. Although production of the legume-based pastures decreases in the dry season cycles, the production level still exceeds 3 L/animal and day, when a large number of small livestock holder do not produce any milk at all. Dry matter yields of the different grass and legume components in the 5 grazing cycles are presented in Table 71.

Table 69. Average milk production (L) per grazing cycle in four different grass-legume associations at Wibuse, San Dionisio, Nicaragua, 2002-2004.

Association with *Arachis pintoi*	Average milk production (L) per grazing cycle Max 2002	 Max/Min 2002/2003	 Min 2003	 Max 2003	 Min 2003/2004
B. brizantha cv. Toledo	259	224	193	530	367
B. Brizantha cv. Marandú	195	289	184	557	358
B. decumbens	209	239	198	419	353
B. humidicola (dictyoneura)	158	151	156	395	212*

(*species significantly different at p<0.05; Ryan-Elliot-Gabriel-Welsh Multiple Range Test)

Table 70. Average milk production (L) per animal and day of four different grass-legume associations at Wibuse, San Dionisio, Nicaragua, 2002-2004

	Average milk production (L) per animal and day				
Association with *Arachis pintoi*	Max 2002	Max/Min 2002/2003	Min 2003	Max 2003	Min 2003/2004
B. brizantha cv. Toledo	6.17	4.12	3.78	5.41	4.36
B. Brizantha cv. Marandú	5.56	4.44	3.29	5.69	4.26
B. decumbens	5.10	4.43	3.60	5.83	4.20
B. humidicola (*dictyoneura*)	5.62	3.51	3.28	5.44	4.05
Legume-grass association (average)	5.61	4.13	3.49	5.60	4.22
Local pastures (average Wibuse)*	3.0 -3.5	2.0-3.0	0-1.5	3.0 -3.5	0-1.5

(* Results of survey at micro watershed level)

While DM yields observed in *B. brizantha* cv. Toledo (CIAT 26110) were low in the first grazing cycle, the grass showed its full potential and good adaptation throughout the experiment with high dry matter yields, especially in the dry season as compared to *B. brizantha* cv. Marandú (CIAT 6780) and *B. decumbens* (CIAT 606). *B. humidicola* (*dictyoneura*) produced the lowest dry matter yields of all grasses.

The dry matter yields of *Arachis pintoi* differed considerably between the dry and wet season. The legume associates well with *B. brizantha* cv. Toledo, probably due to the higher water availability in this grazing area (see Photo 35). *Arachis pintoi* yields in the association with *B. brizantha* cv. Marandú (CIAT 6780) declined over time to the extent of only representing 2% of the total dry matter on offer. The legume percentage of all other associations levelled out at 10%.

The experiment demonstrated the high production potential of the grass –legume associations evaluated in both seasons. The grass-legume associations evaluated seem to be well adapted to the harsh conditions of the Central American hillsides with the exception of the low yielding *B. humidicola (dictyoneura),* which showed signs of degradation after 3 years of grazing. During field days farmers were especially interested in results with *Brachiaria brizantha* cv. Toledo (CIAT 26110) given that the grass showed rapid regrowth at the beginning of the

Table 71. DM yields (kg/ha) of four different grass-legume associations at Wibuse, San Dionisio, Nicaragua, 2002-2004.

	DM yield (kg/ha)				
Grass-legume associations	Max 2002	Max/Min 2002/2003	Min 2003	Max 2003	Min 2003/2004
Grass component					
B. brizantha cv. Toledo	1028	14962	8827	22416	19919
B. Brizantha cv. Marandú	3446	16608	8506	25478	16170
B. decumbens	4992	14305	8712	16479	14726
B. humidicola (dictyoneura)	2471	11331	6971	10550	12155
LSD (0.05)	2923	ns	ns	10544	ns
Legume component					
B. brizantha cv. Toledo	1112	3709	1028	2307	1817
B. Brizantha cv. Marandú	492	1400	545	619	393
B. decumbens	462	1646	518	1018	1634
B. humidicola (dictyoneura)	848	2157	727	1491	1526
LSD (0.05)	480	1692	ns	1670	ns

rainy seasons, produced high dry matter yields, and associated well with *Arachis pintoi* (Photo 37).

Since new forage materials are now available, e.g. *Brachiaria* hybrids with improved dry season tolerance, preparations began in September 2004 to establish a new grazing experiments in San Dionisio to assess the production potential of these materials in the dry hillsides of Nicaragua. The effect of *Arachis pintoi* on animal production under dry hillsides condition has to be further explored, as well as the search for a drought-tolerant pasture legume that associate well with the new generation of *Brachiaria* grasses.

Photo 37. *B. brizantha* cv. Toledo (CIAT 26110) associated with *Arachis pintoi* CIAT 18744/22160 at Wibuse, San Dionisio, Nicaragua.

4.1.10 Potential and constraints of cowpea (*Vigna unguiculata)* in Honduran hillsides: A farmers' assessment

Contributors: C. Reiber, R. van der Hoek, V. Hoffmann, R. Schultze-Kraft (University of Hohenheim), H. Cruz and M. Peters (CIAT)

Rationale

Deteriorating soil fertility, low crop yields, food shortage and inadequate supply of animal feed in particular during the dry season are some of the problems faced by poor smallholders in the central-northern Honduran hillsides. To address these challenges CIAT investigates the potential of various multipurpose forages for improving smallholder production systems. In this context cowpea (*Vigna unguiculata*) was identified as a promising multipurpose legume that can play an important role for human nutrition, animal feed and soil improvement.

Material and Methods

Cowpea is the most important pulse and staple crop in West and Central Africa, with similar importance as the *Phaseolus* bean in Central America. While in other parts of Honduras cowpea has been cultivated since the time of the first settlers, in 2002 different cowpea accessions were introduced to farmers of the Yorito area. These materials were tested at different altitudes. The objective of this research is to assess the biophysical and socio-economic opportunities and constraints of different cowpea accessions for integration into smallholder farming systems. Considering theories on adoption and diffusion of innovations (with special focus on cover crop technologies) and the hypothesis of this work that the further use of cowpea depends on its relative advantage compared to common bean (or other crop innovations), the following research questions are addressed:

1. What are the most severe problems perceived by farmers?

2. What is farmers' perception of the potentials (benefits/opportunities) and constraints of cowpea?

3. Which accessions are the most promising?

4. What is the potential for adoption and diffusion?

Participatory evaluations structured interviews based on a standardized questionnaire were conducted with all farmers in the Yorito area experimenting with cowpea. The questionnaire focused on the experience farmers had so far had with cowpea (and other "new" crops), on the management of the trials and on the perception of differences (advantages and disadvantages) between cowpea and common bean. During group meetings the taste of different cowpea accessions prepared in different forms and the seed appearance (both consider common bean) were evaluated and ranked. Important crop characteristics (e.g. yield, taste, resistance) were ranked using the matrix ranking method. Farmers' perspectives and assessment criteria were complemented by formal measurements (yield, biomass).

In order to better assess cowpea acceptance and its potential for adoption it was crucial to focus not only on cowpea itself but also on experiences with other 'new' multipurpose leguminous crops (acceptance, constraints and diffusion status), general objectives of farmers, farmers' assessment criteria as well as its complementarity with common bean.

Results and Discussion

The following results were obtained:

1. Most severe climatic and agricultural problems perceived by farmers are: a) Increasing temperatures, decreasing predictability and increased variability of rainfall distribution. Drought, storms and heavy rains tend to become more extreme; and b) decreasing soil fertility and increasing presence of pests and diseases in common bean. This leads to food insecurity for farmers living in the hillsides possessing no or only little (infertile) land and who can't afford buying inputs and/or food.

2. Farmers' perception of the major potential (advantages) of cowpea is the high drought tolerance, low disease infestation, good taste, yield, biomass production and the high acceptance of the green edible pods. Mainly due to its multipurpose use and its similarity to common bean cowpea was assessed as better than other crops like soybean (*Glycine max*), mucuna (*Mucuna spp*.), pigeon pea (*Cajanus cajan*), dolichos (*Lablab purpureus*) and canavalia (*Canavalia ensiformis*).

3. Major constraints (disadvantages) of cowpea were the low adaptability to altitudes above 1500 m.a.s.l. and problems due to 'pulgón' (Aphis craccivora), leaf-cutter ants (Atta spp.) and rabbits.

4. The most promising accessions for grain production were FHIA, IITA 716 and CIDICCO 2; for biomass production as feed and green manure cv. Verde Brazil and IITA 637/1; suitable dual-purpose accessions were CIDICCO 1, CIDICCO 2, CIDICCO 4, IITA 284/2 and IITA 9611.

5. Farmers are still in a trial and evaluation stage. They continue to test different accessions on a small scale. Cowpea has a number of characteristics that can facilitate adoption at a larger scale and diffusion. It matches farmers objectives, it is rather complementary than substitutive, has the potential to remove bottlenecks (food scarcity (esp. March-June), low soil fertility, lack of high quality feed) and farmers regard cowpea as a feasible option to improve their livelihoods.

6. Due to its relative advantages compared to common bean concerning disease and drought tolerance, less input requirements and high biomass production to increase soil fertility and reduce danger of soil erosion, the combination of cowpea and common bean can contribute to increase short-term as well as long-term food security.

Based on the above listed aspects, the potential for adoption and diffusion of cowpea is high, mainly as a complement to common bean.

It is recommended that in order to facilitate further adoption the organizations involved (e.g. FIPAH, DICTA and CIAT) continue to work with farmers in conducting participatory research on cowpea, supply information on its importance and its management (cropping methods, integrated pest control) and look for varieties (not excluding other multipurpose leguminous species) that are more resistant to pests, more tolerant to cold, waterlogging and shade, and mature evenly.

4.1.11 Farmer Participatory Research: Selection and Strategic Use of Multipurpose Forage Germplasm by Smallholders in Production Systems in Hillsides of Central America

Contributors: M. Peters, C. Lascano, A. Schmidt, E. Barrios, H. Cruz, C. Davies, P. Argel, L. H. Franco, L.A. Hernández, J.I. Sanz (CIAT); K. Tscherning, R. van der Hoek, R. Schultze-Kraft, V. Hoffmann (Universität Hohenheim, Germany); C. Burgos (Dirección de Ciencia y Tecnología Agropecuaria (DICTA); M. I. Posas (Servicios Técnicos para el Desarrollo Sostenido (SERTEDESO), Honduras; M. A. Mena (Instituto Nicaraguense de Tecnología Agropecuaria (INTA), Nicaragua; J. Bustamante (Fundación Ecotrópica), W. Sánchez (Ministerio de Agricultura y Ganadería (MAG), C.E: Reiche Instituto Interamericano de Cooperación Agrícola (IICA)/ Deutsche Gesellschaft fuer Technische Zusammenarbeit (GTZ), Costa Rica

Rationale

The goal of the project (funded by BMZ, Germany) was to increase the diversity of forage germplasm based technologies available to small farmers to enhance crop and livestock productivity in the Central American hillsides. Specifically the objective was to adjust participatory methods to develop forage-based technologies adapted to the needs of different types of farmers and farming systems in hillsides environments. The project was executed by a team of researchers from CIAT (Forage Genetic Resources, Geographical Information Systems, Participatory Research and Soils) together with farmers, development projects, Non-Government Organisations (NGOs), NARS and other relevant institutions from the region and Germany.

Some of the main strategies followed by the project were:

1. Farmer Participatory research was combined with strategic and applied research. The project applied and, when needed, developed participatory methods for research with farmers involving the targeting, testing and evaluation of multipurpose forage germplasm in diverse production systems. The participatory research focused on crop-livestock interactions and the development of methods for farmer evaluation of forages with combined animal feed and soil quality traits as well as other key traits to farmers. In the *Promotion of seed multiplication efforts* – with farmers' involvement – we placed special attention to opportunities to increase farm income from seed production. This was designed to promote feedback to researchers and early adoption of technologies, which ensures continued diffusion of developed technologies beyond the project lifespan and areas of direct intervention.

2. Strategic on-station research complemented Participatory research on methods for the simultaneous evaluation of feed and soil quality traits to improve research efficiency in the development of multipurpose forage germplasm.

3. Forage germplasm responding to particular demand of farmers was identified and evaluated and particular emphasis was given to annual legumes for multipurpose uses

4. Targeting of germplasm for different uses and environments integrated information on agro-ecological and socio-economic adaptation generated by researchers thereby improving

the selection of germplasm for specific environments and production systems (details reported in Section 4.7.2).

5. Dissemination of results to smallholder farmers in the hillsides at reference sites and other places in the region through joint work with other relevant organizations (development projects, NGOs, farmer organizations, governmental agencies etc.) focused on extension and application of technologies. The work of these organizations was strengthened by the project through participation in the research process, training in the appropriate use of technologies, and use of specific methodologies.

6. Collaboration of local, country and regional organizations was achieved through active networking. The participating institutions can extrapolate and scale-up project results to other communities in participating countries and other countries of the region and, regarding methodology, outside the region.

Major Findings

Development of participatory research methods to identify and evaluate multipurpose crop (forage) based technologies (PhD thesis of Rein van der Hoek and Luis Alfredo Hernández, Prof. V. Hoffmann, University of Hohenheim)

A key objective of the research conducted in Central America was to understand how interactions between people, biophysical and socio-economic environments affected decision-making processes in selecting forage technologies. The research (and development) process used a range of different approaches and types of experimentation that fostered farmer involvement. Since forage technologies have different management requirements and end-uses, the research identified the approaches that were best able to facilitate the adoption of specific forage technologies.

Figure 47 is a summary representation of the R&D approach employed by the project. The left side presents the initial conditions consisting of the different perspectives (Participatory research) surrounded by the contextual elements consisting of the human environment (Farmers' perspective), the biophysical environment (Farming systems perspective) and the technology intervention (Multipurpose forage crop perspective). The right side displays – and the research procedure as affected by the processes and the expected conclusions.

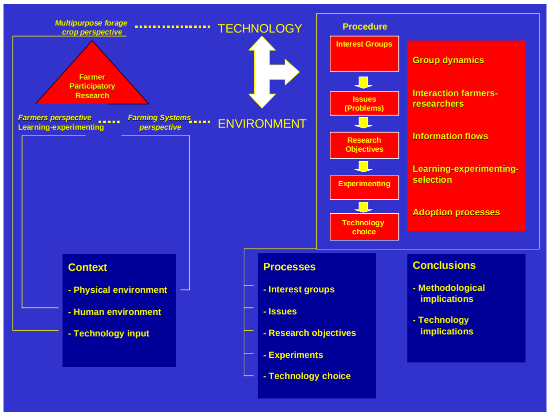


Figure 47. Forage participatory research approach.

The comprehensive methodology included problem identification and prioritization, farming system analysis, formulation of research objectives and implementation and evaluation of experiments. All of these activities were conducted using participatory methods to better appreciate farmers' demands. In the final analysis, the detailed descriptions of processes, methods and approaches were used to define strategies for different farmer interest groups (Note: Under method we understand a specific set of steps to follow to obtain a specific result; under approach we understand a combination of methods and the (philosophical) framework to reach the purpose that smallholder farmers utilize multipurpose forages and technologies suitable to their conditions).

A procedure that is widely applicable and scalable was developed. The procedure integrates the analysis of quantitative and qualitative data from farmer participatory experiments, thus allowing the simultaneous selection of forage materials by farmers and the feed back to and hence better targeting of on-station research. Since the procedure is applicable across countries and not location specific, it can be adapted to widely different production systems. Application and testing in Colombia is underway as part of a different research project.

The procedure comprises the following steps:

a. *Diagnosis, training and planning:* Identification of institutional collaborators and sites and exposing farmers (and technicians) to a range of forage options.
b. *Exposing farmers to different forage options:* A range of methods from nurseries for farmer selection and evaluation to demonstration plots to farmer led experimentation is employed.
c. *Database development.* A central database that includes information on farmer selection criteria across sites was developed.
d. *Selection criteria* for forage technologies, based on farmer input. Farmers refer to these criteria (such as dry season production, ease of establishment) for assessing forage technologies.
e. *The criteria* are then *stratified* according to their similarity to allow a qualitative analysis. I.e. different farmers in different regions utilize different terminology, which need to be translated into and grouped.
f. *Criteria analysis* to determine relative importance using summary statistics (frequencies), Principal Component Analysis (PCA) and/or Factorial Correspondence analysis.
g. *Final analysis and interpretation* of data to identify technologies that match farmers' demands, and consequently are more likely to be adoptable.
h. *Supporting activities:* training modules of high farmer interest complement the R&D process. Seed production technology is an example of such training, along with pasture management.

Farmer involvement in the germplasm selection process increased their knowledge and confidence to progressively assume more responsibility in evaluating technology options. In cycle 1 of the process, farmers were exposed to new forage options. Smaller areas (nurseries) were planted and a large part of the investment risk was assumed by scientists/technicians. According to their limited resources (land, time, capital), farmers selected or rejected technology components, utilizing their own criteria. Through interactions with technician/researchers and experimenting with technologies suitable to their farming conditions farmers gained knowledge and experience on forage germplasm traits and management, leading to an improved farmer ability in employing such technologies on a wider scale at a reduced risk. As a result in cycle 2, farmers increasingly planted larger areas and experimented with more forage species (Figure 48).

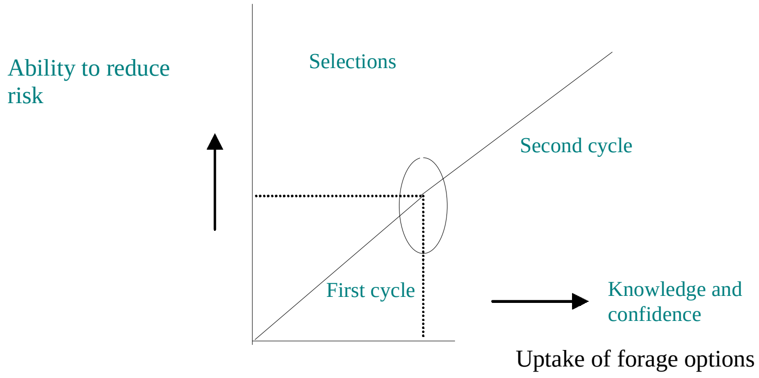


Figure 48. Relation of ability to reduce risk and uptake of forage options over different cycles of a Participatory procedure to forage selection and development.

Another result of the dialogue between farmers and researchers was a build-up of shared knowledge, trust and confidence. In our framework, we assume that knowledge and experience and the ability to take risk are correlated, hence both increase over time as a result of iterative learning process. Farmers were enabled to plant larger areas of chosen technology options, increasingly managed, adapted and adopted by themselves. Technicians/ scientists gain knowledge by monitoring and analyzing these processes. As part of this process, farmers are also enabled to evaluate more complex technologies.

The different selection criteria obtained through farmer participatory evaluation were classified using Principal Component Analysis and Factorial Analysis of Correspondence to reduce variability and identify priority criteria. The Factorial Analysis of Correspondence was particularly suitable to incorporate quantitative and qualitative data. According to a cross tabulation of frequencies across all forage technologies (i.e. grasses, shrubs, herbaceous legumes, and green manures), plant color was the most important criteria in farmer assessment. Across seasons, this parameter was given more importance in the dry season (greater frequency), indicating the importance of alleviating a feed constraint that farmers face during the 5 to 6 dry months. Yield was the next important criterion, followed by cover, leafiness and competitiveness. The analysis then disaggregates the forages into specific technologies, i.e. grasses, shrub legumes, green manure and cover legumes (data not presented in detail, see Annual Report to BMZ 2002 and CIAT Annual Report 2003, page 151-152).

Specific forage-based technologies selected by farmers: Forage technologies were classified into grasses, shrub legumes, short-term multipurpose legumes, and green manures. Over the wet and dry seasons *Brachiaria brizantha* cv. Toledo and *Brachiaria* hybrid cv. Mulato were the grasses that best matched to farmers´ criteria, the latter having a particular high acceptance in the dry season. Farmers preferred the grasses because of their growth, drought resistance and seed production characteristics, which enables them to increase pasture areas and to generate income by selling seed. Even non-cattle owners could plant pastures and produce seed.

The grasses are suitable to altitudes up to 1600 meters. For cut and carry purposes, *Pennisetum* sp. Camerún and King Grass almost always produced satisfactory results, the former was slightly preferred because of the absence of '*guate*', which causes itching while carrying the stalks. Among the shrub legumes, the most preferred species across seasons were *Leucaena leucocephala* CIAT 17263 and *Cratylia argentea* CIAT 18668, the latter especially attractive for its outstanding drought tolerance. There was a limitation of *C. argentea* at higher altitudes and a constraint of slow establishment under alkaline soil conditions. Among the short-term multipurpose legumes, cowpea is of considerable interest to farmers due to its high versatility of use in systems to improve food security, enhance soil fertility and animal nutrition. System trials to define the best temporal niches for cowpea are still underway. Among the forage materials used for green manure, crop residue improvement and green manure *Canavalia brasiliensis* gained increasing acceptance by farmers mainly due to its vigorous growth, drought tolerance and adaptation to a wide range of environments, including soils of very low fertility.

Farmers were less interested in herbaceous legumes for pasture improvement than in grasses, green manure/cover crop and shrub legumes. However *Centrosema pubescens* CIAT 15160 had the highest overall attributes related to farmers´ criteria, with *Centrosema plumieri* being an additional option for the dry season and *Stylosanthes guianensis* CIAT 11844 and *Arachis pintoi* CIAT 22160 potential options for the wet season.

Lessons Learned: Main lessons learned from the project are summarized as follows:

1. Results from participatory evaluations can be analyzed not only qualitatively but also quantitatively. In addition to a more profound understanding of farmer demands, participation allows feedback and better targeting of on-station research results

2. Different entry points for forage technologies need to be evaluated. In Central America, farmers first selected forage grasses followed by shrub legumes and cover crops. Herbaceous legumes for grass-legume mixture are a more complex technology requiring more time for adoption

3. Participatory methods for forage germplasm selection are different from those for more complex technologies such as forages for soil improvement, silage and grass-legume pastures. The latter require more learning processes. A successful uptake of forage germplasm options is, however, an entry point for the adoption of more complex technologies. To achieve wide scale impact, these differences should be acknowledged by future projects with more easily adoptable technologies before moving into more complex ones.

4. A strong demand from farmers to address particular production system constraints along with proven success and good relations between scientists/technicians and farmers encourages farmers to experiment and adapt technologies. Such efforts often continue even in the case of initial failures. In our project, the variable results with the shrub legumes *Cratylia argentea* on soils with higher pH leading in some cases to poor performance at least in the year of establishment serves an example. As farmers recognize the potential of the technology – as it has been demonstrated to function at some sites - they make an effort to improve management or test the technology at other sites.

