Tropical Grasses and Legumes:

Optimizing genetic diversity for multipurpose use

Project IP-5

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

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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

1. Optimized genetic diversity for quality attributes, for host-parasite-symbiont interactions, and for adaptation to edaphic and climatic constraints, for legumes and selected grasses.
2. 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 multiple stress resistance (different spittlebug species, *Rhizoctonia* and aluminum), with high forage quality and high seed production available as a commercial cultivar 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; SROs (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).

PROJECT WORK BREAKDOWN STRUCTURE
2003

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 are developed**	**Grass and legume genotypes with known reaction to pests and diseases and interaction with symbiont organisms are developed**	**Grass and legume genotypes with superior adaptation to edaphic and climatic constraints are developed**	**Superior and diverse grasses and legumes delivered to NARS partners are evaluated and released to farmers**
ACTIVITIES	• Selection of *Brachiaria* hybrids for high crude protein and digestibility • Animal production potential with selected grasses and legumes • Assessment of the potential of legumes with tannins to reduce methane in ruminants • Adjustment of methods for the simultaneous evaluation of tropical legumes for feed and soil improvement	• Pre-selection and propagation of apomictic and sexual *Brachiaria* hybrids • Selection of *Brachiaria* hybrids for resistance to spittlebug • Identification of 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 • Search for useful genes in tropical forage • Genetic control and molecular markers for spittlebug and reproductive mode in *Brachiaria*	• Genotypes of *Brachiaria*, other grasses and *Arachis* with adaptation to edaphic factors • Identification of genotypes of grasses and legumes with dry season tolerance • Shrub legumes with adaptation to acid soils and drought • Grasses with adaptation to poorly drained soils • Nitrification inhibition in tropical grasses	• Partnerships in LAC to undertake evaluation and diffusion of new forage alternatives • Partnerships in Asia to undertake evaluation and discussion of new forage alternatives • Development of methods for participatory evaluation of multipurpose forages in crop-livestock systems • Forage seeds: Multiplication and delivery of experimental and basic forage seed • Enhancing livestock productivity in Latin America • Expert systems for targeting forages and extension materials for promoting adoption of forages • Facilitate communication through journals, workshops and the internet • Update of a Forage Web Site

2.0 Revised Project Log-Frame, 2003

CIAT
Area: **Genetic Resources Research**
Project: **IP-5 Tropical Grasses and Legumes: Optimizing Genetic Diversity for Multipurpose Use** **Project Manager: Carlos E. Lascano**

Narrative Summary	Measurable Indicators	Means of Verification	Important Assumptions
Goal To contribute to the improved welfare of small farmers and urban poor by increasing milk and beef production while conserving and enhancing the natural resource base	• New cultivars of grasses and legumes used by farmers. • Raised productivity of livestock and crops while protecting biodiversity and land in savannas, forest margins and hillsides	Statistics 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 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 to farmers	• 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 an 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 tropicis 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

Research Highlights

- **Mixtures of forage legumes with different tannin types offer the possibility to manipulate methane production in ruminants fed with low quality diets**

We have made progress in defining the potential of tropical saponin-rich fruits to reduce methane emission from rumen fermentation and enhance N utilization by sheep. Results from in vitro and in vivo experiments carried out during the last two years showed that supplementation of fruits of *Sapindus saponaria* improve duodenal microbial protein flow and efficiency of rumen fermentation, and reduces ruminal methane release. This year we confirmed that the inclusion of tannin-rich legumes such as *Calliandra calothyrsus* and *Flemingia macrophylla* in forage-based diets significantly reduces methane release but also negatively affects nutrient degradation and N turnover.

We had hypothesized that to take advantage of the methane suppressing effect of tannin-rich legumes without affecting nutrient degradation and N turnover it was necessary to combine them with legumes low or free of tannin. Our results indicate that *C. calothyrsus* and *F. macrophylla* with high tannin content had similar chemical composition and tannin contents. However, the nutritional value of *F. macrophylla* was higher than that of *C. calothyrsus* when used in combination with a legume of low tannin content as *C. argentea*. Our results also showed that *F. macropylla* was less effective in suppressing methane emission than *C. calothyrsus*.

Future work will concentrate in defining the optimal type and proportion of tannin rich legumes in mixtures to take advantage of their methane suppressing potential without affecting nutrient degradation.

- ***Brachiaria* hybrids with combined resistance to multiple stress factors identified for the first time**

A major objective of the *Brachiaria* Improvement Program is to develop commercial cultivars that combine high level of resistance to abiotic and biotic stress factors. We have for the first time identified apomictic hybrids that combine adaptation to low fertility soils and high aluminum (Al), tolerance to drought and resistance to certain species of spittlebug. These new hybrids are now candidates for further field evaluation as potential cultivars for release.

Hybrid- FM9503-S046-024--- Tolerant to low nutrient supply, resistant to drought and to spittlebug and of high forage quality: Previous results from the entomology group had indicated the *Brachiaria* hybrid FM9503-S046-024 had antibiosis resistance to several spittlebug species (*Zulia carbonaria*, *Z. pubescens*, *Aenolamia reducta* and *Manaharva trifissa*) and tolerance to other species (*A. varia*). This year results from field studies in the Matazul Farm in the Llanos of Colombia indicated that after 3 years the *Brachiaria* hybrid FM9503-S046-024 was not only rapid in its establishment but that it also performed well over time in a low fertility-acid soil with low initial fertilizer application (kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S). Its superior performance in leaf biomass production was associated with its ability to acquire greater amounts of nutrients, particularly Ca and Mg from the soil solution. This hybrid was also very responsive to higher fertilizer application as revealed by live shoot biomass and total forage yield. Results after three years also indicate that under field conditions (Matazul, Llanos of Colombia) the *Brachiaria* hybrid FM9503-S046-024 has had an outstanding performance in the dry season (4 months dry) as indicated by a high proportion of green leaves as a result of its ability to acquire nutrients under water deficit conditions. Finally, results from grazing trials indicate that milk with Brachiaria hybrid FM9503-S046-024 is similar to the milk yield recorded in cv Mulato, which is known to have high forage quality.

Hybrid - BR02NO1372--- Tolerant to low nutrient -high Al and resistant to some species of spittlebug: For the last two years, we have implemented screening procedure to identify Al-resistant *Brachiaria* hybrids that were preselected for spittlebug resistance. Last year, we 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. This year we evaluated Al resistance of the most promising *Brachiaria* hybrids that are resistant/tolerant to spittlebug and found that BR02NO1372, with antibiosis resistance to *A. reducta* and tolerant to *Z. carbonaria*, had the lowest root mean diameter with and without Al in the solution. This hybrid has a superior total root length and fineness of roots than *B. decumbens* cv Basilisk (most widely planted commercial *Brachiaria* cultivar), which is well adapted to soils with low fertility and high Al but susceptible to spittlebug.

- **An antifungal protein was isolated from the seeds of a tropical forage legume**

An array of plant defense mechanisms can be triggered upon wounding or perception of microorganisms, including the synthesis of proteins and peptides that have antifungal activity. Various antifungal and/or antibacterial proteins such as chitinases, thionis, ribosome-inactivating proteins and permatins have been detected in seeds. In the last 4 years we have been examining a number of tropical forage legumes for antifungal properties. Among these examined, seeds of *Clitoria ternatea* exhibited a strong *in vitro* antifungal activity on the test fungus *Rhizoctocnia solani*. The crude extract from seed of *C. ternatea* with high antifungal activity could be eliminated with Pronase E indicating that the active compound is a protein. A new protocol was developed to facilitate the identification of the protein, which turned out to be highly basic (alkaline pI) named Finotin. This protein was active against a number of fungi and bacteria pathogenic on common beans, rice, forage grasses and legumes. The protein was also shown to be very toxic to first instar larvae of bruchid species.

The wide range of effects of Finotin against fungi, bacteria and insects seems to be an important component of the natural defense system of *C. ternatea* known to be free of major pests and diseases. The potential utility of Fenotin will be better defined when the gene coding for the protein is isolated. These results point out once again that tropical grasses and legumes can be an important source of useful genes for many tropical crops.

- **Results from on –farm trials demonstrate the benefits and limitations of *Brachiaria* hybrid cv Mulato**

On-farm evaluation of new forage options offers the opportunity to expose new forage cultivars to different abiotic and biotic constraints, production systems and management strategies. During this year, with financial support from Papalotla, on-farm trials have been established in Colombia and in different countries of Central America and Southeast Asia to validate the utility of *Brachiaria* hybrid cv. Mulato in different cattle systems.

Results confirm high plant vigor at establishment tolerance to certain species of spittlebug and to drought. However, results confirm that cv Mulato does not tolerate poorly drained soils and that in sites with high rainfall *Rhizoctonia solani* heavily attacks it. In sites with low fertility soils cv Mulato has shown symptoms of nutrient deficiencies (e.g., N) after one year indicating that it should be targeted to moderately fertile to fertile soils, in intensive livestock systems or in crop/pasture rotations that are economically profitable for use of fertilizer inputs.

In most location milk yield of cows grazing cv Mulato increased from 1.0 to 2.0 liters per day. Observations carried out in Honduras also indicate liveweight gains of steers of 900 g/an/day with cv. Mulato in contrast to 600 g/an/day with *B. decumbens* cv. Basilisk. In addition, stocking rate has also increased with cv. Mulato in relation to other *Brachiaria* grasses, which translates in more milk and beef per unit of land.

Results in Thailand indicate that cv. Mulato produced very high pure seed yields in small plots (13 kg from 288 m^2 equivalent to 450 kg pure seed/ha), which is considerably higher than yields recorded in Brazil and Mexico (150-200 kg/ha). Trials are being conducted in 2003 with smallholder forage seed producers to see if similar yields can be produced on-farm.

- **Livestock farmers in the llanos of Colombia are obtaining multiple benefits with the utilization of Cratylia as a dry season feed**

In February 2001 we started a 2-year project funded by PRONATTA (Programa Nacional de Transferencia de Tecnologia Agropecuaria) in the piedmont of the Llanos of Colombia to evaluate the utility of *Cratylia argentea* (Cratylia) in smallholder dairy farms. The original idea was for farmers to use Cratylia in a Cut & Carry system but some realized that this system was associated with high labor cost. The alternatives to Cut & Carry of Cratylia that some farmers implemented were silage production and direct grazing all year round using electric fences. Grazing of Cratylia has not caused plant mortality and in some farms a very productive association of the legume with *Brachiaria decumbens* (the grass originally in the plots) was formed.

We had postulated that the main benefit for dairy farmers in the Piedmont would be increased milk production in the dry season and consequently more cash flow. It was interesting however, to learn that farmers saw other benefits when using Cratylia:

a) Possibility of having high quality forage for cows in the middle of the rainy season when pastures were difficult to graze due to high soil moisture
b) Replacement of purchased supplements in the dry season, which has economic implications
c) Possibility of milking cows in the dry season and get higher price for the milk sold
d) Improved body conditions of cows which has been associated with improved reproductive performance

The adoption of Cratylia in the Piedmont of the Llanos of Colombia is an ongoing process being promoted not only by extension people who received training from the Project but also by enthusiastic farmers who have experimented and seen the benefits of the legume in their farms. To enhance adoption of Cratylia we are promoting commercial seed production with farmer groups in different regions of Colombia.

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

1.1 Selection of *Brachiaria* hybrids for high crude protein and digestibility

Highlight

- Developed a NIRS calibration equation to measure crude protein in *Brachiaria* hybrids

1.1.1 Calibration of NIRS for N in *Brachiaria*

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

Rationale

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

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

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

We had developed a narrow – based NIRS equation to predict IVDMD in *Brachiaria* hybrids, which had high precision as indicated by low SE of the calibration (0.98). In addition, estimates of IVDMD using NIRS had a high correlation (r = 0.73 to 0.80) with IVDMD values obtained with the two-stage Tilley and Terry in vitro procedure. This year we were interested in developing NIRS equations to estimate crude protein (CP) in *Brachiaria* hybrids.

Results and Discussion

We applied the NIRS calibration equations for CP with leaves of 50 *Brachiaria* hybrids that form part of a population (*B. ruziziensis* x *B. brizantha* cv. Marandu) used to develop molecular markers for spittlebug. For this we harvested only leaves from replicated (3) pots in the greenhouse. Results showed a high correlation between values of CP estimated using two NIRS equations (Table 1). The CP values ranged from 8.7 to 18.6 % in the first sampling and from 5.4 to 13.9 % in the second sampling. The higher CP content in the first sampling was related to the younger leaf tissue in the genotypes used in the analysis.

These results indicate that the NIRS equation we developed to screen *Brachiaria* hybrids for CP are adequate given the high correlation with protein values measured in the laboratory in tissue of different regrowth.

Table 1. Correlation between values of crude protein (CP) in leaves of *Brachiaria* hybrids measured in the laboratory and with NIRS using two equations (NIRS 1 and NIRS 2).

Sampling Period	No of Samples	CP Lab vs CP NIRS 1	CP Lab vs CP NIRS 2
1 *	150	0.96	0.97
2 **	150	0.92	0.91

* 90 days regrowth
** 168 days regrowth

1.1.2 Variation in quality attributes in *Brachiaria* hybrids

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

Rationale

We had reported previously very low or no correlation between IVDMD values obtained in samples of the same *Brachiaria* population cut at different times. We had postulated that this lack of correlation had to do with the sampling procedure (whole plant instead of leaves) 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).

This year we 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

Results and Discussion

Results showed a significant genotype x sampling interaction for CP, which was not the case for IVDMD (Table 2). It was also interesting to observe that for CP the variance associated with genotype was 3 times greater than the variance associated with the interaction genotype x sampling period, which was not the case for IVDMD. This is contrary to what would be expected from previous results.

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 sampling periods (CP: r = 0.30; IVDMD: r = 0.01) The reasons for these discrepancies could be related to large differences among genotypes in physiological maturity, known to affect forage quality.

Thus in order to select *Brachiaria* hybrids for quality parameters such as CP and IVDMD we still need to develop a standard sampling procedure, which includes fertilizer management and a uniform chronological or physiological age.

For this reason this year we transplanted 50 *Brachiaria* genotypes in the field in Quilichao in replicated (3) plots (spaced plants). Initially we will cut the plants at a uniform height and after 4 weeks of growth will harvest leaves in two different sampling periods without the application of fertilizer.

All samples (leaves) will be analyzed for CP and IVDMD using NIRS. Subsequently we will repeat the measurements of CP and IVDMD on plants with and without N fertilization.

Table 2. Variation in crude protein (CP) and in vitro digestibility (IVDMD) in *Brachiaria* hybrids

Sampling Period	CP (%)		IVDMD (%)	
	Mean	Range	Mean	Range
1*	13.1	8.7 – 18.6	70.0	59.5- 77.6
2**	10.6	5.4- 13.9	70.6	64.8- 73.7
Significance (P)				
Genotype	0.0001 (5.9552)***		0.0434 (9.8191)***	
Sampling Period	0.0001 (485.1408)		0.0308 (32.2752)	
Genotype x Sampling Period	0.0028 (2.9564)		0.0948 (9.0229)	

* 90 days of regrowth
** 168 days of regrowth
*** Values in parenthesis are the Error Mean Square

1.2 Animal Production potential with selected grasses and legumes

Highlight

- Milk yield with an advanced *Brachiaria* hybrid selection with resistance to some species of spittlebug was as high as in pastures of Mulato and higher than in pastures of other commercial *Brachiaria* cultivars

1.2.1 Milk yield with new *Brachiaria* hybrids

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

Rationale

We had reported that milk yield of cows grazing the commercial *Brachiaria* hybrid cv Mulato and the experimental *Brachiaria* hybrid FM 9503-S046-024 was higher than in the recently released *B. brizantha* cv Toledo. As observed in previous experiments, MUN (Milk Urea Nitrogen) was greater in cows grazing Mulato and the new hybrid FM 9503-5046-024 as compared with *B. brizantha* cv Toledo. Thus we concluded quality of the *Brachiaria* hybrid FM 9503-5046-024 was similar to the quality to the first released hybrid cv Mulato as reflected by milk yields.

This year we wanted to reconfirm the milk yield potential of the experimental hybrid FM 9503-S046-024 as compared to commercial *Brachiaria* cultivars.

Two grazing trials were carried out in a rainy period (September 24 to November 4, 2002) and in a dry period (November 5 to December 16, 2002) using 2 cows/ha. In each experiment a total of 6 cows (3 Holstein and 3 Zebu Crossbreds in mid lactation) arranged in a 3 x 3 Latin Square were used to measure milk yield in pastures that were mowed 3-4 weeks prior to grazing. Each period was of 14 days of which 7 were for adjustment to the treatment and 7 for measurement of milk yield milk composition parameters and pasture attributes.

In the two experiment we had the following treatments: T1: *B. brizantha* cv Toledo (control), T2: *B. decumbens* cv Basilisk and T3: *Brachiaria* hybrid FM 9503-S046-024.

Results and Discussion

Our results did not show a significant interaction of cow group and pasture for milk yield, so mean values across cow types are presented in Table 3. In the first experiment in the dry season we observed that milk yield was higher (10%) in the new *Brachiaria* hybrid FM 9503-S046-024 as compared to *B. decumbens* cv Basilisk and *B. brizantha* cv Toledo. In the second experiment during the rainy season milk yield was 18 to 27% higher in cows grazing the new *Brachiaria* hybrid FM 9503-S046-024 as compared to the two commercial cultivars.

In the two experiment MUN (Milk Urea Nitrogen) values were greater in cows grazing the new hybrid FM 9503-S046-024 as compared with *B. decumbens* cv Basilisk and *B. brizantha* cv Toledo. These higher MUN values have been consistently associated with higher CP in the leaf tissue of the new hybrid.

Our results from this year confirm that the *Brachiaria* hybrid FM 9503-S046-024 with resistance to some species of spittlebug has a higher quality than commercial cultivars and a similar quality to the commercial *Brachiaria* hybrid cv Mulato as reflected by milk yield. This new hybrid is currently under regional evaluation and particular attention is being placed on its seed production potential.

Table 3. Milk yield of cows grazing *Brachiaria* pastures in a dry and wet period (Quilichao Research Station).

Pastures	Dry Period		Wet Period	
	Milk Yield (kg/cow/d)	MUN (mg/dL)	Milk Yield (kg/cow/d)	MUN (mg/dL)
B. decumbens cv Basilisk	5.4 b	6.8 a,b	5.1 b	5.2 b
B. brizantha cv Toledo	5.5 b	5.8 b	5.5 b	4.5 b
Brachiaria hybrid FM 9503-S046-024	6.0 a	7.6 a	6.5 a	6.8 a

a, b means with different letters are different ($P<0.05$)

1.3 Assessment of the potential of legumes with tannins to reduce methane in ruminants

Highlights

- The supplementation with *S. saponaria* reduced daily methane release from sheep by over 10%.
- The addition of legumes with high levels of tannins to forage-based supplements reduced methane release per unit of organic matter fermented
- Supplementation with molasses reduced the negative nutritional effects of high concentrations of condensed tannins in legume by enhancing N turnover.

We have made considerable progress in defining the potential of saponin-rich fruits to reduce methane emission from rumen fermentation and enhance N utilization by sheep. The results of experiments carried out during the last two years showed that the fruits of *Sapindus saponaria* are valuable in supplementing tropical forage-based diets since they are effective in improving duodenal microbial protein flow and efficiency of rumen fermentation, and in suppressing ruminal methane release.

This year we confirmed that the inclusion of tannin-rich legumes such as *Calliandra calothyrsus* and *Flemingia macrophylla* in forage-based diets significantly reduces methane release but also inhibits nutrient degradation and N turnover. It was hypothesized that to take advantage of the methane suppressing effect of tannin-rich legumes without affecting nutrient degradation and N turnover, it was necessary to combine them with legumes free of tannin. This hypothesis was partially confirmed this year. However, the effects of legume mixtures were dependent on the type and proportion of tannin rich legumes included in the diet. Although *C. calothyrsus* and *F. macrophylla* presented similar tannin contents, the inclusion of these legumes in mixtures resulted in considerably different effects on rumen fermentation, and *F. macrophylla* was less effective in suppressing methanogenesis than *C. calothyrsus*. Thus, we still need to define the optimal type and proportion of tannin rich legumes in mixtures to take advantage of their methane suppressing potential without affecting nutrient degradation.

1.3.1 Rumen fermentation parameters and methane production with legumes of contrasting quality

Contributors: H.D. Hess (ETH Zurich), M. Kreuzer (ETH Zurich), A. Machmüller (ETH Zurich), R. Beuret (ETH Zurich), M. Lötscher (ETH Zurich),, C.E. Lascano (CIAT)

Rationale

Previous *in vitro*-experiments showed that the inclusion of fruits of *Sapindus saponaria* into tropical diets may suppress methane release by over 10% and that supplementation with leaves from the shrub legume *Cratylia argentea* increased methane release by 300%. However, no information was available on the effect of these tropical forage resources on methane release *in vivo*. Thus a respiratory chamber experiment was carried out to study the influence of a supplementation with fruits of *S. saponaria* and leaves of *C. argentea* on energy utilization and methane release of sheep fed a basal diet of *Brachiaria dictyoneura*.

Materials and Methods

Three basal diets with contrasting forage quality were included. The traditional diet consisted of a low quality hay (*Brachiaria dictyoneura* cv. Llanero), in the other two diets 33.3%, respectively sun-dried leaves of the shrub legume Cratylia argentea replaced 66.7% of the grass hay. All three diets were fed either with a concentrate containing 25% *S. saponaria* or with a control concentrate without *S. saponaria*. Animals were offered 60 g/kg $BW^{0.75}$ of forage and 20 g/kg $BW^{0.75}$ of concentrate per day. Six castrated growing lambs of the Swiss White Hill breed with a initial body weight of 30.1±2.8 kg were allotted to one of six treatments in a complete Latin Square design with 3 × 2 factorial arrangement of treatments (3 basal diets × 2 concentrates) and the 6 experimental periods were of 21 days each. The first 12 days of each experimental period were used for adaptation, days 13 to 20 for measurement of forage intake and total collection of feces and urine and day 21 for blood and rumen liquid sampling. Respiratory measurements were carried out during two 22.5 h-periods on days 19 and 20.

The forage required for the trial was harvested and dried at CIAT's research station in Quilichao (Cauca valley, Colombia) and fruits of *S. saponaria* were collected in the rural area near Cali (Cauca valley, Colombia). Forage and fruits were then shipped to Switzerland and stored at the ETH. The two concentrates were formulated to contain similar amounts of protein, fiber and energy. Approximately 180 kg of each concentrate were mixed and pelleted at the Institute of Animal Sciences at the ETH.

Results and Discussion

The two concentrates offered were consumed completely by all animals throughout the experiment and total DM and OM intakes per kg of metabolic body weight ($BW^{0.75}$) were not affected ($P>0.05$) by type of concentrate. Total DM and OM intakes responded linearly ($P<0.001$) as legume proportion increased, with the highest DM and OM intakes occurring in the treatments with 2/3 of legume in the basal diet. Intake of CP did not vary with type of concentrate (P>0.05) but responded linearly (P<0.001) to dietary legume proportion and increased from 5.98 g/kg $BW^{0.75}$ in the grass-alone treatments to 13.91 g/kg $BW^{0.75}$ in the 2/3-legume treatments.

Apparent total tract digestibilities of OM, CP, NDF and ADF were reduced (P<0.01) by supplementation with *S. saponaria* and digestibilities of OM, NDF, ADF were linearly reduced with increasing legume proportion. Apparent digestibility of CP, however, increased linearly and quadratically (P<0.001) as legume proportion increased.

Rumen fluid pH did not vary (P>0.05) with dietary treatment and averaged 6.76. Rumen fluid ammonia concentration was not affected (P>0.05) by type of concentrate but responded linearly (P<0.001) as dietary legume proportion increased. In the grass-alone treatments rumen ammonia concentration was as low as 2.45 mmol/l and increased to a level of 13.61in the 2/3-legume treatments.

Total VFA concentration was higher (P< 0.01) when the concentrate containing *S. saponaria* was supplied than with the control concentrate but did not vary (P>0.05) with dietary legume proportion. Supplementation with *S. saponaria* reduced (P<0.001) the molar proportions of iso-Butyrate and iso-Valerate but had no effect (P>0.05) on the other VFA. The molar proportion of acetate responded linearly to increasing legume proportion and decreased from 0.73 in the grass-alone treatments to 0.69 in the 2/3-legume treatments. The molar proportions of propionate, iso-Butyrate, n-Valerate and iso-Valerate increased linearly (P<0.001) as dietary legume proportion was raised. The acetate-to-propionate ratio tended to be lower (P=0.078) in the treatments with *S. saponaria* supplementation and decreased linearly (P<0.001) as legume proportion increased.

Total bacteria count was increased (P<0.01) by *S. saponaria* supplementation but showed no clear trend due to dietary legume proportion (P>0.05). Total ciliate protozoa count was reduced by over 50% in the treatments with *S. saponaria* but did not vary (P>0.05) with legume proportion. No significant interactions of dietary legume proportion and *S. saponaria* supplementation on rumen fluid characteristics were found (P>0.05).

Energy intake did not vary (P>0.05) with type of concentrate but responded to dietary legume proportion. Gross energy (GE), digestible energy (DE) and metabolizable energy (ME) intakes increased linearly (P<0.001) as legume proportion increased. Feeding the *S. saponaria* containing concentrate increased (P<0.001) energy losses through faeces, reduced energy losses through urine (P<0.05) and methane (P<0.01), had no effect (P>0.05) on energy expenditure (heat energy) and tended to increase (P=0.080) total energy losses. Energy losses through faeces, urine and heat, as well as total energy losses increased linearly (P<0.001) with increasing legume proportion, whereas energy losses through methane tended to decrease (P=0.075) with increasing legume proportion. Energy retention did not vary (P>0.05) with dietary treatments. No significant interactions of dietary legume proportion and *S. saponaria* supplementation on energy balance were found (P>0.05).

Daily methane release per kg of body weight was reduced (P<0.001) by 9% on average by *S. saponaria* supplementation and was not affected (P>0.05) by dietary legume proportion. Compared to the control concentrate, the *S. saponaria* containing concentrate reduced (P<0.05) methane release relative to CO_2 production and tended to reduce (P=0.063) methane release relative to OM digested but had no effect (P>0.05) on methane release relative to NDF digested. With increasing legume proportion, methane release relative to CO_2 produced and OM digested decreased (P<0.01), and methane release relative to NDF digested increased linearly (P<0.001). Methane release relative to energy retained was reduced by 30% (P<0.05) when *S. saponaria* was supplemented bud did not vary (P>0.05) with legume proportion. In contrast, methane release relative to N retained was not affected (P>0.05) by *S. saponaria* and decreased linearly (P<0.05) with increasing legume proportion. On average, methane release per gram of N retained was reduced by 60% due to legume supplementation. Haematocrite values and activity of liver enzymes (ASAT and GLDH) were not affected by dietary treatments and averaged 34.9%, 80.2 U/l and 10.3 U/l, respectively. This indicates that the dietary proportion of *S. saponaria* tested in this experiment did not affect the health status of sheep. The fact that interactions were mostly insignificant indicates that supplementation of *S. saponaria* fruits is a useful means to reduce methane emission from sheep fed tropical grass-alone and legume-supplemented diets. Legume supplementation represents an environmentally friendly way to increase animal performance of tropical livestock, since it was shown to improve N retention and to reduce methane release relative to body protein retention.

1.3.2 Effect of mixture of legumes with and without tannins on in vitro rumen fermentation parameters and methane production

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Rationale

A previous in vitro experiment had shown that the supplementation of a low-quality grass diet (*Brachiaria dictyoneura*) with *Cratylia argentea* drastically increased organic matter degradation, N turnover and methane release per unit of organic matter degraded, whereas the partial replacement of *B. dictyoneura* by *Calliandra calothyrsus* significantly reduced organic matter degradation, N turnover and methane release. Thus it was hypothesised that, to take advantage of the methane suppressing effect of *C. calothyrsus* without affecting nutrient degradation and N turnover, it was necessary to combine tannin-rich legumes with legumes free of or low in tannins. To test this hypothesis three Rusitec-experiments were performed (see also Activity 1.3.1 in this section) to evaluate the effect of supplementing legumes with contrasting contents of condensed tannins on methane production and N turnover in vitro.

Materials and Methods

In each experiment four basal diets were evaluated in four replicates. In experiment 1 the effect of the inclusion of *C. argentea* (0, 25, 50 and 100% of diet DM) in a diet of *C. calothyrsus* was investigated. In experiment 2 the effect of a partial replacement (50% of diet DM) of *F. macrophylla* by *C. argentea*, *C. calothyrsus* or by a mixture of both legumes was evaluated (Table 4). The daily dry matter supply was maintained constant at 15 g DM.

Table 4. Composition (% of DM) of the experimental diets

	Experiment 1			
Diet	1	2	3	4
Calliandra calothyrsus	100	75	50	-
Cratylia argentea	-	25	50	100
	Experiment 2			
Diet	1	2	3	4
Flemingia macrophylla	100	50	50	50
Calliandra calothyrsus	-	50	25	-
Cratylia argentea	-	-	25	50
	Experiment 3 (Activity 3.3)			
Diet	1	2	3	4
Brachiaria dictyoneura	100	50	50	50
Calliandra calothyrsus	-	50	25	-
Cratylia argentea	-	-	25	50

Results and discussion

In experiment 1, ammonia concentration in the fermenter fluid increased (P<0.001) from 0.81 mmol/l to 13.02 mmol/l when the proportion of *C. argentea* in the diet was increased from 0 to 100%. Counts of ciliate protozoa and bacteria also varied with the composition of the diet and were highest (P<0.05) with 50 and 100% *C. argentea*, intermediate with 25% *C. argentea* and lowest with 100% *C. calothyrsus*. Similarly, total bacteria counts were lowest with 100% *C. calothyrsus* and increased linearly (P<0.01) with increasing dietary proportions of *C. argentea*.

Apparent degradation of nutrients was also related to the level of *C. argentea* in the diet and was lowest in the diet with *C. calothyrsus* alone and increased linearly (P<0.001) with the proportion of *C. argentea*. Apparent organic matter degradation was twice as high in the diet of *C. argentea* alone (35.5%) as in the diet with *C. calothyrsus* alone (17.4%).

Daily methane release increased linearly (P<0.001) from 0.16 to 3.53 mmol/d when the proportion of *C. argentea* increased from 0 to 100%. When 25 or 50% of *C. calothyrsus* were replaced by *C. argentea* no changes occurred (P>0.05) in apparent crude protein degradation and only minor changes were observed in ruminal N turnover. Only the complete replacement of *C. calothyrsus* clearly increased apparent crude protein degradation and enhanced ruminal N turnover. These results suggest that the use of mixtures of *C. calothyrsus* and *C. argentea* is no means to improve ruminal N turnover without increasing methane release. This contrasts with the observations made in the third experiment (Activity 3.3) where the supplementation of low-quality *B. dictyoneura* with a mixture of *C. calothyrsus* and *C. argentea* (50:50) improved the nutritional quality of the diet and increase the ammonia concentration in the fermenter fluid without enhancing methane emission relative to organic matter degraded.

Therefore we suggest that the effects of legume mixtures on rumen fermentation not only depend on the quality and proportion of the different legumes, but also on the remaining components of the diet. Consequently, the evaluation of legume mixtures has to be done in combination with grasses, which represent the most important diet ingredient for ruminants in tropical smallholder livestock systems.

In experiment 2, ammonia concentration in the fermenter fluid was not affected (P>0.05) when 1/2 of *F. macrophylla* was replaced by *C. calothyrsus*, but increased (P<0.05) by 50% when 1/4 of the tannin rich legumes was replaced by *C. argentea*. With 50% of *C. argentea* in the diet, the ammonia concentration was 280% higher (4.6 mmol/l) than with *C. calothyrsus* alone (1.2 mmol/l).

Count of rumen ciliate protozoa was not affected (P>0.05) by the composition of the diet, but total bacteria count was increased (P<0.05) by 45% due to the inclusion of *C. argentea*. A small (-7%) but significant reduction in apparent organic matter degradation was recorded when 1/2 of *F. macrophylla* was replaced by *C. calothyrsus*.

The inclusion of 1/4 of *C. argentea* increased (P<0.05) apparent organic matter degradation by 13%, and with 1/2 of *C. argentea* in the diet, organic matter degradation increased (P<0.05) by 38% relative to the control (*F. macrophylla* alone). Methane release per gram of organic matter apparently degraded, decreased by 24% when 1/2 of *F. macrophylla* was replaced by *C. calothyrsus* and increased (P<0.05) by 27% and 57%, respectively, when 1/4 and 1/2 of *C. argentea* were included in the diet.

When 1/2 of *F. macrophylla* was replaced by *C. calothyrsus* no changes occurred (P>0.05) in apparent crude protein degradation and ruminal N turnover. In contrast, the inclusion of *C. argentea* in the diet resulted in a clear improvement of crude protein degradation and N turnover.

Overall, the replacement of 1/2 of *F. macrophylla* by *C. calothyrsus* resulted in only minor changes in rumen fermentation. Apparent organic matter degradation and methane emission were slightly reduced due to *C. calothyrsus* but the other major fermentation parameters remained unchanged. This suggests that both legumes had similar (negative) effects on rumen fermentation.

In contrast, the inclusion of *C. argentea* in the mixtures with the tannin-rich legumes enhanced rumen fermentation, as indicated by higher ammonia concentration and total bacteria count in the fermenter fluid and by improved apparent nutrient degradation and increased methane emission, both in absolute terms (mmol/d) and relative to organic matter degraded. It's worth mentioning, that apparent crude protein degradation in the mixture of *F. macrophylla* with *C. argentea* (50:50) was twice as high as in the mixture of *C. calothyrsus* with *C. argentea* in experiment 1 (27% vs. 14%). This indicates that *C. calothyrsus* and *F. macrophylla* have similar effects on rumen fermentation when used alone or in combination with each other, but contrasting effects are observed, when these tannin-rich species are mixed with a legume free of or low in tannins.

In general our results suggest that even though *C. calothyrsus* and *F. macrophylla* have similar chemical compositions and tannin contents, the nutritional value of *F. macrophylla* is higher than that of *C. calothyrsus* when these species are used in combination with a good-quality legume, but that is less effective in suppressing methane emission than *C. calothyrsus*.

1.3.3 Effect of supplementing low quality grasses with legumes and soluble carbohydrates on in vitro rumen fermentation parameters and methane production

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Material and Methods

In this in vitro experiment a grass-alone and three legume supplemented (50% of DM) diets were evaluated. The legume supplements consisted of *C. calothyrsus* (100%), *C. argentea* (100%) or a mixture of both legumes (1:1). All four basal diets were evaluated with and without the addition of sugarcane molasses (10% of DM). The eight treatments were tested during 4 × 10 day periods (n=4).

Results and Discussion

The pH of fermenter fluid averaged 6.97 and showed only minor variations between basal diets and due to the addition of molasses. The replacement of 50% of the basal diet by *C. calothyrsus* reduced ($P<0.05$) apparent degradability of N from 350 mg/g (grass-alone diet) to 80 mg/g without affecting fermenter fluid ammonia ($P>0.05$) (Figure 1). Compared to the grass-alone diet, the inclusion of *C. argentea* did not affect ($P>0.05$) apparent degradability of N but drastically increased fermenter fluid ammonia ($P<0.05$) from 1.0 mmol/l (grass-alone diet) to 4.2 mmol/l. When the legume mixture was included, apparent degradability of N was reduced ($P<0.05$) but fermenter fluid ammonia was increased ($P<0.05$).

As expected, the addition of molasses reduced ($P<0.001$) ammonia concentration with all diets. The effect of molasses on apparent N degradability was dependent on the kind of legume supplementation. While no effect was observed ($P>0.05$) when molasses was added to the grass-alone diet and the diets containing *C. argentea*, the addition of molasses to the diet supplemented with *C. calothyrsus* alone, drastically increased apparent N degradability from -45 to 203 mg/g (interaction between diet and molasses, $P<0.001$).

The reasons for this unexpected increase are not well understood but could be related to the higher availability of fermentable energy and hence increased microbial activity when molasses was added, or to the inactivation of condensed tannins due to the formation of complexes between tannins and soluble carbohydrates. Even though we do not completely understand this phenomenon, it is highly interesting, because it indicates that supplementation with molasses could be an alternative to partially reduce the negative

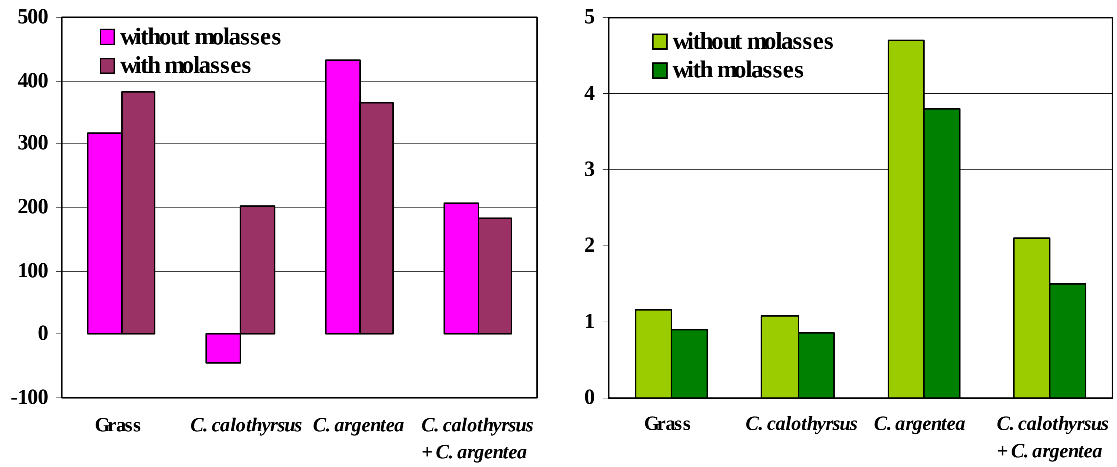

Figure 1. Apparent rumen degradability of nitrogen (mg/g) (on the left) and ammonia concentration (mmol/l) (on the right) observed in Rusitec-fermenters supplied with a grass-alone and with legume supplemented (50% of DM) diets. The legume supplements consisted of C. calothyrsus (100%), C. argentea (100%) or a mixture of both legumes (50:50). All diets were evaluated with and without the addition of sugarcane molasses (10% of DM).

nutritional effects of feeding legumes with high concentrations of condensed tannins.

The supplementation with *C. calothyrsus* alone reduced the apparent degradability of organic matter and neutral detergent fiber (P<0.05) (Figure 2, left). The supplementation with *C. argentea*, in contrast, increased organic matter and fiber degradation (P<0.05). Supplementation with the legume mixture increased organic matter degradation (P<0.05) and had no effect on fiber degradation (P>0.05), indicating that the negative effects of supplementing *C. calothyrsus* on fiber degradation can be avoided when this legume is supplied in combination with a legume low in tannins.

Daily methane release (-50%) and methane release relative to organic matter degraded (-40%) were reduced (P<0.05) by supplementation with *C. calothyrsus* alone (Figure 2), which agrees with the results from a previous experiment. Supplementation with *C. argentea* alone increased (P<0.05) daily methane release (+100%) and methane release relative to organic matter degraded (+40%), which is also in agreement with the results of the previous experiment.

When the low-quality grass diet was supplemented with the mixture of *C. calothyrsus* and *C. argentea*, daily methane release was increased (P<0.05) by 30% but methane release relative to organic matter degraded remained unaffected (P>0.05). Interactions between basal diet and molasses addition were mostly insignificant, except for apparent N degradation (see above). On average among all diets, molasses addition increased (P<0.05) organic matter degradation and methane release and reduced (P<0.05) fiber degradation.

Results of this experiment confirmed the methane suppressing potential of the tannin-rich *Calliandra calothyrsus* and suggest that supplementing mixtures of legumes with high and low contents of condensed tannins could be a useful alternative to improve nutrient supply and ruminal organic matter degradation avoiding the dramatic increase in methane release typically observed when low-tannin legumes are supplemented alone. Additionally, results indicate that supplementation with molasses could be an alternative to partially reduce the negative nutritional effects of feeding legumes with high concentrations of condensed tannins as it enhances N turnover.

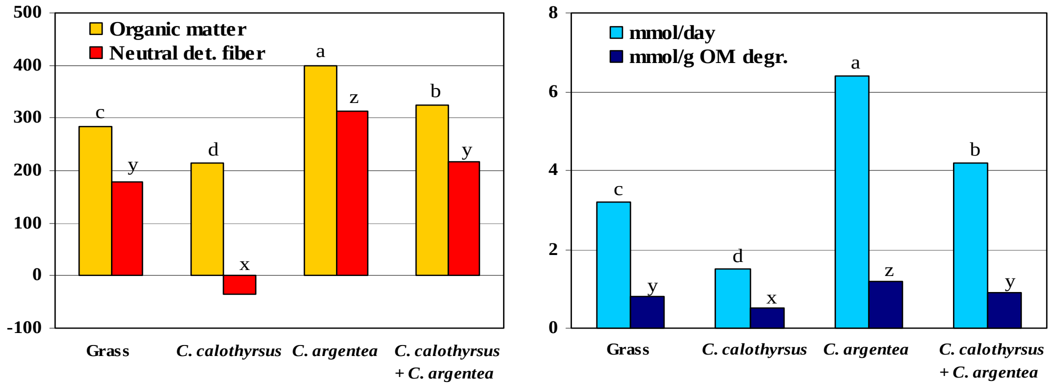

Figure 2. Apparent degradability of organic matter and neutral detergent fiber (mg/g) (on the left) and methane release (on the right) observed in Rusitec-fermenters supplied with a grass-alone and with legume supplemented (50% of dry matter) diets. The legume supplements consisted of *C. calothyrsus* (100%), *C. argentea* (100%) or a mixture of both legumes (50:50).

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

Highlights

- Mixing tannin free legumes with tannin containing legumes is a possibility to reduce the adverse effects of tannins
- PEG application to bind tannins had positive effects on anaerobic protein degradation but not in the aerobic degradation experiments

Rationale

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

In mixed crop-livestock production systems, legume quality is a key factor for obtaining maximum benefits in terms of rate and extent of dry matter loss and N release in the rumen or in the soil. Consequently, Animal Nutritionists and Soil Scientists have been interested in defining plant quality parameters that are correlated with the loss of dry matter and the release of nutrients from tropical legumes. However, studies on quality of legumes often have been carried out in an independent manner and there has been little information sharing on methodological aspects between soil and animal nutrition research.

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

Ruminant animals also decompose plant material through microbes that degrade plant protein and cell wall constituents to ammonia, amino acids and energy for the host animal. To assess the extent and rate of dry matter loss from plant material used as a feed resource, samples are incubated with rumen microbes using *in-vitro* systems or *in situ* techniques. Principles are similar to the nylon litterbag method used for soil studies. However, soil and rumen processes involved in plant degradation have fundamental differences namely an anaerobic aqueous environment and much faster degradation rates in the rumen compared with soil. Despite these differences, the extent and rate of dry matter loss and nutrient release from plants in the two processes is affected by plant chemical composition, e.g. N, lignin and condensed tannins content.

To test the applicability to aerobic soil- related process of anaerobic methods used to assess quality of forages for ruminants the following studies were carried out:

a) The effect of mixtures of legumes with contrasting quality on anaerobic N degradation and aerobic N release
b) Effect of binding tannins in legumes with PEG on anaerobic degradation in the rumen and aerobic decomposition on the soil
c) Effect of binding tannins in legumes with PEG on anaerobic N degradation and aerobic N release

1.4.1 The effect of mixtures of legumes with contrasting quality on anaerobic N degradation and aerobic N release

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Materials and Methods

Two tropical shrub legumes with contrasting quality *Indigofera constricta* Rydb., (Indigofera, no tannin content), and *Calliandra* sp. nov. (Calliandra, high soluble tannin content), were selected. Freeze-dried plant material was ground to 1 mm and mixed in the following proportions (based on air dry matter basis): 100:0, 75:25, 50:50, 25:75, 0:100 (Calliandra : Indigofera).

Chemical characterization of plant material: All samples were subjected to the following chemical analysis: N, C, P, NDF, ADF, IADF, lignin, soluble and bound condensed tannins (sCT and bCT) utilizing standard protocols.

For measuring N release during aerobic degradation, a leaching tube experiment was carried out. Glass leaching tubes (length 15 cm; diameter 5 cm) were filled sequentially with a piece of glass wool, followed by a thin layer of acid-washed sand and a mixture of air-dried soil (45 g)/ acid-washed sand (1:1). A sample of 200 mg of each species and treatment, ground to pass a 1 mm screen, was added on the surface of the soil-sand mixture. A further layer of 10 g sand covered the plant material to avoid soil disturbance during the addition of the leaching solution. The soil-sand mixture was brought to approximately 80% water holding capacity (WHC). Leaching was carried out applying 100 ml leaching solution per tube (1 *mM* $CaCl_2$, 1 *mM* KH_2PO_4, 1 *mM* $MgSO_4$) after 7, 14, 28, 42, 70, 84, 112 and 140 days. Leachates were analyzed for mineral N content (NO_3-N and NH_4-N).

For measuring anaerobic degradation the *in-vitro* dry matter digestibility method (IVDMD) and the gas production method were used.

Results

The measured quality parameters of the legume mixtures were compared with estimated values. Estimated values of legumes mixtures were calculated from measured values obtained in analysis of sole samples of Calliandra and Indigofera. The results of sole legume samples were then fitted to the different proportions of Calliandra-Indigofera mixtures. Example: To estimate the value for the 50% Calliandra/50% Indigofera mixture the following formula was

applied:
Value $_{estimated}$ = (Value$_{Calliandra}$/2 + Value$_{Indigofera}$/2).
The estimated values indicate the theoretical or additive value, which should be obtained in mixing two legume materials. If measured and estimated values show no significant differences, it is assumed that no interaction occurred during mixing. If measured and estimated values are significantly different, it is assumed that interaction occurred. Interaction in this case means, that plant components of one species reacted with plant components of the other species.

Chemical characterization of mixed plant material: The NDF, ADF, Lignin and IADF content of the tissue decreased linearly with increasing proportion of Indigofera. Soluble CT content decreased with increasing proportion of Indigofera, while N content and IVDMD increased with increasing proportion of Indigofera in the mixture.

In the mixture of 25% Calliandra and 75% Indigofera (Call25/Ind75) measured values of IVDMD were significantly higher ($P<0.001$) than estimated values. Interactions were also observed for the sCT content, which was lower ($P<0.01$) than the estimated sCT content and for the bCT content, which has higher ($P<0.05$) than estimated (Figure 3). In the mixtures (Call25/Ind75) and (Call50/Ind50) the measured IADF content was lower ($P<0.05$) than estimated. For ADF, NDF, Lignin and N measured and estimated values were similar indicating that no interactions occurred.

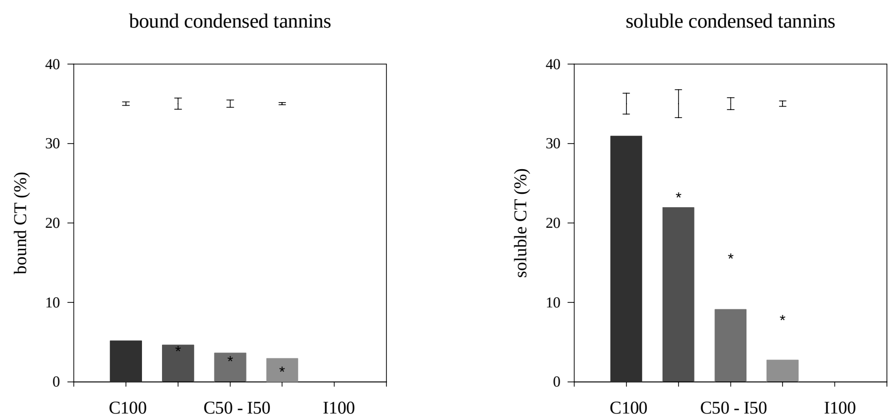


Figure 3. Condensed tannin content of Calliandra (C) and Indigofera (I) mixed in the following proportions: 100:0, 75:25, 50:50, 25:75, 0:100 from Calliandra (left side) to Indigofera (right side) * = estimated value, bars = measured value, I = SE of measured values

Leaching tube experiment In the aerobic leaching tube, net N mineralization rate(k) showed no differences ($P>0.01$) between Ind100, Call25/Ind75 and Call50/Ind50. Highest N mineralization for these treatments was observed during week 2 to 6 of the experiment. Maximum N mineralization was higher ($P<0.01$) for pure Indigofera than for the Call25/Ind75 and the Call50/Ind50 treatment. Extent of N mineralization showed similar values for the Call100 treatment and the Call75/Ind 25 treatment. In both cases N immobilisation was observed. The measured extent of N mineralization was lower ($P<0.01$) than the estimated extent of N mineralization in treatment Call50/Ind50 (Figure 4) indicating that this mixture did not resulted in additive effects on extent of mineralization.

Anaerobic gas production and N degradation: Gas production rate (k) and extent of gas production increased significantly ($P<0.01$) with increasing proportions of Indigofera in the mixture. The measured gas production in the mixture of Call50/Ind50, was significantly ($P<0.01$) higher than the estimated value. Measured *in-vitro* dry matter degradation was also higher than the estimated value in the Call25/Ind75 treatment. In contrast, in the Call75/Ind 25 treatment, measured degraded N

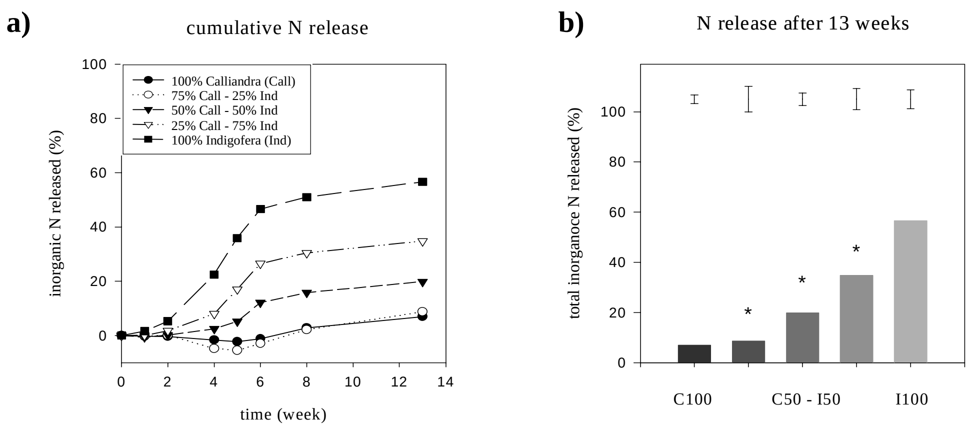


Figure 4. a) Cumulative N release during 13 weeks and b) extent of total released N in the aerobic leaching tube experiment. Calliandra and Indigofera mixed in the following proportions: 100:0, 75:25, 50:50, 25:75, 0:100 from Calliandra (C) to Indigofera (I)

* = estimated value, bars = measured value, I = SE of measured values

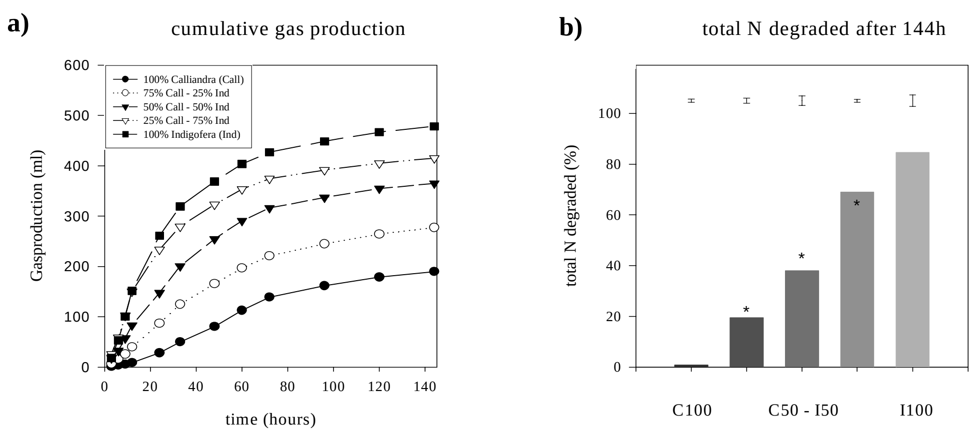


Figure 5. a) Gas production during 144 h and b) total degraded N anaerobic gas production experiment Calliandra and Indigofera mixed in the following proportions: 100:0, 75:25, 50:50, 25:75, 0:100 from Calliandra (C) to Indigofera (I)

* = estimated value, bars = measured value, I = SE of measured values

was significantly lower than estimated degraded N (Figure 5).

Discussion

In the aerobic system the extent of measured mineralized N was lower than estimated for the mixture Call50/Ind50. Stronger N immobilization was observed in the Call75/Ind25 than in Call100. Thus it seem, that mixing prunings of the two legumes altered N mineralization, at the expense of a reduced total amount of mineralized N. In the long run, the remaining N as result of mixing legumes might contribute to the formation of humic and fulvic substances. The strong immobilization of N found in Call75/Ind 25 relative to Call100 might be due to a rapid increase of microorganisms nourished by soluble and easily available C components provided from the 25% of Indigofera.

This may have lead to the incorporation of N by microorganisms, an effect known as short term N-lack. When the mixture consisted of more than 50% of the high-tannin legume Calliandra, the amount of sCT increased sharply in the mixture and reached the additive value. However, no short -term positive effects in N release were observed with any of the legume mixtures during the duration of this study.

Mixing the low quality legume *Calliandra* sp. with the high quality legume *Indigofera constricta* improved the extent of gas production (Call50/Ind50), *in-vitro* dry matter degradation and IVDMD (Call25/Ind75). On the other hand, measured degraded N was not different (P>0.01) from estimated N for Call50/Ind50 and Call25/Ind75. Thus no N loss occurred due to the CT complexation suggesting that a wide range of other tannin-binding compounds such as carbohydrates present in Indigofera may have spared proteins from binding with tannins. In the Call75/Ind25 mixture the sCT content were very high and as a consequences inhibited N degradation.

Soluble and bound condensed tannins did not show an additive behaviour in the 25Call/75Ind and 50Call/50Ind mixtures, which could explain some of the positive and negative effects on the aerobic and the anaerobic systems. For example in the 50Call/50Ind mixture, there was a positive interaction in the anaerobic system as indicated by higher amount of gas production than was estimated for the mixture. Thus it seems that the anaerobic system copes more effectively with the adverse effects on nutrient release or degradation of high sCT content. The high diversity of microorganisms in the rumen liquid may allow a quick adaptation to changing substrates due to the short residence time of the nutrients in the rumen. Degradation processes in the soil are more long term and composition of microorganisms may not change as quick as in the rumen.

1.4.2 Effect of binding tannins in legumes with PEG on anaerobic degradation in the rumen and aerobic decomposition on the soil

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

Materials and Methods

Plant samples were collected from four shrub legume species with contrasting qualities (nil, medium and high tannin content) namely: *Indigofera constricta* Rydb., (Indigofera), *Leucaena leucocephala* (Lam.) de Wit. CIAT 21245 (Leucaena) and 2 provenances of *Calliandra calothyrsus* Meisn: San Ramon CIAT 22310 (San Ramon) and Patulul CIAT 22316 (Patulul). Two provenances of Calliandra, San Ramon and Patulul were taken because of differences in the chemical structure of the extractable tannins. While San Ramon's condensed tannins comprise mainly of gallocatechin/epigallocatechin (producing prodelphinidin with butanol/HCL), Patulul contains mainly catechin/epicatechin subunits producing procyanidin with butanol-HCl Leucaena is known to have condensed tannins composition similar to Patulul. Indigofera is a tannin free species.

Use of Polyethylenglycol (PEG) (application by soaking the entire plant material): Whole freeze-dried leaves were placed in plastic containers and were soaked with PEG solution to obtain a 5 % PEG treatment (+ PEG). For the control treatment (- PEG) the plant material was soaked with de-ionized water.

Chemical characterization of plant material: All samples were subjected to the following chemical analysis: N, C, P, NDF, ADF, IADF, lignin, soluble and bound condensed tannins (sCT and bCT) and ash utilizing standard protocols.

For measuring aerobic degradation a greenhouse litterbag experiment was carried out. Litterbags (nylon mesh bags, 10x10 cm, mesh size 1,5 mm) were filled with 4.5 g of whole plant material and closed with a nylon thread. Litterbags were placed on the surface of soil in plastic pots filled with 2 kg of soil. During the experiment, water-holding capacity (WHC) in the pots was maintained at 60% by weighting the pots weekly and adding water accordingly. Bags were collected at 7, 14, 28, 56, and 112 and were dried for 3 days at 40 °C.

The weight of plant material in each litterbag was recorded and ground in a Wiley mill to pass a 1 mm screen.

For measuring anaerobic degradation the *in-vitro* dry matter digestibility method (IVDMD) and the gas production method were used.

Results

Litterbag experiment: Decomposition rate, averaged over all treatments, was highest for Indigofera, followed by Leucaena, Patulul and San Ramon (Table 5). The extent of decomposition showed significant differences between species following the same order as the decomposition rate (Table 6). The order of decomposition rate and extent was as follows: Indigofera (high N, no CT, low lignin) > Leucaena (intermediate N, intermediate CT, high lignin) > Calliandra Patulul and San Ramon (intermediate N, high CT, high lignin).

Averaged over all species, differences in the extent of decomposition between the +PEG and –PEG treatments were observed. Surprisingly most remarkable differences in dry matter loss between +PEG and –PEG were observed in the species Indigofera and Leucaena (Table 6). In contrast, PEG treatment had no influence on dry matter loss of San Ramon and Patulul.

Gas production experiment: Gas production rate and extent was significantly different between species, being highest for Indigofera, followed by Leucaena, San Ramon and Patulul (Tables 5 and 6). In addition, *in-vitro* dry matter degradation was different between species. Further, gas production rate and extent were different between forage tissue treated and not treated with PEG. Likewise, *in-vitro* dry matter degradation showed significant differences due to PEG. Unexpectedly, *in-vitro* dry matter degradation was higher (P<0.001) for +PEG than for the -PEG treatment in the tannin free Indigofera.

Digestibility: IVDMD were significantly different among species and in between PEG treatments. Interaction between species and treatments was observed. Likewise, dry matter degradation, and IVDMD were difference (P<0.001) between +PEG and -PEG treatment for the tannin free species Indigofera (Table 6).

Table 5. Decomposition rate (k_D) and gas production rate (k_G) of Indigofera, Leucaena and Calliandra San Ramon and Patulul with and without PEG treatment

Plant species	Treatment	k_D	k_G	
Indigofera constricta	+ PEG	-0.00725	0.065	
	- PEG	-0.00512	0.060	
Leucaena leucocephala	+ PEG	-0.00389	0.047	
	- PEG	-0.00222	0.040	
Call – San Ramon	+ PEG	-0.00112	0.038	
	- PEG	-0.00093	0.033	
Call – Patulul	+ PEG	-0.00192	0.038	
	- PEG	-0.00077	0.027	
Significance (p) of effects of		SED		SED
Species			***	0.0005
Sample pre-treatment			***	0.0004
Interaction			***	0.0007

SED = Standard error of the difference between means
*** = (P<0.001)

Discussion

Soluble condensed tannins (sCT) were rapidly reduced in whole leaves during aerobic decomposition in litterbags as shown in Figure 6. As also observed in other decomposition studies, leaching is likely the most important form of sCT loss during decomposition.

Contrary to expectations, losses of sCT in Patulul or Leucaena were not affected by PEG. It is possible, that PEG-tannin complexes were not formed by the application of PEG to the whole leaves. In other words, during the decomposition period in the soil with whole leaf material, PEG only covered the leaves and did not enter into leaf cells vacuoles where tannins are located.

In the case of the two Calliandra provenances there was clear effect of PEG on sCT as indicated by slower loss of sCT in San Ramon as

Table 6. Extent of decomposition (E_D), gas production (E_{Gas144}) and *in-vitro* dry matter degradation ($IVDMD_{Theod}$) and IVDMD of Indigofera, Leucaena and Calliandra San Ramon and Patulul with and without PEG treatment.

		aerobic		anaerobic					
Plant species	**Treatment**	E_D		E_{Gas144}		DM degradation		IVDMD	
Indigofera constricta	+ PEG	47.72		495.32		94.73		98.15	
	- PEG	57.81		478.46		89.12		92.59	
Leucaena leucocephala	+ PEG	62.28		375.02		68.66		87.82	
	- PEG	75.38		348.52		58.46		77.24	
Call – San Ramon	+ PEG	85.83		268.20		49.93		56.88	
	- PEG	95.89		184.18		37.23		37.80	
Call – Patulul	+ PEG	88.61		266.19		46.75		63.84	
	- PEG	90.81		173.30		43.36		44.92	
Significance (p) of effects of			SED		SED		SED		SED
Species		***	3,39	***	8,58	***	0,610	***	0,520
Treatments		**	2,40	***	6,07	***	0,431	***	0,367
Interaction		n.s.	4,80	***	12,14	***	0,862	***	0,735

SED = Standard error of the difference between means
** = (P<0.01), *** = (P<0.001), n.s. = (P>0.05)

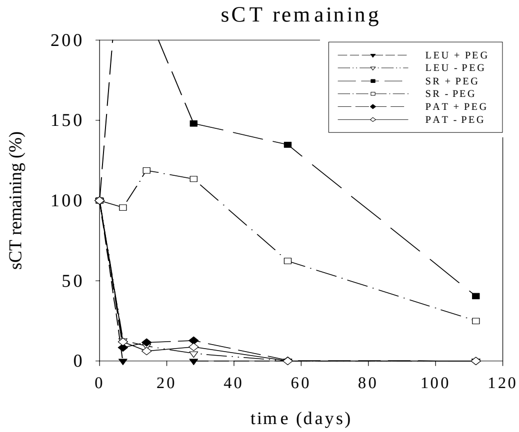


Figure 6. Remaining sCT content in litterbags filled with leave material of Leucaena (LEU), San Ramon (SR) and Patulul (PAT), + PEG and –PEG during decomposition of 113 days

compared to Patalul. This might be due to the different composition of condensed tannins in the two provenances. Gallocatechin and Epigalloctechin, which are only present in San Ramon, usually leach more slowly than Catechins, due to their higher solubility. The low solubility of Gallocatechin and Epigalloctechin allowed to complex with PEG.

Both, Indigofera and Leucaena have very soft leaves in comparison to Calliandra (San Ramon and Patulul). The highly hydroscopic property of PEG may have had a stronger impact on the larger and thinner leaves than on small and tough leaves. This could have made leaves more accessible to microorganisms. It could be also hypothized, that the hydroscopic property of PEG influenced cell vacuoles of Leucaena cells and produced the rapid decline of sCT, which was not the case in San Ramon. It is possible that the hygroscopic nature of PEG had an influence on digestibility of the tannin-free species Indigofera.

1.4.3 Effect of binding tannins in legumes with PEG on anaerobic N degradation and aerobic N release

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

Material and Methods

Plant samples were collected from four shrub legume species with contrasting qualities (nil, medium and high tannin content) namely: *Indigofera constricta* Rydb., (Indigofera), *Leucaena leucocephala* (Lam.) de Wit. CIAT 21245 (Leucaena) and 2 provenances of *Calliandra calothyrsus* Meisn.: San Ramon CIAT 22310 (San Ramon) and Patulul CIAT 22316 (Patulul). For chemical characteristics of tannins in legumes see 1.4.2.

Use of Polyethylenglycol (PEG) (application in ground material): Freeze-dried leave material was ground to pass a 1 mm screen and mixed carefully with PEG solution to receive a PEG application at 7%. Control treatments were mixed with the same amount of de-ionized water.

Chemical characterization of plant material: All samples were subjected to the following chemical analysis: N, C, P, NDF, ADF, IADF, lignin, soluble and bound condensed tannins (sCT and bCT) and ash following standard protocols. For measuring N release during aerobic degradation the leaching tube experiment was carried out as as described in 4.1. For the measuring of anaerobic degradation, the *in-vitro* dry matter digestibility method (IVDMD) and the gas production experiment were carried out.

Results

Leaching tube experiment Significant differences between species were observed for the rate (data not shown) and extent (Table 7) of N release. No differences between + PEG and – PEG treatments were observed either for rate or for extent of N release. For the N release rate an interaction between species and treatments was observed.

Table 7. Extent of released N (E_N) and degraded N (E_{Ndeg}) of Indigofera, Leucaena and Calliandra San Ramon and Patulul with and without PEG treatment.

		Released N (aerob)		Degraded N (anaerob)	
Legumes	**Treatment**	**E_N**		**$E_{N\,deg}$**	
Indigofera constricta	+ PEG	68.13		85.77	
	- PEG	64.87		84.81	
Leucaena leucocephala	+ PEG	41.95		66.12	
	- PEG	34.75		41.39	
Calliandra– San Ramon	+ PEG	34.44		27.68	
	- PEG	35.96		15.11	
Calliandra– Patulul	+ PEG	30.07		25.82	
	- PEG	27.85		7.32	
Significance (p) of effects of			SED		SED
Specie		***	4,34	***	0,937
Treatments		n.s.	3,07	***	0,662
Interaction		n.s.	6,14	***	1,320

SED =Standard error of the difference between means
*** = ($P<0.001$), n.s. = ($P>0.05$)

Gas production experiment Significant differences between species were observed for the extent of degraded N (Table 7). In addition, + PEG and -PEG treatment were significantly different. The tannin free control plant showed no differences due to PEG application.

Discussion

The application of PEG affected N degradation in the anaerobic gas production experiment as expected, but did not show the expected effect in the aerobic leaching tube experiment. In the leaching tube experiment results showed that the application of PEG lowered the sCT in legumes to nill. It is evident, however that these differences in the sCT content due to PEG at time 0 had no influence on N release over time. It was expected that in the +PEG treatment, mineralization would have been higher than in the –PEG treatment, due to the tannin binding capacity of PEG.

It is postulated, that PEG-tannin complexes were not formed in the ground material placed in the leaching tubes given that PEG and sCT need a liquid medium to complex, which was absent during the application of PEG to the ground material. The formation of tannin-protein complexes may have only taken place in the test tubes during the extraction phase in the tannin assay.

In conclusion, both experiments showed that PEG is not suitable for aerobic decomposition and mineralization experiments in which the objective is to assess the effect of tannins from legumes on degradation and N release. This study confirmed however, that PEG is useful to study the effect of tannins in feed quality of tropical legumes.

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

2.1 Pre-selection and propagation of apomictic and sexual *Brachiaria* hybrids

Highlights

- The *Brachiaria* improvement program continued to advance during 2003. A new cycle of the sexual population was sown, propagated, and established in field nurseries in two sites in Colombia. Over 3,000 seedlings are included in these two nurseries.
- A set of 64 "pre-selected" SX x AP clones was propagated for detailed assessment of reaction to three Colombian spittlebug species, reaction to *Rhizoctonia* foliar blight, and aluminum tolerance.
- Open-pollinated (OP) progenies of these pre-selections were established in a field trial to assess reproductive mode.

2.1.1 Pre-select and propagate sexual x apomictic hybrids from field nursery for spittlebug screening

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

The *Brachiaria* improvement program is based on recurrent, cyclical improvement of a synthetic tetraploid, sexual population. A two-year cycle is currently used. Phenotypic selection among several thousand genotypes is conducted sequentially, first on visual assessment of unreplicated spaced plants in field nurseries at two contrasting sites, and subsequently on more detailed assessment of specific traits such as resistance to spittlebugs, reaction to *Rhizoctonia* foliar blight, and tolerance to aluminum. Each cycle, a set of selected sexual clones is identified for recombination to produce the subsequent cycle of the sexual population, and also to cross with elite apomicts to generate hybrid populations from which superior hybrid apomictic clones can be selected for possible development into commercial cultivars.

In 2002, a set of over 2,000 SX x AP (sexual-by-apomictic) hybrids were generated, propagated, and established in field trials. These were culled to 64 by simple visual assessment by 01 Jan 2003. These 64 pre-selections were propagated for more detailed assessment of reaction to spittlebugs, *Rhizoctonia* foliar blight, and Al tolerance. OP progenies were established for assessment of reproductive mode.

2.1.2 Evaluation of sexual populations and pre-selection of clones for spittlebug screening

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

Spittlebug resistance remains a prime-breeding objective. Only sexual clones with resistance to several spittlebug species are included as parentals of the sexual population. This year, just over 3,000 sexual (SX) recombinants were established in field nurseries in April and May 2003. The population is being culled down to approx. 1,000 "pre-selections" by periodic visual evaluation. By year's end, we shall have a list of "pre-selections", which will be propagated for screening for resistance to three Colombian species of spittlebug.

As of reporting date (08 September 2003), a large number of plants have been culled, based on undesirable phenotype in the testing sites.

Vegetative propagation from CIAT-Quilichao to CIAT headquarters will begin in October. Propagation for spittlebug screening will commence in early January 2004.

2.1.3 Propagation of pre-selected sexual x apomictic and sexual hybrids to screen for *Rhizoctonia* reaction

Contributors: J.W. Miles, S. Kelemu, and C. Zuleta (CIAT)

Rhizoctonia foliar blight is a devastating disease of susceptible *Brachiaria* genotypes. The current *Brachiaria* breeding populations have generally high levels of susceptibility. Reliable, massive screening methodology has been lacking. Recent developments in refinement of screening methodology are promising. An initial test population includes 64 "pre-selected" SX x AP genotypes generated in 2002, plus 20 promising hybrid clones involving the highly *Rhizoctonia*-resistant *Brachiaria brizantha* accession CIAT 16320 as pollen parent.

In 2002, a set of over 2,000 SX x AP (sexual-by-apomictic) hybrids were generated, propagated, and established in field trials. These were culled to 64 by simple visual assessment by 01 Jan 2003. These 64 pre-selections were propagated for more detailed assessment of several traits, including reaction to *Rhizoctonia* foliar blight. Also in 2002, a set of 387 hybrids involving the highly *Rhizoctonia*-resistant *B. brizantha* accession CIAT 16320 as pollen parent were established in the field at CIAT-Quilichao. Twenty-nine of these hybrid clones were pre-selected on visual assessment in this trial. The aim is to identify sexual clones among the hybrids with CIAT 16320 that are *Rhizoctonia*-resistant so as to introgress genes for resistance into the synthetic tetraploid, sexual *Brachiaria* breeding population.

Refinements are still being made in screening methodology. A detached-leaf technique is being used. Preliminary results suggest that this method will be adequate at least to identify the most resistant genotypes with reasonable reliability. The method is quick (results in 5 days) and cheap. Only a very small amount of plant material is needed. Of the 93 genotypes being tested, a selected sub-set of 36 has been identified. Reactions of the genotypes in this group are being reconfirmed, while refinements in the screening technique itself are being made.

2.1.4 Propagation of pre-selected SX x AP clones for screening of Al tolerance in solution culture

Contributors: J.W. Miles and I.M. Rao (CIAT)

Candidates for commercial cultivars need to be assessed for reaction to aluminum toxic soils. Sixty-four clones were pre-selected from an initial set of over 2,000 hybrids produced in 2002.

"Pre-selected" hybrid clones were vegetatively propagated and delivered to the plant nutrition group for screening.

2.1.5 General adaptation and agronomic traits of sexual seedlings and pre-selection for Al tolerance

Contributors: J.W. Miles and I.M. Rao (CIAT)

Tolerance to high soil aluminum levels is a desirable attribute in *Brachiaria* cultivars. It is one of the traits we are attempting to incorporate in bred cultivars.

Owing to inherent difficulties in assessment of Al tolerance, rapid screening of large (~1,000 genotypes) populations has not been possible. We are attempting this year to make "pre-selections" earlier (October), so that vegetative propagation from the field at Quilichao can be initiated opportunely to provide timely availability of adequate volumes of plant material for aluminum screening.

We are on schedule to begin propagation of "pre-selections" in October.