Future Research Needs: The project identified a need to further develop forage alternatives for the dry season. A potential for silage and hay technologies adapted to smallholder situations has become evident. Either livestock or non-livestock owners can produce these silages and hays. Research is needed to define the usefulness and acceptability by farmers of such technologies through on-station and on-farm studies. Further research is also needed into the facilitation of farmer innovation, focusing on the adaptation of new forage-based products to smallholder farm constraints, and innovations that facilitate a market between producers and end users. Success in this area would have an impact on income generation and livelihoods of smallholder farmers. The efficiency of approaches facilitating innovation versus more traditional extension approaches needs to be evaluated (i.e. promotion of innovation versus promotion of adoption). Such research should be cognizant of the changing role of NARS in technology delivery. It is anticipated that through the facilitation of innovation, better-adapted forage technologies will emerge and demands for alternative technologies articulated.

The two above mentioned research questions are the core of the recently funded BMZ proposal ‘Demand-Driven Use of Forages in Fragile, Long Dry Season Environments of Central America to Improve Livelihoods of Smallholders’.

4.1.12 Potential and constraints for animal feed as an objective of poor farmers in Central-America

Collaborators: R. van der Hoek (University of Hohenheim), M. Peters (CIAT), V. Hoffmann (University of Hohenheim), and H. Cruz Flores (CIAT)

Rationale

Multipurpose forage crops can play an important role in improving the environmental and socio-economic sustainability of smallholder production systems in fragile environments. However, since the forage technology development framework has not been sufficiently applicable for poor farmers, adoption of especially legumes has been generally low (Peters *et al.*, 2001). In a participatory research effort with smallholder farmers in Honduras, focused at forage-based technologies, food security turned out to be the main selection criterion whereas animal feed was secondary. Since animal feed related activities (farmer-led forage seed systems, production of dry season feed) have been identified as promising income generating options for poor farmers in the hillsides of Central-America, a further analysis was carried out to identify the (mainly household related) factors inducing or inhibiting farmers to opt for production of animal feed.

Materials and methods

A group of 150 farmers with different levels of resource endowment representing the typical maize and beans based agricultural system of central Honduras conducted over 200 experiments in their own fields with several grasses (e.g. *Brachiaria brizantha*), leguminous crops (mainly several varieties of *Vigna unguiculata*) and shrubs (e.g. *Cratylia argentea*).

The choice of research methods and parameters was determined simultaneously by both farmers and researchers. A dichotomous logistic regression model was used to examine the variables influencing the inclusion of animal feed as an objective (Table 72). The independent variables were identified by a Principal Component Analysis.

Results and Discussion

Altitude had no significant influence on the inclusion of animal feed production as an objective. Full or semi landownership increased the chance of feed being an objective by 24%, controlling for other variables in the model (p=. 005).

Farmers who depend on purchased maize from outside are 17% more willing to include feed production as an objective than those who are self sufficient in maize production (p=. 025). Every extra 100 kg/ha urea application on maize increases the chance of feed being an objective by 22% (p=. 025). A yield increase of 100 kg/ha maize augments the chance of feed being an objective by 1% (p=. 033). An increase of one unit of cattle increases the chance of feed being an objective by 2% (p=. 001).

In summary, results indicate that farmers owning land, applying fertilizer and owning cattle are more likely to include animal feed as a research and production objective than the poorer farmers, except for those who are not self-sufficient in maize. Farmers without full decisive power over their land are reluctant to engage in animal feed production for sale in the market. Whereas research and development work can continue to be directed at all farmer categories in Central-American hillsides, special attention is justified for farmers without full land ownership and those who depend on outside acquired basic grains for their food security.

Table72. Definition of variables used in animal feed regression model.

$$\ln(\text{ObjectiveFeed}) = \beta_0 + \beta_1\text{Altitude} + \beta_2\text{LandTenure} + \beta_3\text{BuyMaize} + \beta_4\text{Ureamaiz} \; \beta_5\text{MaizeYield} + \beta_6\text{CattleNr} + e_i$$

Variable	Definition
Objective Feed	1: yes, 0: no
Altitude	1: low (< 800 masl), 0: other (? 800 masl)
Land Tenure	Land tenure: 1: full or semi land ownership, 0:
Buy Maize	Maize bought for consumption: 1: yes, 2: no
Urea maize	Level of urea application on maize (kg/ha)
Maize Yield	Maize yield (kg/ha)
Cattle Nr	Number of cattle

4.1.13 Multilocational analysis of a collection of cowpea targeted to multipurpose needs of clients in hillsides of Central America and Colombia

Contributors: M. Peters, A. Schmidt, P. Argel, H. Cruz, C. Davies, L. H. Franco and G. Ramirez (CIAT)

Rationale

A Genotype x Environment experiment was conducted to test the performance of cowpea selected accessions across sites and to identify the most resilient accessions to recommend over a wide range of environments. An adaptability index was calculated to assess the response of a set of 14 cowpea accessions accross different climates and soils in Honduras, Nicaragua, Costa Rica and Colombia. Accessions best adapted across environments but responding to improved environmental conditions were IT90K-284/2, IT89KD-391, IT95K-1088/4, IT95K-1088/2, IT86D-716, IT93K-637/1 (Figure 49). In farmer participatory evaluations in Honduras and Nicaragua IT90K-284/2 was selected as one of the most preferred materials due to superior agronomic performance in terms of forage and grain yield (dual purpose type).

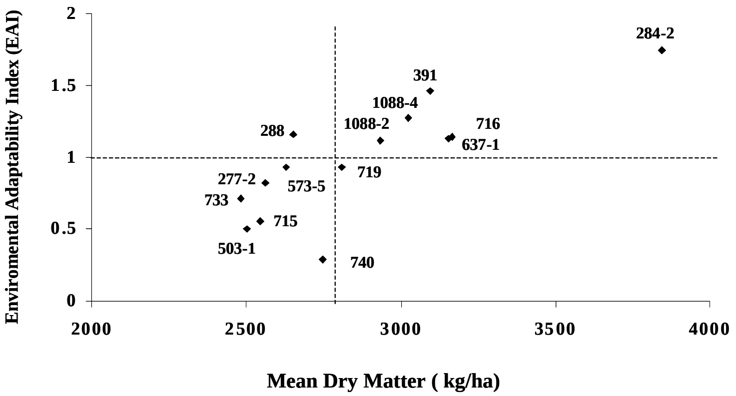


Figure 49. Adaptability Index for dry matter production of a collection of *Vigna unguiculata* evaluated in contrasting environments in Honduras, Nicaragua, Costa Rica and Colombia.

4.1.14 Promotion of seed multiplication and scaling of forage options in Hillsides of Honduras and Nicaragua

Collaborators: H. Cruz, M. Peters, A. Schmidt, L.H. Franco, G. Giraldo, (CIAT), C. Burgos (DICTA), M. Mena (INTA) and C. Davies (CIAT)

Rationale

The adoption of forage technologies is intimately related to the availability of good quality seed at reasonable prices. Therefore, taking into account the current seed market in Central America, the promotion of seed supply systems with a focus on farmer-led enterprises is one of our strategies for scaling up selected forage technologies. At the same time, seed production offers a means of income for small farmers.

Honduras

A farmer group in Honduras working now under their own label – ‘PRASEFOR’ (Productores

Artesanales de Semillas Forrajeras) produces seed since 2002; this farmer- led seed enterprise was established with very limited financial support (i.e. less than US $ 2.000), hence the approach could easily be replicated at other locations. The farmers in PRASEFOR, with assistance from NARS and NGO partners, are constantly exploring market demands. Seed quality and packaging were identified as important criteria to meet seed production targets for the local and wider market. In 2002, production in Honduras began with 400 kg of seed. During 2003 a total of 1,200 kg of *Brachiaria brizantha* cv. Toledo was produced by the 15 members of PRASEFOR on 17 ha, and for 2004 the production target is >1.6 tons. The warehouse to store seed was finished during 2004.

Since the formation of this enterprise, farmers have doubled their seed production areas and are still expanding. The total market value of the seed produced in 2003 reached 14,600 US$, turning seed production into a highly profitable operation for theses farmers. Though the initial focus was on grass seed production (*B. brizantha* cv. Toledo), the enterprise is now exploring the feasibility of diversifying into seed production for the shrub legume *C. argentea,* annual legumes such as cowpea for the elaboration of feed concentrates, and maize for silage.

Nicaragua

In Nicaragua we are also promoting farmer-led forage seed systems with groups of farmers in Pantasma, Jinotega. With technical assistance from INTA, a total of 5 farmers harvested 110 kg of classified seed of *Brachiaria brizantha* cv. Toledo in December 2003. Half of the seed where sold in the region, the rest was utilized by the group to expand their own pasture areas. During 2004, based on these experiences, INTA established in all regions seed multiplication plots in collaboration with farmers adding up to 7 ha of *Cratylia argentea* and 4.5 ha of *Brachiaria brizantha* cv. Toledo. Together with INTA's seed production unit (UNISEM) a forage seed production plan covering all regions of Nicaragua (Table 73) was developed focusing mainly on grasses with high demand (*B. brizantha* cv. Marandu, cv. Toledo, *P. maximum* cv. Tanzania) and *Cratylia argentea*. It is expected that at least one farmer group/ enterprise will be formed in each region providing good quality seed at reasonable prices. In contrast to the above mentioned groups, the PES ("Pequeña Empresa de Semillas") at San Dionisio, Matagalpa, which normally multiplies maize and bean seed, addressed the growing demand for *Vigna unguiculata* (cowpea) and *Canavalia brasiliensis* harvesting 50 kg of cowpea and 15 kg of *Canavalia brasiliensis* to be sown by farmers in the postrera season 2004. Areas will be expanded in 2004/2005 and *Lablab purpureus* will be also included on the multiplication list in San Dionisio. Discussions with larger scale seed distributors in Central America have been initiated to enhance the demand for farmer-led seed production.

In summary, seed production offers an income opportunity to smallholder farmers. For farmer-led seed enterprises to be sustainable over time, they need to be based on a sound business plan. On the other hand, for sustained uptake of forage technologies, a focus on interested farmers and the sustainability of forage seed enterprises is crucial. Support to farmers in provision of seed and free inputs should be limited; in particular not to compete with farmer-led enterprise activities. Rather than large and financial support, there is a need to invest time with farmers and in providing basic seed material for initiating small plot on farm testing and seed multiplication.

Scaling forage technologies in Honduras and Nicaragua:

Scaling and adoption of forage options depends to great extent on availability of good quality seed, socio-economic constraints and farmer knowledge about characteristics and specific features of forage technology options. The approach developed by the project since 2000 combines these elements with participatory selection procedures.

Table 73. Seed production plan (UNISEM-INTA-CIAT) 2005-2008 for Nicaragua.

Year	2005		2006		2007		2008		Total	
Species/Region	Area (ha)	Seed (Kg)	Area (ha)	Seed (Kg)	Area (ha)	Seed (Kg)	Area (ha)	Seed (Kg)	Area (ha)	Seed (Kg)
Marandú										
Pac. Norte	5	100	5	150	5	150	5	150	20	550
Pac. Sur	5	100	5	150	5	150	5	150	20	550
Las Segovias	0	0	0	0	0	0	0	0	0	0
C. Norte	0	0	0	0	0	0	0	0	0	0
C. Sur	0	0	0	0	0	0	0	0	0	0
Total	10	200	10	300	10	300	10	300	40	1100
Tanzania										
Pac. Norte	5	400	5	850	5	1300	5	1750	20	4300
Pac. Sur	4	320	4	680	4	1040	4	1400	16	3440
Las Segovias	0	0	0	0	0	0	0	0	0	0
C. Norte	0	0	0	0	0	0	0	0	0	0
C. Sur	0	0	0	0	0	0	0	0	0	0
Total	9	720	9	1530	9	2340	9	3150	36	7740
Toledo										
Pac. Norte	0	0	0	0	0	0	0	0	0	0
Pac. Sur	8	360	8	880	8	1400	8	1920	32	4560
Las Segovias	10	450	10	1100	10	1750	10	2400	40	5700
C. Norte	10	450	10	1100	10	1750	10	2400	40	5700
C. Sur	10	450	10	1100	10	1750	10	2400	40	5700
Total	38	1710	38	4180	38	6650	38	9120	152	21660
Cratylia										
Pac. Norte	2	0	3	800	3	2000	5	3200	13	6000
Pac. Sur	4	0	4	1600	4	3200	4	4800	16	9600
Las Segovias	0	0	0	0	0	0	0	0	0	0
C. Norte	5	0	5	2000	5	4000	5	6000	20	12000
C. Sur	2	0	3	800	3	2000	5	3200	13	6000
Total	13	0	15	5200	15	11200	19	17200	62	33600

In Tables 74 and 75 we show results from the adoption of forage options at our reference sites in Nicaragua and Honduras.

In Nicaragua, uptake of forage options is gaining speed. More than 250 farmers in San Dionisio are testing and employing new forage options. Preferred options are *B. brizantha* cv. Toledo and *Brachiaria* hybrid cv. Mulato. However, there is a recent interest in the cover legume *Canavalia brasiliensis* and the shrub legume *Calliandra calothyrsus*, the latter to be used as fuel wood.

In Honduras, approximately 600 farmers are now testing and employing forage options in about 100 ha. The largest areas are planted with *Brachiaria* hybrid cv. Mulato (CIAT 36061) and *B. brizantha* cv. Toledo. The increase in area of Toledo has been driven by the availability of seed mainly through PRASEFOR. Though areas planted are still small, there is an increasing farmer interest in *Cratylia argentea, Vigna unguiculata* and *Lablab purpureus.*

Projects led by the NARS and NGO partners are now employing and adapting the participatory methods developed by CIAT with funds from BMZ. For example, INTA in Nicaragua, is currently establishing in all regions germplasm sites and validation plots which are frequently visited by farmer groups. Other institutions,

Table 74. Distribution of forage materials at reference site San Dionisio, Nicaragua, 2002-2004.

Material	2002		2003		2004		Total	
	Area (ha)	Farmers No.	Area (ha)	Farmers No	Area (ha)	Farmers No.	Area (ha)	Farmers No.
B. decumbens	0.2	4					0.2	4
B. brizantha 26110	1.5	37	5.4	19			6.9	56
B. hibrido 36061	1.8	44	4.1	14	0.7	8	6.6	64
C. calothyrsus			0.3	35			0.3	35
Vigna unguiculata			1.4	35	0.9	28	2.3	63
Canavalia brasiliensis			1.4	35	0.5	6	1.4	41
Total	3.5	85	12.6	138	2.1	42	16.9	263

mainly development projects and NGOs, have initiated similar work to adopt new forage-based technologies. Increasing requests from these projects and organizations for forage germplasm and larger amount of seed, especially of *Cratylia argentea*, are addressed through the already existing farmer-led seed enterprises and/or our Seed Unit in Atenas, Costa Rica. As a result, we have reached now more than 2500 farmers in Honduras and Nicaragua. In 2004, a project was initiated in Colombia utilizing the participatory methods developed in the BMZ funded project. To foster further uptake and expansion, a set of extension publications were published. In addition, an interactive internet-based access to some of the extension publications is in development.

Both the application of results by participating institutions and uptake of forages have exceeded our expectations. An unforeseen success was the formation of income generating, self sustained farmer-led seed enterprises in Honduras. Seed multiplication and area expansion increased very quickly, driven by farmer-seed production. In addition, the utilization of results in other core and special project activities of CIAT and its partners was not anticipated at such a scale.

Table 75. Distribution of forage materials at reference site Yorito, Honduras, 2001-2004

Material	2001		2002		2003		2004		Total	
	Area (ha)	Farmers No.	Area (ha)	Farmers No.	Area (ha)	Farmers No.	Area (ha)	Farmers No.	Area (ha)	Farmers No.
A. gayanus 621	0.2	6			0.1	2			0.3	8
B. dictyoneura 6133	0.6	12							0.6	12
B. brizantha 26110	1.1	25	16.3	19	3.4	37	0.7	6	21.5	87
B. hibrido 36061			12.3	6	45.1	23	0.2	1	57.6	30
P. maximum 16031	0.7	17							0.7	17
P. purpureum	0.6	16	0.5	3					1.1	19
A. pintoi 22160	0.1	1					1.2	2	1.3	3
C. argentea 18668	2.3	15	3.4	24	0.8	6	4.1	9	10.6	54
C. pubescens 15160	0.1	2							0.1	2
L. leucocephala 17263	0.6	11							0.6	11
Lablab purpureus	0.1	1			0.3	30	0.3	11	0.7	42
Vigna unguiculata			0.2	2	1.3	195	5.1	62	6.6	259
S. guianensis 184	0.1	6							0.1	6
M. pruriens IITA BENIN	0.1	1			0.1	1			0.2	2
Canavalia. brasiliensis					0.1	3			0.1	3
Canavalia ensiformis					0.1	6			0.1	6
Total	6.4	113	32.7	54	51.3	303	11.6	91	102	561

4.1.15 Improved dry season feeding systems for smallholder dairy cattle in the hillsides and high mountainous tropics of LAC

Contributors: H.D. Hess (ETH Zurich), A. Schmidt (CIAT), C. Perez (Intercooperation), C.A. Gómez (National Agricultural University of Peru), H.M. Romero (ETH Zurich), H.-R. Wettstein (ETH Zurich), F. Holmann (CIAT/ILRI) and M. Kreuzer (ETH Zurich)

Rationale

This project builds on major outputs of two preliminary activities realized during 2003, in which three major problems related to dry season feeding of dairy cattle in the hillsides and high mountainous tropics of LAC were identified: (i) large gaps in knowledge about effects of feeding measures in the dry season and of high altitude on milk composition; (ii) huge7 variation in availability and quality of feed resources depending on rainfall distribution which directly affects income of smallholder dairy farmers; and (iii) insufficient exchange of information and lack of coordination of current projects of local research and extension institutions.

To address these problems a project was designed with the specific aim of developing more efficient feeding systems in different agro-ecological zones of tropical Latin America, based on local knowledge and locally available and introduced feed resources. We expect to demonstrate how these systems contribute to sustained milk production and improved milk quality during the dry season and reduce the dependence on purchased supplements.

Planned activities

The objective of the project will be achieved through (i) assessing the availability and quality of local forage and feed resources, (ii) identifying experimentally the effects of dry season feeding (independent of high altitude and cow genotype) in milk production and quality, and (iii) designing optimal forage management strategies for dry season feeding in several target regions in Nicaragua and Peru. The participatory assessment of utility and viability of alternative dry season feeding options will be a transversal issue throughout this activity. Involvement of farmers in all proposed activities will allow the simultaneous evaluation of biophysical aspects for research purposes and of utility and viability of the new options for the end-users. By means of including NGOs and various participatory approaches, dissemination of scientifically derived results will take place.

Results and Discussion

Major outputs of a stakeholder workshop held in Managua

The results of a consultation workshop in Nicaragua identified the following factors related to the inadequate nutrition of livestock in the dry season: (a) low availability and quality of forage, (b) limited diversity of forage resources, (c) deficient supplementation with mineralised salts, (d) inadequate use of agro-industrial by-products, (e) lack of knowledge about the nutritional value of feed ingredients, (f) deficient management of pastures and other forage resources, and (g) a lack of dry season feeding options which are adapted to the diverse agro-ecological and socio-economical conditions. On the other hand, the participants were able to identify some 30 available dry season feeding options, of which only three were considered to be widely adopted at present. Namely these were the use of maize and sorghum straws, supplementation with cut and carry grasses and supplementation with purchased grass hay. The reasons for the low adoption rates of the other potential dry season feeding alternatives were high cost and lack of knowledge on the nutritional quality of the feeds and the requirement of animals. In addition to the technical problems, it was clear from the workshop made also clear that there is insufficient exchange of information and a lack of coordination of current projects of national research and extension institutions.

Major results of a survey conducted in Peru

A survey was conducted to collect data and perform analyses on the status of feed resources and milk production and quality on four different farms at contrasting altitudes in the Peruvian Highlands (3200 to 4250 m a.s.l.) during the rainy and the dry season. On three of the experimental farms included in the study, cattle production was based on cultivated pastures (perennial ryegrass and white clover) and one farm relied on native grassland. Forage quality (described as crude protein and fiber content) of cultivated pastures was higher ($P<0.01$) than that of native grassland. On average, milk production per cow was 20% lower ($P<0.05$) in the dry season as compared to the rainy season, but differences among individual farms were large.

While the decrease in milk yield on the most intensively managed farm at 3200 m a.s.l. was only about 10%, milk yield was 30% lower on the most extensively managed farm at 4250 m a.s.l. during the dry season than during the rainy season. Also milk composition was affected by season. Fat, protein and casein concentrations were lower ($P<0.01$) during the dry season than during the rainy season. Differences among farms were large and the decrease in milk constituents was much more pronounced on the extensively managed farm at 4250 m a.s.l. than on the remaining farms. On this farm, fat concentration decreased by over 20% and protein and casein concentration by 10% during the dry season. Because both milk yield and milk quality were decreasing at the same time, the unfavorable effects of the dry season were amplified. When processing the milk to cheese or similar dairy products, the loss of income is multifold as (i) less milk, (ii) less cheese yield due to the lower fat and protein content of milk, and (iii) cheese quality may be impaired due to the less favorable milk renneting properties associated with reduced protein (casein) content. Efficient dry-season feeding strategies are required to overcome this unfavorable situation.

4.2 Partnerships in Asia to undertake evaluation and diffusion of new forages alternatives

Highlights

- The Forages and Livestock Systems Project (FLSP) Laos entered into an expansion phase. District teams doubled the number of villages in which they work a year early than expected. By the end of the 4th rainy season the project expects to be working in >105 villages and with >1300 farmers. New extension approaches have been developed and new cases of impact are emerging and being documented.

- Following successful adoption of forage technologies at project sites in Southeast Asia, the Livelihood and Livestock Systems Project (LLSP) is developing participatory methodologies to improve livestock/forage production systems, including marketing constraints and opportunities.

- The *Brachiaria* Mulato hybrid was by CIAT to Southeast Asia in 1996 as part of a large *Brachiaria* variety trial. In Thailand, The Thai Department of Livestock Development (DLD) selected Mulato and seed production trials were initiated in 2003 with 7 small farmers. This year 1793 farmers planted 700 ha of Mulato to produce 140 tons of seed thanks to a guaranteed market by Papalotla.

4.2.1 The Forages and Livestock Systems Project (FLSP)

Contributors: Viengsavanh Phimphachanhvongsod (Researcher, National Ag. & Forestry Research Institute), Viengxay Photakoun (Researcher, National Ag. & Forestry Extension Service), Peter Horne (Agronomist and Team Leader, CIAT) and John Connell (Extension and Agroenterprise specialist, CIAT)

Rationale

CIAT has been conducting forage research in Southeast Asia since 1992, commencing with forage varietal selection and evaluation, both in experimental plots and on farms, in seven countries. One main outcome of this work was the identification of ~40 broadly adapted and robust forage varieties with demonstrated potential to deliver significant impacts on smallholder farms throughout the region. The outcomes of this research are documented in several CIAT publications (Horne and Stür, 1999; Stür et al., 2000; Stür and Horne, 2002; Stür et al., 2002).