2.1.6 Establishment of replicate sets of open pollinated sexual plants in field nurseries in Puerto López (Matazul) and CIAT-Quilichao

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

The *Brachiaria* breeding strategy is based on recurrent, cyclic improvement in a synthetic tetraploid, sexual breeding population.

A new breeding cycle was initiated in 2003, with the generation of a large (>3,000) recombinant population from 22 parental clones selected from the previous cycle. Recombinant seedlings were established in the greenhouse and vegetatively propagated to establish two, un-replicated, space-planted field nurseries. These nurseries were established in April and May, 2003. Visual assessment of these two nurseries allows culling of plants with undesirable phenotypes. By year's end, no more than 1,000 "pre-selected" individuals will remain. These will be propagated for more detailed assessment of reaction to spittlebugs, *Rhizoctonia*, and aluminum.

Field nurseries were successfully established. Several ratings of plant merit have been conducted. Beginning in October, "pre-selected" plants will be propagated to CIAT-Palmira for further evaluations.

2.2 Selection of *Brachiaria* hybrids for resistance to spittlebug

Highlights

- A mass rearing of *Aeneolamia reducta* was successfully established which will facilitate simultaneous evaluation of *Brachiaria* genotypes for resistance to different species of spittlebug.
- Four hybrids showing high levels of antibiosis resistance to *Aeneolamia varia*, *A. reducta*, and *Zulia carbonaria* were identified.
- Several hybrids showing field resistance to *Zulia pubescens* and *Mahanarva trifissa* were identified

2.2.1 Continuous mass rearing of three spittlebug species in Palmira and Macagual

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

This is a continuous activity. A permanent supply of insects is essential in the process of evaluating genotypes for resistance to spittlebug. Progress made in the logistics of mass rearing of nymphs and in obtaining eggs from adults collected in the field has allowed us to screen *Brachiaria* genotypes for simultaneous resistance to six major spittlebug species: *Aeneolamia varia*, *A. reducta*, *Zulia carbonaria*, *Z. pubescens*, *Mahanarva trifiss*a, and *Prosapia simulans*. Insect material produced in our mass rearing facilities is used for greenhouse evaluations in Palmira and field evaluations in Caquetá.

2.2.2 Glasshouse screening of *Brachiaria* genotypes for spittlebug resistance

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

2.2.2.1 Greenhouse screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species (*Aeneolamia varia, A. reducta, Zulia carbonaria, Z. pubescens*)

Rationale

The correct identification of resistant hybrids is an essential step in the process of breeding superior *Brachiaria* cultivars at CIAT. This is why assessment of resistance to spittlebug received special attention in 2003. Based on results obtained in 2001 and 2002, simultaneous but independent screening for resistance to three key spittlebug species was fully implemented.

Materials and Methods

A set of 64 "pre-selected" SX x AP (sexual-by-apomictic) hybrids received from the Breeding Program were evaluated in the glasshouse for resistance to *Aeneolamia varia, A. reducta,* and *Zulia carbonaria*. Test materials were compared with six checks fully characterized for resistance to one or more spittlebug species. 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 reached the fifth instar stage or adult emergence occurred. Plants (5 per genotype) were scored for symptoms using the damage scale (1, no 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 the 1-5 scale) and reduced percentage survival (<30%). Those genotypes showing resistance to two or more spittlebug species were reconfirmed in replicated nurseries (10 replications per genotype per spittlebug species).

Results and Discussion

The preliminary screening revealed that 10 of the 64 hybrids showed acceptable levels of resistance to at least two spittlebug species (Table 8). As in previous occasions, fewer genotypes showed antibiosis resistance to *Z. carbonaria.*

Table 8. Sexual-by-apomicitic hybrids selected in 2003 for reconfirmation of resistance to three spittlebug species. Means of five replicates per genotype per spittlebug species

Genotype	Damage scores			Percentage nymph survival		
	Aeneolamia varia	*Aeneolamia reducta*	*Zulia carbonaria*	*Aeneolamia varia*	*Aeneolamia reducta*	*Zulia carbonaria*
			Hybrids			
BR02NO/0419	1.2	1.6	2.1	3.3	10.0	23.3
BR02NO/0465	1.8	1.1	1.4	30.0	0.0	20.0
BR02NO/0638	1.2	1.6	2.6	23.3	36.7	-
BR02NO/0643	1.1	1.9	3.2	3.3	40.0	-
BR02NO/0644	1.4	1.3	2.2	33.3	20.0	80.0
BR02NO/0649	1.4	1.2	1.6	16.7	3.3	53.3
BR02NO/0756	3.0	1.2	2.0	-	0.0	26.7
BR02NO/0812	2.3	1.6	2.4	-	16.7	56.7
BR02NO/1372	2.4	1.6	1.6	-	20.0	56.7
BR02NO/1485	1.3	1.7	2.5	13.3	20.0	66.7
			Checks[a]			
FM9503/4624 (T)	3.5	2.2	1.5	85.0	25.0	11.7
CIAT 6294 (R)	2.0	1.4	2.5	26.7	25.0	56.7
SX0NO/0102 (R)	1.0	1.1	1.1	0.0	0.0	0.0
CIAT 36062 (R)	1.1	1.2	1.4	1.7	0.0	16.7
CIAT 0606 (S)	5.0	4.3	4.1	95.0	80.0	68.3
BR4X/44-02 (S)	4.9	4.8	3.5	90.0	73.3	70.0
LSD 5%	1.21	1.23	0.94	28.8	22.2	23.7

[a] Classified according to their reaction to *Aeneolamia varia* (S, susceptible; R, resistant; T, tolerant).

Further testing with 10 replications per genotype per insect species allowed us to identify four hybrids combining antibiosis resistance to *A. varia*, *A. reducta*, and *Z. carbonaria* (Table 9). Levels of resistance in this case were comparable to those exhibited by the resistant checks CIAT 36062 and 'Marandú' (CIAT 6294).

Table 9. Sexual-by-apomictic hybrids selected in 2003 for high antibiosis resistance (<30% nymphal survival) to three spittlebug species. Means of 10 replicates per genotype per spittlebug species

Genotype	Damage scores			Percentage nymph survival		
	Aeneolamia varia	*Aeneolamia reducta*	*Zulia carbonaria*	*Aeneolamia varia*	*Aeneolamia reducta*	*Zulia carbonaria*
			Selected hybrids			
BR02NO/0419	1.0	1.1	1.8	0.0	0.0	5.0
BR02NO/0465	1.0	1.0	1.1	10.0	1.7	18.3
BR02NO/0756	1.4	1.2	1.8	18.3	3.3	21.7
BR02NO/0812	1.0	1.5	2.4	6.7	8.3	28.3
			Checks[a]			
FM9503/4624 (T)	1.6	1.8	1.9	51.7	21.7	30.0
CIAT 6294 (R)	1.2	1.9	2.7	25.0	21.7	63.3
SX01NO/0102 (R)	1.0	1.0	1.1	1.7	0.0	1.7
CIAT 36062 (R)	1.0	1.0	1.5	0.0	0.0	6.7
CIAT 0606 (S)	4.9	4.4	3.8	96.7	68.5	46.7
BRU4X/44-02 (S)	4.8	4.1	3.8	96.7	55.0	75.0
LSD 5%	0.36	0.65	0.68	16.8	17.8	26.3

[a] Classified according to their reaction to *Aeneolamia varia* (S, susceptible; R, resistant; T, tolerant).

2.2.2.2 Field screening of *Brachiaria* accessions and hybrids for resistance to four spittlebug species (*Aeneolamia varia, Zulia carbonaria, Z. pubescens, Mahanarva trifissa*)

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 a technique that allows us to properly identify resistance under field conditions. Evaluating for resistance under field conditions is important because it allows us 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, eight major screening trials (six with *Zulia pubescens*, two with *Mahanarva trifissa*) were conducted in Caquetá in 2003.

Results and Discussion

In Table 10 we highlight the results of evaluating 32 hybrids for resistance to *Z. pubescens* in comparison with six checks well known for their reaction to *Aeneolamia varia*. As in previous occasions, there was a significant negative correlation (r = -0.452; $P < 0.001$; n = 2273) between damage scores and tiller ratios. This means that damage scores are useful in predicting tiller losses resulting from intense insect damage. Selected hybrids in Table 10 showed significantly lower damage scores and significantly higher tiller ratios than the susceptible checks CIAT 0606 and BRUZ4X/44-02.

The same set of 32 hybrids was evaluated for field resistance to *Mahanarva trifissa.* Results are shown in Table 11. Resistant hybrids exposed to *M. trifissa* performed significantly better than the checks both in terms of damage scores and tiller ratios.

Table 10. Field resistance to *Zulia pubescens* in selected *Brachiaria* hybrids and checks. Means of six trials, 10 replicates per genotype per trial.

Genotype	Damage scores	Tiller ratio[a]
	Hybrids	
BR00NO/1494	2.0	1.04
BR00NO/0755	2.0	0.92
BR00NO/1392	2.0	0.91
BR00NO/1032	2.1	0.89
BR00NO/0604	2.1	0.88
BR00NO/1076	2.1	0.88
BR00NO/1295	2.0	0.88
BR00NO/0036	2.0	0.87
BR00NO/0042	2.1	0.96
BR00NO/0029	2.1	0.86
	Checks[b]	
FM9503/46/024 (T)	1.1	1.04
CIAT 6294 (R)	1.1	1.04
CIAT 36062 (R)	1.1	1.01
CIAT 6133 (T)	1.8	0.92
CIAT 0606 (S)	3.6	0.46
BRUZ4X/44-02 (S)	3.9	0.47
LSD 5%	0.13	0.12

[a] Tillers per plant at the end of the infestation process/tillers per plant at the beginning of the infestation process
[b] Classified according to their reaction to *Aeneolamia varia* (S, susceptible; R, resistant; T, tolerant).

Table 11. Field resistance to *Mahanarva trifissa* in selected *Brachiaria* hybrids and checks. Means of two trials, 10 replicates per genotype per trial

Genotype	Damage scores	Tiller ratio[a]
	Hybrids	
BR00NO/0587	2.1	1.16
BR00NO/1494	2.1	1.07
BR00NO/1392	2.0	1.06
BR00NO/0106	2.0	1.01
BR00NO/0078	2.1	1.00
BR00NO/0049	2.1	0.97
BR00NO/1733	2.1	0.96
BR00NO/0235	2.1	0.96
	Checks[b]	
FM9503/46/024 (T)	1.1	0.98
CIAT 6294 (R)	1.1	1.22
CIAT 36062 (R)	1.0	1.03
CIAT 6133 (T)	1.8	0.99
CIAT 0606 (S)	3.8	0.41
BRUZ4X/44-02 (S)	4.3	0.28
LSD 5%	0.19	0.16

[a] Tillers per plant at the end of the infestation process/tillers per plant at the beginning of the infestation process
[b] Classified according to their reaction to *Aeneolamia varia* (S, susceptible; R, resistant; T, tolerant).

2.3 Identification of host mechanisms for spittlebug resistance in *Brachiaria*

Highlights

- Finalized studies on mechanisms of resistance to five species of spittlebug: *Aeneolamia varia*, *A. reducta*, *Zulia carbonaria*, *Z. pubescens*, and *Mahanarva trifissa*
- Finalized studies on the effect of simultaneous infestation by two or more spittlebug species on resistance expressions in selected *Brachiaria* genotypes
- Initiated studies on interactions between strains of five spittlebug species and resistance expression in selected *Brachiaria* genotypes (genotype x species x strain interactions)
- Initiated studies on mechanisms of resistance to adults of five spittlebug species
- Initiated studies on possible sub-lethal effects of resistance on adults of *Aeneolamia varia*

2.3.1 Mechanisms of resistance of Brachiaria genotypes to five spittlebug species

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

Rationale

We have shown in previous reports that resistance to one spittlebug species does not necessarily apply to other species. We have also shown that the mechanisms of resistance vary. In 2003 we finalized the characterization of antibiosis and tolerance to *Aeneolamia reducta*, the most important species in the Caribbean zone. What follows is a summary of what we know about host plant resistance mechanisms to five major spittlebug species present in Colombia.

Materials and Methods

Several experiments were conducted and are reported herein. As test materials we used four germplasm accessions well known for their reaction to *Aeneolamia varia*: the susceptible checks CIAT 0606 and CIAT 0654 and the resistant checks CIAT 6294 ('Marandú') and CIAT 36062 (a hybrid-derived clone). These four host genotypes were also used to compare their resistance to other spittlebug species. CIAT 0654 and CIAT 36062, highly susceptible and resistant, respectively, were used in antibiosis studies. Tolerance studies were conducted with CIAT 0654, CIAT 6294, and CIAT 36062. *A. varia*, *A. reducta*, and *Z. carbonaria* were mass-reared on plants of CIAT 0654 in a screen-house. Mature eggs were used to infest test plants in the different experiments. In the case of *Z. pubescens* and *M. trifissa*, large numbers of adults were collected in the field with a sweep net and transferred to muslin cages in a screen-house to feed on potted plants of CIAT 0654. Adults were allowed to oviposit and eggs were separated from the soil. As with other spittlebug species, test plants were infested with mature eggs. All tests were conducted in a glasshouse at a mean temperature of 24^{o}C (range, 19-27^{o}C) and mean relative humidity of 75% (range, 70-90%).

To evaluate antibiotic effects, cohorts of no fewer than 900 individuals of each of the five species under study were established on each of two host genotypes well characterized for their reaction to *A. varia*: CIAT 0654 (highly susceptible) and CIAT 36062 (highly resistant). Cohorts were established by infesting 150 single-plant units with 6 eggs of the respective species per unit as described above. Following eclosion, a sample of two or three tubes per host genotype was examined daily to determine the fate of 12 or 18 individual insects. Nymphal instars and their duration were determined from measurement of the width of the head capsule of every nymph recovered (dead or alive). Survival rates were calculated. The dry weight of each nymph was recorded. Daily sampling continued until all surviving nymphs reached adulthood.

To study tolerance we initially compared the response of the susceptible CIAT 0654 and the *A. varia*-resistant CIAT 6294 ('Marandú') to increasing levels of infestation with nymphs of *A. varia*, *M. trifissa*, *Z. carbonaria*, and *Z. pubescens*. *A. reducta* was not included in these studies. Thirty-day-old plants of CIAT 0654 and CIAT 6294 were exposed to 0, 2, 3, 5, 7, or 10 nymphs per plant of each of the spittlebug species. The 48 host genotype- insect species-infestation level treatment combinations were randomly assigned to single-plant experimental units within 10 complete blocks. Plants were infested with mature eggs and the infestation was allowed to proceed until all nymphs were mature or adult emergence occurred. Plants were then scored for damage using the 5-point scale described above and the percentage nymphal survival recorded. Aboveground dry weight of plants was recorded following drying in an oven at 40°C. Percent weight loss was calculated (relative to the uninfested controls). We calculated functional plant loss indexes for each infestation level based on plant weight loss and damage response. Using the same general methodology, we conducted one more tolerance test in which plants of CIAT 0654 (susceptible) and CIAT 36062 (resistant to *A. varia*) were submitted to increasing levels of infestation (0, 2, 3, 5, 7, or 10 nymphs per plant) with each of the following species: *A. varia*, *A. reducta*, *M. trifissa*, *Z. carbonaria*, and *Z. pubescens*. We used a randomized complete block design with 10 repetitions per species-infestation level-host genotype combination. Damage scores, nymph survival, and above ground plant dry weights were recorded. Functional Plant Loss Indices were calculated.

All data were analyzed using the general linear model procedure. Means were separated by least significant difference (LSD: á = 0.05) only when the overall *F* test was significant (á = 0.05). Percentage nymph survival was transformed to arcsine square root of proportion; percentages of dry weight loss were transformed to square root. Means and standard errors of untransformed data are presented. Antibiotic effects for the different spittlebug species were assessed by comparing nymphal instar duration and nymph weight between the susceptible and resistant host genotypes by paired *t*-test. To compare survivorship of nymphs on susceptible and resistant host genotypes, median survival times were calculated using the Kaplan-Meier test. The Cox-Mantel survival test was used to compare survival distributions on susceptible and resistant host genotypes. Tolerance to the different spittlebug species was assessed by comparing mean percentage survival, mean damage scores, and mean percentage plant dry weight loss of five infestation levels between the susceptible and resistant host genotypes by paired *t*-test within spittlebug species.

Results and Discussion

Antibiosis tests. Relative to the susceptible control, CIAT 0654, there was a significant delay in development time of nymphs of *A. varia*, *A. reducta*, and *Z. pubescens* reared on CIAT 36062 (Figure 7). No such effect was found in the case of *Z. carbonaria*. Mortality of second instars of *M. trifissa* was so high, that we were unable to calculate developmental times for this species.

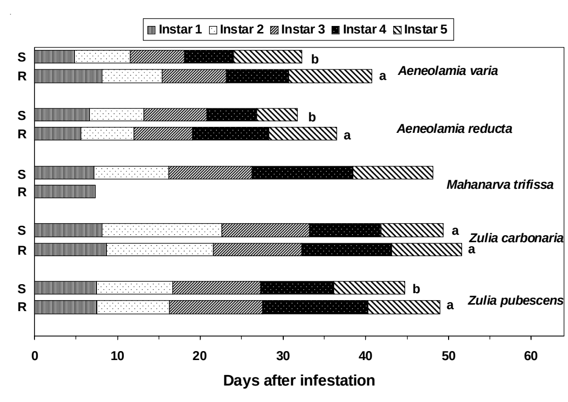


Figure 7. Duration of nymphal instars of five spittlebug species reared on susceptible (S, CIAT 0654) or resistant (R, CIAT 36062) *Brachiaria* genotypes. Bars with the same letter do not differ ($P < 0.05$). Pair-wise comparison by *t*-test within species. *Mahanarva trifissa* was not analyzed due to very high mortality of second instars.

Nymphal survival on the resistant CIAT 36062 was least for *M. trifissa* and greatest for *Z. carbonaria*. Survival of all species was less on CIAT 36062 than on the susceptible CIAT 0654. The Kaplan-Meier survival test revealed significant effects (no overlapping confidence intervals) of the resistant genotype on the median survival times of *A. varia*, *A. reducta*, and ***M. trifissa*** and, to a lesser extent, *Z. pubescens* populations. Survival time of *Z. carbonaria* was not affected by the resistant genotype. Calculation of the Cox-Mantel survival statistic showed differences at the 1% level of significance between CIAT 0654 and CIAT 36062 (Table 12) in terms of survival rates for *A. varia*, *A. reducta*, and *M. trifissa*, and at the 5% level of confidence for *Z. pubescens* (a lower level of antibiosis). No difference was found in the case of *Z. carbonaria* meaning that there is no antibiosis to this species in CIAT 36062. This was confirmed when survival rates of *Z. carbonaria* and *Z. pubescens* on CIAT 36062 were compared. The Cox-Mantel survival test statistic (2.8) was positive and significant at the 1% level, indicating that CIAT 36062 is more favorable to *Z. carbonaria* than to *Z. pubescens*.

Table 12. Survivorship parameters for nymphs of five spittlebug species reared on susceptible (CIAT 0654) or resistant (CIAT 36062) *Brachiaria* genotypes

Spittlebug species	Number tested		C^a
	On CIAT 0654	On CIAT 36062	
Aeneolamia varia	480	480	4.8**
Aeneolamia reducta	420	480	6.4**
Mahanarva trifissa	708	246	9.7**
Zulia carbonaria	744	720	1.4ns
Zulia pubescens	648	648	2.2*

**, Significant at the 1% level; *, significant at the 5% level; ns, not significant

[a]C is the test statistic for the Cox-Mantel two-sample survival test (CIAT 0654 versus CIAT 36062).

Antibiosis to *A. varia*, *A. reducta*, and *Z. pubescens* in CIAT 36062 was also manifested by the reduced weight of surviving 4th and 5th instar nymphs, and adults. No effect on nymphal or adult weight of *Z. carbonaria* was detected. Other manifestations of antibiosis were the occurrence of minute 2nd, 3rd, and 4th instar nymphs, staggering of developmental times, and reduced spittle production by surviving nymphs. Also, we found that nymphs reared on CIAT 36062 usually leave the spittle and wander over the soil surface, eventually dying of dehydration. We found no deformation of nymphs or adults nor did we detect obvious disruptions in the molting process. The level of antibiosis resistance in CIAT 36062 clearly differs by spittlebug species and can be classified as follows: very high for *M. trifissa*, high for *A. varia* and *A. reducta*, moderate for *Z. pubescens*, and absent for *Z. carbonaria*.

Tolerance tests. CIAT 6294 expressed clear antibiosis to *A. varia* and *M. trifissa* as the mean nymphal survival of five infestation levels was significantly lower than the mean for the susceptible control CIAT 0654 (Figure 8). However, survival of *Z. carbonaria* or *Z. pubescens* nymphs was high on both genotypes at all levels of infestation, indicating lack of antibiosis in CIAT 6294 to these two species. These results were consistent with those obtained in resistance reconfirmation tests. CIAT 6294 plants suffered less damage and less plant dry weight loss than the susceptible control at all levels of infestation (Figure 9). As in previous studies, visual damage scores predicted biomass loss. Since survival of the *Zulia* spp. nymphs did not differ between the genotypes, we interpret the lower damage scores and lower plant dry weight losses caused by *Z. carbonaria* and *Z. pubescens* on CIAT 6294 as tolerance.

At all levels of infestation, survival of nymphs on CIAT 36062 was much less than on the susceptible control for *A. varia*, *A. reducta*, and *M. trifissa*, but only moderately less for *Z. pubescens*. *Z. carbonaria* nymphs survived equally well on the two genotypes (Fig. 3). Thus, expression of antibiosis in CIAT 36062 was dependent on spittlebug species. CIAT 36062 suffered significantly less damage (expressed as damage scores or plant weight loss) than the susceptible control at all levels of infestation with *Z. carbonaria* (Figure 9). Since *Z. carbonaria* nymphs survived equally well on both genotypes, we interpret the mechanism of resistance to *Z. carbonaria* in CIAT 36062 as tolerance.

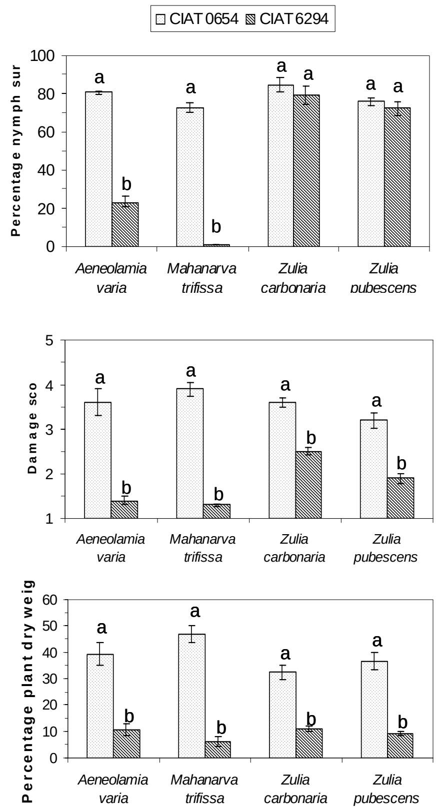


Figure 8. Response of susceptible (CIAT 0654) or resistant (CIAT 6294) *Brachiaria* genotypes to attack by nymphs of four spittlebug species. Means (± SEM) of five levels of infestation. Bars with the same letter do not differ ($P < 0.05$). Pair-wise comparison by *t*-test within spittlebug species

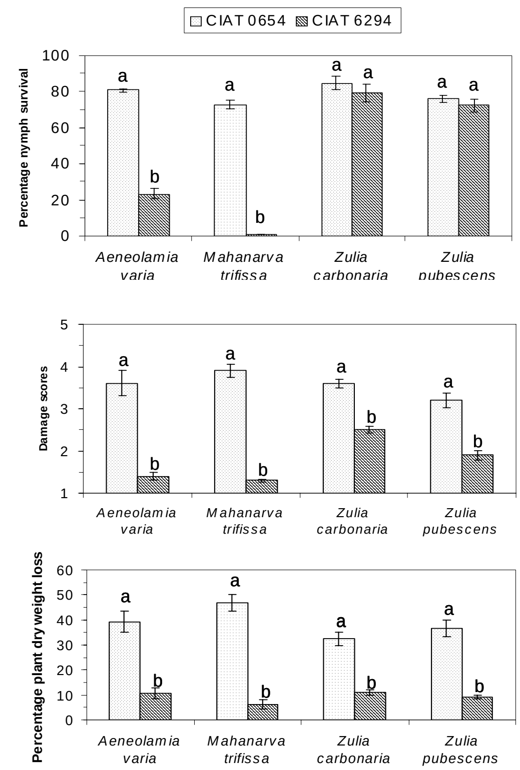


Figure 9. Response of susceptible (CIAT 0654) or resistant (CIAT 36062) *Brachiaria* genotypes to attack by nymphs of five spittlebug species. Means (± SEM) of five levels of infestation. Bars with the same letter do not differ ($P < 0.05$). Pair-wise comparison by *t*-test within spittlebug species

We also calculated a functional plant loss index to measure tolerance to both *Z. carbonaria* and *Z. pubescens*. Losses were highest for the susceptible control, CIAT 0654, at all levels of infestation (Figure 10). Losses caused by both species on CIAT 6294 and on CIAT 36062 were lower at all infestation levels. These results suggest the presence of true tolerance to *Z. carbonaria* in CIAT 6294 and CIAT 36062, true tolerance to *Z. pubescens* in CIAT 6294 and a combination of tolerance coupled with antibiosis as mechanisms of resistance to *Z. pubescens* in CIAT 36062.

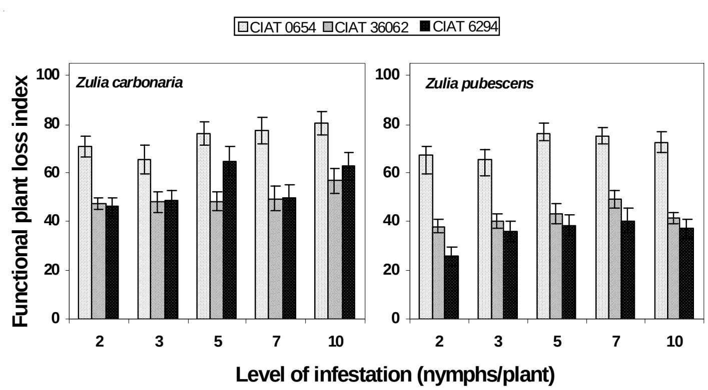


Figure 10. Functional plant loss indices (percentage) for susceptible (CIAT 0654) or resistant (CIAT 36062, CIAT 6294) *Brachiaria* genotypes exposed to five levels of infestation with each of two spittlebug species.

2.3.2 Effect of infestation with two species of spittlebug on resistance expression in selected *Brachiaria* genotypes

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

Rationale

As explained in the 2002 Annual Report, we can identify spittlebug nymphs with absolute precision by means of RAPD-PCR DNA analysis or by comparison of esterase banding patterns. Using these techniques we have been able to detect mixed infestations in commercial fields and to measure percentage survival of different species when mixed infestations by two or more species occur. This in turn has allowed us to study how different species combinations affect resistant expressions in selected resistant or susceptible genotypes.

Materials and Methods

In 2003 we measured the effect of single species infestation as opposed to mixed infestations by infesting plants with eggs of two or more spittlebug species in different proportions. The infestation was allowed to proceed until adult emergence occurred. Plants were then scored for damage and the surviving nymphs were collected and identified to species level by comparison of esterase banding patterns or, in some cases, by RAPDs-PCR analysis. Percentage survival was calculated for each spittlebug species.

Results and Discussion

We will highlight results of studies on the effect of mixed *Aeneolamia reducta* - *Zulia carbonaria* infestations. These are two of the most important spittlebug species present in Colombia. As shown in Figure 11, when the resistant genotype CIAT 36062 is exposed to *Zulia carbonaria* alone or when *Z. carbonaria* predominates in the mixture, damage scores increase so that the genotype is classified as intermediate resistant rather than resistant. This was not the case with the hybrid SX01NO/0102, the most resistant hybrid tested to date for resistance to five spittlebug species.

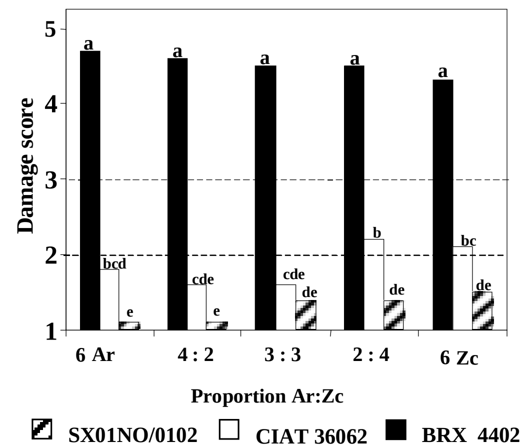


Figure 11. Damage scores recorded on susceptible (S) and resistant (R) *Brachiaria* genotypes exposed to individual or simultaneous attack by nymphs of *Aeneolamia reducta* (Ar) or *Zulia carbonaria* (Zc). Dotted lines represent cut-off points for resistance (< 2) and intermediate ratings (2 -3) in a 1 - 5 damage score scale. Bars accompanied by the same letter represent means that are not significantly different at the 5% level by LSD.

Most important, we detected significant and differential antibiosis effects (Figure 12) when mixed populations of *A. reducta* and *Z. carbonaria* in different proportions were used to infest plants of the resistant genotypes CIAT 36062 and SX01NO/0102 (Figure 12). At all levels of infestation, survival of *A. reducta* on both resistant genotypes was significantly reduced to levels below the cut-off point for resistance rating (< 30%). On the contrary, the survival of *Z. carbonaria* nymphs on CIAT 36062 was significantly higher, in some cases well above the 50% level used to classify genotypes as susceptible. The hybrid SX01NO/0102 showed intermediate resistance to *Z. carbonaria* at two of the levels of infestation tested. Again, these findings emphasize the need to characterize resistance to as many species as possible and illustrate the need to breed for multiple antibiosis resistance.

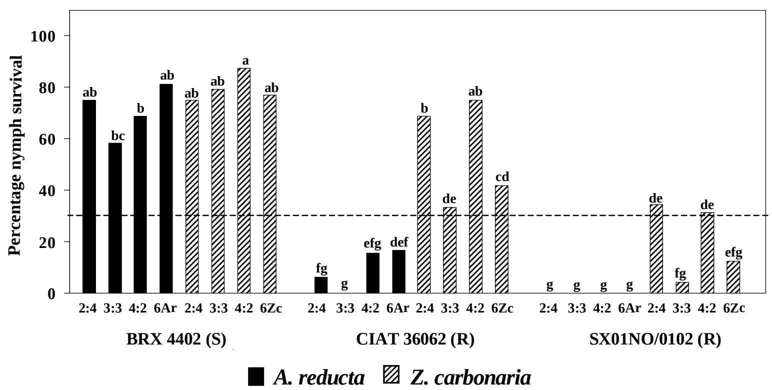


Figure 12. Levels of antibiosis (reduced percentage nymph survival) detected when plants of susceptible (S) or resistant (R) *Brachiaria* genotypes were infested with *Aeneolamia reducta* or *Zulia carbonaria* or combinations thereof (*A. varia*: *Z. carbonaria*). The dotted line represents the cut-off point for resistance rating (< 30% percentage survival). Bars accompanied by the same letter represent means that are not significantly different at the 5% level by LSD.

2.3.3 Interactions between strains of five spittlebug species and resistance expression in selected *Brachiaria* genotypes

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

Rationale

The protective properties of insect-resistant cultivars may be overcome by the development of resistance-breaking strains of a given insect species. These are insect populations that possess an inherent genetic capability to overcome plant resistance. Typically, biotypes develop as a result of selection from the parent population in response to exposure to the resistant cultivar. It may occur that the genetic capability of an insect to overcome resistance is so great that resistance is nullified before the resistance cultivar is grown in a large geographical area. This is why it is important to obtain information on the reaction of resistant genotypes to as many geographical strains of a given insect pest as it is possible. We initiated a series of experiments aimed at measuring the response of resistant cultivars to populations of *A. varia*, *A. reducta, Z. pubescens, Z. carbonaria,* and *M. trifissa* collected in several different areas of Colombia. For the first time, we also generated information on resistance to *Prosapia simulans* (Walker).

Materials and Methods

All trials were conducted using test materials well known for their reaction to *Aeneolamia varia*. We evaluated the susceptible checks CIAT 0606 and CIAT 0654, the resistant checks CIAT 6294 and CIAT 36062, and two new sexual hybrids SX01NO/0102 and SX01NO/0233 classified as highly resistant to *A. varia* in previous studies. The *A. varia*-CIAT colony combination was used as the standard check in all trials. Screening for resistance was conducted using standard methodologies. Plants were infested with six eggs per plant of the respective spittlebug species-geographical combination and the infestation was allowed to proceed without interference until all nymphs reached the fifth instar stage or adult emergence occurred. Plants (20 per genotype) were scored for symptoms using the damage scale (1, no damage; 5, plant dead) developed in previous years. Percentage nymph survival was calculated.

Results and Discussion

We have conducted four trials. At this point, we will highlight results obtained with geographical strains of *A. varia* and *Z. pubescens*. We will also report on our first-ever screening for resistance to *P. simulans*.

The reaction of six genotypes to attack by nymphs of three strains of *A. varia* is shown in Table 13. No significant genotype x strain interaction was detected for damage scores or percentage nymph survival, meaning that resistance ratings did not change when the genotypes were exposed to different strains of *A. varia*. Similarly, no significant genotype x strain interaction was detected when susceptible and resistant genotypes were exposed to attack by nymphs of *Z. pubescens* (Table 14).

Table 13. Reaction of selected *Brachiaria* genotypes to strains of *Aeneolamia varia* from two geographical areas of Colombia

Origin of strain	Genotypes[a]					
	BRUZ4X-44-02	CIAT 0606	CIAT 6294	CIAT 36062	SX01NO/0102	SX01NO/0233
	Damage scores					
Florencia, Caquetá	3.9b	3.8b	1.2a	1.3a	1.1a	2.0a
V/vicencio, Meta	4.2a	3.7b	1.3a	1.1a	1.0a	1.3b
CIAT colony	4.9a	4.6a	1.3a	1.1a	1.0a	2.2a
Mean	4.3A	4.0A	1.3C	1.2C	1.0C	1.8B
	Percentage nymph survival					
Florencia, Caquetá	75.8b	86.6a	17.5b	0.0c	2.5b	26.7b
V/vicencio, Meta	90.8a	71.1b	35.8a	9.2a	6.7a	14.2c
CIAT colony	86.1a	87.1a	19.8b	4.7b	6.3a	40.7a
Mean	84.2A	81.6A	24.4B	4.6C	5.2C	27.2B

Means of 20 replicates by genotype by insect strain. For each variable, means within a column followed by the same lowercase letter, and means within a row followed by the same uppercase letter are not significantly different at the 5% level by LSD.

Table 14. Reaction of selected *Brachiaria* genotypes to strains of *Zulia pubescens* from three geographical areas of Colombia

Origin of strain	Genotypes					
	BRX 44-02	CIAT 0606	CIAT 6294	CIAT 36062	SX01NO /0102	SX01NO /0233
	Damage scores					
Darién, Valle	3.9a	4.2a	2.0a	1.0b	1.1a	1.5b
Popayán, Cauca	3.8a	3.3b	2.1a	1.2ab	1.1a	1.3b
S. José de Fragua, Caquetá	4.3a	4.1a	2.1a	1.4a	1.3a	2.6a
Mean	4.0A	3.9A	2.1B	1.2C	1.2C	1.8B
	Percentage nymph survival					
Darién, Valle	71.6a	55.9ab	34.1b	2.8b	0.9c	4.6b
Popayán, Cauca	44.3b	45.5b	47.5a	11.6a	6.7b	5.0b
S. José de Fragua, Caquetá	74.1a	69.0a	34.2b	8.8a	21.6a	47.2a
Mean	63.3A	56.8A	38.6B	7.7D	9.7D	18.9C

Means of 20 replicates by genotype by insect strain. For each variable, means within a column followed by the same lowercase letter, and means within a row followed by the same uppercase letter are not significantly different at the 5% level by LSD.

In Table 15 we summarize results of our first screening for resistance to *Prosapia simulans*. Susceptible (CIAT 0606, BRX-44-02) and resistant genotypes (CIAT 6294, CIAT 36062, SX01NO/0102, and SX01NO/0233) differed for damage scores for all spittlebug species tested (Table 15). *P. simulans* caused more damage than *A. varia* and *M. trifissa* on the resistant genotype CIAT 6294 ('Marandú'). Using our resistance classification, CIAT 6294 would be classified as resistant to *A. varia* and *M. trifissa* (damage scores: 1-2) but intermediate to *P. simulans* (damage scores: 2.1-3.0). SX01NO/0233 was intermediate to all three species tested. Survival of nymphs of *A. varia* and *M. trifissa* was significantly lower on the *A. varia*-resistant genotypes than on the susceptible controls CIAT 0606 and BRX-44-02 (Table 15). Survival of *P. simulans* nymphs was significantly higher on CIAT 6294 than on the other resistant genotypes suggesting that antibiosis resistance to this species is absent in 'Marandú'. Using our resistance classification, CIAT 6294 would be classified as susceptible (> 50% survival) to *P. simulans*. The relatively low levels of damage caused by *P. simulans* on CIAT 6294 could be the result of tolerance to this species.

Table 15. Response of selected *Brachiaria* genotypes to attack by nymphs of three spittlebug species

Spittlebug species	Spittlebug species		
	Mahanarva trifissa	*Aeneolamia varia*	*Prosapia simulans*
	Damage scores		
BRX-44-02	4.2aA	4.9aA	4.3aA
CIAT 0606	3.5bB	4.6aA	4.3aA
CIAT 6294	1.2cB	1.3cB	2.4bA
CIAT 36062	1.1cA	1.1cA	1.3cA
SX01NO/0102	1.0cA	1.0cA	1.5cA
SX01NO/0233	2.1bcA	2.2bA	2.3bA
	Percentage nymph survival		
BRX-44-02	55.0aB	86.1aA	90.8aA
CIAT 0606	36.0aC	87.1aA	79.2bA
CIAT 6294	0.0bC	19.8cB	65.0bA
CIAT 36062	0.0bB	4.7dA	14.2cA
SX01NO/0102	0.0bA	6.3dA	6.7dA
SX01NO/0233	34.5aA	40.7bA	5.0dB

Means of 20 replicates by genotype by insect species. For each variable, means within a column followed by the same lowercase letter, and means within a row followed by the same uppercase letter are not significantly different at the 5% level by LSD.

2.3.4 Mechanisms of resistance in Brachiaria to adults of five spittlebug species and sub-lethal effects of antibiosis on adults of spittlebug

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 is known about possible direct effects of antibiotic genotypes on the biology of adults. Even less is 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*.

Materials and Methods

Initially, we conducted two experiments aimed at measuring how feeding on an antibiotic genotype affects the biology of adults of *A. varia*. Later on, we initiated a comprehensive series of experiments aimed at determining whether antibiosis to nymphs has an adverse effect on the biology of resulting adults. For this, a number of life tables will be constructed. Treatment combinations are shown in Table 16.

This work is in progress. Results will be presented in full in 2004.

Table 16. Treatment combinations to study possible sub-lethal effects of intermediate and high levels of nymphal antibiosis on adults of *Aeneolamia varia.*

Nymphs reared on:	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 susceptible to *A. varia*. CIAT 6294 and CIAT 36062 show intermediate and high levels of antibiosis resistance to nymphs of *A. varia,* respectively.

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

Highlights

- Detection and identification of a nitrogen fixing/growth promoting bacteria associated with *Brachiaria*.
- Development of a quick inoculation method in *Brachiaria* for *Rhizoctonia solani*.

2.4.1 Development of a new inoculation method for *Rhizoctonia solani* in *Brachiaria*

Contributors: C. Zuleta, S. Kelemu, J. Miles, I. Rao (CIAT)

Rationale

Rhizoctonia foliar blight, caused by *Rhizoctonia solani* Kühn, 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 is the most widely known species of *Rhizoctonia* with a wide host range. The fungus is a basidiomycete and does not produce any asexual spores (conidia). Occasionally the fungus produces sexual basidiospores. Out in nature *R. solani* reproduces mainly asexually and exists as vegetative mycelia and/or dense sclerotia. The pathogen primarily infects below ground plant parts in a number of plant species, but can also infect above ground plant parts such as pods, fruits, and leaves and stems as is the case with *Brachiaria*. In *Brachiaria*, infected leaves first appear water-soaked, then darken, and finally turn to a light brown color (see symptom Photo 1). Lesions may coalesce quickly during periods of prolonged leaf wetness and temperatures between 21 and 32 ^{o}C.

The pathogen's sclerotia can survive in soil and on plant debris for several years. These sclerotia can germinate and produce hyphae that can infect a wide range of host plants. The infection process is enhanced by the production of many different extracellular enzymes that degrade components of plant cell walls. As the plant cells die due to infection, the hyphae continue to grow and colonize dead tissue, eventually forming sclerotia (eg. see Photo 1). New inocula are produced on or in host tissues, and a new cycle is repeated when plant hosts or other substrates become available.

The ability to uniformly induce disease and measure resistance accurately is crucial in a breeding program for developing resistant cultivars. The objectives of this study are to: 1) develop a rapid and reliable artificial inoculation method that allows uniform disease development in genetically susceptible genotypes of *Brachiaria*, 2) accurately measure resistance for subsequent use in identifications of resistant materials among *Brachiaria* genotypes.

Photo 1. Foliar blight symptoms caused by *Rhizoctonia solani* on leaves of *Brachiaria*. Note mature brown and young white sclerotia on the leaves.

Materials and Methods

Plant materials: Two *Brachiaria* genotypes CIAT 16320 (resistant to *R. solani*) and a susceptible CIAT 36061 (cv. Mulato) were used for developing a new inoculation method.

Inoculum preparation: Sclerotia of *R. solani* originally isolated from *B. brizantha* CIAT 6780 was germinated on potato dextrose agar (PDA) at 28 ^{o}C. Mycelial discs (0.5 mm in diameter) were cut out of the actively growing 2 days old PDA culture.

New inoculation method: Mature leaves were detached and cut to 12 cm in length. Two leaves (one from CIAT 16320 and another of CIAT 36061) were placed in each plastic Petri dish of 15 cm in diameter containing a 2-mm- thick filter paper (see Photo 3). Ten-ml sterile distilled water was applied to the filter paper to keep it moist and to create humidity. A mycelial disc was placed in the middle of each leaf. The Perti dish was closed with its lid and sealed with a parafilm tape. All plates containing inoculated leaves were placed on a laboratory bench with access to sunlight through a large glass window and incubated at room temperature that ranged 26-29 °C. Control leaves were inoculated with pure agar discs free from *R. solani*. Three replications were made for each treatment.

For comparison, whole plants were inoculated in the greenhouse using previously described methods (IP-5 Annual Report 2002) that were slightly modified. CIAT 16320 and CIAT 36061 (each 20 plants) generated from tillers were inoculated with mycelial discs of *R. solani*. A mycelial disc was placed in contact of each plants stem just 2 cm above the soil level and wrapped with parafilm to secure the contact. Each inoculated plant was kept inside a plastic column to keep them separated from each other (see Photo 3). The inoculated plants were kept in a humidity chamber with 2 hours of mist application per day for 10 days. They were then evaluated for resistance using visual estimation based on Harsfall-Barratt rating system (1-11, where 1 = 1-3%, 2 = 4-6%, 3 = 7-12%, 4 = 13-25%, 5 = 26-50%, 6 = 51-75%, 7 = 76-87%, 8 = 88-93%, 9 = 94-97%, 10 = 98-99%, and 11 = 100%; as well as using measurements of chlorophyll content.

Photo 2. Plastic columns separating individual plants after inoculation with *Rhizoctonia solani* in greenhouse tests.

Evaluation of resistance: Disease reaction was assessed at 0, 120, 144 and 168 hr after inoculation. Three different measurements were taken. 1) disease lesion size (both width and length). These measurements were presented as percent of the total leaf size (both width and length); 2) chlorophyll content of each leaf. Thirty measurements were taken across each leaf with chlorophyll meter SPAD 502 (Minolta), and an average of these 30 measurements recorded which gives a good indication of the chlorophyll content of the leaf being evaluated (the values are calculated based on the amount of light transmitted by the leaf in 2 wavelength regions in which the chlorophyll absorption is different); 3) Chlorophyll fluorescence. Chlorophyll fluorescence transients emitted from leaves were measured by a plant efficiency analyzer) PEA; Hansatech Ltd., King's Lynn, Northfolk, UK). Light was provided by an array of six high intensity light-emitting diodes (650 nm wavelength) that were focused onto the leaf surface to be evaluated (providing a red light of a peak 650 nm wavelength which is absorbed by the chloroplasts of the leaf). The values recorded correspond to a ratio of the variable fluorescence divided by the maximum fluorescence that is automatically calculated during measurement.

Results and Discussion

The appearances of disease symptoms on susceptible leaves start 48 hr after inoculation. Symptoms fully develop throughout the inoculated leaf of the susceptible genotype (CIAT 36061) 120 hr after inoculation (see Photo 3). The experiment was repeated three times and the data obtained were consistent in all the three experiments.

There are clear distinctions in values (lesion size, chlorophyll content and chlorophyll fluorescence) between CIAT 16320 (highly resistant) and the susceptible CIAT 36061 (Table 17). In addition, there is strong correlations (r=0.88) between measurements of lesion size and chlorophyll content as well as those of lesion size and photosynthesis efficiency, indicating that either one of the measurements can be used to measure resistance (see Figures 13 and 14). There was also a high correlation (r=0.88) between the chlorophyll content and fluorescence measurements.

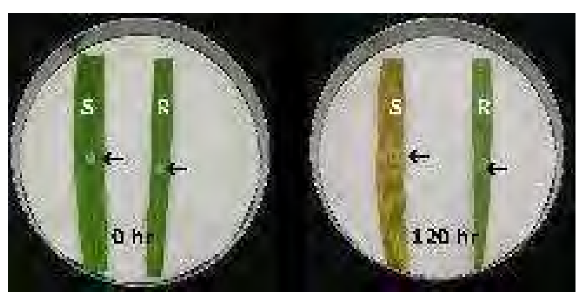


Photo 3. Reactions of detached *Brachiaria* leaves of CIAT 36061 (S; susceptible) and CIAT 16320(R; resistant) to *Rhizoctonia solani*, at the time of inoculation (0 hr) and 120 hr. after inoculation. The arrows indicate the inoculation point where a potato dextrose agar (PDA) mycelial disc was placed.

Table 17. Disease reaction measurements for two contrasting genotypes of *Brachiaria* inoculated with *Rhizoctonia solani* at various hours after inoculation.

Brachiaria CIAT No.	Hour after inoculation	Lesion size (% of total leaf area)	Chlorophyll content (SPAD)	Chlorophyll fluorescence Fv/Fm
16320	0	0.0	48.1	0.84
36061	0	0.0	43.8	0.84
16320	120	1.1	39.2	0.82
36061	120	46.4	9.0	0.40
16320	144	1.5	32.0	0.79
36061	144	53.9	6.7	0.08
16320	168	2.5	24.6	0.74
36061	168	60.1	5.8	0.00

Lesion size measurements are time consuming and provide no additional advantage in resistance measurement. Measuring fluorescence values is more time consuming than chlorophyll content measurement. Because these values are highly correlated with each other, we selected chlorophyll content values as a measure of resistance to foliar blight disease.

Disease reaction data on live plants in greenhouse tests are comparable to those on detached leaves in Petri dishes. Chlorophyll content measurements of one of the middle infected leaves and the visual rating system were correlated (r=0.70).

This new inoculation and resistance evaluation method we developed has several advantages: 1) it is rapid: disease reactions can e evaluated within 120 h (5 days) after inoculation as compared to 10-15 days in GH whole plant inoculations; 2) reproducible: results are consistent among repeated experiments; 3) highly uniform among replications: there is little or no variation among leaf replications of the same genotype; 4) very little space on a laboratory bench, compared to a whole green house room for whole plant assays, is needed for the same number of materials to be evaluated; 5) less labor and material cost since no soil preparation, planting pots, transplanting, watering and maintaining plants are needed, 6) leaf tissues can be cut directly from the breeding population for evaluation without the need to propagate tillers; 7) chlorophyll content measurement for the evaluation of resistance is faster than disease lesion measurement and yet provides more consistent quantification than commonly used visual rating methods which can vary from person to person and can be subjective to individual judgments; 8) comparable to disease reaction data on whole plants in green house tests.

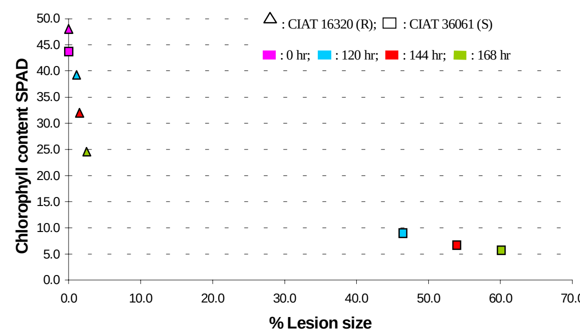


Figure 13. Chlorophyll content and percentage foliar blight lesion size of *Brachiaria* CIAT 16320 (resistant) and CIAT 36061 (susceptible) detached leaves inoculated with *Rhizoctonia solani*

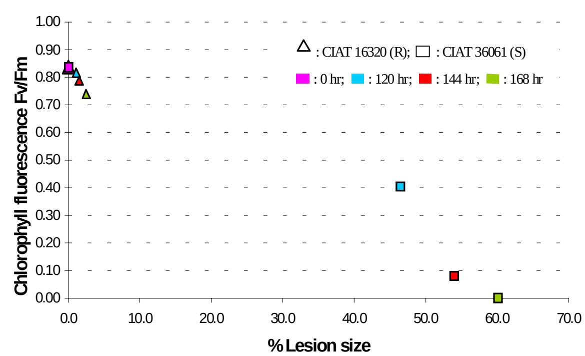


Figure 14. Chlorophyll fluorescence values and percentage foliar blight lesion size of *Brachiaria* CIAT 16320 (resistant) and CIAT 36061 (susceptible) detached leaves inoculated with *Rhizoctonia solani*

2.4.2 Characterization of pathogen associated with bacterial wilt disease in Brachiaria

Contributors: M. Rodriguez and S. Kelemu (CIAT)

Rationale

We have previously reported a bacterial wilt disease of *Brachiaria* and its casual agent, (Zuleta et al., 2002. Manejo Integrado de Plagas y Agroecologia 64:41-47). *Xanthomonas campestris* pv. *graminis* infects a number of cultivated forage grasses. Some of the first symptoms are chlorotic/ necrotic stripes along the leaves. As the disease advances, the whole leaf may die. Under severe conditions, the whole plant may turn yellow and die. Another typical symptom is wilting and curling of leaves without any discoloration or lesions, which result in quick plant death.

We have demonstrated that the pathogen is seed transmitted and is also transmitted vegetatively (AR-2001). Although the disease is not economically important to date, it is important for quarantine purposes.

Sixty-seven isolates of *X. campestris* pv. *graminis* have been collected from sites in Colombia from various genotypes of *Brachiaria* in order to determine pathogenicity and genetic diversity.

Families of repetitive DNA sequences such as repetitive extragenic palindromic (REP), enterobacterial repetitive intergenic consensus (ERIC), and box elements (BOX), which are present in all prokaryotes can be used for bacterial fingerprinting. Polymerase chain reactions (PCRs) based on these repetitive sequences, collectively designated as rep-PCR, have been used to assess variation among pathovars as well as to differentiate strains of the same pathotype of *Xanthomonas* species. In this study, we used rep-PCR with REP, BOX and ERIC primers to evaluate the genetic diversity of *X. campestris* pv. *graminis* isolates.

Materials and Methods

Bacterial isolates: A total of 67 independent colonies of X*anthomonas campestris* pv. *graminis* were collected from naturally infected species of *Brachiaria* at Carimagua, Santander de Quilichao, Popayán, and Palmira. Leaves were cut into small pieces (approximately 1 $cm^{2)}$ and surface-sterilized in1% NaOCl solution for 2 min and in 70 % ethanol for 1 min. They were then rinsed with sterile deionized water, and macerated in sterile water. A dilution series of the macerated suspension was plated on nutrient agar for selection of independent bacterial colonies. Their pathogenicity was confirmed by inoculating a susceptible material (hybrid *Brachiaria* CIAT 36062). Selected colonies were grown in nutrient broth with shaking (200 rpm) at 28 ^{o}C. They were stored in 30% glycerol at -20 ^{o}C for use in further studies. Two isolates (CIAT 46 and CIAT 469) of *X. axonopodis* pv. *manihotis* were included as control.

DNA isolations: Bacterial cells were grown overnight in Luria broth medium in a shaker (200 rpm) at 28^{o}C. Cells were collected in microcentrifuge tubes by centrifugation (8000 rpm for 10 min) and discarding the supernatant. The cells were re-suspended in 600-µl TE (50mM Tris, pH 8 and 50mM de EDTA) and stored at –20 ^{o}C for 10 min, and subsequently thawed at room temperature. Sixty-µl of a freshly prepared lysozyme (10 mg/mL in 25 mM Tris pH 8) and a 6-µl RNase (stock concentration 10mg/ml) were added to the cell suspension and incubated 15 min at room temperature and transferred to ice for another 15 min. A 120-µl STEP solution (0.5% SDS, 50mM Tris pH 7.5 and 280-ìg of proteinase) was added and incubated at 37 ^{o}C for an hour. Subsequently, 216-µl of ammonium acetate solution (7.5 M concentration) was added and mixed well. The solution was precipitated with phenol: chloroform: isoamyl alcohol (25:24:1) The supernatant was treated twice with equal volume of chloroform: isoamyl alcohol (24:1). The DNA was then precipitated with isopropanol and centrifuged. The DNA pellet was washed with 70% ethanol, air-dried and re-suspended in 100-ìl sterile distilled water.

DNA amplifications: The following primers were used: 1) ERIC (enterobacterial repetitive intergenic consensus sequence) ERIC-1R: 5'ATG TAA GCT CCT GGG GAT TCA C 3', ERIC –2: 5'AAG TAA GTG ACT GGG GTG AGC G 3'; 2) BOXA1R (Box element sequence): 5' CTA CGG CAA GGC GAC GCT GAC GCT GAC G 3'; 3) REP (repetitive extragenic palindromic sequence) REP1R-I: 5' IIII CGI CGI CAT CIG GC 3', REP2-I : 5' ICG ICT TAT CIG GCC TAC 3'

Each 25-µ reaction mixture contained 30 ng template DNA, 3mM $MgCl_2$, 1.2 (for BOX) and 2 (for ERIC and REP) pmol each primer, 200-ìM each of the four dNTPs, 1 U of Taq-DNA polymerase, 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 0.1% Tritón X-100 and 10% (v/v) DMSO (dimethyl sulfoxide).

Amplifications were performed in an automated thermocycler (MJ Research Inc, MA) with an initial denaturation (3 min at 94 ^{o}C), followed by 35 cycles of denaturation (30 s at 92 ^{o}C, annealing ((1 min at 50 ^{o}C for ERIC and BOX; at 40 ^{o}C for REP), and extension (8 min at 65^{o}C), with a final extension (10 min at 65^{o}C).

RAPD-PCR: Amplifications were carried out with 7 primers from Operon Technologies, Inc. with codes and sequences as follows OPA-01 (5'-CAGGCCCTTC –3'), OPA-02 (5'-TGCCGAGCTG –3'), OPA-03 (5'-AGTCAGCCAC-3'), OPA-04 (5'-AATCGGGCTG-3´), OPAJ-11 (5´-GAACGCTGCC-3´), OPC-02 (5´-GTGAGGCGTC-3´), OPD- 03 (5´-GTCGCCGTCA-3´). The reaction had a total volume of 20-µl with 30 ng DNA, 3-mM MgCl2, 0.5-µM primer, 0.26-mM of mixture of dNTPs, 50-mM KCl, 10-mM Tris-HCl (pH 8.8), 0.1% Tritón X-100 and 1 U Taq-DNA polymerase.

Amplifications were performed in an automated thermocycler (MJ Research Inc, MA) with an initial denaturation (2 min at 94 ^{o}C), annealing (5 min at 28 ^{o}C), denaturation (1 min at 94 ^{o}C) followed by 45 cycles of denaturation (20 s at 92 ^{o}C), annealing (1 min at 35 ^{o}C), and extension (1 min at 72 ^{o}C), with a final extension (7 min at 72 ^{o}C).

***Data analysis*:** rep-PCR fingerprints were converted to binary form (presence =1; absence = 0) and similarity coefficients for pairs of strains were calculated NTSYS (Numerical Taxonomy and multivariate Analysis system) version 2.02 (Exeter Software), using SIMQUAL with the Dice coefficient and were subjected to unweighted pair group method (UPGMA) cluster analysis. The same data matrix was subjected to multiple correspondence analysis (MCA) and analyzed using CORRESP Procedure of SAS/STAT Software.

Results and Discussion

Multiple correspondence analysis of the combined data matrix generated using REP-PCR, ERIC-PCR and BOX-PCR resulted in 3 groups with an average similarity index of 78% (Figure 15). Isolates *Xc* 44 and *Xc* 45 that were collected in Carimagua appear to be clonal. The same was true with isolates *Xc* 49 and *Xc* 50 that were collected in Palmira.

Group 1 consists of 30 isolates with an average similarity index of 52%. Group 2 had 15 isolates with a 50% similarity index. The two control isolates from cassava were clustered within group 2. Group 3 consisted of 24 isolates and had a high similarity index of 89%.

Multiple correspondence analysis of the RAPD data set resulted in three groups of isolates as well (Figure 16). The first dimension clearly separated group 2 from groups 1 and 3, whereas dimensions 2 and 3 did not contained 40 isolates with 84% similarity index. Group 2 consisted of 25 isolates that were all collected in Palmira. Group 3 had only 4 isolates.

Both RAPD and rep-PCR generated multiple bands. However, correlation between these two techniques was low (r = 0.36). RAPD data did not differentiate the two isolates of *X. axonopodis* pv. *manihotis*. The same was the case with ERIC-PCR. However, with BOX primers, as well as the combined data of BOX and REP-PCR separated the two distinct isolates from those of *X. campestris* pv. *graminis*. These results hint that rep-PCR can be used to develop rapid diagnostic tools for *Xanthomonas* pathovars. In addition, both RAPD and rep-PCR revealed genetic diversity among isolates of *X. campestris* pv. *graminis*. In light of this, caution has to be taken in germplasm movement in order not to transfer new isolates of this seed-borne pathogen from one location to another.

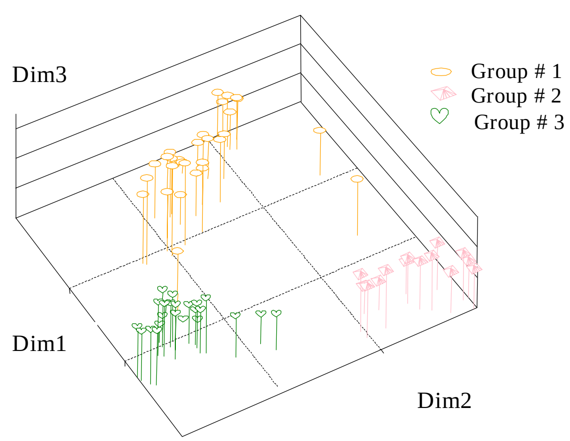


Figure 15. Multiple Correspondence Analysis (MCS) by combining the rep-PCR fingerprints of 69 isolates of *Xanthomonas* obtained with each of the two primers REP and ERIC, and BOX primer.

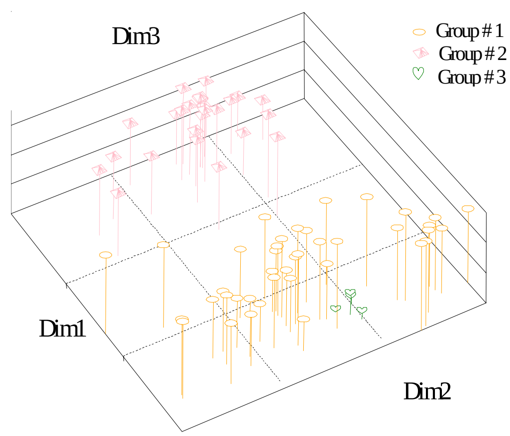


Figure 16. Multiple Correspondence Analysis (MCS) of RAPD-PCR data conducted with 69 *Xanthomonas* isolates.

2.4.3 Resistance in Brachiaria to *Xanthomonas campestris* pv. *graminis* (*Xcg*)

Contributors: M. Rodriguez and S. Kelemu (CIAT)

Rationale

Breeding host plants for disease resistance is the most important, cheapest and practical method of disease control. The financial, environmental and social benefits of using resistant cultivars of important crops are big. By combining the genes for resistance from various different genotypes, a formidable host resistance should be built against evolving races of the pathogen. The serious obstacle to this could be the variability encountered in the pathogen.

Although, there is currently no breeding program in IP-5 to combat this disease, we examined some genotypes of *Brachiaria* for their reaction to *X. campestris* pv. *graminis*. The objective of this study is to identify some sources of resistance as well as to evaluate important genotypes of *Brachiaria*.

Materials and Methods

Inoculum preparation and plant inoculation: Bacterial cells from a single colony of each isolate were grown in tubes containing freshly prepared nutrient broth (Difco), and incubated with shaking at 200 rpm, 28 C, overnight. Bacterial cells were collected by centrifugation at 4,000 rpm for 20 minutes. The medium was removed and bacterial cells re-suspended in sterile distilled water and adjusted to an optical density of $OD_{600} = 0.1$. Sterilized scissors were immersed in the bacterial suspension and used to cut leaves of *Brachiaria* plants. Leaves of control plants were cut with scissors immersed in sterile distilled water. All plants were placed in humidity chambers maintained at 27 C and RH of 70% for 48 hours. They were then moved to a growth chamber at 28-30 C and photo- period of 12 hours, or in the green house until symptoms were expressed.

Plant evaluation: Selected *Brachiaria* accessions and hybrids were evaluated for their reactions to *X. campestris* pv. *graminis*. Plants that showed any visible wilt symptoms within 15 days after inoculations were rated as susceptible (S), and those that maintained "healthy" appearance were rated resistant (R).

Results and Discussion

Thirteen *Brachiaria* genotypes, BRO-02-193, BRO-02-415, BRO-02-445, BRO-02-465, BRO-02-968, BRO-02-1045, BRO-02-1405, BRO-02-1474, CIAT 16322, CIAT 26110, CIAT 26990, CIAT 36061, 36062, were tested for their reaction to *X. campestris* pv. *graminis*. Seventeen isolates of the pathogen were used to inoculate each of the genotypes. Three genotypes, CIAT #16322, 26110 and 26990, showed no disease symptoms after inoculations with each of the 17 isolates. CIAT 36062 was the most susceptible of the genotypes evaluated, being infected with 16 of the isolates. Isolates of *X. campestris* pv. *graminis* that infect *Brachiaria* exhibit a wide range of genetic diversity. Pathogenic variation reveal that a wide range of pathotypes exist within the pathogen population. It is encouraging to note that high levels of resistance exist in *Brachiaria*, and it is possible to combine the available resistance in a breeding program.

2.5 Elucidate the role of endophytes in tropical grasses

Highlights

- First evidence that endophyte infection can improve dry season performance under field condition by improving the uptake of N, P and K acquisition by two accessions of Brachiaria brizantha.

2.5.1 Endophyte seed transmission studies in *Brachiaria*

Contributors: H. Dongyi (South China University) and S. Kelemu (CIAT)

Rationale

Brachiaria is a pan-tropical genus of grasses with about 100 species. The fungus *Acremonium implicatum* can develop an endophytic association that is mutually beneficial with *Brachiaria* species.

DNA from isolates of *A. implicatum* was amplified using 10-base random primers. Primer OPAK 10 (Operon Technology Inc.) amplified bands including a 500-bp product common to all of the isolates tested. This fragment has been cloned and sequenced. Based on this sequence data, several primers were designed and synthesized. A primer pair designated P1 (5'-TTCGAATGATAAGGCAGATC-3' and P4 (5'-ACGCATCCACTGTATGCTAC-3') amplified a 500-bp product with template DNA from isolates of *A. implicatum* in pure cultures and in tissues of *Brachiaria* infected with *A. implicatum*. No amplification product was detected in plants free from *A. implicatum* or using DNA of non-endophytic fungi or the bacterium *Xanthomonas campestris* pv. *graminis*, a pathogen of species of *Brachiaria* (Kelemu et al., 2003. Molecular Plant Pathology 4: 115-118).

This primer pair was used to conduct seed transmission studies in plants with and without *A. implicatum*. We report here the results of *A. implicatum* transmission studies in seedsand seedlings of *Brachiaria*. Preliminary data have been reported in IP-5 annual report 2002. The primer pair amplified a 500-bp product with template DNA of seeds harvested from *A. implicatum* infected *Brachiaria* plants, but no amplified products were observed with DNA of seeds from endophyte-free plants.

Materials and Methods

Endophyte elimination: The fungicide Folicur® was used to generate endophyte-free *Brachiaria* clones. Twenty or more plantlets were propagated from a mother plant naturally or artificially infected with the endophyte. Half of these plantlets were soaked in a solution of 0.6 mL/L of Folicur® (250 g a.i./L) for 6 h to eliminate the endophyte, and the other half were left untreated to serve as controls. All plantlets were individually planted in small pots and placed in the greenhouse. Plants were examined 4-6 weeks after treatment for the presence or absence of *A. implicatum*.

DNA isolations: Fresh mycelia of endophyte isolates cultured on PDA plates, endophyte-infected or endophyte-free plant leaves, or seeds were collected and macerated in liquid nitrogen for genomic DNA isolation. Genomic DNA was extracted using the DNeasy™ Plant Mini Kits (QIAGEN, Valencia, CA) according to the manufacturer's instructions.

PCR Amplifications: Specific primers P1 (5'-TTCGAATGATAAGGCAGATC-3') and P4 (5'-ACGCATCCACTGTATGCTAC-3') were used in the PCR reactions. Amplifications were carried out in a Programmable Thermal Controller (MJ Research, Inc.), programmed with 44 cycles for genomic DNA of endophyte pure cultures or plant leaves, and 54 cycles for DNA from *Brachiaria* seeds, of a 30 sec denaturation step at 94°C (3 min for the first cycle), follwed by 1 min at 65°C, and primer extension for 1 min (10 min in the final cycle) at 72°C. The amplification products were separated by electrophoresis in a 1.0% agarose gel (Bio-Rad), stained with ethidium bromide and photographed under UV lighting.

Seed samples were collected from plants confirmed to be endophyte-infected or endophyte-free using the PCR tests with template DNA isolated from plant tissues, and fungal endophyte isolation on culture media.

Results and Discussion

Acremonium implicatum forms a symbiotic endophytic association with at least some of the economically important *Brachiaria* species. We sought to ascertain whether endophytic *A. implicatum* could be seed-transmitted in *Brachiaria*. Twenty tillers were vegetatively propagated from a single, endophyte-infected mother plant. Ten tillers were treated with the fungicide Folicur® to eliminate the endophyte while the remaining ten tillers were untreated. Seeds were harvested individually from these genetically identical plants, with or without the endophyte. Some of the seeds were germinated and seedlings grown in the glasshouse. A polymerase chain reaction (PCR)-based method developed previously uses a pair of endophyte-specific primers to amplify a single DNA fragment of about 500 bp. DNA both from remnant seeds and from 2-month-old seedlings was amplified with these primers to detect presence of the endophyte. The diagnostic DNA fragment was consistently amplified in DNA of seeds harvested from the endophyte-infected plants and DNA from seedlings grown from seeds harvested from endophyte-infected plants, but not from seeds or seedlings originating from fungicide treated endophyte-free plants. We conclude that *A. implicatum* can be transmitted through seeds.

The primer pair, P1/P4, allows the precise and rapid detection of *A. implicatum* in *Brachiaria* plants and permits a differentiation between endophytic and non-endophytic fungi (Kelemu et al., 2003. Molecular Plant Pathology 4: 115-118). A single band of about 500-bp in all examined isolates of *A. implicatum* was amplified. Endophyte-containing and endophyte-free plants were also consistently differentiated using this primer combination (data not shown). Seeds were collected from plants whose tissue samples were used as well as other plants. All seed DNA from endophyte-containing plants had a 500-bp amplified product. No amplification product was detected with seed DNA from endophyte-free plants (Figure 17).

Seedlings generated from seed samples of endophyte-containing and endophyte-free plants had consistently tested positive or negative, respectively, for the diagnostic 500-bp amplified product (Figure 18). From these results, we concluded that *A. implicatum* maintains its symbiotic association with species of *Brachiaria* through seed transmission.

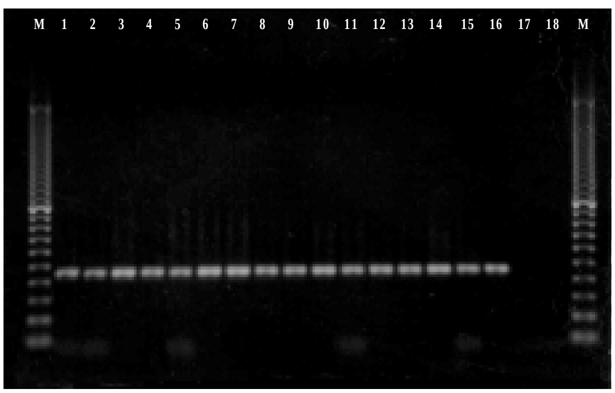


Figure 17. Specific detection of *Acremonium implicatum* in seeds harvested from endophyte-infected *Brachiaria* plants using polymerase chain reaction (PCR) with primer pair P1/P4. **Lanes 1-16**, template DNA extracted from seeds of endophyte infected *Brachiaria* hybrids SX99/3488 (8), SX99/0275 (14), BR99NO/4132 (22), FM9201/1873 (29), BR99NO/4015 (37), BR99NO/4132 (39), *B. decumbens* CIAT 606 (42), BRUZ4X/4402 (44), FM9201/1873 (48), SX99/0731 (52), *B. brizantha* CIAT 16320 (32a), FM9503/S046/024 (45), *B. brizantha* CIAT 26110 (15), *B. brizantha* CIAT 6780 (56), *B. brizantha* CIAT 6780 (68), and *B. brizantha* CIAT 6780 (111), respectively. **Lanes 17,18**, DNA extracted from seeds of endophyte-free plants of *B. brizantha* CIAT 16320 (32-25) and *B. brizantha* CIAT 16320 (32-29); **lanes M**, 100-bp ladders. *B. brizantha* CIAT 26110 (15), *B. brizantha* CIAT 6780 (56), *B. brizantha* CIAT 6780 (68), *B. brizantha* CIAT 6780 (111) were artificially infected. All others were naturally infected.

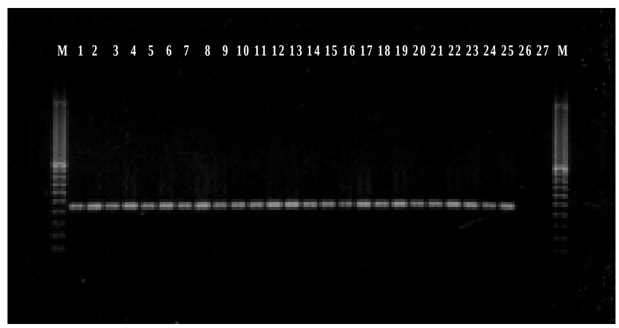


Figure 18. Specific detection of *Acremonium implicatum* in seedlings generated from seeds of endophyte-infected and endophyte-free *Brachiaria* plants using polymerase chain reaction (PCR) with primer pair P1/P4. **Lanes 1-7**, seedlings from seeds harvested from naturally endophyte-infected plants SX99/3488 (8), BRN99NO/4132 (22), BRN99NO/4132 (39), *B. decumbens* accession CIAT 606 (42), BRUZ4X/4402 (44), FM9201/1873 (48), SX99/073 (52), respectively; **lanes 8-17**, seedlings generated from seeds of ten artificially infected *B. brizantha* CIAT 26110 (15) plants; **lanes 18-25**, seedlings generated from seeds of eight naturally infected *B. brizantha* CIAT 16320 (32) plants; **lanes 26 & 27**, seedlings generated from seeds of two endophyte-free *B. brizantha* CIAT 16320 (32-25) plants; **lanes M**, 100-bp ladders.