In 2000, CIAT secured funding from the Australian Agency for International Development (AusAID) for a five year project to integrate forage and improved livestock management strategies into upland farming systems of Laos using participatory research approaches. The project works with 36 partner staff from national, provincial and district government agencies, conducting research and extension aimed at:

- Increasing income by improving the productivity of small and large livestock;
- Increasing labor efficiency and reduce women's workloads in the livestock production systems;
- Enhancing sustainable cropping systems by increasing soil fertility and reducing soil erosion; and,
- Sustaining livestock production within the national policy of stabilizing shifting cultivation

Progress with forage technology development

In the first field season (June 2001), the project supported small scale testing on farms during which time farmers tested the forage varieties, sorted out which ones they preferred and wanted to expand. This was a time when the DAFO and PAFO staff also learned about the varieties, their environmental adaptation and the process of working in partnership with farmers.

Building on the experiences of the first year, the second field season (June 2002) was a period of expansion based on targets set by the project (e.g. the number of villages was doubled and the number of farmers tripled) or targets set by farmers (based on the desire to get large enough areas of forages to have some significant quantities of feed for their animals). Farmers sometimes (but not always) reduced the number of varieties and, more significantly, started to look for ways of utilizing the forages to help resolve current problems or to develop new opportunities. During that second year the project challenged field staff with new villages**,** new technologies and many new farmers to encourage them to move away from a dependence on the very intensive one-to-one processes that had been used in the first field season and move more towards farmer group processes.

Leading up to the third field season of the project (June 2003), some interesting, sometimes novel, often unexpected impacts had started to emerge. The district staff had become very familiar with the processes of working with farmers. Indeed these processes are now becoming their 'comfortable norm' back to which they will retreat naturally, given the support of their organizations. Further expansion will not now be driven by project targets but by impacts. This focus on "impacts driving extension" will continue for the next two wet seasons.

This fourth year (2004) was a year for expansion based on impacts. District teams will double the number of villages in which they work which will mean we almost reach the target number of villages for the project a year earlier than expected. A major focus of this year will be on developing different extension approaches for new farmers and experienced farmers; for new villages and experienced villages. New cases of impacts are emerging and being documented as we go (>20 have been documented so far). We are engaging with the development community to provide another avenue of expansion of impacts. We are also about to embark on a doubling of the numbers of experienced staff. By the end of this fourth wet season we expect to be working in >105 villages with >1300 farmers. District staff estimate that about 350 of these farmers will be experiencing significant livelihood impacts from intensifying their livestock systems. By the end of this season, the project will be supporting the most experienced and confident farmers as field extension workers to help with the expansion of impacts to more people and more villages. The expansion that has occurred in the first four field seasons of the project is summarized in Figure 50.

The total number of farmers working on forage technologies with the FLSP is expected to rise from 803 in 2003 to >1300 in 2004. Of this total, about half are expected to come from new villages. This is a reflection in a change in strategy of the project in 2004.

In 2003, the project focused on:

- Consolidating emerging impacts.
- Expansion to new farmers through emphasis on impacts.
- Emergence of second level problems (such as nutrient decline in cut forage plots) and opportunities (such as improving livestock management to maximizing market returns).
- Documenting cases of impacts for further future expansion.
- An extension approach based on cross visits, village planning meetings and working with separate production groups in villages (small animals, large animals).

In 2004, the project aimed to significantly expand to new villages while consolidating the progress made in old villages. The challenge was how to ‘ramp up’ the expansion beyond the steady increase from year to year that has happened so far? This steady increase has been necessary so that (i) farmers and district staff can learn about technologies and methods and (ii) impacts can be consolidated. Now that both of these have been achieved, we have the chance to scale out.

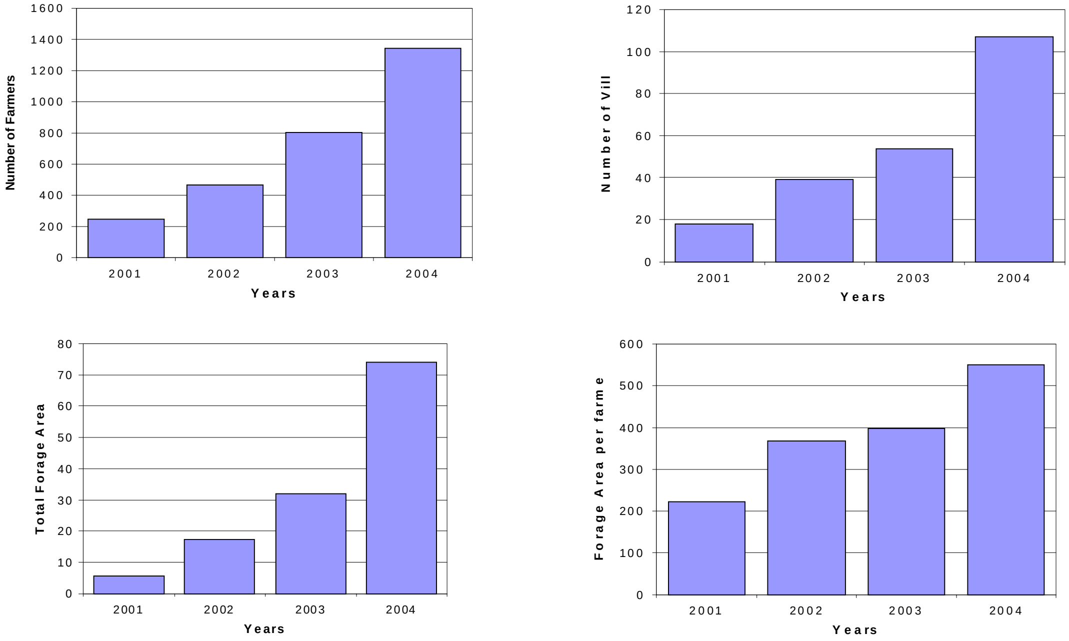


Figure 50. Expansion of number of farmers and area planted with forages as a result of interventions by the FLSP.

The varieties preferred by farmers at this time are *Brachiaria brizantha* cv Marandu, *Brachiaria* hybrid cv Mulato", *Panicum maximum* "Simuang" and *Stylosanthes guianensis* "Stylo 184". Other productive varieties but with particular uses or adaptation are the grasses *Andropogon gayanus* "Gamba", *Paspalum atratum* "Terenos" and *Setaria sphacelata* "Solander" and the legume *Gliricidia sepium* "Retalhuleu".

4.2.2 The Livelihood and Livestock Systems Project (LLSP)

Contributors: Werner Stür, Francisco Gabunada, Phonepaseuth Phengsavanh, Jindra Samson and John Connell (CIAT)

Rationale

The Asian Development Bank (ADB) funded 'Livelihood and Livestock Systems Project' (LLSP) started in January 2003 for a period of three years. The LLSP is a collaborative research for development project bringing together livestock researchers and extension workers in seven countries in Southeast Asia. The purpose of the project is to improve (i) sustainable livelihoods of smallholder farmers in the uplands through intensification of crop-livestock systems, using farmer participatory approaches to improve and deliver forage and feed technologies, and (ii) delivery mechanisms for the dissemination of these technologies.
The LLSP follows the Forages for Smallholders Project (FSP), which developed forage technologies with smallholder farmers and disseminated these to other farmers in target districts in partner countries in Southeast Asia. The activities of the new project are broader as it works with farmers to maximize the benefit from having planted forages through the development of improved livestock production systems (with emphasis on feeding), analysis of production and marketing constraints and opportunities, and the efficient dissemination of new technologies to new areas and farmers. In each partner country, the project collaborates with a national research and/or development agency (Table 76).

Table 76. National coordinating agencies collaborating with the LLSP

Country	National Coordinator	Agency
Cambodia	Dr. Sorn Sam	National Animal Health and Production Investigation Centre, Department of Animal Health and Production, Phnom Penh.
PR China	Prof. Yi Kexian	Chinese Academy of Tropical Agricultural Science (CATAS), Danzhou, Hainan.
Indonesia	Mr. Yakob Pangedongan (National coordinator) Mr. Djodi Suparto (Liaison officer)	Livestock Services of East Kalimantan, Samarinda, East Kalimantan Directorate General of Livestock Services, Ministry of Agriculture, Jakarta.
Lao PDR	Mr. Bounthavone Kounnavongsa	National Agriculture and Forestry Research Institute (NAFRI), Vientiane.
Philippines	Mr. Eduedo Magboo	Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD), Los Baños, Laguna.
Thailand	Dr. Chaisang Phaikeaw	Department of Livestock Development, Ministry of Agriculture and Cooperatives, Bangkok.
Viet Nam	Mr. Le Hoa Binh	National Institute of Animal Husbandry (NIAH), Ministry of Agriculture and Rural Development (MARD), Hanoi.

Within countries, one or more provinces and districts are involved in the project with site coordinators (provincial or district) and several extension workers involved at project sites. A summary of sites and collaborating staff is provided in Table 77.

Table 77. LLSP project sites and partners

Country	Main project sites	Main collaborators	Agencies
Cambodia	Kampong Cham province	Mr. Chea Socheat	Provincial Agriculture and Forestry Office, Kampong Cham
Indonesia	East Kalimantan province	Mr. Yacob Pangedongan and Mr. Ibrahim	Provincial Livestock Services, East Kalimantan and District Agricultural and Livestock Extension offices
Lao PDR	Savannakhet province	Mr. Bounmy Phewvankham	Provincial Livestock Office, Savannakhet
P.R. China	Hainan province	Mr. Yi Kexian and Mr. Tang Jun	Chinese Academy of Tropical Agricultural Sciences
Philippines	Bukidnon and Misamis Oriental provinces	Dr. P. Asis, Ms. N. Jacutin, Ms. G. Cania, Ms. J. Saguinhon	City Veterinary Office Cagayan de Oro and Municipal Agricultural Offices in Bukidnon
Thailand	Nakornratchasim	Ms. Ganda Nakamanee and Dr. Chaisang Phaikaew	Animal Nutrition Research Center Pakchong, DLD
Viet Nam	Daklak and Tuyen Quang provinces	Mr. Nguyen Van Ha, Dr. Truong Tan Kanh and Ms. Vu Hai Yen	Provincial Agricultural Office and Tay Nguyen University, Daklak and District and Provincial Department of Agriculture and Rural Development Tuyen Quang

4.2.2.1 Integrated feeding systems for livestock that optimize the use of improved and indigenous fodders and crop residues, and farm labor

The previous FSP project successfully introduced forage accessions to Southeast Asia (>500 accessions), conducted nursery and regional evaluations to select a set of approximately 50 broadly adapted, robust forage varieties, and evaluated these with smallholder farmers in a broad range of farming systems using a farmer participatory approach. The combination of well-adapted forage varieties and a participatory approach was successful in introducing forages to farmers in systems where no forages had previously been planted, and farmers relied purely on naturally occurring feed resources. As farmers started to integrate forages into their farming systems (e.g. contour hedges, intensive plots near houses, etc.) and use them in a variety of ways (e.g. night feeding for cattle, grasses for fish), the project and its partners started to disseminate forages to other farmers and new areas, using tools such as cross visits, development of multiplication systems (seed and vegetative), and training of extension workers and key farmers. Many of these farmers are expanding their forage areas and receive benefits, such as reduced labor needs for feeding livestock, improved income from sale of animals and manure, improved soil fertility through application of manure, and improved soil conservation by planting of contour hedgerows and cover crops.

However, few farmers are intensifying or expanding animal production and many achieve only a moderate level of animal productivity. Some farmers are feeding insufficient quantities of feed, most farmers are feeding forages that are too old (poor quality) and almost all farmers are feeding insufficient amounts of protein (e.g. protein supplements of forage legumes) to achieve high levels of animal production. At sites with extremely low soil fertility, such as in East Kalimantan, planted and native forages are lacking in minerals required for animal production, leading to poor animal production and susceptibility to diseases. Planting forages has changed the way animals are raised; with many benefits and advantages for farmers. There has been a big increase in the confinement of animals in pens or tethering near houses as farmers now have a feed resource located conveniently near the house enabling them to provide at least supplementary feed to their animals. They do not have to spend time bringing animals to far-away fields for grazing when they are busy with other tasks, and they can collect manure easily, which can be sold or used on their own crops. Also, parasites are less of a problem and animals have less contact with other animals thus limiting the spread of diseases. On the other hand, animals can no longer select the feed they eat but depend on the farmer for selecting the diet and as animals were previously allowed to graze freely farmers have limited knowledge of feed requirements. These issues require a lot of farmer (and extension worker) learning as well as the introduction of new technologies.

The project conducted a review of the most important livestock systems at each site and selected one or two of these systems for intervention by the project. These include cattle fattening, cow-calf production, dairy cattle, cattle for draught and saving, rabbit production, goat production, dairy buffalo, fish production, fresh forage for sale and forage seed for sale. At each site, collaborators held consultations with the communities and formed farmer groups interested to work with the project. Together, they analyzed the production system and identified constraints and opportunities for improvement. They then designed farmer experimentation to evaluate options or designed farmer field school-type training to improve the selected livestock production systems. These are currently being carried out, and results of farmer experimentation and their impact on production improvement will be reported in the next Annual Report.

The main objective of farmer experimentation is farmer (and technician) learning and generating farmers' interest in improving feeding systems; not the collection of accurate data as these is known and predictable. The main strategic output is the process and methods of working with farmers and extension services to intensifying and improving livestock production systems. The project is attempting to combine farmer experimentation with ways of disseminating the generated information by included key farmers and extension workers from other areas with similar livestock production systems in the design, monitoring and analysis of farmer experiments as well as any training associated with experimentation. At one site in Daklak, Viet Nam, site partners also conducted field days and produced videos and television programs of the farmer experimentation.

4.2.2.2 Improved methods to develop forage feed systems and extend them to new farmers, optimizing the use of M&E for feedback to others in the community

An analysis of the process of participatory technology development and dissemination methods and tools used by LLSP partners commenced with three workshops in Viet Nam and is continuing in other countries. Methods clearly vary between sites and countries, with factors such as capacity and enthusiasm of the extension worker, institutional support (and 'culture') for participatory approaches, distance to 'successful forage-feed system examples' and the need for capacity building emerging as important determinants of success. Three scenarios are emerging:

- Dissemination within villages or districts: The simplest form of dissemination is by assimilation; where farmers learn from other farmers nearby who are already at an advanced level of developing forage and feed technologies. This is most successful when farmers are already experiencing significant positive impacts of improve livestock feeding systems, and where there are enthusiastic, well-trained extension workers in the area who actively facilitate dissemination through field days, cross visits and farmer-to-farmer learning.

- Dissemination to new villages or districts in the same geographical region (e.g. same province): This requires an additional process of 1) identifying new areas with high potential, 2) winning institutional and political support for working in the new villages or district, 3) training of extension workers in the new area in feed and livestock technologies and in participatory approaches, and 4) establishing forage multiplication sites in the new area to ensure access to planting materials. This is relatively simple if successful farmer examples of improved feed and livestock technologies are available in nearby districts, and cross visits, field days and trainings can be arranged in the successful areas. Once a small number of farmers have started to evaluate improvement options and are experiencing benefits then similar methods and processes as described in (1) can be applied. Other options to create awareness of successful technologies are the use of radio, television and printed media.

- Dissemination to new villages or districts in a different geographical region: Added challenges are that there are no easily accessible examples of successful feed and livestock technologies nor trained extension workers or farmers nearby, and involvement at the national level is likely to be required for selection of new areas and winning of institutional support. In this situation, many of the most successful methods and tools such as cross visits and farmer-to-farmer extension are not immediate available and new examples (and capacity) need to be developed before dissemination can be successful.

The three workshops held in Viet Nam documented and reviewed forage technology development and dissemination at LLSP (and previous FSP) sites in Daklak and Tuyen Quang provinces. The first workshop with key partners from LLSP sites in Daklak and Tuyen Quang was held in Nha Trang from 6-9 January 2004. During the workshop, the basic methodology employed at the two sites was described and discussed.

This first workshop was followed with site visits by John Connell, Francisco Gabunada and Le Hoa Binh and with more comprehensive discussions and workshops with a wider range of extension workers in Tuyen Quang from 8-11 June and Daklak from 14-16 June 2004.

Results showed that adoption and spread of forage technologies was faster in areas with more intensive, market-oriented agriculture. In these areas, farmers consider cattle / livestock production as an income-generating enterprise (not as a means of accumulating capital) and farmers are more willing to invest in inputs for livestock production. The types of forages adopted in these areas were high quality, productive varieties requiring external inputs (organic/inorganic fertilizers, management).

In both provinces forage and livestock technology has reached a point where the farmers themselves visit the extension workers to ask for technical assistance. Another aspect for the rapid spread of forage technologies in intensive areas was the relatively close distance between farms and households. This proximity aided a rapid spread of successful technologies and information from farmer to farmer without major inputs by extension workers. Often, extension workers could advise information or technology seeking farmers simply to go and visit other farmers or a village where they have achieved a lot of progress already without the extension workers. This encouraged farmers to seek informal contact and they often bought planting material from the farmers they visited thus providing an additional incentive for experienced farmers to share their experiences and advice.

The spread of forage technologies was slower in more extensive agricultural systems and areas in both Daklak and Tuyen Quang provinces. Often, farmers in these areas kept livestock in extensive grazing systems, accepting seasonal variation of communal feed availability as inevitable. They tend to adjust to this situation by manipulating cattle numbers or simply accepting the fact that their animals become thin during the dry season when there is little feed available. Moving towards a more intensive type of livestock production with at least some stall-feeding requires a significant change in attitude and production system.

Also, farms and houses are located further from each other, thereby slowing the flow of informal and direct exchange of information and technologies between farmers. Extension worker need to invest a lot of time and effort into organizing farmers and providing information and advice.

In both provinces, it was observed that there was little opportunity for feedback from farmers to the extension services. What tends to happen is that the knowledge and experience of extension workers and the farmers who first worked with the extension workers were used as basis for generation and promotion of technologies. As forage technologies became successful, a lot of effort went into expanding these technologies to new districts and more farmers.

The methods used were a simplified form of participatory diagnosis, selection of interested farmers, organization of cross visits by some key farmers, provision of planting material and some basic training for extension workers and farmers. There was little follow up after the initial provision of planting material as the same procedure was followed in other districts.

The experiences of farmers planting forages and utilizing these for improving animal production was not harnessed nor did these farmers receive a lot of support to assist them with maximizing the benefits from having intensified livestock production. This limited the progress of innovation and improvement of farmers' livestock production.

Based on the experiences with documenting dissemination methodologies in Viet Nam, the Project will conduct similar workshops at selected LLSP in other countries and compare these to the results from Viet Nam.

4.2.2.3 Increased capacity in DMCs, at different levels, to expand the use of improved forage and feed systems, and respond to local needs

The LLSP builds capacity of project partners in many different ways. These include:

- Annual project review and planning meetings of national coordinators and selected site collaborators to review progress, share experiences, discuss project strategies and develop work plans for each site.
- National and/or provincial review and planning workshops where project staff and country coordinators facilitate sharing of experiences, review activities, plan future activities and provide relevant training at project sites to enable our collaborators to carry out the next phase in their work plan.

- National and local training courses for site collaborators (extension workers and key farmers) on particular aspects such as forage agronomy and utilization, participatory approaches, animal nutrition and health by national and site coordinators and supported by project staff.
- International training courses for national coordinators and selected site collaborators on subjects important to the project such as agroenterprise development. Participants to these courses are expected to develop and organize training courses in their countries.
- Mentoring of all project partners by project staff (and experienced partners) through site visits, cross visits to other sites and short-term attachments to experienced staff.

Building the capacity of project partners to be able to work with farmers to improve forage-feed systems and disseminate these to other farmers requires skills and knowledge that are difficult to learn in formal training courses. We found that the most effective way of building the capacity of site partners is to keep the formal part of training course short (review experiences, discuss options for improvement, present additional options/knowledge/skills), then go into the field and demonstrate new skills, ask participants to practice with other farmer groups, get back together and review experiences and discuss difficulties and ways of overcoming these difficulties. Participants then return to their own sites and apply their new skills. A follow-up training is then planned which reviews progress and takes site partners to the next level. This type of programmed, on-the-job training can be fully integrated with review and planning workshops and are clearly targeted at the needs of our local partners and the needs of the project.

A list of training courses by project staff is presented in Table 78. Additionally, site collaborators carry out a large number of training courses for extension workers and farmers. For example, project partners in PR China conducted two training course for 50 farmers and 20 extension workers at CATAS. In Viet Nam, site partners in Daklak and Tuyen Quang held training courses for technicians, extension workers and key farmers, who in turn held training courses for a large number of farmers. All site partners as part of their regular farmer groups meetings and extension activities carried out training of farmers in forage establishment, management and utilization.

The project also supports training in Agricultural English for key project partners. Four national / site collaborators attended a 6-week course at the International English Language Training Center in Vientiane, Lao PDR, from 13 October to 21 November 2003. Participants were Mr. Tang Jun (Site coordinator, CATAS, P.R. China), Mrs. Vu Hai Yen (Site coordinator, Tuyen Quang, Viet Nam), Mr. Yacob Pangendongan (National coordinator, Livestock Services of East Kalimantan, Indonesia) and Mr. Bounthavone Kounnavongsa (National coordinator, NAFRI, Lao PDR). All participants benefited greatly from attending this language course and the course has already resulted in improved communication with LLSP staff and with other project partners. Part of the training was a 1-week visit to FLSP sites in northern Lao PDR to learn and discuss technology development and the process of working with farmers in this project with FLSP collaborators.

4.2.2.4 Comparison of development opportunities, and market and logistic constraints, for intensification of smallholder livestock systems across sites in five countries

Following the participation of five LLSP members in the Southeast Asian Course on “Sustainable agro-enterprise development in a micro-regional context” in Viet Nam in early 2003, the first part of a market study was conducted in Daklak, Viet Nam, from 9-18 December 2004. The study was aimed at providing a better understanding of the livestock production to market chain at project sites in Daklak. The study commenced with a series of

Table 78. Training courses, workshops and cross visits organized by the LLSP.

Date	Title	Staff involved as resource person	Place	No. participants	
				Male	Female
2-6 Jun 03	Participatory livestock research and development training course (organized by PCARRD)	F. Gabunada	Small Ruminant Center, Central Luzon State University, Philippines	9	8
23-27 Jun 03	LLSP planning workshop and write-shop	F. Gabunada	Cagayan de Oro City, Philippines	18	11
1-3 Sep 03	Training course on forage selection and establishment	P. Phengsavanh	Animal Health and Production, Kampong Cham, Cambodia	10	3
4-5 Aug 03	Planning workshop with LLSP collaborators	W. Stür, F. Gabunada	Penajam, Indonesia	15	3
18-23 Aug 03	Cross visit of FLSP partners in Tuyen Quang	P. Phengsavanh, F. Gabunada	Tuyen Quang, Viet Nam	15	2
23-26 Sep 03	Training course on animal nutrition and experimentation with small farmers	F. Gabunada	BPLB, Sempaja, Indonesia	13	1
11-14 Nov 03	Training course on participatory diagnosis and evaluation	J. Samson, P. Phengsavanh	Kampong Cham, Cambodia	13	1
22-29 Nov 03	Cross visit of Tang Jun, Vu Hai Yen and Yacob Pangedongan to FLSP sites	P. Phengsavanh	Luang Phabang, Lao PDR	2	1
11-14 Dec 03	Livestock market study workshop	J. Samson, P. Phengsavanh	Ea Kar & M'Drak District, Viet Nam	38	25
15-17 Dec 03	Annual Review and planning workshop of LLSP Philippines	F. Gabunada	Cagayan de Oro City, Philippines	8	8
6-12 Oct 03	Training course on forage seed production for LLSP collaborators from Viet Nam	C. Phaikaew, G. Nakamanee	Mukdahan Animal Nutrition Station, Thailand	6	4
7-8 Jan 04	Dissemination methodology workshop	F. Gabunada, W. Stür, J. Connell, P. Phengsavanh, J. Samson	Tuyen Quang and Daklak Province, Viet Nam	9	3
21 – 27 Jan 04	Training course on developing forage technologies with small farmers	F. Gabunada	BPLP in Sempaja, Samarinda, Indonesia	12	3
15-19 Mar 04	Training course on production system analysis and planning workshop for field staff	F. Gabunada	East Kalimantan, Indonesia	8	2
6-10 Apr	Training course on	F. Gabunada,	CATAS, PR. China	20	2

meetings with the key stakeholders involved in livestock production and marketing in Daklak. These included (1) authorities such as agricultural planners, credit providers, extension services and provincial and local government representatives, (2) livestock farmers from project sites, and (3) traders. Each group was met separately to keep participant numbers for each meeting to a manageable size, avoid potential conflicts between stakeholders and allow focused discussion. The meetings were held over 3 days with each meeting lasting half a day with wrap-up sessions and summaries following each meeting. The meetings were facilitated in an informal way with open-ended and probing questions, and the use of a range of PRA tools. Farmer and trader groups identified a range of constraints to production and marketing with considerable differences in perception between the two groups. For example, farmers felt that trader were paying low prices for their animals while traders explained that farmers often try to sell old, thin and sick animals which have a low value. They are willing to pay high prices for good-quality animals and reasonable prices for thin animals as long as they look like they can be fattened before marketing or sold on to other farmers. Farmers tend to have few options on how to sell their cattle since there are no local markets and transport for small number of cattle to the provincial markets is too expensive. Local traders buy individual cattle from farmers for some time and only transport them once they have a large number of animals assembled. Farmers have limited knowledge about current market prices and the trader bases the sale price on weight estimates.

In June 2004, the second phase of the market study was conducted in Ea Kar district, Daklak. The LLSP team conducted three separate feedback meetings – one for each stakeholder group (farmers, traders and local government) – to present the results (problem identification) of the first phase of the market study which was conducted in December 2003. During the meetings, the problems/issues identified by each stakeholder group during the first phase of the market study was presented and each issue was opened for discussion and brainstorming of options for addressing the identified issues. The meetings were very positive and participation was active and constructive. Farmers were keen to immediately start evaluating production improvement options, traders were offering to train farmers in judging quality and weight of animals, and entering in partnerships with farmers to ensure a steady supply of good-quality animals, and government agreeing to support activities with credit and investigating the possibility of establishing livestock marketing opportunities. Table 79 shows the issues identified in phase 1 and possible solutions and actions identified during the feedback meetings.

During the meetings, the idea of forming a stakeholder committee was raised to coordinate and take forward the ideas and proposed actions generated during the meetings. This was accepted and each stakeholder group elected representatives to this stakeholder committee (SC). Membership of the SC comprises 4 farmers, 1 trader, 1 bank representative, 1 extension officer (who will also represent the local government) and a representative of the LLSP. The formation of the SC was supported by the chairman and vice chairman of Ea Kar district; they expect the SC to develop policy recommendations, which enhance livestock development in the district. The first Stakeholder Committee meeting was held on 26 June 2004 with representation from the LLSP. The Head of the Extension Office was elected to coordinate activities of the SC. The SC discussed its role, objectives and official status, and decided to apply for registration of the group with the People's Committee to ensure that the SC is well integrated into the development process of the district. The date of the next meeting was set for 10 July 2004. The formation of the SC is critical to ensure the continuation of activities started during the LLSP, but requiring a longer-term commitment. Also it ensures that the outputs generated are clearly contributing to the development strategy of the local government. The lessons learnt from the Daklak market study experience will be documented in a comprehensive report and plans are being prepared to conduct similar production to consumption studies at some other LLSP sites.

Table 79. Result of feedback meetings with farmers, traders and government in Ea Kar, Daklak

Problems	Solutions	Opportunities	Actions
Traders' issues:			
• Farmer always ask for very high price • Farmers don't exactly know about the price. Prices are always changing • Lack of capital • Lack of place where to buy & to sell • Low supply of cattle (farmers always want to keep their cattle for reproduction to increase number) • Farmers lack knowledge/technology to raise good quality cattle • Access to capital Traders can borrow from the local bank, but the loan is not enough for them to buy substantial number of cattle to gain good profit. They also find it difficult to borrow from the bank due to the many processes & requirements.	• If farmers have the capital & the capability/knowledge to raise good quality animals, then possibly they can keep the best breeder to produce more calves. • Traders think that the authorities should support them by developing good policies / projects where both farmers & traders can buy & sell (trading place) good quality animals • The authorities should provide easier access to capital to help the farmers & the traders in buying animals • Improve knowledge of farmers on cattle production and management so that traders can buy more improved type of animals and achieve a more steady supply to meet the demand of the market	• Demand for cattle is higher than the supply because • lack of capital for both traders & farmers • lack of technology to produce quality animals • farmers prefer keeping the thin animals for their farm use • Traders are willing enough to discuss possible solutions with the different players. • There are some companies & traders who are willing to lend capital to farmers, so that they can benefit together.	• Generate information on prices • Improve market information through extension officers by training farmers on how to measure the weight of the cattle • EW train farmers how to recognize the breed / quality / type of cattle • EW bring together farmers & traders to discuss & understand each other about the activities involved in buying/selling of cattle • Develop the skills on cattle production (raising) in the village
Farmers' issues:			
• Price of cattle for breeding is high, farmers can't afford to buy enough • Farmers don't know how to measure the weight of cattle • Farmers would like to know the price trends (when is the price at its highest & lowest). • Lack of feed for cattle • Farmers don't know how to buy good quality cattle • Farmers find it difficult to look for good quality cattle for breeding • Lack of skills to plan the activities on raising cattle • Farmers find it difficult to forecast the price	• Establish a market place for cattle • Provide studies to bring information on the market (in general) • Organize a group of people who are interested to raise cattle • Train farmers how to measure the cattle, how to get the weight of the beef • Help the farmer to sell the cattle by using a scale as basis of weight		• Train the farmer about the technology (e.g. animal health, nutrition, production / breeding, forage technology) • Train and guide the farmers to develop plans on how to raise better cattle, suitable quantity of animal, amount of feed needed, types of feed, animal health & animal housing, etc. • Train farmers to make plans on how to compute for economic benefits, right timing to raise & sell cattle in order to maximize benefit • Formation of farmer interest groups on cattle production. So they can help each other to exchange information on technology techniques, market and get capital (credit) easily from banks • Attend seminar on how to loan money from the bank and how to use the money to get benefits

4.2.3 Seed Production of new Brachiaria hybrids in Southeast Asia

Contributors: Chaisang Phaikaew, Ganda Nakamanee, Michael Hare (DLD) and Semillas Papalotla

Thailand

Several species of the grass genus *Brachiaria* have high potential as a source of feed for livestock production in the tropics. All of these species, however, have significant limitations. For example, one of the most common varieties, *Brachiaria decumbens* “Basilisk”, grows well in the dry season, but produces very little seed in most areas of Southeast Asia. *Brachiaria ruziziensis* “Ruzi” produces high yields of good quality feed in the wet season, but is poorly adapted to the long dry season and soon dies out. In the mid 1980s, CIAT scientists started a breeding program to try to combine the best characteristics of different *Bracharia* species into new hybrids. The first of these was released in 2001 in a public-private partnership between CIAT and the international seed company, Papalotla. This *Brachiaria* hybrid, known as Mulato, combines the best qualities of its parents, *Brachiaria ruziziensis* and *Brachiaria brizantha*, into one plant. That is, it has both excellent dry season tolerance and produces higher quality feed than most *Brachiaria* varieties. Most significantly, whilst it is a hybrid, a peculiarity of the reproductive biology of the *Brachiaria* genus (‘apomixis’) means that the seed collected from this hybrid remains true to the parent. Thus, it is a hybrid that does not lock smallholder farmers into regularly buying seed from large companies, as is the case with most hybrid crops, such as hybrid corn.

Mulato is ideally suited to moderately fertile to fertile soils, in intensive livestock systems or in crop/pasture rotations. It does not grow well in very infertile soils or waterlogged areas. Recent research in Colombia has shown that cows grazing Mulato can produce an extra 1.0 to 2.0 liters of milk per day compared with cows grazing other grass varieties. In Honduras, steers grazing Mulato gained 900 g/day compared with 600 g/day on *B. decumbens* cv. Basilisk. The *Bracharia* Mulato hybrid was first introduced by CIAT to Southeast Asia en 1996 as part of a large *Brachiaria* variety trial in Thailand. The Thai Department of Livestock Development (DLD) identified the *Brachiaria* hybrid cv. Mulato as the most promising variety for livestock production in the seasonally wet-dry climates and poor soils of the northeast. They commenced seed production trials on-station in 2000 and, because of the promising results, commenced on-farm trials in 2003 with 7 smallholder farmers near Khon Kaen. On the strength of the results, Papalotla provided a guaranteed market in 2004 that allowed 1793 farmers to plant 700 hectares for seed production (Photo 38). An estimated 140 tons of seed will be produced primarily for export to Latin America, India and other Asian countries. Farmers producing Mulato seed will earn 25% more income than producing seed of Ruzi grass. One limiting characteristic of Brachiaria hybrid cv Mulato is its low seed yields (<180 kg/ hectare). A new Brachiaria hybrid (Mulato 2), which is agronomically very similar to Mulato except that it produces double the seed yields, has been developed by CIAT. Ubon Ratchatani University and Papalotla are working with 105 farmers in 2004 to produce an estimated 10 tons of seed of Mulato 2.

Photo 38. Thai farmer harvesting Mulato seed

Lao PDR

The potential for production of seed from the hybrid Brachiaria hybrid cv Mulato in the uplands of Lao PDR, both as a cash crop and as an alternative to shifting cultivation for smallholder farmers, is high. The biophysical conditions are nearly ideal (and similar conditions occur in relatively few other areas of the world), there is a strong market and the seed crop requires relatively simple management methods that are ideally suited to smallholder farming. Madeleen Husselman, an MSc. Student from Wageningen Agricultural University, is evaluating this potential with small plot and on-farm trials on the Bolovens Plateau in southern Lao.

4.3 Partnerships in Africa to undertake evaluation and diffusion of new forage alternatives

Highlights

- CIAT, ILRI and ICRAF reviewed ongoing forage research activities in East and Southern Africa and outputs that could be achieved in short term were identified. Principles for a long-term forage research strategy for the region were defined.

- CIAT, ILRI and EARO jointly evaluated forage germplasm with farmers in highly degraded highland of Ethiopia. Farmers preferred Napier, lablab and vetch. Farmers produced significant amount of planting materials and seeds with their own resources, to expand the forage areas in the next planting season.

4.3.1 Development of strategic alliances

Contributor: R. Roothaert (CIAT-PRGA/ILRI)

The development and scaling out of forage technologies are a common objective for three CGIAR centres which operate in East and Southern Africa: CIAT, ILRI and ICRAF. CIAT has bred and selected improved forage germplasm for the low altitude, humid, and sub-humid agro-ecological zones. CIAT has also demonstrated success with participatory approaches and large numbers of farmers adopting forages in LAC America and SE Asia. ILRI forage germplasm is well suited for the arid, semi-arid, and highland agro-ecological zones. ICRAF has demonstrated success with woody forage legumes in the highlands of East, Central and Southern Africa.

Delegates form the three centres met in Nairobi, December 2003, to discuss a common strategy for research on forage technologies for East and Southern Africa. The areas for collaboration were grouped into (1) important activities, which have been identified by the centres in projects and proposals already, and (2) long term strategies.

The following activities and outputs are expected to be implemented in the short term:

- Development of improved forage germplasm on-station and participatory work to deploy forages in collaboration with partners.
  - CIAT – ILRI collaborative work has started with NARS and NGOs in Ethiopia, Malawi and Uganda, in 2003.

- Development of seed supply systems as an important prerequisite for the scaling process.
  - In Malawi, the Department of Agricultural Research Services (DARS) established one hectare of forage seed multiplication plots at the Chitedze Agricultural Research Station, in 2003-2004. More than 100 grass, herbaceous and woody legume species and accessions obtained from ILRI, CIAT, ICRAF, and CSIRO have been planted.

  - In Uganda, plans were made for establishing central seed multiplication systems through the National Agricultural Research Organisation (NARO) in collaboration with Makarere University, in 2004. The NARO stations selected for multiplication are Mukono (intensive, sub-humid systems), Mbarara (grazing systems) and Serere (semi-arid systems). In Mbarara, 20 species and accessions from CIAT and ILRI were established in 2004.
  - Vegetative propagation of selected grasses and herbaceous legumes has started by two farmer groups in Tororo, Uganda in 2004.

- Development of forages for monogastric animals and fish as an important research issue.
  - A farmer group in Ukwe, Malawi, planned the evaluation of maize, molasses, and legume forage for pig raising, in 2004.

- Linking farmers to markets.
  - Careful choice of locations and production systems is necessary and clear definition of strategic research issues that need to be addressed to make proposals attractive to donors.
  - Market chain analysis has been conducted with communities in Uganda and Malawi for livestock enterprises, starting in 2003.

- GIS supported targeting of forage germplasm.

- Training in forage agronomy, seed systems, participatory methods and market development.
  - A training on participatory research methods was held in Ethiopia, in 2003.

- Monitoring and Evaluation of Forage / Livestock projects, with emphasis on improving livelihoods.
  - ME systems have been developed with partners and communities in Uganda.
  - An ME workshop was conducted in 2004 in collaboration with ILRI for the DFID fodder innovation project in Nigeria. Participants learned about participatory methods for process monitoring, developed partnerships, and made regional ME plans.

The following points were related to a long term forage research strategy for East and Southern Africa:

- Working through existing networks should be utilized wherever possible, instead of creating new ones.
  - A regional interest group on participatory research methods for feed and forage systems have been established with IARs, NARS and NGOs in Ethiopia, Kenya and Uganda. Funding is being sought through the Association for Strengthening Agricultural Research in East and Central Africa (ASEARECA). The group intends to work in close association with the ASARECA Animal Agriculture Network (AAARNET).

- Involvement in Challenge Programs, such as African Sub-Saharan Challenge Program and Water and Food Challenge program.

- Define the role of forages in systems and how does forage research relate to livestock research.
  - To revise characterization done in the region, e.g. systems related to forages and feeds, leading to define demand; relative importance of forages in contrast to feeds and other aspects such as animal health.

- Define the comparative advantage of ILRI, ICRAF, CIAT to work jointly on some of the issues coming out of characterization, in contrast to other research and development stakeholders.

- Process and technology research need to go hand in hand.

- Opportunities for scaling out and a poverty focus should be defined before implementation of larger initiatives.

4.3.2 Partnerships and adaptive forage research in Ethiopia

Contributor: R. Roothaert (CIAT-PRGA/ILRI)

Rationale

In 2003, a pilot project was started with ad hoc resources from ILRI, CIAT and the Ethiopia Agricultural Research Organisation (EARO), to work with communities in the degraded watershed of Mt. Yerer. The purpose was to introduce improved NRM technologies towards better and sustainable livelihoods. Forage technologies were chosen as an option towards alleviating the chronic feed shortage for draught and dairy animals, and for the potential of reversing land degradation. Severe gully erosion and nutrient depleted soils were major problems for food production and infrastructure. Although many forage options for the Ethiopian highlands had been developed on-station in the past, very little research had been carried out with farmers. The objectives of the study were to develop integrated forage technologies with farmers, and to evaluate improved forages through farmers' perceptions.

Materials and Methods

Meetings were held with committee members of two Peasant Associations (PA), Yerer Selassie and Gende Gorba, representing 600 and 1200 households, respectively. Discussions were held about PRA conducted a year before, and the potential to address some of the described problems through forage technologies. A field visit and workshop was organised for each PA for interested farmers to view forage plots at the nearby ILRI station, Debre Zeit, and to plan participatory trials on-farm. The PA committees selected 57 farmers, of whom 10 women farmers, to participate in the experiment. The farmers came from 6 villages. The altitude varied from 1900 – 2100 masl. Soils were heavy vertisols at the lower altitudes, and sandy loam at higher altitudes. Average annual rainfall is 815 mm, mostly falling within June – September.

During the planning workshops with farmers, they suggested a range of niches for the forages to be planted: in the backyard within the compound, in the fenced area adjacent to the compound, along contours in the field, and in the gullies. For this season, however, everyone wanted to plant either in the compound or in the adjacent fenced areas, because they valued the experiments too much and did not want them to be disturbed by stray livestock. They also preferred a controlled environment for seed and planting material production, so that they could plant larger areas in the unprotected fields the following season. The materials were planted between 20 and 30 June 2003. The forage species and amounts made available to farmers are listed in Table 80.

The performance of the forages was evaluated in terms of (1) germination and survival, (2) establishment in the early growth phase, (3) forage yield or biomass, and (4) general farmer preference. Technicians visited each farm between 30 Sept. and 20 Oct. 2003, when the rains had stopped, to facilitate farmers' evaluations.

Table 80. Forage species and planting materials distributed to farmers in six villages of Mt. Yerer watershed, 2003.

Species and accession	Number distributed per farmer
Napier grass (*Pennisetum Purpureum*) acc. 14984	200 stem cuttings
Setaria (*Setaria sphacelata*) acc.142	25 root splits
Vetiver grass (*Vetiveria zizanioides*)	25 root splits
Vetch (*Vicia dasycarpa*) 6213	100 grams
Lablab (*Lablab purpureus*) 6529	100 grams
Neonotonia (*Neonotonia wightii*) 6762	100 grams
Macroptyloma axillare, 6756	100 grams
Pigeon pea (*Cajanus cajan*) 11560	100 grams
Sesbania (*Sesbania sesban*) 15019	100 grams
Leucaena pallida (14203)[1]	5 seedlings
Leucaena diversifolia (14193)[2]	5 seedlings
Calliandra (*Calliandra calothyrsus*) 15143	5 seedlings
Tagasaste (*Chamaecytisus palmensis*) 15378[3]	5 seedlings

[1] Four villages only: Buti, Korke, Babugaya, G/Gorba
[2] Two villages only: Mekanna, Godetti
[3] In Korke village (high altitude) only.

A matrix drawn on a manila sheet of paper was used to evaluate the planted species against the four criteria. Some follow up visits were made during the season. Final feedback and planning meetings with the farmers in the two PA were held on 15 and 17 Dec. 2003.

Results

The species that scored best for germination, survival, establishment, and early biomass production were lablab, vetch, and napier (Table 81). Pigeon pea and setaria also received a mean score of above 7 for germination, survival, and establishment. The tree and shrub species took longer time to establish, as could be expected. Napier, lablab and vetch ranked highest for overall preference (Figure 51).

Pigeon pea, setaria and vetiver followed after that. Although only 19 % of the original contact farmers were women, 34 % of the respondents were women, indicating a gradual shift of involvement in the experiment from men to women. Disaggregating the responses by sex did not show any differences in terms of rating for germination, survival, establishment, and early biomass production. The six most preferred species were also the same for men and women. Women showed higher preference for tagasaste, and lower preference for calliandra and *L. pallida* than the men did.

Farm visits and meetings provided additional qualitative information about preferences of forages. Farmers had started to feed small quantities of feed to their animals and assessed palatability. Napier, vetch and lablab were the most palatable species. Setaria, vetiver and pigeon pea were also fed, and ranked slightly lower in palatability. Farmers also mentioned the importance of being able to produce seeds or planting materials. Almost every farmer had produced vegetative planting materials of napier and setaria for expansion in the next season. Out of the 34 farmers who attended the feedback meetings, 14 farmers had multiplied vetiver; 13, 9 and 8 farmers respectively had collected seeds of pigeon pea, vetch and lablab by December 2003. Additional criteria were mentioned: pigeon pea was often appreciated for its dual purpose, food and feed; napier was appreciated for its good feed value.

In terms of planning for the next season, many suggested that they would expand vetch and lablab to the cropping areas, as these places would be protected during the growing season. After that these areas could be grazed. They would also be able to produce enough seeds of these crops to continue planting in the following seasons. In addition, they requested seeds of oats from ILRI, so that they could experiment with intercropping vetch and oats. They had heard about this technology from other farmers. Farmer visits in January 2004 revealed that lablab was regrowing after harvest, well into the dry season. It was also continuing to produce significant amount of seeds.

Discussion

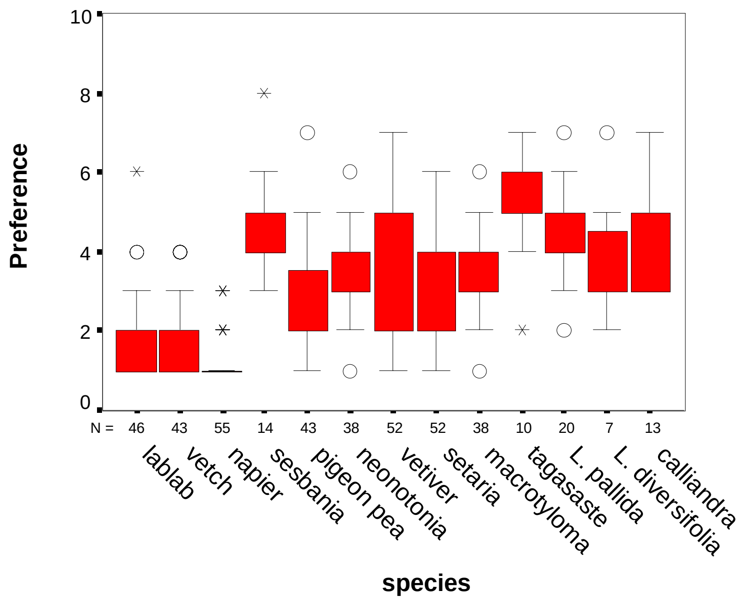
One season of participatory evaluation showed that six out of thirteen species were clearly performing better and more preferred by farmers. These were napier, lablab, vetch, pigeon pea, setaria and vetiver. The shrubby species didn’t rank high, but farmers also mentioned that it was too early to conclude anything about those species, because seedlings were very small and they take long to establish.