2.5.2 Distribution of endophytes in different plant parts of Brachiaria

Contributors: H. Dongyi (South China University), T. Sakai (JICA), and S. Kelemu (CIAT)

Rationale

Endophytic fungi often develop a systemic association with their hosts. Several reports demonstrated that endophytic fungi, such as *Epichloë* and *Neotyphodium*, could be distributed in leaf sheaths, leaf blades, stems, roots, seeds and embryos of their grass hosts.

Although endophytes infect their hosts systemically, the concentration of hyphae is not uniform throughout parts of infected plants. Some parts of endophyte-infected plants can even be endophyte-free. Using tissue staining and culturing methods, endophytic fungus *A. implicatum* was observed in leaf sheaths and seeds of *Brachiaria*. These two methods, however, are time consuming and unreliable for endophyte distribution studies in different parts of the plant, especially where fungal mycelia are sparsely distributed. We have developed a rapid and sensitive PCR-based method for specific detection of *A. implicatum* in tissues of *Brachiaria* (Kelemu et al., 2003. Molecular Plant Pathology 4: 115-118). We used this method to determine the distribution of *A. implicatum* in various parts of *Brachiaria* plants.

Materials and Methods

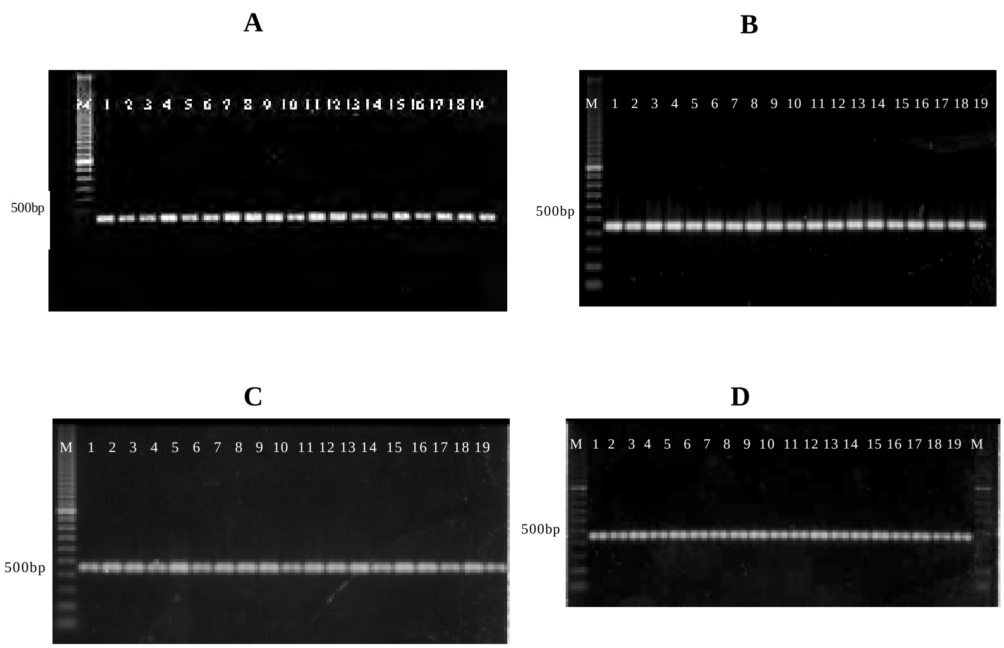
DNA isolation: Leaf sheaths, leaf blades, stems, roots, seeds, embryo and endosperm of seeds were collected from endophyte-infected or endophyte-free plants and macerated separately in liquid nitrogen for genomic DNA isolation. Genomic DNA was extracted using the DNeasy™ Plant Mini Kits (QIAGEN, Valencia, CA).

PCR amplifications: Composition of PCR reactions (20 mL) were 1x PCR buffer, 3mM $MgCl_2$, 0.25mM dNTPs, 0.5 ìM primer P1 and P4, 1U Taq DNA polymerase, 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, 65°C 1 min; step 4, 72°C 1 min; step 5, go to step 2 for 44 cycles (for genomic DNA of leaf sheaths and leaf blades) or 54 cycles (for genomic DNA from stems, roots, seeds, embryos and endosperms); then 72°C 10min. The amplification products were separated by electrophoresis in a 1.0% agarose gel (Bio-Rad), stained with ethidium bromide and photographed under UV lighting.

The distribution of *A. implicatum* in plant tissues was determined by the presence or absence of a diagnostic 500-bp amplification product.

Results and Discussions

A diagnostic 500-bp amplification product was observed in all examined leaf sheaths, leaf blades, stems, and roots of *Brachiaria* plants infected with *A. implicatum* (Figures 19 A, B, C, D). The amplification product was also detected in whole seeds, embryo, and endosperm of seeds (data not shown). These results indicate that *A. implicatum* is distributed in the plant parts described above. Johnson et al. (1985, Plant Disease 69:200-202) described the concentration and distribution of Epi*chloë typhina* in tall fescue individual plants with decreasing order in leaf sheaths, seeds, crows, stems, leaf blades, and roots. Because amplifications with template DNA from leaf sheaths, leaf blades and fresh seeds generate the diagnostic 500-bp product with just 45 cycles, as opposed to 55 cycles with DNA from roots and stems, it is likely that mycelial concentrations and distributions in *A. implicatum*/*Brachiaria* associations have a similar trend as those reported in Epi*chloë typhina*/ tall fescue. There was no obvious difference in sensitivity with genomic DNA from leaf blades and sheaths, although isolations on culture media is more routinely and successfully done from leaf sheaths than leaf blades.



Figures 19 A, B, C, D. Specific detection of *Acremonium implicatum* in leaf blades (Figure A), leaf sheaths (Figure B), stems (Figure C), and roots (Figure D) collected from endophyte-infected *Brachiaria* plants using polymerase chain reaction (PCR) with primer pair P1/P4. **Lanes 1~19**: *Brachiaria* hybrid plants SX99/3488 (8), SX99/0275(14), BR99NO/4132 (22), SX99/1513 (23), FM9201/1873 (29), BR99NO/4015 (37), BR99NO/4132 (39); *B. decumbens* accession CIAT 606 (42); *B*. hybrids BRUZ4X/4402 (44), FM9201/1873 (48), SX99/0731(52), FM9503/S046/024 (19); *B. brizantha* accession CIAT 16320 (32a); *Brachiaria* hybrid FM9503/S046/024 (45), SX99/2341(47); *B. brizantha* accession CIAT 26110 (15), *B. brizantha* 6780 (56), *B. brizantha* 6780 (63), *B. brizantha* 6780 (111), respectively. *Brachiaria* hybrid FM9503/S046/024 (19), *B. brizantha* CIAT 16320 (32a), *Brachiaria* hybrid FM9503/S046/024 (45), *Brachiaria* hybrid SX99/2341(47) were naturally infected with isolates we have previously characterized. Plant *B. brizantha* CIAT 26110 (15), *B. brizantha* CIAT 6780 (56), *B. brizantha* CIAT 6780 (63), and *B. brizantha* CIAT 6780 (111) were artificially infected with an isolate (EB 6780(201) of *A. implicatum.* All remaining plants were naturally infected with yet to be isolated and characterized strains.

2.5.3 Effect of fungal endophytes on pathogens *in planta*

Contributors: H. Dongyi (South China University) and S. Kelemu (CIAT)

Rationale

Several *in vitro* studies have demonstrated that *Acremonium* endophytes and *Epichloë typhina* cultures exhibit antifungal activity. White and Cole (1985, Mycologia 77:487-489; 1986, Mycologia 78:102-107) reported that an *Acremonium* spp. from *Festuca*, *A. coenophialum* (now renamed *Neotyphodium coenophialum*) from tall fescue, and *A. lolii* (renamed *N. lolii*) from perennial ryegrass inhibited mycelial growth of seven different fungi including *Rhizoctonia* spp. in culture. Siegel and Latch (1991, Mycologia 83:529-537) examined the effect of a series of isolates of A*cremonium sp., E. typhina, Phialophora*-like sp. and *Gliocladium*-like sp. on mycelial growth of several grass pathogens in agar culture. Their results indicate that individual isolates of the same species differed in their growth inhibition activities of grass pathogens.

Although many endophyte isolates show antifungal activities *in vitro*, there are only a few reports on resistance to pathogens conferred by endophytes *in planta*. Reduction of tall fescue seedling density due to *Rhizoctonia zeae* was inversely correlated with endophyte (*N. coenophialum)* infestation level of the seed lot (Gwinn and Gavin, 1992, Plant Disease 76:911-914). Plant protection by *E. typhina* against *Cladosporium phlei*, the causing pathogen of purple leaf spot of timothy grass, was reported (Greulich et al. 1999, Ann. Phytopathol. Soc. Jpn. 65:454-459).

Apart from providing direct resistance to fungal pathogens, endophytes can reduce the spread of viral diseases by deterring insect vectors such as the aphid *Rhopalosiphum padi.*

Drechslera sp. and *Rhizoctonia soloni* are the most important pathogens of species of *Brachiaria*. Our earlier results showed that *A. implicatum* infected plants had fewer and smaller disease lesions caused by *Drechslera* sp. than did genetically identical endophyte-free plants (Kelemu et al., 2001, Canadian Journal of Microbiology 47:55-62). Some genotypes of *Brachiaria* are resistant to *R. solani*. We speculate that *A. implicatum* may contribute to some of this resistance to foliar blight disease caused by *R. solani*.

Materials and Methods

Culture maintenance: All endophytic or pathogenic fungi were cultured and maintained as described by Kelemu et al. (2001, Canadian Journal of Microbiology 47:55-62).

Antifungal extractions from endophyte cultures: Mycelia/conidia were collected from 27 colonies (about 20 mm in size) of *A. implicatum* isolate EH32a grown on potato dextrose agar (PDA). This was macerated in 50 mL sterile distilled water and centrifuged at 12000 rpm for 30 minutes. The supernatant was lyophilised and re-suspended in 9 mL sterile distilled water. This extract was then filter sterilized using 0.22 ìm pore-size nylon membranes. The 9 mL filtrate was then divided into 3 parts of 3 mL each. The first part was heat treated at 100 ºC for 20 minutes. The second portion was treated with pronase (2.0 mg/mL final concentration) and incubated at 37 ºC for 4 hours. The third portion was left in its natural state as control.

Antifungal activity tests: Filter paper discs were soaked with 400-ìl endophyte mycelial/conidial extract prepared as described above. These were placed on PDA-containing petri dishes individually inoculated with *Drechslera* sp. and *R. solani* as shown in Photo 4. These were incubated at 28 ºC in the dark for 3 –5 days.

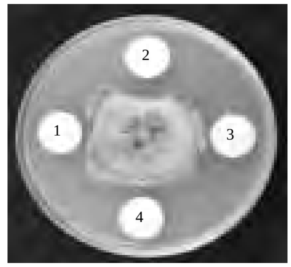
Plant inoculation and disease evaluations: Young tillers from genetically identical endophyte-infected and endophyte-free *B. brizantha* CIAT 6780 or CIAT 16320 were transplanted individually in pots. Plants were inoculated with mycelial agar discs removed from actively growing *R. solani* cultures, by placing the discs in contact with the plant stems just above the soil level and wrapping them with parafilm to secure the contact. Inoculated plants were maintained at high relative humidity in the greenhouse. The upward progression of disease spread and symptoms was measured as distance from the inoculation point.

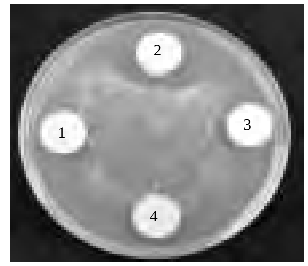
Results and Discussion

In *in vitro* inhibition tests, most of the 11 *A. implicatum* strains showed antifungal activities although they differ in the inhibition zone area they generated (data not shown). Strains EB 6780(501) and EH 32a showed strong inhibition to both *Drechslera sp.* and *R. solani.*

With *in vivo* tests, endophyte-infected *B. brizantha* CIAT 6780 and CIAT 16320 plants showed more resistance (exhibited as slower upward disease progression) to foliar blight disease than their endophyte-free counterparts at the early stages of infection (7 days after inoculation). Using the Harsfall-Barratt visual rating system (1945. Phytopathology 35:655), disease severity was 4% and 25% on CIAT 16320 and *B. brizantha* CIAT 6780, respectively; as opposed to 25% and 39% on their endophyte-free counterparts, respectively. We concluded that *A. implicatum* contributes to *Rhizoctonia* foliar blight resistance in these two genotypes of *Brachiaria*. It is also important to note that those isolates that exhibited strong inhibitory activities in *vitro* contributed to *in planta* resistance.

Extracts from *A. implicatum* strain EH32 showed strong inhibition to *Drechslera sp* and *R. solani* (Photos 4a and 4b). Extracts treated with heat or pronase lost their antifungal activity. Further extensive studies are needed to determine the nature of the antifungal activity in *A. implicatum*.





Photos 4. Growth inhibition of *Drechslera* spp. (a) and *Rhizoctonia solani* (b) by cell-free culture extracts of *Acremonium implicatum* strain EH32a. Filter paper discs 1-3 were soaked with cell-free extracts of *Acremonium implicatum* strain EH32a. Filter paper discs # 1, #2, #3, and # 4 were soaked with heat-treated extracts, extracts in their natural state, extracts treated with pronase, and sterile distilled water, respectively.

2.5.4 Drought tolerance in endophyte-infected Brachiaria accessions under field conditions

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

Rationale

Previous research conducted in the greenhouse with soil-grown plants showed 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). Last year, 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*. This year we report the preliminary results from that trial based on measurements conducted in the rainy season and dry season.

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 uptake were measured at the end of wet season (November 2002) and dry season (March 2003).

Results and Discussion

At 6 months after establishment, i.e., at the end of rainy season, the endophyte-infected plants (E+) showed significantly lower values of leaf biomass (Figure 20a) in both accessions of *Brachiaria brizantha* (CIAT 6780 and CIAT 26110). Between these two accessions, CIAT 26110 was more productive. Results on shoot nutrient uptake also showed that uptake of N, P, K, Ca and Mg was greater with uninfected (E-) plants than that of endophyte-infected (E+) plants (Figure 20b).

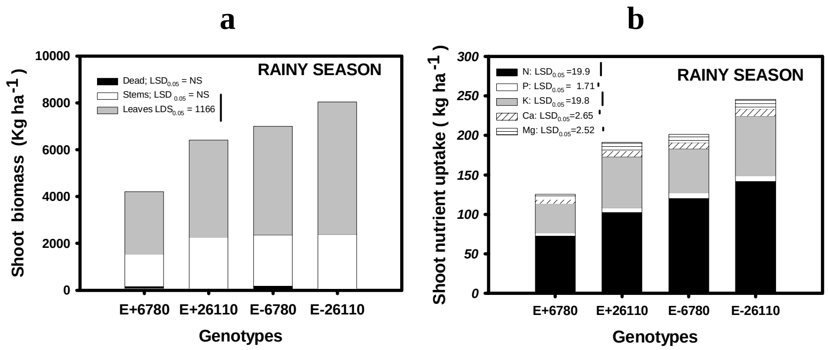


Figure 20. Influence of endophyte infection on (a) shoot biomass production and (b) nutrient uptake of two accessions of *Brachiaria brizantha* CIAT 6780 and CIAT 26110 at 6 months after establishment (at the end of rainy season). E+ are endophyte-infected plants while E- are endophyte-free plants.

In contrast to the results at the end of rainy season, at 10 months after establishment, i.e., at the end of dry season, the endophyte infected plants showed significantly greater values of green leaf biomass (Figure 21a) in both accessions of *Brachiaria brizantha* (CIAT 6780 and CIAT 26110). This observation is consistent with the results from the greenhouse study where the benefits of endophyte infection were noted only under severe drought stress. Results on nutrient uptake at the end of dry season also showed that the uptake of N, P and K was greater with endophyte-infected plants than that of uninfected plants (Figure 21b).

This on-going field study indicated that endophyte infection could improve dry season performance by improving the uptake of nutrients by two accessions of *Brachiaria brizantha*. Further field evaluations for the next two years are needed to cofirm the role of endophytes in improving dry season tolerance of *Brachiaria* grasses.

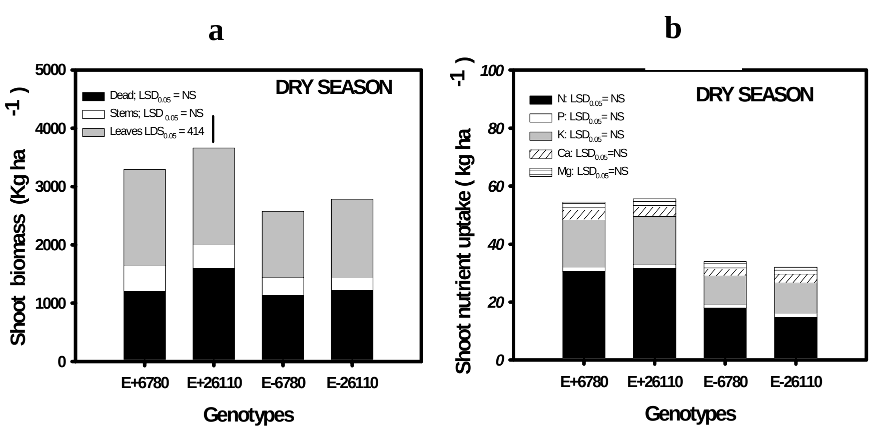


Figure 21. Influence of endophyte infection on (a) shoot biomass production and (b) nutrient uptake of two accessions of *Brachiaria brizantha* CIAT 6780 and CIAT 26110 at 10 months after establishment (at the end of dry season). E+ are endophyte-infected plants while E- are endophyte-free plants.

2.6 Association of bacteria with Brachiaria genotypes

Highlight

- Identification of a nitrogen-fixing bacteria associated with Brachiaria hybrid CIAT 36062
- Isolated an endophytic bacteria in the Brachiaria hybrid CIAT 36062

2.6.1. Search for nitrogen fixing bacteria associated with species of *Brachiaria*

Contributors: C. Zuleta, R. Sedano and S. Kelemu (CIAT)

Rationale

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). Tropical forage grasses and grasslands could be ideal for investigating associations with nitrogen fixing bacteria because of their perennial nature and low chemical inputs including fertilizers. The main objectives of this initiative are to: 1) look for endophytic and rhizospheric bacteria responsible for nitrogen fixation in association with species of *Brachiaria*, 2) identify and characterize both plant growth promoting and nitrogen-fixing bacteria that also result in healthier plants.

Because nitrogen fixation is performed by diverse groups of prokaryotic organisms, detection of a marker gene which is unique and is required for nitrogen fixation may be useful to conduct our studies. The *nifH* gene (encodes nitrogenase reductase) has been used with a number of PCR primers that amplify the gene from microbes and other samples by a number of researchers.

Materials and Methods

Bacterial isolates: Isolates of the genera *Rhizobium* of *Bradyrhizobium* were used as positive controls. A bacterium which was consistently isolated from *Brachiaria* CIAT 36062 in 1999, and which we suspected might have a role in fixing nitrogen was included in the test. An isolate of *Xanthomonas campestris* pv. *graminis* (isolate 1015), the causal agent of bacterial wilt of species of *Brachiaria*, was used as a negative control. Bacterial isolates include the following: 1) *Bradyrhizobium* 3101 isolated from forage legume *Centrosema* (Colombia), 2) *Bradyrhizobium* 2469 isolated from forage legume *Desmodium* (Colombia), 3) BR97-155 CBT, unidentified bacterium isolated from *Brachiaria* BR97-155 (Colombia), 4) 16445 CBT, unidentified bacterium isolated from *Brachiaria* CIAT 16445 (Colombia), 5) 16497 CBH, unidentified bacterium isolated from *Brachiaria* CIAT 16497 (Colombia), 6) FM97-383 CACT, a bacterium isolated from *Brachiaria* FM97-383 (Colombia), 7) *Rhizobium* 668 isolated from *Phaseolus vulgaris,* 8) BR97-1371, a bacterium isolated from *Brachiaria* CIAT 36062 (Colombia), 9) *Xanthomonas campestris* pv. *graminis* isolated from *Brachiaria* 1015.

DNA extractions from bacteria: DNA extraction was conducted 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. The quality of DNA was checked on 1 % agarose gel.

DNA isolations from soil samples: A protocol described by Porteous et al. (1997. An improved method for purifying DNA from soil for polymerase chain reaction amplification and molecular ecology applications. Technical note. Molecular Ecology. 6: 787-791) was used to isolate DNA from soil. The method in general involves lysis of microbial cells, sonication, precipitation, and various steps of cleaning.

Plant DNA extraction: A method described by Dellaporta et al (1983. A plant DNA mini-preparation: version II. Plant Molecular Biology Reporter 1: 19-21)

Nested PCR Amplification: 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.

Results and Discussion

DNA extraction from bacterial cells: DNA extracted from bacterial cells is shown in Figure 22.

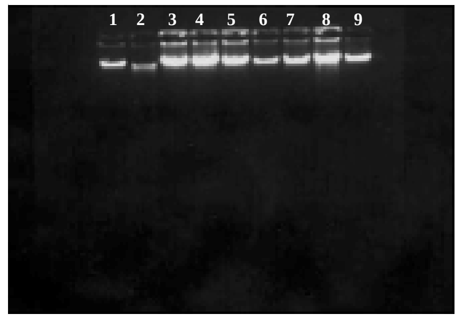


Figure 22. DNA isolated from: 1) *Bradyrhizobium* 3101, 2) *Bradyrhizobium* 2469, 3) unidentified bacterium BR97-155 CBT, 4) unidentified bacterium16445 CBT, 5) unidentified bacterium 16497 CBH, 6) unidentified bacterium FM97-383 CACT, 7) *Rhizobium* 668, unidentified bacterium. BR97-1371, 9) *Xanthomonas campestris* pv. *graminis* 1015.

Nested PCR amplifications:.Amplified products of approximately 370-bp size were amplified with template DNA from nitrogen-fixing bacteria *Rhizobium* and *Bradyrhizobium*, as well as from those randomly picked bacterial colonies isolated from *Brachiaria* CIAT 16445, *Brachiaria* CIAT 16497, and *Brachiaria* FM97-383 (Figure 23). Template DNA from a randomly picked bacterial colony from *Brachiaria* CIAT 36062 amplified a product with approximately 210-bp size (Figure 23). No amplification products were observed with DNA from the pathogen *X. campestris* pv. *graminis* (Figure 23; lane 9) and with that of a bacterium isolated and selected from *Brachiaria* BR97-155 (Figure 23; lane 3).

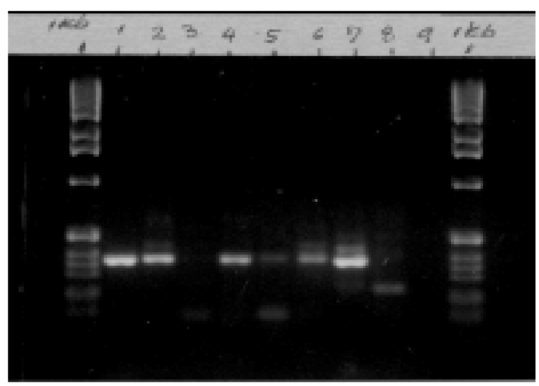


Figure 23. Nested PCR amplification products with three primers of sequences of *nifH* gene. Lanes 1-9, *Bradyrhizobium* 3101, *Bradyrhizobium* 2469, BR97-155 CBT, 16445 CBT, 16497 CBH, FM97-383 CACT, *Rhizobium* 668, BR97-1371, *Xanthomonas campestris* pv. *graminis* 1015 (negative control), respectively. Size markers 1kb ladder.

Brachiaria hybrid CIAT 36062 (BR97-1371) is of particular interest because of its maintenance of green color in the absence of nitrogen input. We have plants of this hybrid in pots in the glasshouse for the last 4 years with no application of nitrogen fertilizer, but are still green. We, therefore, concentrated on this hybrid and isolated independent bacterial colonies from roots, leaves, stems, and soil around the plant roots. Roots were sectioned into three parts: superficial (next to stems), middle and bottom parts. Pieces plant tissues were surface sterilized, macerated in sterile distilled water and plated on nutrient agar for bacterial isolations. Cells from individual bacterial colonies (random colony selection was based on colony color and morphology) were transferred to fresh nutrient agar for further increment. Nested PCR amplification with DNA of these colonies resulted in various size products ranging between 200-1000 bp sizes (Figure 24). Two colonies (Figure 24; lanes 7, 15) gave no amplification products. Some bacterial colonies isolated from the bottom part of the root, the leaf and stem generated strong amplification products with the same size as that produced by nitrogen-fixing bacteria used as positive controls (Figure 24).

In this study, the application of nested PCR amplifications of the *nifH* gene provided us with the first clue that there are bacteria associated with *Brachiaria* hybrid CIAT 36062 involved in nitrogen fixation. These bacteria exist in higher concentration around/in the roots and in the soil around the plant roots than in the leaves and stems. Using these preliminary results as a basis, we intend to conduct more detailed studies to understand the association and to exploit its field application.

DNA isolated from soil samples taken from the surface, middle and bottom part of the potted plants all generated strong amplification products with the same size as those produced by nitrogen-fixing bacteria used as controls (Figure 25).

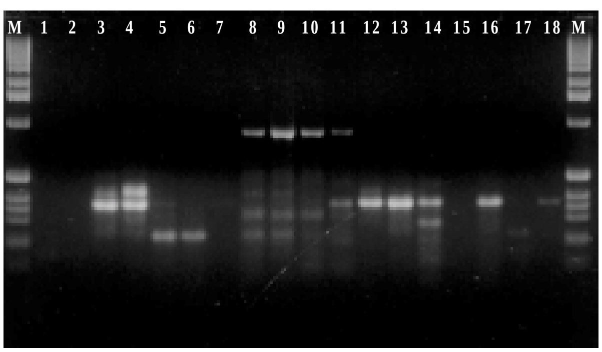


Figure 24. Nested PCR amplification products with three primers of sequences of *nifH* gene. Lane 1) negative control with no template DNA, 2) *Xanthomonas campestris* pv. *graminis* (negative control), 3) *Bradyrhizobium* 3101 (positive control), 4) *Rhizobium* 668 (positive control); lanes 5-7, bacteria isolated from the top part of the root (next to the stem) of *Brachiaria* CIAT 36062 – colonies 1, 2, 3, respectively1; lanes 8-10, bacteria isolated from the middle part of the root – colonies 1, 2,3, respectively; lanes 11-13, bacteria isolated from the bottom tip of the root – colonies 1, 2, 3, respectively; lanes 14-18, bacteria isolated from leaf -1, stem -B1, stem -A2, stem -A3, stem –C, respectively; M=1Kb

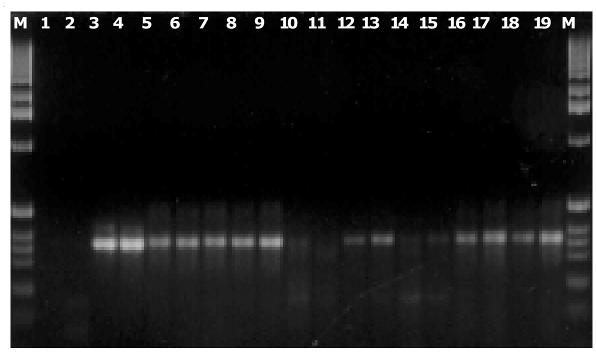


Figure 25. Nested amplifications of the *nifH* gene from soil samples of pots, where *Brachiaria* CIAT 36062 plants have been growing for four years, and bacterial cultures. Lane 1, control with no template DNA; lane 2) *Xanthomonas campestris* pv. *graminis* 1015 (negative control); lane 3) *Bradyrhizobium* 3101 (positive control), 4) *Rhizobium* 668 (positive control); lanes 5-19, surface soil-1, surface soil-2, middle-level soil-1, soil from bottom part of pot-1, soil from bottom-part of pot-2, leaf of CIAT 36062 plant 1, leaf of CIAT 36062 plant 1, leaf of CIAT 36062 plant 2, leaf of CIAT 36062 plant 2, leaf of CIAT 36062 plant 3, leaf of CIAT 36062 plant 3, root of CIAT 36062 plant 1, root of CIAT 36062 plant 3, root of CIAT 36062 plant 3, respectively. Total microbial and plant DNA was extracted from the plant tissues for amplification. M=1Kb ladder.

2.6.2 Bacterial endophytes isolated from *Brachiaria*

Contributors: C. Zuleta, R. Sedano, and S. Kelemu (CIAT)

Rationale

Endophytic bacteria are bacteria that reside in plant tissues without causing any visible harm to the plant. These bacteria can be isolated from surface-sterilized plant tissue or extracted from internal plant tissue. Different bacterial species have been isolated from a single plant. Although the primary point of entry for many of these bacteria is the root zone, aerial plant parts like flowers and stems may also be entries. Once inside a plant, they may be localized at the point of entry or spread throughout. They have been reported to live within cells, in the intercellular spaces or in the vascular system.

Soil bacteria of the genera *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* are of great agricultural importance, because of their ability to fix atmospheric nitrogen in a symbiosis association with legumes. Populations of rhizobia can survive in the soil as saprophytes in the absence of legumes. In recent years, the natural habitat of rhizobia was extended to the roots of gramineous plants. *Rhizobium leguminosarum* bv. *trifolii* was reported to exist inside the roots of rice plants grown in rotation with clover in Egypt (Yanni et al. 1997. Plant Soil 194:99-114), without forming root nodules or nodule-like structures. Various other N_2-fixing endophytic bacteria, known as plant growth-promoting rhizobacteria (PGPR), such as *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in sugarcane, *Azoarcus* spp. in Kallar grass (*Leptochloa fusca*), and *Azospirillum* spp. in maize and rice have been reported. *Herbaspirillum seropedicae* has also been found in association with maize, sorghum and other gramineous plants. Sugarcane plants inoculated with a wild type strain of *A. diazotrophicus* had a higher nitrogen content those inoculated with a *nif* mutant strain or uninoculated controls in nitrogen-deficient conditions.

The objectives of this study were: 1) to isolate nitrogen-fixing endophytic bacteria associated with *Brachiaria*; 2) to identify these bacteria; 3) to characterize them using *nif* gene primers.

Materials and Methods

Bacterial isolation: Leaf, stem and root tissues of *Brachiaria* CIAT 36062 (grown in pots in the green house) were collected and cut into 3-5- cm long sections (roots were first washed in tap water before sectioning them). They were then surface sterilized in 1% NaOCl for 2 min, in 70% ethanol for 1 min, and rinsed 3 times in sterile distilled water. The tissues wee separately macerated in 1-mL sterile distilled water in mortar and pestle. Fifty-µl of the macerated solution was spread uniformly on agar nutrient medium (Difco Lab., Detroit, MI) and incubated at 28 ºC until bacterial colonies appeared.

DNA isolation: DNA extraction was conducted 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. The quality of DNA was checked on 1 % agarose gel.

Nested PCR Amplification: 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.

Bacterial identification: Three bacterial colonies (codes 36062-H4 [isolated from leaf]; 36062-R2 [isolated from root]; 36062-V2 [isolated from stem]) that tested positive for *nif* were sent to Microbial ID, Newark, DE for identification. The company's identification is based on Similarity Index which expresses how closely the fatty acid composition of the unidentified sample compares with the mean fatty acid composition of the strains used to create the library entry. An exact match of the fatty acid composition results in a similarity index of 1.000.

Results and Discussion

The three bacterial isolates 36062-R2, 36062-H4, and 36062-V2 consistently isolated from *Brachiaria* CIAT 36062 in roots, leaves and stems, respectively, tested positive in nested PCR amplifications (Figure 26. 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 (Luz, W.C., http://www.ag.auburn.edu/argentina/pdfmanuscripts/luz.pdf, accessed on 05 August, 2003). The analysis matched isolate 02-36062-H4 with *Agrobacterium rubi* at 0.845 similarity index. The name *A. rubi* is synonymous to *Rhizobium rubi* (Young et al. 2001. Int. J. Syst. Evol. Microbiol. 51:89-103). 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).

Future research will include: 1) isolation and characterization of more endophytic bacteria and identifications based not only on fatty acid composition, but also on morphology and DNA based; and 2) the role of these bacteria in plant development.

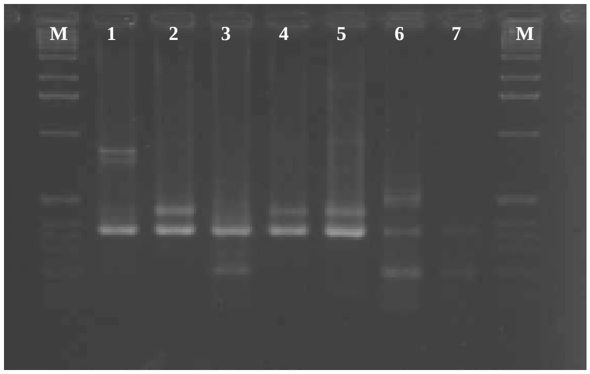


Figure 26 Nested PCR amplifications, using *nif* gene primers, with template DNA of bacterial isolates. Lanes1-3 bacterial isolates 36062-H4, 36062-R2, 36062-V2, isolated from leaf, root and leaf sheath tissues of *Brachiaria* CIAT 36062, respectively. Lane 4, positive control *Bradyrhizobium* isolate 2469; lane 5, positive control *Rhizobium* isolate 49; lane 6, negative control *Xanthomonas campestris* isolate 1015; lane 7, nested PCR cocktail control; lanes M, 1 kb-ladder.

2.7 Search for useful genes in tropical forage

Highlights

- Isolated a protein (Fenotin) from a tropical forage legume (*Clitorea ternatea*) that has antifungal, antibacterial and insecticides properties

2.7.1 Isolation of an antifungal protein in seeds of a tropical legume

Contributors: G. Segura, C. Cardona, and S. Kelemu (CIAT)

Rationale

An array of plant defense mechanisms can be triggered upon wounding or perception of microorganisms, including the synthesis of proteins and peptides that have antifungal activity. Like plants, bacteria, insects, fungi, and mammals synthesize a number of antifungal proteins and peptides (small proteins). Plant seeds use strategies to germinate and survive in the soil that is densely inhabited with a wide range of microfauna and microflora. Various antifungal and/or antibacterial proteins such as chitinases, β-glucanases, thionins, ribosome-inactivating proteins and permatins have been detected in seeds.

In this study, we examined a number of tropical forage legume seeds for antifungal properties. Among those examined, *Clitoria ternatea* (L.) seeds exhibited strong antifungal activity on the test fungus *Rhizoctonia solani* in vitro. Among other traits, *C. ternatea* is: 1) adapted to a wide range of soil conditions; 2) drought resistant; 3) practically free of diseases and pests.

We report here the isolation, purification, and characterization of a peptide from seeds of C. ternatea with antifungal, antibacterial and insecticidal properties.

Materials and Methods

Biological materials: *C. ternatea* CIAT 20692 samples were initially obtained from germplasm collection maintained by CIAT's genetic resources unit. Once antifungus activity was determined, we planted the remaining seeds on field plots at CIAT headquarters in Palmira for large quantities of seed production. The test fungus *R. solani* originally isolated from Centrosema pubescens CIAT 5596 was maintained as air-dried sclerotia produced on potato dextrose agar (PDA).

Seed extraction: Seeds (3 g) of *C. ternatea* CIAT 20692 were surface sterilized in 70% ethanol (4 min), in 2.5% NaOCl solution for 15 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 30 mL sterile distilled water with sterile mortar and pestle. The macerated solution was filtered through several layers of cheese cloth. The filtrate was then centrifuged in Eppendorf tubes (1 mL) at 13,000 x g for 30 min. The supernatant was used to determine antifungal activity bioassay.

Antifungal activity bioassay: Three thick filter (#7) paper discs were placed on PDA containing petri dishes. A 300-µl seed extract filtrate was carefully applied onto one of the filter paper discs, where as sterile distilled water (300-µl) was pipetted onto the second filter paper. A single sclerotium of R. solani was then placed in the center of the plate and incubated at 28 ºC. Evaluations were made after two days of incubation.

Determination of the nature of active extract: The extract was either treated with pronase (2 mg/mL final concentration) and incubated at 37 ºC for 2 h; or heat treated at 100 ºC for 5 min.

Protein gel electrophoresis (IEF and SDS-PAGE): Samples were cleaned and concentrated (typically 10-fold) by ultrafiltration with Centricon-3 membrane tubes (3,000-molecular-weight cutoff; Amicon). They were then analyzed by isoelectric focusing (IEF) in ultra-thin-layer polyacrylamide gels (Serva Fein-biochemica GmbH & Co). The samples were loaded in triplicates on the same gel, leaving enough space between them for cutting the gel in three equal parts once the run was complete. One was stained with Coomassie Brilliant Blue R250 to visualize the proteins; the second was neutralized in a buffer and then lightly coated with PDA by pouring a warm PDA before it solidified; the third was neutralized and then over-imposed on the Coomassie-stained triplicate and gel areas corresponding to individual stained protein bands were cut out for further antifungal activity tests.

Samples were also analyzed by SDS-PAGE (separating gel: 12% total acrylamide, 0.3% bis-acrylamide; stacking gel: 4% total acrylamide, 0.2% bis-acrylamide).

Isolation and purification of antifungal protein: Proteins were extracted from10 g seeds macerated in 100 mL sterile distilled water for protein purification. The macerated suspension was filtered through several layers of cheese cloth and centrifuged at 13,000 x g for 30 minutes. The supernatant was deprived of low-molecular-weight solutes by ultrafiltration with Centricon-3 and then concentrated by lyophilization. The lyophilized powder was re-suspended in (1/10th of the original volume) sterile distilled water. The sample was resolved by preparative granulated bed isoelectric focusing (Bio-Rad Laboratories) with pH range of 3.5-9.5, and according to the manufacturer's instructions. The gel was divided into approximately 0.7 cm wide sections, which were scooped out and placed in microcentrifuge tubes. Proteins were eluted by centrifuging the fractions in microcentrifuge tubes.

Insect rearing and feeding tests: Tests were conducted with two species of bruchids that are key pests of stored beans around the world: the Mexican bean weevil, Zabrotes subfasciatus (Boheman), and the bean weevil, *Acanthoscelides obtectus* (Say). Techniques to maintain insect cultures of the bruchids were identical to those described by Cardona et al. (1989. J. Econ. Entomol. 82: 310-315). All experiments were conducted at 27^{o}C and 70% RH in a controlled environment chamber.

To test for possible insecticidal effects of the protein on both bruchid species, "artificial" seeds were prepared with flour of the commercial, highly susceptible bean variety 'ICA Pijao'. Artificial seeds were prepared by following, without modifications, the technique devised by Shade et al. (1986. Environ. Entomol. 15: 1286-1291) for the cowpea weevil, *Callosobruchus maculatus* (F.). Briefly, beans were soaked, the testae were removed and the flour was dried and milled. The flour was then reconstituted in Teflon molds, lyophilized, then hydrated at room temperature. Artificial seeds were coated with gelatin and infested as if they were intact.

The purified protein was mixed with flour of 'ICA Pijao' at different concentrations (0, 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, and 5% w/w). Infestation procedures were as follows: for Z. subfasciatus, seeds were infested with at least eight pairs of bruchids per seed. After five days, seeds were examined under a dissecting microscope and 5-6 eggs were left per seed by destroying the excess with a needle. For A. obtectus, seeds for each protein concentration were infested with 5-6 neonate larvae per seed. Larval penetration was subsequently checked to guarantee correct mortality counts. All artificial seeds were individually evaluated in glass vials. Percent adult emergence and days to adult emergence until the last insect emerged were the parameters recorded, although insect survival has been expressed in terms of percent mortality. At the end of the trial, when no more adult emergence occurred, the instar of dead larvae within the seed was determined by measuring the width of the head capsule after dissection of the seeds.

Statistical analysis: Concentration-mortality responses were estimated by means of probit regression analysis (SAS Institute1989). The Statistix package (Analytical Software 2000) was used for analysis of variance performed with data on days to adult emergence.

Results and Discussion

Antifungal activity: The crude extract from seeds of *C. ternatea* CIAT 20692 showed strong antifungal activity on the test fungus *R. solani* (Photo 5). This activity could be eliminated by treatment with Pronase E (Photo 6), indicating that the active compound is a protein. The activity was heat stable (Photo 7). Seeds release this heat stable proteinaceous antifungal compound after mechanical disruption of their seed coat (Photo 8) or after germination (data not shown).

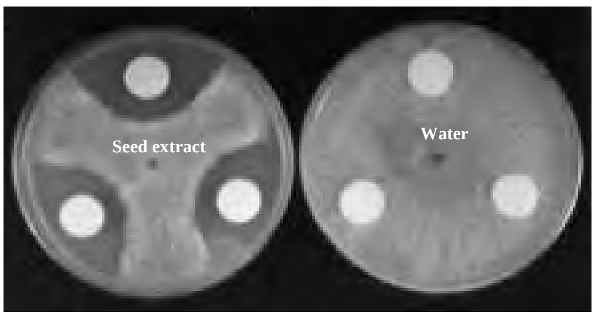


Photo 5.Growth inhibition of *Rhizoctonia solani* by seed extract from *Clitoria ternatea* CIAT 20692 on potato detrose agar plates incubated at 28 ºC for 2 days. Seed extract filtrate (300-µl) was applied on each of the three filter paper discs, whereas the control plate had discs with equal volumes of sterile distilled water. A single sclerotium of *R. solani* was placed on the center each plate.

Photo 6. Elimination of antifungal activity of extracts from seeds of *Clitoria ternatea* CIAT 20692 after treatment with pronase E. A single sclerotium of *R. solani* was placed on the center each plate containing potato dextrose agar and incubated at 28 ºC for 2 days. Seed extract filtrate (300-µl), either treated with pronase E or untreated, was applied on each of the three filter paper discs, whereas the control plate had discs with equal volumes of sterile distilled water.

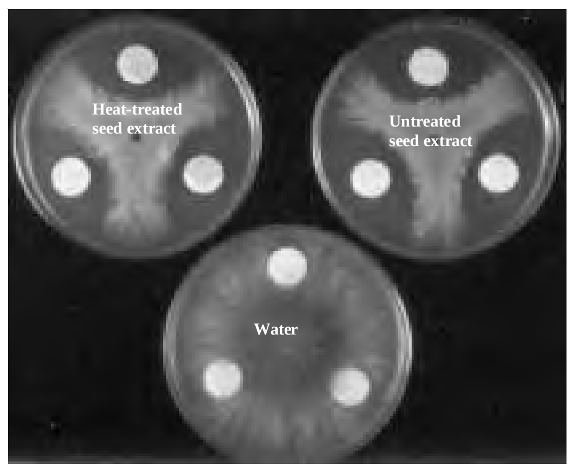
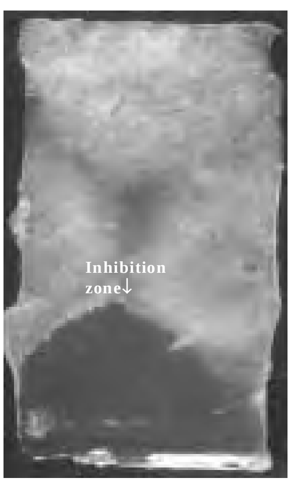


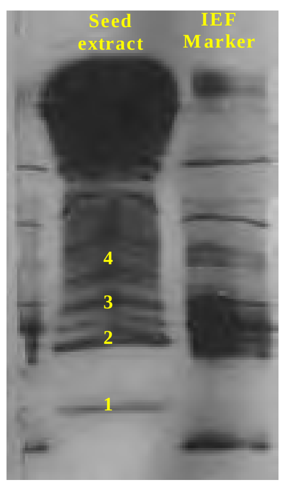
Photo 7. Heat stability of antifungal activity of extracts from *Clitoria ternatea* CIAT 20692 seeds after boiling for 5 min. A single sclerotium of *R. solani* was placed on the center each plate containing potato dextrose agar and incubated at 28 ºC for 2 days. Seed extract filtrate (300-µl), either boiled or untreated, was applied on each of the three filter paper discs, whereas the control plate had discs with equal volumes of sterile distilled water.

Photo 8. Seeds release an antifungal compound after mechanical disruption of their seed coat. Note that a single seed can release a compound with substantial antifungal activity against the test fungus *Rhizoctonia solani*.

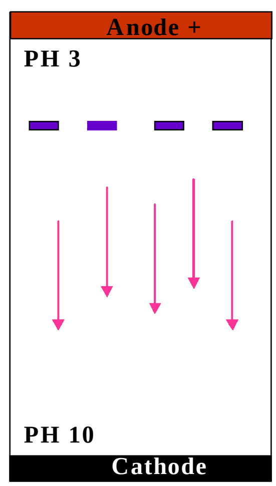
Identification of antifungal protein and purification: Resolving the seed extract by isoelectric focusing gel revealed a number of proteins. Thus, identifying the specific protein (s) responsible for the antifungal activity seemed a daunting task. To reduce the complexity of that task and to facilitate the identification of the specific protein of interest, we created a new protocol which involves: 1) resolving the seed extract by an IEF gel, 2) neutralizing the gel to eliminate the pH gradient, 3) lightly and uniformly coating the gel with warm PDA, 4) inoculating the gel/PDA composition with R. solani sclerotia, 5) wrapping it with Saran wrap to avoid loss of moisture and incubating it at 28 ºC for 2 days. This protocol, in fact, had greatly facilitated our task. R. solani grew uninhibited in the large portion of the gel/PDA composition, but was inhibited in the area where proteins with alkaline pI run (Photo 9).The specific antifungal protein was identified by cutting out ultra-thin-layer polyacrylamide gel areas corresponding to individual protein bands in a duplicate Coomassie-stained gel. The sliced gels were each macerated in 100-µl sterile distilled water in Eppendorf tubes and used for antifungal activity. The results of these tests show that a highly basic protein (numbered 1 in Photo 9) was responsible for the antifungal activity (Photo 10).



IEF gel replica coated with PDA and inoculated with *Rhizoctonia solani*



Coomassie blue-stained IEF gel for selection of protein bands



IEF gel pH gradient

Photo 9. Extracts of *Clitoria ternatea* seeds resolved by isoelectric focusing (IEF) gel. *Rhizoctonia solani* growth inhibition zone indicated that the protein (s) responsible for antifungal activity were in the alkaline part of the gel. A triplicate IEF gel was superimposed on an identical Coomassie-stained IEF gel. Gel areas corresponding to the stained bands 1-4 were cut out for identification of the antifungal protein.

The peptide was well separated from the other proteins on IEF gels, making the purification procedure using the preparative granulated bed isoelectric focusing a relatively easy task. Five of the fractions showed activity with decreasing intensity starting from the highly alkaline pI (isoelectric point) [Photo 11]. The active protein which was mostly recovered in fraction 1 was named Finotin. Both SDS-PAGE (Photo 12) and IEF gels (data not shown) showed that fraction 1 is pure and free of other proteins.

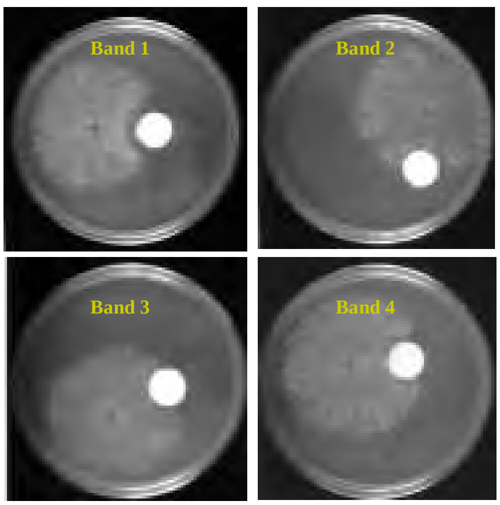


Photo 10. Identification of specific antifungal protein band. The protein band with the most alkaline pI (isoelectric point) numbered 1 in Figure Finotin 4 demonstrated growth inhibition of the test fungus *Rhizoctonia solani*.

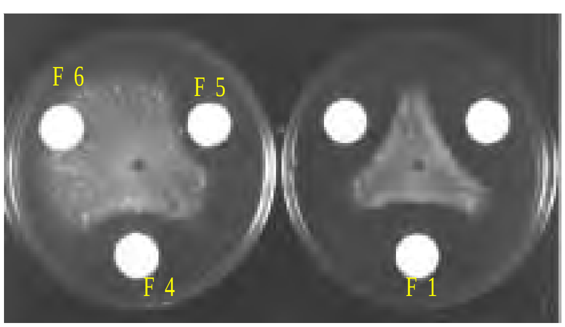


Photo 11. Purification of an antifungal protein from seeds of *Clitoria ternatea* using preparative granulated bed isoelectric focusing. Five fractions scooped from the gel starting from the alkaline part of the gel as # F1 demonstrated antifungal activities, with F5 having the least growth inhibitory activity against *Rhizoctonia solani*.

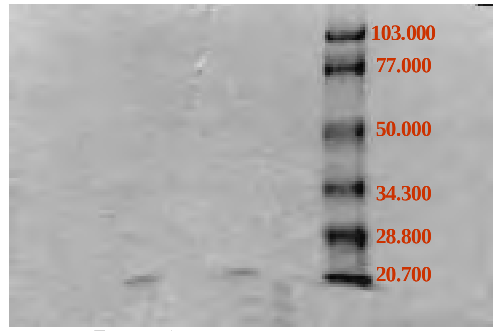


Photo 12. SDS-PAGE analysis of purified protein fraction from *Clitoria ternatea* seeds.

Antifungal/antibacterial activity: Finotin was active against a number of fungi and a bacterium pathogenic on common beans. It inhibits fungi pathogenic on a number of plants, including rice, beans, *Brachiaria*, and *Stylosanthes*, that we tested so far. *Colletotrichum lindemuthianum* and *Xanthomonas axonopodis* pv. *phaseoli* from common beans; *Lasiodiplodia theobromae* and *Colletotrichum gloeosporioides* from *Stylosanthes* spp; *Helminthosporium* spp. and *Pyricularia grisea* from rice; and *Rhizoctonia solani* from Brachiaria were all inhibited in growth by Finotin in culture. We have yet to determine the mode of action of Finotin.

Insecticidal activity: Mortality on artificial seeds prepared with the susceptible background bean flour ('ICA Pijao') was very low (less than 3%) for both *Z. subfasciatus* and *A. obtectus*. Enrichment of artificial seeds with increasing levels of the test protein led to an increase in mortality reaching maximal levels (100% larval mortality) at the dosage of 5% in the case of *Z. subfasciatus* and 1% in the case of *A. obtectus*. Probit analysis (Table 17) showed that the protein is highly toxic to both bruchid species with LC_{50} values that can be considered low (less than 2%). The LC_{50} value for *A. obtectus* (0.36%) was ca. four times lower than that for *Z. subfasciatus* (1.21) meaning that the protein is more toxic to *A. obtectus*. The protein is very toxic to first instar larvae of both bruchid species: dissection of infested seeds revealed that up to 75% of the larvae did not reach the second instar stage.

Table 17. Toxicological responses of bean bruchids *Zabrotes subfasciatus* and *Acanthoscelides obtectus* to a purified protein (Finotin) isolated from seeds of *Clitoria ternatea*

No. tested	LC_{50} (95% FL)[a]	LC_{95} (95% FL)	Slope ± SEM	χ^2
		Zabrotes subfasciatus		
147	1.21 (0.99 - 1.47)	2.88 (2.17 - 5.21)	4.3 ± 0.88	2.78
		Acanthoscelides obtectus		
155	0.36 (0.28 - 0.43)	0.77 (0.61 - 1.28)	4.9 ± 0.96	0.84

[a] FL, fiducial limits

Concentration responses in terms of days to adult emergence are shown in Table 18. Developmental times of those few insects that survived the different protein concentrations were prolonged. There was a definite dosage response: the higher the dosage the longer the developmental time. This is further proof of the toxicity of the protein to both bruchid species.

The protein Finotin showed growth inhibitory effects against fungi, bacteria and insects. This wide range of activity strongly suggests that this peptide is an important component of the natural defense system of *C. ternatea*. The preferential release of this peptide during seed germination or seed damage can contribute to the protection of the emerging seedlings from soil-borne pathogens. Interestingly, the expression of this peptide is not restricted to seeds, but it also occurs in other parts of *C. ternatea* such as roots upon drought stress in greenhouse tests (data not shown). However, it is not clear whether this peptide plays any role in drought tolerance, a trait which *C. ternatea* has. This and other potential roles played by Finotin are yet to be determined once the gene encoding Finotin is isolated.

Table 18. Effect of increasing concentrations of a purified protein isolated from seeds of *Clitoria ternatea* on the biology (days to adult emergence) of the bean bruchids *Zabrotes subfasciatus* and *Acanthoscelides obtectus*

Protein concentration (% w/w)	**Days to adult emergence**	
	Zabrotes subfasciatus	*Acanthoscelides obtectus*
0.0[1]	43.1e	34.4c
0.0625	45.0e	33.8c
0.125	51.5d	35.2c
0.25	55.6c	49.4b
0.5	57.3c	63.4a
1.0	72.7b	N.E.
2.0	80.0a	N.E.
5.0	N.E[2].	N.E.

Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD. ANOVA on data testing for differences among dosages (protein concentrations). For *Z. subfasciatus*: F = 106.9; df = 6,21; P < 0.001; for *A. obtectus*: F = 184.9; df = 4,16; P < 0.001.
[1] Background flour prepared with 'ICA Pijao'
[2] N.E., no adult emergence, 100% larval mortality.

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

Highlight:

- Found two putative effector proteins that may be contribute to the antibiotic action of the resistant plants to spittlebug

2.8.1 Reproductive mode of new *Brachiaria* hybrids by progeny trial

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

SX x AP *Brachiaria* hybrid populations ought to segregate approximately 1:1 for reproductive mode. Only apomictic clones are candidates for development to commercial cultivar status. A reliable means of assessing reproductive mode is by progeny test. The progeny of an apomictic

clone will be uniform and provide a first seed increase of promising genotypes.
Over 2,000 SX x AP seedlings were produced in 2002. These were propagated and established in two un-replicated field nurseries. They were culled down to 64 genotypes on the basis of periodic visual assessment in the field. OP seed was hand-harvested from the 64 "pre-selected" plants in late 2002. This seed was germinated in mid-2003 and 20 random seedlings per progeny were transplanted to the field on August. Where 20 seedlings were not obtained, all available seedlings were transplanted.

2.8.2 Identification of genes induced during the defense response of *Brachiaria* to spittlebug

Contributors: C. Romero, I. F. Acosta, J. Miles, C. Cardon,a and J. Tohme (CIAT- SB2 Project)

Rationale

The molecular basis of plant defense responses to insects is a challenging area whose understanding should make feasible the use of natural immunity in economically important plants. Although molecular biology has recently been incorporated in the exploration of these defense mechanisms, it has been mainly limited to studying the interaction between dicotyledonous plants and herbivorous chewing insects. Herein we focus in the defense responses of a monocot that exhibits a conclusive resistance to a xylem-sucking insect, an interaction that is poorly understood at the molecular level given the peculiarity of this feeding habitat. One of the possible approaches to get closer to such a system without previous molecular data is the characterization of transcriptional changes during the plant response to the insect attack.

Materials and Methods

The subtractive hybridization was performed as described in the 2002 Annual Report, where we also showed the results of two 96-well pilot libraries (Annual Report, 2002). Then, we decided to construct two additional libraries of 384 wells in order to expand the coverage of the subtractive product and to avoid the RNA ribosomal artifacts. Four bands corresponding to rRNA generated in the cDNA synthesis were identified by size when the product of the subtraction was run in an acrylamide gel. The rest of the smear observed in this gel was excised and cloned to create the new libraries.

Similarity searches were performed in the GenBank using the BLASTX algorithm. The matching sequences were searched in annotated databases such as TAIR (The Arabidopsis Research Institute), GRAMENE, SWISS-PROT, and ENZYME in order to determine their putative functions. More specific information was obtained in secondary databases as InterPro (*Integrated Resource of Protein Families*), Pfam (*Protein Families Database*), PRINTS (*Protein Motif Fingerprint Database*), AraCyc (*AraCyc: Arabidopsis thaliana Biochemical Pathways*), and CDD (*Conserved Domain Database*, at NCBI). In these databases we found functional information such as precise biochemical roles, metabolic pathways and redundant proteins (other proteins with the same function). Furthermore, we found structural information such as protein motifs and domains contained in the predicted *Brachiaria* proteins. Finally, in some cases we performed two pair alignments of amino acid sequences using the pair BLAST algorithm to confirm the structural relationship between elements isolated from *Brachiaria* and the proteins previously reported in resistance studies in other species.

A macroarray experiment was carried out in order to test the differential expression of the isolated transcripts. The clones of the four libraries were arrayed using a 384-well pin replicator on duplicated nylon membranes and grown on LB-agar medium overnight. The bacterial colonies were denaturalized and the free DNA was UV-crosslinked. These filters were hybridized with radioactivity labeled cDNA from infested plants and from non-infested plants and exposed to autoradiograph films.

Results and Discussion

The cloning strategy to avoid rRNA sequences in the new libraries -which have been partially screened at this point- was successful since the proportion of these artifacts decreased from 40% to 5%. Bringing back together the results of last year's pilot libraries, a total of 240 clones yielded readable sequences, which corresponded to 74 unique transcripts. No match was found for 26 (35%) of them in the GenBank; these may constitute a reservoir of new genes, absents in model species. Seven more sequences were not considered further (e-values over 10^{-7}). Fifteen out of the remaining 41 sequences are similar to genes that have been shown to be part of defense responses in other plant-insect or plant-pathogen interactions (see Table 19). These sequences can be divided in six main groups according to their presumed roles in defense (see Figure 27)

Table 19. Putative functions assigned to the transcripts isolated. Sequences related with defense responses in other systems are shown in bold

Putative Function	E value	Score
O-Methyltransferase	3.00E-89	329
Hypothetical Protein 1	1.00E-71	269
Ornithine carbamoyltransferase (OCTase)	**5.00E-67**	**233**
Cysteine Proteinase	**7.00E-63**	**241**
Phospholipase C (A)	**8.00E-58**	**213**
Hypothetical Protein 3	1.00E-52	206
dTDP-glucose 4-6-dehydratase	4.00E-53	206
Chlorophyll a/b-binding protein (CAB)	5.00E-50	196
Phospholipase C (B)	**1.00E-49**	**195**
Omega-3 fatty acid desatura	**4.00E-47**	**187**
CBL-interacting protein kinase (CIPK)	**1.00E-41**	**168**
Tubulin alpha	2.00E-41	168
Caffeoyl Coenzyme A 3-O-Methyltransferase 1	**4.00E-39**	**161**
MADS Box	**1.00E-37**	**148**
Glutathione S-conjugate ABC transporter	3.00E-35	147
Xyloglucan endotransglycolase	**2.00E-34**	**145**
Lypoxigenase A (LOX)	**3.00E-34**	**145**
Lypoxigenase B (LOX)	**3.00E-34**	**144**
Unknown protein 3 (TMS1d)	2.00E-31	132
Fructose bisphosphate aldolaseÊ	1.00E-27	122

Putative Function	E value	Score
Ca dependent mitochondiral carrier protein	7.00E-27	119
NAD+ dependent isocitrate dehydrogenase subunit 1	1.00E-25	116
Sequence associated to Pi2 (RG64 RFLP marker)	3.00E-24	119
Developmental Protein	3.00E-22	104
Elongation factor 1-alpha	6.00E-21	100
Unknown protein 2	2.00E-20	98
Pur-alpha 1Ê	**3.00E-17**	**88**
SCARECROW	**3.00E-17**	**88**
Putative replication proteinÊ	4.00E-16	84
Extracellular lipase 3 (A)	2.00E-15	55
Cold acclimation protein	8.00E-14	77
60s ribosomal protein L13	9.00E-14	76
Hypothetical Protein 6	7.00E-12	71
S-adenosylmethionine synthetaseÊ	**2.00E-11**	**69**
Hypothetical Protein 4	2.00E-10	66
Caffeic acid O-methyltransferase	**1.00E-09**	**62**
Carbonic Anhydrase	3.00E-09	146
Metalloprotease	**1.00E-08**	**57**
Unknown protein 1	1.00E-07	56
Steroid 22-alpha-hydroxylase (cytochrome p450)	**2.00E-07**	**56**
Extracellular lipase 3Ê(B)	2.00E-07	57

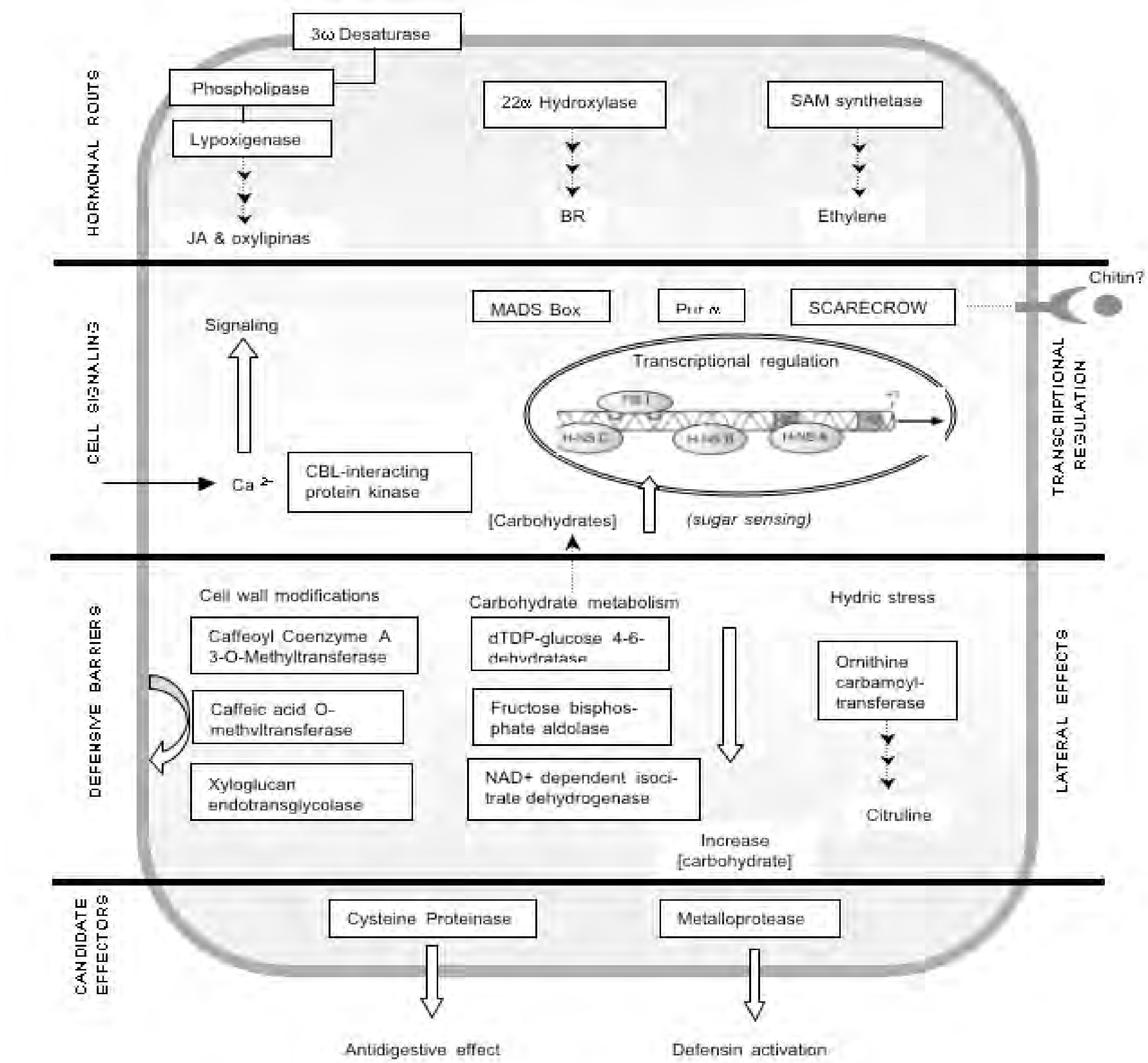


Figure 27. Summary of the candidate defense-related genes and cellular processes found in a *Brachiaria* resistant line in response to the interaction with the spittlebug (*Aeneolamia varia*). They can be divided in 6 main functional groups discussed in the text.

1. **Hormone Biosynthesis Pathways:** The first group contains sequences coding for putative enzymes that catalyze important steps in the biosynthesis of oxylipins, brassinosteroids and ethylene.

   Oxylipins are the most important hormones in the systemic response to wounding and insects. Plants deprived of Phospholipase (Turner *et al.*, 2002), Fatty acid desaturase (Martin *et al.*, 1999) and Lypoxigenase (Bell *et al.*, 1995) are not able to produce Jasmonic Acid (JA) and are incompetent to activate defense mechanisms such as proteinase inhibitors, defensins and thionins.

   Brassinosteroids have been mainly studied in cell division and plant development but recently they were assigned a role as systemic defense hormones in responses to virus, bacteria and fungi in both dicots and monocots (Nakashita *et al.*, 2003).

Although the role of **Ethylene** is widely recognized in plant-pathogen interactions, its effects can differ in different situations. The emission of ethylene has been proved as a mechanism of communication between organisms that attracts natural enemies of herbivores and causes unwounded leaves to initiate ethylene biosynthesis as a positive feedback loop (Arimura *et al.*, 2002).

2. **Cell signaling.** The presence of a putative CIP kinase can be explained by two different processes: a) this protein may interact with CBL, a plant calcium sensor, in the signaling cascade activated under a spittlebug attack. Ca+ signaling in responses to wounding and pathogens has been well documented. b) CIP kinases contain a SNF1 domain, suggesting a possible role in gene regulation controlled by cytoplasmic carbohydrate concentration (sugar sensing), an activity that may be related with the rapid change in metabolism that should occur to supply the energy requirements of the defense responses (Rolland *et al.*, 2002).

3. **Transcriptional Regulation.** SCARECROW is a transcription factor that is rapidly induced upon perception of the elicitor N-acetylchitooligosaccharide. Moreover, its mRNA is induced in rice upon fungal infection but not in the presence of bacterial pathogens (Day *et al.*, 2003). Both evidences suggest that this transcription factor may be involved in responses to enemies that contain chitin, which could be a way to regulate defenses against a broad but specific range of organisms (fungi and arthropods).

4. **Lateral effects (water stress).** The ornithine carbamoyltransferase participates in the synthesis of citrulline, a precursor amino acid of arginine. Some plants accumulate citrulline under water stress in order to increase the concentration of compatible solutes (those that do not alter the electrostatic equilibrium and thus do not disrupt the catalytic properties of enzymes). In this way, the plant enhances its capacity to absorb water decreasing its hydric potential. The production of citrulline in *Brachiaria* in its interaction with the spittlebug may be due to the dramatic alteration of the hydric state caused by this sucking insect on the xylem vessels.

5. **Defensive barriers.** Transcripts for 3 putative enzymes implicated in lignin biosynthesis and cell wall modification were detected in this study. This phenomenon is frequently found in response to pathogens to confine them to the site of infection and avoid their dispersion (Ye *et al.*, 2001), an action whose importance in the defense against an insect seems less obvious. The expression patterns of these elements agree with previous evidences because they have shown to be induced by wounding, ethylene and brassinosteroids.

6. **Effector mechanisms.** Finally, we found two sequences that encode putative proteins that may participate more directly in the reduced survival of the insect in resistant varieties (antibiosis): A metalloprotease with a domain similar to that of proteases involved in the activation of defensins (Liu *et al.*, 2001), this, in turn, may have been previously induced by the oxylipins.
   A cysteine protease highly homologous to one in maize that induces the disruption of the peritrophic matrix in the gut of a caterpillar. Maize callus transformed with the gene encoding this protein reduce growth of the insect as the resistant plant does. The effect of the cysteine proteinase is probably the perforation of the digestive tube (Pechan *et al.*, 2002), demonstrated by electron microscopy, an event that decreases nutrient absorption and facilitates invasion by pathogens.

The macroarray experiment ran to confirm the differential expression of the transcripts isolated showed an encouraging percentage of 5% of false negatives. Consistently the putative functions of these non-differential sequences were unrelated with defense mechanisms, therefore they have not been taken in to account for the discussion.

In summary, in this work we show the results of isolation of differentially expressed sequences in the resistant *Brachiaria* hybrid CIAT 36062 when challenged with *Aeneolamia varia* nymphs. This was achieved by a subtractive hybridization technique and a rigorous sequence analysis to identify putative functions of the isolated transcripts. Sequencing analysis of ~240 clones from the subtractive library revealed that they corresponded to 74 unique expressed genes. Putative functions were assigned to 41 transcripts through sequence similarity searches and the predicted proteins were classified in eight functional groups. These functional groups fall into three biosynthetic pathways of important plant signaling hormones: cell signaling; transcriptional regulation; cell wall modification and the homeostasis of the plant during the water stress caused by the insect. Finally, we found two putative effector proteins that may be contribute to the antibiotic action of the resistant plants on the insect.

Conclusions

Our results shed the first lights on the molecular mechanisms that determine resistance of a monocotyledonous plant to a xylem-sucking insect.

New and exciting experiments should be designed to complement our findings and to test the assumptions that transcript sequence information has provided. In the short-term these are our goals:

- Exogenous applications of jasmonic acid and brassinolide hormone to susceptible varieties in order to determine phenotypic changes in defense capacities
- Real Time PCR amplification to quantify expression of these transcripts between plants infested by different spittlebug species in order to detect common central defense mechanisms.
- Use of Virus Induced Gene Silencing (VIGS) to evaluate the function in defense responses of the differentially expressed genes.
- A new subtractive hybridization to detect constitutive mechanisms of resistance by comparing gene expression of susceptible and resistant plants both exposed to the insect. A more subtle infestation method will be used and the experiment will span the first 24 hours post-infestation.

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

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

Highlights

- Tolerance to low phosphorus (P) in the *Brachiaria* hybrid cv. Mulato involves two major strategies: (1) increasing the ability to use P efficiently by inducing phosphohydrolases (APase and RNase) in shoots with P deficiency; and (2) enhancing sugar catabolism and subsequent synthesis of amino acids and organic acids in leaves under P deficiency.
- Phenotypic characterization of *Brachiaria decumbens* x *Brachiaria ruziziensis* population showed that genotypes more severely affected by Al toxicity have smaller root systems (shorter root length) that are made up of thicker roots (greater root diameter) and are more heavily branched (more root tips per unit dry weight).
- Two hybrids (BR02NO1372 and BR02NO1621) were identified with greater level of Al resistance than other hybrids generated in the *Brachiaria* breeding program.
- Showed that the *Brachiaria* hybrid, FM9503-S046-024 performed well into the fourth year after establishment in the Llanos and its superior performance at 40 months after establishment was associated with its ability to acquire greater amounts of nutrients, particularly Ca and Mg from low fertility soil.
- Accesions of *Arachis pintoi* CIAT 18744 and 22172 were superior to the commercial cultivar, CIAT 17434 in acquiring phosphorus (P) from from less available P-pools in a low-P oxisol, under growth chamber conditions. The superior performance of CIAT 18744 and 22172 was related to greater P-acquisition efficiency rather than greater P-use efficiency
- Three accessions of *Arachis pintoi* (CIAT 18744, 18751 and 22159) were superior under field conditions than the commercial cultivar (CIAT 17434) in terms of persistence with low fertilizer application
- High farmer preferences for commercial *Brachiaria brizantha* cvv. Toledo, Marandú and La Libertad
- Recorded high and similar DM yields for both *Brachiaria* hybrid FM 9503-S046-024 and *Brachiaria* hybrid cv. Mulato

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, M.E. Buitrago, A. Arango, D. F. Cortes, G. Gallego, E. Gaitán, and C. Plazas (CIAT), M. Nanamori, T. Shinano, T. Yamamura, M. Osaki (Hokkaido University, Japan)

Studies 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 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 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*.

3.1.1.1.1 Surface charge density of root apices as a potential factor contributing to aluminum resistance of *Brachiaria decumbens*

Contributors: A.L. Chaves, M.E. Recio, P. Wenzl**,** J. Tohme and I.M. Rao

Rationale

There is a pronounced difference in aluminum (Al) resistance between *B. decumbens* and *B. ruziziensis* (Wenzl et al., 2001). Previous results demonstrated that this difference is not restricted to Al^{3+} ions but is also observed for trivalent lanthanide ions (see IP-5 Annual Report, 2002). We hypothesized that a less negative (or even positive) surface charge of root apices of *B. decumbens* compared to those of *B. ruziziensis* could be the underlying physiological mechanism.

Materials and Methods

Seeds of *B. decumbens* and *B. ruziziensis* were germinated in 200 µM $CaCl_2$ (pH 4.2) for 4 – 5 days. Homogeneous seedlings, with root lengths between 2 and 3 cm, were transferred to continuously aerated solutions containing 200 µM $CaCl_2$ (pH 4.2) and various concentrations of different cationic or anionic toxicants. The seedlings were left to grow in the glasshouse for 3 days. At harvest, root lengths were measured and root elongation was calculated by subtracting the root length at transfer. Relative root elongation (RRE) values were computed by referencing root elongation values against root elongation in the toxicant-free reference treatment.

Results and Discussion

Figure 28 displays RRE values of the two species exposed to various concentrations of a variety of cationic and anionic toxicants. Data points below the diagonal indicate that *B. decumbens* is more resistant than *B. ruziziensis*; data points above the diagonal indicate greater susceptibility of *B. decumbens* compared to *B. ruziziensis*. The left panel clearly shows that *B. decumbens* is more resistant to all the trivalent cations tested. The latter would be consistent with root apices of *B. decumbens* having fewer negative surface charges than those of *B. ruziziensis*. This is because a lower negative surface charge density would entail lower concentrations of cationic toxicants in the vicinity of root surfaces.

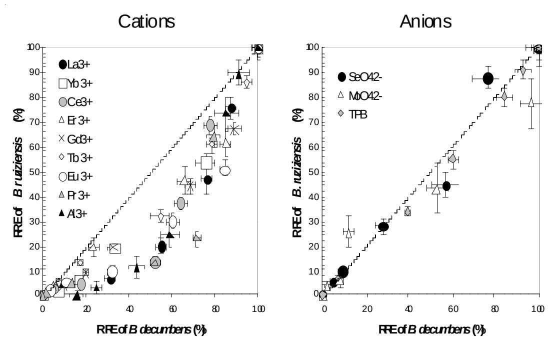


Figure 28. Comparison of relative root elongation (RRE) of *B. decumbens* and *B. ruziziensis* exposed to cationic (left panel) or anionic toxicants (right panel). Cationic toxicants included lanthanum (La^{3+}), ytterbium (Yb^{3+}), cerium (Ce^{3+}), erbium (Er^{3+}), gadolinium (Gd^{3+}), terbium (Tb^{3+}), europium (Eu^{3+}), praseodymium (Pr^{3+}), and aluminum (Al^{3+}). Anionic toxicants included selenate (SeO_4^{2-}), molybdate (MoO_4^{2-}) and tetraphenylborate (TPB). All values are means ± SE of 36 seedlings measured in three independent experiments.

A lower negative surface charge density, however, should also make *B. decumbens* more sensitive to anionic toxicants than *B. ruziziensis*. The right panel of Figure 1, however, does not appear to confirm this prediction because the data points do not cluster above the diagonal. Results from a greater variety of anionic toxicants are required; yet these data do not appear to be consistent with the idea that Al resistance of *B. decumbens* is due to reduced electrostatic attraction of Al ions to the surface of root apices. Instead they appear to point to a mechanism that may be based on binding to cellular ligand(s). Work is in progress to further characterize this generic cation-resistance mechanism.

3.1.1.1.2 Physiological components of acid soil adaptation in a population of *Brachiaria ruziziensis* × *Brachiaria decumbens* hybrids

Contributors: M.E. Buitrago, M.E. Recio, A.L. Chaves, P. Wenzl, J. Tohme, J.W. Miles and I.M. Rao

Rationale

Last year we confirmed that a *B. ruziziensis* × *B. decumbens* hybrid population segregates for two physiological components that are important for growth in infertile acid soil: root growth vigor and Al resistance. We therefore continued to evaluate most of the 274 hybrids. The cumulative data from ten harvests are now ready to be combined with molecular marker data to identify QTLs underlying these traits.

Materials and Methods

Stem cuttings of hybrids and parents were rooted in a low ionic strength nutrient solution in the glasshouse during 9 days. Equal numbers of stem cuttings 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 $AlCl_3$ 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, average root diameter and number of root apices. The root growth data from ten harvests were log-transformed because such growth data tend to be log-normally distributed (Causton and Venus, 1981). They were then adjusted for harvest mean (based on the differences between harvest means and overall mean) and the dry weight of the stem cuttings (using linear regression).

Results and Discussion

The B. ruziziensis × B. decumbens hybrids showed a broad range of root growth vigor. A considerable number of individuals were superior to well-adapted *B. decumbens*, perhaps as a result of heterosis induced by the interspecific cross (see large number of data points to the right of *B. decumbens* in Figure 29). By contrast there were only few individuals whose roots elongated as poorly as those of *B. ruziziensis*.

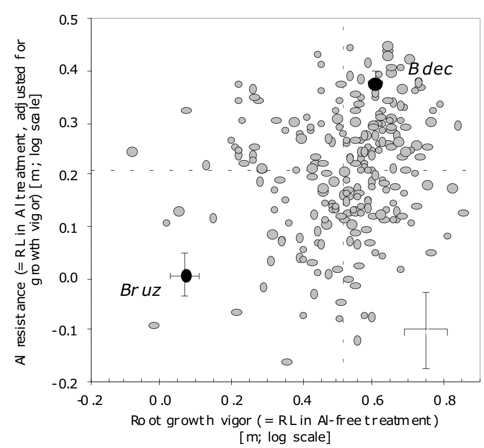


Figure 29. Segregation of root growth vigor (x axis) and Al resistance (y axis), both measured based on root elongation (RL = root length) in a *B. ruziziensis* x *B. decumbens* hybrid population. Al resistance was quantified by adjusting RL of plants in the Al treatment for their RL when grown without Al (see text for further details). Data points representing parents are highlighted with large black symbols. Error bars in the lower right corner denote the average SE of the hybrid population.

Because root growth vigor varies among hybrids, the inhibitory effect of Al on root length (RL) is not easy to measure, and RL in the Al treatment has to be properly referenced. We tested two approaches. First, we calculated relative root length values by comparing – for each pair of stem cuttings distributed among the two treatments – RL in the Al treatment vs. RL in the treatment without Al (the logarithm of the ratio was used for this purpose). The resulting "Al resistance index" was not correlated with root growth vigor. We therefore also tested adjusting RL values in the Al treatment for RL values in the Al-free treatment using linear regression.

The two approaches were compared using the degree of correlation among three parameters describing different effects of Al toxicity as a criterion. These were: (i) inhibition of root elongation (see above), (ii) lateral swelling of roots (resulting in a greater root diameter, RD), and production of a greater number or short laterals (resulting in a higher specific root tip number, sRT = number of root tips per unit dry weight). The RD and sRT parameters had been computed based on the two approaches described for RL. The three parameters were more tightly correlated in the second approach, thus suggesting superior data quality. The latter was also corroborated by the fact that the two parents were closer to the two extremes of the distribution. We therefore used the linear regression approach to present the data in Figure 29.

Figure 30 displays the relationship among the three different Al-toxicity parameters used above. Genotypes more severely affected by Al toxicity have smaller root systems (shorter RL) that are made up of thicker roots (greater RD) and are more heavily branched (more root tips per unit dry weight, sRT). It may be possible to increase the robustness of detecting Al-resistant genotypes by computing the principal component of these three parameters to create "a composite Al resistance index" that captures the various Al-induced changes in root growth and architecture in a single parameter.

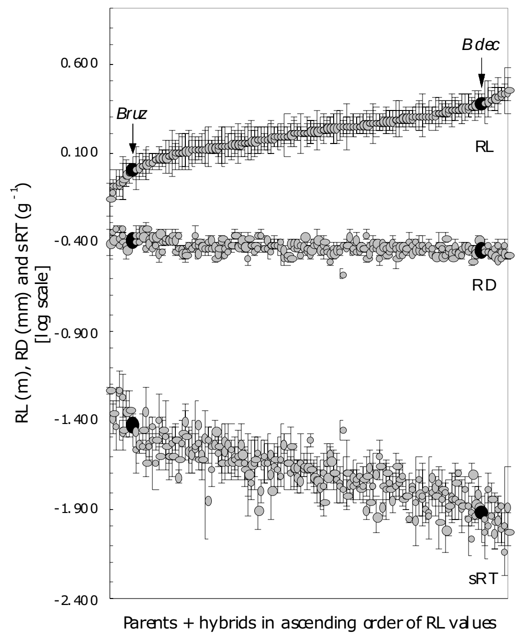


Figure 30. Comparison of three parameters that reflect Al resistance: root length (RL), average root diameter (RD), and number of root tips per gram of root dry weight (sRT = specific number of root apices). The parameters were measured with rooted stem cuttings in an Al-toxic solution. They were adjusted for harvest mean, the effect of the dry weight of stem cuttings used, and root growth vigor measured with another set of stem cuttings in an Al-free reference treatment (see x axis in Figure 1). Data points representing parents are highlighted with large black symbols.

3.1.1.1.3 Identification of candidates genes associated with aluminum resistance in *Brachiaria*

Contributors: A. Arango, D. F. Cortes, G. Gallego, P. Wenzl, I.M. Rao, M. Ishitani and J. Tohme

Rationale

Last year we reported on the identification of individuals of a *Brachiaria ruziziensis* x *Brachiaria decumbens* hybrid population with contrasting degrees of Al resistance. This suggested the feasibility of isolating candidate Al-resistance genes from root apices based on a comparison of gene expression patterns between an Al-resistant and an Al-sensitive bulk of hybrids. This year we pursued this approach.

Materials and Methods

Rooted stem cuttings of *B. decumbens*, *B. ruziziensis* and a group of hybrids with contrasting Al resistance levels were cultivated as described in "Evaluating traits associated with acid soil adaptation in a population of *Brachiaria ruziziensis* × *Brachiaria decumbens* hybrids" (see activity 3.1.1.1.2). Root apices collected at various harvests were pooled to create 6 samples for RNA extraction: two Al-resistant bulks of hybrids (grown with +/-Al), an Al-sensitive bulk of hybrids (grown with Al), two *B. decumbens* samples (grown with +/-Al) and a *B. ruziziensis* sample (grown with Al). Total RNA was isolated, mRNA was captured with magnetic beads, and ds-cDNA was synthesized using anchored oligo(dT) primer/adapters. To minimize cross-hybridization among genes belonging to the same family, 3'-UTRs were amplified by suppressive PCR after simultaneous digestion of cDNAs with MseI and MspI followed by adapter ligation. The resulting amplicons were then mixed in various combinations and subjected to three rounds of differential subtraction chain (DSC) (Luo et al., 1999).

Results and Discussion

Figure 31 displays the 3'-UTR amplicons obtained from the six mRNA samples. Control experiments confirmed that only 3'-UTR fragments and no internal gene fragments had been amplified (i.e. suppressive PCR was successful). In addition, sequencing of random 3'-UTRs fragments identified homologies to 3'-regions of known genes, including one coding for a root-specific protein. As a result of the double digestion with two 4-bp cutters, the fragments are small. This should have minimized cross hybridization among different genes during the subsequent subtractive hybridization steps.

The 3'-UTR fragments remaining after three rounds of subtractive hybridization were separated on a polyacrylamide gel. Distinct bands were visible in some combinations of tester and driver amplicons (Figure 32). This appears to suggest that the DSC procedure may have been successful.

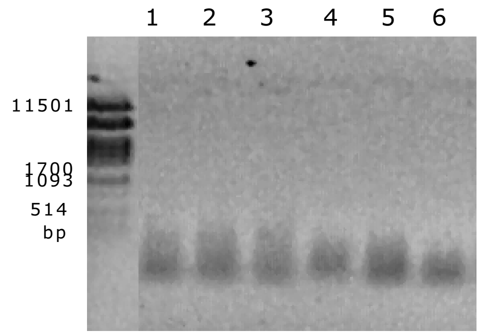


Figure 31. 3'UTR amplicons obtained from six cDNA samples: **1**, Al-resistant F_1 bulk grown at 200 µM Al^{3+}; **2**, Al-resistant F_1 bulk grown without Al^{3+}; **3**, Al-sensitive F_1 bulk grown at 200 µM Al^{3+}; **4**, *B. decumbens* grown at 200 µM Al^{3+}; **5**, *B. decumbens* grown without Al^{3+}; **6**, *B. ruziziensis* grown at 200 µM Al^{3+}.

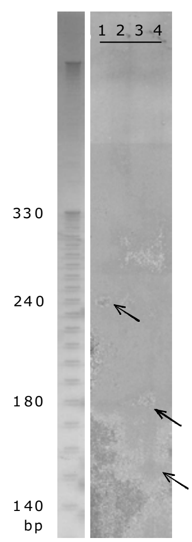


Figure 32. 3-UTR fragments remaining after three rounds of DSC. The numbers refer to the four combinations of tester and driver amplicons listed in Table 19.

Bands will be excised from the gel, cloned and sequenced. In addition, random fragments from the second round of subtraction will be cloned and sequenced. Depending on their redundancy they will be used to fabricate cDNA microarrays for screening for differentially expressed genes.

Table 19. Combinations of tester and driver cDNA samples used for subtractive hybridization.

Combination	Tester		Driver	
	Genotypes	Treatment	Genotypes	Treatment
1	Al-resistant F_1 bulk	200 µM Al^{3+}	Al-sensitive F_1 bulk	200 µM Al^{3+}
2	Al-resistant F_1 bulk	200 µM Al^{3+}	Al-resistant F_1 bulk	no Al^{3+}
3	*B. decumbens*	200 µM Al^{3+}	*B. ruziziensis*	200 µM Al^{3+}
4	*B. decumbens*	200 µM Al^{3+}	*B. decumbens*	no Al^{3+}

3.1.1.1.4 Isolating genes from root apices of *Brachiaria decumbens* that enhance Al resistance of yeast

Contributors: P. Wenzl, E. Gaitán, I.M. Rao and J. Tohme

Rationale

Some genes that when transformed into plants increase their resistance to Al, were originally identified based on their ability to enhance Al resistance of yeast. We used this approach to identify candidate Al-resistance genes in root apices of *B. decumbens* grown in the presence of Al.

Materials and Methods

Total RNA was extracted from root apices of rooted *B. decumbens* stem cuttings grown in a solution containing 200 µM $CaCl_2$, 200 µM $AlCl_3$ (pH 4.2). After capture of mRNA with magnetic beads, ds-cDNA was synthesized, ligated to adapters, size-fractionated and PCR-amplified. PCR products from the > 2 kb and < 2 kb fractions were ligated separately to linearized pYES2 plasmid and transformed into *E. coli*. The libraries obtained were amplified on plates. Plasmids extracted were mixed at a 1:1 ratio and re-transformed into yeast. Transformants were plated on a medium containing enough Al to arrest growth of yeast cells transformed with empty plasmid. Plasmids from the most quickly growing colonies were isolated, re-transformed into *E. coli*, and extracted for further characterization.

Results and Discussion

Approximately 100 yeast colonies were obtained from two million cfu plated on the Al medium (= 0.005 %). The plasmids isolated from 48 well-growing colonies were digested with a mixture of restriction enzymes to identify clones that had been isolated more than once, thus minimizing the chance of selecting false positives for further analysis. This fingerprinting experiment identified nine clones that had been isolated at least twice from different colonies. Work is underway to sequence and characterize inserts from these clones.

3.1.1.2 Low phosphorus tolerance mechanisms in *Brachiaria*: Phosphorus recycling and photosynthate partitioning in the *Brachiaria* hybrid

Contributors: M. Nanamori, T. Shinano, T. Yamamura, M. Osaki (Hokkaido University, Japan) and I. M. Rao (CIAT)

Rationale

The ongoing *Brachiaria* breeding program at the Centro Internacional de Agricultura Tropical (CIAT), conducted in collaboration with the Empresa Brasileira de Pesquisa Agropecuaria

(EMBRAPA), has generated several promising genetic recombinants (genetically recombined apomictic or sexual hybrids). Field evaluation of these hybrids identified one apomictic hybrid, FM9201/1873 (from *Brachiaria ruziziensis* clone 44-06 and *Brachiaria brizantha* cv. Marandú), as productive when grown on P-deficient, low-fertility, acid soils in both wet and dry seasons. This hybrid, the world's first commercial *Brachiaria* hybrid, was recently released in Mexico as cv. Mulato, and is being adopted by farmers in the tropics. Before this study, we had previously evaluated the *Brachiaria* hybrid's tolerance of low P supply, and found that it had very high P-use efficiency (g of biomass produced per mg of P uptake). Our current study aims to determine the hybrid's mechanisms for tolerating low P supply, that is, the physiological basis of its high P-use efficiency. We quantified the effects of P deficiency on phosphohydrolases and carbon metabolism in its leaves, and compared them against the values for the rice (*Oryza sativa* L.) cultivar Kitaake, which is also relatively tolerant of low P and low pH conditions.

Materials and Methods

Brachiaria hybrid cv. Mulato and rice (*Oryza sativa* L. cv. Kitaake) were grown hydroponically under greenhouse conditions (43°3' N, 141°2' E, altitude 17 m; maximum temperature 32°C; minimum temperature 16°C; average photoperiod during experiment = 14.8 h light and 9.2 h darkness; maximum photon flux density = 1550 µmol m^{-2} s^{-1}). Seedlings were pre-cultured in a 56-L vessel, containing a nutrient solution. After 1 week in pre-culture, 5 plants were transplanted to 56-L vessels containing nutrient solutions, each with one of three levels of P concentration (0 µM, 6 µM, and 32 µM) for 2 weeks. The experimental design was a randomized complete block with 3 replicates. *Brachiaria* hybrid and rice were cultured in different containers. Phosphorus concentration was adjusted every day, as was the pH (5.2 ± 0.1). Phosphorus depletion in each treatment was monitored daily to make sure that transient P depletion is not a major problem during plant growth. The P levels selected represent P deprived, low P and adequate P conditions for plant growth. The nutrient solution was completely renewed once a week. Three plants were pooled for one replicate, and each experiment conducted with three replicates (obtained from three different containers). Half of the collected plants were dried in an oven at 105°C for 3 days and weighed. The other half was frozen in liquid nitrogen and stored at -80°C until analysis of inorganic phosphate (P_i) and enzyme activities. Dried samples were digested with sulfuric acid and hydrogen peroxide to determine total N and P.

We used the Schmidt-Thannhauer-Schneider method with slight modifications to fractionate P compounds in plants. Phosphorus concentration in each fraction was measured by the molybdate-blue method. APase, RNase, PEPC, PEPP and PK activities were measured according to published methods. APase activities were assayed in sodium acetate buffer (pH 5.6), using *p*-nitrophenylphosphate as substrate. One unit of APase activity was defined as the activity that liberated 1 µmol of *p*-nitrophenylphosphate per minute. RNase activities were assayed in sodium acetate buffer (pH 5.6), using 250 µg of yeast RNA as the substrate. One unit of RNase activity was defined as the activity that liberated the amount of soluble nucleotide corresponding to one unit of nucleotide per minute. One unit is the amount of nucleotide that has an A_{260} of 1.0 in a volume of 1.0 mL. Values are the means ± SE of three replicates.

A lyophilized leaf sample (about 100 mg) was used to extract organic acids, after agitating with 5 mL of 0.01 N HCl for 1 h. After centrifuging at 3000 *g* for 10 min, the supernatant was collected and then filtered, using a 45-µm membrane filter. The organic acids were then analyzed, using capillary electrophoresis. A lyophilized leaf sample (about 50 mg) was incubated with 80% (v/v) ethanol at 40°C for 17.5 h to extract glucose, fructose, and sucrose. Carbohydrate contents were measured with an F-kit purchased from Roche Diagnostics Corporation.

For the experiment on ^{14}C partitioning analysis, we used plants grown under either the 0 µM P or the 32 µM P treatment for 2 weeks after pre-culture. $^{14}CO_2$ was generated by adding 30% PCA into $NaH^{14}CO_2$ (18.5 kBq). $^{14}CO_2$ was fed to the plants for 5 min in a vinyl package under natural lighting (photon flux density was immediately in liquid nitrogen. The sample was lyophilized and stored at -20°C until analysis for different fractions. ^{14}C assimilated in shoots was fractionated into amino acids, organic acids, phosphate esters, sugars, and residue, using the ion-exchange columns. The residual fraction is considered to consist of protein, starch, and constituents of cell walls such as cellulose, hemicellulose, and lignin. All of the fractions were concentrated, using a rotary-evaporator at 40ˆC, then making up their volumes to 10 mL by water. From each sample, 1 mL was mixed with toluene scintillator (DPO 4 g, POPOP 200 mg, and nonion 300 mL, made up to 1 liter with toluene) and placed in darkness overnight. Radioactivity was measured, using a liquid scintillation counter.

Results and Discussion

Plant growth and nutrient status: Results on changes in total P concentrations in the nutrient solution in each container within 24 h showed that the 3 levels of P treatment 0 µM P, 6 µM P and 32 µM P provided conditions of complete P deprivation, low P supply and adequate P supply, respectively, during the treatment period of 2 weeks. Maximum amount of P depletion of up to 85% to 100% was observed at 2 weeks after treatment with 6 µM P while it was only up to 40% with 32 µM P treatment.

Although plant growth decreased with P deficiency, the difference between the treatments 6 µM P and 32 µM P was not significant (Table 20). The total dry weight (DW) of the *Brachiaria* hybrid decreased by 33% in the 6 µM P treatment under P deficiency, and lower P supply had greater effect on leaf and stem growth than on root growth. Rice also decreased its growth with P deficiency, but its growth in the 0 µM P treatment was greater than that of the *Brachiaria* hybrid. This was because rice seed is larger and contains more P, and therefore contributed to a higher accumulation of P in rice seedlings before they were subjected to low-P treatments.

However, P-use efficiency (the inverse of P concentration) was greater in the *Brachiaria* hybrid (1.85 g DW per 1 mg P) than in rice (1.61 g DW per 1 mg P) (Table 21). Thus, in the 0 µM P treatment, the *Brachiaria* hybrid showed greater tolerance of low concentrations of tissue P, compared with rice. In contrast, P-use efficiency in the 32 µM P treatment was greater

Table 20. Dry weight (DW) among plant parts of a Brachiaria hybrid and rice grown at three different levels of phosphorus (P) supply.

Species	Treatment (µM P)	DW (g per plant)			
		Leaf	Stem	Root	Total plant
Brachiaria hybrid	0	0.425 a	0.248 a	0.438 a	1.11 a
	6	0.707 b	0.395 b	0.539 a	1.64 ab
	32	0.799 b	0.439 b	0.449 a	1.69 b
Rice	0	0.400 a	0.583 a	0.365 a	1.35 a
	6	0.487 b	0.645 a	0.403 a	1.53 a
	32	0.542 b	0.700 a	0.363 a	1.61 a

Means followed by the same letter within the column are not significantly different, according to Duncan's multiple range test ($P \leq 0.05$).

Table 21. Nitrogen and P contents in different plant parts of a *Brachiaria* hybrid and rice grown at three different levels of P supply.

Nutrient	Species	Treatment (μM P)	Concentration (mg per g DW)				P-use efficiency (g DW per mg P)
			Leaf	Stem	Root	Total plant	
	Brachiaria						
Nitrogen	hybrid	0	44.7 [ab]	43.3 [b]	32.0 [ab]	39.6 [a]	
		6	42.0 [a]	37.3 [a]	29.2 [a]	38.6 [a]	
		32	45.2 [b]	38.3 [a]	33.1 [b]	40.2 [a]	
	Rice	0	33.4 [a]	15.1 [a]	18.0 [a]	21.3 [a]	
		6	44.9 [b]	24.6 [b]	23.9 [b]	30.8 [b]	
		32	50.5 [c]	30.7 [b]	29.6 [c]	37.1 [c]	
	Brachiaria						
Phosphorus	hybrid	0	0.44 [a]	0.62 [a]	0.58 [a]	0.54 [a]	1.85 [a]
		6	1.69 [b]	1.94 [b]	1.78 [b]	1.78 [b]	0.56 [b]
		32	11.0 [c]	6.92 [c]	8.35 [c]	9.26 [c]	0.11 [c]
	Rice	0	0.74 [a]	0.57 [a]	0.56 [a]	0.62 [a]	1.61 [a]
		6	2.03 [b]	1.83 [b]	1.78 [b]	1.88 [b]	0.53 [b]
		32	6.29 [c]	7.00 [c]	5.40 [c]	6.40 [c]	0.16 [c]

Means followed by the same letter within the column are not significantly different, according to Duncan's multiple range test ($P \leq 0.05$).

in rice than in the *Brachiaria* hybrid. Apparently, the mechanisms of P-use efficiency differ between the two test crops. Although P-use efficiency was different, dry weight in the 32 μM P treatment was similar for the two test crops, indicating that both crop plants would serve as good models for understanding how different plant mechanisms function in relation to P nutrition. Nitrogen concentration was stable in the *Brachiaria* hybrid, regardless of P treatment, whereas it decreased markedly in rice with P deficiency (Table 20). Phosphorus concentration decreased markedly in both test crops with P deficiency (Table 21), which was lower at 0 μM P treatment, especially in leaves of the *Brachiaria* hybrid. Inorganic P (P_i) concentration, expressed on the basis of dry weight also decreased markedly with P deficiency in both test crops (Table 21), with P_i concentration being higher in leaves than in roots. P_i concentration in stems was higher in rice than in the *Brachiaria* hybrid, although when P was supplied at 6 μM P or 32 μM P, P-use efficiency was similar for both the *Brachiaria* hybrid and rice.

Fractions of phosphorus compounds: Acid-soluble P_i accounted for a large part of total P in both species under P-sufficient conditions (Figure 33). But the ratio of acid-soluble P_i to total P decreased drastically with P deficiency in plant parts (leaf, stem, and root) and total biomass, especially in rice (Table 22). In the 0 μM P treatment, the ratio of acid-soluble P_i accounted for 47% and 17% in the Brachiaria hybrid and rice, respectively. However, in both test crops, the RNA:P ratio increased with P deficiency as acid-soluble P_i decreased.

Phosphohydrolase activity: We measured APase and RNase activities as P-recycling enzymes to discover whether they have any role in increasing the efficiency of acquired P in the plant. APase and RNase activities were both induced by P deficiency. The *Brachiaria* hybrid had higher APase activity, especially in shoots, than did rice. Specific activity of APase in leaves was 5.2 to 7.7 times higher in the *Brachiaria* hybrid (Figure 34A) than in rice (Figure 34B) and, in stems, 2.7 to 3.5 times higher. APase activity in roots was noticeably induced in both test crops when they were grown without P. RNase activity in leaves and stem was also higher in the *Brachiaria* hybrid than in rice (Figures 34C and 34D), while being slightly lower in roots. RNase activities in *Brachiaria* hybrid leaves were 4.4 to 5.8 times higher than in rice leaves and 1.7 to 5.4 times higher in stems.

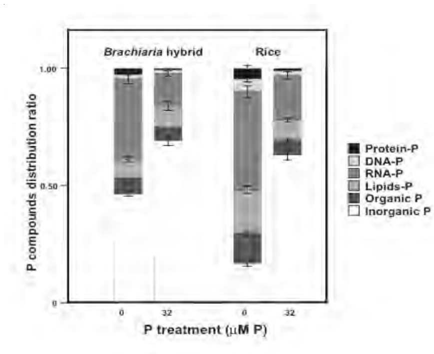


Figure 33. Distribution of P compounds in the *Brachiaria* hybrid and rice under three levels of P supply. Plants, 7 to 8 weeks old, were grown in P-deficit nutrient solution for 2 weeks. Phosphorus in plants was fractionated into the following six fractions: acid-soluble inorganic P, acid-soluble organic P, lipids-P, RNA-P, DNA-P, and residual P. Most of the residual-P fraction is considered to consist of protein P. Values are the means ± SE of three replicates.

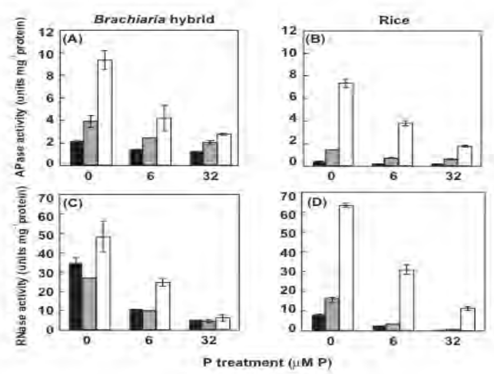


Figure 34. APase (A, B) and RNase (C, D) activities of a *Brachiaria* hybrid (A, C) and rice (B, D). Plants were grown at three different levels of P for 2 weeks. APase and RNase activities were measured in leaves (black bars), stem (hatched bars), and roots (white bars).

Organic acid concentration in leaves: Total organic acid concentration was higher in the *Brachiaria* hybrid than in rice (Figure 35). With P deficiency, organic acid concentration dropped by 37% in the Brachiaria hybrid and by 55% in rice. Results on organic acid composition showed thatoxalate and fumarate were the main organic acids in the *Brachiaria* hybrid, whereas oxalate concentration was high in rice only in the 32 µM P treatment. In rice, the marked decrease in oxalate was accompanied by increased malate and citrate concentrations in leaves.

Carbohydrates in leaves: The concentrations of glucose, fructose, and sucrose dropped with P deficiency (Figures 36A, 36B, 36C, respectively). These decreases were more prominent in the *Brachiaria* hybrid. Starch (Figure 36D), glucose, and fructose concentrations were higher in the *Brachiaria* hybrid than in rice, with sucrose concentration being higher in rice (Figure 36C). The level of starch decreased in the *Brachiaria* hybrid with P deficiency while it increased in rice.

Table 22. Contents of P_i and P_i:total P ratio in different plant parts of a Brachiaria hybrid and rice grown at three levels of P supply.

P_i parameter	Species	Treatment (µM P)	Leaf	Stem	Root	Total plant
P_i concentration (mg per g DW)	*Brachiaria* hybrid	0	0.13^{a}	0.08^{a}	0.06^{a}	0.09^{a}
		6	0.73^{a}	0.22^{a}	0.30^{b}	0.47^{a}
		32	7.43^{b}	3.48^{b}	4.63^{c}	5.66^{b}
	Rice	0	0.13^{a}	0.08^{a}	0.05^{a}	0.08^{a}
		6	0.57^{b}	0.24^{a}	0.31^{a}	0.36^{b}
		32	3.61^{c}	4.05^{b}	3.04^{b}	3.67^{c}
P_i:total P ratio (%)	*Brachiaria* hybrid	0	29.6^{a}	14.1^{a}	11.3^{a}	17.5^{a}
		6	43.5^{b}	11.1^{a}	16.7^{a}	26.2^{b}
		32	67.1^{c}	50.2^{b}	56.0^{b}	61.1^{c}
	Rice	0	17.0^{a}	13.7^{a}	8.5^{a}	13.6^{a}
		6	27.8^{b}	13.3^{a}	17.3^{a}	19.1^{b}
		32	57.4^{c}	57.8^{b}	56.2^{b}	57.4^{c}

Means followed by the same letter within the column are not significantly different, according to Duncan's multiple range test ($P \leq 0.05$).

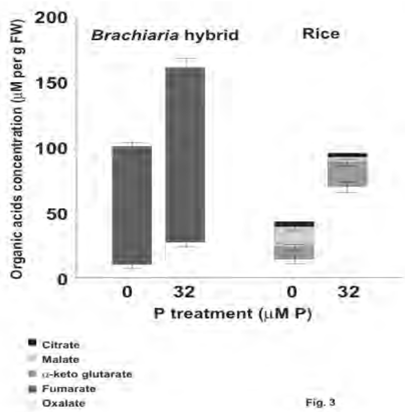


Figure 35. Organic acid concentration in *Brachiaria* hybrid and rice leaves. Plants were grown with or without P for 2 weeks. Organic acid content was measured, using capillary electrophoresis. We identified each peak of an organic acid by comparing the peaks with standards. Contents were determined by comparing the peak area of the sample with that of the corresponding standard. Values are the means ± SE of three replicates.

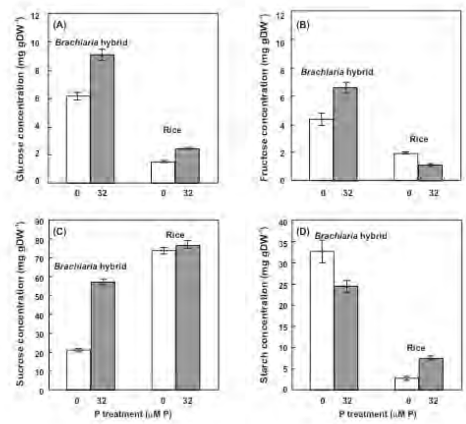


Figure 36. Carbohydrate concentration in *Brachiaria* hybrid and rice leaves: (A) glucose, (B) fructose, (C) sucrose, and (D) starch concentrations. Plants used for the assays were grown with P (white bars) or without P (hatched bars) for 2 weeks. Carbohydrate concentration Values are the means ± SE of three replicates.

Enzyme activities catalyzing the PEP-consuming reaction: The three enzymes that catalyze the PEP-consuming reaction in glycolysis are PEPC, PEPP, and PK. Phosphoenolpyruvate carboxylase (PEPC) activity was greater in the *Brachiaria* hybrid than in rice (Table 23). In rice, it increased by a factor of 2.4 with P deficiency, whereas it did not change in the *Brachiaria* hybrid. In both test crops, phospho*enol*pyruvate phosphatase (PEPP) activity was induced by P deficiency: 5.6 times in the *Brachiaria* hybrid and 6.0 times in rice, although in leaves, it was higher in the *Brachiaria* hybrid than in rice. Pyruvate kinase (PK) activity in rice was 1.6 times higher in the 0 μM P than in the 32 μM P treatment. Although PK activity in the *Brachiaria* hybrid was also higher in the 0 μM P treatment, it was not significant.

^{14}C partitioning: In both test crops, photosynthetically fixed carbon pools were mainly distributed to sugars, and the ^{14}C distribution ratio to sugars increased with P deficiency (Figure 37). The effect of P deficiency on ^{14}C partitioning to sugars was larger in rice than in the *Brachiaria* hybrid. The distribution ratio to sugars was 68% in rice and 50% in the *Brachiaria* hybid in the 0 μM P treatment. The ^{14}C distribution ratio to the amino acid and organic acid pools was greater in the *Brachiaria* hybrid than in rice, and slightly increased with P deficiency in the *Brachiaria* hybrid. The ^{14}C distribution ratio to phosphate esters markedly decreased with P deficiency in both test crops. The ^{14}C distribution ratio to the residue fraction, which is supposed to contain protein, starch, and nonstructural carbohydrate pools, increased with P deficiency in both test crops.

Table 23. Influence of P deprivation on enzyme activities that are invo metabolism in leaves of *Brachiaria* hybrid and rice.

Species	Treatment	Activity (μmole min^{-1} mg	
	(μM P)	PEPC	PEPP
Brachiaria hybrid	0	4.39[a]	0.218[a]
	32	3.79[a]	0.039[b]
Rice	0	0.04[a]	0.060[a]
	32	0.09[b]	0.010[b]

Plants used for the assays were grown with or without phosphorus for 2 effect of phosphorus concentration in the medium was determined sepa *Brachiaria* hybrid and rice by t-test (P ≤ 0.05).

Results from this study show that tolerance of low P in the *Brachiaria* hybrid and rice involved marked differences in P recycling and carbon metabolism. For the *Brachiaria* hybrid, low-P tolerance involved two major strategies: (1) increasing the ability to use P efficiently by inducing APase and RNase in shoots with P deficiency; and (2) enhancing sugar catabolism and subsequent synthesis of amino acids and organic acids in leaves under P deficiency. For rice, strategies for low-P tolerance differed by involving (1) decreased carbon flow to amino acids and organic acids, and decreased N concentration; and (2) improved partitioning of photosynthates to sucrose, combined with restricted sugar catabolism.

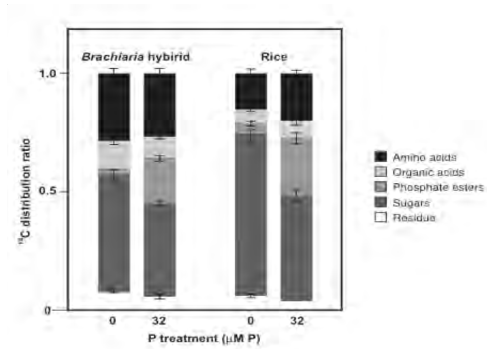


Figure 37. Distribution of photosynthetically assimilated ^{14}C in the *Brachiaria* hybrid and rice. $^{14}CO_2$ (18.5 kBq) was fed to P-deficient or P-sufficient plants under natural lighting. After $^{14}CO_2$ was fed for 5 min, only shoots were harvested and used for assays. ^{14}C assimilated in shoots was fractionated into amino acids, organic acids, phosphate esters, sugars, and residue, using the ion-exchange columns. The residual fraction is considered to consist of protein, starch, and constituents of cell walls such as cellulose, hemicellulose, and lignin. Values are the means ± SE of three replicates.

3.1.1.3 Screening of *Brachiaria* hybrids for resistance to aluminum

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

Rationale

For the last two years, we have implemented screening procedure to identify Al-resistant *Brachiaria* hybrids that were preselected for spittlebug resistance.Last year, 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. 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 the most promising *Brachiaria* hybrids that are resistant/tolerant to spittlebug.

Materials and Methods

A total of 79 genotypes (including 54 new hybrids and 24 checks that included previous selections together with 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 hybrids were screened for spittlebug resistance (C. Cardona, personal communication; also see Output 2 and Activity 2.2 of this report). Stem-cuttings were rooted in a low ionic strength nutrient solution containing $CaCl_2$ (200µM), selected for uniformity and transferred to a solution containing 200 µ*M* $CaCl_2$ (pH 4.2) and exposed to 2 levels of $AlCl_3$ (0 and 200µM). The solution was replaced every third day, and total root length and root biomass were measured after 21 days of Al treatment (Photo 13). Root architecture was measured using WINRHIZO software program. Results reported are mean values from 3 experiments.

Results and Discussion

As reported for the past 2 years, results on total root length per plant 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 (Figure 38).

Photo 13. Different steps involved in screening for aluminum resistance: (a) growing of individual genotypes in soil; (b) cultivation of vegetative propagules in nutrient solution for inducing rooting; (c) generation of vegetative propagules with 4 to 5 cm long roots for evaluation; (d) treatment with or without aluminum in nutrient solution for 3 weeks; and (e) quantitative evaluation of root traits at the end of 3 weeks of treatment (Photos are from the MS thesis work of Adriana Arango).

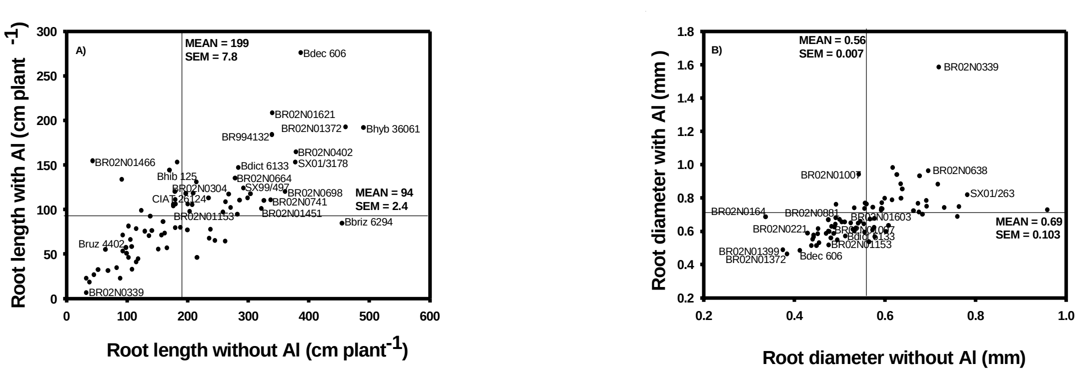


Figure 38. Identification of Al resistant hybrids of *Brachiaria* based on (A) total root length and (B) mean root diameter. Total root length and mean root diameter were measured after exposure to 0 or 200 ˜M $AlCl_3$ with 200 ˜M $CaCl_2$ (pH 4.2) for 21 days. Genotypes with (A) higher values of root length were identified in the upper box of the left hand side and (B) lower values of mean root diameter (fine roots) with no or high Al in solution were identified in the lower box of the left hand side. SEM = standard error of the mean.

Among the 54 new hybrids tested, 2 hybrids (BR02NO1372 and BR02NO1621) showed greater level of Al resistance based on total root length per plant (Figure 39). Among these two hybrids, BR02NO1372 showed greater fine root development than CIAT 606 in the absence of Al in solution (Figure 39). Another hybrid, BR02NO402 also showed moderate level of Al resistance based on root length value with the presence of Al. Exposure to Al decreased the mean value of total root length of the 79 genotypes from 199 to 94 cm plant^{-1}. Among the checks tested, 2 sexual hybrids (SX99/7075 and SX01/1378) that were selected before for Al resistance also showed moderate level of Al resistance. Among the apomictic hybrid checks cv. Mulato (CIAT36061) and BR99/4132 showed greater level of Al resistance.

Results on mean root diameter showed that the hybrid BR02NO1372 had the lowest values under both with and without Al treatments (Figure 39). This hybrid was superior to CIAT 606 in fine root development. One of the hybrids, BR02NO339 showed very high values of mean root diameter of about 1.6 mm (coarse root system) with Al in solution.

The relationship between total root length and mean root diameter in the presence of Al in solution showed that 2 hybrids (BR02NO1621 and BR02NO1372) were outstanding in developing fine root system (Figure 39). The same relationship without Al in nutrient solution indicated that the hybrid BR02NO1372 is superior to CIAT 606 in terms of total toot length The mean values of root diameter decreased from 0.69 to 0.56 mm with the exposure to Al in nutrient solution. of mean root diameter).

Results on spittlebug resistance of these hybrids are reported in section 2.2 of this report. The hybrid BR02NO1372 was selected as one of the spittlebug resistant hybrids. Two other hybrids (BR02NO402; BR02NO794) that showed moderate level of Al resistance were also found to be moderate in resistance to spittlebugs. This set of hybrids are being evaluated under field conditions to determine the mode of reproduction (sexual or apomictic) using progeny test. The hybrids that combine desirable attributes with apomictic mode of reproduction will be candidates for further field evaluation as potential cultivars for release. The promising sexuals could be used in recurrent selection to generate superior hybrids of *Brachiaria*.

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.

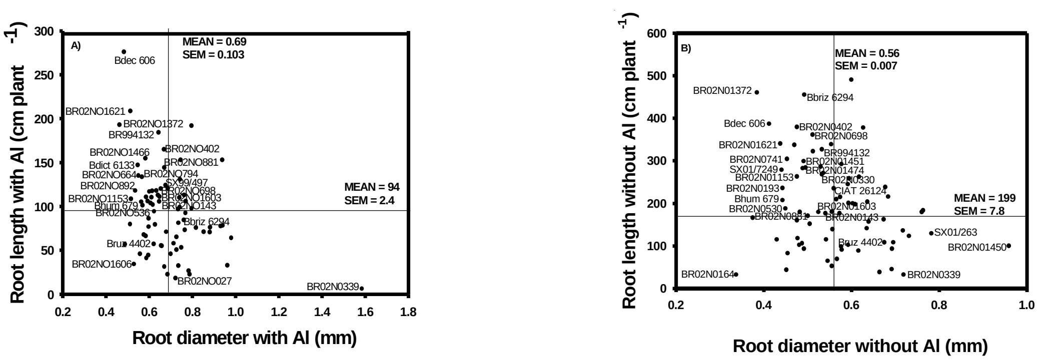


Figure 39. Relationship between total root length and mean root diameter of 78 genotypes of *Brachiaria* with (A) presence or (B) absence of aluminum in solution. Gentoypes that develop finer root system were identified in the upper box of the left hand side.

3.1.1.4 Identification of plant attributes in *Brachiaria* associated with persistence under low nutrient supply

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

Rationale

A field study is completed this year at Matazul Farm in the Llanos of Colombia. The main objective was to identify genetic recombinants of *Brachiaria* with tolerance to low nutrient supply and evaluate plant attributes that contribute to superior adaptation. Results obtained from this field study at 28 months after establishment indicated that the *Brachiaria* hybrid, FM9503-S046-024 was not only rapid in its establishment but also performed well into the third year after establishment. Its superior performance was associated with its ability to acquire greater amounts of nutrients, particularly Ca and Mg from low fertility soil. This year we report the results on the performance of genetic recombinants in terms of their growth and persistence under low fertility acid soil conditions at 40 months 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*. The trial was planted as a randomized block in split-plot arrangement with two levels of initial fertilizer application (low: kg/ha of 20P, 20K, 33Ca, 14 Mg, 10S; and high: 80N, 50P, 100K, 66Ca, 28Mg, 20S and micronutrients) as main plots and genotypes as sub-plots. Live and dead forage yield, shoot nutrient composition, shoot nutrient uptake and leaf and stem TNC (total nonstructural carbohydrates) were measured at the end of the wet season (November 19, 2002), i.e., at 40 months after establishment. Maintenance fertlizer (half the levels of initial application) was applied in July 2001.

Results and Discussion

As expected, application of high amounts of maintenance fertilizer at 2 years after establishment improved forage yield of most of the genotypes compared with low fertilizer application (Figure 40). At 40 months after establishment, live forage yield with low fertilizer application ranged from 0 to 2004 kg/ha and the high values of forage yield were observed with three germplasm accessions (CIAT 26110, 26318 and 6294) and one spittlebug resistant genetic recombinant, FM9503-S046-024 (Table 24). This spittlebug resistant genetic recombinant not only showed rapid establishment but also maintained greater level of forage production over time for the past 3 years with low initial fertilizer application. It also was very responsive to higher fertilizer application as revealed by live shoot biomass and total forage yield. As expected, the performance of one of the parents, BRUZ/44-02 was very poor compared with other parents and genetic recombinants. These results are similar to those observed at 28 months after establishment (IP-5 Annual Report, 2002). The values of leaf to stem ratio were markedly superior with the genetic recombinants than the parents and other germplasm accessions with both levels of fertilizer application (Figure 40). This is an important attribute for improving animal production through *Brachiaria* breeding activities.

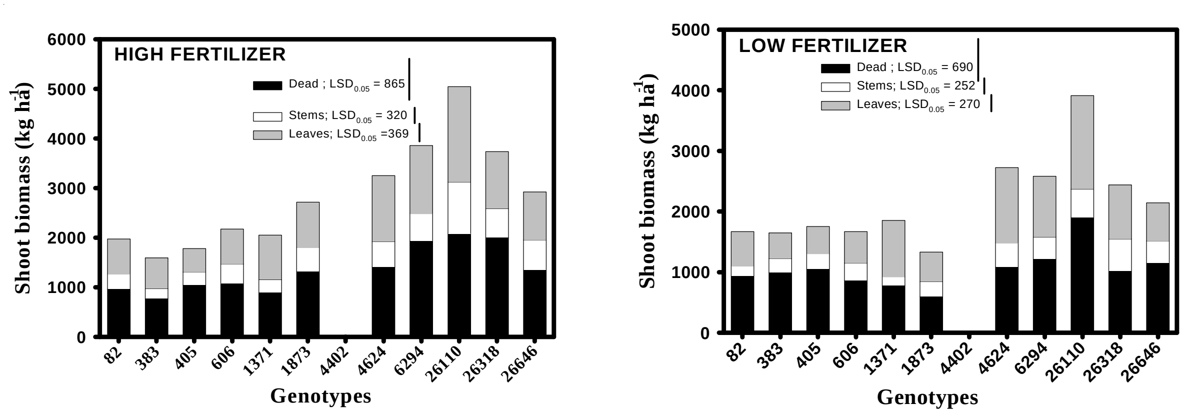


Figure 40. Genotypic variation as influenced by fertilizer application in dry matter distribution among leaves, stems and dead biomass of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 40 months after establishment (November 2002) LSD values are at the 0.05 probability level.

Among the genetic recombinants, FM9503-S046-024 was outstanding in production of green leaf biomass with both low and high fertilizer application (Figure 40). One of the germplasm accessions, CIAT 26110 was outstanding in its leaf biomass production and its production was almost 2 times greater than that of the mean value of 12 genotypes tested. These results are consistent with the observations made in previous years.

Shoot N uptake with low fertilizer application was greater for one accession (CIAT 26110), one parent (CIAT 6294) and two genetic recombinants (FM9503-S046-024, FM9301-1371) (Figure 41). These two genetic recombinants were also outstanding in their ability to acquire greater amounts of P, K, Ca and Mg from low fertilizer application when compared with other genetic recombinants (Figure 41). The ability of these two hybrids and CIAT 26110 to acquire Ca from soil with low fertilizer application treatment was particularly outstanding compared with other hybrids and germplasm accessions. This ability to acquire greater amounts of Ca from acid soil (Table 24) could not only contribute to its superior persistence on acid infertile soils but also could contribute to greater forage quality and animal production. Among the parents, *B. Brizantha*, CIAT 6294 was superior in nutrient acquisition from low and high fertilizer application (Figure 41). Shoot nutrient uptake by CIAT 26110 was outstanding with both low and high fertilizer application (Figure 41).
Production of live forage yield was associated with significantly lower contents of nutrients (P, Ca and Mg) in stem tissue of *Brachiaria* genotypes, particularly under low fertilizer application, indicating the importance of efficient mobilization of nutrients from stems to leaves and efficient utilization of nutrients for the production of green forage (Table 24). This could be an important physiological mechanism for superior performance with low fertilizer application. Leaf ash (mineral) and stem ash contents were also negatively associated with green forage yield indicating lower mineral status and greater nutrient use efficiency under low fertilizer treatment.

Results from this field study indicated that the *Brachiaria* hybrid, FM9503-S046-024 was not only rapid in its establishment but also performed well into the fourth year after establishment. Its superior performance at 40 months after establishment was associated with its ability to acquire greater amounts of nutrients, particularly Ca and Mg from low fertility soil.

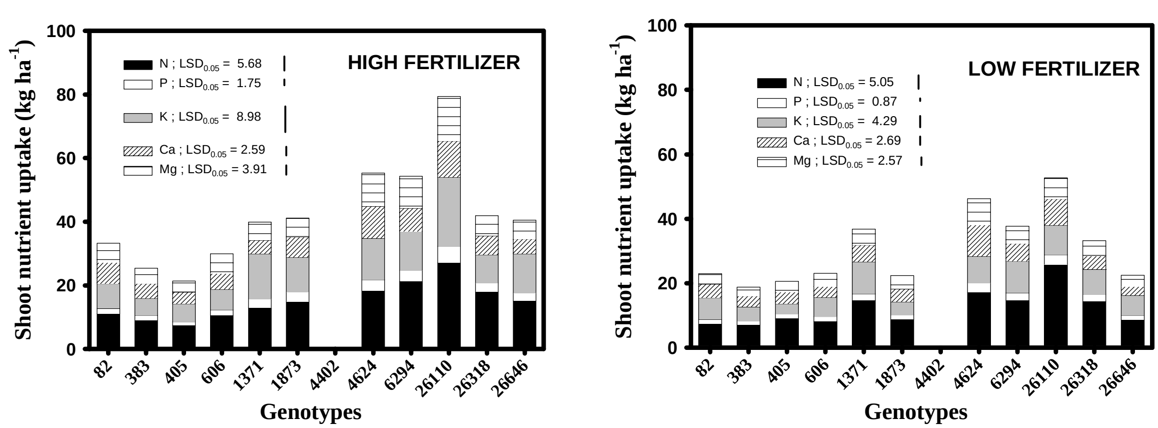


Figure 41. Genotypic variation as influenced by fertilizer application in nutrient (N, P, K, Ca, Mg) uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 40 months after establishment (November 2002) LSD values are at the 0.05 probability level.

Table 24. 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)	0.89***	0.94***
Dead shoot biomass (t/ha)	0.61***	0.76***
Leaf biomass (t/ha)	0.95***	0.97***
Stem biomass (t/ha)	0.76***	0.92***
Leaf N content (%)	0.19	-0.20
Leaf P content (%)	-0.29*	0.03
Leaf K content (%)	-0.30*	-0.10
Leaf Ca content (%)	-0.22	-0.33*
Leaf Mg content (%)	-0.37**	-0.08
Leaf TNC content (mg g^{-1})	-0.39**	0.02
Leaf ash content (%)	-0.20	-0.15
Stem N content (%)	-0.22	-0.49**
Stem P content (%)	-0.37**	-0.06
Stem K content (%)	-0.06	0.04
Stem Ca content (%)	-0.36*	-0.57***
Stem Mg content (%)	-0.39**	-0.32*
Stem TNC content (mg g^{-1})	-0.05	-0.35*
Stem ash content (%)	-0.22	-0.01

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

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

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

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 and nutrient uptake were measured at 18 months after establishment (November 2002).

Results and Discussion

At 18 months after establishment, live forage yield with low fertilizer application ranged from52 to 2049 kg/ha and the high values of forage yield were observed with one spittlebug resistant genetic recombinant, FM9503-S046-024 and one parent (CIAT 6294). With high initial fertilizer application also these two genotypes were outstanding in shoot biomass production (Figure outstanding in shoot biomass production (Figure 42). Among the 4 hybrids tested, 4624 was outstanding in its adaptation to low intial fertilizer application. It is important to note that CIAT 6294 had greater amount of dead biomass and stem biomass under low fertilizer application (Figure 42).

Results on shoot nutrient uptake indicated that the two hybrids, 4624 and 4015 were superior to CIAT 606 under low fertilizer application (Figure 43). Nutrient acquisition by the hybrid 4624 was also greater than the rest of the test materials with high initial fertilization. These results are consistent with previous field study conducted on the same farm.

The performance of the 4 hybrids in comparison with two parents with maintenance fertilizer application will be monitored for the next 2 years in terms of forage yield and nutrient acquisition.

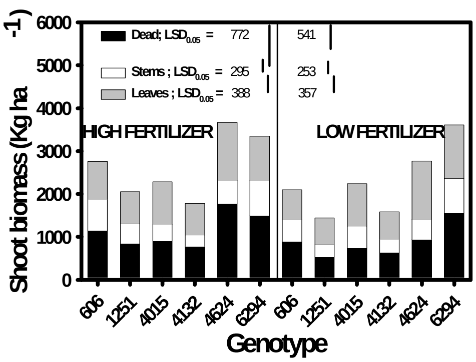


Figure 42. Genotypic variation as influenced by fertilizer application in shoot biomass production (forage yield) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 18 months after establishment (November 2002). LSD values are at the 0.05 probability level. NS = not significant.

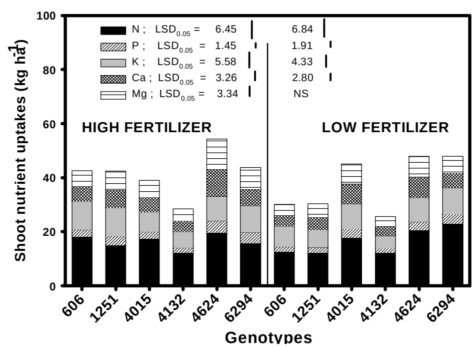


Figure 43. Genotypic variation as influenced by fertilizer application in nutrient uptake (N, P, K, Ca and Mg) of two parents (CIAT 606, 6294) and four genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 18 months after establishment (November 2002). LSD values are at the 0.05 probability level. NS = not significant.

3.1.2 Field trials in Costa Rica, Nicaragua and Honduras for participatory evaluation of *Brachiaria* hybrids in comparison with commercial cultivars

Contributors: Beatriz Sandoval and Marco Lobo (INTA-MAG), Pedro J. Argel, Guillermo Pérez, A. Schmidt, H. Cruz, L.A. Hernandez, L.H. Franco, M. Peters, J. Miles and I. M. Rao (CIAT)

Farmers' participation is very important in the process of identifying and selecting promising forage germplasm, because this group bases their selection criteria on particular plant attributes not necessarily observed by the researchers. Thus, in the selection process, farmers' participation complements additional experimental information generated on the same plots by technicians in charge of the experiments. Participatory evaluation of *Brachiaria* hybrids and accessions is being carried out in hillsides of Costa Rica, Honduras and Nicaragua.

Rationale

As part of the BMZ-GTZ project on developing aluminum resistant *Brachiaria* hybrids, last year we initiated field studies in Costa Rica, Nicaragua and Honduras for evaluation of new hybrids of *Brachiaria* along with commercial *Brachiaria* cultivars in participation with farmers. Farmers' participation is very important in the process of identifying and selecting promising forage germplasm, because this group bases their selection criteria on particular plant attributes not necessarily observed by the researchers. Thus, in the selection process, farmers' participation complements additional experimental information generated on the same plots by researchers in charge of the experiments. 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 use in crop-livestock systems.

Materials and Methods

Costa Rica - A field trial was established on 30 August 2002 in the area of Puriscal (Cantón Mora) in a farm called El Rodeo. National program (INTA) staff responsible for managing the trial are Ing.Beatriz Sandoval and Marco Lobo from MAG of Costa Rica. The design is a complete randomized block with 3 replications. The plot size was 2 m long x 4 m wide. The texture of the soil is loamy with the following chemical characteristics: pH 4.6, Al (0.8 ppm), Ca (6.2 cmol/kg), Mg (1.76 cmol/kg), K (0.46 cmol/kg), Zn (0.6 mg/kg), Cu (5.0 mg/kg), Fe (52 mg/kg); SOM (5.7%). The *Brachiaria* species planted had the following CIAT numbers: 606, 26110, 26990, 26318, 26646, 6133, 36061, 6789, 36062, 26124 and Mixe (no CIAT number). Among the 11 genotypes planted, CIAT 36061 and CIAT 36062 are apomictic hybrids. During 2003 we continued the participatory evaluation of promising lines and hybrids of *Brachiaria* at the locality of El Rodeo (Cantón Mora, Costa Rica). A number of plant attributes including forage yield, dry matter distribution and nutrient uptake are being monitored.

Nicaragua - The experimental site was chosen in Ubú Norte (12° 58' 44" N, 84° 54' 23" E, 261 masl) in the Region Autonoma del Atlantico Sur (RAAS) where acid soils are predominant. Soil characteristics are: SOM 6.61%, pH 5.66, P(BrayII) 3.22, K (meq) 0.55, Ca (meq) 5.5, Mg (meq) 2.42. 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") 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 (5 x 4m) were sown early October 2002 upon the beginning of the second rainy season. Plot establishment 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. Two fertilization treatments were applied upon plot establishment. Fertilization levels were adjusted to soil analysis results. The first agronomic and participatory evaluation was conducted in August 2003 (7 weeks after standardization cut).

Honduras - The trial planted in the Yorito reference site in Honduras (neutral to slightly acidic soils, 6 months dry season) used the same experimental design as reported for the Costa Rica trial. Data for the participatory evaluation was analysed using correspondence factorial analysis (CAF; see section 4.1.9 of this report).

Results and Discussion

All *Brachiaria* lines established well, however differences existed in plant height 109 days after planting within and between *Brachiaria* species. Table 25 shows that *B. brizantha* cv. Marandú (CIAT 6780), cv. Toledo (CIAT 26110), one line of this species called Mixe, as well as *Brachiaria* hybrid cv. Mulato (CIAT 36061) and

Table 25. Plant height of *Brachiaria* lines established for participatory evaluation at El Rodeo farm (Puriscal, Costa Rica) 109 days after planting.

Species	Plant height (cm)
B. brizantha (Mixe)	67.8 a*
B. brizantha cv. Marandú (CIAT 6780)	66.7 a
B. hybrid cv. Mulato (CIAT 36061)	60.6 a
B. brizantha cv. Toledo (CIAT 26110)	56.7 a
B. decumbens cv. Basilisk (CIAT 606)	55.5 a
B. brizantha CIAT 26124	51.1 ab
B. brizantha CIAT 26990	49.4 ab
B. brizantha cv. La Libertad (CIAT 26646)	44.2 abc
B. hybrid CIAT 36062	30.5 bc
B. humidicola cv. Llanero (CIAT 6133)	25.3 c
B. brizantha CIAT 26318	24.5 c

*p<0.001 (Duncan's Multiple Range Test)

B. decumbens cv. Basilisk (CIAT 606), among others, had well developed plants and more vigorous establishment than other lines under test. However, *B. humidicola* (synonymous: *B. dictyoneura*) cv. Llanero is a highly stoloniferous species that covers very well the soil and establishes well although produces shorter plants.

Participatory evaluation: Two groups of farmers formed by 8 and 12 participants and coming from the localities of La Guácima and El Rodeo, were selected to participate in separate sessions for participatory evaluation of the trial, as illustrated in Photo 14. One session was carried out in February (dry period) and another in May (beginning of the wet season).

Photo 14. Participatory evaluation of Brachiaria species and hybrids established in El Rodeo, Costa Rica

The variables plant cover, leafiness, leaf texture and color, were chosen to measure degree of preference and probabilities of adoption of the *Brachiaria* germplasm under test. The data were pooled and statistically analyzed following a logistics regression model suggested by L. A. Hernández (CIAT, 2000).

A summary of the results is presented in Table 26. Farmers showed special preference for the commercial materials *B. brizantha* cv. Toledo, Diamantes 1 (Marandú) and La Libertad, particularly referred to plant cover and abundance of leaves. Cultivar Mulato had a medium acceptance for the same variables but was rated high for leaf texture and color. Similar preference was showed by *B.* hybrid CIAT 36062, although in this case plant cover was rated low. Other entries had low to medium acceptance for the variables measured, including the commercial line *B. decumbens* cv. Basilisk.

An additional participatory evaluation will be conducted next October, and the experiment will continue under evaluation for another growing season.

Participatory evaluation in Nicaragua

The first agronomic evaluation (Table 27) showed no significant fertilizer effect on plant height, soil cover and dry matter yield. This was mainly due to greater availability of exchangeable cations. There were significant differences among accessions/ hybrids. However, no accession/

Table 26. Farmer preferences of ten species and hybrids of *Brachiaria* established for evaluation at the locality of El Rodeo, Costa Rica (Information supplied by Beatriz Sandoval and Marco Lobo from INTA).

Species	Plant cover	Abundance of foliage	Foliage texture and color
B. decumbens cv. Basilisk (CIAT 606)	medium*	low	medium
B. brizantha cv. Diamantes 1 (CIAT 6780)	high	high	medium
B. brizantha cv. La Libertad (CIAT 26646)	high	high	medium
B. hybrid CIAT 36062	low	medium	high
B. hybrid cv. Mulato (CIAT 36061)	medium	medium	high
B. brizantha (Mixe)	low	low	medium
B. brizantha cv. Toledo (CIAT 26110)	high	high	high
B. humidicola cv. Brunca (CIAT 6133)	low	high	low
B. brizantha CIAT 26124	low	high	medium
B. brizantha CIAT 26990	medium	medium	low

* The groups high, medium and low presented significant differences between them ($p<0.05$, Chi-square Test).

Table 27. Plant height, soil cover and DM yield of 14 *Brachiaria* accessions and hybrids in Ubú Norte, Nicaragua

Parameter	Plant height (cm)		Soil cover (%)		DM yield (g/m^2)	
Fert. Level						
- High	88	ns *	64	ns	802	ns
- Low	82		60		747	
No. CIAT						
606	90	bcd	76	abc	578	cd
654	71	cde	48	de	538	cde
679	32	e	17	e	-	-
6133	55	ef	30	ef	339	de
6780	126	a	89	ab	1000	bc
16322	98	bcd	88	ab	646	cd
26110	108	ab	65	bcd	1682	a
26124	99	bc	89	ab	1239	ab
26318	90	bcd	34	ef	854	bcd
26646	75	cde	48	de	505	cde
26990	87	bcd	60	cd	695	bcd
36061	99	bc	93	a	1546	a
36062	70	de	32	ef	440	cde
"Mixe"	89	bcd	90	ab	775	bcd

(* $P \leq 0.05$; Duncan's Multiple Range Test; ns = not significant)

hybrid interactions with fertilizer levels were detected. *Brachiaria brizantha* cv. Toledo (CIAT 26110) and *Brachiaria* hybrid cv. Mulato (CIAT 36061) produced the largest amount of dry matter yield, followed by *B. brizantha* (CIAT 26124) and *B. brizantha* cv. Marandú (CIAT 6780). The lowest yields were obtained from *B. humidicola* (CIAT 679) and *B. dictyneura* (CIAT 6133) due to their slow and insufficient 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). The tallest plants were produced by *B. brizantha* cv. Toledo (CIAT 26110) and *B. brizantha* cv. Marandú (CIAT 6780).

Prior to agronomic data collection, a farmer group (7 pers.) evaluated the plots in accordance with their own criteria. Their preference ranking resulted as follows: *B. brizantha* cv. Marandú (CIAT 6780), *B. brizantha* cv. Toledo (CIAT 26110), *Brachiaria* hybrid cv. Mulato (CIAT 36061), *B. brizantha* (CIAT 26990), *B. brizantha* (CIAT 26124), *B. brizantha* (CIAT 16322), *B. decumbens* (CIAT 606), *B. brizantha* "Mixe", *B. ruzizensis* (CIAT 654), *B. brizantha* (CIAT 26318), *B. brizantha* cv. La Libertad (CIAT 26646), *B*. hybrid (CIAT 36062), *B. dictyoneura* (CIAT 6133), and *B. humidicola* (CIAT 679). The applied criteria were plant height, soil cover, foliage production, leave size and color. While the high ranking of *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 and green leaves. The fact that this year both materials were heavily sold on the seed market could have influenced the ranking. Accessions such as *B. brizantha* CIAT 26990, 26124, 16322 were classified as less productive because of their leaf size. All other materials were rated low due to low soil cover or plant height.

Through additional application of fertilizer it may be possible for detecting accession/hybrid interactions with fertility levels. Agronomic data and farmers' ranking presents a similar first picture of the materials established in Ubú Norte. Further evaluations will generate better conclusions.

Participatory evaluation in Honduras

Vigor, flowering time, competitiveness, earliness, robustness, growth, foliage abundance, rooting capacity, color and drought tolerance were the criteria employed by farmers in assessing forage grasses. The global analysis for wet season showed that the first 4 Dims (row coordinates factors) explained 86.9 % of the variation, a high percentage in analyzing participatory work.

Growth, vigor and time of flowering were the most important criteria defined in Dim 1. Cover and Competitiveness were the most important criteria in Dim 2. Color and earliness were the most important criteria in Dim 3, while robustness was the most important criteria defined in Dim4.

The global analysis for the dry season showed that the first 3 Dims explained 87 % of the variation. Abundance of foliage, drought tolerance and robustness were defined in Dim 1, while rooting capacity, softness, and leafiness were the most important parameters in Dim2. Color and cover were the most important parameters in Dim3. Hence, for farmers it is important that independent of season, forages have good establishment and growth and compete effectively with weeds. In the dry season leaf color as an indicator of drought tolerance, i.e. staying green and retaining leaves becomes an important criterion for farmer selection.

In the dry season *Brachiaria* hybrid cv. Mulato (CIAT 36061) and *B. brizantha* cv Toledo (26110) were the forage options most preferred by farmers; *B. brizantha* CIAT 6780 and *Brachiaria* hybrid CIAT 36062 had an intermediate preference. In the wet season *B. brizantha* cv Toledo, *B. brizantha* CIAT 6780 and *Brachiaria* hybrid CIAT 36062 were the most preferred materials; *Brachiaria* hybrid cv. Mulato obtained an intermediate classification. Over seasons *Brachiaria brizantha* cv. Toledo, *B. brizantha* CIAT 6780, *Brachiaria* hybrid cv Mulato (CIAT 36061), and *Brachiaria* hybrid (CIAT 36062) were the grasses best responding to farmers' criteria.

Preferences were based on farmer evaluation of materials under cutting in small plots. For a final assessment, an evaluation including the effect of the grazing animal on the grasses may be required. Preliminary results from this multilocational participatory evaluation of *Brachiaria* genotypes indicate that farmers' criteria for identifying the promising genotypes include rapid establishment, vigor, competetiveness, leaf color and texture, and green leaf production in the dry season. Further evaluations are planned for evaluating persistence.

3.1.3 Edaphic adaptation of *Arachis pintoi*

3.1.3.1 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

Field studies conducted in Caqueta, Colombia and greenhouse studies conducted at CIAT-Palmira indicated significant genotypic variation in P acquisition and utilization in *A. pintoi*. In order to identify P-efficient *Arachis pintoi* genotypes and to define specific mechanisms contributing to P efficiency, a Ph. D. theis work is in progress at the University of Goettingen in collaboration with CIAT. Two growth chamber studies were conducted and results from the first study to determine genotypic differences among ten accessions of *A. pintoi* in P-acquisition and utilization from low P soil were reported last year. Results from this study indicated that the commercial cultivar (CIAT 17434) is relatively less adapted to low P supply in soil. This study also confirmed the previous results obtained under field conditions that the accession CIAT 22159 is better adapted to very low P supply in oxisols. Results from the second study that included 3 contrasting accessions and aimed at identification of specific physiological mechanisms of P efficiency are reported below.

Material and Methods

A growth chamber trial was conducted at Institut for agricultural chemistry – Goettingen, Germany. An 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 L^{-1}) was used for evaluating the P efficiency of 3 accessions of *A. pintoi* (CIAT 17434, 18747, 22172) in a pot experiment. A low P-adapted commercial peanut, *A. hypogea* cv. AK-12/24, from India was used as a crop control. Treatments were arranged in a complete randomised block design with 3 replications. Three levels of P supply were used and three harvests (30, 60 and 90 days after planting) were conducted in order to evaluate the influence of the plant growth on the P-influx. Among the 3 *A. pintoi* accessions tested, CIAT 17434 is a commercial forage cultivar in Latin America.

Plastic pots of 4 L capacity were filled with 2.7 kg air-dry soil at a bulk density of 1.2 g cm^{-3}. Three levels of P supply (0, 50 and 1000 mg kg^{-1}) as $Ca(H_2PO_4)_2.H_2O$ were given. Basal nutrients were applied (mg kg^{-1}) to each pot:, 50 K as K_2SO_4, 40 Mg as $MgSO_4$, 0.2 B as H_3BO_3, 0.1 Mo as $(NH_4)_6Mo_7O_{24}$ and 100 N as $Ca(NO_3)_2$ was applied every 30 days. Two weeks before sowing, water was added to get a moisture content of 25% w/w. Seeds were sown directly in the pots. One pot for each P treatment was kept unplanted to measure soil moisture evaporation losses from the pots. Plants were thinned at the earliest to maintain 4 plants in each pot. The soil surface in each pot was covered with a layer of quartz sand (1 to 2 cm) to avoid the formation of a superficial crust due to the watering. The pots were watered daily and water was added to maintain the soil with 60% of its water holding capacity. Pots were kept 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.

At harvest, quartz sand was removed form the soil surface and shoots were cut above the soil surface. The dry weight of shoot was recorded. Roots were carefully separated by washing the soil on a sieve with 200 µm mesh width. Roots were cleaned of any foreign material, surface moisture was removed by centrifuging and root fresh weight was determined. Subsamples of around 0.5 g were preserved in 20% ethanol for root length measurements using line intersection method. To determine P concentration in plant tissue, plant 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 were determined. Data were subjected to an analysis of variance using the SAS computer program. Least-significant differences were calculated by an F-test. A probability level of 0.05 was considered statistically significant.