There is a keen interest to expand forages on-farm. Grass splits will already be sufficiently available form the farms, and they could be established along contours in the cropping land. Farmers want to plant herbaceous legumes in the cropping land, but would probably still need to be helped initially with some seeds.

Farmers appreciated the ILRI-CIAT facilitation of the on-farm research. They wanted some more information about intercropping technologies. Perhaps the most rewarding outcome of the research was the following comment expressed during a group meeting: “At the start both parties were fearing each other. We were a bit suspicious; we did not believe that

Table 81. Mean scores of germination, establishment and yield of forage species rated by 57 farmers in Mt. Yerer watershed, 2003. 10 = highest, 1 = lowest.

Species		Germination/ Survival	Establishment	Yield/ Biomass
lablab	Mean	8.65	8.61	8.20
	S.d.	2.1	2.2	2.4
vetch	Mean	8.30	8.58	8.30
	S.d.	2.3	2.1	2.2
napier	Mean	8.87	8.87	8.44
	S.d.	1.9	2.1	2.3
sesbania	Mean	6.43	5.79	4.21
	S.d.	2.5	2.2	1.7
pigeon pea	Mean	7.51	7.56	6.00
	S.d.	2.1	2.6	2.4
neonotonia	Mean	5.89	5.87	4.76
	S.d.	2.6	2.5	2.1
vetiver	Mean	7.35	5.94	4.96
	S.d.	2.2	2.3	2.3
setaria	Mean	7.88	7.19	5.94
	S.d.	2.4	2.5	2.4
macrotyloma	Mean	5.89	5.87	4.76
	S.d.	2.6	2.5	2.1
tagasaste	Mean	6.90	4.80	3.60
	S.d.	3.0	2.4	1.2
L. pallida	Mean	5.15	3.70	2.75
	S.d.	3.7	2.4	1.7
L. diversifolia	Mean	5.71	5.71	5.29
	S.d.	2.4	2.0	1.5
calliandra	Mean	5.23	4.38	3.85
	S.d.	3.3	2.1	2.1
Total	Mean	7.37	6.98	6.08
	S.d.	2.7	2.7	2.8



Key:

Red box: Contains 50 % of observed responses (farmers)

Line in red box: Median
O: Outlier
*** : Extreme value**
N: number of responses (farmers)

Figure 51. Distribution of farmers' general preference ranking of forage species tested on their farms, Mt. Yerer, 2003. 1 = highest preference, 10 = lowest preference.

ILRI [and CIAT] would actually help us, or that ILRI [and CIAT] would follow up on the initial activities. ILRI [and CIAT] scientists were also suspicious about the farmers; they did not believe that farmers would have enough land to plant forages. Now that we have worked together for a whole season, those suspicions have gone. We are all appreciating the forage experiment and we will be able to work much better together in future."

4.4 Forage Seeds: Multiplication and delivery of experimental and basic forage seeds

Highlights

- More than 1 t of seed was produced by the Seed Unit at CIAT. Seed distributions totaled 222 kg.
- A total of 1084 kg of experimental and basic seed was either produced in the Atenas Seed Unit or procured from associated collaborators. The bulk of the seed distributed was formed by *Cratylia argentea* (195.0 kg) and *Brachiaria* hybrid cv. Mulato (740.5 kg)

4.4.1 Multiplication and delivery of forage seeds in the Seed Unit of Palmira

Contributors: A. Betancourt; J. Muñoz and J.W. Miles (CIAT)

The delivery mechanism for our technology (in the form of improved germplasm) is seed. For many of the materials we are developing, no commercial seed supply exists. While we seek to encourage private initiative in supplying seed, we recognize that in the early stages of development a need for seed for experimental purposes and initial distribution can only be met by internally generated supplies. The Project maintains a modest seed multiplication and processing capacity to meet this demand.

Seed multiplication field plots are established and maintained at headquarters (CIAT-Palmira) and at substations at CIAT-Popayán and CIAT-Quilichao. Final seed processing and all aspects of seed distribution are handled at CIAT headquarters, where routine seed quality determinations are also conducted.

More than 1 t of seed was produced by the small seed multiplication unit at CIAT (Table 82). Nearly half of the total (545.7 kg, total for 12 accessions) was seed of *Cratylia argentea*. Significant quantities of *Lablab purpureus* (189.25 kg, 50 accessions) and *Canavalia brasiliensis* (132.0, 1 accession) were also produced. Smaller quantities of seed of 16 species (30 accessions) completed the total.

Seed distribution was only about one-fifth of the total produced (222.34 kg: Table 83). A total of 153 samples was distributed to 10 different countries and a diversity of categories of users including public agricultural research institutions, NGOs, public universities, private producers' organizations, and individuals.

These samples were sent to ten (10) countries: Bolivia (3); Costa Rica (1); Germany (14); Honduras (2); Nicaragua (2); Philippines (1); Uganda (7); Venezuela (1); Virgin Islands (1); and Colombia. Within Colombia, seed was distributed to collaborators in: CIAT (23); NGO (1); Universities (1); Private individuals (68); Agroamazonia (1); Agrogenética Global (1); Umata (15); CENIPALMA (3), Futuro Verde (1); COOLECHERA (1); DELAGRO (1); Fundamaz (1); Corpoica (4).

Table 82. Seed multiplication at the CIAT-Quilichao, CIAT-Popayán, and CIAT-Palmira experimental stations. (September 2003 to September 2004), totals by species.

Genus *	Species	Number of Accessions**	Harvest (Kilograms)
Brachiaria	*brizantha*	10	89.000
Brachiaria	*lachnantha*	1	3.500
Brachiaria	cv. Mulato	1	7.000
Brachiaria	sp.	3	27.500
Calliandra	*calothyrsus*	4	8.700
Canavalia	*brasiliensis*	1	132.000
Centrosema	*macrocarpum*	1	3.500
Centrosema	*molle*	1	10.000
Cratylia	*argentea*	12	545.700
Desmodium	*heterocarpon*	1	30.000
Flemingia	*macrophylla*	4	36.800
Lablab	*purpureus*	50	189.246
Leucaena	*leucocephala*	1	34.000
Mucuna	*pruriens*	1	11.000
Pueraria	*phaseoloides*	1	1.000
Stylosanthes	*guianensis*	1	0.400
Total		**93**	**1,129.346**

*16 Genera
** 93 distinct genetic materials (accessions)

Table 83. Seed dispatched from CIAT forage seed multiplication unit (September 2003 to September 2004).

Genus	Kilograms	Number of samples
Brachiaria	0.678	8
Cajanus	0.024	1
Calliandra	0.114	3
Canavalia	0.5	1
Centrosema	1.774	3
Cratylia	57.100	30
Desmodium	101.299	22
Flemingia	0.124	6
Lablab	39.066	23
Leucaena	1.620	6
Mucuna	0.050	1
Pueraria	0.024	1
Stylosanthes	0.468	2
Vignas sp.	19.5	46
Total	**222.341**	153

4.4.2 Multiplication and delivery of selected grasses and legumes in the Seed Unit of Atenas

Contributors: Pedro Argel and Guillermo Perez (CIAT)

Rationale

Seed multiplication activities of promising forage germplasm continued during 2004 at the Atenas Seed Unit (Costa Rica) in collaboration with the Escuela Centroamericana de Ganadería (ECAG). The seed produced is destined to support advanced evaluations and promotions of forage germplasm both by CIAT's projects and regional research/development institutions.

From September 2003 through August 2004 a total of 1084.1 kg of experimental and basic seed was either produced at Atenas or procured from associated collaborators. The bulk of the seed was formed by *Cratylia argentea* (195.0 kg), *Brachiaria* spp. (9.5 kg), *Brachiaria* hybrid cv. Mulato (740.5 kg), *Arachis pintoi* (12.5 kg), *Leucaena* spp. (10.0 kg), *Centrosema* spp. (5.7 kg), *Panicum maximum* (8.9 kg) and *Paspalum* spp.(3.40 kg). Small quantities of experimental seed was also produced of *Canavalia brasiliensis,Vigna* spp., *Chamaechrista rotundifolia* sp. *grandiflora* and other forage species.

During the period September 2003-August 2004 a total of 379.7 kg of experimental and basic seed was delivered by the Seed Unit of Atenas (Costa Rica).

In Table 84 we show that 49 seed requests were received from 9 countries, where most of the requests came from Costa Rica, the host country

Table 84. Countries, number of requests and amount of experimental/basic forage seed delivered by the Unit of Atenas (Costa Rica) during the period September 2003-August 2003.

Country	No. of Requests	Forage species (kg)			
		Brachiaria spp.	*Arachis pintoi*	*Cratylia argentea*	Other species
Brasil	1	0.5		0.2	
Colombia	2	0.1			1.1
Costa Rica	32	24.1	4.7	20.6	29.2
Guatemala	2		105.0	18.0	
Nicaragua	7	6.7	0.3	44.3	9.0
Panamá	2	6.0		0.5	0.4
Perú	1	1.0			
Puerto Rico	1		7.5		
Venezuela	1	0.5			
Total	49	38.9	117.5	183.6	39.7

of the forage project. However, a significant amount of experimental seed was delivered to Guatemala (123.0 kg) and to Nicaragua (51.3 kg), both countries involved in forage projects with the participation of CIAT.

A high amount of basic and experimental seed of the promising shrub *Cratylia argentea* (183.6 kg) was delivered, and of *Brachiaria* species, particularly of cv. Mulato, the new hybrid of this genus that is being promoted regionally with the assistance of the private sector.

4.5 Enhancing livestock productivity in Central America

Highlights

- Approximately 30% of pastures in Honduras are in a severe state of degradation, loosing 284,106 MT of fluid milk and 48,271 MT of beef (live weight) annually, equivalent to 48% of the annual production of milk and to 37% of beef. In economic terms, these losses in milk and beef yields are worth US$63 and US$48 million annually, respectively

- A large demand for high quality hay for feeding during the dry season in Honduras and Nicaragua was identified. Production of hay silage in plastic bags in the wet season for use in the dry is an alternative that will be examined by the Forage Project in the future.

4.5.1 Estimation of the trade-offs of livestock productivity and income with pastures under different stages of degradation in Honduras

Contributors: F. Holmann (CIAT/ILRI), P. J. Argel, L. Rivas, D. White (CIAT), R. D. Estrada (CIP/CIAT), C. Burgos (DICTA), E. Perez (ILRI), G. Ramirez, and A. Medina (CIAT)

Rationale

Objectives of this study were to: (a) estimate milk and beef yields obtained from cows grazing pastures in different stages of degradation; (b) estimate income losses as a result of the degradation process; (c) estimate the proportion of pasture areas found in each stage of degradation within the six administrative regions of Honduras; and (d) identify different strategies and costs to recuperate degraded pastures.

Materials and Methods

Data came from two surveys executed during a workshop carried out in March 2004. The subjective perceptions of 25 livestock producers and 8 extension agents of the 6 administrative regions of Honduras were obtained to estimate the losses of animal productivity within the farm, region, and country. A 4-level scoring of pasture degradation was defined – where 1 was for the best condition (i.e., non-apparent degradation) and 4 was for the worst (i.e., severe degradation). Regressions, explaining the animal productivity losses at each level of pasture degradation, were generated according to the subjective and descriptive information (Figures 52, 53).

Results and Discussion

Comparing the perception of the degraded areas, producers considered that in Honduras the extent of pasture degradation is lower compared with extension agents. According to producers, 29% of the pasture area in the country is at Level 1 (i.e., no degradation) compared with only 19% of extension agents. Moreover, producers perceived a lower proportion of pastures with a level of severe degradation (i.e., Level 4, 27%) in comparison with almost 31% perceived by extension agents. In the intermediate degradation levels (i.e., Levels 2 and 3), both groups were similar. The country is sacrificing milk and beef production due to the process of pasture degradation. According to estimations from producers, Honduras is loosing 284,106 MT of fluid milk and 48,271 MT of beef (live weight) annually in the pasture areas found in Level 4 (i.e., severe degradation), equivalent to 48% of the annual production of milk and to 37% of beef. In economic terms, these losses in milk and beef yields are worth US$63 and US$48 million annually, respectively. The perception of extension agents is even more alarming. Honduras could produce 66% more milk and 50% more beef annually if livestock producers renovated their pastures before they reached level 4, equivalent to US$94 million in less revenue from milk sales and US$66 million from less beef sales. Both groups perceive that pastures, in an early stage of degradation (i.e., Level 2), are more economical, practical and rapidly to recuperate. Also, as the process of degradation advances (i.e., to Levels 3 and 4), both cost and time of recuperating such pastures increases significantly. According to producers, the recuperation of a pasture from Level 4 to Level 1 costs $140/ha and takes almost half year (i.e., 5.6 months). Extension agents estimate this cost of recuperation 27% higher ($178/ha) with 5% more time (i.e., 5.9 months). At the national level, to recuperate all pasture areas found in Level 4 would cost $57 million according to producers and $84 million according to extension agents. However, this amount represents, in the opinion of producers, 51% of the $111 million, and according to extension agents, 52% of the $160 million in milk and beef sales not received annually due to lower animal production from cows grazing Level 4 pastures. Producers perceive that grasses spend proportionately less time in going from Level 1 to 2 (i.e., 2.9 years) and as the process of degradation continues, pastures remain longer at advanced degraded levels (i.e., 3.1 years in going from level 2 to 3, and around 4.0 years in going from level 3 to 4). Moreover, producers think that the average productive life of improved grasses is about 10 years, while extension agents think that grasses degrade faster, with an average productive life of 8.4 years, 16% less than producers. According to producers and extension agents, pastures degrade at an annual rate of 10% and 12%, respectively. With these rates, Honduras would maintain its current level of degradation between levels 2.48 and 2.65. However, the renovation of pastures at an annual rate of 10-12% does not solve the problem, but maintains it.

Producers argued that the current financial situation does not allow the necessary cash flow to renovate their plots, and the option of credit is not viable since real interest rates are high (i.e., 10%). After simulating this scenario, it was demonstrated that farmers are able to generate the additional income necessary to pay a credit, but only if this credit is taken with interest rates similar to those found in the international market (i.e., 3%). In order to eliminate the degraded areas found in Level 4 at the country level, it is necessary a one-time investment of $57 million. The benefit obtained from this investment would result in a daily increase of 156,000 liters of milk and 26,500 kilograms of beef, equivalent to $22 millions/yr. Therefore, there are significant economic and productive incentives for the private and public sectors to develop and execute a plan of action to recuperate pasturelands in advanced stages of degradation.

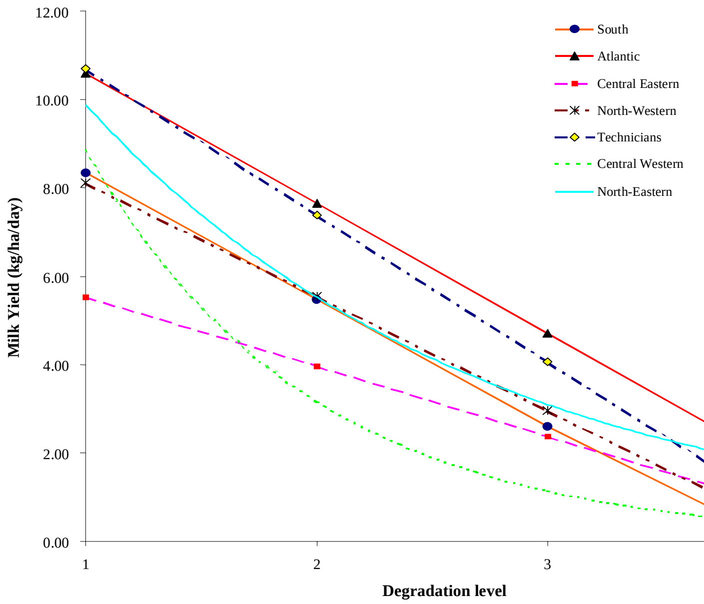


Figure 52. Perceived milk yield by level of degradation.

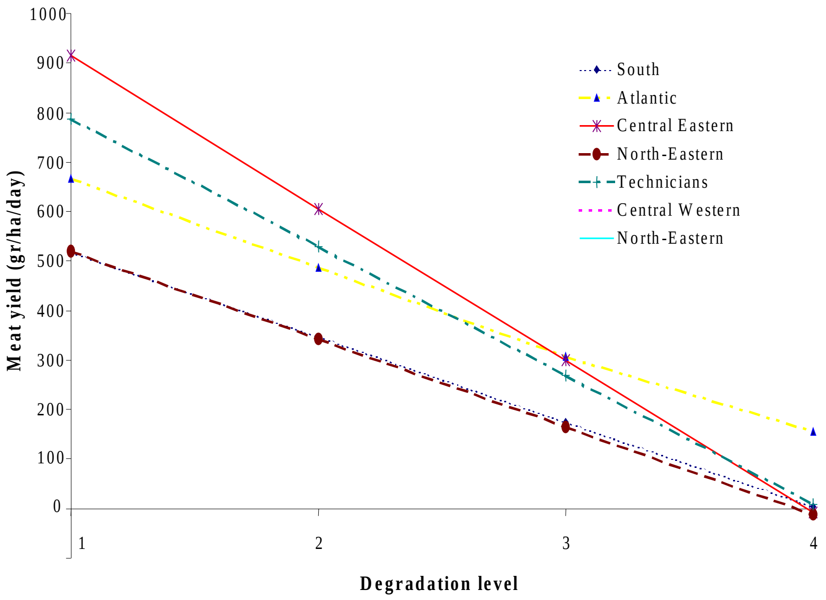


Figure 53. Perceived beef yield by level of degradation

4.5.2 Demand for forage technologies for dry season feeding in Nicaragua and Honduras

Contributors: S. Fujisaka (CIAT), F. Holmann (CIAT/ILRI), M. Peters, A. Schmidt (CIAT), C. Burgos (DICTA), M. Mena (INTA), M. I. Posas (SERTEDESO), H. Cruz (CIAT), C. Davis (INTA), and B. Hincapié (CIAT)

Rationale

Smallholders with dual- purpose (milk and meat) cattle in much of Honduras and Nicaragua are faced with a long (4-8 month) dry season in which livestock feed is scarce and/or expensive. As a result, they produce substantially less milk in the dry as compared to the wet season. Livestock feed alternatives in the dry season range from native pasture and crop-residue grazing to silage and cut-and-carry forages to purchase inputs, with the higher costs of the more productive alternatives serving as a disincentive to higher dry season output. CIAT, ILRI, and partners in Honduras and Nicaragua are working with producers to develop effective, affordable, and adoptable alternatives for dry season livestock feeding systems. Diagnostic surveys were conducted as an initial step in order to understand animal feeding systems within a whole farm context. Among other objectives, the surveys were designed to estimate the demand for forage-based products such as ("little bag") hay and silage and improved forage germplasm.

Material and Methods

The diagnostic surveys were conducted in May 2004 in areas of Honduras (Juticalpa) and Nicaragua (Ocotal-Somoto-Esteli) featuring long dry seasons. Teams in each country consisted of 10-12 persons from NARS (DICTA, INTA) an NGO (SERTEDESO), ILRI and CIAT. Interviews were conducted by team members working in pairs. The surveys consisted of interviews of 65 livestock owners in Honduras and 53 in Nicaragua using informal structured open-ended interviews. No written/printed interview form was employed. Interviewers were asked to conduct thoughtful, interactive conversations open to new topics and issues rather than a follow-the-recipe form-filling exercise.

The team used this diagnostic survey as a training exercise to replicate it in different regions of both countries during the months of June and July. In August, ILRI and CIAT scientists will travel to the region to discuss, synthesize and consolidate the information from the other regions and a final draft of the study is expected to be ready by October 2004. The following preliminary results are from the initial survey:

Results and Discussion

Proportions of the different sizes of livestock operations encountered in the two countries were essentially the same: 15% small (1-12 head of cattle), 55% medium (13-70), and 17% large (>70) in Honduras and 17% small, 58% medium, and 25% large in Nicaragua. The largest herd sizes were encountered in Honduras where large holders had a mean of 178 head compared to 148 in Nicaragua (a difference possibly due to sampling error). Total farm size was somewhat larger in Nicaragua where small holders (recall that relative classes are based on numbers of cattle and not land holding) had 18, medium had 48, and large had 226 ha. Honduran small operations had 11, medium had 38, and large had 85.8 ha.

Almost all farms cropped maize and beans, with largest areas farmed by medium operators in both countries: small operators dedicated roughly 3, medium about 5, and large roughly 4 ha to annual crop cultivation (Table 85). Some farmers reported limiting basic grain production to meeting household consumption needs as a response to low prices paid for maize and beans.

Livestock feeding systems consisted of different combinations of alternatives from a reliance on natural pastures for low input-output systems to reliance on such sources as silage of forage maize and sorghum, cut-and-carry forages, hay, and concentrates for higher input-output systems (Table 85). Each of these components is considered in order from least to most intensive in terms of cash and labor requirements. Each is also considered in terms of possible future related research (synthesized in Table 85).

Based on producers' estimates, wet season milk production in Honduras and Nicaragua is probably quite similar across scales of operation. Large-scale producers in Honduras had the highest dry season outputs, however, reflecting inclusion of more favorable areas surveyed and intensification in those areas based on use of remittances, with intensification in the form of greater use of concentrates, cut-and-carry, purchased hay supplements, and silage of forage maize and sorghum. A contrasting situation was encountered in Nicaragua where small holders (compared to medium and large) obtained the highest production per lactating female: among small holders were those who maintained very few milk cows, often of improved breeds (Holstein) and provided them with intensive care. It appears that, overall dry season milk production will remain significantly lower than wet season production—in spite of high economic incentives in the dry season—because of the relatively high cost of increasing dry season production, including the opportunity costs of land, labor, and capital associated with increasing dry season outputs. However, research may be able to change the equation by offering lower-cost alternatives for the currently used dry season animal feed forms of improved pasture, hay, forage trees, concentrates, cut-and-carry forages, and silage of forage maize and sorghum.

Table 85. Farm characteristics, milk production, and animal feeding by farm size in Honduras and Nicaragua.