Results and Discussion

With no external supply of P (P-0) to an infertile oxisol, the commercial cultivar (CIAT 17434) was least productive over time (30, 60 and 90 days after planting) in terms of shoot biomass compared with the other 2 accessions (22172 and 18474) and commercial cultivar of peanut, *A. hypogea* (Figure 44). Among the 4 genotypes tested, CIAT 18744 was outstanding in shoot biomass production with different levels of P supply. This observation is consistent with field observations made at Carimagua (Oxisol) and Caqueta (Ultisol) where the commercial cultivar (CIAT 17434) was slow in establishment. Response to increase in P supply in terms of shoot growth was also markedly greater with *A. pintoi* accessions than A. hypogea. This was mainly due to belowground pod development and growth in peanut over time (Table 28). This greater development of pods in peanut had contributed to greater P uptake into the pods particularly with high P supply. Results on shoot growth rate over time showed that the accession CIAT 18744 was outstanding with P-0 treatment in exhibiting 100% of the growth rate of P-1000 between 60 and 90 days of growth (Figure 45).

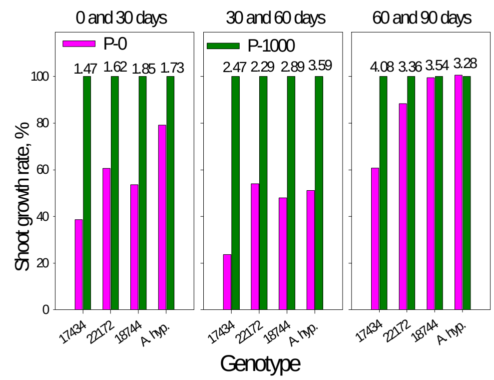


Figure 45. Genotypic variation as influenced by phosphorus supply (P-0 and P-1000) in shoot growth rate of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* grown for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Absolute values of plant growth rate (grams of dry weight per plant per month) with P-1000 are shown at the top of the bars for each genotype.

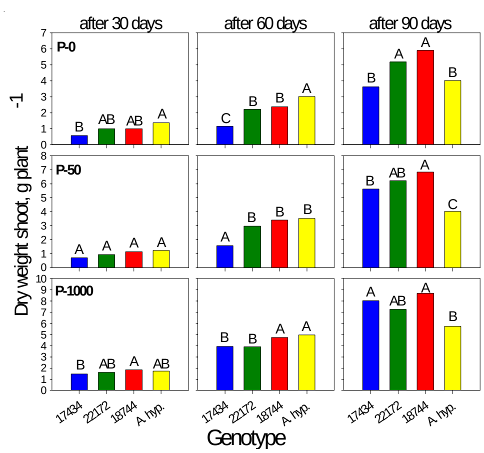


Figure 44. Influence of phosphorus supply (P-0, P-50 and P-1000) on genotypic variation in shoot biomass (forage) production of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* grown for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

Differences in P use efficiency (g of shoot biomass produced per mg of P uptake in the plant) were not consistent among the 3 accessions of *A. Pintoi* indicating that internal P utilization efficiency is not that different among the accessions during establishment (Figure 46). But shoot P uptake was significantly greater with CIAT 18744 and 22172 than the commercial cultivar 17434 (Figure 47). This greater acquisition of P by these two accessions contributed to their superior performance under P-0 treatment. The shoot P uptake of peanut variety was markedly lower due to greater pod development (Table 28). The superior acquisition of P by CIAT 18744 and 22172 was not related to their root system development as revealed by the relationship of root length to shoot biomass ratio (Figure 48). These results on root length to shoot biomass ratio indicate that the greater

Table 28. Production of pods and their contribution to P uptake.

Genotype	Dry weight of pods (g plant^{-1})			Pod P uptake (mg plant^{-1})		
	P-0	P-50	P-1000	P-0	P-50	P-1000
60 DAP						
CIAT 17434	-	-	-	-	-	-
CIAT 18744	-	-	-	-	-	-
CIAT 22172	-	-	-	-	-	-
A.h AK-12/24	0.57	2.1	1.36	1.34	6.67	5.38
90 DAP						
CIAT 17434	0.05	-	-	0.1	-	-
CIAT 18744	-	-	0.05	-	-	0.1
CIAT 22172	-	-	-		-	-
A.h AK-12/24	10.37	11.03	11.31	22.57	33.67	47.48

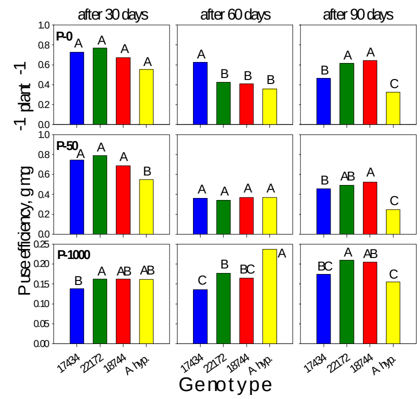


Figure 46. Influence of phosphorus supply (P-0, P-50 and P-1000) on genotypic variation in P use efficiency of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* grown for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

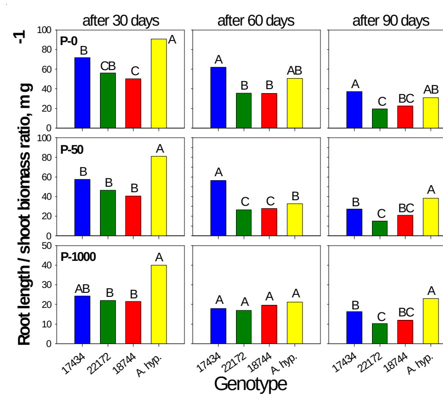


Figure 48. Influence of phosphorus supply (P-0, P-50 and P-1000) on genotypic variation in root length/shoot biomass ratio of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* grown for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

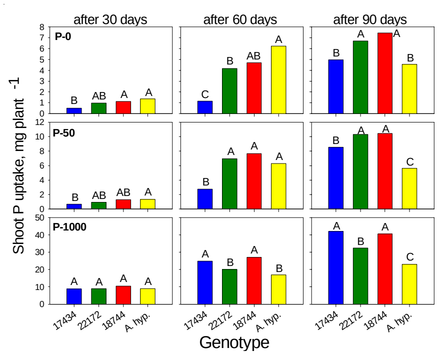


Figure 47 . Influence of phosphorus supply (P-0, P-50 and P-1000) on genotypic variation in shoot P uptake of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* grown for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

ability to acquire P from P-0 treatment by these 2 accessions could be related to biochemical changes induced in the rhizosphere. For example exudation of organic acids or enzymes that can mobilize less available P forms in the rhizosphere.

Measurements of P influx at different times after planting showed that there was significantly greater P influx in CIAT 18744 and 22172 than CIAT 17434 between 30 and 60 days of growth with P-0 and P-50 treatments (Figure 49). Measurements of P concentration in soil solution showed that CIAT 18744 was particularly outstanding in increasing P level in soil solution with P-0 treatment. Further research work is in progress to evaluate the role of root exudation in this increase in P concentration in soil solution.

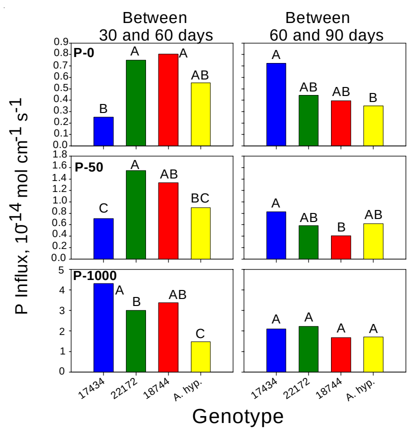


Figure 49. Influence of phosphorus supply (P-0, P-50 and P-1000) on genotypic variation in P influx of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* grown for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age.

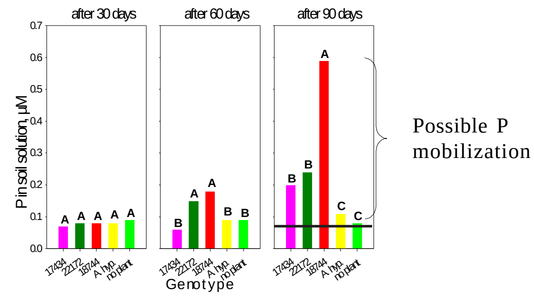


Figure 50. Influence of 3 accessions of *Arachis pintoi* and 1 accession of *Arachis hypogea* on P concentration in soil solution when grown with no phosphorus supply (P-0) for 30, 60 and 90 days in a clay loam oxisol in a growth chamber. Means are different (P<0.05) if letters above bars are different within P supply level at a given age. DAP = days after planting.

This study conducted under controlled environmental conditions in a growth chamber indicated that the rapid establishment and greater P efficiency of CIAT 18744 and 22172 are associated with their greater ability to acquire P from P-deficient soil but not due to their ability to utilize acquired P. (Figure 50).

3.1.3.2 Field evaluation of promising accessions of *Arachis pintoi* in the Llanos of Colombia

Contributors: I. M. Rao, M. Peters, C. Plazas and J. Ricaurte

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 two sites (Piedmont and Altillanura) in the Llanos of Colombia. The site in Piedmont is close to La Libertad (CORPOICA Experimental Station) and the soils in this region are relatively more fertile than in the Altillanura. The site in Altillanura is at Matazul farm where the soils are relatively infertile (sandy loam). 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 May, 2001. The trial was planted in Piedmont as monoculture. We expect multiple use for this legume in the Piedmont area (e.g., cover legume in plantations). 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 28 months after establishment (September 2003) 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 and Discussion

A visual assessment of vigor and soil cover was carried out at 27 months after establishment showed that under both low and high initial fertilization, CIAT 18751 showed greater vigor and soil cover.

At 28 months after establishment of the trial, the response in terms of shoot biomass production with fertilizer application was greater with the commercial check, CIAT 17434 and CIAT 22160 (Figure 51). Overall the performance of CIAT 18744 and CIAT 18751 and CIAT 22159 was better than the other accessions. A number of plant attributes including nutrient uptake are being monitored to evaluate persistence. This field study indicated that the *Arachis pintoi* accessions CIAT 18744, 18751 and 22159 are superior to the commercial cultivar (CIAT 17434) in terms of persistence into third year after establishment with low amounts of initial fertilizer application.

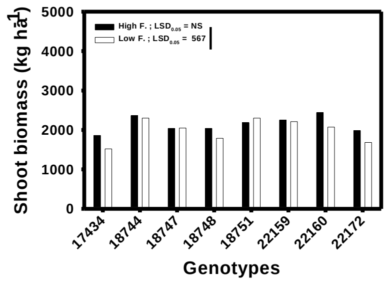


Figure 51. Genotypic variation in forage yield of 8 accessions of *Arachis pintoi* at 28 months after establishment (September 24, 2003) in forage yield (kg/ha) as influenced by initial level of fertilizer application to a clay loam oxisol at La Libertad, Piedmont.

3.2 Identication of genotypes of grasses and legumes with dry season tolerance

Highlights

- Superior performance of one germplasm accession (CIAT 26110) and one hybrid (FM9503-S046-024) of *Brachiaria* which maintained greater proportion of green leaves during dry season in the Llanos of Colombia, is associated with greater acquisition of nutrients under water deficit conditions.

3.2.1 Field evaluation of *Brachiaria* hybrids for drought tolerance in a subhumid environment of Costa Rica

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

Rationale

Commercial lines of the genus *Brachiaria* are widely used in the tropics as forage plants. However given the diversity of ecosystems and systems of production a broad field still exists for *Brachiaria* lines of high forage quality and better adaptation to drought, waterlogged sites and poor soils. More recently new hybrids of *Brachiaria* are available from a breeding program carried out at CIAT headquarters, thus it is worth to investigate the climate and soil adaptation as well as the potential yield of this germplasm as compared with traditional lines of *Brachiaria*.

Material and Methods

Six *Brachiaria* hybrids were direct planted using acid scarified seeds in a randomized block design with four replicates at Atenas, Costa Rica. Plot size was 2 m wide x 3 m long (6 m^2) and the planting distances were respectively 0.50 m between plants and rows; plants were thinned to two per site two weeks after seedling emergence and plant height and plant cover measured 16 weeks after planting. Dry matter yield was measured by cutting 9 central plants at 10 cm height after 5 and 8 weeks re-growth respectively during the wet and dry periods; a total of 6 evaluation cuts during the wet period and 3 cuts during the dry period have been carried out up to date. The experiment will finalize at the end of the next dry period for a total of two years of evaluation. The site is located in a subhumid environment with a total annual rainfall of 1600 mm, and 5 to 6 months dry from December to May. The soils are Inceptisol of medium fertility with pH 5.0 and low P and low aluminum content.

Results and Discussion

All *Brachiaria* hybrids established well and no re-planting of the plots was necessary. The accession *Brachiaria* hybrid CIAT 36061 (cv. Mulato) presented high seedling vigor and two weeks after planting all experimental plots of this line had more developed plants than the other hybrids. It has been also observed that following evaluation cuts this hybrid produces rapid re-growths, comparable only to those observed in *Brachiaria* hybrid CIAT 46024.

Plant growth and DM yields: Four months after establishment cv. Mulato had significantly taller plants ($p<0.05$) than other *Brachiaria* hybrids under evaluation (Table 29). However at this date, plant cover was not hundred percent for any hybrid and it was very similar between hybrids with the exception of CIAT 4015 that had significantly less soil cover. None of these hybrids is truly stoloniferous, thus it cannot be expected that under a cutting regime as it was the case in this experiment, a complete soil cover will be observed.

Total DM yields have been very similar between cv. Mulato and the *Brachiaria* hybrids BR99NO/4015, C BR99NO/4132 and FM9503-SO46-O24 (Table 30). This tendency has been observed for both the wet and dry season yields ($p<0.1$). However, the hybrid CIAT FM9503-SO46-O24 had a tendency to produce more yield during the dry season, which is a desirable characteristic of this promising line. Less DM yield have been recorded for the hybrids BR98NO/1251 and CIAT 36062, which seem to be poorly adapted to the Atenas conditions.

Table 29. Plant height and plant cover of *Brachiaria* hybrids 4 months after establishment at Atenas, Costa Rica

Hybrid	Plant height (cm)	Plant cover (%)
CIAT 36061 (cv. Mulato)	73.5 a*	85.3 a*
BR99NO/4132	35.4 b	80.9 ab
FM9503-SO46-O24	44.9 b	74.7 ab
CIAT36062	36.9 b	73.3 ab
BR98NO/1251	40.3 b	68.0 ab
BR99NO/4015	42.7 b	57.3 b

Although total DM yields were not statistically different between the commercial *Brachiaria* hybrid cv. Mulato ($p<0.1$) and the highly promising new hybrid CIAT 46024 (Table30), there was a clear tendency of the latter to produce more yield at each evaluation cut along

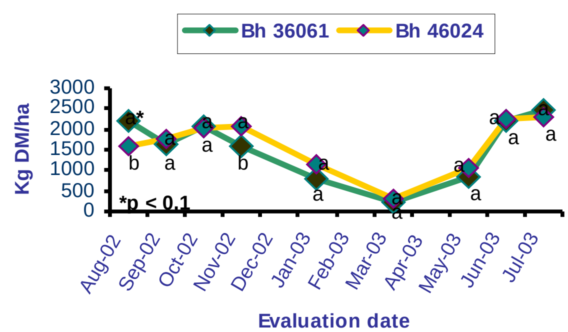


Figure 52. Dry matter (DM) yields under cutting of *Brachiaria* hybrid CIAT 46024 and cv. Mulato (CIAT 36061) from August 2002 through July 2003 in Atenas, Costa Rica.

Table 30. Dry matter yields during the wet and dry periods of *Brachiaria* hybrids established in Atenas, Costa Rica (mean of 6 cuts carried out every 5 weeks during the wet period and mean of 3 cuts carried out every 8 weeks during the dry season)

Hybrid	Mean dry matter yields (kg/ha)		
	Wet	Dry	Total
CIAT 36061 (cv. Mulato)	2030.9 a*	634.2 ab	1570.5 a
FM 9503-S046-024	2000.2 a	832.6 a	1611.9 a
BR99NO/4132	1890.1 ab	588.2 ab	1456.8 ab
BR99NO/4015	1530.8 abc	612.6 ab	1230.9 ab
BR98NO/1251	1360.3 bc	438.8 b	1054.7 b
CIAT 36062	1290.8 c	486.1 ab	1027.8 b

* $p<0.1$ (Tukey's Studentized Range Test)

the reported evaluation period, as shown in Figure 52. At the first evaluation cut in August 2002, cv. Mulato produced significantly more yield than CIAT 46024, but during subsequent cuts the latter consistently produced more DM yields even during the dry period from December 2002 to May 2003. These yields, although statistically similar, may however have some importance under forage utilization, given that grazing animals would have more forage available in paddocks planted with the hybrid CIAT 46024 in sites with prolonged dry season like Atenas.

3.2.2 Genotypic variation in dry season tolerance in *Brachiaria* 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, 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 fourth 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) were measured at the end of the dry season (44 months after establishment; March 3, 2003). Maintenance fertlizer (half the levels of initial application) was applied at the beginning of the wet season of 2001 (July, 2001).

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 53). At 44 months after establishment (4 months after dry season – March 3, 2003), live forage yield with low fertilizer application ranged from 0 to 329 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 4 parents, CIAT 6294 was outstanding in live forage and dead biomass production with low fertilizer application. A spittlebug resistant genetic recombinant, FM9503-S046-024 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 leaf to stem ratio values of one of the genetic recombinants (BR97NO-0082) were markedly superior to other genotypes under low and high levels of initial fertilizer application (Table 31).

The superior performance of the accession CIAT 26110 with low fertilizer application was mainly attributed to its ability to produce green leaf biomass during dry season (Figure 53). Results on leaf and stem N content indicated significant differences among genetic recombinants, parents and accessions with both levels of fertilizer application (Table 32). But shoot N uptake with low fertilizer application was markedly greater for the hybrid, FM9503-5046-024 (Table 32; Figure 54). With high fertilizer application, the hybrid FM9503-5046-024 and CIAT 26110 were outstanding in shoot N uptake. Shoot uptake of P, K, Ca and Mg was also greater with the hybrid FM9503-5046-024 and CIAT 26110 (Tables 33 and 34; Figure 54). Among the parents, CIAT 6294 was superior in P, K, Ca and Mg acquisition from both low and high fertilizer application.

Correlation analysis between green leaf biomass produced in the dry season and other shoot attributes indicated that superior performance with low and high fertilizer application was associated with greater stem biomass indicating the importance of stem reserves for production of green leaf biomass (Table 35). No significant negative association was observed between green leaf biomass and level of nutrients in green leaves. But significant negative association was observed between green leaf biomass production and stem P

Table 31. 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 44 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

Genotype	Live shoot biomass		Leaf to stem ratio		Total forage yield	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
			(kg/ha)-			
Recombinants:						
BR97NO-0082	121	145	1.5	2.3	544	848
BR97NO-0383	130	227	1.5	0.7	969	1040
BR97NO-0405	116	181	1.1	0.5	1734	1759
FM9201-1873	136	238	1.1	1.1	460	1181
FM9301-1371	180	226	0.9	1.2	612	938
FM9503-5046-024	269	386	0.9	0.8	1323	1814
Parents:						
CIAT 606	100	108	1.4	0.7	870	758
CIAT 6294	326	288	0.6	0.6	2145	2093
BRUZ/44-02	0	0	.	.	0	0
CIAT 26646	88	198	0.8	1.0	1115	2397
Accessions:						
CIAT 26110	203	454	1.4	0.4	2536	3474
CIAT 26318	185	314	1.6	0.3	2498	2865
Mean	160	231			1309	1615
LSD *(P=0.05)*	156	171			1146	808

Table 32. 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 44 months after establishment (at the end of the dry season - March 2003). LSD values are at the 0.05 probability level.

Genotype	Leaf N content		Stem N content		Shoot N uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	---------- (%) ---------		---------- (%) -----------		---------- (kg/ha) --------	
Recombinants:						
BR97NO-0082	2.02	2.21	0.84	0.91	1.75	2.64
BR97NO-0383	2.32	1.94	0.87	0.59	2.15	2.49
BR97NO-0405	1.98	2	1.03	0.63	1.79	1.89
FM9201-1873	1.78	2.02	0.97	0.55	1.91	3.06
FM9301-1371	2.42	2.16	1.15	0.98	3.07	3.63
FM9503-5046-024	2.26	2.02	0.85	0.5	3.63	4.77
Parents:						
CIAT 606	2.1	2.16	0.72	0.65	1.51	1.46
CIAT 6294	2.1	1.97	0.66	0.54	3.94	3.03
BRUZ/44-02	-	-	-	-	-	-
CIAT 26646	1.53	1.64	0.69	0.44	0.78	2.05
Accessions:						
CIAT 26110	2.32	2.14	0.68	0.51	3.18	4.36
CIAT 26318	2.18	2.27	0.45	0.4	2.80	2.65
Mean	2.08	2.05	0.78	0.61	2.41	2.98
LSD *(P=0.05)*	0.47	0.33	0.44	0.26	1.99	1.97

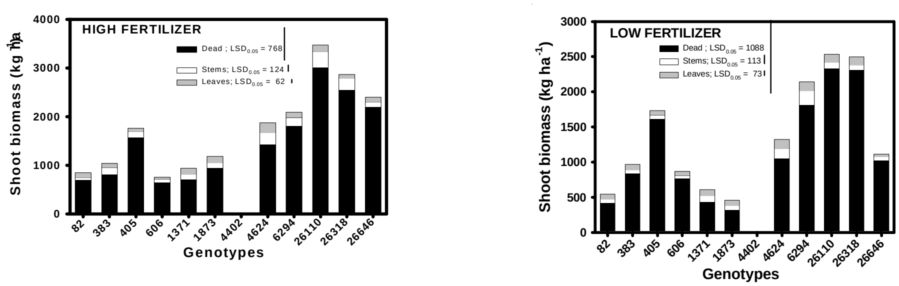


Figure 53. Genotypic variation as influenced by fertilizer application in dry matter distribution among green leaves, stems and dead biomass of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

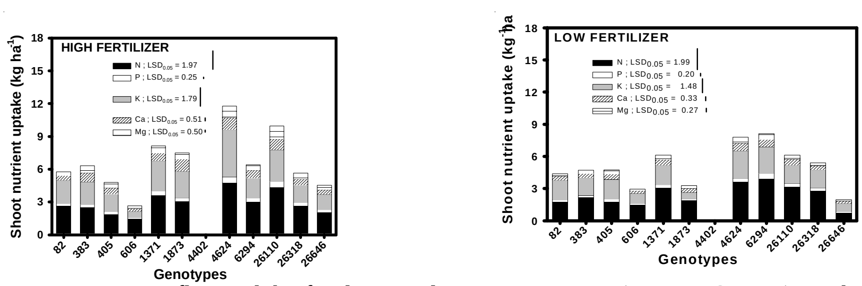


Figure 54. Genotypic variation as influenced by fertilizer application in nutrient (N, P, K, Ca, Mg) uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

and Ca content. The usefulness of this trait for evaluating dry season tolerance needs further research work. Stem ash (mineral) content was also negatively associated with green leaf biomass. Results from this field study indicated that the superior performance of one germplasm accession (CIAT 26110) and one genetic recombinant (FM9503-S046-024) which maintained greater proportion of green leaves during dry season in the Llanos of Colombia, was associated with greater acquisition of nutrients under water deficit conditions.

Table 33. Genotypic variation as influenced by fertilizer application in leaf P content, stem P content and shoot P uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season - March 2003). LSD values are at the 0.05 probability level.

Genotype	Leaf P content		Stem P content		Shoot P uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
	---------- (%) ---------		---------- (%) ----------		------- (kg/ha) -------	
Recombinants:						
BR97NO-0082	0.19	0.18	0.13	0.09	0.20	0.22
BR97NO-0383	0.18	0.17	0.09	0.08	0.19	0.26
BR97NO-0405	0.30	0.24	0.10	0.10	0.24	0.26
FM9201-1873	0.14	0.18	0.08	0.07	0.15	0.33
FM9301-1371	0.21	0.20	0.11	0.11	0.28	0.36
FM9503-5046-024	0.19	0.20	0.08	0.06	0.34	0.50
Parents:						
CIAT 606	0.15	0.17	0.08	0.05	0.12	0.10
CIAT 6294	0.24	0.20	0.09	0.05	0.47	0.31
BRUZ/44-02	-	-	-	-	-	-
CIAT 26646	0.18	0.17	0.09	0.06	0.12	0.23
Accessions:						
CIAT 26110	0.23	0.23	0.07	0.06	0.32	0.53
CIAT 26318	0.20	0.20	0.06	0.06	0.27	0.29
Mean	0.20	0.19	0.09	0.07	0.25	0.31
LSD *(P=0.05)*	0.07	0.05	0.03	0.04	0.20	0.24

Table 34. Genotypic variation as influenced by fertilizer application in shoot K uptake, shoot Ca uptake and shoot Mg uptake of genetic recombinants, parents and other germplasm accessions of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 44 months after establishment (at the end of the dry season - March 2003). LSD values are at the 0.05 probability level.

Genotype	Shoot K uptake		Shoot Ca uptake		Shoot Mg uptake	
	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer	Low Fertilizer	High Fertilizer
			(kg/ha)			
Recombinants:						
BR97NO-0082	1.82	2.14	0.35	0.41	0.25	0.34
BR97NO-0383	1.67	2.10	0.39	0.82	0.30	0.63
BR97NO-0405	1.81	1.59	0.52	0.54	0.39	0.50
FM9201-1873	0.59	2.41	0.36	1.11	0.24	0.57
FM9301-1371	1.85	2.79	0.49	0.69	0.43	0.64
FM9503-5046-024	2.56	4.34	0.70	1.08	0.55	1.08
Parents:						
CIAT 606	0.93	0.58	0.21	0.24	0.16	0.24
CIAT 6294	2.50	1.85	0.69	0.68	0.54	0.50
BRUZ/44-02	-	-	-	-	-	-
CIAT 26646	0.74	1.42	0.20	0.41	0.14	0.40
Accessions:						
CIAT 26110	1.7	2.88	0.52	0.98	0.42	1.18
CIAT 26318	1.71	1.60	0.35	0.53	0.28	0.53
Mean	1.63	2.16	0.44	0.67	0.34	0.60
LSD *(P=0.05)*	1.48	1.79	0.33	0.51	0.27	0.50

Table 35. Correlation coefficients (r) between green leaf biomass (t/ha) and other shoot traits of *Brachiaria* genotypes grown with low or high fertilizer application in a sandy loam oxisol in Matazul, Colombia.

Shoot traits	Low fertilizer	High fertilizer
Live forage yield (t/ha)	0.85***	0.77***
Total forage yield (live + dead) (t/ha)	0.57***	0.66***
Dead biomass (t/ha)	0.48***	0.57***
Stem biomass (t/ha)	0.93***	0.94***
Leaf TNC content (mg g^{-1})	0.13	-0.08
Leaf ash content (%)	-0.04	0.04
Stem N content (%)	-0.32	0.08
Stem P content (%)	-0.41*	0.04
Stem K content (%)	-0.27	0.21
Stem Mg content (%)	-0.25	0.11
Stem TNC content (mg g^{-1})	-0.13	0.23
Stem ash content (%)	-0.24	0.14

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

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

Contributors: I. M. Rao, J. Miles, C. Plazas, J. Ricaurte and R. Gracía

Rationale

Previous research on evaluation for dry season tolerance in *Brachiaria* grasses indicated that the superior performance of the *Brachiaria* hybrid, FM9503-S046-024 which maintained greater proportion of green leaves during moderate dry season in the Llanos of Colombia, was associated with lower levels of K and N content in green leaves. The main objective of this field study was to evaluate dry season tolerance of the more recent hybrids of *Brachiaria* in comparison with their parents when grown 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 and nutrient uptake were measured at the end of dry season (march 2003; 4 months of drought stress), i.e., at 22 months after establishment of the trial. The trial was managed with strong and frequent mob grazing at 2 months interval.

Results and Discussion

At 22 months after establishment (4 months after dry season), live forage yield with low fertilizer application ranged from 65 to 235 kg/ha and the highest value of forage yield was observed with the hybrid 4624 (Figure 55). Differences in dry matter distribution among the hybrids and parents indicated that the parent CIAT 6294 was superior to other genotypes in terms of shoot biomass

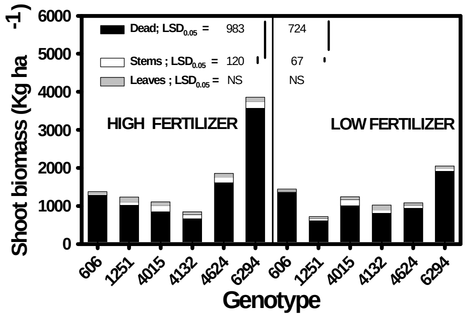


Figure 55. Genotypic variation as influenced by fertilizer application in dry matter distribution among green leaves, stems and dead biomass of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 22 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

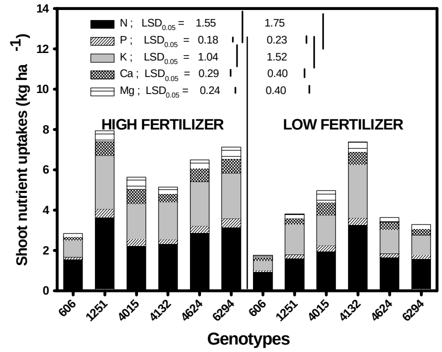


Figure 56. Genotypic variation as influenced by fertilizer application in nutrient (N, P, K, Ca, Mg) uptake of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 22 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level. NS = not significant.

production with both low and high initial fertilizer application. This parent produced greater dead biomass under both low and high initial fertilizer application. The dry matter distribution pattern of the some hybrids such as 4015 was in contrast with that of the parent CIAT 6294. These hybrids produced lower amounts of dead biomass and a greater proportion of aboveground biomass was in green leaves. Another hybrid, 4132 had markedly lower stem biomass compared with green leaf biomass.

The hybrid 4132 was also outstanding in its ability to acquire nutrients, particularly from low application of fertilizer (Figure 56). Results on nutrient uptake also showed that two other hybrids 1251 and 4624 were also superior in their ability to acquire nutrients. The results obtained on green leaf and stem N and P contents also indicated the superior nutritional quality of the hybrid 1251 under both high and low initial fertilizer application (Figure 57). The dry season performance of the 4 hybrids will be monitored for the next 2 years in comparison with the two parents in terms of forage yield and nutrient acquisition.

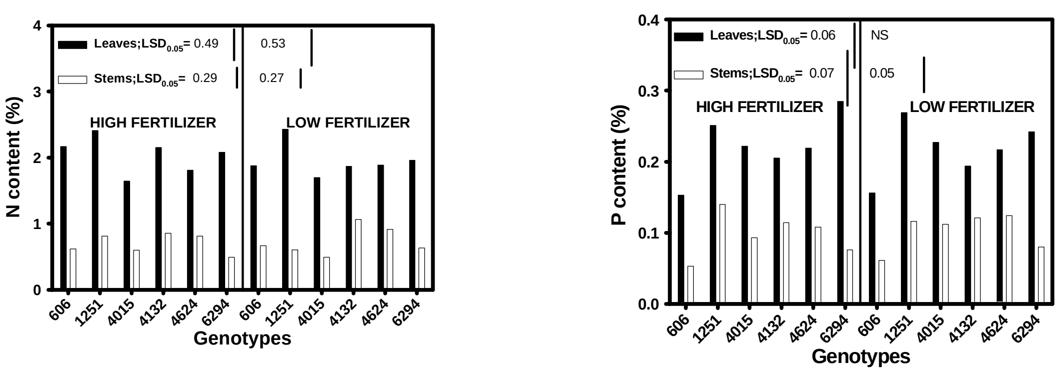


Figure 57. Genotypic variation as influenced by fertilizer application in nitrogen (N) and phosphorus (P) content of leaves and stems of two parents (606, 6294) and 4 genetic recombinants (1251, 4015, 4132, 4624) of *Brachiaria* grown in a sandy loam oxisol at Matazul, Colombia. Plant attributes were measured at 22 months after establishment (at the end of the dry season – March 2003). LSD values are at the 0.05 probability level.

3.3 Shrub legumes with adaptation to acid soils and drought

Highlights

- *Accessions of* Cratylia argentea *with superior productivity relative to* C. argentea *cv Veraniega were identified*
- Accessions of *Flemingia macrophylla* with digestibility and productivity superior to Control CIAT 17403 were identified
- RAPD molecular markers for *Cratylia argentea* and *Flemingia macrophylla* were identified

3.3.1 Genetic diversity in the multipurpose shrub legumes *Flemingia macrophylla* and *Cratylia argentea*

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Little variation in nutritive value among *Cratylia* accessions was observed. CIAT 18674, 22375, 22406, 22408 and 22409 had higher dry matter yields in the dry and wet seasons than CIAT cv Veranera (CIAT 18516/18668). These accessions, collected in the states of Goias and Mato Grosso in Brazil, were selected for seed multiplication given that they had DM production higher than 3 t/ha per cut, good seed production capacity and equal or superior digestibility and crude protein content than Veranera.

Three semierect accessions CIAT 18437 from Indonesia, and 21083 and 21090 from Thailand were superior in comparison to CIAT 17403 (from Thailand). They had digestibility values >48% and dry matter yields >2 t/ha. More detailed analysis of two subsets showed that accessions with higher feed quality in terms of digestibility have lower fiber and condensed tannin contents than low-quality accessions.

For *Cratylia argentea* analysis of origin, agronomic morphological and molecular marker information did not identify correlations between the clusters obtained in the different approaches. In the case of *Flemingia macrophylla*, clustering obtained by molecular marker information correlated well with morphological information and grouped accessions according to their different growth types.

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* and *C. argentea* with three 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; (b) germplasm origin information; and (c) molecular markers (RAPDs).
3) To assist future germplasm collections on methodology, geographical focus and genetic erosion hazards.

Material and Methods

Agronomic characterization and evaluation*:* Space-planted, single-row plots in RCB design with three replications were established in Quilichao in March 1999 (*Cratylia argentea*, 39 accessions) and March 2000 (*Flemingia macrophylla*, 73 accessions). Additionally two replications were sown for seed production and morphological observations.

The following parameters were measured in the trials: vigor, height and diameter, regrowth, incidence of diseases, pests and mineral deficiencies, and dry matter yield during wet and dry seasons. For the analysis of nutritive value, crude protein content and *in vitro* dry matter digestibility (IVDMD) of the entire collections were analyzed. For the morphological evaluation, qualitative and quantitative parameters were measured, such as days to first flower, days to first seed, flower color, flowers per inflorescence, flowering intensity, pod pubescence, seeds per pod, seed color, leaf area, peduncle length, etc.

For *F. macrophylla*, a more detailed analysis of nutritive value was conducted of a representative subset (25 accessions), which included high, intermediate and low nutritive value accessions. The groups were selected based on crude protein content and IVDMD. The chemical analysis comprised fiber (NDF, ADF, N-ADF), extractable and bound condensed tannin (ECT, BCT) content and astringency (protein binding capacity). Monomer composition of the extractable condensed tannin fraction (procyanidin:prodelphinidin:proelargonidin ratio = C:D:P) was determined with high-performance liquid chromatography (HPLC). In the latter case, results were extremely variable both between laboratory replicates and among field repetitions.

In order to give at least an idea of the monomer composition of extractable condensed tannins in *F. macrophylla* accessions, results from only five accessions, which were consistent between duplicates and among repetitions, are reported. Additionally, another subset of 10 accessions (9 high-quality accessions (18437, 18438, 21083, 21090, 21092, 21241, 21580, 22082, 22327) and CIAT 17403) was sampled 4, 6 and 8 weeks after cutting, to investigate the effect of age on digestibility as well as on protein, fiber and condensed tannin content and astringency.

Based on data referring to the morphological, agronomic and feed quality variation of all accessions a core collection will be created, using multivariate statistic tools (Principal Component Analysis and Cluster Analysis).

Analysis of available origin information: Based on ecogeographical information on origin of accessions, a core collection was created, hypothesizing that geographic distance and environmental differences are related to genetic diversity. The analysis was conducted with FloraMap™, a GIS tool developed by CIAT, which allows the production of climate probability models using Principal Component Analysis (PCA) and Cluster Analysis.

Genetic analysis by molecular markers (RAPDs): Efforts made in genetic analysis showed that common manual DNA extraction methods did not work well with *F. macrophylla* and *C. argentea*. A modified protocol, which was used to extract DNA showed promising initial results. However, frequent degradation, contamination and partial digestion of DNA occurred, due to secondary plant compounds, probably polyphenols. In preliminary trials with a commercial extraction kit instead, the DNA purity was higher but partial digestion continued to be a severe problem. Various studies with amplified fragment length polymorphism markers (AFLPs), the method of choice, did not succeed and finally studies using this methodology could not be completed. Instead, random amplified polymorphic DNA (RAPD) markers, which do not require enzymatic digestion, were successfully employed.

A total of 47 RAPD 10-mer primers (Operon Technologies, Alameda, CA, USA) were screened as single primers for the amplification of RAPD sequences. Primers with highest levels of polymorphisms were repeated to test for reproducibility and those that produced polymorphic, distinct and reproducible bands were chosen for RAPD analysis. Multiple Correspondence Analysis (MCA) was performed on a matrix created based on the presence (1) or absence (0) of amplified bands. Subsequently, cluster analysis was performed on the coordinates obtained by MCA. Dendrograms were generated using UPGMA method. Nei's coefficient was used as estimator of similarity between accessions in order to generate between- and within-group similarity tables. Diversity was estimated using Nei's H and G_{ST} estimators.

Data analysis and synthesis: Individual and combined data analyses of all generated information was carried out using multivariate statistics. We have applied principle component analysis in all data sets (agronomic, morphological, geographical and molecular). In addition, cluster analysis was performed and the resulting clusters were compared to identify similarities.

Results and Discussion

Agronomic characterization and evaluation*:* Results from evaluations per season carried out for *Cratylia argentea* and *Flemingia macrophylla* indicated considerable phenotypic and agronomic variation in the collections studied. Data for *C. argentea* and *F. macrophylla* have been presented already in previous reports. For *C. argentea*, IVDMD varied between 59 and 69% and crude protein content between 18 and 24%. Mean dry matter production was 2.2 (range 0.8 to 5.2) t/ha and 1.93 (range 0.6 to 3.3) t/ha in the wet and dry season, respectively. Dry season yields were relatively high and confirm the good adaptation of *C. argentea* to dry conditions. There was a pronounced effect of season on some agronomic and quality traits. DM production was higher in the rainy season than in the dry season whereas ADF was higher in the dry than in the wet season. A season x genotype interaction was detected for IVDMD. The cluster analysis dendrogram (Ward's Method) was truncated at the 6-group level. The detailed agronomic characteristics of each group are listed in Table 36. Group 4 was the agronomically most promising cluster. It contained three accessions with the highest DM production (3.2 t/ha in the rainy and 2.4 t/ha in the dry season), CP content, regrowth and plant diameter values. The highest dry matter yields (2.4 to 3.8 t/ha) were recorded in accessions 18674, 22375, 22406, 22408 and 22409 from groups 4 and 6. Productivity of these accessions was higher than yields of the cultivar released in Costa Rica (cv. Veraniega) and Colombia (cv. Veranera) - an accession mixture of CIAT 18516/18668 (yield 1.9 and 3 t/ha). In addition to the higher yield, these accessions also had equal or superior digestibility values (65 to 69%) and crude protein content (20 to 24%) in comparison to CIAT 18516/18668 (IVDMD 64 to 67%, CP 21 to 24%). Based on high forage yield and good seed production potential we selected CIAT 18674, 22375, 22406, 22408 and 22409 for seed multiplication and regional testing (Table 37).

Table 36. Identification of *Cratylia argentea* accessions of agronomic interest. * *rainy/dry season value*

Group 1 (average/low yields, low regrowth, high dry season digestibility, high CP, high ADF):* CIAT 22382, 22390, 22392, 22393, 22394, 22396, 22399, 22411
Group 2 (high/low yields, low regrowth, high dry season digestibility, average CP, high ADF): CIAT 18675, 22380, 22383, 22384, 22386, 22387, 22391
Group 3 (average/low yields, low regrowth, average digestibility, lower CP than group 1, high ADF): CIAT 18672, 22376, 22378, 22381
Group 4 (very high/average yields, good regrowth, high digestibility, high CP, low ADF, low NDF): CIAT 22374, 22375, 22406
Group 5 (high/average yields, good regrowth, average digestibility, average CP, high ADF): CIAT 18676, 18957, 22373, 22400, 22410, 22412
Group 6 (very high/average yields, good regrowth, high digestibility, high CP, low AFD, higher NDF than group 4): CIAT 18516, 18667, 18668, 18671, 18674, 22379, 22404, 22407, 22408, 22409

Table 37. Selected promising *Cratylia argentea* accessions and the two control accessions CIAT 18516/18668. Data of two evaluation cuts with 8 weeks of regrowth per season. IVDMD = *in vitro* dry matter digestibility, CP = crude protein.

Accession number	DM production (t/ha) [a]		IVDMD[b] (%)		CP[c] (%)		Seed production[d] (g/plant)
	Rainy	Dry	Rainy	Dry	Rainy	Dry	
18674	3.82	2.44	65	65	20	23	153
22375	3.12	2.41	65	66	21	23	255
22406	3.54	2.59	64	66	21	22	152
22408	3.25	2.45	69	67	21	22	153
22409	3.11	2.62	66	68	22	24	97
18516 (Control)	3.06	2.04	64	67	21	24	18
18668 (Control)	2.39	1.91	64	65	21	23	110

[a] Plant density 10 000 plants/ha
[b] Two-stage technique (Tilley & Terry 1963)
[c] Kjeldahl nitrogen x 6.25 (AOAC 2003)
[d] 15 months after sowing

In *F. macrophylla*, accessions evaluated differed in IVDMD, DM production, ECT, tannin extractability (ECT/total CT) and astringency (protein binding capacity) whereas CP and BCT showed only minor variability. IVDMD varied from 28 to 58% and crude protein content from 13 to 25%. Mean dry matter production was 2.08 t/ha in the wet and 1.18 t/ha in the dry season. The chemical composition of 25 *F. macrophylla* accessions with contrasting digestibility varied greatly among accessions and in response to harvest season (Tables 38 and 39).

Total condensed tannin content ranged from 1.5 to 16.7% in the rainy season and from 1.8 to 22.4% in the dry season. Astringency ranged from 1.7 to 6.8 (PBE) in the rainy season and from 2.4 to 7.9 in the dry season. The acetone-extractable CT among accessions ranged from 0 to 19.4%, whereas the content of acetone-bound CT ranged from 1.3 to 3.3%. The ECT represented 0% of total condensed tannins in CIAT 21090 but 95% in CIAT 20616. Positive correlations were found between ECT and astringency ($r_{rainy} = 0.712$, $r_{dry} = 0.721$, $P < 0.01$). IVDMD was negatively correlated with ECT ($r_{rainy} = -0.694$, $r_{dry} = -0.576$, $P < 0.01$) and astringency ($r_{rainy} = -0.632$, $r_{dry} = 0.548$, $P < 0.01$).

The monomer composition of the extractable CT fraction in 5 accessions *F. macrophylla* was

Table 38. *In vitro* digestibility, fiber and crude protein content of a representative subset of *F. macrophylla*. Data of one evaluation cut in the wet season and one in the dry season. n = no. of accessions evaluated, IVDMD = *in vitro* dry matter digestibility, CP = crude protein, NDF and ADF = neutral and acid detergent fiber, N-ADF = nitrogen bound to acid detergent fiber.

Forage quality[a]	IVDMD[b] (%)		CP[c] (%)		NDF[d] (%)		ADF[d] (%)		N-ADF[d] (%)	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
High (n=6)	52.8	48.0	22.4	21.6	33.0	33.8	20.3	23.7	11.1	11.2
Medium (n=12)	46.4	43.7	21.3	20.5	34.6	36.1	23.7	24.9	12.0	11.2
Low (n=7)	42.4	40.1	20.5	20.2	34.6	35.9	23.1	24.4	11.5	12.0
Minimum	39.9	36.8	17.0	17.6	29.5	31.2	17.0	21.5	9.1	6.6
Maximum	56.2	51.3	24.4	23.6	39.3	39.8	27.6	29.2	15.4	16.9
Mean	46.8	43.7	20.9	20.7	34.2	35.6	22.7	24.5	11.7	11.5

[a] high: average IVDMD ≥48%. intermediate: ≥43-47%, low: <43%
[b] Two-stage technique (Tilley & Terry 1963)
[c] Kjeldahl nitrogen x 6.25 (AOAC 2003)
[d] van Soest et *al.* 1991, Robbins et *al.* 1987

Table 39. Condensed tannin content and composition in 25 *F. macrophylla* accessions with contrasting digestibility. Data of one evaluation cut in the wet season and one in the dry season. ECT = acetone-extractable condensed tannins, BCT = acetone-bound condensed tannins, PBE = protein-binding entities, ND = not detectable. n.a. = not available.

CIAT No.		**ECT[a] (%)**		**BCT[a] (%)**		**Extractability (ECT/TotalCT %)**		**Astringency[b] (PBE)**	
		Wet	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**
18437	High Quality	4.2	7.6	2.33	2.65	64.54	74.05	4.21	4.57
18438	High Quality	0.2	1.6	1.76	2.02	12.00	44.81	3.33	3.78
20065	High Quality	7.7	9.7	1.30	2.24	85.47	81.26	4.84	n.a.
21083	High Quality	0.1	0.0	1.36	1.81	7.48	0.00	1.65	2.39
21087	High Quality	7.2	6.6	1.57	1.98	82.04	76.95	4.40	6.44
21090	High Quality	ND	ND	1.57	1.99	0.00	0.00	2.03	2.53
17403	Intermediate Quality	4.3	9.6	2.12	2.43	67.08	79.83	4.52	4.99
20622	Intermediate Quality	12.3	13.5	3.27	1.20	79.05	91.83	4.60	5.85
20744	Intermediate Quality	11.5	13.2	1.91	1.75	85.77	88.29	4.39	5.79
20975	Intermediate Quality	13.5	14.5	2.59	2.01	83.90	87.80	6.77	6.33
20976	Intermediate Quality	13.4	16.7	1.85	1.33	87.90	92.62	6.09	5.33
21092	Intermediate Quality	6.6	3.8	1.53	2.21	81.11	63.35	2.81	3.55
21249	Intermediate Quality	9.0	10.7	2.18	2.59	80.55	80.51	4.44	5.70
21529	Intermediate Quality	9.3	10.3	2.90	3.32	76.19	75.64	4.88	5.15
21982	Intermediate Quality	8.0	12.5	1.87	2.90	81.13	81.16	6.03	7.70
21992	Intermediate Quality	7.8	12.0	2.19	2.37	78.17	83.51	5.12	7.91
22082	Intermediate Quality	0.3	0.3	1.86	1.96	11.85	11.31	2.73	2.88
J 001	Intermediate Quality	7.1	15.8	2.55	1.73	73.63	90.15	4.11	5.58
17407	Low Quality	7.3	11.5	2.13	2.25	77.41	83.58	5.46	5.33
19457	Low Quality	8.5	11.6	2.76	2.65	75.51	81.46	5.80	6.74
20616	Low Quality	5.9	15.7	2.00	0.84	74.59	94.92	4.71	5.97
20621	Low Quality	14.2	17.1	2.54	0.96	84.78	94.67	5.42	5.88
21241	Low Quality	10.0	8.8	2.16	2.11	82.22	80.62	3.81	6.13
21580	Low Quality	9.4	5.7	1.79	2.07	84.06	73.22	5.49	5.07
21990	Low Quality	12.4	19.4	2.30	2.98	84.38	86.67	6.37	6.95
High		3.9	5.1	1.6	2.1	41.9	46.2	3.4	3.9
Intermediate		8.6	11.1	2.2	2.2	73.9	77.2	4.7	5.6
Low		9.7	12.8	2.2	2.0	80.4	85.0	5.3	6.0
Mean		7.9	10.3	2.1	2.1	68.0	71.9	4.6	5.4

[a] Butanol-HCl (Terrill et *al.* 1992, Barahona et *al.* 2003)
[b] Radial diffusion assay (Hagerman 1987, Lareo et *al.* 1990)

quite variable due to accession but not due to season of the year (Figure A). In four accessions (CIAT 20621, 20744, 20975 and 20976) prodelphinidin made up more than half of the proanthocyanidins (range from 49 to 79%). The second most important constituent was propelargonidin, which ranged from 16 to 38%. Procyanidin was only present in small proportions (0 to maximum 16%). It was interesting to observe that in CIAT 21092 propelargonidin represented 82% of total proanthocyanidins in the rainy season and 95% in the dry season. Procyanidin was absent and prodelphinidin was less than 20%.
The five accessions for which we have reliable data on monomer composition of ECT are not representative of the entire *Flemingia* collection in terms of forage quality. However, four of them had very high ECT concentrations (13-17%) whereas CIAT 21092 presented relatively low ECT levels (7 and 4% in the rainy and dry season, respectively). The latter had an exceptionally high propelargonidin proportion but totally lacked cyanidin, which could indicate a relationship between monomer composition and forage quality.

Analysis of a subset of 10 high-quality accessions (including control) showed that forage quality varied over time. Patterns were different in the

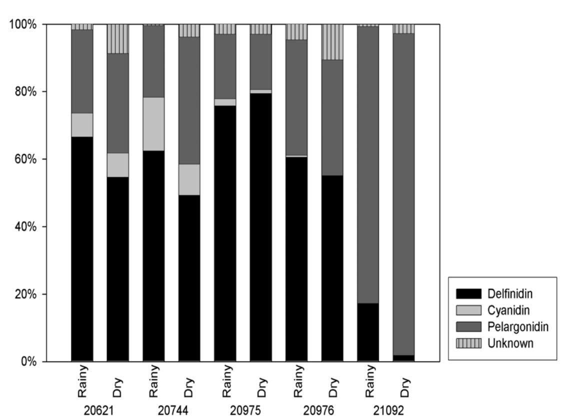

Figure 58. Monomer composition (procyanidin: prodelphinidin: propelargonidin ratios (C: D: P)) of the ECT fraction of five *F. macrophylla* accessions in rainy and dry season.

rainy and dry season for both the averaged values of the 10 accessions and for individual accessions (Figure 59). Correlations found in this analysis confirmed the negative correlations between IVDMD and ECT and IVDMD and astringency. Season had a large effect on IVDMD, DM production, plant height and diameter (higher in the rainy than in the dry season) and ADF, NDF, ECT and astringency (slightly higher in the dry season than in the rainy season). Extractability (percentage ECT of total CT) was relatively stable between harvest seasons (differences <10%). Only in six accessions (CIAT 17403, 18438, 20616, 20622, 21092 and J 001) differences up to 33% between rainy and dry season were found. No genotype x season interactions were detected for DM production and regrowth.

The accessions with the highest average *in vitro* dry matter digestibility were CIAT 18437, 18438, 21083, 21090, 21092 and 21241. The most productive accessions were CIAT 7184, 21090, 21241, 21248, 21249, 21519, 21529, 21580 and CPI 104890 with a total DM production >3.5 t/ha in the rainy and >2 t/ha in the dry season. Among the materials superior to CIAT 17403 (digestibility 36%, DM production 1.5 t/ha in the dry season) CIAT accessions 18437, 21083 and 21090 were identified for further testing as promising materials for dry season supplementation because they combined high digestibility with high productivity and low extractable condensed tannin content. These accessions had digestibility values > 48% and dry matter yields > 2 t/ha. Extractable condensed tannin content was 4.2 and 7.5% in the rainy and dry season for CIAT 18437 and nil in CIAT 21083 and 21090. However, their low seed production in the site where they were evaluated can limit their value.

The dendrogram (Ward's Method) was truncated at the 7-group level, explaining 72% of variation. The detailed agronomic characteristics of each group are listed in Table 40. Group 4 was the one that had the most promising accessions from an agronomic point of view. It contained eight accessions (7 semierect, 1 'tobacco') with the highest digestibility values of the collection (51% in

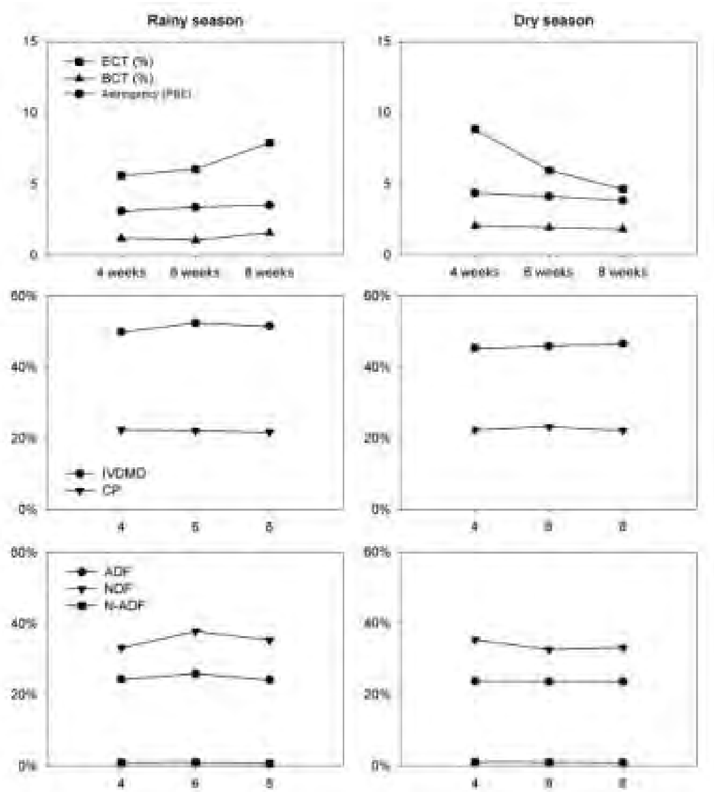

Figure 59. Variability in IVDMD, CP, fiber and tannin content of 10 *Flemingia macrophylla* accessions after 4, 6 and 8 weeks of regrowth in the rainy and dry season. ECT, BCT = acetone-extractable and bound condensed tannin, PBE = protein binding entities, IVDMD = *in vitro* dry matter digestibility, CP = crude protein, NDF and ADF = neutral and acid detergent fiber, N-ADF = nitrogen bound to ADF

the rainy and 47% in the dry season) and high DM production (2.6 t/ha in the rainy and 1.2 t/ha in the dry season). The three selected promising accessions CIAT 18437, 21083 and 21090 were contained in this cluster.

Genetic analysis by molecular markers (RAPDs): Out of 47 random primers tested, 9 were chosen that produced 171 RAPD bands ranging from 4 to 18 polymorphic bands per primer. Eight primers were selected for *Flemingia macrophylla* (D01, D04, D15, I07, J04, J06, J07, J12), and six for *Cratylia argentea* (D15, G12, I07, J06, J07, J12) (Table 41). Clustering of 47 *Cratylia argentea* and 1 *C. mollis* (outgroup) accessions resulted in 5 groups (Figure 60), plus two genetically very distinct materials: "yacapani" (the only prostrate *C. argentea* accession) and *C. mollis* (data not shown here). Group 1 included 28 accessions and group 2 twelve. Group 3 comprised accessions 884 and CIAT 18668 and 22389. Group 4 was conformed of CIAT 22386 and 22387. Group 5 contained only CIAT 18674, one of the two agronomically most promising accessions. No correlation was found between the clustering according to RAPD polymorphisms and agronomic, morphological or geographical characteristics. Analysis of genetic diversity within accessions revealed high variability. Nei and Li similarity between groups often was as high or higher than within groups (Table 42). This could indicate either seed contamination of accessions and/or outcrossing during multiplication in the field. Research on reproduction of *C. argentea* is urgently required to determine the rate and impact of outcrossing in this species.

Clustering of 111 *Flemingia macrophylla* and 2 *F. paniculata* (outgroup) accessions resulted in six groups (Figure 61), distinguishing well among the different morphotypes of this species, which have been described in the morphological evaluation (Photo 15, see also CIAT Annual Report 2002).

Group 1 included the two *F. paniculata* accessions. Group 2 was conformed by 55 of the 111 *F. macrophylla* accessions, which - with the exception of CIAT 20065 (prostrate) - belongederect growth type. Group 3 was composed of 23 semierect-1 and one "tobacco"-type accession and group 4 comprised 3 semierect-2 accessions. Group 5 included 8 prostrate, 14 semierect-1, 4 "tobacco" and 2 erect accessions and group 6 contained one semierect-2 and one semierect-1 accession.

No correlation was found between the clustering based on RAPD polymorphisms and agronomic or geographical characteristics. On the other hand, RAPD analysis proved to b useful for the identification/distinction of the different *F. macrophylla* morphotypes. It is suggested that the employment of the more powerful AFLP markers would detect higher polymorphisms within the morphotypes of this species.

Photo 15 . Four *F. macrophylla* morphotypes: 1=erect, 2=semi-erect 2, 3=prostrate, 4='tobacco'

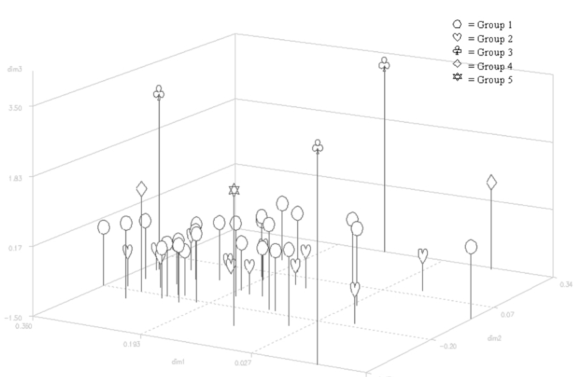

Figure 60. Tridimensional representation of five groups (without outgroup and prostrate accession "yacapani") resulting from clustering (UPGMA) of 47 *Cratylia argentea* and 1 *C. mollis* accessions according to molecular marker information (RAPDs).

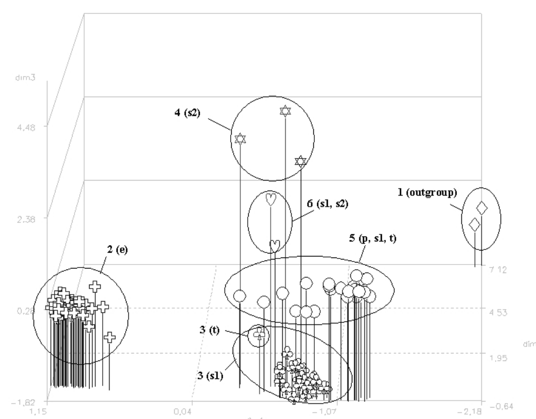

Figure 61. Tridimensional representation of the groups resulting from clustering (UPGMA) of 111 *Flemingia macrophylla* and 2 *F. paniculata* accessions according to molecular marker information (RAPDs). e = erect, s1 and s2 = semi-erect 1 and 2, p = prostrate, t = "tobacco" morphotype.

Table 40. Identification of *Flemingia macrophylla* accessions of agronomic interest. * *rainy/dry season value*

Group 1 (average/low digestibility, low yields, low regrowth, average CP, low vigor, low plant height):* CIAT 20973, 20977, 21080, 21086, 21994, 22053, 22087, 22090
Group 2 (average/low digestibility, average/low yields, high regrowth, average CP, low vigor, low plant height): CIAT 20979, 21079, 21990, 21993, 22285
Group 3 (high/low digestibility, low yields, average regrowth, high/average CP, low vigor, low plant height): CIAT 18048, 20972, 20976, 20978, 20980, 20982, 21982, 21991, 21992, 21995, 21996, 22327
Group 4 (high digestibility, high/average yields, good regrowth, high/average CP, high vigor, average plant height): CIAT 18437, 18438, 20975, 21083, 21087, 21090, 21092, 22082
Group 5 (average digestibility, high yields, average regrowth, high CP, high vigor, high plant height): CIAT 801, 7184, 20622, 20625, 20626, 20631, 20744, 21241, 21248, 21249, 21519, 21529, 21580, C104890, I15146, J001
Group 6 (like group 5, but lower yields, lower vigor and lower plant height): CIAT 19453, 19454, 19797, 19798, 19799, 20065
Group 7 (low digestibility, high/average yields, average regrowth, high CP, high vigor, average plant height): CIAT 17400, 17403, 17404, 17405, 17407, 17409, 17411, 17412, 17413, 18440, 19457, 19800, 19801, 19824, 20616, 20617, 20618, 20621, 20624

Table 41. Oligonucleotide primers employed in RAPD analysis, their sequence, number of bands obtained and percentage of polymorphic bands per species (% PBS).

Primer code	Sequence (5' to 3')	Number of bands		PBS (%)
		Polymorphic	Monomorphic	
Flemingia macrophylla				
D 01	ACCGCGAAGG	7	1	
D 04	TCTGGTGAGG	5	0	
D 15	CATCCGTGCT	18	1	
I 07	CAGCGACAAG	4	0	
J 04	CCGAACACGG	8	1	
J 06	TCGTTCCGCA	14	2	
J 07	CCTCTCGACA	13	0	
J 12	GTCCCGTGGT	6	2	
Total		***75***	***7***	***91.5***
Cratylia argentea				
D 15	CATCCGTGCT	10	4	
G 12	CAGCTCACGA	17	1	
I 07	CAGCGACAAG	17	4	
J 06	TCGTTCCGCA	13	1	
J 07	CCTCTCGACA	8	3	
J 12	GTCCCGTGGT	9	2	
Total		***74***	***15***	***83.1***

Table 42. Nei similarity within and between groups resulting from clustering (UPGMA) of 47 *Cratylia argentea* and 1 *C. mollis* accessions according to molecular marker information (RAPDs)

Group	N	1	2	3	4	5	6	7	Total
1	28	0.825	0.814	0.759	0.774	0.769	0.487	0.413	
2	12		0.839	0.721	0.764	0.720	0.515	0.404	
3	3			0.757	0.748	0.754	0.457	0.388	
4	2				0.717	0.757	0.479	0.400	
5	1					1.000	0.426	0.433	
6	1						1.000	0.444	
7	1							1.000	
Total	**48**								**0.776**

3.3.2 Evaluation of a core collection of *Desmodium velutinum*

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

Tropical shrub legumes of high forage quality are available for medium to high-fertility soils (e.g. *Leucaena leucocephala*), which is not the case for germplasm with adaptation to acid, low-fertility soils in drought-prone environments. 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 these mostly concentrate on one or two accessions. However, the available information indicates that this legume that produces forage of high quality has the potential to adapt to drought and (acid) low-fertility soils.

The proposed research attempts to explore the genetic diversity of *D. velutinum* in terms of morphology, yield and quality parameters through the evaluation of the world collection (138 accessions) held in CIAT, which come from a wide range of environments mainly in Asia and to a lesser extent in Africa. A core collection based on agronomic and morphological parameters and origin information, using GIS tools, will be identified for more detailed regional evaluation.

A total of 138 accessions of *Desmodium velutinum*, mostly originating from Asia, were planted at Quilichao. Plants were transplanted from jiffy pots into single-row plots, with 4 replications, in a randomized block design. Dry matter yield, drought tolerance and forage quality are the main parameters being measured. Currently the trial is in the establishment phase (Photo 16). Next year we will report results for a wet and for a dry period.

Photo 16. Plots of *Desmodium velutinum* established at Quilichao

3.4 Grasses with adaptation to poorly drained soils

Highlight

- Established a field trial to define adaptation of selected grasses to poorly drained conditions.

3.4.1 Field evaluation of *Brachiaria* and *Paspalum* genotypes in poorly and well drained sites in Costa Rica (Atenas)

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

Rationale

The adaptation and response of forage plants to different climates and soils, as well as pests and diseases is variable. It is well known that some genotypes growth well in poor acid soils, other need medium to high fertility soils, and other do not perform well in heavy poorly drained soils. The expression of genetic combination of characters is so wide and variable that it is impossible to anticipate or predict the adaptation and potential production of a forage plant. For this reason field tests are necessary to characterize promising forage germplasm, particularly those related with tolerance to drought and poorly drained soil conditions.

Material and Methods

Seedlings of the forage species *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 46024, CIAT 4015 and CIAT 36062, were transplanted for evaluation in a site with variable slope. The soil is a heavy clay with the following characteristics: pH 5.6 (medium), 0.4 meq/100 ml of Al content (medium), high content of Ca (26.9), high of Mg (10.4) and medium of K (0.44). Phosphorous content is very low (4 ppm), low in Zinc (2.5 ppm) and medium respectively in Mn (27.5), Cu (16.3) and Fe (39.7). Linear plots of 6 m long were used in a split plot design with 4 replicates. Main plot was formed by the forage species and subplots formed by different gradients of soil humidity, namely poorly drained, moderately drained and well drained area. Forage seedlings grown in jiffy pots of 45 days of age were established the 10th of July at a distance of 0.50 m between plants along a row perpendicular to the slope line for a total of 12 plants per plot.

By mid September a dike will be built at the lower part of the plot to create variable gradients of soil humidity. It is expected to have a waterlogged condition, a moderately drained area and a well-drained area. Mean plant height will be measured at the commencement of the waterlogged conditions, and plant mortality, vigor and growth will be measured every two weeks for a period of 16 weeks following the created soil moisture conditions. Forage DM yield will be determined for each species at the end of the observations at the different soil moisture conditions.

Results and Discussion

All *Brachiaria* lines and cv. Pojuca established well. Growth two months after planting shows vigorous plants along the slope line of the plots and none pests or diseases have been present. A measure of plant height immediately before the construction of the dike shows considerable variation in plant development: cv. Mulato (81.7 cm), *B. brizantha* (Mixe, 75.0 cm), cv. Pojuca (72.2 cm), *Brachiaria* hybrid CIAT 46024 (71.7 cm), *Brachiaria* hybrid CIAT 36062 (71.7 cm), *B. brizantha* CIAT 26124 (68.9 cm), CIAT 26318 (63.9 cm), CIAT 26990 (58.3 cm) and *Brachiaria* hybrid CIAT 4015 (48.3 cm).
This experiment will continue under evaluation during the present wet season and results will be report next year.

3.5 Legumes for multipurpose use in different agroecosystems and production systems

Highlights

- Accessions of *Vigna unguiculata* (cowpea) with good performance across sites in Central America, Haiti and Colombia were identified.
- Selected out of 17 accessions of *Vigna unguiculata* received from IITA, 5 accessions for acid soils (IT86D-715, IT89KD-288, IT6D-733, IT89KD-391 and IT95K-1088/4) and 5 for neutral soils IT95K-1088/4, IT86D-719, IT95K-1088/2, IT93K-637/1and IT96D-740); some promising local accessions identified for further testing.
- Three accessions of *Lablab purpureus* (CPI 34777, CPI 106471 and CPI 52535) were selected for high yield on acid and neutral soils.

3.5.1 Evaluation of annual legumes for drought tolerance in Central America

Contributors: Axel Schmidt (IP-5), Steve Hughes (SARDI, Australia), Clark Davies (IP-5), Michael Peters (IP-5), Luis Alfredo Hernández (SN-3)

Rationale

Farmers' adaptive capacity to cope with increasing drought incidents in Central America depends to a large extent on the availability of drought tolerant crops and forages. Limited by 6-7 months of dry season (November to May/June; 750-1100 mm/year), smallholders in the hillsides of Nicaragua are looking for crop varieties and forage species which safeguard their harvests and permit to feed animals during the dry season. Multipurpose legumes adapted to these dry conditions can play a significant role in enhancing livestock feed during the dry season and, if integrated in cropping systems, they may also improve soil fertility through nitrogen fixation and suppress weeds when used as cover crops (mulch).

Species requiring smaller amounts of water for considerable biomass production (feed, cover, green manure) might even offer the possibility to extent the growing season beyond the two traditional growing periods "*primera*" from May/ June to September and "*postrera*" from September to November.

To match farmers' demand for such plant materials, it is necessary to screen available germplasm for adaptation to local conditions and for plant characteristics which allow for the integrations of these selected species into the traditional maize-beans cropping system. In order to improve adoption such germplasm evaluation and selection should be preferably based on farmers' criteria and in collaboration with them.

Material and methods

In collaboration with the South Australian Research and Development Institute (SARDI) and its Australian Medicago Genetic Resource Center, a set of 30 annual legume species (Mediterranean region – see Table 43) were selected for an adaptation experiment at the SOL SECO site (the Spanish acronym for Supermarket of technologies for hillsides – dry) in San Dionisio, Matagalpa, Nicaragua. The site is known by local farmers as the driest place in San Dionisio and is shared by other CIAT projects and national collaborators for the screening of different crops for their drought tolerance The selection criteria were: low water requirement, fast growth and soil cover, annual growth cycle, ease of establishment, late flowering and no seed shattering.

The experiment was reestablished in October 2002 since the first establishment failed due to the unusually early beginning of the dry season in October 2001. The experiment was designed as randomized blocks with 3 replicates. Seeds were pre-germinated and seedlings transplanted in the 4.5 m x 1.5 m plots with an overall planting distance of 0.5 m between plants. The experiment protocol included periodical evaluations (every two weeks) of field emergence, plant height, ground cover, drought tolerance, flowering patterns, biomass production, seed production, incidents of pests and diseases, and for farmer selected species forage quality parameters.

Results and Discussion

Although rainfall conditions favored plant establishment this time, plant development was slow and most of the accessions died within three weeks after transplanting. Only *Hedysarum coronarium* (SARDI 32506) and *Hedysarum flexuosum* (SARDI 35361) showed some adaptation potential, but died during the first dry weeks. Since no accession showed promising signs of adaptation during the two years under characterization and evaluation, the collection will be dropped form the work plan.

Since the issue of drought tolerant multipurpose legume germplasm remains important, work will focus in 2004 on the evaluation of CIAT's collection of *Vigna umbellata* (rice bean), a plant species which raised high expectation this year among farmers during a small experiment at the SOL-Wibuse site in San Dionisio. While CIAT's *Vigna umbellata* collection (8 accessions) is diverse in its origin (Central America, Colombia, Africa, Southeast Asia), the collection was never evaluated under field conditions for drought tolerance and multiple purposes.

Table 43: Available germplasm passport data of annual legume species established at SOL SECO site in San Dionisio, Nicaragua.

SARDI Acc. No.	Genus	Species	Alt.	Lat.	Long.	Precip. (mm/a)	Origin
367	Trifolium	purpureum	60	33° 54' N	35° 28' E	890	Lebanon
687	Trifolium	hirtum	300	32° 33' S	118° 13' E		Australia
1011	Trifolium	vesiculosum					Italy
3400	Medicago	arabica					USA
4715	Trifolium	clypeatum		36° N	27° E		Greece
5036	Tetragonolobus	purpureus		45° 30' N	73° 36' W		Canada
5045	Trigonella	balansae					Sweden
6125	Trifolium	apertum	50	45° 02' N	39° 00' E		Russia
8687	Lotus	peregrinus	140	32° 42' N	35° 12' E		Israel
12701	Lotus	edulis	700	36° 30' N	03° 45' E	650	Algeria
12953	Lotus	maroccanus	91	34° 03' N	06° 33' W	425	Morocco
14935	Trifolium	spumosum	400	37° N	30° E		Turkey
17357	Trifolium	diffusum					USA
22575	Hymenocarpos	circinnatus	150	39° 41' N	19° 45' E	1100	Greece
24228	Hedysarum	carnosum	200	35° 50' N	09° 12' E	300	Tunisia
24578	Trifolium	isthmocarpum	250	34° 05' N	05° 00' W	750–1000	Morocco
32202	Trigonella	***Calliceras***					Canada
32233	Medicago	scutellata					Australia
32506	Hedysarum	coronarium					Morocco
32511	Tetragonolobus	palaestinus					Spain
33621	Trifolium	alexandrinum					Australia
33816	Vicia	sativa					Australia
33842	Lotus	ornithopodioides					Czechoslovakia
33884	Trifolium	incarnatum					Unknown
34647	Medicago	polymorpha					Turkey
35361	Hedysarum	flexuosum		35° 48' N	05° 45' W	750-1000	Morocco
35644	Trifolium	salmoneum	120	35° 07' N	35° 38' E	400	Israel
36323	Medicago	polymorpha					Australia
36400	Trifolium	squarrosum					Unknown
36441	Trifolium	michelianum					Bulgaria

3.5.2 Evaluation of a core collection of *Vigna unguiculata* for multipurpose uses in Colombia, Nicaragua, Honduras, and Costa Rica.