	Honduras			Nicaragua		
	Small (n = 10)	Medium (n = 36)	Large (n = 19)	Small (n = 9)	Medium (n = 31)	Large (n = 13)
Farm characteristics						
Cattle (head)	9	43	178	7	29	148
Farm size (ha)	11	38	86	18	48	226
Cropped (ha)	2.8	4.7	3.8	3.1	5.3	4.0
(% of farm)	26	12	4	18	11	2
Head/ha	0.5	0.8	0.9	0.2	0.5	0.5
Milk production						
% No dry season milk	20	3	0	33.0	10	0
Number cows milked	4.3	12	41	1.6	10	40
Milk dry season (liters)	3.2	4.2	5.4	5.1	2.1	3.3
Milk wet season (liters)	-	-	-	6.8	5.1	6.7
Dry/wet milk ratio	-	-	-	75	41	49
Animal feeding						
Crop residues (%)	60	40	10	66	84	77
Rice straw (%)	-	-	-	44	42	46
Rent land (%)	40	20	10	22	26	42
Improved pasture (ha)	1.0	5.3	57	1.1	4.4	89
(% of farm)	9	14	66	6	9	86
Forage trees (%)	Low	Low	low	56	71	77
Concentrate (%)	30	30	60	11	48	69
Hay (%)	10	30	50	20	10	55
Cut-carry (%)	20	30	70	11	35	62
Irrigated land (%)	10	10	30	0	24	42
Forage maize/sorghum	-	-	-	78	68	92
Silo (%)	0	10	40	0	10	42

Collaboration with the various development projects and NGO efforts will also be appropriate: their efforts are dealing with, among others, potable water (wells and hand pumps), subsidized motorized pump use for limited irrigation, household sanitation (outhouses), biogas for cooking, introduction of new forage materials, house construction, and reforestation and aforestation.

4.6 Impact of forage research in LAC

Highlights

- The area planted with *Brachiaria* cultivars during 1990 to 2003 amounts to 6.5% of the total area of permanent pastures in Mexico, 12.5% in Honduras, 1% in Nicaragua, 18.7% in Costa Rica, and 0.1% in Panamá. Species of *Brachiaria* dominate the forage seed market. During the last 5 years, 84% of all grass seed sales in Mexico and Honduras, 90% in Nicaragua, 85% in Costa Rica, and 97% in Panama have been of *Brachiaria* species (i.e. *B. decumbens* and *B. brizantha* cv. Marandú).

- The net present value (NPV) of technological benefits from the adoption of *Brachiaria* hybrids resistant to spittlebug was estimated at US$4166 million, of which 54% would be generated by additional beef production and the rest by milk. Most of the benefits were concentrated in Mexico, US$2831 (68%); followed by Colombia, US$960 million (23%), and Central America, US$363 million (9%). The NPV is equivalent to 44% of the value of beef and milk produced in 2003, ranging between 16% in Honduras and 78% in Nicaragua.

4.6.1 Impact of the adoption of grasses from the *Brachiaria* genus in Mexico and Central America

Contributors: F. Holmann (CIAT/ILRI), L. Rivas, P. J. Argel (CIAT), and E. Perez (ILRI)

Rationale

In Latin America and the Caribbean (LAC) there has been an important effort to develop new pastures technologies to increase livestock productivity for the extensive systems prevailing in the tropical lowlands. This multi-national and inter-institutional effort was initiated through the International Network for the Evaluation of Tropical Pastures (RIEPT, by its name in Spanish), which operated from 1976 to 1996 under CIAT leadership. RIEPT became a platform for institutions to train technicians, share forage material from existing gene banks, study the behavior of new germplasm under different environments, and established the exchange of scientific information to extrapolate research results (Toledo, 1982). RIEPT trained 645 agronomists from 24 countries in LAC in subjects related to forage agronomy and pasture evaluation.

The training was key for the success of RIEPT because these professionals carried out evaluations of new and improved forages under contrasting ecosystems and provided feedback. In addition, during this period participating institutions in RIEPT released 11 selected grasses as commercial cultivars, most of them from the *Brachiaria* genus, as well as 16 forage legume cultivars (CIAT, 2003). In Central America and Mexico these cultivars were released between 1990 and 1996. Forage evaluation activities in this region continues at present through a joint research agenda between CIAT and ILRI through special projects as well as between CIAT and the private seed sector. Of all pasture cultivars released, grasses from the *Brachiaria* genus currently dominate the market. Namely, about 84% of all grass seed sales in Mexico and Honduras, 90% in Nicaragua, 85% in Costa Rica, and 97% in Panama during the last 5 years (Holmann et al., 2004). The objective of this study was to estimate the adoption of *Brachiaria* grasses released through RIEPT on the basis of seed sales during the period 1990-2003 to assess its impact in terms of animal productivity and income of adopters.

Material and Methods

The methodology to estimate the adoption of *Brachiaria* grasses on the increase in animal productivity and income was from Sáez and Andrade (1990), which estimated the planted areas based on the volumes of seed marketed in each country. To calculate the marginal production of milk and beef due to the adoption of *Brachiaria*, the planted area was multiplied by the difference in productivity between the traditional and the improved technology (Holmann et al., 2004). In addition, the marginal value of milk and beef due to this adoption was obtained by multiplying the marginal production of milk and beef by the producer prices received during the period 1990 to 2003.

Results and Discussion

As observed in Figure 54, during the first years (i.e., first half of the 90's) the adoption was low because grasses were recently released and as a result, there was little knowledge and information among producers on productivity responses with these new options. However, as the planted areas with Brachiaria cultivars expanded and became more familiar to producers, seed sales grew rapidly up to being exponential at the beginning of the millennium, behaving similarly to the theoretical pattern of adoption.

Areas planted with Brachiaria grasses. It is assumed that most of this seed was allocated to renovate pastures in advanced stages of degradation or naturalized pastures with low productivity. The largest volumes of seed sales and planted areas correspond to Mexico (9,100 mt of seed with 2,616,130 ha planted). Costa Rica is the country in Central America with the largest seed sales and planted areas (1,692 mt of seed and 437,516 ha planted), followed by Honduras (671 mt and 186,788 ha), Nicaragua (134 mt and 35,822 ha) and Panamá (40 mt and 10,952 ha). During this period the annual increase rate in seed sales was respectively 32% in Mexico, 62% in Honduras, 45% in Nicaragua,

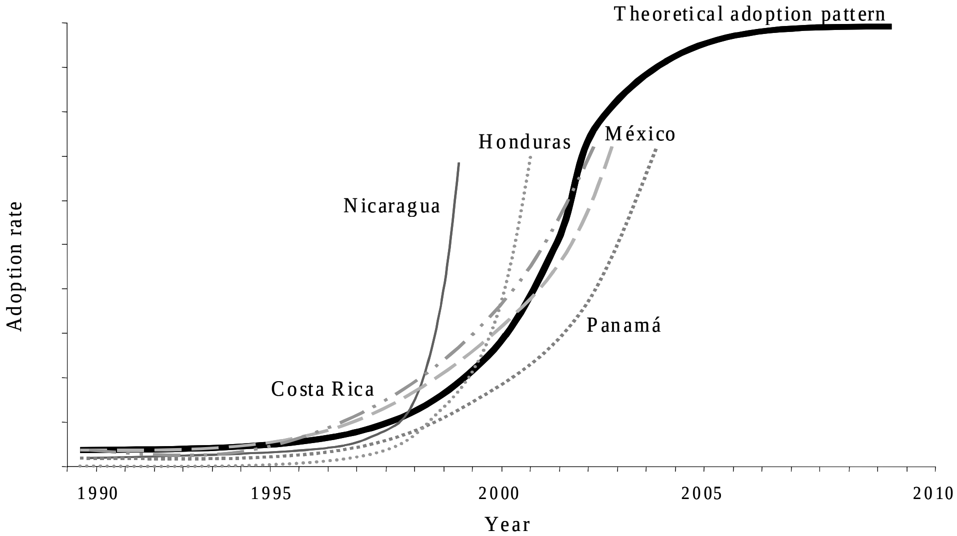


Figure 54. Theoretical adoption pattern of new forage technologies and adoption curves in México and Central America based on *Brachiaria* seed sales (Holmann et al., 2004).

39% in Costa Rica, and 54% in Panamá. Total area planted with Brachiaria cultivars during this period amounts to 6.5% of the total area of permanent pastures in Mexico, 12.5% in Honduras, 1% in Nicaragua, 18.7% in Costa Rica, and 0.1% in Panamá.

Additional milk and beef production. The main beneficiary from the adoption of Brachiaria cultivars has been Costa Rica, since more than 55% of the national milk production and almost 18% of the beef produced in 2003 was due to the marginal increase in the productivity of Brachiaria pastures compared to the traditional technology from degraded or naturalized grasses. These benefits are followed by Mexico where the increase in productivity from the adoption of Brachiaria cultivars amounts to 24% of the national milk production and 5% of the production of beef. In Honduras the marginal increase in milk and beef production is equivalent to 25% and 12% of national production, respectively. In Nicaragua and Panamá the adoption of Brachiaria grasses has been the lowest in the region. As a result, the additional increase in milk in Nicaragua and Panama amount to 11% and 5% of the national production, respectively. In the case of beef production, the additional increase in Nicaragua and Honduras has been 2% and 1% of domestic production, respectively. These figures suggest that adopters of Brachiaria grasses are producers oriented toward dairy and to a lesser proportion, to beef. Given the production systems existing in this region, it can be argued that the main adopters of these grasses have small and medium been dual-purpose livestock farmers.

Conclusions

The underlying hypothesis addressed by RIEPT was that lack of adaptation of commercial cultivars selected in other continents could be overcome by the selection of locally adapted forage germplasm. This in turn required a large effort on multi-locational screening of germplasm for adaptation to prevailing biotic and abiotic constraints. Likewise, the participation of the private sector, through commercial seed enterprises, was key to ensure wide adoption of improved forages. The results presented in this section indicate that the investments of public funds in Central America and Mexico to support a forage evaluation R&D network paid of in terms of adoption of improved grasses and increased supply of beef milk, staple food commodities for consumers across income levels in the region. The process of adoption of new Brachiaria cultivars has been stimulated by the availability at reasonable prices of commercial seed produced elsewhere and sold regionally by local seed companies. The region dos not have comparative advantages for Brachiaria seed production, particularly in terms of soil and climatic conditions and the availability of proper technology. However, grass seed production is a large commercial activity in countries such as Brazil, and much of the Brachiaria cultivars released locally, are readily taken by Brazilian companies as new seed products to be commercialized in response to an increasing forage seed demand along the region.

4.6.2 Potential Economic Impact from the adoption of new *Brachiaria* grasses resistant to spittlebugs in livestock systems of Colombia, Mexico and Central America

Contributors: L. Rivas (CIAT) and F. Holmann (CIAT/ILRI)

Rationale

Cattle raising in Tropical Latin America is one of the main productive activities in the region. Its economic importance is based on the use of significant quantity of available lands from all agro-ecosystems, from the contribution to the food supply, and because of its importance for employment and income generation, especially among small and medium farms dedicated to dual-purpose systems (i.e., beef and milk production).

One of the main constraints faced by producers is the limited forage supply regarding to quantity and quality, which is more critical during the dry season. *Brachiaria* grasses are a partial solution to this problem, because of the broad scope of adaptation, the tolerance to acid and infertile soils, and the high level of productivity, compared to other alternative forage materials. These African grasses were disseminated in the continent during the 1960s and 70s, particularly *B. decumbens*, the most utilized species: it is estimated that nearly 40 million hectares are currently planted with this variety.

Pasture research led by CIAT and many national institutions during the 80s and 90s, contributed with new *Brachiaria* species with various characteristics and uses, that were incorporated with incomparable success, in livestock systems of the Latin American lowlands. *B. brizantha*, *B. dictyoneura*, *B. humidicola* and *B. ruziziensis* are some of the forage materials released by research institutions in the region. Despite its indisputable advantages, the *Brachiaria* genus presents limitations because of its low tolerance to intense and prolonged droughts and its high susceptibility to spittlebug, a pest that causes considerable economic losses to the cattle-raising industry. Thus, most recent research in the *Brachiaria* breeding program has focused on the development of a second generation of *Brachiaria* grasses: outstanding agronomic characteristics, establishment vigor, good sprout capacity, high yield, high nutritional quality, good seed production, resistant to *Rhizotocnia* and to multiple spittlebug species. The results of this effort have conveyed to the recent release of Mulatto grass, the first hybrid of the *Brachiaria* genus obtained by CIAT's genetic improvement program.

In the waiting list of the second generation of *Brachiaria grasses* is the hybrid #4624 (CIAT 36087), to be released in 2005, having a similar forage quality as Mulato and with all the attributes defined for the second generation of *Brachiaria* grasses. Moreover, several other hybrids are in advanced stages of evaluation and close to being released as commercial cultivars.

Materials and Methods

The potential economic impact of the adoption of new *Brachiaria* grasses on the livestock systems was evaluated using the Economic Model MODEXC. Two regions were considered in Colombia: the Northern Coast and the Eastern Plains. In Mexico, the tropical region; and in Central America, its six constituent countries. The model estimates the economic benefits attributable to the utilization of the new materials, disaggregating per country, region, ecosystem, system of production and large social groups (both consumers and producers). It works with two types of parameters: the technical ones that characterize the new technology and its process of dissemination, and the economic ones representing the conditions of supply and demand in the markets of products (beef and milk) affected by the technical change.

The technical parameters are based on previous research projects from the countries considered in this study, including the opinion of experts in the subject. The economic ones were set based on various studies about the beef and milk marketing in the region.

The benefits of the new technology (from the year 2007) were calculated for a period of 20 years and the results were expressed in terms of the net present value (NPV) and annuities (A). The estimates were made using alternatively an economic framework of open and closed economy.

Results and Discussion

In a closed economy, without international trade, the NPV of the technological benefits was estimated at US$4166 million, of which 54% would be generated by marketing beef and the rest by milk. Most of the benefits were concentrated in Mexico, US$2831 (68%); followed by Colombia, US$960 million (23%), and

Central America, US$363 million (9%). In order to have criteria on the extent of the estimated technological benefits, the value of the beef and milk yield during 2003 was calculated in the reference countries. The NPV is equivalent to 44% of the value of that year, ranging between 16% in Honduras and 78% in Nicaragua. The results show the great importance of the dual-purpose livestock production system. In most countries, more than half of the technological benefits were generated in this system: Colombia 70%, Central America 62%, and Mexico 50%.

When a country is self-sufficient and the surplus resulting from the technical improvements is marketed domestically, the benefits are transferred to the consumers, who are favored with the reduction in prices, making possible for them to increase the consumption. In the current case of a closed economy, consumers would capture 83% of total benefits. Trade liberalization implies a re-distributive process favoring producers. Export purchases increase total demand and restrain the fall of domestic prices. In an open-market economy, the share of benefits to producers would rise to 46%.

Research investment is conceived as a primary mechanism to achieve two of the most basic social goals: 1) poverty reduction and the improvement of equity, and 2) the promotion of economic growth. Having this premise, in order to establish to what extent this technical change contributes to the fulfillment of these goals, the acquired benefits were estimated for the most vulnerable population groups: a) The two quintiles of poorer population, representing 40% of total population, and b) the smaller producers. In both schemes, open or closed economy, both groups receive more than one-fourth of the benefits of the technical change, 27% and 31%, respectively. This is equivalent to a NPV ranging between US$1137 - 1303 millions.

Despite the definition of the levels of critical variables, especially those associated with the productivity and the adoption of the new *Brachiaria* grasses, conservative criteria were considered in order to avoid overestimating the benefits; it is important to evaluate the sensitivity of these, against undesirable changes of those variables. For this purpose, three alternative scenarios were established: 1) The reduction of 50% of the area cultivated with new *Brachiarias*, 2) the reduction of 10% in the yields of the new materials, and 3) the increase of 50% in the total time of adoption.

It is concluded that the most critical variable in the determination of the amount of benefits is yield (productivity) of the new technology, in terms of beef and milk per hectare. The elasticity of the benefits regarding the yields was estimated at 2.2 for Colombia and 1.8 for Central America and Mexico. This suggests that if the yield declines by 1%, the reduction of the social benefits is more than proportional.

The social benefits are less elastic with regard to the area planted with new *Brachiaria* grasses or the time of adoption. For example, in Colombia, if the area with improved materials declines in one percentage point, the benefits will diminish at approximately six tenths of one point. In all the proposed alternative scenarios, the investment in the development of these new pastures turns economically attractive, despite the adverse circumstances proposed in those scenarios.

The technological benefits expressed as an annuity (a fixed payment received for a specific number of years) shows that the investment for the development of new forage options is very low, less than US$ 20 million, compared with the annual benefits resulting from the use of these new materials.

4.7 Expert systems for targeting forages and extension materials for promoting adoption of forages

Highlights

- Information on adaptation, uses and management of over 200 forage species was gathered from the literature, research reports and memories of experienced forage agronomist and was incorporated in the SoFT database and selection tool.

- The spatial decision support system coined CaNaSTA (Crop niche selection for tropical forages) was compared with three existing forage knowledge bases and tested with forage experts. Results indicated several strengths of CaNaSTA: (a) facilitates for data input, (b) allows spatial variability to be made explicit and (c) the score and ranking system allows more suitable forage species for a given niche to be considered first.

- It was demonstrated that using GIS, sites could be found where multiple forage species with distinctive distribution might be brought together to coexist as part of a strategy for in situ conservation of forage species.

4.7.1 Selection of Forages for the Tropics (SoFT) – a database and selection tool for identifying forages adapted to local conditions in the tropics and subtropics

Contributors: B.C. Pengelly (CSIRO), B.G. Cook (QDPI), I. J. Partridge (QDPI), D.A. Eagles (CSIRO), M. Peters (CIAT), J. Hanson (ILRI), S. D. Brown (CSIRO), J. L. Donnelly (CSIRO), B. F. Mullen (CSIRO) , R. Schultze-Kraft (University of Hohenheim), A. Franco (CIAT) and R. O'Brien (Curtin University of Technology)

Rationale

Rising populations and incomes in developing countries are likely to double demand for livestock products by 2020 (Delgado et al. 1999). This strong demand has potential to improve profitability for farmers but will require improved animal feeding in both semi-intensive crop-livestock and more extensive livestock systems. Forages are commonly the most cost-effective option to supply feed demands, particularly for ruminant livestock, but also for pig and poultry production. Selecting the most suitable forages for the local system and conditions is critical. Smallholder and even larger-scale farmers depend heavily on advice from extension and development agencies, and from seed companies, but this advice is often limited by inexperience and the difficulty in accessing reliable information. Expert information on an extensive range of tropical forages is now readily available through the SoFT database.

Database development and structure

Forage research over the last 50 years has identified many useful tropical grasses and legumes. Information on their adaptation and use has resided in peer-reviewed literature, research reports with limited distribution and, often most importantly, in the memories of forage agronomists with decades of experience.

The SoFT database has accessed these information sources to define the adaptation and use of over 200 forages, and has integrated this knowledge into a user-friendly database.

The database has four main features: (i) information in fact sheets on the adaptation, uses and management of forage species, cultivars and elite accessions; (ii) a selection tool built on LUCIDä that enables easy identification of best-bets based on 19 criteria (Table 86); (iii) a bibliography of more than 6,000 references and

Table 86. Selection criteria available in the SoFT database for selecting the most suitable forages for environments and uses.

Climate/farming system attributes	Soil environment attributes	Plant attributes
Latitude x altitude	Soil pH	Plant family (legume or grass)
Rainfall (average annual)	Level of available soil Al/Mn	Life cycle
Length of dry season	Level of soil salinity	Growth form
Inundation	Soil drainage	Stem habit
Intended forage use	Soil texture	Cool season growth
Grazing pressure	Soil fertility	Frost tolerance (foliage damage)
Shade environment		

abstracts on forage diversity, management and use; (iv) a collection of photographs and images of species to help in their identification and use. The database selection tool is an expert system based on the experiences of more than 50 forage specialists who have worked for many years in tropical and subtropical regions of Africa, Tropical USA, Central and South America, South and South-east Asia and Australia.

Conclusions

This year members of the SoFT project summarised information on tropical forage adaptation and use from available literature and experiential sources. On CD and the Internet, the SoFT database will allow researchers and advisors to select those forages most suitable for local conditions. It is also a valuable teaching tool for colleges and universities. Updates of SoFT will be undertaken by CIAT.

4.7.2 CaNaSTA – Crop Niche Selection for Tropical Agriculture, a spatial decision support system

Contributors: R. O’Brien (Curtin U. of Technology), M. Peters (CIAT), R. Corner (Curtin U. of Technology) and S. Cook (CIAT)

Rationale

Farmers in the developing world frequently find themselves in uncertain and risky environments, often having to make decisions based on very little information. Risks for smallholder farmers are often critical because of their poverty. In addition, in the tropics and subtropics, the natural environment is spatially and temporally variable and often harsh, thereby increasing the uncertainty faced by these farmers. Forage-based technologies are an excellent option for intensifying meat and milk production.

The research aims to improve forage adoption decisions in the developing world, thereby increasing sustainable intensification and ultimately contributing to increased sustainable world food production and the alleviation of under-nutrition.

A spatial decision support can facilitate the decision process by making available relevant data and knowledge. Spatial Decision Support Systems (SDSS) work with explicitly spatial data, and outputs usually include maps. An SDSS has been developed called CaNaSTA (Crop Niche Selection for Tropical Agriculture) (*canasta* is Spanish for basket, and the tool aims to offer a basket of options to farmers).

Materials and Methods

The engine used to develop CaNaSTA is Bayesian probability modeling tool. The following main criteria were used for selecting the model: (a) the ability to work with small datasets, (b) the ability to work with expert knowledge and (c) the ability to predict a range of species’ responses. In addition, a low structural complexity is required as well as ease of communication and the ability to implement the DSS spatially.

Probability calculations

In Bayesian probability modeling, if Y is a response variable, then the prior probability of Y is denoted $P(Y)$. $P(Y, X)$ is the joint probability, and $P(Y \mid X)$ is the conditional probability of Y given X, i.e., P(forage adapts | rainfall is suitable) denotes the conditional probability of a forage adapting given that rainfall is suitable. Conditional probability can be calculated from prior and joint probability values (Eq. 1). Posterior probability of an outcome Y occurring given a number of events is proportional to the conditional probability of each event occurring, assuming all variables are conditionally independent (Eq. 2).

$$P(Y \mid X) = \frac{P(Y, X)}{P(Y)} \tag{1}$$

$$P(Y \mid X^1, X^2, ..., X^n) \propto P(Y)\prod_k \left(\frac{P(Y \mid X^k)}{P(Y)}\right) \tag{2}$$

In CaNaSTA, $P(Y)$ is the probability of adaptation, with values of ‘excellent’, ‘good’, ‘adequate’ or ‘poor’. The predictor variables X^k are elevation, annual rainfall, length of dry season, soil pH, soil texture and soil fertility. Model outputs include a score value based on the probability distribution and a certainty value associated with the distribution, derived from trials data and expert knowledge, including the forage knowledge base SoFT. Stability measures are derived from changes in distribution when variables change states. From these, a ranked list of recommended species is calculated, along with suitability maps.

Results and Discussion

Results from CaNaSTA when compared with results from three existing tropical forage knowledge bases and direct elicitation from forage experts, highlighted a number of strengths of the tool. Firstly, species are not automatically excluded when one variable is unsuitable, as all other variables may be highly suitable. Secondly, the score and ranking system allows more suitable species to be considered first, rather than the user being presented with an unranked list of all species, which fit the criteria. Finally, CaNaSTA produces suitability maps dynamically; most other available knowledge bases do not have inherent spatial functionality and maps can only be produced on an ad-hoc basis.