Contributors: M. Peters, L. H. Franco, A. Schmidt, H. Cruz Flores, P. Argel, P. Avila, B. Hincapié, G. Ramírez, (CIAT) 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 low fertility soils 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 grain, hay for 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.

3.5.2.1 Evaluation of new cowpea accessions in Quilichao and Palmira, Colombia

In 2003, a new collection comprising 32 breeding lines obtained from IITA (B.B. Singh), complemented with 5 accessions locally used in Honduras and Nicaragua were evaluated in Quilichao and Palmira. The objective was to compare the effect of climate and soil on performance and to identify accessions with broad adaptation, which is key for Central American hillsides with highly variable soil and climatic conditions (Photo 17). Standard agronomic evaluation methods were employed as in previous years.

This year we report results from Quilichao only as the trial in Palmira is still in the establishment phase. In Quilichao (acid soils) no incidence of pests and diseases was reported. In table 44 results on vigor, soil cover and dry matter yield are presented.

Mean dry matter yields (1416 kg/ha) were lower than recorded with previous collections in Quilichao (mean yield 2229 kg/ha), possibly as a result of favorable climatic conditions during growth period. However, yield differences ($P \leq 0.05$) between accessions were measured at harvest in the pre-flowering stage (8 weeks after planting). Accessions IT97K-825-3 IT97K-461-4, IT89KD-288, IT98K-131-2 and 9611 had the highest yields with equal or more than 1800 kg/ha DM. After sampling, cowpea biomass was incorporated in the soil to measure the effect on a succeeding maize crop.

Photo 17. Plots of Cowpea (*Vigna unguiculata*) at Quilichao

Highly significant (P≤0.01) differences between accessions were measured for *in-vitro* dry matter digestibility (IVDMD), phosphorus (P) and potassium (K) content, but not for protein content (CP). In general results confirm cowpea as a very high quality material with dry matter digestibilities above 83% and protein contents ranging between 20 and 24 %. Several accessions had digestibilities above 88%, among these the high yielding IT98K-131-2, IT97K-825-3 and 9611 (Table 45).

Table 44 . Vigor, soil cover (%) and dry matter yield (kg/ha) of *Vigna unguiculata* in Quilichao, 2003

Accessions	Vigor	Soil Cover (%)	DM kg/ha
	1 – 5	8 weeks	8 weeks
IT97K-825-3	4.3	95	2067
IT97K-461-4	3.0	73	2040
IT89KD-288	4.0	90	1893
IT98K-131-2	4.3	92	1800
9611	5.0	100	1800
IT99K-429-2	4.0	87	1780
IT98K-476-8	4.0	88	1727
IT99K-1122	4.0	93	1720
IT97K-1069-2	4.3	93	1673
IT98K-205-8	3.3	80	1667
IT99K-1060	3.0	78	1660
IT97K-494-3	3.3	78	1653
IT99K-216-24-2	4.0	87	1567
IT97K-570-18	4.6	95	1553
Cidicco 2	4.6	97	1520
IT97K-356-1	3.6	87	1487
FHIA	3.3	72	1480
IT98K-390-2	4.6	97	1433
IT98K-391-2	4.0	90	1400
IT98D-1399	3.3	82	1340
Cidicco 1	4.6	93	1327
YT98K-406-2	4.0	87	1320
IT97K-818-35	4.0	90	1320
IT98K-412-8	3.3	77	1307
IT96D-610	3.3	82	1287
IT99K-7-14	2.6	68	1267
IT98K-428-3	3.6	87	1240
IT98K-412-13	4.0	92	1233
IT95K-52-34	4.6	95	1200
IT99K-409-8	3.6	77	1193
IT97K-1069-6	3.6	83	1147
IT98K-506-1	2.6	68	1120
IT98K-311-8-2	4.3	93	1067
IT99K-7-21-2-2	3.0	70	1020
IT97K-819-118	2.6	65	940
Cidicco 4	4.3	85	913
Cidicco 3	4.0	87	873
IT97K-499-38	2.5	65	780
Mean			1416
MSD (P≤0.05)			976.23

Table 45. Fodder quality of accessions of *Vigna unguiculata* grown in Quilichao, 2003.

Accessions	Forage			
	IVDMD	Protein	P	K
		%		
IT98K-131-2	89.8	21.0	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
9611	88.8	20.2	0.16	1.44
FHIA	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	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	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	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	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	5.74	0.08	0.71
	($P \leq 0.01$)	n.s.	($P \leq 0.01$)	($P \leq 0.01$)

3.5.2.2 Evaluation of cowpea in Honduras

In 2002, an experiment was established in the SOL Yorito site (neutral pH) to evaluate a core collection of cowpea. *Lablab purpureus* DICTA was included for comparison. In the 2002 Forages Annual Report dry matter yields of the cowpea accessions were reported, hence here the green manure effects on a succeeding maize crop are presented.

Highest maize grain yields were obtained following accessions IT93K-637/1, IT86D-719, 9611, IT6D-733, IT93K-573/5 and IT90K-284/2. Maize grain yields in these treatments were above 2 t/ha and higher than obtained with N-fertilization. Maize grain yields following incorporation of the accession IT93K-637/1 were almost double as compared to the non-fertilized treatment (N 0). The green manure effect of the different accessions cannot be explained by dry matter production of cowpea materials (Table 46).

3.5.2.3 Evaluation of cowpea in Nicaragua

In 2002, a collection of cowpea (*V. unguiculata*) was established in the Sol Seco site in San Dionisio (for evaluation of drought tolerance). Standard agronomic evaluation methods were employed, to evaluate accessions planted in a Randomize Complete Block Design with 3 replications and to determine effects of.

Highly significant differences (Pd≤0.01) were recorded both for biomass as well as grain yields (table 47). The local cowpea type ‘Café’, IT90K-284/2, type ‘INTA’, type ‘SF libre’ and ‘Negro’ provided the highest dry matter yields with up to 4.7 t/ha while highest cowpea grain yields were measured for local types ‘Negro’, ‘Café’ and ‘Rojo’.

From the results it appears that there are local materials available in Nicaragua with a production superior to most introduced lines under the dry conditions at the SOL Seco site. The performance of these lines across environments would need to be studied. Green manure effects on a succeeding maize crop are currently being investigated.

3.5.2.4 Genotype x Environment interactions

An adaptability index to assess the response of a set of 14 cowpea accessions across different climates and acid to alkaline soils in Honduras, Nicaragua, Costa Rica and Colombia was calculated. 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 62). In view of their wide adaptation across environments these accessions will be multiplied and selected for further comparison with local varieties and new collections for use as fodder. For utilization as green manure, based on data from only 2 sites, accession IT90K-284/2 is the most promising material; however, data from other 2 sites in Nicaragua and Colombia will be key to confirm the superiority of this accession.

Table 46. Dry matter yields of *Vigna unguiculata* (cowpea) genotypes before incorporating into the soil and maize grain yields after incorporation of cowpea (Yorito, Honduras. 2002 – 2003)

Treatment	Cowpea	Maize	
	Biomass	Grain yield	DM Total
		Kg/ha	
IT93K-637/1	8397	2575	4905
IT86D-719	7422	2329	4571
9611	11440	2250	4401
IT6D-733	5349	2230	4179
IT93K-573/5	6866	2083	3837
IT90K-284/2	12132	2071	4179
N80*		2016	3571
IT93K-503/1	4484	2000	3798
IT86D-716	8134	1825	3742
Lablab purpureus DICTA	7368	1802	3310
N40		1790	3139
IT90K-277/2	6370	1742	3579
IT89KD-288	8086	1639	3718
IT96D-740	3229	1635	3238
IT89KD-391	10158	1619	3583
FHIA	3663	1607	3318
IT86D-715	5110	1603	3250
CIDICCO3	8718	1599	3528
CIDICCO1	5010	1591	3810
CIDICCO2	10724	1556	3206
IT95K-1088/2	7992	1500	3089
IT95K-1088/4	9208	1373	3071
N0		1357	2710
Mean	7545	1822	3649
LSD ($P \leq 0.05$)	4243	1030	1281.6

Table 47. Dry matter and grain yields of *Vigna unguiculata* (cowpea) genotypes before soil incorporation in San Dionisio (Sol Seco), Nicaragua 2002.

Treatment	Cowpea	
	Biomass DM kg/ha	Grain yield Kg/ha
Café	4730	2268
IT90K-284/2	3063	1243
INTA	2780	1180
SF Libre	2627	1060
Negro	2463	2630
Rojo	1920	2123
IT93K-503/1	1763	1290
IT86D-716	1737	1300
IT95K-1088/4	1727	1103
IT93K-637/1	1613	1053
IT86D-715	1467	747
IT95K-1088/2	1140	923
IT89KD-391	1057	980
IT86D-719	1000	1077
IT93K-573/5	927	500
IT96D-740	900	730
IT6D-733	563	797
IT89KD-288	377	533
IT90K-277/2	263	117
Mean	1690	1138
LSD ($P \leq 0.01$)	2268.6	1689.7

*Maize grain yields in N120, N160 and N200 treatments were similar to the N80 treat

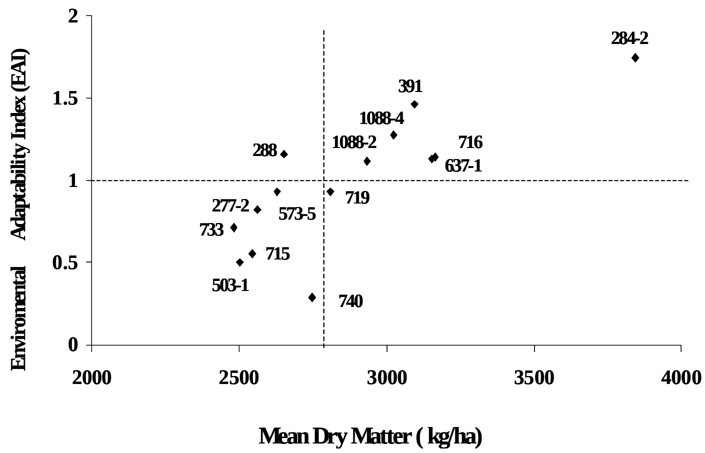

Figure 62. Adaptability Index for Dry matter production of a collection of *Vigna unguiculata* evaluated in contrasting environments in Honduras, Nicaragua, Costa Rica and Colombia

3.5.3 Evaluation of *Lablab purpureus* for multipurpose uses

Contributors: M. Peters, L. H. Franco, B. Hincapié, A. Schmidt, H. Cruz Flores, P. Argel and G. Ramírez (CIAT)

Lablab purpureus (syn. *Dolichos lablab* L.) is a legume with high potential as green manure and fodder in the tropics. Some commercial lines have been available to farmers for some time, but considerable variability exists within this genus that merits characterization with the aim to identify more productive and better-adapted lines. Under this consideration, new lines from a core collection from ILRI/CSIRO are under evaluation in the subhumid environment of Colombia and Central America.

Our main objective with the collection is to select accessions with broad adaptation to different soils and climate conditions in tropical America. However, of immediate interest is to evaluate the Lablab collection in acid and neutral soils to define niches of Lablab for green manure and fodder (especially for hay or deferred feed), with emphasis on Central America where soils are highly variable in pH.

3.5.3.1 Evaluation of collection *Lablab* in contrasting sites

Quilichao – Acid Soil: Most accessions had soil covers above 70% 12 weeks after establishment, showing strong ability to compete with weeds. At the 16 week sampling period, soil cover of some accessions was lower than at 12 weeks, due to defoliation triggered by flowering in early flowering material and attack by leaf eating insects at the beginning of the dry period.

No interactions between accession and harvest date were statistically detected ($P<0.05$); however, it was evident that for late flowering material dry matter yields increased over time while for some early flowering materials yields were reduced at the 16 week compared to the 12 week sampling. This has implications for the utilization of Lablab materials as green manure as different combinations of time available for green manure and Lablab material can address different temporal niches in the farming system.

Highest dry matter yields were recorded for accessions I-14442, CPI-106471, CPI-52535, CPI-52535, CPI-52535 ,I-11615, cv. Highworth and CPI-36903 with more that 3 t/ha 16 weeks after planting (Table 48).

Table 48. DM yield (kg/ha), vigor and soil cover (%) of *Lablab purpureus* accessions in Quilichao, 2003

Accessions	Soil cover (%)		DM yield (kg/ha)	
	12 Weeks	16 Weeks	12 Weeks	16 Weeks
I-14442	92	97	3153	4600
CPI-106471	92	83	2573	4467
CPI-52535	88	63	3720	4127
21603	97	97	2693	3367
I-11615	47	35	1693	3233
Cv. Highworth	88	80	2340	3160
CPI-36903	77	57	2360	3047
CPI-34777	92	65	3273	2650
I-11632	70	45	2413	2587
I-6533	73	77	1793	2513
CQ-2975	83	90	2327	2360
CPI-67639	77	73	1860	1973
L-987	87	78	2200	1950
I-14437	75	77	1560	1760
CPI-35894	50	20	1800	1560
Mean	79	69	2384	2918
LSD ($P \leq 0.05$)			2670.12	3060.16

Palmira-Neutral soils: As in Quilichao leaf eating insects attacked Lablab accessions at the beginning of the dry period. Dry matter yields, soil cover and vigor of Lablab accessions was better in Palmira than in Quilichao, confirming earlier results that *Lablab purpureus* responds to better soil conditions and higher pH. Average soil covers were above 90% both at the 12 and 16 week sampling, with mean dry matter yields above 4 t/ha and 7 t/ha 12 and 16 weeks alter planting, respectively (Table 49).

Costa Rica: Twelve lines of *Lablab purpureus* were established for evaluation of adaptation in a randomized complete block design with three replicates at Atenas, Costa Rica. Plot size was 2 m wide x 1.5 m long (3 m^2), and the planting distances were 0.25 m and 0.50 m between plants and rows respectively. Plots were fertilized with 50 kg/ha of P_2O_5, 50 kg of K_2O, 20 kg of S and 20 kg of Mg. Plant emergence and plant vigor and cover were measured respectively 4 and 6 weeks after planting. The experiment will continue under evaluation to measure DM yield and forage quality.

The experiment was initially planed for a plot size of 8 m^2 (3 m wide x 2.5 m long), but we had to adjust the size of the plots to the availability of seed quantities. Plant emergence was acceptable and there was no need for replanting, thus over 87% plant emergence was observed 4 weeks after planting as Table 50 shows. Differences in plant cover and vigor have been also observed up to date, and cv. Highworth and the lines CPI-67639 and L-987 have shown good growth and plant development. Less vigor and have shown the lines CPI-106471, 21603, CQ-2975 and CPI-34777, while other lines are intermediate in plant development.

Some variability in plant vigor has been observed within identical lines, but we are not sure if this is due to seed quality or caused by particular soil conditions. So far, low incidence of leaf eater insects, as well as foliar diseases have been observed.

Table 49. DM yield (kg/ha), vigor and soil cover (%) of *Lablab purpureus* accessions in Palmira, 2003

Accessions	Soil cover (%)		DM yield (kg/ha)	
	12 Weeks	16 Weeks	12 Weeks	16 Weeks
CPI 106471	75	90	6713	8567
CPI 52535	100	97	5500	8053
CPI 36903	97	83	4120	7633
I-11632	98	93	4213	7573
I-14437	100	95	4788	7487
I-6533	98	98	4427	7453
I-14442	100	98	4247	7340
CPI 34777	98	83	5853	7140
I-11615	90	80	4613	7060
CPI 67639	100	98	5580	6547
Highworth	100	95	4493	6527
CQ-2975	98	100	3813	6480
CIAT 21603	88	90	3420	6253
L-987	100	100	4920	6073
CPI-35894	100	65	3860	5273
Mean	96	91	4704	7031
LSD ($P \leq 0.05$)			2304.09	3073.92

Table 50. Plant emergence, plant vigor and cover of *Lablab purpureus* lines established in Atenas, Costa Rica

Accessions	**Plant Emergence (%)**	**Plant Cover(%)**	**Plant Vigor (%)***
21603	95.8	25.0	2.3
CPI-106471	90.3	35.0	2.3
CPI-34777	87.5	38.3	2.8
CPI-36903	93.1	45.0	3.0
CPI-52535	100.0	43.3	3.2
CPI-67639	100.0	61.7	4.3
CQ-2975	98.6	22.5	2.3
Highworth	100.0	62.5	4.3
I-11632	87.5	42.5	3.0
I-14442	97.2	55.0	3.7
I-6533	90.3	55.0	3.8
L-987	88.9	57.5	4.3

* Visual rating (1 poor vigor, 5 highly vigorous plant)

3.5.3.2 Effect of lablab accessions as a green manure

In the 2002 Forages Annual Report data on biomass production of *Lablab purpureus* in Quilichao and Palmira were reported. In this report we summarize the effect of different accessions of Lablab on a succeeding maize crop on neutral to alkaline soils in Palmira.

Significant differences ($P \leq 0.05$) between accessions were measured for total maize biomass yield but not for maize grain yield.

Accessions CPI 34777, CPI 106471, CIAT 21603, CPI 52535, cv. Highworth and I-11615 resulted in maize grain yields above 4 t/ha, substantially higher than the maximum maize grain yield of 2.5 t/ha recorded with N160 - fertilization. Maize yields following lablab did not appear to be directly related to biomass production of lablab (Table 51).

Table 51. Dry matter yield (kg/ha), vigor and soil cover (%) of *Lablab purpureus* accessions and grain and dry matter yield of a following maize in Palmira, 2002-2003.

Treatment	*Lablab purpureus*			Maize	
	Vigor	Soil cover (%)	DM yield (kg/ha)	Grain	Total
	1 a 5	12 Weeks		(kg/ha)	
CPI 34777	5	100	5293	7902	22400
CPI 52535	5	100	4733	6116	20215
CPI 52544	4	97	3840	5789	19884
CPI 100602	4	97	3513	5773	19858
I-11630	5	100	4353	5706	15353
I-14411	5	100	3460	5525	17076
CPI 29398	4	97	4167	5261	17417
CPI 36903	4	97	3093	5240	17778
CQ-2975	5	100	4187	5157	17264
CPI 96924	3	63	3160	5101	14424
I-14437	4	100	4413	4916	19437
I-11615	3	87	4480	4897	16513
I-11613	4	90	3220	4823	18327
CPI 35894	3	90	3713	4740	16240
CPI 99985	3	93	3387	4704	14414
I-14442	4	100	3067	4580	18751
cv. Highworth	5	97	4620	4556	16323
I-6533	4	97	4033	4467	17458
I-14441	5	96	4040	4406	19951
CPI 106471	5	100	5053	4244	20201
CPI 106548	4	87	3200	4091	17343
CPI 76998	4	100	3613	3753	17097
CPI 67639	4	93	3673	3609	14952
CIAT 21603	5	100	4853	3387	15758
Endurance	3	80	2653	3267	12844
CPI 81626	3	93	4213	3257	13594
cv. Rongai	4	100	4113	2761	13219
L-987	5	100	4227	2745	12948
NI60				2474	8961
cv. Koala	3	80	3067	2106	16534
N80				2002	7756
N120				1747	8460
N0				1300	8597
N40				1105	6589
Mean		94	3919	4185	15813
LSD ($P \leq 0.05$)			1505	NS	11537

In view of the heterogeneity of the variances between the different treatments for yields of a succeeding maize crop, a smaller group of 19 treatments (14 accessions and including all 5 fertilizer control treatments) with relatively high homogeneity of variances within treatment was selected. With this smaller group highly significant ($P \leq 0.01$) differences in maize grain and total biomass yield were detected. *Lablab purpureus* CPI 34777, CPI 52535, I-11630 and I-14441 as green manure resulted in significantly ($P \leq 0.01$) higher maize grain yield than with N-fertilization (Table 52).

Table 52. Dry matter yield (kg/ha), vigor and soil cover (%) of *Lablab purpureus* accessions and grain and dry matter yield of a following maize in Palmira, 2002-2003.

Treatment	*Lablab purpureus*			Maize	
	Vigor	Soil cover (%)	DM yield (kg/ha)	Grain	Total
	1 a 5	12 Weeks		(kg/ha)	
CPI 34777	5	100	5293	7902	22400
CPI 52535	5	100	4733	6116	20215
CPI 52544	4	97	3840	5789	19884
CPI 100602	4	97	3513	5773	19858
I-11630	5	100	4353	5706	15353
I-14411	5	100	3460	5525	17076
CPI 29398	4	97	4167	5261	17417
CPI 36903	4	97	3093	5240	17778
CQ-2975	5	100	4187	5157	17264
CPI 96924	3	63	3160	5101	14424
I-14437	4	100	4413	4916	19437
I-11615	3	87	4480	4897	16513
I-11613	4	90	3220	4823	18327
CPI 35894	3	90	3713	4740	16240
CPI 99985	3	93	3387	4704	14414
I-14442	4	100	3067	4580	18751
cv. Highworth	5	97	4620	4556	16323
I-6533	4	97	4033	4467	17458
I-14441	5	96	4040	4406	19951
CPI 106471	5	100	5053	4244	20201
CPI 106548	4	87	3200	4091	17343
CPI 76998	4	100	3613	3753	17097
CPI 67639	4	93	3673	3609	14952
CIAT 21603	5	100	4853	3387	15758
Endurance	3	80	2653	3267	12844
CPI 81626	3	93	4213	3257	13594
cv. Rongai	4	100	4113	2761	13219
L-987	5	100	4227	2745	12948
NI60				2474	8961
cv. Koala	3	80	3067	2106	16534
N80				2002	7756
N120				1747	8460
N0				1300	8597
N40				1105	6589
Mean		94	3919	4185	15813
LSD ($P \leq 0.05$)			1505	NS	11537

Among the lablab accessions CPI 34777 and CPI 52535 are the most promising materials, producing high biomass yields and having a high green manure effect on succeeding crops. CPI The accession 34777 has rapid growth and needs to be utilized quickly while CPI 52535 can occupy niches with longer time spans for harvesting the forage and grain. Eventhough yields of Lablab are generally higher on soils with a pH above 5.0, these two accessions were among the highest yielding in the Lablab collection on both acid and alkaline soils. Both accessions are included in a G X E experiment to assess performance over a wider range of environments (see below).

3.6 Nitrification inhibition in tropical grasses

Highlights

- Found substantial differences in total and specific NI (nitrification inhibition) activity among tropical grasses.
- Developed and tested a simple protocol to quantify the impact of addition of root exudates from plants on nitrification inhibition of incubated soils.

Last year, we showed the feasibility of using a bioassay (with recombinant Nitrosomonas europaea) that detects nitrification inhibitory activity in plant (such as root exudates or tissue extracts) or soil samples (such as soil water extracts). Using this bioassay we have shown that root exudates from B. humidicola inhibit nitrification. The inhibitory activity of the root exudates increased with the plant age (mostly because of the increase in root mass) and showed a sigmoid pattern; subsequently, NI activity in root exudates declined as the plants reach the maturity stage.

This year we report our ongoing research activities on methodology development, and comparative evaluation of other tropical grasses for the ability to inhibit nitrification. The other ongoing research activities in this collaborative project include: isolation of the active compound responsible for NI activity in B. humidicola, mechanisms underlying the inhibition of nitrification in root exudates, and factors that regulate the expression of NI activity. Results from these activities will be reported next year.

3.6.1 Bioassay – Further improvements and refinements in the methodology

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

The bioassay methodology was adopted from Izumi et al. (1998), which was initially developed to detect nitrification inhibitors in the municipal-waste water treatment plants. This methodology has gone through improvements to get reliable and stable measurements in detecting inhibitory effect on nitrification from root exudates, tissue extracts and soil-water extracts. Of the various factors, one of the most important is the incubation temperature of the bacterial culture with the test compound (i.e. root exudates) for the bioassay measurements. The bioassay appears to be at its best in detecting the inhibitory activity at 15^{O}C; the bioassay's ability to detect inhibitory effect from a known inhibitor (allylthiourea) decreased with an increase in incubation temperature. Also, the room temperature where the luminometer is located should be maintained close to 20 to 22^{O}C to obtain stable measurements.

The Nitrosomonas culture age of 6 to 7 days is found to be the optimum stage for the bioassay measurements; beyond this, the response to known inhibitor (Allyl thiourea) decreased with the culture age. These modifications are now part of the bioassay methodology (for the other details on the methodology please see CIAT, 2002 - IP-5 Annual Report) to evaluate nitrification inhibitory activity of the plant samples and will improve our ability to generate reliable and stable measurements for this research project.

3.6.2 Root exudates – Development of sample processing and preparation protocols for the determination of nitrification inhibitory activity using a bioassay

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

In most cases root exudates when collected from the plant roots (by keeping intact plant roots in deionised/distilled water for 24 hours) needs to be condensed several fold (about 50 to 100) before they can be used for the determination of NI activity. We have noticed several times that the contamination of chloride (either from water that is used for collecting root exudates or from the soil-water extracts) interferes with the inhibitory activity measurements. To avoid chloride contamination problems in the root exudates and soil-water extracts, we have developed a sample preparation protocol where the root exudates sample is evaporated to dryness using a rotari-evaporator at 45^{O}C, and then extracted with methanol; the methanol extract is further evaporated to dryness and then re-dissolved in dimethyl sulfoxide. Using this sample preparation protocol, we could eliminate completely the problems of chloride interference, as chloride does not dissolve in methanol.

Using several root exudates samples from B. humidicola, we have shown that the NI activity from the root exudates or plant tissue extracts can be recovered into the methanol extract. The remaining portion of the root exudates did not show inhibitory activity (data not shown). This sample preparation protocol has now become a standard procedure for processing the root exudates samples for the determination of NI activity.

3.6.3 Comparative evaluation of six tropical grass species for the ability to inhibit nitrification from acid soil

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

Rationale

Collaborative research with JIRCAS colleagues has shown that *B. humidicola* CIAT 679 inhibits nitrification of ammonium and reduces the emission of nitrous oxide into the atmosphere (IP-5 2001 Annual Report). Given these findings with one genotype of *B. humidicola*, there is a need to determine the extent of genetic variation among tropical grasses in their ability to inhibit nitrification and reduce emissions of N_2O. 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 constraints but also can protect the environment. Given the vast areas under *B. humidicola* in the tropics, reductions in net emissions of N_2O could have important environmental implications. The main objective was to quantify differences among several tropical grasses to inhibit nitrification and associated reductions in N_2O emission under greenhouse conditions using infertile acid soil. Also we intend to correlate nitrification inhibition with root biomass and length, and to monitor nitrate and ammonium levels in the soil after addition of ammonium –N as fertilizer.

Materials and Methods

A sandy loam oxisol from the Llanos (Matazul) of Colombia was used to grow the plants (4 kg of soil/pot). 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 6 different tropical grasses were used as test plants at two levels of ammonium sulfate application (0 and 100 kg/ha). The grasses included: *B. humidicola* cv. *Humidicola*; *B. decumbens* cv. *Basilisk*; *B. dictyoneura* cv. *Llanero*; *B.* hybrid cv. *Mulato*; *B. brizantha* cv *Marandu*; *P. maximum* cv. *Common*. Two control treatments were included: soil without plants that had no application of ammonium sulfate or had application of ammonium sulfate. The experiment was arranged as a completely randomized block design with 4 replications. Plants were allowed to grow for 7 weeks and were cut to 10 cm to stimulate regrowth for 3 weeks and were cut again at 10 cm height to allow regrowth and to simulate grazing effects under field conditions. After another week (at 11 weeks) of regrowth, ammonium sulfate was applied in solution at a rate equivalent to 100 kg $N\text{-}NH_4$ per hectare. To favor a greater development of plant roots in the pots, which could increase the NI effects, the plants were allowed to grow for 13 weeks more (at 24 weeks) before making a second application of ammonium sulphate at the same dose. Plants grew for 4 weeks more before final harvest (at 28 weeks). Thus the total length of the experiment was 7 months. At the end of the experiment, plants were harvested and separated into shoot and roots. Root length was measured using a root length scanner. Dry matter content and N status of both shoot and roots was determined.

Results and Discussion.

Results from the comparative evaluation of six tropical grasses (that are predominantly grown in South America) indicate that substantial levels of NI (Nitrification inhibitory) activity is present in the root exudates of other Brachiaria grasses in addition to *B. humidicola* (Table 53). However, among Brachiaria grasses tested in this study, NI activity is substantially lower in *B. brizantha* cv. Marandú. It is interesting to note that a complete absence (below the detectable limit to our bioassay) of NI activity from the root exudates of *P. maximum*.

The total NI activity (i.e. NI activity from four plants pot^{-1}) of *B. humidicola*, *B. decumbens*, B. *dictyoneura* and *Brachiaria* hybrid cv. Mulato were similar based on our bioassay estimations (Table 53). Nevertheless, the NI activity of the root exudates needs to be confirmed further using soil incubation studies.

The presence of substantial levels of NI activity in the root exudates of *Brachiaria* hybrid cv. Mulato has immediate practical implications as this hybrid has a huge potential for being planted in large areas of South America. Also, the implications of such high levels of nitrification inhibition (if confirmed further from the soil incubation studies and field studies) in *Brachiaria* hybrid cv. Mulato opens the possibility to screen for this trait in the Brachiaria improvement program. The lack of nitrification inhibition ability in *P. maximum* is an interesting issue as this is the levels of N inputs.

Large differences in specific NI activity among the tropical grasses were found in this study, with *B. humidicola* exhibiting the highest level of inhibition (Table 54). Furthermore, the total and specific NI activities were substantially lower in the treatment without ammonium in all the Brachiaria grasses tested. Similar trends (data not shown) were found when the specific activity was calculated using total root length instead of root dry weight that is shown in Table 54.

The immediate task is to characterize and quantify this phenomenon further in grasses that have contrasting abilities in nitrification inhibition (e.g., *B. humidicola* vs. *Panicum maximum*) both under glasshouse and field conditions. This would help us understand the potential impact of this relatively new phenomenon in nitrogen dynamics in pasture systems. We also need 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. Whether the presence of ammonium nitrogen (or any form of nitrogen) near the root zone of the soil acts as a triggering factor for the release of inhibitory compounds or if the plant nitrogen status regulates the NI activity in the root exudates, are some the other issues that need to be resolved through further research work.

Table 53. Nitrification inhibitory activity (total NI activity pot^{-1}) of the root exudates from 6 tropical grasses grown under two levels of N fertilization. Plants were grown for seven months before being used for collecting root exudates.

Pasture species	N Fertilizer Treatment	NI activity (in AT units pot^{-1})	SD
B. humidicola	With ammonium supply	81.95	7.35
B. decumbens	With ammonium supply	77.39	5.33
B. dictyoneura	With ammonium supply	70.54	11.30
B. hybrid Mulato	With ammonium supply	84.52	11.20
B. brizantha	With ammonium supply	49.62	13.33
P. maximum	With ammonium supply	-9.43*	8.80
B. humidicola	Without ammonium supply	13.04	6.46
B. decumbens	Without ammonium supply	21.43	1.28
B. dictyoneura	Without ammonium supply	19.22	1.90
B. hybrid cv. Mulato	Without ammonium supply	23.19	1.45
B. brizantha	Without ammonium supply	23.91	0.36
P. maximum	Without ammonium supply	1.81	2.39

Note: Ammonium was supplied as ammonium sulfate at 100 kg ha^{-1} rate. NI activity is expressed as AT units; One AT unit is defined as the inhibitory activity caused by the addition of 0.44 µM of allylthiourea 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 - nitrification was stimulated by the root exudates. SD = standard deviation.

Table 54. Specific NI (Nitrification inhibitory) activity (NI activity g^{-1} root dry wt) of the root exudates from 6 tropical grasses grown under two levels of N fertilization.

Pasture species	N Fertilizer Treatment	Specific NI activity	SD
B. humidicola	With ammonium supply	17.30	0.34
B. decumbens	With ammonium supply	12.31	0.27
B. dictyoneura	With ammonium supply	5.93	0.68
B. hybrid *Mulato*	With ammonium supply	10.16	2.26
B. brizantha	With ammonium supply	8.35	2.20
P. maximum	With ammonium supply	-1.67	1.34
B. humidicola	Without ammonium supply	2.87	1.17
B. decumbens	Without ammonium supply	7.22	2.19
B. dictyoneura	Without ammonium supply	2.90	0.31
B. hybrid Mulato	Without ammonium supply	6.46	0.45
B. brizantha	Without ammonium supply	6.75	0.40
P. maximum	Without ammonium supply	0.69	0.75

3.6.4 Development of an incubation protocol to assess nitrification inhibition by addition of root exudates to soils.

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

Rationale

According to the results presented in the preceding section, root exudates from various tropical grasses have differential ability to reduce nitrification as demonstrated by the bioassay. As indicated before, the next sequential step is to assess the effect of application of root exudates directly to soil in relation with Nitrification Inhibition. Given that the process of collecting and concentrating root exudates is time and labor intensive, and that it is difficult to generate large amounts of root exudates, it is desirable to develop a methodology to apply root exudates to small amounts of soil in conditions that allow a proper monitoring of inorganic nitrogen in the soil as well as reliable monitoring of fluxes of nitrous oxide. This later process is especially difficult to follow, given the inherent variability in gas fluxes from soil even under controlled incubation conditions.

The purpose of a recent scientific internship of Marco Rondon at JIRCAS laboratories was to develop and test a simple incubation methodology to quantify the effect of application of root exudates to soils on nitrate and ammonium levels and on fluxes of nitrous oxide. As nitrous oxide fluxes are highly dependent on the moisture content of the soil, the method needs to maintain moisture content at levels of 50-60% of water filled pore space (WFPS), which is considered optimum for nitrification in most soils (Del Grosse et al., 2002) After trying various alternatives, a suitable method was developed and tested. The final protocol is described here, and some results using it are presented.

Description of the method

Plastic syringes (20 ml) are used both as incubation containers and as gas collection chambers for monitoring gas fluxes. Ten g of air-dried soil are loaded inside the syringe on top of an inert fine nylon (100 μm mesh), which allows gas exchange but prevents soil to move out of the syringe. On top of the soil surface, another 2 discs of nylon mesh are placed to hold the soil in place and serve as barriers to reduce moisture evaporation from the soil surface. The nylon barrier also serves to isolate the soil from the syringe piston during the gas collection process. Once the soil and nylon discs are placed inside the syringe, the syringe is tapped several times until the soil redistributes itself into a minimum volume. Water, fertilizer solution or the root exudates to be tested are then added to the soil to reach 60% WFPS. Water is sprinkled slowly and uniformly on top of the nylon mesh with a fine syringe needle. The liquid progressively moistens the soil by capillary movement and after about one hour, the soil is well mixed with the liquid without a need for further disturbances. This procedure allows a very effective control of the moisture content in the syringes, which otherwise, is a very time consuming and error causing procedure. Temperature is maintained stable during the incubation time by placing the syringes in an incubation chamber. During the incubation time, the soil can freely exchange gases with the surrounding air. The combination of controlled temperature and moisture content permits reliable and reproducible monitoring of gas fluxes.

At the time of collecting gas samples, syringes are removed from the incubation chamber. A Teflon valve is attached to the lower end of the syringe. The syringe piston is inserted into the barrel and the piston is depressed fully (without compressing the soil) to force the air inside the soil pore space to move outside the syringe. The soil air is replenished with fresh air by moving backwards the syringe piston. This procedure is repeated four times to warrant a complete exchange of the original air inside the soil pores. Once this is done, the gas sample corresponding to time zero could be collected. Then, the piston is raised at a predetermined height inside the syringe, to provide a preset soil to air volume ratio. The needle valve is closed creating a tight sealed chamber. The chamber is allowed to exchange gases during a determined gas collection period, typically one hour. After this time, the air inside the chamber could be directly analyzed by gas chromatography or transferred into pre-evacuated glass vials to be analyzed at a later time. Once the sample is collected, the syringe piston is removed to allow the soil to exchange again gases freely with the surrounding air. The procedure permits an easy way to adjust the air to soil volume ratio, so very low fluxes of gases could be detected. The procedure was tested repeatedly and good reproducibility in gas measurements was obtained.

3.6.5. Effect of application of root exudates on inorganic nitrogen and fluxes of nitrous oxides from incubated soils.

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

Materials and methods

The procedure described in activity 3.8.4 was used to study the effect of application of root exudates on fluxes of N_2O from incubated soils. Root exudates were obtained from intact plants grown in solution media, which were transferred from the nutrient media into de-ionized water. Plants were allowed to exudate during 24 hours and the resulting root exudates were concentrated using a rotovapor at 45C.

Experimental treatments included:

1. Blank (W): Application of water
2. Control (AS): application of aqueous solution of ammonium sulfate at a rate of 91 ug $N\text{-}NH_4$/ g soil
3. Root exudates from *B. humidicola* at low concentration (BL). Exudates were concentrated from 30 liters into 900 ml with a rotovapor.
4. Root exudates from *B. humidicola* at high concentration (BH). Exudates were concentrated from 30 liters into 200 ml with a rotovapor.
5. Root Exudates from soybean at low concentration (SNB). Exudates were concentrated from 30 liters into 900 ml.

Treatments with root exudates received the same dose of ammonium sulfate as the Control AS. The ammonium was dissolved in the root exudates prior to adding the solution to soils.

A fertile Andisoil from Tsukuba was used. Soil was collected from the top 20 cm in the field, air dried and then sieved with a 2 mm mesh. Soil was well mixed and 10 g sub samples were loaded into plastic syringes. The corresponding treatment liquid (Water, ammonium sulphate, root exudates + AS) was then applied to the syringes. 3.6 ml of aqueous solution was used in each syringe, to raise the soil moisture content to 60%WFPS. Once wetted, the syringes were kept at 21C in an incubation chamber for 24 days. These syringes were subsequently used to monitor fluxes of N_2O over time, according to the procedure described above (activity 3.8.4). Gas samples were collected at 5, 9, 14, 19 and 24 days after ammonium sulphate application. 8ml of the gas sample were transferred into a 4ml preevacuated glass vial. Analysis of N_2O was performed typically within 2 days after collection using a Shimadzu 14B GC equipped with an ECD detector, and a stainless steel column packed with Porapaq Q80 mesh.

Gas fluxes were calculated by linear regression of chamber headspace concentration vs. time. The results are presented as arithmetic means of three replicates.

For monitoring of nitrate and ammonium levels, parallel samples of soil were incubated. Due to limited availability of root exudates, only 2 grams of air-dried soil was used for monitoring of inorganic N. The vials received the same treatments as the syringes and incubation was started at the same date. KCl extracts were made of the incubated soil in the vials at the same dates of gas collection. 20 ml of 2M KCl were used to extract the 2 g of soil in each vial. Shaking time was 1 hour. The KCl extract was filtered immediately after shaking using Whatman 5C filter paper that was prewashed with KCL. Extracts were maintained refrigerated until the time of analysis. Analysis was done colorimetrically using an Autoanalyzer.

Results and Discussion

As expected, (Figure 63), addition of water or aqueous solutions to air dried soil resulted in a temporary increase in fluxes of N_2O. The addition of root exudates from soybean strongly promoted the fluxes of the gas from the soil during the initial week of incubation compared to the Control treatment. Recent research (G. V. Subbarao, personal communication) has shown that root exudates from soybean also promote nitrification in the bioassay medium. This may be the result of addition of readily available carbon sources in the root exudates. The large peak of N_2O could be caused by rapid denitrification. Reports from the literature (Azam et al., 2002), indicate that when nitrogen is added to the soil, in addition to nitrogen sources, microbial respiratory activity is greatly enhanced and this may result in a rapid depletion of oxygen in the soil micropores which favors the formation of anaerobic microsites where denitrification may take place. After one week, the initial peak of nitrous oxide decays and soils that received root exudates from B. *humidicola* at high concentration, show subsequently lower emissions of nitrous oxide.

In Figure 64, accumulated net fluxes of nitrous oxide for a period of 24 days are presented. Exudates from soybean also result in appreciable higher net accumulated fluxes of N_2O during the incubation period. Whether these fluxes are resulting from enhanced nitrification or denitrification needs to be clarified in further studies. No appreciable reduction in fluxes of the gas appear to occur as a consequence of the addition of root exudates from *B. humidicola* at low concentration, suggesting that the added dose of the active compound was probably not high enough to interact with all the population of nitrifying bacteria in the soil. In contrast to this, the addition of exudates of *B. humidicola* at high concentration resulted in a net decrease of around 45% of total emissions of N_2O relative to the control treatment.

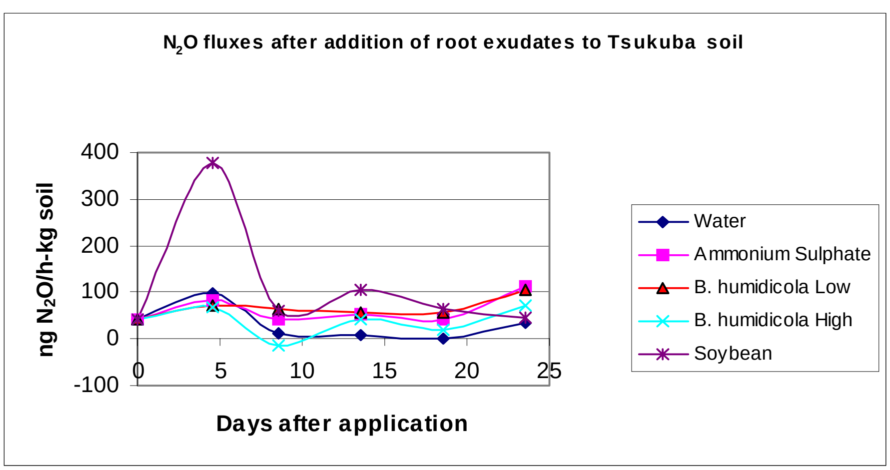

Figure 63 Fluxes of nitrous oxide following addition of root exudates from different species to incubated Andisol.

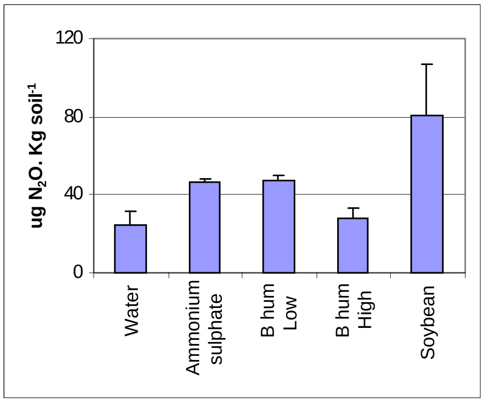

Figure 64 Accumulated fluxes of nitrous oxide over a 24-day incubation period.

Similar trends were observed in levels of ammonium in the soils, (Figure 65). Ammonium levels were consistently higher in the *B. humidicola* treatment indicating reduced nitrification rate, while consistently lower levels were found in the soybean exudates treatment confirming promoted nitrification.

The nitrification inhibition seems to be more effective between 5 and 9 days as indicated by the slope of the graphs in Figure 65. After two weeks of incubation, the curves show similar slopes suggesting that the residual effect of the root exudates could be of around two weeks. This is shorter residual time than the 3 weeks

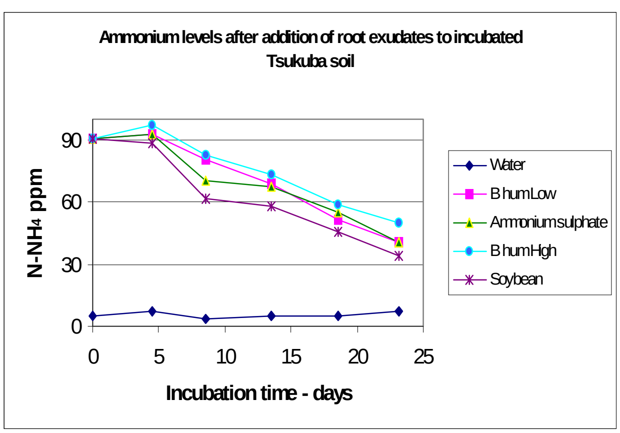

Figure 65. Ammonium evolution on incubated soils after receiving root exudates.

suggested by previous studies in pots (Ishikawa et al, in press), and could be an indication of low additions of the active compound with the root exudates as compared with concentrations generated by the root system in pot experiments or in the field.

Results from this study suggest that there is a minimum threshold concentration of root exudates (active compound) required to effectively reduce nitrification in soils. Once the active compounds in root exudates that are responsible for NI activity are identified, it would be possible to conduct more controlled experiments to add known amounts of the active compound to the soils.

The procedures developed and tested here, have proven indeed to be easy, and reliable for monitoring fluxes of nitrous oxide and appropriate to follow the levels of inorganic nitrogen in the soil. These methods could be applied to test the effect of additions of root exudates from different plant species to soils on Nitrification Inhibition and associated fluxes of N_2O and would contribute to rapid advances in this field of research.

The method is being currently used to assess the effect of application of various root exudates into soils of contrasting chemical and physical characteristics including oxisols from Colombian savannas and inceptisols from Andean hillsides. Results will be reported next year.

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

4.1 Partnerships in LAC to undertake evaluation and diffusion of new forage alternatives

Highlights

- Results from on-farm trials demonstrated the benefits and limitation of *Brachiaria* hybrid cv Mulato
- Adoption of Cratylia by smallholders in the llanos of Colombia is being promoted by farmers who have experienced the multiple benefits of this legume as a dry season feed
- Forage species to feed livestock in cut/carry systems and for green manure were selected and multiplied for use in hillsides of Haiti
- Scaling of new forage alternatives is an active process in hillsides of Honduras and Nicaragua being promoted by NARS, NGOs and farmers groups
- The first farmer-led forage seed enterprise (PRASEFOR—Productores artesanales de semilla forrajera) was formed in Honduras
- Scaled up the testing of green manures with selected legumes in different production systems in Hillsides of Nicaragua

4.1.1 Evaluation in Central America of *Brachiaria* hybrid cv Mulato

Collaborators: Conrado Burgos (DICTA), Heraldo Cruz (DICTA/CIAT), Dionisio Soto (INTA, Nicaragua), Axel Schmidt (CIAT), Marco Lobo and María Mesén (INTA, Costa Rica), Bolívar Pinzón, Rubén Montenegro and Luis Hartentains (IDIAP)

Rationale

During the last years the availability of new forage options has increased in Central America, particularly new cultivars of the genus *Brachiaria* and *Panicum.* Usually commercial seed companies with limited participation of local research and development institutions carry out promotion of these materials. The involvement of national institutions in on-station and on-farm processes of validation and promotion of the new forage options offers the chance to generate reliable information and expose the new cultivars to different biotic and abiotic stresses and production systems. During the last year this strategy has been utilized to evaluate *Brachiaria* hybrid cv. Mulato.

Material and Methods

As mentioned in the 2002 Annual Report of IP5, a protocol for the validation/promotion of *Brachiaria* hybrid cv. Mulato was developed and proposed to national institutions of Panamá, Nicaragua, Costa Rica and Honduras. As a result dual purpose and beef cattle farms were established with the grass in the region of Yoro, Yorito, Victoria and Olancho (Honduras); León, Chinandega, Posoltega and San Dionisio (Nicaragua); Puriscal, Miramar, San Jerónimo and Orotina (Costa Rica) and Bugaba, Gualaca and Boquerón in Panamá. Commercial plantings have been carried out in these countries and in Guatemala, since the validation exercise has been accompanied by the availability of commercial seed from Semillas Papalotla from Mexico.

Results and Discussion

Successful establishment of cv. Mulato was reported from every site in the different countries were the grass was planted. High seed quality, high seedling vigor, good soil preparation and adequate weather conditions accounted for the excellent establishment of the grass. The absence of major pests and diseases also contributed to the adequate establihsment of cv. Mulato.

On-farm establishment: One of the outstanding characteristics of cv. Mulato is the quickness of the establishment given the high seedling vigor that the plant has. Table 55 shows that in farms in Honduras, plant height and cover had a mean of 0.80 m and 80% respectively, less than two months after the grass was planted. This observation was also made in other countries of the area. As a result of rapid establihsment, farmers could make use of the paddocks much earlier than the time experienced with other grasses.

Table 55. Number of plants, plant height and plant cover of *Brachiaria* hybrid cv. Mulato established in dual-purpose cattle farms of Honduras. (Information supplied by Conrado Burgos and Heraldo Cruz of DICTA and DICTA/CIAT respectively).

Farm/Site	Days after planting	Plants/m^2	Mean plant height (m)	Mean plant cover (%)
La Laguna/Yorito	54	17	0.40	65
Las Brisas/Sulaco	54	23	1.05	95
Las Delicias/Victoria	51	28	1.00	90
Ojo de Agua/Victoria	48	12	0.80	85
Don Pedro/Victoria	49	20	0.75	80
Mean	**51**	**20**	**0.80**	**83**

On-farm monitoring: Milk production has been monitored in dual-purpose cattle farms with cv. Mulato in several countries of the region including Honduras (Table 56). In every site milking cows grazing the grass increased individual daily milk production; for instance in the case of Honduras the increase has ranged from 1.0 to 2.0 liters/day. Additional to this and because of more DM production, the stocking rate has also increased in pastures of cv. Mulato as compared to other *Brachiaria* grasses like *B. decumbens* cv. Basilisk. As a result milk production also increased per unit of land.

Table 56. Area planted, milk production and milk daily increase of dual-purpose cows grazing *Brachiaria* hybrid cv. Mulato in dual-purpose cattle farms of Honduras. (Information supplied by Conrado Burgos and Heraldo Cruz of DICTA and DICTA/CIAT respectively).

Farm/Site	Area planted (ha)	Number of milking cows	Grazing period (days)	Mean daily milk/cow (kg)	Mean daily milk increase/cow (kg)
La Laguna/Yorito	2.0	12	28	5.4	1.7
Las Brisas/Sulaco	3.0	17	37	4.0	1.0
Las Delicias/Victoria	3.0	10	30	9.6	1.6
Ojo de Agua/Victoria	2.0	14	24	11.3	1.7
Don Pedro/Victoria	2.5	6	45	4.0	2.0

Controlled grazing trial: A grazing trial was established by IDIAP (Instituto Panameño de Investigación Agropecuaria) during 2002 at the Experimental Station Gualaca in Panamá. The site is at 100 masl and located in a 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.

Cultivar Mulato was established by direct seeding after controlling the existing vegetation with herbicides. 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 rotational grazing scheme of of 3/21 days of grazing/rest. Five young steers with a mean of 205 kg liveweight were used to give an initial stocking rate of 2.5 AU/ha. Animal liveweight gains during the dry period from December 2002 to March 2003 were 363 g/day, while that during current wet period (April to September, 2003) the mean liveweight gain has been 781 g per animal/day. This liveweight gains are relatively high given that usually in this site animal lose weight during the dry period and have liveweights gains around 500 g/day in other *Brachiaria* species during the wet period. The high gains are the result of the good forage quality of cv. Mulato, particularly in terms of digestibility and N content. Forage availability has been 798 kg DM/grazing/cycle during the dry period and 1792 kg DM/grazing/cycle during the wet period. This experiment will continue under evaluation. Observations carried out in Honduras indicate liveweight gains of 900 g/an/day for animals grazing cv. Mulato in contrast with a liveweight gain of 600 g/an/day for animals grazing *B. decumbens* cv. Basilisk. This observation will continue under conditions of a commercial beef cattle farm.

4.1.2 On-farm evaluation of new grasses for livestock systems in Colombia

Contributors: C. Plazas, John W. Miles, P. Argel and C. E. Lascano (CIAT)

4.1.2.1 On–farm evaluation of *Brachiaria* cv Mulato

An important activity in the Forage Project is the on farm evaluation in different regions of Colombia of the *Brachiaria* hybrid cv Mulato. After one year of evaluation the following observations can be made:

1. In the well-drained savannas Llanos of Colombia with soils of low fertility Mulato should be part of a crop –pasture rotation system. One option is to establish Mulato with Maize, as was the case in the Costa Rica farm. In this farm the maize harvested covered 80% of the cost of establishment of Mulato and a very productive pasture was left behind.

2. In the piedmont of the llanos with soils of higher fertility than in the well-drained savannas the use of Mulato requires the application of fertilizer. In the Isla farm Mulato shows very high animal productivity and regrowth after intensive defoliation with the application of chicken manure.

3. In the north coast of Colombia with more fertile soils the performance of Mulato is very good and could be an alternative to grasses with very poor performance in the dry season as is the case of *Bothriochloa* sp., which is the predominant grass in many cattle regions with 6 month dry season.

4. In both the Llanos and the north coast we observed in Mulato and damage caused by the insect *Antonina graminis*, but the problem seems to be easily managed by intensive defoliation.

4.1.2.2 Release of *Brachiaria* hybrid cv Mulato

A major event this year was the release in Colombia of the *Brachiaria* hybrid cv Mulato by a private seed company (Semillano). The field day to introduce cv. Mulato to farmers in the Llanos was held on Tuesday, 17 June. CIAT staff from headquarters, from Central America and from the Llanos gave a series of short presentations to an audience of 80-90 participants, mostly farmers. Presentations were followed by a visit to the Costa Rica farm, where a 15-ha Mulato pasture was established (with maize) last year (Photo 18). The seed company had Mulato seed (from Papalotla) for sale, in attractive 1-kg, sealed tins. The aggressive promotion of Mulato by Semillano is impressive, as they also had a similar event in the north coast (Turipana-Corpoica) to present cv Mulato to farmers (Photo 19).

Photo 18. *Brachiaria* hybrid cv Mulato established with maize in the Llanos of Colombia (Costa Rica farm)

Photo 19. *Brachiaria* hybrid cv Mulato pasture in the north coast of Colombia (Turipana Research Station-Corpoica)

4.1.3 On-farm evaluation and diffusion of shrub legumes for dairy systems in the Llanos of Colombia

Contributors: C. Plazas and C. Lascano (CIAT)

In February 2001 we started a 2-year project funded by PRONATTA (Programa Nacional de Transferencia de Tecnologia Agropecuaria) in the piedmont of the Llanos of Colombia to evaluate the utility of *Cratylia argentea* (Cratylia) in smallholder dairy farms. We were particularly interested in defining with farmers different uses of Cratylia as a supplement for milking cows in the dry season and in developing a seed supply system as a means of promoting the use of Cratylia in the region.

To implement the project we selected 14 farms in which the main activity was milk production. With active farmer participation we evaluated in the different farms the use of Cratylia for cut and carry, direct grazing and seed production. A total of 12.5 ha were planted in the 14 farms (range 0.12 to 1.0 ha) out of which 6 ha were for cut and carry, 3 ha for direct grazing and 3.5 ha for seed production.

In what follows we report results on performance of Cratylia in the different farms, on milk yield with supplementation of Cratylia and on seed production. Finally we discuss some of the lessons learned in the project.

Agronomic performance and quality of Cratylia. In the different farms were we established Cratylia we measured seasonal yield and leaf: stem ratio in the forage harvested. In the wet season the mean green dry matter (GDM) yield across farms was 3337 Kg/ha of which 67% was leaf (Figure 66). Plants averaged 1.21 m of height at the time of harvest, which was carried out every 45 to 55 days. In the dry season forage yield of plants of 1.12 m averaged 600 K GDM with 52% leaf (Figure 67).

In one farm we measured yield of plants with and without an uniformization cut (20 cm above ground surface) following grazing. Results showed that days of regrowth forage yield was greater (490 vs. 787 Kg DM/ha) with plants that were not cut but the leaf proportion was considerably less (29 vs. 54%). In the dry season crude protein (CP) content of Cratylia averaged 22% as compared with 6.5% in the grass available in the pastures (Figure 68). The CP content of Cratylia in the dry season has been similar to *G. sepium*, which is a well known legume used by few farmers in the region to supplement milking cows.

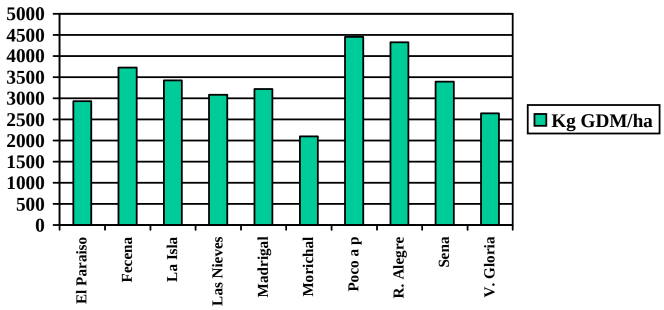

Figure 66. Forage yield (green dry matter) of *Cratylia argentea* harvested during the wet season in different farms in the Piedmont of the Llanos of Colombia

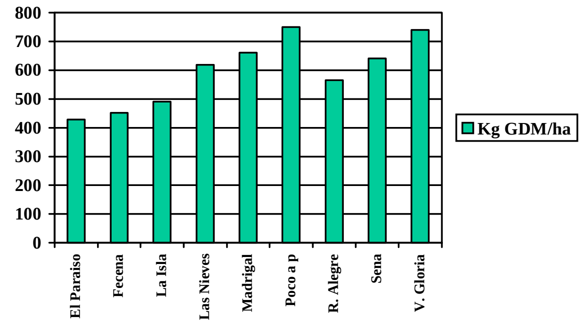

Figure 67. Forage yield (green dry matter) of *Cratylia argentea* harvested during the dry season in different farms in the Piedmont of the Llanos of Colombia

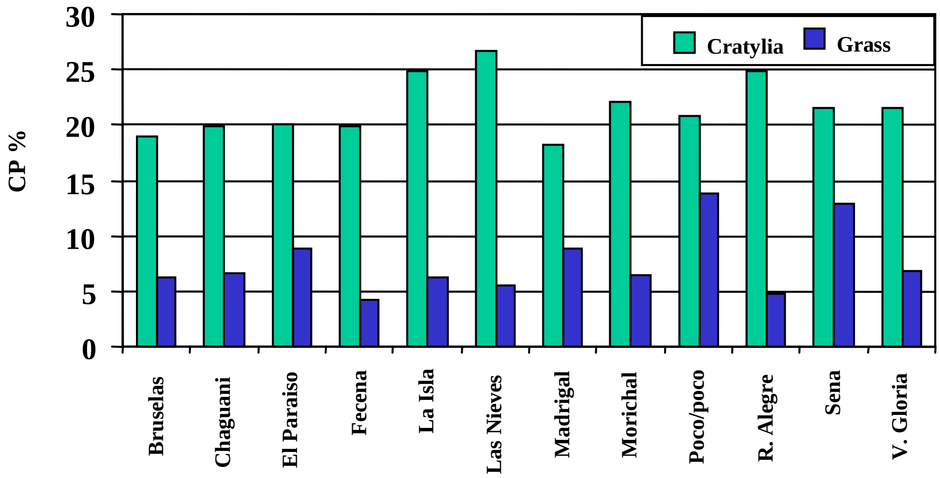

Figure 68. Crude protein (CP) content in *Cratylia argentea* and in the grass available in pastures grazed by milking cows during the dry season (Piedmont- Llanos of Colombia)

The combination of Cratylia with maize silage, an initiative of some farmers, has as expected increased CP content of the silage (7 to 10%) and this has reflected in more milk yield.

Milk yield of cows supplemented with Cratylia

It has not been easy to measure the effect of supplementing Cratylia on milk yield during the dry season given that the farmers do not want to loose money as they clearly see the benefit of the legume. In the period December 2001 to May 2003, we measured milk production in the same cows with and without supplementation of Cratylia in several farms in the project. Results showed that in some cases there where was positive response to the legume whereas in other cases there was no response (Table 57).

Table 57. Milk yield of grazing cows with and without supplementation of *Cratylia argentea* **(Piedmont- Llanos of Colombia)

Farm	No of Cows	Grass (l/cow/d)	Grass + Cratylia (l/cow/d)
1	7	8.5	8.5
2	10	8.0	8.5
3	14	5.2	6.3
4	6	5.9	6.0
5 (AM Milking)	8	6.6	8.0
5 (PM Milking)	8	6.3	6.0
6	10	6.9	6.5

**Measurements carried out from December 2001 to May 2003)

In spite of this, farmers are very impressed with the improved body condition of cows receiving Cratylia after the dry season, which should have a positive effect on reproduction. In addition, farmers see other benefits of feeding Cratylia as discussed bellow.

Seed Production of Cratylia. One important goal of the project was to establish seed multiplication plots of Cratylia to allow diffusion of the technology in and outside the region. In seed plots established in 7 farms we were expecting to harvest 650 kg of clean seed based on yields obtained in Costa Rica. In February of this year we began the seed harvest and only harvested 100 kg. The seed yield per plant varied from 25 g to 67 g with an average of 47 g/plant for 4 farms.

During the dry months (February and March) 90% of the seed harvested was of excellent quality and germination, but once the rains started in April the quality of the seed dropped significantly (from 90% to 40% good seed).

The cost of production of the seed harvested was in the order of $US 4.50 /kg, which makes attractive the activity of seed multiplication given that the current price of Cratylia seed in the market varies from US $ 14 to 15/ kg.

In spite the high potential economic benefits of producing Cratylia seed in the Piedmont of the Llanos of Colombia, it became clear that it was not an ideal region for this activity given the low yields obtained associated with a short rainy season. As a result a Cratylia Network was established with participants from contrasting regions in Colombia. One initial objective of the Network is to define seed production potential of Cratylia in contrasting eco-regions (north coast, Magdalena Valley, Coffee zone) of Colombia.

Lessons Learned. Given the participatory approach used in the project farmers were able to define alternative uses of Cratylia, which at the end were quite different from those initially suggested by the technical staff in the project (Photo 20).

A

B C

Photo 20. Different uses of *Cratylia argentea* in smallholder dairy systems in the Piedmont of the Llanos of Colombia (A= Cut & Carry; B= Silage; C= Direct grazing)

The original idea was for farmers to use Cratylia in a Cut & Carry system but some realized that this system was associated with high labor cost. The alternatives to Cut & Carry of Cratylia that some farmers implemented were silage production and direct grazing all year round using in electrical fences. Grazing of Cratylia has not caused plant mortality and in some farms a very productive association of the legume with *Brachiaria decumbens* (the grass originally in the plots) was formed.

Through farmer experimentation we have also learned that it is possible to reduce cost of establishment of Cratylia by intercropping maize or other crops such as tomatoes, and cucumbers.

Finally, one of the most important lessons learned is that criteria of farmers to assess the utility of forage plant like Cratylia may be quite different from the criteria of researchers. We had postulated that the main benefit for dairy farmers in the Piedmont would be increased milk production in the dry season and consequently more cash flow.

It was interesting however, to learn that farmers saw other benefits when using Cratylia:

a) Possibility of having high quality forage for cows in the middle of the rainy season when pastures were difficult to graze due to high soil moisture
b) Replacement of purchased supplements in the dry season which has economical implications
c) Possibility of milking cows in the dry season and get higher price for the milk sold
d) Improved body conditions of cows which has been associated with improved reproductive performance

The adoption of Cratylia in the Piedmont of the Llanos of Colombia is an ongoing process which is being promoted not only by extension people who received training from the Project but also by enthusiastic farmers who have seen the benefits of the legume in there farms. In spite of this, adoption of Cratylia in the Piedmont and other regions of Colombia could be enhanced if commercial seed was available. There is a need to identify regions in Colombia suitable for Cratylia seed production and farmers willing to invest money and effort on seed multiplication.

4.1.4 Evaluation of multipurpose legumes as green manures in the Llanos of Colombia

Contributors: C. Plazas, M. Peters, Luis H. Franco and B. Hincapie (CIAT)

Rationale

One of the aims of the Forage Project is to develop alternative green manures for maize and rice based systems (Photo 21) in the Llanos of Colombia. It is expected that suitable legumes will reduce the need for external inputs (herbicides, fertilizer) and thus make the crops more competitive.

Photo 21. Rice following green manure incorporation in the llanos (C.I. La libertad)

Materials and Methods

Two types of potential green manures were selected for different temporal niches. 1) Rapidly establishing legumes to be used as green manures (incorporated 80-90 days after planting) and 2) Slower establishing species to be utilized for fallow improvement. The following species and accessions were evaluated:

Green manures	Fallow improvement
Vigna unguiculata (IT86D-716)	*Cajanus cajan* CIAT 913
Vigna unguiculata (IT6D-733)	*Centrosema macrocarpum* CIAT 5713
Vigna unguiculata (IT89KD-288)	*Centrosema pubescens* CIAT 15160
Vigna unguiculata cv. Cabecita negra.	*Canavalia brasiliensis* CIAT 17009
Mucuna pruriens CIAT 9349	*Pueraria phaseoloides* CIAT 9900
Stylosanthes guianensis CIAT 11844	*Stylosanthes guianensis* CIAT 11844
Stylosanthes guianensis CIAT 11833	*Stylosanthes guianensis* CIAT 11833
Stylosanthes guianensis CIAT 184	*Stylosanthes guianensis* CIAT 184
Stylosanthes guianensis population 3	

Green manure in upland rice: Results on the performance of green manures were summarized. in the AR-2002. In Figure 69, the residual effects of the green manure on a 2nd rice crop (1 year after green manure incorporation) are presented. Results indicated no significant green manure effects on rice yield. Differences in yield of rice due to green manure species were only evident with the application of N (40 kg/ha). This confirms previous results that there are no positive effects of using short term green manure on upland rice systems, for the first crop in the piedmont area of the llanos.

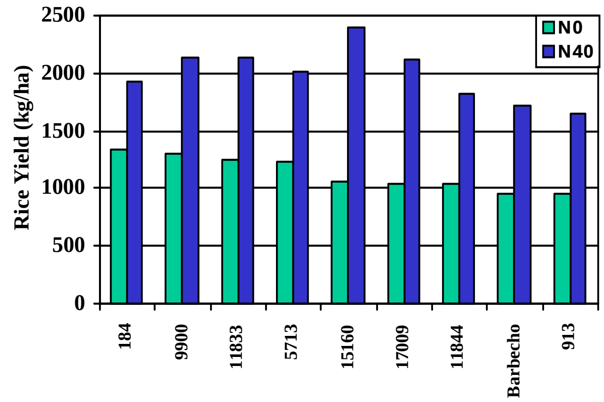

Figure 69. Residual effects of green manure as affected by N fertilization on rice grain yield, C.I. La Libertad, Piedemonte, Llanos Orientales de Colombia, 2003.

Short- term green manure species for maize and rice cultivation: In view of the increasing importance of maize in the Altillanura of Colombia, short-term green manure species were tested for their effect on succeeding maize. The experiment was established on an Oxisol at the Hacienda Matazul. 7 accesions of legumes were sown, i.e.: *Mucuna pruriens* CIAT 9349, *Vigna unguiculata* IT89KD-288, *Vigna unguiculata* IT6D-733, *Vigna unguiculata* var. Cabecita negra, *Stylosanthes guianensis* CIAT 184, *Stylosanthes guianensis* CIAT 11833 and *Stylosanthes guianensis* Población 3.

All materials were incorporated at the same time (after 11 to 12 months). Results indicated that short-term use of legumes planted after native savanna does not have any effect on subsequent maize in the Altillanura. It is possible that after continuous cultivation of maize the effect of planting short-term legumes as green manure may be beneficial.

4.1.5 Participatory evaluation of improved forages for multipurpose use in hillsides of Haiti

Contributors: Pedro Argel (CIAT), Garline Amboise, Mirtho Jerome, Levael Eugene, and Jean Osmy Chéry (CIAT/HAP) and Luis H. Franco (CIAT)

To contribute to the objective of HAP, the Tropical Forages Project relied on the large number of herbaceous and woody forage species held in the Genetic Resources Unit of CIAT and on preliminary results from the Hurricane George Recovery Program on adaptation of different grasses and legumes to contrasting soils types in Haiti. Methodology developed by CIAT for participatory selection of forages for smallholder systems were to be used to define grass and legume options suitable to environmental and socio-economic conditions in hillsides of Haiti. Demonstration to farmers of the value of improved forages to feed livestock and to improve soil fertility for crop production was to be a major component of CIAT's Forage Adaptive Research Strategy. These activities would be complemented with the development of sustainable seed delivery systems of improved forages.

The methodology proposed for the HAP in Haiti was as follows:

Multilocational Testing. Different grass and legume species pre- selected on the basis of previous knowledge on adaptation to calcareous and acid soils, and potential utility to feed livestock and to enhance soil fertility are evaluated in small plots with farmer participation. Staff from different NGO's collaborating with HAP will be trained in forage agronomy and in participatory methods. Representative plots in the HAP target areas will be selected to establish a wide range of pre-selected forage species using methodology developed by CIAT. Farmers will be involved in the selection of grasses and legumes using there own selection criteria, which will then be compared with criteria used by researchers. The main output of this activity will be the selection with farmers of adapted "elite" grasses and legumes for subsequent on –farm testing and seed multiplication.

On- farm Testing. Elite grasses and legumes will multiplied by CIAT to assess their value as feed resources for bovines and goats and as covers to enhance soil fertility for annual crops. Farms with plots in different stages of degradation will be selected in the HAP target areas to plant selected grasses and legumes. Grasses will be evaluated for ease of establishment, seasonal biomass production, and acceptability to livestock when offered at different stages of maturity. Legumes will be evaluated for ease of establishment, ground cover, seasonal biomass production, seed quality, acceptability to livestock and effect on soil fertility through yields of an indicator crop (maize and /or beans). The main output of this activity will be the demonstration in selected and representative farmer's fields of the utility (biological and economical) of improved grasses and legumes to feed livestock and to improve soil fertility for annual crop production.

Effective Seed Delivery System. Seed of selected commercial grass and legume cultivars will be multiplied and purchased outside of Haiti through linkages to be developed with the private sector in Haiti. This will allow good quality –low cost seed availability to farmers in a sustainable manner. Seed multiplication of selected grasses and herbaceous and woody legume specie not commercially available in the regional market will be mainly carried out in Haiti through contracts with NGO's that have the capacity to manage seed plots, process and store seed.

A strategy to involve local Farmer Groups in seed multiplication of selected grasses and legumes will be promoted as an other means of providing income and allowing effective diffusion of improved forages. The main output of this activity will be a mechanism in place for diffusion and adoption by farmers of improved forages for multipurpose use.

After 1-½ years of work in HAP the funds for CIAT work were cancelled in midyear with short notice and consequently we could not meet our objectives. In what follows we present a summary of the accomplishments and difficulties encountered in establishing forage trials in different regions of the hillsides of Haiti.

Forage trials in the Jacmel region

It has been difficult for the field staff of the project in Haiti to establish the forage trials that were planned. Sev eral reasons account for that situation. Small and medium-size farmers have been reticent to yield their plots to set up forage trials because they believe that by doing so, they:

a) Would not have enough land to grow cash crops like corn, beans or vegetables
b) Will be kept out of their plots for too long a period of time (two years on the average) according the protocol for the forage trials

c) Will be denied the possibilities to get their usual revenues and crop production from their small piece of land

In spite of all the difficulties, the field staff succeeded establishing three forage trials during the first cycle and another three during the second cycle (Table 58). The main objective of the forage research activities was to identify forage species that would:

a) Adapted to local conditions,
b) Contribute to improve the soil fertility level,
c) Provide feed to livestock, and
d) Serve as living barriers in order to protect the soil and reduce the level of erosion on hillsides.