Conclusions

Incorporating spatial capabilities into an agricultural DSS, as in CaNaSTA, facilitates data input, allows more informative output of results, and allows spatial variability to be made explicit, both of results and of uncertainties related to the results. Even with limited data, results can be obtained which support the technician farmer’s decision-making process. When uncertainties are made explicit, technicians working with farmers can then make less-risky decisions by taking these uncertainties into account. Providing access to decision support through an SDSS, such as CaNaSTA, ensures that the information is delivered in a consistent and robust manner. Trial data and expert knowledge previously inaccessible to farmers are made available so that decisions taken are better informed.

4.7.3 Identifying areas for field conservation of forages in Latin American disturbed environments

Contributors: Michael Peters, Glenn Hyman and Peter Jones, with collaboration from B. Hincapié, G. Ramirez, G. Lema, V. Soto and E. Barona (CIAT)

Rationale

An attractive new idea has come out of CIAT this year. Originally proposed by Dr D. Debouck; the conservation of critical germplasm on roadsides in Latin America has many attractions. We therefore investigated the feasibility of this approach using key forage legume species that have been identified in CIAT research and that definitely fulfill the role of conservation premia.

In situ conservation can complement *ex situ* conservation that can be very costly. It also allows for continuing evolution and adaptation of plant species. However, to be successful, *in situ* conservation projects need to pay attention to the socio-economic components of biodiversity (Brush 2000). In their original conception, large areas must be set-aside for all time, so conventional *in situ* conservation can be even more expensive than germplasm collections. Here we concentrate on field conservation of some species that could be considered a subset of *in situ* conservation and could avoid many of the high costs of complete set aside conservation.

Many forage plants have evolved in environments affected by human or animal activity, it is highly likely that they can be conserved in disturbed environments with less restriction of use than required in natural reserves. The number of natural reserves is limited; thus the approach that we suggest could complement conservation in natural habitats.

Latin America has only 3% of the world's roads, with less than 20% paved. However, there are over 10,000 km of *freeways*, and there is a growing trend towards the development of public toll roads managed by the private sector. The verges of main roads in Latin America are mainly publicly owned and are herbaceous. Often they are cut or grazed to maintain low, disturbed vegetation as part of normal road maintenance. Apart from establishment and monitoring, the costs for maintenance of roadside forage conservation areas are not envisaged to be much greater than those that road maintenance authorities currently incur. We recognize that in some areas the roadsides are cultivated. These areas would not be selected when identified in further analysis.

Materials and Methods

We used DIVA, and FloraMap® software to determine two probable distributions. DIVA deals only with the actual presence of a sighting of the species and therefore maps the *known* area of extent. FloraMap on the other hand creates a probability model of the *possible* distribution and so maps the extent of environments that could potentially be host to the species in question. This difference was the crux of our analysis. We postulated that there were two main reasons for trying to conserve species on roadsides. The first was to establish populations of genetic diversity within their area of origin, the second to establish mass reservoirs of the germplasm wherever they could be established. Both of these are credible aims. Even though the second might be much harder to achieve. We selected forage legume species based on size of collections held in CGIAR germplasm banks, importance, and knowledge of species. *Stylosanthes* sp. is probably the most researched and widely distributed tropical forage legume genus, with *S. guianensis* (Aublet) Sw. and *S. scabra* Vogel best adapted to acid soils, and the pH neutral *S. hamata* (L.) Taubert probably best known. These are complemented with *S. viscosa* Sw. and *S. capitata* Vogel. *Centrosema pubescens* Benth., *C. macrocarpum* Benth., and *C. brasilianum* (L.) Benth. have been researched in depth. *C. pubescens* is broadly distributed in the tropics as feed, cover crop, and green manure. More recent interest in forage legumes has been in *Arachis pintoi*, showing initial adoption as pasture in grass legume associations and as cover in plantations. *Aeschynomene histrix* Poiret has been adopted in West Africa as forage and for improved fallow.

We defined two scenarios; first, to conserve the germplasm within the known area of its abundance, second, to form mass reservoirs where it would be likely to be adapted.

The DIVA analysis gave us the clearest indication for the first scenario, but left us wanting on the second. FloraMap results gave us the best indication for the second case. FloraMap produces probability surfaces for the potential distribution of a species. In order to find the most promising areas to search we had to combine the probability surfaces.

Combining probability surfaces. Determining the best *in situ* conservation sites for the greatest number of the 10 species examined in this study requires the combination of the 10 probability surfaces. We tried a number of different ways to combine the probabilities; most were unsatisfactory, but one appeared to give us what we needed:

$$p = \frac{1}{n}\sum_{i=1}^{n} (p_i \geq a) \quad (1)$$

Equation 1 forms an index from the number of species where the probability, p_i, exceeds a threshold, a. We decided to use a threshold probability of 0.5, and used this equation to map species richness. Because this index if freed from actual accession observation, a low or zero value indicates low probably of diversity, and not just lack of knowledge as in the case of DIVA.

Defining potential road verges. We defined the potential for establishing *in situ* conservation sites along roadsides using the digital road maps of Latin America and the Caribbean at 1:1,000,000, from the Digital Chart of the World (DCW). We improved on this map, where possible, by adding finer scale data from national road maps, supplementing the DCW, and allowing us to verify the road conditions. These maps were then overlaid on the species richness maps to determine the probable sites with accessible lengths of roadside environment suitable for the conservation of the maximum number of species together.

Results

Evaluating diversity in the areas of origin: Scenario 1: Most of the 10 forage species studied were collected in Central America, Colombia, Venezuela, and south and eastern Brazil (Figure 55). The map shows four areas where six or more forage species were found within the same 1-degree grid cell. These include an area in Central Panama near the town of Anton, an area in eastern Venezuela between Cumana on the coast and El Tigre to the south, and two areas in Brazil from Salvador westwards into Bahia State and in central Mato Grosso. The Venezuelan site showed the highest concentration of forage species richness.

Evaluating diversity of mass reservoirs outside areas of origin: Scenario 2: We produced individual distribution maps for each species, including the accession points used to calibrate each model. For the ten species distributions, we combined the probability distributions using Equation 1. The result, demonstrated in Figure 56, shows the concentration of species having a probability greater than 50% of finding an environment similar to areas of origin. We overlaid this map on the road distribution, and selected those areas where significant numbers of pixels with high species richness index also showed major road access. This eliminated two significant areas—the first in Western Brazil, where access is limited, and the second in Venezuela, south of the Orinoco, which is likewise highly remote.

Discussion

Can we form stable associations of legume species? Results from work with legume mixtures show that through compensation and complementation of individual legume species, mixtures are seasonally and temporally more stable than single stands. The complementarity of species signifies that although some less competitive species may reduce in the sward over time, a stable mixture of several species in a sward can be formed. Successful legumes have included *Stylosanthes*, tree legumes and niche legumes such as forage *Arachis* species. Their characteristics varied greatly but, with some exceptions, they demonstrated persistence, vigor and longevity under grazing or cut-and-carry systems, ease of establishment (with the exception of *Leucaena*), and either high seed yield or ease of vegetative propagation.

Genetic drift within the populations. Genetic drift is expected in the populations once established), but by selecting areas most climatically similar to the areas of origin we hope

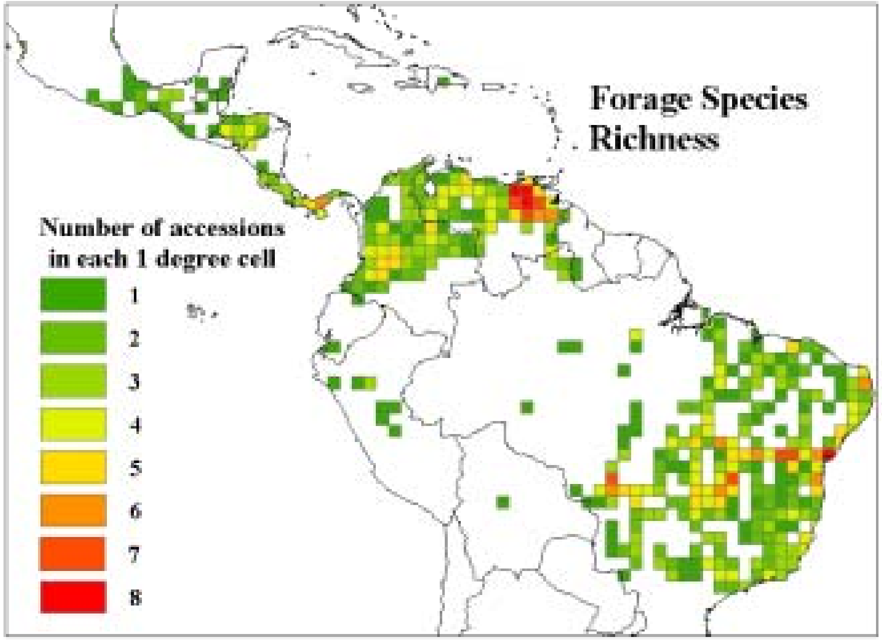


Figure 55. Species richness of 10 forage species in Latin America.

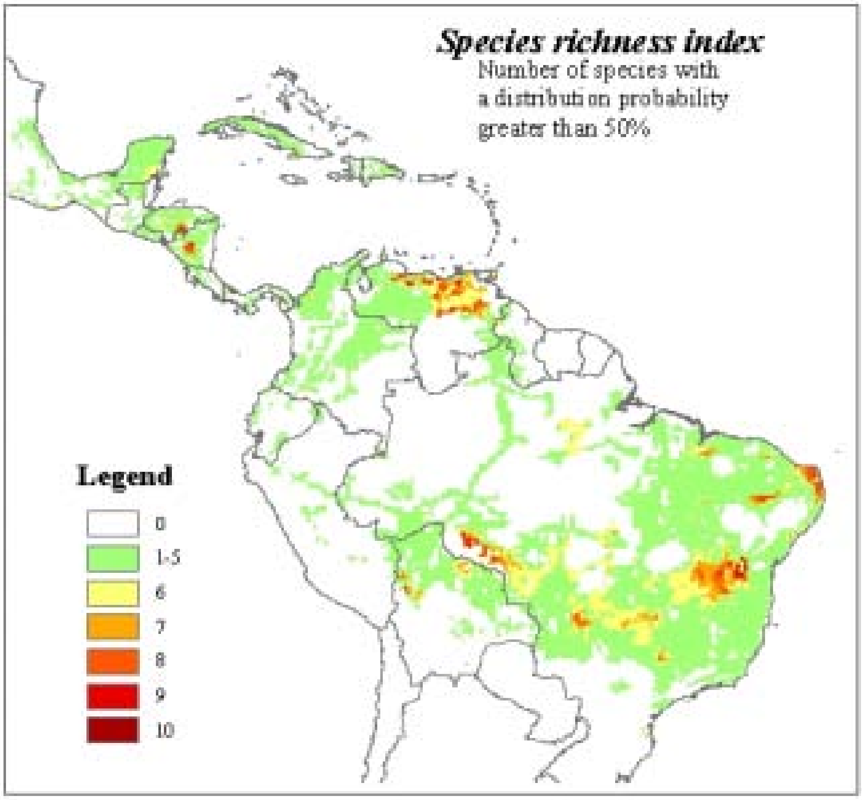


Figure 56. Potential areas for *in situ* conservation outside areas of origin. The concentration of species having a probability greater than 50% of finding an environment similar to areas of origin.

that this will be minimized. Legume persistence in tropical pastures can be problematic under heavy grazing pressure, but is much easier to control under light grazing There are a few exceptions to this rule since legumes such as *Arachis pintoi* and *Desmodium hetercarpon* subsp. *ovalifolium* require heavy grazing for persistence. Road verges often can have a variety of these characteristics. We propose that test ecotypes could be taken from a core collection of each species to ensure including the full range of available genetic variation. A general problem emphasizing areas of species richness is that they may miss outliers of plant species with particular characteristics. Conversely, an area suitable for all 10 species may mean that some or all will be adapted marginally, resulting in rapid selection and genetic drift. We have attempted to limit this possibility by requiring a high threshold probability of 0.5 for the inclusion of a species in the diversity index, but we accept that the roadside sites will not guarantee full conservation of a collection. Having a range of sites may be necessary to conserve as much diversity as possible.

The difference between FloraMap and DIVA.

The coincidence between the species richness maps produced by the DIVA and FloraMap methods in those areas where actual existence of accessions occurred gives us confidence in the FloraMap analysis. The advantage of the latter method is that it can predict potential areas without the necessity of actually having observed the plants there. However, the DIVA analysis guarantees that all species actually have been present in the area selected, and may be the most appropriate for strict conservation in areas of known origin.

Conservation in areas of origin

Our results suggest that four areas could hold suitable sites for *in situ* conservation in the areas of origin for eight forage species (Figure 55). Since establishing conservation plots in these areas of origin involves limited risk, our maps identify priority areas that can guide conservation specialists and policymakers. Detailed mapping and local knowledge of these potential sites is needed to plan for pilot projects. The potential sites that we identified can be studied in greater detail using remote sensing data. High-resolution imagery or air photos possibly could provide useful information, even of the verges. Soil information is needed to assess the diversity of soil environments for conservation sites. If a detailed soil map of the area is available, this assessment could be carried out in the office. Eventually, researchers and local experts will need to carry out field reconnaissance to verify whether the identified sites could be set up as conservation areas.

Mass reservoirs, not necessarily in areas of origin

An advantage of having an *in situ* conservation area with public access in an area where forage legumes have a niche in farming systems is that the populations will serve as an ongoing adaptation trial. Eventually, farmers will be able to select promising ecotypes well suited to their area, directly from the conservation area. Thus, mass reservoirs can be seen to fulfill two roles, that of conservation and that of selection of adapted material. These could be viewed as conflicting ends, and may require careful balancing. Detailed studies under controlled conditions are necessary to assess implications of such reservoirs. The area around El Tigre, Venezuela, and in Bahia State in Brazil may provide ideal study grounds since they combine areas suitable for conservation in the areas of origin (Figure 55) and for mass reservoirs (Figure 56), hence reducing the risks of genetic drift, and introduction of harmful weeds.

Conclusions

This study used temperature, precipitation, elevation, transportation infrastructure, and species passport information for selecting possible locations for *in situ* conservation of plant genetic resources on roadsides. We have shown that, using geographic information systems (GIS) technology, sites can be found where multiple forage species with distinctive distributions might be brought together to coexist. There may be biological problems to overcome and we have outlined some of them with possible solutions and caveats. However, it would appear that there could be good precedent for putting together easily maintained mixtures of legume species. The actual operation of this type of conservation will require local support; we feel this is eminently feasible and should be tried. If it can be made to work with forage legumes, it might be an option with other colonizing species, and a low cost solution to the conservation of valuable wild crop relatives.

4.8 Facilitate communication through journals, workshops, and the Internet

Highlights

- As the Journal Pasturas Tropicales celebrates its 20th Anniversary it continues to be an important vehicle for forage researchers in LAC to publish their work. During 2004, three volumes with 20 research papers from Brazil, Colombia, Mexico and Paraguay were published.

- The Forage Web site continues to be an excellent media to reach large audiences interested in Forage R&D. Late last year we launched the Spanish Forage Web in order to reach researchers and development workers in LAC. Half of the hits (32,631) recorded in 2004 were for the Spanish Web version.

4.8.1 Publication of Pasturas Tropicales

Contributors: A. Ramírez (Independent Publisher) and Carlos Lascano (CIAT)

During 2004 three issues, corresponding to Volume 26, were published. The contributions of research papers from Brazil (13), Colombia (5), Mexico (1) and Paraguay (1) are summarized in Table 87. As in previous years, the number of contributions has been high. Twenty-two documents are pending to be published in coming volumes. This high number of contributions is an indicative of researcher's preferences and of the high international visibility of the forage related publication.. This year Pasturas Tropicales is celebrating its twentieth anniversary of circulation. This, in addition to the fact of having been qualified by Colciencias in its Index Series of Colombian Scientific Magazines, consolidates even more its position as an effective means of communicating forage research in Tropical Latin America. Timely circulation of the magazine was an important advance during 2004. This was accomplished by transfering the tasks of editing and designing the magazine to staff of CIAT's Forage Project.

Table 87. Topics and number of contributions published in Tropical Pastures during 2004.

Topics	26(1)	26(2)	26(3)	Institution	Country
Covers	_	1	_	Corpoica	Colombia
Pastures Rehabilitation	1	2	2	Embrapa-Ro. (3) Corpoica (1), CIAT (1)	Brazil Colombia
Seeds Phenology	_	1	_	INIFAP	Mexico
Silvopastoril systems	_	2	_	Embrapa-Agrobiología	Brasil
Forage quality- trees			1	CIAT	Colombia
Establishment	_	1	_	UdeA	Colombia
Intake	1	_	_	Embrapa-Amazonia	Brazil
Fertilization	3	_	_	Embrapa-Ro (1), Univ. Rural de Rio Janeiro (2)	Brazil
Establishment	1	_	_	Embrapa-Agrobiology	Brazil
Animal production	_	_	1	Embrapa-Amazonia	Brazil
Impact of forage adoption	_	_	1	CIAT	Colombia
Simulation growth model	_	_	1	ESALQ	Brazil
Colection of Arachis	_	_	1	USDA-Univ. do Paraná	Paraguay
Total	6	7	7		

4.8.2 Update on the Forage Web Site

Contributor: Simone Staiger and B. Hincapie (CIAT)

The Tropical Forages Project, web site is the result of teamwork between all Project members, under the general Web site coordination of the Communications Unit and with the support of both the Systems and the Information and Documentation Unit. The Web site has allowed us to disseminate our research results extensively and to promptly communicate important news. Late last year we launched the version in Spanish of the Forage Web page in order to reach a large audience in LAC (universities, research institutes, donors, and the scientific community in general). The site is accessible under the URL http://www.ciat.cgiar.org/forrajes/index.htm

In Figure 57 we show the number of hits in the period October 2003 – August 2004, for the English and Spanish versions. A total of 64,459 hits were recorded out of which half (32,631) were for the Spanish version.

In Table 88 we summarize downloads of different documents placed in the web page. To date their has been 106, 038 documents downloaded, the most popular being the Technical Bulletin of *Brachiaria brizantha* cv. Pasto Toledo with 27,077 downloads, followed by the recently launched Index of Pasturas Tropicales.

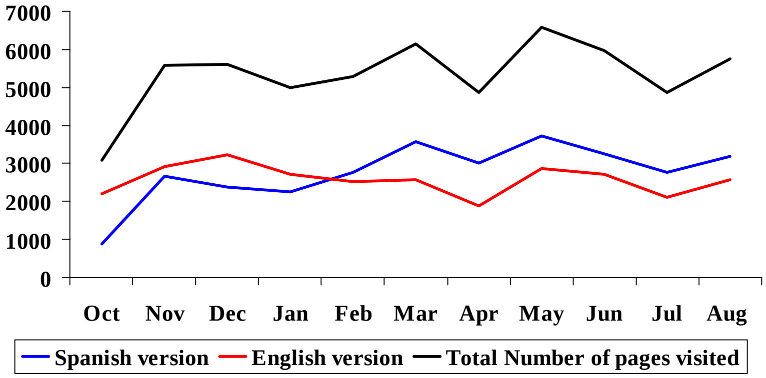


Figure 57. Number of visits to the Forage Web sites (English and Spanish) during the October 2003 to August, 2004 period.

Table 88. Download of documents in the Tropical Forages Web site (October, 2003 to August 2004).

Downloads	Number
Technical Bulletin: Cultivar Pasto Toledo	27077
Pasturas Tropicales Indice de Autores y Especies Forrajeras	26612
Technical Bulletin: Cratylia argentea cv Veranera	24165
Methods: Evaluación Pasturas con Animales	12010
Annual Report 2003	7459
Producción Artesanal de Semillas de Pasto Toledo	4551
Informe MADR 2003	4164

Annex

List of Publications

Refereed Journal (published, In press and submitted):

Abreu, A.; Carulla J.E.; Kreuzer, M.; Lascano C.E.; Díaz T.E.; Cano A.; Hess H.D. 2003. Efecto del fruto, pericarpio y extracto semipurificado de saponinas de *Sapindus saponaria* sobre la fermentación ruminal y la metanogénesis *in vitro* en un sistema RUSITEC. Revista Colombiana de Ciencias Pecuarias, 16:147-154.

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Awards to Staff in the Project

- **Franco Luis H.:** Outstanding Support Staff (OSSCA), CIAT, December 2003
- **Wenzl, P., A. L. Chaves, G. M. Patiño, J. E. Mayer and I. M. Rao.** 2002. Aluminum stress stimulates the accumulation of aluminum-detoxifying organic acids in root apices of *Brachiaria* species. J. Plant Nutrition and Soil Science 165: 582-588. Outstanding Research Publication Award (ORPA), CIAT, December 2003.
- **Reiber Christoph:** Ruthenberg-Preis 2004 Potential and constraints of cowpea (*Vigna unguiculata*) in Honduran hillsides. A farmers' assessment. Master-thesis, University of Hohenheim. Field work with CIAT in Honduras
- **Pabón, A**., **G. Sotelo, and C. Cardona:** "Francisco Luis Gallego Award" to the best paper presented by an undergraduate student. XXX Congress of the Colombian Entomological Society, 2003.