Table 58. Forage trials established in the Jacmel region

Trials	Zone	Planting date	Altitude	Growing stage
Cowpea Lines (4 + Check)	Cap-Rouge	June 13, 02	700	Normal
Grasses (5 + Check)	La Vallée	May 17, 02	800	Poor emergence
Live Barriers (4 +2 Checks)	Fd Jn-Noël	April 5, 02	1225	Destroyed by repeated heavy rains
Shrub Legumes (6)	La Vallée	May 17, 02	800	Normal, was planted twice
Grasses (5 + Check)	La Revoir	August 23	800	Poor emergence
Shrub Legumes (6)	La Revoir	August 23	800	Poor emergence
Herbaceous Legumes (4 Species)	Cap-Rouge	August 16	700	Good germination
Shrub Legumes (6)	Cap-Rouge	August 29	700	Good germination

In Table 59 we present initial results on plant emergence, vigor and plant cover during the first 9 weeks of the trial. All cowpea lines established well, although the local line was showing better adaptation followed by the introduced lines 391, 716, 277-2 and 1088-4. The second aspect of the cowpea trial involves the planting of corn in half of the plots after incorporating the cowpea biomass into the soil. The second half of the cowpea plot will be used to measure cowpea grain yield. To estimate the N contribution of cowpea to the soil, maize will be sowed using six (6) nitrogen doses within three (3) replications. Finally, since farmers indicated that they could not spare there plots used for crop production to carry out forage research work, we decided to plant test forages along the borders of the plots. It was a decision that many farmers welcomed with interest.

Table 59. Mean seedling emergence, plant vigor and cover after 9 weeks of growth of five Cowpea accessions established in Cap-Rouge

	Week 2	Week 4		Week 6		Week 9		
Entry	Emergence (%)	Vigor	Cover (%)	Vigor	Cover (%)	Vigor	Cover (%)	Height (cm)
391	19.7	3.3*	28.3	3.5	35.3	3.7	41.7	29.0
716	17.3	2.3	16.7	2.8	22.7	3.2	28.3	20.7
1088-4	19.0	3.3	25.7	2.8	27.3	2.7	30.3	22.3
277-2	19.3	3.0	26.7	3.2	29.7	3.3	41.0	20.1
Local	17.7	3.3	30.0	3.7	41.3	4.0	51.7	19.7

* Vigor is rated as 1=poor vigor; 5=highly vigorous plant

Forage Trials in the Cap Haitien region

Evaluation of Cowpea as grain and green manure

This trial was established at the locality of El Matador, near Dondon. Data for biomass yields were collected and looked pretty interesting; and the biomass has been already incorporated into the soil. Among others, the accession IT86D -716 showed high yield performance as shown in Table

60. Corn was just being planted afterwards to complete the second phase of the trial. In a participatory evaluation session the accession IT86D-716 ranked first based on the selection criteria that had been set up for the exercise. A few farmers indicated their concern about the labor and the cost to incorporate the biomass into the soil at the pre-flowering stage.

Table 60. Yields of Cowpea (*Vigna unguiculata)* lines established as grain and green manure in Haiti.

IITA No.	Foliage green yield (kg/ha)
IT86D-716	3533 a
IT90K-277/2	3000 ab
Local line	2033 bc
IT89KD-391	2000 bc
IT95K-1088/4	1666 c

a, b, c Means followed by the same letter are not statistically different (P<0.05)

Bertin Site: The forage accessions did not germinate well due to drought conditions and probably to the methods of planting. They were re-planted again at mid-September 02. Some species like *Panicum maximum* cv. Mombasa and *B. brizantha* cv. Toledo performed quite well.

Foundation Vincent Site: The trials set up at Foundation Vincent did not germinate well and consequently the material was planted several times. Seedling emergence was quite acceptable in a re-planting carried out in September, but it has been noted also the urgent need to set up a fence around the trial plot to stop stray goats coming from a nearby neighborhood that feed on the vegetative materials of the trial.

In Table 61 we show results of one evaluation carried out approximately one year after planting. The legumes *Arachis pintoi* and *Stylosanthes guianensis* failed to establish, due mainly to the unreliability of the rains and the grazing of the plots by uncontrolled goats. Nevertheless the legumes *Centrosema macrocarpum* and *C. pubescens* established well and had acceptable plant cover and vigor one year after planting.

The grasses P. *maximun* cvs Mombasa and Tanzania showed good plant vigor, as well as the *Brachiaria* cvs Toledo and Mulato. At the evaluation date, plant height of these grasses was close to 1 m per plant that indicates the possibility of utilizing them in a cut and carry system, which is widely used in Haiti and considered as a viable alternative for animal feeding in the country.

Table 61. Mean plant height, cover and fresh weight at first cut of legumes and grasses established at Foundation Vincent (North of Haiti)

Species	CIAT No.	Plant height (cm)	Plant cover (%)	Fresh weight (g/m^2)
C. macrocarpum (Ucayali)	25522	34.5	76.8	1.4
C. pubescens	15160	44.0	85.0	2.4
B. brizantha (Toledo)	26110	98.3	76.3	11.3
P. maximum (Tanzania)	16031	93.5	97.8	7.8
P. maximum (Mombasa)	-	97.0	87.0	5.5
P. atratum (Pojuca)	26986	96.8	82.0	3.3
B. hybrid (Mulato)	36061	104.5	78.0	9.0
B. decumbens (Basilisk)	606	91.3	79.7	5.3
P. purpureum (Elephant)	-	115.3	78.8	13.0

As a result of the evaluation of different forage species in Haiti we selected for on regional testing and seed multiplication a number of forage species:

a) Grasses: *Panicum maximum* cv Mombaza (for better soils and for cut and carry), *Brachiaria decumbens* cv Basilisk (for poor soils and for grazing) and *Brachiaria brizantha* cv Toledo (for poor soils, for cut and carry and for grazing).
b) Herbaceous perennial legumes: *Centrosema pubescens* CIAT 15160 and *Centrosema macrocarpum* CIAT 25522 (for low fertility soils for forage and/or long- term green manure).
c) Woody legumes: *Cratylia argentea* (for poor soils and for cut and carry as dry season feed)
d) Herbaceous annual legumes: Cowpea (IITA-716 for the North and IITA- 1088 for the South East for short-term green manures, for forage -hay or grain).

If funding becomes available to continue forage/ livestock work in Haiti we would need to concentrate on: a) evaluation with farmers of grasses, herbaceous (annual and perennial) and woody legumes selected in Haiti based on adaptation to biotic and abiotic constraints in hillsides and incorporation of selected forage species in current farming systems, b) development of forage conservation technologies for dry season feeding that are appropriate for small farmers and c) development of seed systems to promote diffusion of improved forages.

4.1.6 Participatory introduction of improved forages in smallholder dairy systems in Hillsides of Nicaragua

Contributors: Axel Schmidt (IP5), Clark Davies (IP5), Michael Peters (IP5), Luis Horacio Franco (IP5), Pedro Argel (IP5), Luis Alfredo Hernández (SN3)

Rationale

Scaling up of research results is considered as the most important issue to be addressed in current R&D activities due to increasing pressure from donors and civil society that money spent in agriculture must result in a lasting impact on the lives of the rural poor. Furthermore, it was recognized that many relevant technologies and approaches are not achieving their full potential impact due to low levels of adoption, which has led to more emphasis on the effectiveness of research to produce adoptable technological options. There is a need not only to increase impact, but also to show good quality research results, which attract different stakeholders in the R&D environment (e.g. NGOs, farmer associations) in order to achieve a high degree of adoption in combination with a faster dissemination process.

The BMZ funded project 'Farmer Participatory Research in Action: Selection and Strategic Use of Multipurpose Forage Germplasm by Smallholders in Production Systems in Hillsides of Central America' has had several achievements: a) development of a technology package made up of different components such as participatory diagnosis and stakeholder analysis, b) availability of forage germplasm for farmer participatory evaluation and selection, c) on-farm animal production trials including economic analysis, d) complementary training modules (participatory evaluation and selection, forage utilization/management, participatory monitoring and evaluation), and d)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. 1000 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.

Materials and Methods

CIAT was contracted to 1) 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 and 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 seed production system within the project in order to secure long term sustainability and adoption of the selected forage options.

Results and Discussion

The project started in late August 2002 with the selection of the three sites and their preparation. Nursery plots were sown at the beginning of October. A range of grasses (18), herbaceous legumes (12) and shrub legumes (9) species were established across three different sites with three replicates (Table 62). At one site, Ubú Norte (Photo 22), a fertilizer treatment was applied in order to demonstrate fertilizer effects on yield of grass species. Not all of the 367 plots could be established in 2002 due to unusual high precipitation, which made the establishment difficult. Establishment if the forage species was completed after the dry season in June 2003.

Table 62. Forage options established in target zones in Nicaragua

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		

Forage options were presented to selected farmer groups at all three sites. Agronomic and participatory evaluations are being followed on a regular basis. As expected, farmers initial interest was on grass species, in particular the new cultivars *Brachiaria brizantha* cv. Toledo (CIAT 26110) and *Brachiaria* hybrid cv Mulato (CIAT 36061). Since cv. Mulato was introduced commercially into Nicaragua in 2003, a field day was organized in the target area in collaboration with the seed company Papalotla in order to facilitate specific information on the new grass option. Apart from the mentioned *Brachiaria* species, *Paspalum atratum* and *Paspalum plicatulum* raised farmers’ interest because of their good establishment even under the difficult

Photo 22. Grass plots at reference site Ubú Norte, Nicaragua

Photo 23. *B. brizantha* cv. Toledo (CIAT 26110) pasture in Ubú Norte, Nicaragua

waterlogging conditions in 2002 and their high biomass production.

The project also facilitated the establishment of plots with a smaller selection of forage entries in different locations across the target zones. These plots are managed directly by farmer groups under the supervision of Technoserve, an extension company (30 technicians) also 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 in 2003. CIAT's Seed Unit in Atenas, Costa Rica, played a vital role in this effort.

In order to demonstrate milk production potentials of the new forage options on offer, large grazing plots (1-2 ha) (Photo 23) were established in each zone. *B. brizantha* cv Toledo (CIAT 26110) and *B.* hybrid cv. Mulato (CIAT 36061) was chosen for this effort. Parts of the plots were established with association of grasses and *Arachis pintoi* CIAT 18744. Since September 2003 milk production is being recorded in comparison to the traditional pastures available in the area and preliminary results indicate a milk increase of 15%. This has to be confirmed through further grazing cycles. Nevertheless, the involved farmer spread the news, which is now triggering interest in the new forage options. Additional field days will be organized to address forage agronomic characteristics and pasture management issues.   The establishment of *Cratylia argentea* for dry season feed purposes resulted very difficult due to high precipitation and the inherent slow initial growth of the shrub legume. Only at the Ubú Norte site, a uniformity cut could be applied so far. Further investigation involving farmers is necessary for defining factors associated with the establishment of *Cratylia* in order to speed up initial growth and reduce the risk of failure under different environmental conditions.

Training is a major activity in the project. This includes training of project technicians (30) but also of a small group of key farmers. So far, 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) (Photo 24).

Photo 24. Measuring pasture yields in order to calculate stocking rates – Training course on pasture management in Matiguás, Nicaragua, April 2003

Courses on dry season feeding and seed multiplication will follow in October, November and December 2003. Training on seed production will be of major importance since formal seed markets in Nicaragua are only beginning to evolve with prices often beyond the economic ability of farmers. Furthermore, only grass seed of few selected species is available so far. Farmers interested in seed production are currently being organized with the intention to establish a seed production group similar to PRASEFOR (see 4.1.8) in Honduras. A visit was made to the Yorito, Honduras in March 2003 (Photo 25) in order to expose interested farmers from Nicaragua to the seed multiplication and processing experiences of farmers in Honduras. The visit was complemented by a seed-training course at CIAT's Seed Unit in Atenas, Costa Rica. Efforts were made to initiate the seed production of *B. brizantha* cv. Toledo, *Arachis pintoi* and *Pueraria phaseoloides*. CIAT will continue to facilitate this process.

Although establishment of the different forage options was not as fast as anticipated, the effects of the new basket of options are already visible. High interest is evolving among farmers who in the past have spent little time and effort on improving feed resources for their animals. Natural pastures were the main source of nutrition for livestock. Now, farmers establish their forage plots and buy seed on their own account. Further effort will be necessary to maintain the participatory focus of the project. Technicians will receive a further training course in participatory methodologies in order to avoid traditional top down extension practices which could prove detrimental to the farmer-led process being implemented.

Photo 25. Mobile seed cleaner demonstrated during training visit to PRASEFOR group at Yorito, Honduras, March 2003

4.1.7 On-farm evaluation of green manures in hillsides of Nicaragua

Contributors: Campos Verdes (San Dionisio, Nicaragua), Axel Schmidt (IP-5), Caroline Dohmeyer (University of Hohenheim), Erik Sindhoj (PE2-TSBF), Michael Peters (IP-5), Clark Davies (IP-5), Edmundo Barrios (PE-2)

Rationale

Farmers are increasingly concerned of the increased price of inputs input used for agriculture production such as fertilizers. At the same time soil fertility on farmer fields is decreasing and weeds become a larger problem over time. In order to overcome these limitations and backed up by CIAT, the local farmer organization "Campos Verdes" initiated a project to introduce, evaluate and promote the use of cover crops and green manures (CCGM) in the communities of San Dionisio in 2001.

The CCGM legumes may significantly contribute to enhanced soil fertility, water and soil conservation and weed suppression. Some of these green manure legumes can be used as forage or even 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 the demonstration of the multiple uses offered by CCGM including their drought tolerance, participatory learning about CCGM, their management within the local community and the identification of key farmers to provide feedback on local soil organic matter management.

Materials and Methods

A workshop was held in San Dionisio in April 2001 to which all members of Campos Verdes had been invited. A total of 27 farmers participated in the event and the proposed project was presented and discussed.

Sites with different soil and climate conditions throughout San Dionisio were identified and CCGM options discussed. Farmers chose *Mucuna pruriens*, *Canavalia ensiformis* and *Lablab purpureus* as CCGMs for the experiment. At the end of September 2001 the experiments were established on 8 farms in different communities of San Dioniso (Table 63).

The experiments consist of seven treatments, which were arranged, in a randomized block design with 3 replicates at each site. The treatments are summarized in Table 64. CCGM legumes were sown in maize plots (4 x 4 m) at the traditional bean sowing distance (0.4 x 0.4 m).

Table 63. Location and site description of on-farm cover crop/green manure experiments in San Dionisio, Nicaragua.

Farmer	Community	Latitude	Longitud	Altitude	Observations
D. Salgado	Piedra Colorada	12° 49' 47.2 N	85° 51' 51.1" W	504	River valley
A. Castro	Susuli central	12° 48' 29.2" N	85° 50' 24.5" W	564	Slope
J. Hernández	Susuli arriba	12° 47' 48.0" N	85° 50' 05.2" W	565	Steep slope
V. Cebilla	Corozo	12° 47' 02.2" N	85° 52' 17.6" W	484	Slope
J. Orozco	Carizal	12° 47' 08.2" N	85° 54' 15.0" W	715	Moderate slope
J. Jarquín	Piedras Largas	12° 43' 32.6" N	85° 49' 43.1" W	474	Slope
J. Hernández	Jícaro	12° 46' 19.2" N	85° 50' 15.6" W	530	Very steep slope
E. Ochoa	Ocote arriba	12° 45' 23.2" N	85° 53' 17.3" W	735	Slope

Table 64. Treatments included in on-farm cover crop/green manure experiments in San Dionisio, Nicaragua.

Treatment	Year 2001*	Year 2002
1	Maize	Maize without N-fertilizer (Control)
2	Maize	Maize with low N-fertilizer level
3	Maize	Maize with high N-fertilizer level
4	Maize	Maize with very high N-fertilizer level
5	Maize with *Mucuna*	Maize without N-fertilizer
6	Maize with *Canavalia*	Maize without N-fertilizer
7	Maize with *Lablab*	Maize without N-fertilizer

* Cover crops/green manures were sown into existing maize plots in September when the maize was entering its mature stage.

The evaluation of the legumes sown as green manures was carried out on a monthly basis. All CCGMs were kept in the maize plots throughout the dry season 2001-2002 and maize was planted into the CCGM mulch on the onset of the wet season 2002. Fertilizer treatments were applied and plant height, biomass production and maize yields were recorded at harvest in November 2002. Field days were held throughout the participating communities in order to demonstrate and discuss practical management issues with farmers. By discussing soil fertility issues farmers to provide feedback on local soil organic matter management were identified.

In September 2002 a German student from the University of Hohenheim initiated a survey on local soil organic matter management among the identified key farmers in San Dionisio. Farmers using any kind of organic matter to improve soil organic matter contents were interviewed and their experiences and techniques documented. In collaboration with the PE-2/TSBF –Project additional information on farm nutrient balances were also collected.

Results and Discussion

The absence of rainfall at the beginning of October 2001 affected the CCGM establishment and biomass production at all sites, reducing potential fertility effects on the subsequent maize crops. The effect of green manures was small at high-altitude sites such as Ocote arriba, Carrizal and the Susuli sites on the more humid eastern zone of San Dionisio. On the other hand, plots at Susuli central were lost due to massive leave cutting ants infestation. Plant growth at the Jicaro and Piedras largas sites were heavily influenced by early drought conditions and biomass production was very low.

In Table 65 we show plant height, soil cover and biomass production of all green manure species prior to the maize crop. Maize plants sown after cowpea suffered from a dry spell in August 2002 and became stunted. Subsequently, these sites had to be eliminated from the experiment. Reliable data where only obtained from the Carrizal and Susuli sites. Data recorded by farmers at Corozo, Piedra Colorada and Ocote arriba were found to be unreliable due to inconsistencies and anticipated harvest.

Table 65. Plant height, soil cover and biomass production of green manure species prior to maize crops at San Dionisio (15 weeks after establishment)

Location	Species	Plant height (cm)	Soil cover (%)	Biomass (g/m^2)
Ocote arriba	*Mucuna pruriens*	30	100	456
	Canavalia ensiformis	55	100	325
Piedra colorada	*Mucuna pruriens*	30	75	178
	Canavalia ensiformis	55	40	68
	Lablab purpureus	55	60	103
Susuli arriba	*Mucuna pruriens*	42	100	388
	Canavalia ensiformis	67	100	299
Carrizal	*Mucuna pruriens*	40	100	255
	Canavalia ensiformis	57	75	108
	Lablab purpureus	15	40	89
Corozo	*Mucuna pruriens*	25	50	122
	Canavalia ensiformis	40	60	77
	Lablab purpureus	12	40	60
Susuli Central	-	-	-	-
Jicaro*	Cowpea IITA 637-1	41	80	389
	Cowpea IITA 284-2	39	60	175
	Cowpea IITA 719	36	50	194
Piedras largas*	Cowpea IITA 637-1	51	80	299
	Cowpea IITA 284-2	44	100	234
	Cowpea IITA 719	46	80	253

* Data recorded 9 weeks after establishment

Results from Carrizal indicate that the application of CCGMs can replace the local high fertilizer application level (N high = 92 kg 15-15-15) in maize crops. *Mucuna pruriens* and *Lablab purpureus* show a slightly better effect on plant height, biomass production and maize yield than *Canavalia ensiformis* (Table 66).

At the Susuli site effects were not as pronounced as at Carrizal. This was expected since the Susuli site was heavily hit by hurricane Mitch in 1998 when much of the top soil layer was lost. Production data are somewhat below the average production level for San Dionisio, which, might be attributed to experimental, and data recording errors in this farmer-led experiment.

Partial replacement of chemical fertilizer in San Dionisio can be achieved through the use of CCGMs as demonstrated in this experiment. While differences in organic matter contents were not obtained, it could be shown that soil fertility is affected by CCGMs when grown before the long dry season. This is especially important for the improvement of the prevailing maize-bean system that is running down more and more soil fertility at hillsides locations such as San Dionisio.

Table 66. Plant height, biomass production and maize yield under different fertilizer and cover crop/green manure treatments at San Dionisio, Nicaragua

Location	Treatment	Plant height (cm)		Biomass (g/m2)		Maize yield (g/m2)	
Carrizal	N 0	105	e*	341	e	43	d
	N low	140	d	545	d	89	c
	N high	162	c	806	b	93	b
	N S-high	188	a	1362	a	125	a
	Canavalia	165	c	681	c	92	b
	Mucuna	177	b	885	b	111	b
	Lablab	175	b	843	b	105	b
Susuli arriba	N 0	80	b	255	c	23	b
	N high	100	a	634	a	77	a
	Canavalia	95	a	514	b	66	a
	Mucuna	98	a	620	a	74	a

N 0 = no fertilization; N low = 46 kg 15-15-15; N high = 92 kg 15-15-15; N S-high = 92 kg 15-15-15 + 100 kg urea)

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

The survey on the soil organic matter management in San Dionisio revealed that certainly the majority of the interviewed farmers had positive experience growing CCGMs, but only few continued to grow them. Reasons indicated were: lack of time, lack of land, the invading growing habit especially of *Mucuna* sp. and the difficulty of obtaining seed.

Mucuna sp. was the CCGM, which was grown most in the target area. The majority of the farmers visited, who cultivated *Canavalia*., *Lablab,* and/or *Mucuna,* did grow CCGMs in monoculture. The majority of the interviewed farmers had at least once experiences with organic compost. Because of high investments in time and effort, farmers use organic compost only in high value crops. Mainly the farmers growing organic coffee used compost regularly. Animal manure was not mentioned as a source of organic matter improvement.

In general the survey indicated that farmers consider CCGMs as beneficial, but lack sufficient information and consciousness to adopt these technologies. Without doubt, projects on CCGMs have to take into account the greater necessity to work in a participatory framework in order to offer real alternatives in complex production systems.

Complemented by outputs of further CIAT activities on soil fertility, the above described on-farm CCGMs and survey results were presented at a soil improvement workshop in December 2002. During the workshop farmers suggested an integrative project approach capitalizing on obtained results from the different activities presented since the year 2000. A project proposal was developed combining the findings of the work with multipurpose forage germplasm (cowpea, *Canavalia brasiliensis*, *Mucuna pruriens*, *Lablab purpureus*) with production system alternatives. In collaboration with local farmer organizations (Cooperativa Sueños Realizados y Campos Verdes) CIAT developed a project proposal for a small grant from FUNICA, which was approved in May 2003. Different systems alternatives are validated on 31 farms in the Wibuse-Jicaro watershed for a time period of two years. Erosion is monitored in the large validation plots (20 x 20 m) and reliable data for economic analyses are recorded. Farmer field days on a regular basis ensure the on-going debate on project objectives and evolving problems. Participatory evaluations complement agronomic data collections. The initiated project can be seen as a further step to scale our activities in CCGM from plot levels to watershed levels.

4.1.8 Forage seed production by small farmers in Honduras and Nicaragua

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The adoption of new forage technologies are intimately connected to the availability of good quality seed at reasonable prices. Small farmers, often located in remote areas, do not have the economic ability to buy commercial seed and have no direct access to seed markets. Furthermore, not all recently developed forage germplasm is available through conventional market channels. In order to overcome this traditional bottleneck for adoption, we supported farmer groups in initiating their own forage seed production schemes for selected species such as *Brachiaria brizantha* (CIAT 26110) cv. Toledo and *Cratylia argentea* (CIAT 18668). The objective was not only to increase seed availability for participating farmers and their communities, but also to connect these farmers through a value-added forage product (seed) to local, regional and national markets. Furthermore, results from these production efforts complement our knowledge about seed production potentials under different environmental conditions in Central America.

In December 2002, a small but dedicated farmer group (12 farmers) in Yorito, Honduras, harvested 350 kg of *B. brizantha* cv. Toledo in close collaboration with DICTA and CIAT. This was a slightly lower seed yield than farmer had expected. The group, already working under their new own label – "PRASEFOR" (Productores Artesanales de Semillas Forrajeras), began to explore marketing opportunities at the beginning of 2003. Market demands such as seed quality and packaging requirements were identified. Quickly, demand for seed from PRASEFOR exceeded supply (Photo 26). While some seed was sold on the local market or were kept for the expansion of production area in 2003, FONDEAGRO, a development project in Nicaragua, which assists 1000 small livestock holders in the Matiguás region, bought most of the available seed.

On the bases of this success, the group defined a seed production target of 1,600 kg for 2003. This means further expansion of production areas (to a total of 20 ha), additional training in production aspects, post-harvest technology, seed quality, storage and marketing. First contacts were made with the formal seed sector to explore sale potentials.

A comparable development is taken place in Pantasma, Jinotega, Nicaragua, where CIAT in collaboration with INTA is working on the formation of a group similar to PRASEFOR. A total of 5 farmers started out to established cv. Toledo in 2002 but failed to produce seed at the end of the year. Further technical assistance was provided for the group and a training course will be conducted prior to the harvest in December 2003. The already established and working small farmer seed enterprise PES San Dionisio, experienced in the production of bean and maize seed, will be collaborating in post-harvest technology and marketing. It remains to be seen if production levels in grasses will allow for competitive prices on the Central American seed market, where Toledo seed is now offered for more favorable prices. Forage legume seed, in contrast, seem to enter a non-competitive market offering good opportunities for small scale farmer seed enterprises.

In view of these changing market opportunities farmers in Nicaragua with interest in seed production were encouraged to plant the shrub legume *Cratylia argentea*, since demand is constantly raising and seed supply was again short throughout 2002-2003. A total of 6.9 ha of Cratylia were established on-farm at different locations (Managua, Cuidad Dario, Siuna, Rivas, San Juan del Sur, El Suace, Posoltega, Santo Tomás, Jinotega, Esteli, Condega) (Photo 27) throughout Nicaragua which complement the already established plots at CIAT's reference site in San Dionisio, Matagalpa (1.6 ha). While seed harvesting and post-harvest operations are technically less demanding for Cratylia, first considerable seed production can only be expected in the second year after establishment. Plots in San Dionisio will be harvested in January 2004.

A major advance for the work in seed production was the completion of the regulation on seed production in which CIAT was heavily involved. Standards and guidelines are now in place for seed certification, import and export, sale and quality aspects. INTA and MAGFOR will assist in achieving the certification of seed produced by small farmers.

Photo 26. A farmer group (12 farmers) in Yorito, Honduras, harvested 350 kg of *B. brizantha* cv. Toledo in close collaboration with DICTA and CIAT

Photo 27. *Cratylia argentea* establishment for seed production in Nicaragua

4.1.9 Farmer participatory research in action in Central America

Contributors: L. A. Hernández Romero, M. Peters, A. Schmidt, L.H. Franco and G. Ramírez (statistician), with the collaboration of NARS (C. Burgos, DICTA Honduras, M. Mena, INTA Nicaragua, W. Sanchez, MAG Costa Rica) and NGO (M. Posas, SERTEDESO Honduras, J Bustamante, Fundación Ecotropica Costa Rica) partners, technicians (H. Cruz and C. Davies) and many farmers in Honduras, Nicaragua and Costa Rica

Rationale

Forage germplasm in its multiple uses - for example as feed, for the suppression of weeds, maintenance and improvement of soil fertility and for erosion control - could play an important role in improving the well being of the small and medium size farmers in Central American hillsides. However, adoption - particularly of forage legumes - has been limited, possibly due to lack of direct interaction with the farmers. Therefore it is necessary to develop forage technologies with farmers, using participatory approaches. To address this issue, CIAT in collaboration with NARS, NGO's and farmer groups is identifying forage options with farmer participation.

We anticipate that the work in Central America will contribute to the development to a strategy by NARS for the diffusion of forage-based technology for small farmers. Thus the interaction with national partners – alongside the farmers – will be of paramount importance to the success of the approach.

4.1.9.1 Farmer Criteria for Selecting Forages

Utilizing farmer criteria, the procedure for participatory selection of forages described in the 2002 Annual Report was analyzed using a different statistical procedure. Utilizing an updated database containing 1623 records from Honduras, Nicaragua, and Costa Rica (data collection until July 2003), correspondence factorial analysis (CFA) was employed and compared with results from Principal Components Analysis (PCA, see Annual Report 2002).

The classification of criteria and technologies using factorial analysis of correspondence is a classical method used to analyze large matrix of data that includes qualitative variables (Guinochet, B.1973. Logistic regression, survival analysis and the Kaplan-Meier curve. J. American Statistical Association 83: 214-225). This is the case for matrixes of numbers of coexistence between sets, i.e. sets of technologies and the set of criteria given (in this case by diverse producers groups). The analysis consists in calculating the distance between elements of each one of the sets, taking as pairs (clones and related criteria with the respective rankings).

Following the sequence of analysis described in the 2002 Forages Annual report (see also Annual report 2001-2003 Project SN-3, page 115) the procedure used for this analysis included the following steps: a) validation of criteria for forage technologies, based on farmer input, and b) determination of relative importance of criteria using relative frequencies and CFA for different forage technologies evaluated (i.e grasses, herbaceous, legumes, shrubs legumes and green manures).

According to a cross tabulation of frequencies, across all forage technologies, plant color was the most important criteria in farmer assessment. Across seasons this criteria was given more importance in the dry season (greater frequency). Yield was the next important criteria followed by cover, leafiness and competitiveness. Similar results have been found with PCA analysis utilizing a smaller data set (928 records) in 2002.

Disaggregated analysis according to specific forage technologies

Grasses: In the wet season, the most important criteria were: color, yield, leafiness, cover, competitiveness, reproductive capacity, height, and softness (frequencies in descending order).

The global analysis for the wet season showed that, the first four Dims (row coordinates factors) explained 86.2 % of the variation, a high percentage in analyzing participatory work. Height, competitiveness, and yield were defined in Dim 1, while cover, softness, and reproductive capacity were the most important parameters in Dim 2. Leafiness was the most important criteria in Dim3, while color was the most important criteria defined in Dim 4.

The global analysis for the dry season showed that the first three Dims explained 92.80 % of the variation.

Color, yield, leafiness, and softness were defined in Dim 1, while competitiveness and reproductive capacity were the most important parameters in Dim 2; cover was defined in Dim 3

These results indicate that for farmers it is important that grasses have good establishment and yield and compete effectively with weeds, regardless of season of the year. In the wet season, height was an important additional criterion in grasses. Color is important throughout seasons indicating healthy productive plants in the wet season and drought tolerance in the dry season. Confirming the results shown in the 2002 Annual Report, *Brachiaria brizantha* cv. Toledo and *Brachiaria* hybrid cv. Mulato were the grasses best meeting farmers 'criteria, the latter having a particular high acceptance in the dry season. Other forage options attractive to farmers were *Panicum maximum* cv Camerún and *B. brizantha* CIAT 26646. For king grass there was a close relation between height and preference probably related to the use of this material for cut and carry.

Herbaceous pasture legumes: For herbaceous legumes 3 Dims explained 91.2 % and 98.6 % of variation in the rainy and dry season, respectively. Among the legumes, *Desmodium ovalifolium* CIAT 33058, *Centrosema plumieri* DICTA and *Arachis pintoi* 22160/17844 were the options for the dry season meeting most criteria by farmers. When taking into account both seasons of the year, the selection criteria used by farmers to select pasture legumes included cover, color, competitiveness, yield and leafiness. Comparable tendencies were found with PCA in 2002. However, subsequent analysis with farmers showed that preference of farmers for herbaceous pasture legumes is low, as at least in our sites farmers since the main interest of farmers are new grasses, followed by shrub legumes and legumes for green manures.

Shrub legumes: For shrub legumes the first three Dims explained 96.7 % and 94.7 % of variation in the rainy and dry season, respectively. Most important farmer selection criteria were in descending order color, leafiness, yield, reproductive capacity and (fuel) wood. The most preferred species across seasons were *Leucaena leucocephala* CIAT 17263 and *Cratylia argentea* CIAT 18668. *Calliandra calothyrsus* CIAT 22316 was the most preferred accession for providing (fuel) wood. Similar results were found in 2002 using PCA analysis.

Green manure legumes: For the green manure legumes, three Dims explained 82.1 % of the variation in the wet season. Due the annual nature of most species dry season results were not measured. Criteria of major importance in selection of forages are in descending order: color, competitiveness, yield, cover and leafiness. The species that met most of these criteria were *Lablab purpureus*, *Mucuna pruriens* and *Pueraria phaseoloides*.

In response to the above findings, in subsequent participatory experiments *Canavalia brasiliensis* and *Vigna unguiculata* were introduced as options that meet farmers criteria for green manures; farmers are quickly taking up these materials for further testing, as described in sections 4.2.1 and 4.2.2. New accessions of *Lablab purpureus* were introduced in 2003, but no information of farmer preferences exists yet.

4.1.9.2 Scaling of forage options in hillsides of Honduras and Nicaragua

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In Tables 67 and 68 we show results from uptake of forage options in Honduras and Nicaragua, over the past 2 to 2.5 years.

In Honduras now more than 400 farmers are now testing and employing various forage options, sown on about 180 ha, indicating a steady increase over time. The largest areas are planted to *Brachiaria* hybrid Mulato (CIAT 36061) and *B. brizantha* cv. Toledo; increase in area for the latter is driven by seed multiplication through the farmer-led seed enterprise PRASEFOR (see Section 4.1.8) or purchase of the materials now known to farmers from commercial seed producers. Though areas are still small there is an increasing farmer interest in *Cratylia argentea* and various cowpea (*Vigna unguiculata*) and Lablab accessions. In Nicaragua uptake of forage options by farmers also is gaining speed, with now more than 150 farmers testing and employing forage options on their farms. Preferred options are *B. brizantha* cv. Toledo and *Brachiaria* hybrid cv. Mulato. There is a recent interest in testing *Calliandra calothyrsus*, utilization as fuel wood, which is an important selection criteria.

Table 67. Distribution of forage materials in Honduras, 2001-2003

Forage Species	Honduras							
	2001		2002		January – August 2003		Total	
	Area (ha)	Farmers (No.)	Area (ha)	Farmers (No.)	Area (ha)	Farmers (No.)	Area (ha)	Farmers (No.)
A. gayanus 621	0.24	6			0.09	2	0.33	8
B. dictyoneura 6133	0.52	12					0.52	12
B. brizantha 26110 *	1.11	25	16.29	19	3.29	37	20.69	81
B. hybrid 36061			12.30	6	45.10	23	57.4	29
P. maximum 16031	0.68	17					0.68	17
P. purpureum cv. Camerún	0.64	16	0.51	3			1.15	19
A. pintoi 22160	0.04	1					0.04	1
C. argentea 18668	0.89	15	3.44	24	0.83	6	5.16	45
C. pubescens 15160	0.04	2					0.04	2
L. leucocephala 17263	0.55	11					0.55	11
L. purpureus	0.10	1			0.03	8	0.13	9
S. guianensis 184	0.12	6					0.12	6
M. pruriens IITA BENIN	0.10	1			0.04	1	0.14	2
V. unguiculata (Verde Brazil)			0.14	2	0.29	8	0.43	10
V. unguiculata CIDICCO 1					0.12	17	0.12	17
C. brasiliensis CIAT17009					0.013	3	0.013	3
C. ensiformis					0.063	6	0.063	6
Local cowpea					0.035	3	0.035	3
L. purpureus 106471					0.004	4	0.004	4
L. purpureus 34777					0.009	7	0.009	7
L. purpureus 52535					0.011	7	0.011	7
L. purpureus L987					0.004	4	0.004	4
P. vulgaris					0.002	1	0.002	1
V. unguiculata 1088/2					0.041	11	0.041	11
V. unguiculata 1088/4					0.021	3	0.021	3
V. unguiculata 284/2					0.063	16	0.063	16
V. unguiculata 573/5					0.026	6	0.026	6
V. unguiculata 637/1					0.030	6	0.030	6
V. unguiculata 715					0.034	8	0.034	8
V. unguiculata 740					0.021	6	0.021	6
V. unguiculata 9611					0.152	28	0.152	28
V. unguiculata CIDICCO 2					0.090	11	0.090	11
V. unguiculata CIDICCO 4					0.149	26	0.149	26
V. unguiculata FHIA					0.182	33	0.182	33
Total	5.03	113	32.68	54	50.74	291	88.45	458

***Note:** Additionally seed of *B. brizantha* CIAT 26110 (cv. Toledo) sold by PRASEFOR amounts to an additional 14 ha sown in Sulaco, Victoria, Yorito and Yoro. Through commercial seed sale of Toledo another 80 ha were established.

Table 68. Distribution of forage materials in Nicaragua, 2002-2003

Forage Species	2002		2003		Total	
	Area (ha)	Farmers No.	Area (ha)	Farmers No.	Area (ha)	Farmers No.
B. decumbens	0.16	4			0.16	4
B. brizantha 26110	1.48	37	5.35	19	6.83	56
B. hybrid 36061	1.76	44	4.1	14	5.86	58
C. calothyrsus			0.28	35	0.28	35
Total	3.4	85	9.73	68	13.13	153

4.2 Partnerships in Asia to undertake evaluation and diffusion of new forages alternatives

Highlights

- Documented and published the impact of the Project "Forages for Smallholders" in Southeast Asia
- Doubled (247 to 467) in a two year period the number of farmers planting forages in Laos mostly as a result of direct farmer experience rather than promotional efforts
- Farmers in Laos raising pigs see benefits of feeding *Stylosanthes guianensis*: labor saving, and increased survival and growth rates of piglets
- Launched a new project funded by ADB (Improving livelihoods of upland farmers using participatory approaches to develop more efficient livestock systems) for countries in southeast Asia

4.2.1 Completion of Forages for Smallholders Project in Southeast Asia

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Rationale

The project "RETA 5866 (Fourth Agriculture and Natural Resources Research at CGIAR Centers: Developing Sustainable Forage Technologies for Resource - Poor Upland Farmers in Asia"), in short called the "Forages for Smallholders Project" (FSP), started in January 2000. It was funded by the Asian Development Bank for a period of three years, and had an extension of 6 months. The objectives of the project were to:

A) Develop sustainable forage technologies for resource-poor farmers in upland farming systems in Asia.
B) Strengthen the capacity of National Agricultural Research Systems in the Bank's Developing Member Countries to develop and deliver these technologies to farmers.

The FSP was co-coordinated by the Centro International de Agricultura Tropical (CIAT), which is part of the Consultative Group on International Agricultural Research (CGIAR). The implementing agencies in the participating countries were:

China:	Tropical Pasture Research Center (CATAS), Hainan
Indonesia:	Dinas Peternakan, Samarinda and Directorate General of Livestock Services (DGLS), Jakarta
Lao PDR:	National Agriculture and Forestry Research Institute, NAFRI, Vientiane
Philippines:	Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD), Los Baños, Visayas State College of Agriculture (ViSCA) and Department of Agriculture, Region 10

Thailand:	Department of Livestock Development, Ministry of Agriculture and Cooperatives, Bangkok
Vietnam:	National Institute of Animal Husbandry (NIAH), Ministry of Agriculture and Rural Development (MARD), Hanoi

In what follows the main accomplishments of the FSP are summarized.

4.2.1.1 Development of sustainable forage technologies for resource-poor farmers in upland farming systems

In most countries, periodic surveys of a sample of farmers were carried out to assess changes in forage technologies, areas planted, and multiplication materials and seeds produced. The farmer for these surveys had been selected randomly, although weighed by districts or barangays.

In Lao PDR surveys covered 100% of the farmers. Average area of forages cultivated per household varied from 188 m^2 (Lao) to 4,210 m^2 (Thailand), Table 69. The largest area of forages by site is in Daklak, Vietnam, due to many farmers adopting and relatively large areas per farmer.

Table 69. Areas of forage cultivated in sample areas according to the most recent surveys, and extrapolation of forages in whole project area.

Country and site	Sample size	Total forage area (m^2)	Forage per household (m^2)	No. of house-holds involved	Area extrapolated to households involved (ha)
Philippines					
Malitbog	24	31,418	1,309	385	50.4
Cagayan de Oro	31	48,514	1,565	372	58.2
Cebu and Leyte				670	96.3
Impasugong and Manolo Fortich				236	33.9
Indonesia					
East Kalimantan	30	63,083	2,103	740	155.6
Vietnam					
Tuyen Quang	99	70,510	712	761	54.2
Daklak	60	81,882	3,625	976	353.8
China					
Hainan	30	36,627	1,221	176	21.5
Thailand					
Nakornratchasima	19	80,000	4,210	276	116.2
Lao PDR					
Luang Prabang and Xieng Khuang[1]	343	64,400	188	343	64.5
Total				4,935	1005

[1]FLSP (AusAID) has been operating in Xieng Khuang.

Impact in East Kalimantan, Indonesia

The study was conducted with about 85 farmers in three villages: Makroman, Sepaku and Samboja. Livestock systems varied from stall-feeding improved goats (Makroman), grazing under coconuts on improved pasture by Bali cattle (Samboja), to grazing and stall-feeding improved forages to Ongole cattle (Sepaku).

Forage availability: Before introduction of new forages, some farmers already used King grass for cut and carry. The participatory work on forages with farmers resulted in four technology changes: 1) reduction or disappearance of grazing on communal rangeland and increased pasture fencing (Samboja); 2) prolonged pasture time at home-plot (Sepaku); 3) pasture fencing in upland and old rice fields (Sepaku) and 4) planting of forage in contour lines and as cover crop associated with food and cash crops (Makroman and Sepaku). The average availability of improved forage area was 0.4 ha per farm. Most farmers in Samboja who adopted new forage had enough improved pastures to provide enough fodder for that their cattle. In Sepaku and Makroman, forage availability was still not high enough for optimum livestock production.

Ruminant productivity: Introduction of new forage species increased off-take of animals due to shorter inter-parturition periods, in all species and breeds. Twinning rate in goat increased in some herds. Improvement was also perceived through better body condition of animals resulting in better carcass quality and higher prices paid by butchers. Since new forage introduction in Makroman, farmers have doubled their herd size of goats. Increases in cattle numbers were less pronounced.

Reproduction rate of Bali cattle in Samboja was higher than for cattle and goats in Sepaku and Makroman. Off-take rate (animals sold in a period divided by the mean herd size during the period) in Samboja increased from 21 to 23.5% after adoption of forages. In Makroman en Sepaku, new forages associated to food and cash crops enhanced soil fertility and prevent erosion.

Labor requirements and gender: The adoption of new forage technologies reduced time needed to collect fodder as well as for forage crop maintenance. The spared time was put to good use, either for feeding more animals or by doing more off-farm work. Mean time saved was close to 20%.

The effects of new forage technologies on labour division were generally gender neutral. In one sub-district, youth were less often involved and in another, women were slightly more frequently involved. Sometimes women were more frequently involved in fodder collection as she replaced the husband doing off-farm activities. All genders appreciated new forage introduction positively as the impact on household income was high. To make immigration settlement possible, Indonesia developed infrastructures in swampy mostly uninhabited areas, and the original population also participated in FSP. Unequal income distribution in rural areas couldn't be attributed to FSP. FSP strategy improved fodder balance and increased the possibilities to extend livestock ownership, a major generator of rural income.

Other benefits and economic performance: Manure applied on food and cash crops contributed approximately 40% to farm household income from livestock in Makroman and Sepaku. This indicates the dependency of households on manure for soil fertility maintenance. In these two sub-districts market value of manure was approximately ten times its estimated mineral value. Apart from application on crops, farmers sold manure for cash income and estimated they applied less then 15% of manure on forage crops. As a result, productivity and quality of new forages was low. The use of manure on food and cash crops does not conflict, however, with the overall objectives of FSP, namely to improve livelihoods.

On average, increased ruminant productivity in the study area, in terms of cash income from sales of livestock and manure, resulted in an increased gross margin of 35 % per household. In addition to increased animal productivity, on average 24 % labor input was saved in the new forage systems in terms of days worked per year. The saving of labor amounts to an extra 28 % increased income from the livestock system, when time is valued in money.

The average rural income in the province in 2001 was US$ 184 per capita per year. The average income from livestock in the three villages increased from US$ 73 to US$ 92 per capita per year. This corresponds to an increase of 10.3 % of total income per capita per year. Saved labor could have contributed to another 11 % increase of total income per capita, depending on the availability of casual labor opportunity or the investment of saved labor in farm enterprises.

Impact in Mindanao, Philippines

Livelihood consisted of a variety of crops, dairy cattle, dairy buffaloes, beef cattle and goats and other small livestock. The average number of large ruminants per farm was 4.2.

Forage availability: Before the introduction of new forages, no cutting and carrying were practiced. Animals were tethered on roadsides or fields, or herded on hillside rangelands and fed with residue. The project had a significant effect on the quantity and quality of available forage; farmers who were growing forages derived 67 % of the feed resources from these forages.

Ruminant productivity: Farmers mentioned several benefits of new forage introduction: improved body condition and overall health of animals; increased length and quality of work by draught animals; greater pig and poultry production; larger amounts of available manure due to reduced herding-time and more animals.

Farmers observed increased numbers of offspring and shortened anoestrus after parturition: from three to one month in goats; one year to three months in cattle; and 10 to four months in buffaloes.

Labor requirements and gender: Time spared due to new forages was estimated at 30 minutes to 2 hours per day. This time was either invested in increasing the number of animals, or other farm activities such as tending to vegetables and fishponds, off-farm trading, and roadside food selling. Farmers could also make themselves and an animal available for hire for off-farm work or use the time to attend meetings and pursue administrative problems. More time and energy was available for social activities inside the household and in the community. In some areas, the reduction in time needed in the wet season was limited as the forage for cutting and carrying was often a long way from the village.

Life became more relaxed as it was easier to plan activities when animals were not grazing. The reduction or disappearance of tethering and herding also resulted in less destruction of crops. Consequently the production of maize, banana and vegetables, and the income from animals' work outside the farm, increased.

Most farm households that had ruminants prior to forage adoption increased their herd size after joining FSP. More labor input was required due to the increase in livestock numbers and as a consequence the time available for non-farm work decreased. The increased need for on-farm labor had a negative trade-off on the income from firewood as farmers did not have time to cut in nearby forests. However, this was a positive change for the environment.

The introduction of new forages had a gender effect in the Philippines: the involvement of women and children in tasks like herding and cutting diminished, and men were responsible for more livestock tasks. A large increase in the number of animals owned by early adopters resulted in the need for greater labor input. This created labor in rural areas and reduced labor migration by young people.

Other benefits and economic performance: In the Philippines, improved forage species increased animal production, improved soil conservation and saved farmers time. Net yearly income per household from animal production increased from $54 to $157 in the farming community at Malitbog, and from $68 to $503 in Cagayan de Oro. Planting forages in contour lines increased crop production slightly and contributed another $22.50 to yearly income. The reduction in labor requirements allowed households to make $36 per year from other activities.

Impact in Tuyen Quang, Vietnam

Livelihood consisted of a variety of crops, a variety of animals including fishponds, and several sources of off-farm income. The average number of large ruminants per farm was 0.8. Comparing farmers who had adopted them several years ago with farmers who had started less than one year ago was used to assess the impacts of new forage technologies.

Forage availability: Farmers reported higher yields of forages compared to native grass. The high yields of new forages allowed farmers to keep more animals or to keep animals in zero grazing. Improved forages enabled other farmers to start keeping animals, as they were able to produce sufficient fodder from their small plots. The average estimated contribution of new forages to animals' diets was 53 % during summer and 32 % during winter.

Ruminant and fish productivity: Ruminant productivity increased through faster growth of animals, higher price received for the animals at the market due to better body condition, increased working capacity of draught animals, and increased amounts of manure available. The productivity of fish also increased the period until marketing was reduced from 11 months to nine months due to the availability of new forages.

Labor requirements and gender: Saved time was an important benefit for most farmers keeping ruminants. This was not the case for those who had increased their number of animals, or had just started keeping buffalo or cattle, since the introduction of new forages. The number of labor days per year required for large ruminants reduced from 258 to 149 for farmers with and without forages, respectively. Farmers with forages had saved on average 120 days per year in the ruminant system. The mean number of saved days for fish production was 30 days per year, which corresponds to approximately 40 minutes per day. Two-thirds of adults' saved time was used for productive farm activities. Family members used the remaining saved time for leisure, training and study.

Positive gender effects were significant in Vietnam. Women and children benefited most from the reduction in time spent cutting, carrying and herding. They used this extra time for educational and cultural activities. According to women, forages had a positive effect on other crops due to soil conservation and manure availability. Labor saved from livestock was used to better manage crops, resulting in higher yields. Higher yielding crops then required increased labor time for harvesting and processing. Saved time was invested in a range of farm activities including cash crops like rice, cassava, beans, sugarcane and fruit trees. Activities women appreciated more were planting forages and feeding fish.

Other benefits and economic performance: In Vietnam, improved forage systems also had a pronounced effect on income levels and welfare. Net income from ruminant-fish production systems increased from $99 to $199 per year. Saved time also allowed households to increase their income from other, mainly agricultural, activities. This contributed to an additional yearly income of $52 per household. Farmers were grouped in four income classes; the majority was in the class that earned between US$ 301 and US$ 736 per year per household. An increase of $ 152 from the livestock system therefore corresponds on average to an increase in total household income of 29 %.

Poorer farmers who depended more on livestock due to small land area, benefited most from the improved forages. Improved forages allowed them to keep large ruminants—increasing their income from livestock— and intensify their production systems. Other positive effects on rural development included a reduction in the number of farming conflicts, rehabilitation of barren land and reduced use of pesticides.

4.2.1.2 Scaling-up forage technologies from farm to community and provincial levels

At the end of 1999, about 1750 farmers spread over 19 sites in Indonesia, Philippines, Vietnam and Lao PDR were evaluating forages on-farm, in the FSP project funded by AusAID. In 2000, the ADB funded FSP project adopted several sites from the earlier project: East Kalimantan in Indonesia, Malitbog and Cagayan de Oro in the Philippines, Tuyen Quang and Daklak in

Vietnam, and Luang Prabang in Lao PDR. Some other sites were added in 2000: akornratchasima in Thailand and Hainan in China. A strategy for scaling-out was developed with the focus sites playing a central role. The expertise of researchers and field workers at the focus sites in research on forages and facilitating the technology development was used to train other new staff in neighbouring districts or provinces.

Participatory diagnosis (PD) and planning remained a key activity for starting the technology development process at new sites. Farmers at the focus sites who had been experimenting with forages for several years were asked to host cross-visits and receive farmers from the neighboring villages, districts and provinces. The scaling process within countries in the FSP is shown in Table 70.

Table 70. Scaling out to new sites within countries.

Year	Indonesia (East Kalimantan prov.)	Philippines (Mindanao and Visayas)	Vietnam (Daklak and Tuyen Quang prov.)	Lao PDR	Thailand (Nakornrat-chasima prov.)	China (Hainan prov.)
2000	Penajam Paser Utara[1] Samarinda[1] Kutai Balikpapan Bulungan Berau	Malitbog[1] Cagayan de Oro[1] Impasugong Manolo Fortich	Madrak[1] Ea Kar Han Yen[1] Yen Son Son Duong	Luang Prabang[1]	Sungnuen Sikhew Dankhuntod	Baisha Ledong Danzhou
2001	Penajam Paser Utara Samarinda Kutai Balikpapan Bulungan Berau	Malitbog Cagayan de Oro Impasugong Manolo Fortich Cebu Leyte	Madrak Ea Kar Han Yen Yen Son Son Duong	Luang Prabang Savannakhet	Sungnuen Sikhew Dankhuntod	Baisha Ledong Danzhou
2002	Penajam Paser Utara Samarinda Kutai Balikpapan Bulungan Berau East Kutai Central Kutai	Malitbog Cagayan de Oro Impasugong Manolo Fortich Cebu Leyte	Madrak Ea Kar Han Yen Yen Son Son Duong	Luang Prabang Savannakhet	Sungnuen Sikhew Dankhuntod Pakchong	Baisha Ledong Danzhou Dongfang

[1] Adopted sites from FSP phase 1.

In Table 71 we show an overview of how many PD and cross visits were conducted, how many farmers participated, and how this led to new farmers experimenting with forages on their farms. On average about 18 farmers would participate per PD, and about 77 % of those farmers would start planting forages. Many of those farmers were encouraged by what they saw and heard during the cross visits. As a result, a total of 4200 new farmers have started to grow and experiment with forages during the duration of the project.

Table 71. Scaling-out activities and number of new farmers experimenting with forages.

Year	No. of participatory diagnoses (PD) conducted	No. of farmers participated in PD	No. of new groups	No. of cross visits organized	No. of farmers partic. in cross visits	No. of new farmers planting forages
2000	45	1087	52			748
2001	151	2173	179	187	1330	1537
2002	101	2148	52	141	1833	1870
Total	297	5408				4155

There have been efforts of monitoring what happened to the early and subsequent experimenting farmers in terms of adoption, expansion within their farm, and continuation to grow forages. A large drop out was observed in Luang Prabang, where at the beginning of the wet season in Luang Prabang there were 262 farmers growing forages; eight months later there were 170 farmers remaining. Another big drop out was observed at Malitbog. Some farmers naturally drop out due to various reasons such as:

- Feed shortage was not a problem (e.g. in Luang Prabang).
- They expected livestock dispersal on loan from the government, but no animal was received (e.g. in Malitbog).
- They no longer have ruminant livestock, due to emergency sales.
- They abandon their farm to seek employment elsewhere.
- They get absorbed in other dominating farm activities such as cash crops.
- Forages were overgrown by weeds due to labor pressure in early stage.

As said earlier, there were already farmers growing forages at the focus sites, which were inherited from the previous FSP. Out of those 1750 early farmers, about 800 were found in the focus sites of the new FSP. At he end of 2002 there were 4780 farmers, including early adopters, growing forages at the sites where FSP phase II was active (Table 72). The total number of drop -outs therefore was about 200 (4752 – 800 – 4155).

Table 72. Total number of farmers growing forages at FSP sites at December 2002, per country.

Country	Indonesia (East Kalimantan prov.)	Philippines (Mindanao and Visayas)	Vietnam (Daklak and Tuyen Quang prov.)	Lao PDR (Luang Prabang Prov.)	Thailand (Nakornrat-chasima prov.)	China (Hainan prov.)
No. of farmers	740	1663	1737	160	276	176
Grand total:	4752					

Guidelines for scaling-up forage technologies. The FSP is an example of how a research project started in a conventional way, with little farmer participation before 1995, with on-farm experiments being largely contractual. More than 500 species and varieties of forages were screened in a few locations. From 1995, FSP phase II and I engaged farmers in research on forage with progressing levels of participation. Research went from centralized community plots of well laid out test plots of 10-20 best bet species and varieties to plots on-farm where farmers decided on the species and varieties to test, the lay out of the plots, and the methods of harvesting. There are three new dimensions that this phase of research entered: (1) the move from test plots to forages integrated with other food or cash crops, either as cover crops, intercrops, relay crops, live erosion barriers or live fences, (2) the forage crops were evaluated not only for growth characteristics but also for feed for livestock, (3) larger areas of one or two favorite species or varieties were grown at farm level (Figure 70).

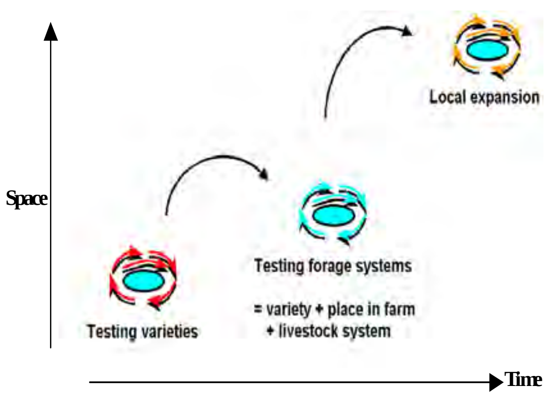

Figure 70. Adoption: from on-farm test plots to on-farm expansion (Adapted from Horne et al., 2000).

The process described in Figure 70 plays within the boundaries of a farm. Scaling out and scaling up, however, implies spreading of technologies across farm boundaries. There are principles to be considered for successful and sustainable scaling out. The FSP has developed a conceptual framework to guide national teams of fieldworkers, scientists, administrators and policy makers in this process (Figure 71).

There is a natural sequence of research activities (stages 1 – 11 of Figure 71). The first step for either starting research at a focus site, or scaling out to a new site is to gather secondary information and to carry out a rapid rural appraisal with a wide range of stakeholders. If a need for forage R&D is perceived, extension workers of the Local Government Units (LGU) are trained in forage agronomy, participatory research, and gender analysis. During these courses, the more active and motivated extension workers, who can effectively lead work in the project, are identified (step 2). The selected extension workers are assisted in their first

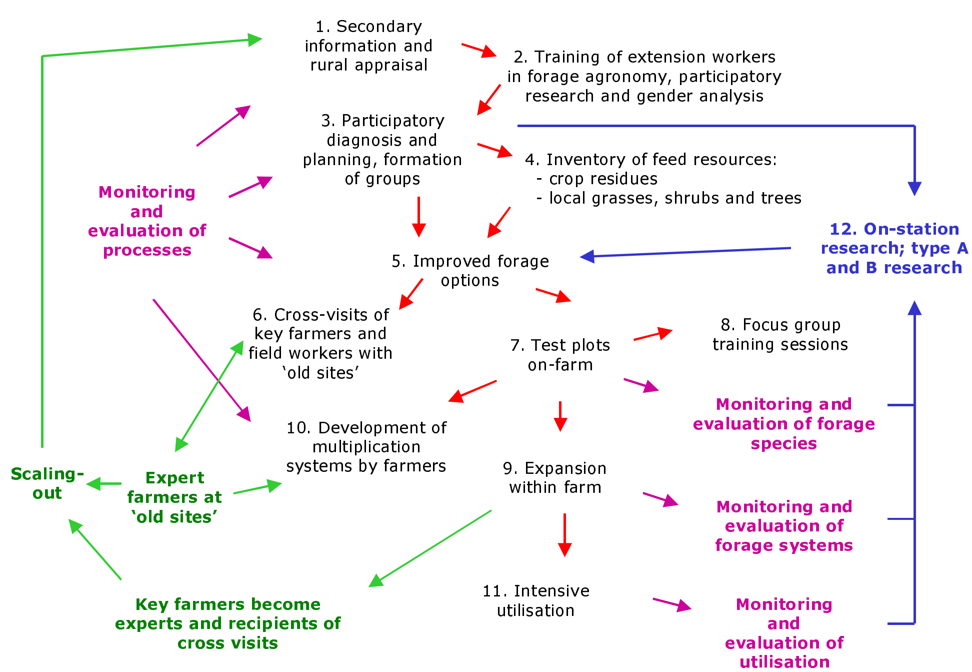

Figure 71. Research and development processes in FSP

participatory diagnosis and planning exercises with their communities (step 3). Community inventory of existing feed resources greatly assists the identification of suitable improved possible forage systems, which are offered to farmers (steps 4 and 5). If focus sites with experienced farmers exist elsewhere, cross-visits are facilitated for farmers to visit them at the new sites, even if it might involve extensive travel (step 6).

About 1 year after initial planting, farmers are likely to perceive effects on animal productivity, soil fertility, or erosion control. Farmer groups are sometimes trained on specific issues relevant to their stage of research or development with forages. Multiplication of planting materials or seeds is an essential step in the scaling out process (steps 7 to 11).

The green arrows in the diagram pointing left indicate the processes of scaling out, whereas the purple arrows indicate the different levels of monitoring and evaluation, which provided feedback to stakeholders, and assisted in identifying strategic research issues (step 12). Many tools were used to facilitate the scaling out process (Figure 72). A case study was written entitled ‘Issues and Strategies for Going to Scale: A Case Study of the Forages for Smallholders Project in the Philippines’ (Roothaert and Kaaria, in press).

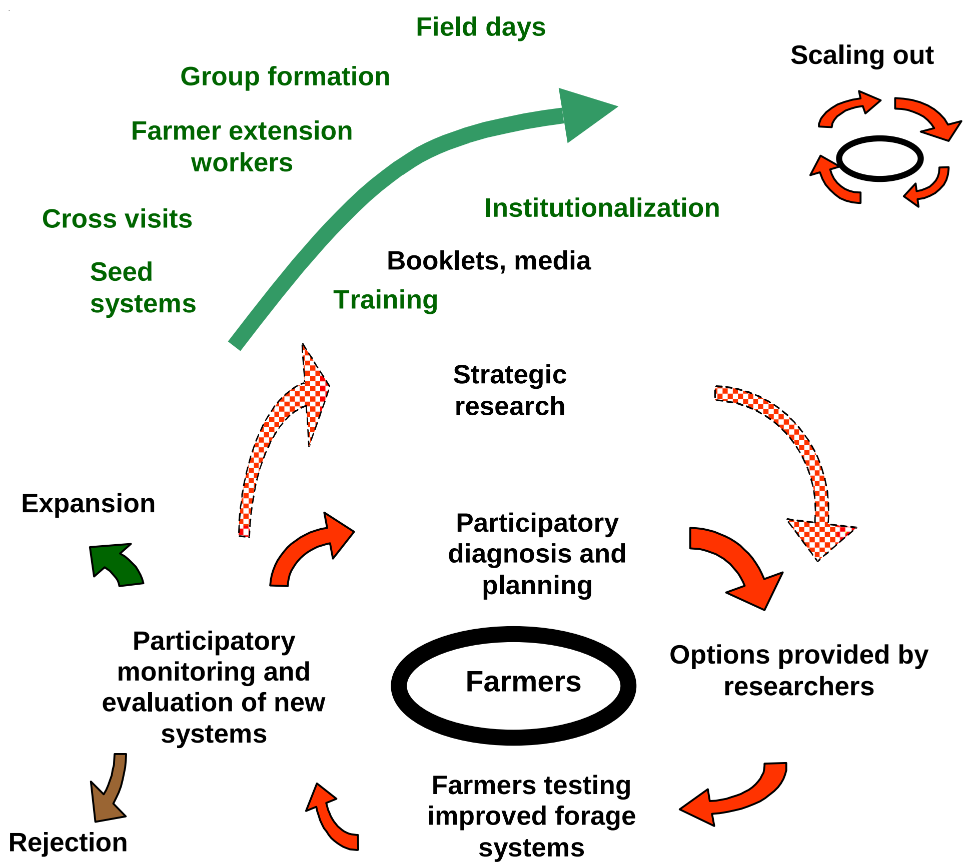

Figure 72. Farmers research process and tools for scaling out.

4.2.1.3 Capability in member countries for developing and disseminating forage technologies using farmer participatory approach (FPA)

Many training activities have been conducted towards capacity building of project staff and their partners in PR China, Indonesia, Lao PRD, Philippines, Thailand and Vietnam. Training activities were either technical or methodological in nature, or a combination of the two. An examples of a technical course is the course on 'Forage growing and animal production', held in September 2001 in Hainan, China. An example of training in methodology is the course on 'Participatory Diagnosis' held in January 2001, in Lao PDR . An example of where both technical and methodological issues were combined in one course is the course on 'Development of forage technologies with farmers' held in June 2001, Samarinda, Indonesia (Table 73).

Table 73. Capacity building on technical and methodological topics.

Type of training	Year	Project staff trained	Farmers trained	Training topics
Forage science and related technologies	2000	3	653	Forage management, animal nutrition, livestock management, legume trees, forage utilization, milk production and processing, forage technologies, forage production, body weight monitoring, agronomy, fodder tree nursery management, goat raising, animal health, duck raising, utilization of fodder trees, seed production, cattle fattening, integrated farming system, conservation farming
	2001	44	1805	
	2002	87	2464	
Participatory research approaches	2000	32	0	PD, participatory development and gender analysis, dissemination of forage technologies, participatory monitoring and evaluation, participatory extension, participatory tools, data recording and analysis
	2001	57	35	
	2002	45	40	
Combination of science and participatory approaches in one course	2000	120	0	Developing forage technologies with farmers
	2001	100	13	
	2002	33	4	
Total 3 years		521	5014	

4.2.2 On-farm evaluation of new grass and legume options for livestock systems in northern uplands of Laos

Contributor: Peter Horne (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" (FLSP). 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 stabilising shifting cultivation

In the first field season (starting June 2001), the project supported small scale testing on farms during which time farmers evaluated forage varieties in small plots and sorted out which they preferred and wanted to expand. This was a time when the district and provincial 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 (starting 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 generally started to look for ways of utilizing the most promising forage varieties to either 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 (starting June 2003), interesting, sometimes novel, often unexpected impacts started to emerge. The district staff had become very familiar with the processes of working in partnership with farmers. Indeed these processes are now becoming their 'comfortable norm' back to which they will retreat naturally, given the support of their organisations. Further expansion will not now be driven by project targets but by IMPACTS. This focus on "impacts driving expansion" will be the focus of the project for the next two wet seasons. By 2004, 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. At the same time the Extension Managers (the bosses of the field staff) will become much more actively involved in the process.

4.2.2.1 Project Review

In August 2002, AusAID sent a technical reviewer to:

i) Assess FLSP progress to date in relation to achievement or likely achievement of project objectives
ii) Identify problems and issues that either presently impact on FLSP implementation or are likely to do so in the future, and suggest cost effective strategies to alleviate any negative impacts and
iii) Make recommendations as appropriate to enhance the quality of FLSP implementation in a manner that does not lead to significant project cost increases.

The review concluded that "the project is on course for meeting its objectives and is pursuing the wider outcomes necessary for the sustainability of the program, as indicated by the following:

- The innovations are appropriate. Agricultural productivity is increasing. Planting forages close to homes, thereby also increasing substantially the productivity of labor, reduces environmental pressure on the uplands.
- The program is farmer-led.

- The project is institutionalizing a participatory, facilitative extension strategy consistent with a farmer-led program whilst ensuring and increasing the technical competence of staff.
- Promotion of a sense of Lao ownership of the program at all levels.
- Food security is increasing.

The review went on to recommend that to continue this process and to accelerate adoption, the project needs to:

i) Expand the extension strategy concept to an enlarged community-based group approach with selected farmers having a training role,
ii) Increase the capacities of extension staff in on-farm analysis of options within smallholder farming systems,
iii) Focus in the coming year on consolidation of impacts and on skills of field staff rather than just on expansion,
iv) Increase its outreach to rural women,
v) Prioritize the issue of nutrient recycling
vi) Implement the proposed strategy of disease minimization to meet farmers' needs for improved animal health

These recommendations defined the project's field activities in 2003, which aimed to:

- Enhance information exchange, (i) between farmers within villages (ii) between villages and (iii) between extension workers and farmers
- Generate genuine impacts not just increases in area.
- Work with focus groups in the field rather than individual farmers
- Work with women's groups
- Develop case studies to quantify impacts not just outputs
- Expand to new farmers and villages only where there is real momentum
- Support district teams with the training, mentoring and resources they need to be able to organize cross visits / field days
- Develop extension materials for scaling out from local successes.

4.2.2.2 Forage Technology Development

As a result of the work done by district, provincial and national staff during the second field season (June-October 2002), the total number of farmers planting forages increased from 247 in 2001, to 467 in 2002 (Table 74). This is almost a doubling in the number of farmers and so a very substantial increase. It is worth looking at where this increase comes from in more detail:

Number of farmers continuing	238
Number of farmers dropping out	9
Total number of new farmers	230
New farmers in 'old villages'	60
New farmers in 'new villages'	170

The number of new farmers in the 'old villages' gives an indication that sustained expansion is occurring, based on farmers' direct experiences. This must be distinguished from the number of new farmers from 'new villages', which is due to the 'promotional efforts' of the district staff being successful. The increase of 60 new farmers in these old villages was about 24% over the number of farmers in the first year. This is a reasonable figure of expansion. There was little to distinguish between the scaling-up between the two provinces.

There were just 170 new farmers (on average slight more than 8 farmers / village) recruited in the new villages this year. This is a much lower figure than last year (on average 14 farmers / village) indicating that the district staff now recognize that they need to start with a number of farmers in the first year which they can reach and support.

The total number of farmers particularly in an early stage of establishment of a new enterprise can hide both good and poor progress. Staff estimated that about 30% of all the farmers were developing significant forage systems and would be enthusiastic supporters of expansion. In any extension process this would be regarded as a positive initial outcome.

Table 74. Number of farmers and forage areas planted in target villages

District and Village	2001		2002			2003					
						Number of farmers who					
	Number of Farmers	Forage area (m^2)	Number of Farmers	New forage area in 2002 (m^2)	Total forage area in 2002 (m^2)	Maintain their area	Increase their area	Are new	Total farmers	New forage area in 2003 (m^2)	Total forage area in 2003 (m^2)
PEK											
Ta	8	7,400	13	7000	14400	9	4	3	16	2700	17100
Phonekham	35	4,500	26	2,850	7350	20	6	3	29	3850	11200
Dong	7	750	14	450	1200	14	0	0	14	0	1200
Xang	12	6,400	17	5,850	12250	14	3	0	17	2450	14700
Mouan	0	0	9	3,800	3800	3	6	4	13	3200	7000
Lek	0	0	15	3000	3000	14	1	0	15	300	3300
Vieng Khouan	0	0	11	1,850	1850	0	10	2	12	3750	5600
Or An	0	0	11	4,850	4850	10	1	1	12	1250	6100
Vieng	0	0	0	0	0	0	0	11	11	1600	1600
Nakhone	0	0	0	0	0	0	0	13	13	3600	3600
Bee	0	0	0	0	0	0	0	22	22	4700	4700
Sub-total	**62**	**19050**	**116**	**29650**	**48700**	**84**	**31**	**59**	**174**	**27400**	**76100**
NONGHET											
Houay Khiling	8	2,500	9	3200	5700	6	3	1	10	1950	7650
Nonghetai	9	1,400	12	1950	3350	12	0	0	12	0	3350
Paklak	8	1,300	10	3050	4350	9	1	1	11	400	4750
Khanganien	14	2,800	14	9950	12750	8	4	0	12	5350	18100
KeoPaTu	0	0	8	2750	2750	3	5	0	8	3800	6550
Pha ‘En	0	0	9	2500	2500	8	1	2	11	1000	3500
Sandon Koe	0	0	7	2950	2950	5	2	1	8	1150	4100
Tham Toum	0	0	8	5550	5550	6	2	1	9	4200	9750
Nongkob	0	0	0	0	0	0	0	6	6	2700	2700
Nongle	0	0	0	0	0	0	0	5	5	2600	2600
Tamseua	0	0	0	0	0	0	0	9	9	1800	1800
Sub-total	**39**	**8000**	**77**	**31900**	**39900**	**57**	**18**	**26**	**101**	**24950**	**64850**
XIENG NGEUN											
Kieuw Chaluang	23	14500	23	1500	16000	17	6	1	24	1200	17200
Kieuw Talun Noi	6	200	12	1400	1600	6	6	10	22	3200	4800
Kieuw Talun Nyai	12	1250	16	4700	5950	9	7	15	31	7600	13550
Phonesaad	7	2050	19	9850	11900	10	9	9	28	8300	20200
Long Or	0	0	8	2850	2850	7	1	5	13	1650	4500
Houay Hia	0	0	8	4100	4100	0	8	6	14	5550	9650
Kieuw Nya	0	0	7	4050	4050	3	4	8	15	6100	10150
Nam Mok	0	0	3	900	900	1	2	17	20	6800	7700
Houay Yen	0	0	0	0	0	0	0	9	9	4800	4800
Silaleck	0	0	0	0	0	0	0	16	16	4700	4700
Ensavanh	0	0	0	0	0	0	0	9	9	2100	2100
Suandala	0	0	0	0	0	0	0	9	9	2100	2100
Sub-total	**48**	**18000**	**96**	**29350**	**47350**	**53**	**43**	**114**	**210**	**54100**	**101450**
LUANG PHABANG											
Long Lao 2	26	4600	26	1200	5800	24	2	7	33	1700	7500
Nong Tawk	9	1500	13	1200	2700	8	5	8	21	1850	4550
Kok Wan	13	650	20	1550	2200	17	3	5	25	1300	3500
Bo Hae	16	1750	18	800	2550	16	2	5	23	1000	3550
Houay Leuk	16	650	17	1300	1950	14	3	3	20	1100	3050
Dansavanh	18	1000	19	950	1950	15	4	9	28	2150	4100
Nadon Koun	0	0	5	600	600	3	2	4	9	1600	2200
Pik Noi	0	0	6	900	900	2	4	8	14	2050	2950
Phadeng	0	0	4	900	900	3	1	5	9	1000	1900
Pik Nyai	0	0	7	1000	1000	4	3	11	18	2250	3250
Pak Pa	0	0	4	1600	1600	3	1	3	7	850	2450
Long Lan	0	0	21	4700	4700	15	6	8	29	2400	7100
Nasao	0	0	0	0	0	0	0	4	4	1400	1400
Paksi	0	0	0	0	0	0	0	13	13	4500	4500
Nounsavad	0	0	0	0	0	0	0	5	5	800	800
Sub-total	**98**	**10150**	**160**	**16700**	**26850**	**124**	**36**	**98**	**258**	**25950**	**52800**
PAK OU											
Had Pang	0	0	11	7000	7000	5	6	20	31	10950	17950
Somsanouk	0	0	5	1900	1900	3	2	10	15	2750	4650
Pakcheck	0	0	3	300	300	0	3	6	9	750	1050
Hadxoua	0	0	0	0	0	0	0	2	2	300	300
Houay Tun	0	0	0	0	0	0	0	3	3	300	300
Sub-total	**0**	**0**	**19**	**9200**	**9200**	**8**	**11**	**41**	**60**	**15050**	**24250**
TOTAL (All Districts)	**247**	**55200**	**468**	**116800**	**172000**	**326**	**139**	**318**	**803**	**147450**	**319450**

During the second field season (June-October 2002), the area of forages increased from 5.5 hectares in 2001, to 17.2 hectares. This is a trebling of the area planted in 2001. This figure can also be examined further;

Total new area of forages	11.7ha
'Old villages' new forage area	5.9ha
'New villages', new forage area	5.8ha

Thus the forage areas within the old villages more than doubled. This can be compared to the increase in farmers in the old villages of just 24%. This is different from the expansion dynamics commonly experienced with crops, where the increase in area tends to lag behind a more rapid increase in the 'number of farmers testing'. The expansion of forage areas in the old villages has come mainly from 'old farmers' expanding their plots, and is an indication of emerging impacts driving expansion.