Thesis Students

BS Thesis

Name	Status	University	Title
Bertrand Ramón Iván	On-going	Universidad Católica Agropecuaria del Trópico Seco, Estelí, Nicaragua	Diagnóstico sobre alimentación de ganado doble propósito en época de verano en comunidades aledañas al municipio de Somoto, Madriz – Nicaragua
Chaves Q. Carlos A.	2004	Universidad de Costa Rica, Costa Rica	Calidad y consumo de *Cratylia argentea* y sorgo forrajero (*Sorghum* sp.) con y sin melaza ensilada en bolsas plásticas
Flores Zenelia	Completed	Universidad Nacional Autónoma de Nicaragua – UNAN CUR, Matagalpa, Nicaragua	Determinación de la composición botánica, disponibilidad y producción de leche en potreros establecidos con pastos *Brachiaria* en asociación con *Arachis pintoi* en la comunidad Wibuse, San Dionisio
Abello Javier F.	On-going	Universidad Nacional, Bogota, Colombia	*Brachiaria* endophytes as gene delivery system
Leiva Aráuz, Oscar Javier & Martínez González Róger	On-going	Universidad Católica Agropecuaria del Trópico Seco, Estelí, Nicaragua	Validación de sistemas de cultivos con introducción de leguminosas como abonos verdes y coberturas sobre la sostenibilidad de sistemas de producción tradicionales en una microcuenca
Mera Álvarez Mónica Lorena	On-going	Universidad Nacional de Colombia, Palmira, Colombia	Effecto de leguminosas forrajeras tropicales ricas en taninos sobre la fermentación ruminal y la producción de metano en un sistema in vitro (RUSITEC)
Miller María Fernanda	On-going	Universidad del Valle, Cali, Colombia	Resistencia de *Brachiaria* spp. al salivazo*:* Efectos subletales de cultivares resistentes sobre los adultos de *Zulia carbonaria* (Lallemand) (Homoptera: Cercopidae)
Real Posada Franklin Rigoberto, Rayo Carazo Omar Antonio, Ramirez Ramirez Edwin José, Lopez Suarez Cheyla Matilde, Romero Duarte Juan Adán, Luna García Álvaro José	On-going	Universidad Nacional Agraria (UNA), Managua, Nicaragua	Survey on dry season feed resources in three different livestock regions of Nicaragua
Rincón Lozano Joisse Dayana	On-going	Universidad Nacional de Colombia, Palmira, Colombia	Evaluación del efecto de la sequia en genotipos de *Brachiaria* bajo condiciones de invernadero
Rosero Jaime	On-going	Universidad Nacional, Palmira	Ensayo Multilocacional de Sistemas de Establecimiento de *Cratylia argentea* cv. Veranera
Sotelo Paola	Completed	Universidad del Valle, Cali, Colombia	Resistencia de *Brachiaria* spp. al salivazo*:* Efectos subletales de cultivares resistentes sobre los adultos de *Aeneolamia varia* (F.) (Homoptera: Cercopidae)

MS Thesis

Name	Status	University	Title
Arango Adriana	Completed	National University, Bogotá, Colombia	Identification of candidate genes for aluminium resistance in *Brachiaria*
Castro Ulises	On-going	Colegio de Postgraduados de Chapingo, Chapingo, Mexico	Mechanisms of resistance to *Aeneolamia albofasciata* and *Prosapia simulans* en *Brachiaria* spp.
Cortés Cortés Javier Eduardo	On-going	Universidad Nacional de Colombia, Bogotá, Colombia	Efecto de los taninos de leguminosas tropicales sobre la degradación in vitro de la proteína con fluido ruminal y pepsina
Husselman Madeleen	On going	Wageningen Agricultural University	Evaluation of potential production of seed from the hybrid Brachiaria “Mulato” with small plot and on-farm trials on the Bolovens Plateau in southern Lao
Monsalve Castro Lina Maria	On-going	Universidad Nacional de Colombia, Palmira, Colombia	Efectos sobre la fermentación ruminal, el flujo de proteína duodenal y la absorción de nitrógeno en ovinos alimentados con leguminosas con y sin taninos
Nieto B. Juan C.	On-going	Universidad de Costa Rica, Costa Rica	Caracterización nutricional de material fresco y ensilado de Maní forrajero (*Arachis pintoi*) cultivado en asocio con Maíz (*Zea mays*) a tres densidades de siembra
Noto Fabio	Completed	Swiss Federal Institute of Technology (ETH), Zurich, Switzerland	Effects of provenance (Colombia and Kenya) on the ruminal fermentation characteristics of *Calliandra calothyrsus* (var. Patulul)
Pabón Alejandro	On-going	Universidad de Viçosa, Brazil	Mechanisms of resistance to *Deois incompleta* and *Notozulia entreriana* en *Brachiaria spp.*
Payan Arlen	On going	Centro Agronómico de Investigación y Enseñanza (CATIE), Costa Rica	Efecto de *Cratylia argentea* sobre la producción animal en la cuenca de Jucuapa, Matagalpa, Nicaragua
Reiber Christoph	Completed	University of Hohenheim	Potential and constraints of cowpea (*Vigna unguiculata*) in Honduran hillsides. A farmers’ assessment.
Ricaurte José Jaumer	On-going	Universidad Nacional de Colombia, Palmira, Colombia	Impact of aluminium tolerant *Brachiaria* genotypes on soil quality characteristics of an oxisol of the altillanura of the Meta Department of Colombia
Stürm Christoph Dominic	Completed	Swiss Federal Institute of Technology (ETH), Zurich, Switzerland	Effects of combinations of legumes with contrasting contents of tannins on in vitro ruminal fermentation
Vivas Nelson	On-going	Universidad Nacional de Colombia, Palmira, Colombia	Evaluación agronómica de 144 accesiones de *Desmodium velutinum* como alternativa forrajera para las zonas de ladera del norte del departamento de Cauca

PhD Thesis

Name	Status	University	Title
Andersson Meike Stephanie	On-going	University of Hohenheim, Germany	Genetic diversity and core collection approaches in the multipurpose shrub legumes *Flemingia macrophylla* and *Cratylia argentea*
Bartl Karin	On-going	Swiss Federal Institute of Technology (ETH), Zurich, Switzerland	Effects of improved feeding systems for dairy cattle in tropical smallholder farms on milk production and quality at high altitudes
Castañeda Nelson	On-going	University of Goettingen, Germany	Genotypic variation in P acquisition and utilization in *Arachis pintoi*
Hernández Luis Alfredo	On going	University of Hohenheim, Germany	A participatory procedure applied to selection and development of forages with farmers
Louw-Gaume Annabé	On-going	ETH, Zurich, Switzerland	Adaptation of *Brachiaria* grasses to low P soils
Mejia Sergio	On-going	Universidad Nacional de Colombia, Palmira, Colombia	Identification of candidate genes responsible for adaptation of tropical forage grass, *Brachiaria* to low phosphorus soils
O'Brien Rachel	Completed	Curtin University, Australia	Incorporating socio-economic data and expert knowledge in complex spatial decision-making
Reiber Christoph	On going	University of Hohenheim, Germany	Encouraging adoption of research-based offerings with contrasting extension approaches
Rincon Alvaro	On-going	Universidad Nacional de Colombia, Bogotá, Colombia	Integration of maize with forages to recuperate degraded pastures in the Llanos of Colombia
Tiemann Tassilo	On going	Swiss Federal Institute of Technology (ETH), Zurich, Switzerland	The forage potential of tanniniferous legumes: Search for sustainable ways to cope with nutritional limitations in smallholder livestock
Tscherning, Karen Joanna	Completed	University of Hohenheim, Germany	Development of methods for the simultaneous evaluation of forage legume for feed and for soil improvement
Van der Hoek Rein	Completed	University of Hohenheim, Germany	Participatory research methods for forage-based technologies in Central-American hillsides

List of Donors

Asian Development Bank
Livelihood and Livestock Systems Project
2003-2005

Australia – ACIAR
Development of a knowledge system for the selection of forages for farming systems in the tropics (co-financed with BMZ)
2002-2005

Australia Curtin University 2001-2004
Incorporating socio-economic data and expert knowledge in representations of complex spatial decision-making (stipend PhD Rachel O'Brien)

Australia – AusAID
Forages and Livestock Systems Project
2000-2005

Common Fund for Commodities (CFC) 2003-2006
Enhancing beef productivity, quality, safety, and trade in Central America (Guatemala, Nicaragua, Honduras)

Colombian Government
Grasses and legumes with high nutritive quality
Grasses and legumes adapted to low fertility acid soils
Grasses and legumes with adaptation to drought, poor soil drainage and resistant to pests and diseases
Integration of improved grasses and legumes in production systems in savannas

FONDEAGRO- Nicaragua 2002-2003
Introducción participativa de forrajes mejorados en sistemas de producción de leche de pequeños productores en Matagalpa, Nicaragua

FUNICA - Nicaragua 2003-2004
Validación de sistemas de cultivos con introducción de leguminosas como abonos verdes y coberturas sobre la sostenibilidad de sistemas de producción tradicionales en una microcuenca, San Dionisio, Nicaragua

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 (special project) 2000-2004
- Development of a knowledge system for the selection of forages for farming systems in the tropics (cofinanced with ACIAR) 2002-2005
- Utilising multipurpose legume diversity to improve soil and feed quality including application in a watershed in the Central American hillsides – PostDoc program A. Schmidt 2004-2007
- Demand-driven use of forages in fragile dry season environments of Central America to improve livelihoods of smallholders. 2004-2007
- An integrated approach for genetic improvement of aluminium resistance of crops on low-fertility acid soils 2001-2004

Germany - Volkswagen Foundation
Research and development of multipurpose forage legumes for smallholders croplivestock
systems in the hillsides of Latin America (with the U. of Hohenheim and CORPOICA
2003-2006

Japan – The Ministry of Foreign Affairs - 1995
- The role of endophytes in tropical grasses
- The tropical Forage Project (core funds)

Semillas Papalotla, S.A. de C.V. 2001-2005
Brachiaria Improvement Program

Switzerland – ETH (Swiss Federal Institute of Technology), Zurich
- Adaptation of Brachiaria to low P – (2003-2005)
- The forage potential of tanniniferous legumes: The search for sustainable ways to cope with nutritional limitations on smallholder systems – (2004-2006)
- Improved feeding systems for smallholder dairy cattle with emphasis on dry season feeding and its effect on milk production and quality (2004-2007)

UK- DFID
Development of a database and retrieval system for the selection of tropical forages for farming systems in the tropics and subtropics, SoFT (with CSIRO, ILRI and QDPI) 2002-2004

List of Collaborators

Austria
E. Heberle-Bors, University of Vienna
R. Albert, University of Vienna

Australia
Bruce Cook, Ian Partridge, QDPI
Bruce Pengelly, Stuart Brown, David Eagles CSIRO
Rob Corner, Curtin University
Sukumar Chakraborty, CSIRO

Brazil
Cacilda Borges do Valle, EMBRAPA/ CNPGC, Campo Grande, MS

Cambodia
Sorn San, NAHPIC

China
Huang Dongyi, South China University
Yi Kexian, CATAS

Colombia
Alirio Martínez M., UMATA Restrepo, Valle del Cauca
Antonio Pérez, Universidad de Sucre
Carlos H. Cardona H., REVERDECER, Restrepo, Valle del Cauca
Carlos Vicente Durán, Universidad Nacional de Colombia, Palmira, Valle del Cauca
Carmen B. de Muñoz, Universidad de la Amazonía, Florencia, Caquetá
Tito Díaz, CORPOICA-Tibaitatá
Daniel Villada, FIDAR, Valle del Cauca
Edgar A. Cárdenas, Universidad Nacional de Colombia, Bogotá
Freddy Parra, CORPOICA, Popayán, Cauca
Henry Mateus, CORPOICA, Barrancabermeja, Santander
Jaime Velásquez, Universidad de la Amazonía, Florencia, Caquetá
Jairo Osorio, CORPOICA, Colombia
Jorge Medrano, Corpoica, Villavicencio
José Restrepo, FIDAR, Valle del Cauca
Juan Carulla, Universidad Nacional de Colombia, Bogotá
Rafael Mora, Universidad de los Llanos, Villavicencio
Rolando Barahona, Corpoica, Bogotá
Silvio Guzmán, Fundación Universitaria San Martín, Barranquilla

Costa Rica
Augusto Rojas, UCR
Beatriz Sandoval, MAG
Carlos Hidalgo, MAG
Carlos Jiménez, UCR
Carlos Zumbado, MAG
Alberto Barbosa, CGUS
Jobny Chávez, Cámara de Ganaderos de Cañas
Corporación de Fomento Ganadero, CORFOGA
Francisco Romero, ECAG
Guillermo Pérez, CIAT
Horacio Chi Chan, MAG
Jesús González, ECAG
Luis Mesén, MAG
Marco Lobo, MAG
María Mesén, MAG
Mauricio Chacón, MAG
Milton Villareal, ITC
Mohammed Ibrahim, CATIE
Ricardo Guillen Montero, MAG
Vidal Acuña, MAG
William Sánchez, MAG

Uganda
Africa 2000 Network
African Highland Initiative, Kampala
National Agricultural Research Organisation (NARO), Uganda

Ethiopia
Areka Agricultural Research Institute, Ethiopia
Ethiopia Agricultural Research Organization (EARO), Ethiopia
FARM Africa, Ethiopia
International Livestock Research Institute (ILRI), Ethiopia
Jean Hanson, ILRI
Land-O-Lakes, Malawi
Melkassa Agricultural Research Center, Ethiopia
Mr. Endale Gebre, EARO, Ethiopia
Mr. Muluneh Minta, EARO, Ethiopia

Malawi
Department of Agricultural Research Service (DARS), Malawi

Germany
Brigitte Maass, University of Goettingen
Norbert Claassen, University of Goettingen, Goettingen
Rainer Schultze-Kraft, University of Hohenheim
Volker Hoffmann, University of Hohenheim
Walter Horst, University of Hannover, Hannover

Guatemala

Asocación de Criaderos de Ganado Brahman, ASOBRAHMAN
Ramiro Pérez, (MAGA)

Honduras

Conrado Burgos, DICTA
Ernesto Ehrler, DICTA
Gustavo Agurcia, FENAGH
J. Jimenez, IPCA
Konrad Uebelhör, Jochen Leitz, gtz
Marlen Iveth Posas Ponce, SERTEDESO
Peter Müller, KFW

India

Michael Blummel, ILRI

Indonesia

Dinas Peternakan Samarinda

Italy

Stephen Reynolds, FAO
Caterina Batello, FAO

Japan

G. V. Subbarao, JIRCAS, Tsukuba, Japan
K. Okada, JIRCAS, Tsukuba, Japan
M. Osaki, Hokkaido University, Sapporo
T. Ishikawa, JIRCAS, Tsukuba, Japan
T. Tadano, Hokkaido University, Sapporo
Y. Saito, National Grassland Research Institute, Kitasaku, Nagano
Sumio Miyawaki- Coordinador, JICA

Kenya

James Smith, ILRI
Dannie Rommney, ILRI
D. Friesen, IFDC-CIMMYT
Steve Franzel, ICRAF
William Thorpe, ILRI

Lao, PDR

Bounthavong Kounnavongsa, NAFRI
Viengsavanh Phimphachanhvongsod, NAFRI
Viengxay Photakoun NAFE

México

Andrei Nicolayevsky, Semillas Papalotla, Mexico, DF
Armando Peralta M., Agroproductos de Iguala, S.A., Iguala, Guerrero
Eduardo Daniel Bolaños Aguilar, INIFAP, Huimanguillo, Tabasco
Eduardo Stern, Semillas Papalotla, Mexico, DF
Francisco Javier Henríquez, INIFAP, Isla, Veracruz
Jorge David Guiot, Semillas Papalotla, Villahermosa, Tabasco

Nicaragua

Alejandro Blandón, INTA
Dionisio Soto, MAG-FOR
Humberto Sánchez, FAGANIC
Instituto de Desarrollo Rural, IDR
José Antonio Molina, INTA
Lino Estrada, Asociación Campos Verdes, San Dionisio
Martin A. Mena, INTA
Octavio Menocal, INTA
Yuri Mongalo, ASODEL

Nigeria

B.B. Singh, IITA
Nicoline de Haan, IITA
Robert Carsky, IITA
S.A. Tarawali, IITA-ILRI
T.O Williams, ILRI

Philippines

Eduedo Magboo, PCARRD
Elsie Gabonada, Impasugong
Francisco Gabunada, ViSCA
Judith Saguinhon, MAO, Malitbog
Leonardo Moneva, Mag-Uugmad Fdn.
Perla Asis, City Veterinary Office, Cagayan de Oro

Switzerland

A.Gaume, ETH, Zurich
A. Oberson, ETH, Zurich
Andrea Machmüller, Methanogenesis, ETH Zurich
Dieter Hess, ETH, Zurich
E. Frossard, ETH, Zurich
Michael Kreuzer, ETH, Zurich

Thailand

Chaisang Phaikaew, DLD
Ganda Nakamanee, PCANRC

United States

C. Schardl, University of Kentucky
James White, Rutgers University

Vietnam

Le Hoa Binh, NIAH
Truong Tan Khanh, Tay Nguyen
University Vu Thi Yen, DARD

Project Staff List

Principal Staff

Lascano Carlos E, Project Manager and Animal Nutritionist
Argel Pedro, Forage Agronomist; San José, Costa Rica
Cardona Cesar, Entomologist
Holmann Federico, Animal Production Systems/ Economics
Horne Peter M., Forage Agronomist
Kelemu Segenet, Pathologist
Miles John, Plant Breeder
Peters Michael, Forage Biologist
Rao Idupulapati, Plant Nutritionist/Physiologist
Schmidt Axel, Forage Agronomist (Systems)
Stur Werner, Asia

Consultants

Wenzl Peter, CAMBIA, Canberra, Australia

Visiting Scientist

Castro Ulises, Colegio de Postgraduados, Chapingo, Mexico
Pabón Alejandro, Universidad Federal de Vicosa, Vicosa, Brasil
Sakai Tomoko, Japan Overseas Cooperation Volunteers (JOCV), JICA, Japan
Mejia Kerguelen Sergio Luis, Federal Institute of Technology (ETH), Zurich, Switzerland

Biometrics Specialist

Ramírez Gerardo

Research Associates and Assistants

Avila Patricia, Forage Quality
Cruz Flores Heraldo, Regional evaluation (Yorito, Honduras)
Davies Salmeron Clark Sydney, Regional evaluation (San Dionisio, Nicaragua)
Franco Luis Horacio, Germplasm
Franco Manuel Arturo, Data Base
Gabunada, Francisco, Regional evaluation, Asia
Hernández Luis Alfredo (from SN3)
Hernández Oscar (contract by gtz Honduras)
Plazas Camilo, Regional evaluation, (Llanos)
Rodriguez Martin, Phytopathology
Sedano Raúl, Phytopathology (until June 2004)
Sotelo Guillermo, Entomology
Zuleta Carolina, Phytopathology (until October 2004)
Phonepaseuth Phengsavanh, Regional evaluation, Asia
Samson Jindra, Regional evaluation, Asia

Specialists

Betancourt Aristipo, Genetics
García Ramiro, Plant Nutrition
Hincapié Belisario, Germplasm

Technicians

Baena Alvaro, Plant Pathology
Bonilla Ximena Patricia, Phytopathology
Córdoba Gilberto, Entomology
Feijoo Fernando, Plant Nutrition
Franco Humberto, Seed Unit (Palmira)
Mera William, Entomology (Caquetá)
Muñoz Perea Jacqueline, Seed Unit (Palmira)
Ospinal Gustavo, Forage Quality
Pareja José Reinaldo, Entomology
Pérez Guillermo, Seed Unit, (Atenas, Costa Rica)
Rojas Castillo Claudia Ximena, Plant Nutrition
Segura Gustavo, Plant Pathology
Vergara Daniel, Genetics (Villavicencio)
Viveros Darío H., Phytopathology

Workers

Amaya José Nelson, Genetics
Aragón José Ever, Forage Evaluation (Quilichao)
Arce Crisanto, Seed Unit, Atenas, Costa Rica
Carabalí Néstor, Forage Evaluation (Quilichao)
García Benilda, Forage Quality
Gutiérrez Pedro José, Genetics (Popayán)
Lasso Jesús, Forage Germplasm
López Luis Alberto, Plant Nutrition
Mora José Manuel, Costa Rica
Mueces Fanny Isabel, Genetics
Recio Maria Eugenia, Plant Nutrition
Hernández Gutiérrez Isled Yomar, Plant Nutrition
Mona Alvarez Héctor Fabio, Plant Nutrition
Trujillo Filigrana Orlando, Forage Quality
Viveros Roso Hernando, Forage Evaluation (Quilichao)
Zúñiga Harold Orlando, Forage Evaluation (Quilichao)

Ph.D. Students

Andersson Meike Stephanie, University of Hohenheim, Germany (In preparation)
Bartl Karin, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland (On going)
Castañeda, Nelson, University of Goettingen, Germany (On going)
Hernández, Luis Alfredo, University of Hohenheim, Germany (On going)
Louw-Gaume Annabé, ETH, Zurich, Switzerland (On going)

Mejia Kerguelen Sergio Luis, Universidad Nacional de Colombia, Palmira (On going)
O'Brien, Rachel, Curtin University, Australia (Completed)
Reiber, Christoph, University Hohenheim, Germany (On going)
Rincón, Alvaro, Universidad Nacional de Colombia, Bogotá (On going)
Tiemann Tassilo, Swiss Federal Institute f Technology (ETH), Zurich, Switzerland (On going)
Tscherning, Karen Joanna, University of Hohenheim, Germany (Completed)
Van der Hoek, Rein, University of Hohenheim, Germany (Completed)

MSc. Students
Arango, Adriana, Universidad Nacional de Colombia (completed)
Castro, Ulises, Colegio de Postgraduados, Chapingo, Mexico. (On going)
Cortés Cortés Javier Eduardo, Universidad Nacional de Colombia, Bogotá (On going)
Husselman Madeleen, Wageningen Agricultural
Monsalve Castro, Lina Maria, Universidad Nacional de Colombia - Palmira (On going)
Nieto Betancur, Juan C., Universidad de Costa Rica. Costa Rica (On going)
Noto Fabio, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland (Completed)
Pabón Alejandro, Universidad de Vicosa, Brasil (On going)
Payan Arlen, Centro Agronómico de Investigación y Enseñanza (CATIE), Costa Rica (On going)
Reiber Christoph, University of Hohenheim, Germany (Completed)
Ricaute, José Jaumer, Universidad Nacional de Colombia, Palmira (0n going)
Stürm Christoph Dominic, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland (Completed)
Vivas, Nelson, Universidad Nacional, Palmira (On going)

Pre-Graduate Students
Abello Javier Francisco, Universidad Nacional de Colombia, Bogotá, Colombia (On going)
Bertrand Ramón Iván, Universidad Católica, Nicaragua (On going)
Chavez Quiroz, Carlos A. Universidad de Costa Rica. Costa Rica. (2004)
Flores Zenelia, Universidad Nacional Autónoma de Nicaragua, Nicaragua (Complete)
Leiva Aráuz, Oscar Javier and González Róger; Universidad Católica Agropecuaría del Trópico Seco, Estelí, Nicaragua
Mera Mónica Lorena, Universidad Nacional de Colombia, Colombia (On going)
Miller, Maria Fernanda, Universidad del Valle, Cali (On going)
Real Posada, Franklin Rigoberto, Rayo Carazo Omar Antonio , Ramirez Ramirez Edwin José, Lopez Suarez Cheyla Matilde , Romero Duarte Juan Adán , Luna García Álvaro José .Universidad Nacional Agraria (UNA), Managua, Nicaragua, (On going)
Rincón Lozano Joisse Dayana, Universidad Nacional de Colombia, Colombia (On going)
Rosero Jaime, Universidad Nacional, Palmira (On going)
Sotelo Paola, Universidad del Valle, Colombia (completed)

Trainees
Calderón Juan Carlos, Student in Practice, Base de Datos (with Systems Unit)
Dohmeyer Caroline, University of Hohenheim, (organic matter survey San Dionisio)
Plata César Aurelio, Student in Practice, Universidad Javeriana, Base de Datos (with Systems Unit)
Schloen Marie, University of Hohenheim, Student in Practice (SoFT, literatura revision *Canavalia brasiliensis)*

Administrative Assistant
Medina Anderson, Consorcio Tropileche

Office Personnel
Burbano Jenny Milena

Secretaries
Arenas Salazar Beatriz
Bonilla Diadema, Philippines
Gómez Quintero Julia
Salazar Marielos, Costa Rica