By comparison, at the start of the third field season (June 2003), the total number of farmers planting forages had increased from 467 in 2002 to 803 (see Table 1). This is another almost-doubling in the number of farmers working with the project, despite the fact that expansion this year was based on nascent demand, not project targets. Once again, it is worth examining this expansion in more detail:

Number of farmers continuing	465
Number of farmers dropping out	2
Total number of new farmers	338
New farmers in 'old villages'	202
New farmers in 'new villages'	136

The number of new farmers in the 'old villages' (202) was a substantial increase, indicating that the experiences of the 'early adopters' are sustaining expansion. This expansion is not, however, expected to continue, as a ceiling of adopters is likely to be reached in each village after three years. This ceiling will vary from village to village but 25% of adopters would be regarded as very successful. The number of new farmers from 'new villages' (which this were supposed to come from nascent demand resulting from cross visits, field days and promotional information) was 136; a doubling of the number from the second year.

The 136 new farmers recruited in the new villages this year averaged slightly more than 9 farmers /village; a much more reasonable number than some of the villages in the first year (some >30). This indicates that the district staff are becoming more comfortable and experienced with the methods and the need to be able to support a small group of farmers early in their learning process.

At the start of the third field season (starting June 2003), the indicative area of forages planted increased from 17.2 hectares in 2002 to 31.9 hectares. This figure can also be examined further:

Total new area of forages	14.7ha
'Old villages' new forage area	10.7ha
'New villages', new forage area	3.0ha

Thus the forage areas within the old villages doubled, as happened in 2002. Of the total number of farmers in the 'old' villages, only 30% were new farmers this year, so the expansion of forage areas in the old villages continues to come mainly from 'old farmers' expanding their plots, and is an indication that the desire for increasing impacts is driving expansion.

The forage varieties that are most popular with farmers are (i) grasses for cut and carry feeding of cattle and buffalo (mainly *Panicum maximum* TD58 "Simuang", *Brachiaria brizantha* CIAT 6780 "Marandu", *Brachiaria decumbens* CIAT606 "Basilisk", *Paspalum atratum* BRA9610 "Terenos" and *Andropogon gayanus* CIAT 621 "Kent") and (ii) *Stylosanthes guianensis* CIAT184 "Stylo184" for feeding pigs. This year the *Brachiaria* hybrid "Mulato" was introduced for evaluation by >150 farmers. Early indications are that farmers are impressed by its rapid growth (and regrowth) and the fact that it can be fed to all animals, but its potential in comparison to the other grass varieties cannot be adequately assessed until after one complete dry season. The figures reported here are outputs of the technical work on forages. The FLSP is also working to report impacts (see later in this report) (Figure 73).

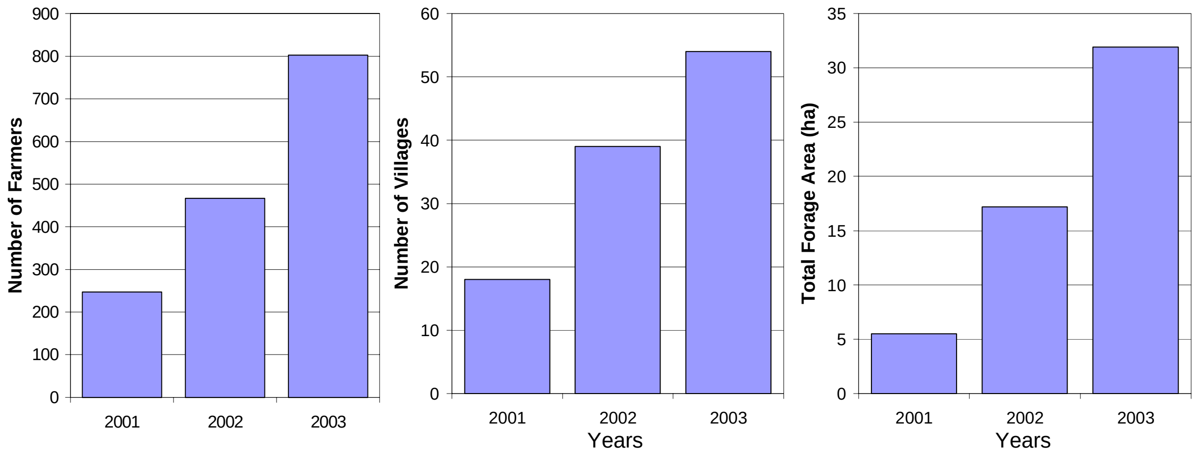

Figure 73. Summary of the expansion occurred in the first three field seasons of the project.

It is worth noting that:

(i) The outputs being reported are a moving target, with changes happening on individual farms for different reasons. Farmers are inherently responsive to the opportunities and constraints within their environments. Forages (and other feed and animal health resources) become just another set of 'cards' they can play in response to the changing interplay of factors in their livelihood systems. Each family and each farm is different.

(ii) The outputs are averages, which hide the huge variation between farmers. Some farmers have rapidly expanded to more than 1ha of forages while others have kept relatively small areas. The project is documenting the cases of expansion to try to understand the opportunities and constraints that have allowed some farmers to do this and others not.

(iii) Large impacts come from relatively small areas of forages. Unlike crops, which are harvested once a year, forages can be harvested 6-8 times per year. Casual observers are usually skeptical that large impacts can come from small areas...but they do…and the FLSP is documenting these from farmers reported experiences.

(iv) Forages are changing the whole dynamic of smallholder livestock systems. In many areas where we work, farmers have traditionally kept livestock as an additional activity to cropping. They are a safety net; a bank. They were often let loose in the forests and grazing lands, with little management. Now, however, these grazing lands are becoming over-utilized. In the past, government extension agencies have suggested farmers improve their livestock management systems but without a reliable feed resource, this was not possible. We are seeing that planted forages are allowing farmers, for the very first time, to start moving into livestock production as a livelihood system not just as livelihood security. That is, they can start cranking up their livestock production to produce cash for buying staple foods rather than expending huge amounts of labor to grow these staple foods. Once again, the FLSP is documenting these cases of impacts to help drive further expansion.

v) Freeing up labor is a major impact of forages. Many of the farmers we work with are flat-out keeping their families fed. In many cases they spend 2-4 hours per day looking for cut feed for their animals. Planting forages can reduce this time to less than one hour per day. Freeing up labor is a key factor in allowing farmers the 'breathing space' to start developing alternatives to their current farming systems in the steep uplands.

4.2.2.3 Development of other Feed Resources

To ensure the potential benefits of introduced forages as a supplement to livestock in villages, the FLSP is also investigating Non-Forage Feed Resources for smallholder livestock systems. In the field season of 2002, four main feed resources were tested: (i) sweet potato varieties (sourced from CIP, Vietnam), (ii) QP maize varieties (sourced from CIMMYT), (iii) cassava varieties (sourced from CIAT) and (iv) new robust lucerne varieties (sourced from an ACIAR project managed by SARDI, Adelaide).

All four technologies were selected because of their potential as feed resources in smallholder pig systems. Rearing pigs is a common household livelihood activity in the northern uplands of Laos, especially among the Hmong ethnic group in which pigs constitute up to 60% of household cash income. It is an activity almost exclusively controlled by women, for whom pigs constitute a savings bank that can be converted to cash as and when required. Feeding is the single most time consuming activity, often taking 2 – 4 hours per day 4 – 7 times per week just to collect tubers, banana stems and leafy vegetables from the forest to supplement meager supplies of maize and rice bran.

Compounding this problem is the fact that all sources of pig feed are in short supply at the start of the wet season and many piglets get thin and die from malnutrition. Recently, farmers in northern Laos working with the FLSP have been reporting substantial benefits from planting forage legumes (especially *Stylosanthes guianensis*) for feeding pigs. These include labor savings in collecting feed at critical times of the year (savings of up to 3 hours per day), higher survival rates of piglets (from 10 – 30% without supplementation to 80 – 100% with supplementation) and greater growth rates of piglets (reaching saleable weight 2 months earlier than unsupplemented pigs).

Of the four technologies, the new sweet potato varieties caused immediate and strong interest among farmers. This was partly because of their immediate impacts on pig feeding but also because of the potential that they can be used as human food. The project decided to expand field evaluation of the sweet potato varieties in 2003 by importing 5 tons of planting material (vines and tubers) of 7 varieties of sweet potato that have been selected by CIP for feeding pigs. By the end of June 2003, >120 farmers in the five districts were growing the seven varieties. Formal evaluations will be conducted at the end of the 2003 wet season. Field evaluations of the other three technologies are to be expanded in 2004.

4.2.2.4 Development of Animal Health and Livestock Management Strategies

To ensure the potential benefits of introduced forages as a supplement to livestock in villages, the FLSP is also developing and evaluating practical approaches to improving animal health in villages that are expanding their forage resources. In late 2002, two animal health consultants identified the three priority health issues in smallholder livestock systems in the north of Laos:

1. *Toxocara vitulorum* infection in buffalo and cattle calves
2. Epidemic diseases (Classical Swine Fever and non-specific bacterial diseases) in pigs
3. Epidemic diseases (Fowl Cholera and Newcastle Disease) in chickens and ducks.

Control of *Toxacara vitulorum* in buffalo and cattle calves is being implemented and monitored in all villages where the project is working. Control methodologies for pig and poultry diseases are more complex and so are being trialled in only 15 villages. The fundamental principle of the strategy is that (i) there are no 'magic bullet' solutions to animal health in villages, (ii) the most promising way to improve productivity in smallholder livestock systems is to combine basic improvements in feeding and management with strategic use of veterinary chemicals and (iii) to drive intensification of livestock systems, we need to document impacts from this strategy on improved animal productivity.

CIAT has no in-house expertise in Asia to implement such a strategy, so in 2003 we worked with the Lao Department of Livestock and Fisheries and CSIRO Animal Health Laboratory (AAHL) to develop a new ACIAR-funded project to work on diseases of smallholder pig production systems and their management. The project, "*Improved diagnostic and control methodologies for major livestock diseases in Lao PDR*" has been approved and will start in July 2003 and operate for 3 years. The following description is extracted from the project document:

> *"The broad objective of the proposed project proposal is to use improvements in husbandry and disease control as an entry point to conduct research on the implementation and impact of CSF vaccination in the village pig production systems. Using the participatory approach for working with farmers on technology adaptation and adoption that has been developed under the AusAID – funded Forages and Livestock Systems Project (FLSP) with CIAT in Laos, the project will introduce a CSF vaccination program to villages and determine the most effective and practical strategy to maintain the level of herd immunity necessary to prevent outbreaks of disease.*
>
> *The Lao – CIAT FLSP will be a key collaborator in the field, having developed innovative ways to link participatory research with extension, thus providing an excellent vehicle for introducing the concept of vaccination and disease control to village pig producers. By working in collaboration with the FLSP, the proposed project will be able to implement vaccination strategies in the field and develop a working system for local animal health workers to improve disease management in village pig production systems. An additional benefit of this collaboration is that the potentially high economic and social benefits of reduced pig mortalities can only be realised through parallel improvements in feed resources, which are being developed by farmers working with the FLSP."*

This project will provide the specific expertise in animal health that FLSP lacks, opening the opportunity for a synergy to develop where the combination of improved health and improved feeding can deliver more sustained impacts to smallholder pig production systems.

A second initiative aimed at providing additional support to our work on forages in Laos was developed in the first half of 2003. A proposal was submitted to the Swiss Agency for Development Cooperation (SDC) to appoint a smallholder livestock systems specialist to work for CIAT in Laos with three broad objectives:

1. to expand the impacts of the FLSP in two new districts using participatory research activities in livestock feeding, management and health
2. to scope the opportunities for new development-oriented livestock systems research and development in the northern uplands of Laos, using Oudomxay and Xieng Khouang provinces as case study areas.
3. to develop the outline of a practical handbook on livestock management options for smallholders in the uplands (with collaboration from ACIAR, ILRI and VSF)

The proposal was approved an the specialist appointed to commence work in Laos, initially for one year, starting in October 2003.

4.2.3 Establishment of a living forage germplasm bank for Southeast Asia in Laos

From 1992 – 1999, CIAT conducted a series of forage germplasm evaluations in Southeast Asia to identify a small suite of broadly adapted and robust varieties that had the potential to deliver substantial impacts to smallholder farmers. Preliminary nursery evaluations of the >600 most promising varieties were conducted in three countries (Malaysia, Indonesia and Philippines). The resulting 100+ varieties were introduced into regional evaluations in six countries targeted for on-farm evaluation and forage development. This added to our understanding of their agronomic performance across a range of climates and soils. As the work progressed, the enormous value of farmer evaluation of forage varieties on their own farms was realized. A smaller suite (~50 varieties) were evaluated using participatory research approaches allowing forages program to base species selection not only on environmental adaptation but also on farmers' needs and opportunities in resource-poor upland areas.

The program also conducted a genotype x environment experiment of the main varieties at 12 sites across 6 countries over two years. Through this lengthy process, ~35 broadly adapted, robust forage varieties suitable for smallholder farmers across the region. Details are available in Horne and Stür, 1999 and Stür et al., 2002.

Since this work was completed CIAT's forages program in Asia has continued to evaluate a small suite of new varieties, either as they become available or for particular niches. The main focus of the program has, however, become the integration of the promising forage varieties into smallholder livestock systems. At the same time, it is essential that the selected varieties are maintained by CIAT, both as seed and as a living forage germplasm resource. Currently seed of all the selected varieties is kept in store at the CIAT office in IRRI, Philippines. To ensure that this seed collection is continually updated and that small quantities of seed and cuttings are available to support regional evaluation, a "Living Germplasm Bank for Southeast Asia" was established in July 2003 at the Lao National Livestock Research Center (LRC), 45 km from Vientiane. CIAT will continue to work with LRC to maintain (and, where necessary, expand) this collection for the benefit of all countries in the region.

4.2.4 Livelihoods and Livestock Systems Project in Southeast Asia

Contributors: Ralph Roothaert and Werner Stür (CIAT)

A new Project entitled 'Improving livelihoods of upland farmers using participatory approaches to develop more efficient livestock systems' (LLSP) is being funded by the Asian Development Bank (ADB), and convened by the Centro Internacional de Agricultura Tropical (CIAT). The Technical Assistance Agreement was signed in January 2003, referred to as RETA No. 6067. The purpose of the project is to:

1) Improve the sustainable livelihood of small farmers in the uplands through intensification of crop-livestock systems, using farmer participatory approaches to improve and deliver forage and feed technologies; and
2) Improve delivery mechanisms in participating DMCs for the dissemination of these technologies.

The project is based on the results of the previous CIAT-ADB project 'Developing sustainable forage technologies for resource poor upland farmers in Asia', in short 'Forage for Smallholders Project (FSP-II)' which is ending in June 2003. The new project builds on previous experiences in the Philippines, Indonesia, PDR. A new country, Cambodia, will join, and a reduced program is envisaged in Thailand and Lao PDR. The project will expand research activities to incorporate integrated feed systems using indigenous forages and crop residues. It will also expand to more farmers in the participating countries and further develop participatory monitoring and evaluation systems to enable community learning and provide feedback. Capacity building will stretch beyond field level to institutional heads to bring about institutionalization of approaches and technologies. Research will also aim to address constraints to increased livestock production beyond the forage and feed components, such as increased commercial orientation. Synergies will be established through existing networks and new collaboration with development projects to accomplish the following outputs:

1. Integrated feeding systems for livestock, that optimise the use of improved and indigenous fodders and crop residues, and farm labour;
2. Improved methods to develop forage feed systems and extend them to new farmers, optimising the use of M&E for feedback to others in the community;
3. Increased capacity in DMCs, at different levels, to expand the use of improved forage and feed systems and respond to local needs;
4. Comparison of development opportunities, and market and logistic constraints, for intensification of smallholder livestock systems across sites in five countries;
5. Improved regional interaction and linkages with national and donor funded development projects that ensure synergistic and multiplier effects.

A meeting to launch the new project was held in Hainan, PR China this year. The meeting provided an opportunity for each country of the FSP-II to show what had been achieved in the last three years, the lessons learned, and the research needs for the new project. Objectives of the new project were presented, related questions were clarifications were discussed, and countries indicated the priorities they would allocate to each objective. Participants grouped by country were given more than a day to develop and fine- tune country research objectives, strategies and workplans. Summaries of the strategies were presented towards the end of the workshop, but detailed workplans would be completed during the first quarter of the project. The ADB senior agricultural specialist provided guidelines for improving indicators that were mentioned in the TA framework. A lot of consideration during the working group sessions went into making the indicators more realistic and closer to the project purpose and objectives.

The management structure will be different from FSP-II. The previous network coordinator, Dr. Ralph Roothaert, is leaving to take up a new position in Africa. The new project management will consist of a team of a senior international scientist, Dr. Werner Stur, and two regional scientists, Mr. Francisco Gabunada and Mr. Phonepaseuth Phengsavanh. Dr. Rod Lefroy will remain the Regional Coordiator of CIAT in Asia, and Ms. Pratima Dayal will be the ADB project officer.

In each country a national coordinator was identified and letters of commitment, otherwise called Memorandum of Agreement (MoA), will be composed in collaboration with the management team and the implementing institutions in each country, before April 2003.

It was agreed that the planning workshops would continue to be held on an annual basis, each time in a different country to enable delegates to directly learn from regional experiences during the field day. The newsletter of the 'Southeast Asia Feed Resources Research and Development Network' (SEAFRAD) will continue to be produced by country editors on a rotational basis, although the timing will be more flexible.

The next two issues will be edited and produced by Mr. Yi Kexian, China. A new name was accepted for the relatively long project title 'Improving livelihoods of upland farmers using participatory approaches to develop more efficient livestock systems', which became 'Livelihoods and Livestock Systems Project' (LLSP). It was accompanied by a new logo, reflecting gender focus, feed resources and livestock systems.

4.2.5 Participatory Evaluation of *Brachiaria* hybrid "Mulato in South East Asia"

In many parts of Southeast Asia (particularly Indochina) the climate is characterized by high annual mean rainfalls (typically 1500-2500mm) but with relatively long and severe dry seasons. This results in highly variable feed availability, which limits the potential for intensification of smallholder systems which rely on locally-available feed resources. The genus Brachiaria includes many varieties that are well adapted to such conditions but there is huge intra-specific variation in adaptation to drought within the genus. One species, *Brachiaria ruziziensis* "Ruzi", is widespread throughout Indochina (especially Thailand) mainly because of its fast growth rates in the wet season and ease of seed production. It performs very poorly, however, in the dry season or on low fertility soils and many farmers re-sow it after 2-3 years. For this reason, CIAT has been assisting work researchers from the Thai Department of Livestock Development (DLD) since 1997 to identify alternative varieties to Ruzi that are both reasonably productive in the dry season and can produce commercial quantities of seed. After four years of trials, the line that emerged as showing most promise (CIAT1873) is the hybrid that was subsequently renamed "Mulato".

In March 2003, the Driector of the Mexican seed company which owns the rights to "Mulato" (Papalotla), visited staff from DLD and CIAT in Thailand to evaluate the work that has been done by DLD and plan joint activities during the wet season of 2003. Experimental work conducted at the Animal Nutrition Research Center at Pakchong indicated that the Thai seed collection system based on hand shaking of seed heads could produce up to double the yields achieved in Mexico. Forage seed production in Thailand is mainly with smallholder producers. There are about 3000 producers in the country. Typically a smallholder producer is contracted to produce seed from 2 *rai* (1 ha = 6.25 *rai*), but often they plant larger areas for sale outside the government system. Seed is mainly produced using intensive harvesting methods (shaken by hand or the seedheads are bagged with nets). Typically yields of 90% pure Ruzi seed are about 80kg/*rai* and the estimated total production last year was ~500 tons. The current subsidised price within the government system for Ruzi seed is 60 Baht/kg (1USD = 40 Baht). In 2003, market demand within Thailand for forage seed exceeded the supply and prices for Ruzi seed in the open market reached 100 Baht/kg. Papalotla agreed to fund DLD to conduct several trials in Thailand to evaluate the forage and seed production potential of "Mulato":

i) On-farm seed production trials near Khon Kaen
   In 2003, 6 farmers are producing Mulato seed on 2 *rai* each. Each farmer will evaluate two seed harvesting methods: shaking seed heads or bagging seedheads. DLD staff will measure
   - seed yield and quality (%MC, Purity, 1000 seed weight, Germination, Viability) compared between two harvesting methods
   - cost of production/rai

In 2004, the same 6 farmers will remove 1 rai of Mulato and replant to compare seed production between "New" Mulato versus "Second Year Stand" Mulato

ii) On-farm utilization trials near Khon Kaen
   In 2003, 2 dairy farmers each planted 1 *rai* Ruzi, 1 *rai* Simuang Guinea (*Panicum maximum* TD58), 1 *rai* Tha Pra Stylo (*Stylosanthes guianensis* CIAT184) and 1 *rai* Mulato. The forages will be irrigated in the dry season and farmers' observations on Mulato compared with the other forages recorded, especially productivity in the wet and dry seasons, regrowth capacity, plant vigour, weed issues and palatability.

iii) On-station seed production trials at Pakchong
   Last year DLD staff harvested 13 kg of pure seed from 288 m^2 of Mulato plots (equivalent to 450kg pure seed/ha). This was part of a trial being conducted to look at nitrogen

application rates and closing date on seed yields. This trial will continue in 2003.

iv) On-farm utilization trials at Pakchong
Four smallholder dairy farmers are evaluating Mulato compared with Ruzi for forage and livestock production on farm. Each planted at least 2 *rai* of Mulato and 2 *rai* of Ruzi. Their observations of both varieties (and measurements made by DLD staff) are being monitored, with reference to productivity in the wet and dry seasons, regrowth capacity, plant vigour, weed issues and palatability.

At the same time CIAT is conducting evaluations of Mulato with smallholder farmers in Laos. Seed was provided by Papalotla and distributed to >200 farmers in July 2003. District staff will record farmers' observations in village focus group meetings. Two students from the National University of Laos are also working with CIAT conducting formal trials with farmers to (i) compare yields of Mulato with four other forage grasses and (ii) conduct preference analyses with farmers about all five varieties. The trials will be continued until the end of the dry season in 2004 to compare the varieties across both wet and dry seasons.

4.3 Development of methods for participatory evaluation of multipurpose forages in crop-livestock systems

Highlights

- Developed a conceptual framework for participatory evaluation and selection of forages for different uses
- A higher proportion of farmers in higher altitude areas in the reference site in hillsides of Honduras are testing legumes (i.e. cowpea) for food (grain) and soil fertility improvement than for livestock feed
- Farmers in the reference site in hillsides of Honduras selected *Brachiaria brizantha* cv Toledo because of drought tolerance and high seed yield (possibility to harvest sees for own use and for sale)

4.3.1 Development of participatory research procedures to identify, test and evaluate multipurpose forage-based technologies

Contributors: R. van der Hoek, V. Hoffmann (University of Hohënheim), M. Peters (CIAT)

Rationale

Research procedures are being developed for identifying, testing and evaluating multipurpose forage based technologies with farmers within the framework of the BMZ/GTZ supported project on participatory research on selection and strategic use of multipurpose forage germplasm in Central American hillsides.

Materials and Methods

A key component of forage research in Central America is to understand the interactions between people, biophysical and socio-economic environments and the decision processes in selection of technologies. The research process is characterized by a large variability of different approaches and types of experimentation that could be employed by farmers.

These are being compared in order to define which processes and approaches may be the most efficient in relation to forage technologies (Figure 74).

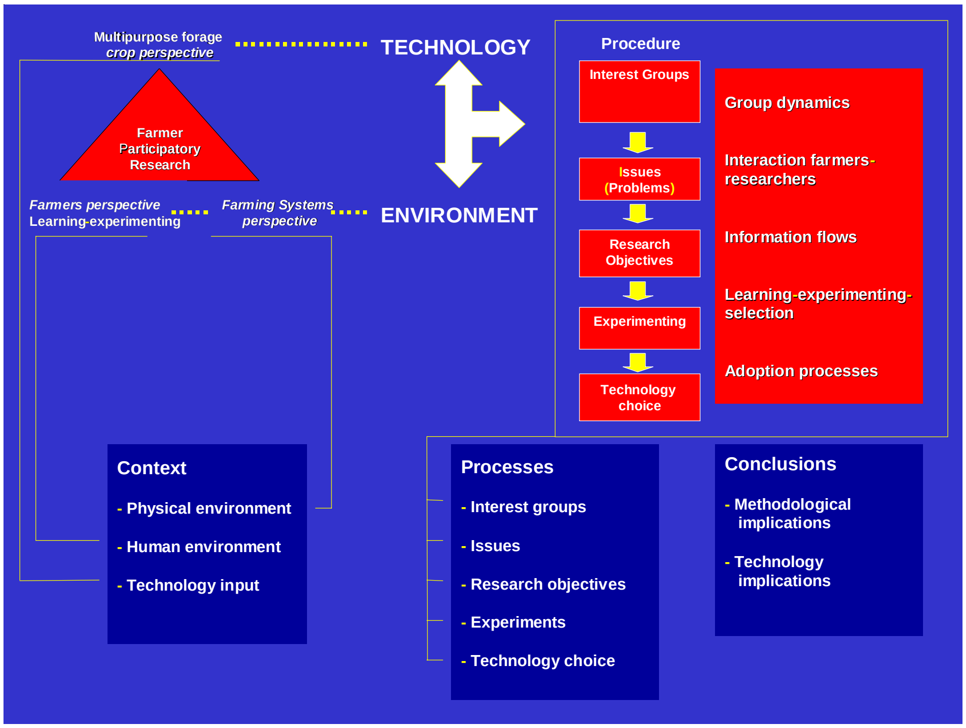

Figure 74. Forage participatory research framework. Left side the main components – i.e. the central theme (Participatory research) surrounded by the contextual elements consisting of the human environment (Farmers' perspective), the biophysical environment (Farming systems perspective) and the technology input (Multipurpose forage crop perspective) – and the research procedure determined by the processes mentioned at the right side.

Results and Discussion

In the department of Yoro, central Honduras, farmers in more than 15 communities have conducted experiments with different types of multipurpose forages like grasses, leguminous cover crops and shrubs in three different agro-ecological zones, characterized by being in different altitudes, during the Primera and Postrera seasons of 2002 and the Primera season of 2003 (Table 75). The methodology being used includes farming system analysis, problem identification and prioritization, formulation of research objectives, implementation and evaluation of experiments, which are carried out using participatory methods and based on farmers' demand. The proportion of farmers testing multipurpose forage -based technologies to enhance animal (cattle) production is relatively low, compared to testing the use of mainly legumes for food and soil fertility maintenance/improvement (Figure 75). This is due to the fact that in the research area only 20% of the farmers possess cattle whereas food security is the main concern of a large proportion of the population, especially those living in the higher areas. For more details, see CIAT Annual Report 2002.

Table 75. Number of experiments per altitude, forage category and growing season (Yoro, Honduras)

Altitude category	Forage category	2002 Primera[1]	2002 Postrera[2]	2003 Primera	Total
Low altitude	Grasses	3		2	5
	Legumes	2	4	11	17
	Shrubs			2	2
Medium altitude	Grasses	3		7	10
	Legumes	5	5	8	18
	Shrubs	5			5
High altitude	Grasses	3		1	4
	Legumes	8	7	11	26
	Shrubs	5		1	6
Total		34	16	43	93

[1] First growing season
[2] Second growing season

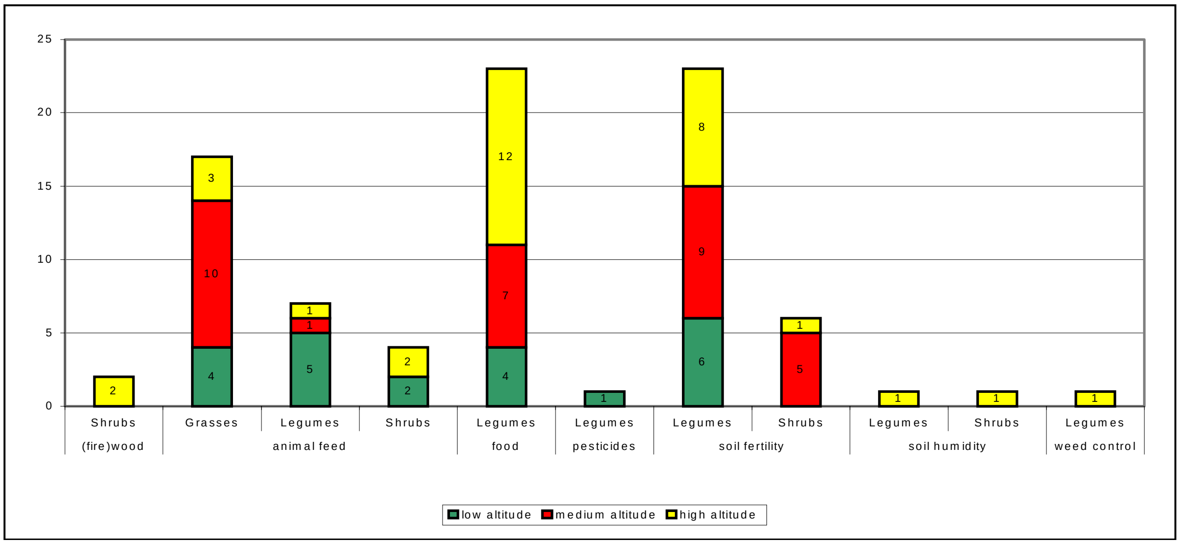

Figure 75. Forage-based experiments for different farmers' objectives, forage categories and altitudes (2002-2003) (Yoro, Honduras)

Of the 90-implemented experiments, 39% were carried out individually (by individual farmers on individual fields), 17% on a semi-collective basis (in which at least part of the work – planting, weeding - is done as a group, but on individual farmers' fields) and 44% collectively (all activities carried out together on fields allocated to the group). Some examples are shown in Table 76.

As mentioned in Figure 74, the major processes involved in the research being carried out are:

- Group processes: relationships between group members, role of "leaders", relationship of the group with the community
- Interactions between farmers and researchers: "neutrality" of facilitator, influence on perceived problems and solutions, role of fringe benefits of intervening institutions
- Information flows: between farmers, between institutions and farmers

Table 76. Some typical examples of experiment objectives and modalities for different altitudes

Altitude	Individual	Semi-collective	Collective
Lower (< 800 m)	Adaptation trial with *Brachiaria brizantha* 26110 "Toledo" for grazing and seed production	None	Comparison trial of different cowpea varieties by youth CIALs as food crop (green pods, grains) and green manure
Medium (800- 1200 m)	Comparison trial of different cover crops (Lablab, Canavalia, Cowpea) as green manure	Adaptation trial of *Cratylia argentea* to improve soil fertility	Comparison trial of *Brachiaria brizantha* 26110 "Toledo" with *Andropogon gayanus* for grazing and seed production
Higher (> 1200 m)	Comparison trial of *Canavalia brasiliensis* with *Canavalia ensiformis* as a green manure	Comparison trial of *Cratylia argentea* with *Calliandra calothyrsus* for animal feed and firewood	Comparison trial of different Lablab varieties for food production and as a green manure

- Learning, experimentation, selection: importance, use of local knowledge, ways of experimenting (try out", informal or formal comparisons, with/without repetitions), choice of experiment sites in relation to perception of land use, monitoring and evaluation of experiments criteria for selecting technologies
- Adoption processes: extent to which farmers invest in new forage based technologies, possibilities/ideas to modify technologies

In the final analysis, the detailed descriptions of these processes will be used to define strategies for the different interest groups.

Collaboration with other projects and institutions: At the request of representatives of a group of 10 CIALs in the municipality of Vallecillo, department of Francisco Morazán, around 20 experiments were started this year in collaboration with IPCA (Investigación Participativa en Centro América). Most of these experiments are focused on improving pastures (using *Brachiaria brizantha* 26110 "Toledo" and *Cratylia argentea*) and comparing cowpea varieties. Because of the availability of natural resources (more fertile soils than in Yorito area) and the proximity to markets (Tegucigalpa) perspectives for further production and processing of multipurpose forages are promising. Furthermore, collaboration has started with the Youth Project (partly implemented by CIAT Communities & Watersheds). Five youth CIALs have implemented around 10 experiments, comparing different cowpea varieties, improving pastures with *Brachiaria brizantha* 26110 "Toledo" and testing *Cratylia argentea* to produce animal feed and improve soil fertility.

4.3.2 Determination of the suitability of different multipurpose forage-based technologies in smallholder farms

Contributors: R. van der Hoek, V. Hoffmann (University of Hohënheim), M. Peters

Rationale

It is well recognized that forage-based technologies can play an important role in improving the environmental and socio-economic sustainability of smallholder production systems in the tropics, especially in situations with a fragile balance between the availability of natural and economic resources and their utilization. Forages can serve multiple objectives, such as provision of animal feed, enhancing soil conservation and maintaining and improving soil fertility. Forage species that are widely adapted, productive and palatable have been identified, but farmer adoption has often been low. One explanation is that too much emphasis has been placed on supply-driven research with little participation of farmers. Hence in this research participatory methods are applied to define forage based technologies suited to smallholder systems.

Materials and Methods

The methodology utilized includes the following elements:

1. Introductory meetings with farmer groups to obtain information on the state of knowledge on forages and its uses and to obtain basic information on farming systems
2. Field visits to forage demonstration sites to inform farmers on the range of options available and their potential utilization
3. Problem ranking with farmers
4. Definition of research and forage priorities with farmers
5. Design and planning of experiments with farmers
6. Execution of farmer-led experiments
7. Identification of suitable forage technologies by farmers
8. Definition of constraints to scaling of forage based technologies

Results and Discussion

Figure 76 shows the farmers' rating of the experiments carried out in 2002. Performance of legumes (*Lablab purpureus*, *Vigna unguiculata*, *Canavalia ensiformis*) was highly variable. Failure in the Primera season (first growing season) was mainly due to seed quality, whereas in the Postrera season (second growing season) the main reasons for failure were adverse weather conditions (first drought, and then a cold period which also caused failure of the Postrera bean crop) and pests like rabbits. Poor performance was caused by the fact that the germplasm was not always suitable for the local conditions like poor soils, poor management and some pests (insects).

Shrubs showed for the most part disappointing results, *Cratylia argentea* being mainly responsible for this. Although looking promising at the *SOL* site in Luquigüe and very much liked by the farmers for its characteristics (high quality fodder, leaves covering the soil improving soil fertility and maintaining soil humidity, producing firewood), the plant does not appear to adapt well to many sites in the region, often being characterized by clayish and alkaline soils. Some other shrubs did well though, like *Cajanus cajan* and *Calliandra calothyrsus*.

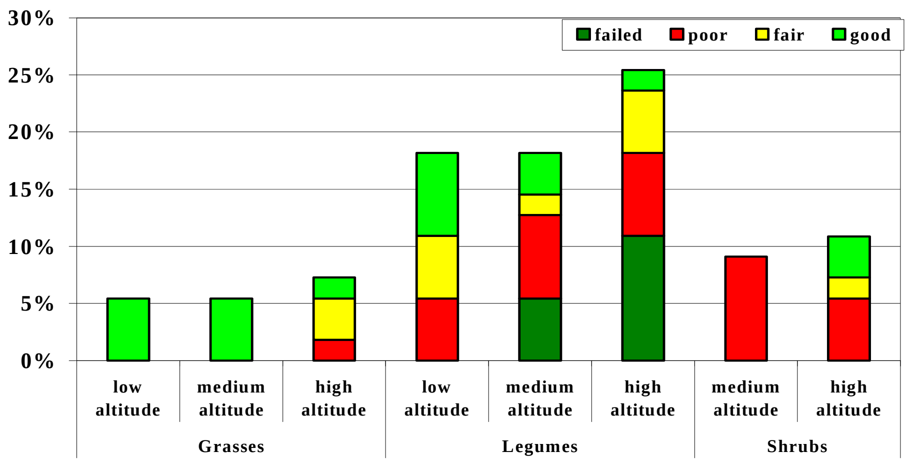

Figure 76. Rating of experiments per crop category (*Primera* and *Postrera* seasons, 2002)

Grasses (*Brachiaria brizantha* cv Toledo , *Andropogon gayanus*, *Pennisetum* spp.cv Camerún and cv King Grass) performed generally well. They were all sown in the main growing season (Primera) and plots were mostly well maintained.

Figures 77 and 78 show for some selected forage farmers' ratings and primary causes of poor performance and failure. The poor performance of *Brachiaria brizantha* 26110 "Toledo" and *Cratylia argentea* in certain plots is mainly due to poor soils and management, whereas the cowpea varieties suffer more from pests and adverse weather conditions. In what follows we present as an example the results of the evaluation of cowpea accessions by a group of farmers in Victoria, Honduras.

Case study: Cowpea trial in Guachipilín, Victoria, Postrera 2002

In March 2002 a group of male farmers of Guachipilín, a hillside village at 1000 masl in the municipality of Victoria, requested "CIAT-forrajes" to start working with them to increase the number of options for animal feed production. After a comparison trial of *Brachiaria brizantha* 26110 cv Toledo and *Andropogon gayanus* in the Primera growing season, they decided to continue with an experiment with five cowpea varieties: 9611, 1088/2, 716 (all via CIAT from IITA), FHIA (from the Foundation Hondureña de Investigación Agrícola, La Lima) and CIDICCO 4 (from CIDICCO, Tegucigalpa) (Figure 79). The cowpea was sown in October in a maize field

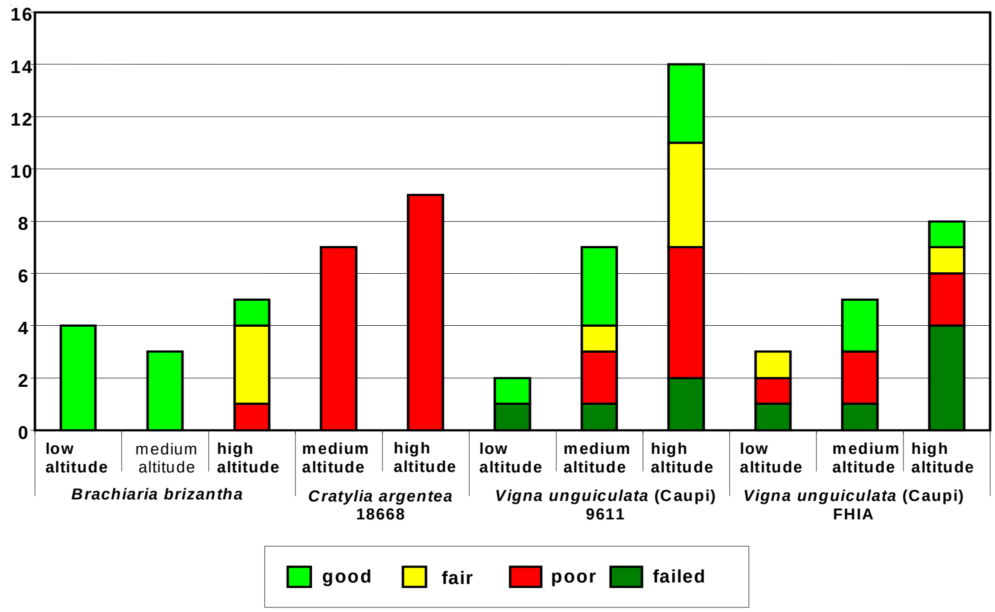

Figure 77. Rating of plots with some selected crops (Primera and Postrera seasons, 2002) (Yoro, Honduras)

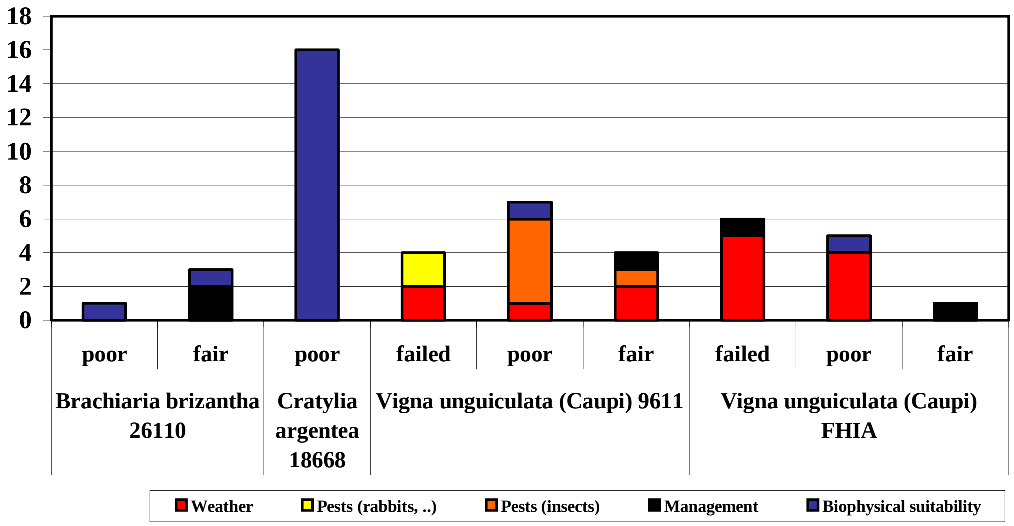

Figure 78. Primary causes of poor performance and failure for some selected crops (2002, number of plots) (Yoro, Honduras)

(which had been planted in June), in the "dead furrows" between the maize plants (Photo 28). At the time of sowing the maize stalks were bent (as usual in this growing stage) which improved the exposure to light for the cowpea. Harvest of both maize and cowpea took place in December, yields were measured and an evaluation was held with the nine group members and six of their wives (the group members themselves suggested that their wives might be interested regarding the characteristics of the plant).

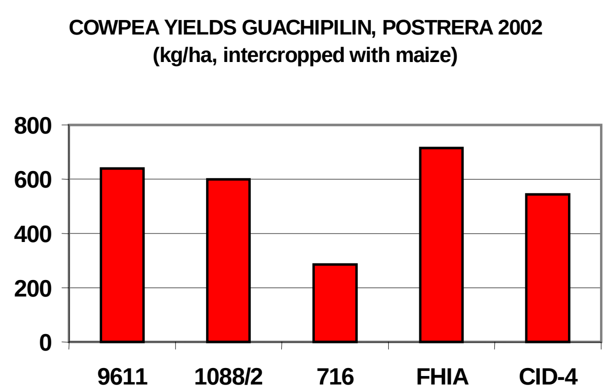

Figure 79. Dry matter yield of five cowpea varieties selected by the farmers at Guachipilin

Some observations were:

- Their most important objective of trying cowpea was production of green manure ("hojas para nutrir la tierra") in rotation with other crops
- Four varieties have good yields and other important advantages: FHIA produces food fast, CIDICCO 4 is resistant against pests and suitable as animal feed, 1088/2 ripens unevenly, allowing picking of green pods (*"habichuelas"*) during a prolonged period, 9611 has various purposes: animal feed, green manure and food. The 716 varieties yielded less because pods started to rot.

Our results with cowpea indicated that:

- Cowpea as green manure is regarded as the most important and promising product.
- Cowpea accession 9611 is the true multi-purpose genotype.
- To make use of the different characteristics of the different cowpea accesions they could be sown in rotation.
- There might be good possibilities to use the seed and leaf meal of cowpea as pig feed ("siempre hay mercado para carne").
- In spite of the bad Postrera season, which caused many crops to fail, yields of cowpea were acceptable and somewhat higher than of common bean.

In general, results of this year look promising given that farmers as well as researchers have learned from their mistakes made last year and as a result, the great majority of 43 experiments underway will be successful. Cowpea (in total 15 accessions) accounts for 40% of the trials, in terms of plots for almost 75% of which some already have been harvested.

Other materials being tested by farmers include grasses (e.g. *Brachiaria brizantha* cv Toledo), legumes (e.g. some accessions of *Lablab purpureus*, *Canavalia ensiformis*, *Canavalia brasiliensis*) and *Cratylia argentea* (to try on some specific more promising niches).

Photo 28. Group of farmers testing cowpea as green manure in maize fields in Honduras

Conclusions

Grasses

- *Brachiaria brizantha* cv Toledo generally performs well with some management in relatively good soils at altitudes up to 1600 masl. Farmers like the grass because of its growth, its drought tolerance and its seed production characteristics, which enables them to increase pasture areas and to generate income by selling seed, even by non-cattle owners.
- *Pennisetum spp.* Camerún and King Grass almost invariable show satisfactory results, the former slightly preferred because of the absence of '*guate'* which causes itching while carrying the stalks.

Herbaceous legumes

- Cowpea is of considerable interest to farmers. Cowpea accessions grown in the Postrera season of 2002 showed in general disappointing results. In spite of this, the farmers continued to grow cowpea and established 130 plots in this Primera season.
- The main reasons to experiment with cowpea are to improve food security (65%), to enhance soil fertility (27%) and to produce animal feed (8%).
- Some pests like lice are prevalent but hardly affect cowpea production. Others are more

damaging, like *zompopo* (an antlike insect which destroys leaves) and rabbits. In general, farmers comment that cowpea is more resistant to pests and diseases than common beans but is susceptible to cold periods, especially at the higher altitudes.

- *Lablab purpureus* varieties show varying results, in some cases being susceptible to pests and lacking vigour at higher altitudes. In 2003, new accessions of Lablab are being introduced, which may perform better under the prevalent conditions.

- Canavalia (both *C. ensiformis* and *C. brasiliensis*) is well adapted, showing vigorous growth, at both lower and higher altitudes.

Shrub legumes

- In the first year of trials, *Cratylia argentea* appears not to be suitable for the higher altitudes in the region, which are predominantly alkaline. Generally, the plants germinated well but showed poor growth and many died off after some weeks. For adaptation to alkaline soils, further studies to test the performance over longer time and the addition of fertilizer to enhance establishment need to be carried out. However, farmers are still interested in *Cratylia* because of its characteristics and some of them have proposed to plant the crop at specific sites, which are more suitable (with slightly acid, sandy soils).
- Some positive results have been obtained with *Cajanus cajan* (intercropped with maize) and *Calliandra calothyrsus*.

4.4 Forage seeds: Multiplication and delivery of experimental and basic forage seed

Highlights

- The Seed Unit of Atenas- Costa Rica and CIAT- Palmira delivered 500 and 1,000 kg respectively, of seed to partners in different countries

- The growth-controlling hormone (Cytokinin) did not improve plant development or seed set when applied to *Brachiaria* hybrid cv Mulato

4.4.1 Multiplication and delivery of forage seeds in the Seed Unit of Palmira (JWM)

Contributors: A. Betancourt; J. Muñoz; J.W. Miles

Seed is the vehicle by which improved plant selections are generally made available to potential users. The tropical forages project maintains a small seed multiplication unit at CIAT headquarters to service seed needs of project members, the wider CIAT community, and, where excess supply is available, to the wider public and private community, free for small quantities, or on a cost basis for larger seed volumes. Seed production plots are established at CIAT headquarters, CIAT-Quilichao, and at CIAT-Popayán. Owing to the small scale of the operation, all harvesting and seed processing is done by hand. Requests for seed are addressed as possible according to seed supplies. One hundred fifteen distinct accessions of 15 species were multiplied and processed for total production of over 900 kg of seed (Table 77). Relatively large quantities of *B. brizantha* (225.8 kg) and *Cratylia argentea* (411.4 kg) were produced, plus much smaller quantities of the other species.

Over one ton of seed was distributed, in 354 individual seed samples sent to eight different countries (Table 78).

Table 77. Seed multiplication at the CIAT-Quilichao, CIAT-Popayán, and CIAT-Palmira experimental stations. (September 2002 to September 2003), totals by species.

Genus	Species	Number of Accessions	Harvest (kilograms)
Arachis	*pintoi*	8	13.400
Brachiaria	*brizantha*	14	225.800
Brachiaria	*decumbens*	2	8.300
Brachiaria	*lachnantha*	1	12.000
Calliandra	*calothyrsus*	4	16.850
Calliandra	sp.	1	0.800
Centrosema	*macrocarpum*	1	10.000
Cratylia	*argentea*	12	411.410
Desmodium	*heterocarpon*	1	42.000
Flemingia	*macrophylla*	1	4.700
Lablab	*Purpureus*	21	45.110
Leucaena	*leucocephala*	2	56.000
Rhynchosia	*schomburgkii*	2	0.650
Stylosanthes	*guianensis*	5	21.400
Vigna	*unguiculata*	40	68.400
Total		115	936.82

Major quantities of *C. argentea*, *Brachiaria* spp., and *Desmodium heterocarpon* were distributed, reflecting interest in recent legume releases. A total of 354 samples, representing 1,015.43 kg were distributed.

Table 78. Seed dispatched from CIAT forage seed multiplication unit (September 2002 to September 2003).

Genus	Kilograms	Number of samples
Arachis	1.04	5
Brachiaria	171.879	64
Cajanus	2.1	2
Calliandra	0.3	1
Canavalia	9.715	3
Centrosema	8.698	7
Chamaecrista	0.002	1
Clitoria	0.002	1
Cratylia	607.999	183
Desmodium	111.931	40
Flemingia	11.785	4
Galactia	0.085	1
Lablab	60.375	16
Leucaena	3.15	4
Mucuna	15.277	8
Neonotonia	0.002	1
Pueraria	3.108	5
Stylosanthes	6.524	6
Vigna	1.533	2
Total	1,015.43	354

These samples were sent to eight (8) countries: Costa Rica (4); Philippines (1); Germany (5); Honduras (4); Nepal (3); Nicaragua (8); USA (4); and Colombia (313). Within Colombia, distribution was to: CIAT (122), NGOs (1), Universities (5), NARs (Corpoica) (5), Private individuals (180). A total of 354 samples, representing 1,015.43 kg were distributed.

4.4.2 Multiplication and delivery of selected grasses and legumes in the Seed Unit of Atenas

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

Seed multiplication activities of promising forage germplasm continued during 2003 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 2002 through August 2003 a total of 418.88 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* (129.95 kg), *Brachiaria* spp. (27.20 kg), *Brachiaria* hybrid cv. Mulato (5.45 kg), *Arachis pintoi* (47.0 kg), *Leucaena* spp. (30.44 kg), *Centrosema* spp. (5.79 kg), *Panicum maximum* (15.18 kg) and *Paspalum* spp.(12.31 kg). Small quantities of experimental seed were also produced of *Desmodium velutinum, Chamaechrista rotundifolia* spp. *grandiflora* and other forage species.

During the period September 2002-August 2003 a total of 494.10 kg of experimental and basic seed were delivered by the Seed Unit of Atenas

(Costa Rica). Table 79 shows that 56 seed requests were received from 6 countries, where most of the requests came from Costa Rica, the host country of the forage project. However, a significant amount of seed was delivery to Nicaragua (196.75 kg) and Haiti (53.40 kg), both countries involved in forage projects with the participation of CIAT. A high amount of experimental seed of *Brachiaria* species was delivered, particularly of cv. Mulato, the new hybrid of this genus that is being promoted regionally with the assistance of the private sector.

Table 79. Countries, number of requests and amount of experimental/basic forage seed delivered by the Unit of Atenas (Costa Rica) during the period September 2002-August 2003.

Country	No. of Requests	Forage species (kg)			
		Brachiaria spp.	*A. pintoi*	*C. argentea*	Other species
Costa Rica	50	118.77	36.00	56.80	24.55
El Salvador	1	4.00			
Guatemala	1	0.10		1.00	
Haití	1	13.40		2.00	38.00
Nicaragua	1	10.50	175.00	10.00	1.25
Panamá	2	1.50			0.80
Total	56	148.27	211.00	69.80	64.60

4.4.3 Effect of cytokinin on seed quality and yield of *Brachiaria* hybrid cv. Mulato

Collaborators: Pedro J. Argel and Guillermo Pérez (CIAT

Rationale

There are environmental factors such as temperature, day length, rainfall distribution, as well as soil fertility that influence flowering and seed set in tropical grasses. Additionally, plants have internal hormonal mechanisms that interact with the environment to control plant growth and differentiation. The grass cv. Mulato grows well in the tropics, particularly in fertile well-drained soils; the flowering is abundant and well synchronized, but the seed set of the plant is very poor which traduces in low seed yields. This is a limitation of this cultivar since commercial seed production becomes very inefficient and increases the cost of the seed produced. This factor contributes to high seed prices to the consumers and obviously reduces the demand and the potential adoption of the grass.
For this reason any attempt to promote seed yields of this grass is worth to investigate.

Methods

The growth-controlling hormone cytokinin was applied to one year old plants of *Brachiaria* hybrid cv. Mulato at the rate of 0, 0.1,0.2 and 0.3 cc/ha. A uniformity cut and the fertilization of the grass with 75, 40 and 50 kg/ha of N P K respectively, and 30 kg/ha of S, were carried out in June at the beginning of the rains. The hormone rates were applied at two different dates: 1st of August (vegetative growth), and the 1st of September (at early spikelet initiation). A split plot design was used were the main plot corresponded to date of hormone application and the subplots to hormone rates. Plot size was 3.37 m^2 and the amount of water used utilizing a backpack spray, corresponded to 545 l/ha. Flower initiation, maximum flowering time, harvesting date, number of inflorescences/m^2, caryopsis content, seed unit weight, seed purity and seed yield were measured.

Results and Discussion

Cytokinins are a group of four or five plant hormonal compounds derivatives of adenine that have actions on plant cell division. Commercially products based on these hormones are available and recommended to improve fruit formation, to increase photosynthetic and respiration rates, to stimulate root development and improve nutrient uptake. In general these compounds stimulate healthy plant development and increase crop yields. However, no significant effect of cytokinin was observed for date of application, or for any of the plant measurement taken on cv. Mulato as Table 80 shows. The rates of cytokinin applied corresponded to 1, 2 and 3 liters/ha of a commercial product, which are within the range of recommended application rates for other crops.

Results showed very low percentages of seed purity, which accounts for the relatively low seed yield of this grass, despite the fact that the plant produces a high number of panicle per square meter. The rates of cytokinins applied did not improve plant development or seed set of cv. Mulato.

Table 80. Effect of cytokinin on seed production and quality of *Brachiaria* hybrid cv. Mulato in Atenas, Costa Rica

Cytokinin (cc/ha)	Plant height (cm)	Panicles (No/m^2)	Seed yield (kg/ha)	Seed purity (%)	Seed unit weight (g/100 seeds)
0.0	73.2	644.3	167.9	15.9	0.79
0.1	72.3	592.9	160.7	16.8	0.78
0.2	71.1	658.1	157.8	15.8	0.78
0.3	70.1	541.0	151.4	16.3	0.76

4.5 Enhancing Livestock Productivity in Latin America

Highlights

- The Colombia dairy sector has become over the last 10 years more productive and competitive but less profitable
- Launched a new project (Enhancing beef productivity, quality, safety and trade in Central America) funded by CFC and led by ILRI
- Small farmers surveyed in different eco-regions in Colombia see dairy cattle as a way out of poverty

4.5.1 Evolution of milk production systems in tropical Latin America and its interrelationship with markets: An analysis of the Colombian case

Contributors: Federico Holmann, Libardo Rivas, Juan Carulla, Luis A. Giraldo, Silvio Guzman, Manuel Martinez, Bernardo Rivera, Anderson Medina, and Andrew Farrow

Rationale

The livestock sector in tropical Latin America (LAC) has been one of the main economic activities within the agricultural sector due to a great extent to abundant areas under savannas appropriate for livestock production. Despite its vast forage resources, livestock production in tropical LAC faces acute problems due to low productivity levels and market changes. In addition, internal discussion exists on the viability of these production systems to compete in a free trade economic environment, especially now that negotiations are under way to join the North American Free Trade Agreement (NAFTA). The aim of this document was to study the evolution of milk producing systems taking Colombia as a case study and to analyze their constraints and opportunities in the context of small producers, technological change, and competitiveness of the regional livestock sector.

Materials and Methods

Data came from a survey to 545 farms in five ecosystems during 2000 to calculate variable costs, income, and to characterize farms by productivity and management practices using multiple correspondence and general linear models. Costs and incomes were estimated based on the methodology described in Holmann et al., (1990). Competitiveness was defined as the permanence capacity in the dairy activity and was measured through the unitary cost of milk and/or beef production. Thus, the lower the production cost, the more competitive the farm is. Profitability was defined as annual net income divided by the number of adult cows.

Technological change was measured through the concept of productivity, expressed as production of milk and beef per cow and per hectare per year.

Twelve technologies and/or management practices were evaluated to quantify their impact on productivity, profitability, and competitiveness, which were: (1) proportion of improved pastures established on the farm; (2) number of grazing paddocks used by milking cows; (3) amount of feed supplements offered to milking cows; (4) reproductive system used; (5) breed group used; (6) number of milkings per day; (7) use of fertilization; (8) use of the irrigation; (9) proportion of mature herd in milk; (10) years of experience producing milk; (11) herd size; and (12) de-worming frequency against both external and internal parasites.

Data was analyzed by production system (i.e., specialized dairy vs. dual-purpose) and by region (i.e., two sites in lowland areas: Caribbean and Piedmont; and three sites in highland areas: Coffee-growing area; Antioquia, and Cundiboyacense altiplanicie).

Results and Discussion

Effect of technological change. Depending on the region where farms were located, farmers that adopted more than two thirds of the area allocated to livestock under improved grasses produced 126% to 309% more milk/ha, had 31% to 350% higher net income/cow/yr, and produced milk at 8% to 13% lower cost than farms with a low proportion of improved forages (i.e., less than one third of livestock area). Farms that had more than 20 grazing paddocks for a more efficient rotation of the milking herd produced 12 to 140% more milk/ha, generated 54% to 133% higher net income/cow/yr, and produced milk at 19% to 27% lower cost compared to farms that had less than 10 grazing paddocks.

The use of strategic feed supplementation to the basal diet of forage had mixed effects. The best economic response to this supplementation in lowland regions was with low quantities (i.e., < 0.5 kg DM/cow/day) of feed supplements while in highland regions was with moderate quantities (i.e., between 0.5 and 2 kg DM/cow/day). The use of fertilization and irrigation increased productivity, but reduced net income and increased production costs in all regions and production systems, except in the Cundiboyacense altiplanicie, which suggested the need to allocate research resources to determine the best economic response to various levels of N_2 and H_2O to different improved grasses under various soil types and conditions.

Farms that practiced twice a day milking produced 83% to 520% more milk/ha, generated 25% to 148% higher net income/cow/yr, and produced milk at 15% to 27% lower costs compared to farms that milked once per day. Farms that de-wormed the milking herd with low frequency (i.e., less than twice/yr) for internal parasites obtained 77% to 128% higher incomes and 8% to 35% lower production costs in comparison with farms that de-wormed with higher frequency (i.e., more than 3 times/yr) although there were not differences in productivity. The amount of years of experience from farmers at producing milk was a key factor to increase profits (38% to 120%), although not productivity. The most competitive and profitable breed group in the dual-purpose system was the crossbred with low (i.e. 24% European - 76% Zebu genes) and medium levels of dairy genes (55% European - 45% Zebu genes) but had lower productivity than the purebred group (i.e. 98% European genes). In the specialized dairy system, the purebred group was slightly more profitable, productive and competitive than the crossbred group with medium level of dairy genes, but this difference was not significant.

Independent of production system or region where farms were located, the increase in competitiveness was in direct relationship with herd size. Thus, as herd size increased, production costs per unit of milk and beef decreased and net incomes per cow increased. However, when this increase in competitiveness with associated with increases in productivity, this trend was not observed, which suggested that highly productive farms were not necessarily profitable. The dual-purpose system was the most profitable one in the Piedmont, Caribbean, and Coffee growing regions while in Antioquia and in the Cundiboyacense altiplanicie the most profitable was the specialized dairy system. Thus, Colombia should have different strategies for research and technology transfer in order to exploit more efficiently the comparative advantages of each region.

Evolution of milk production systems. The Colombian dairy sector has become more productive and competitive, but less profitable. Comparing the evolution of dairy farms with studies 12 years ago (Aldana, 1990), milk production per hectare has increased by 44% in dual-purpose herds and by 14% in specialized dairies. This increase in productivity reduced the milk production cost by 16% and 10% in dual-purpose and specialized dairies, respectively, due to an increase in stocking rate by 15% and 17% in dual-purpose and specialized dairies as well as to an increase in investment in infrastructure and equipment by 258% and 37% in dual-purpose and specialized dairies, respectively. However, net income per hectare during this period decreased by 27% and 69% in dual-purpose and specialized dairies due to a reduction in the producer's price of milk and beef of 22% and 20% in dual-purpose systems, and of 41% and 27% in specialized dairies. Nevertheless, this reduction in price to the producer was never translated in lower prices to consumers, but remained in the hands of supermarkets and milk plants which expanded and modernized with long-life technology. Figure 80 shows the percentage of the price paid for one liter of milk by the consumer that is retained by producers. As observed, producers retained in 1989 about 70% of the final price. However, during the 90's this percentage was systematically reduced to only 37% in 2001. This occurred because the adjustments in the price of milk to producers were always below inflation while the adjustments of milk price to consumers usually surpassed the level of inflation (Figure 81).

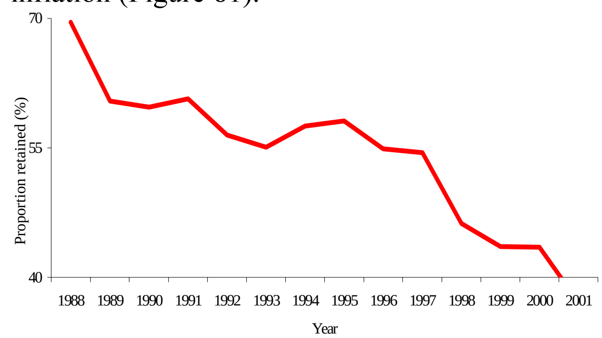

Figure 80. Proportion of the milk price paid by consumers that is retained by producers in Colombia during the period 1989 to 2001.

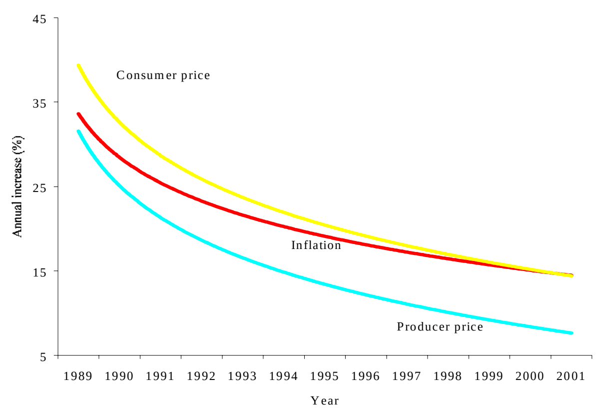

Figure 81. Inflation rate and adjustments in the price of milk paid by consumers and received by producers in Colombia during the period 1989 to 2001.

Development agencies must internalize the fact that policies oriented to markets will be increasingly "oriented to supermarkets". If one adds that in Colombia exists 3 or 4 supermarket chains that control the food retail market, the conclusion is that sectoral policies will need to learn how to deal with a handful of giant companies. This in a huge challenge, and demands an urgent review of ideas and strategies.

The Challenge. The information presented in this case study illustrates the problems and opportunities of the dairy sector in Colombia. However, these systems could represent similar situations in other countries of Latin America. Given the phenomenon of globalization and higher degree of efficiency that these systems are being exposed to, the issues of productivity, technological change, competitiveness, and markets, are critical and of enormous relevance for the performance and survival of the livestock sector in the next decades.

In the coming years, producers cannot limit themselves to participate only in the primary phase of production, but to expand their scope of action to other phases of the market chain to have a higher participation in the formation of milk price and to capture a greater piece of the final price. To achieve this, collective action is required, either through cooperatives or associations, not only to buy or sell at a better price, but also to help small producers to adapt to new patterns with higher levels of competition. Otherwise, the new rules of the game could induce a massive exodus of producers in the short term and in a relatively brief period of time.

Efficiency goes hand in hand with technology and this depends on research and technology transfer. However, public funds allocated to agricultural and livestock research are being reduced (Holmann et al., 2003). The challenge consists that producers in Colombia and Latin America take greater control of livestock research by building alliances with local, regional and international organizations leaders in forage and livestock research. For this it is necessary that producers define and fund their own research agenda.

4.5.2 Enhancing beef productivity, quality, safety, and trade in Central America

Contributors: Federico Holmann, Edwin Perez, Paul Schuetz, Pedro Argel, Carlos Pomareda, and Victor Arrúa

Rationale

Beef is an important commodity in the economies of Central American countries. In 1995, regional beef exports realized more than US$ 126 M and imports cost nearly US$ 17 M. This is especially important to the low-income countries in the region. It is, for example, Nicaragua's most important agricultural activity accounting for 22% of the total Agricultural Gross Domestic Product (AGDP), with exports of fresh and processed meat to El Salvador, Costa Rica, Mexico and the USA among other countries. Guatemala, Honduras and Costa Rica export meat primarily to the USA and Mexico. Total beef exports from the region are showing a declining trend although intra-regional exports increased by 45% in 1998 relative to 1997. However, increases in intra-regional exports are falling behind the total imports by the region. The lost of international markets, particularly the USA, added to the increasing presence of extra-regional competitors and the stagnation of the production and productivity in Central American countries are an indication of the crisis of the beef industry in the region, which affects smallholder producers.

Project Description

This a development-oriented project approved by the Common Fund for Commodities (CFC) that began activities in July of this year, which aims at alleviating poverty by raising smallholder farm productivity, and enhancing trade in beef with improved meat quality and safety. This overall objective is to increase the availability of affordable safe meat products for local consumers and make the Central American beef industry more competitive against imported beef. The project is being executed in the CFC member states Guatemala, Honduras, Nicaragua and Costa Rica.

This is a 4-year project with an estimated cost of US$5 million of which $3.5 millions are being funded by CFC and the remaining $1.5 million from co-funding from counterpart contributions. The project executing agency is the International Livestock Research Institute (ILRI) and the project leader Federico Holmann.

Co-executing agencies are CIAT (Centro Internacional de Agricultura Tropical), SIDE (Servicios Internacionales para el Desarrollo Empresarial), IICA (Inter-american Institute for Cooperation in Agriculture), and CAC (Consejo Agrícola Centroamericano). Local partner organizations are CORFOGA in Costa Rica, MAG-FOR and FAGANIC in Nicaragua, SAG and FENAH in Honduras, and MAGA and ASOBRAHMAN in Guatemala. The project coordinating office is housed within the facilities of the Instituto de Desarrollo Rural (IDR) based in Managua, Nicaragua.

The project's goal is to improve the livelihoods of smallholder producers, make quality safe animal-source foods affordable and available to low income consumers and increase the intra- and inter-regional beef trade in Central America. These activities are conducted as part of the following project components: (1) Improving farm productivity; (2) Beef quality and safety standards and controls; and (3) Project monitoring, impact assessment and dissemination of research products.

The main activities of Component 1 (Improving farm productivity) are:

(a) Identification of technical interventions to improve farm productivity;
(b) On-farm validation of best-bet technologies to improve farm productivity
(c) Implementation of disease surveillance and control measures; and
(d) Strengthening risk analysis capacity to prevent exotic diseases disrupting exports

The main activities of Component 2 (Beef quality and safety standards and controls) are:

(a) Development of food safety and processing procedures along the beef production-to-consumption chain;
(b) Development of a carcass classification systems and regulations for meat quality and safety.

The main activities of Component 3 (Project monitoring, impact assessment and dissemination of research products) are:

(a) Monitoring project progress and assessing the impact of the component activities and providing feedback to stakeholders;
(b) Dissemination of project outcomes and outputs to policymakers and official and private extension agents.

CIAT will participate in the co-execution of Components 1 and 3 in aspects related to increasing on-farm productivity through the introduction of improved-based forages aimed to increase weaning weights of pre-weaned calves and to improve the nutritional status of dams.

The project held an initial planning meeting during July 7-10 in San José, Costa Rica with 20 participants which included representatives from both the private and public sectors from Guatemala, Honduras, Nicaragua, and Costa Rica, as well as from the co-executing agencies. The objectives of the planning meeting were to:

(a) make a presentation of the overall project objectives, goals and activities;
(b) know each other better by making brief presentations of the goals and objectives from each participating institution as related to the project activities;

(c) review the state of the art of the beef sector in the region as well as in each participating country;
(d) present and discuss the project workplan for the first year; and
(e) Discuss operational issues related to the project.

The products from the planning meeting were:

(1) the project workplan for the first year with its corresponding calendar of activities discussed and approved;
(2) Responsibilities from each participant discussed and approved;
(3) Indicators for project monitoring and evaluation selected;
(4) Mechanism for management of project funds discussed and approved; and
(5) Frequency, information required, and format for the technical project reports discussed and approved.

The project will start executing the approved workplan in mid-September with the execution of a baseline study in each of the selected regions, which are: Perez Zeledón in Southern Costa Rica, Boaco and Chontales in Nicaragua, Olancho and Valle del Aguán in Honduras, and Izabal and Baja Verapaz in Guatemala.

4.5.3 The role of livestock in poverty alleviation: An analysis of Colombia

Contributors: Federico Holmann, Libardo Rivas, Juan Carulla, Luis A. Giraldo, Silvio Guzman, Manuel Martinez, Bernardo Rivera, Anderson Medina, and Gerardo Ramirez.

Rationale

The potential of livestock to reduce poverty is enormous. Livestock contribute to the livelihoods of more than two-thirds of the world's rural poor and to a significant minority of the peri-urban poor. The poorest of the poor do not have livestock, but if they can acquire animals, their livestock can help start them along a pathway out of poverty.

Roles of livestock keeping revolve around storing wealth, contributing to food and nutritional security, providing draught power, transport and manure, and serving traditional social functions. In some situations, the "livestock ladder" may allow the poor to progress from modest livestock holdings, such as a few poultry, to acquiring sheep and goats or pigs, or even cattle. Livestock production provides a constant flow of income and reduces the vulnerability of agricultural production. The objective of this study was to understand the perception of agricultural producers in Colombia who currently do not own livestock about the role of cattle in alleviating poverty in their farms.

Materials and Methods

Data came from direct survey interviews during January to June of 2002 to 143 farmers who did not own cattle in the five most important regions of animal production in Colombia to elicit their perception about the role of livestock as a pathway out of poverty. Selected regions were: Piedmont, Caribbean, the Coffee-growing region, the highlands of Antioquia, and the Cundiboyacense altiplanicie.

Results and Discussion

Land Use. Table 81 contains the average farm size and land use by farmers who do not own cattle. Mean farm size varied from 5.7 ha in the Cundiboyacense altiplanicie to 13.4 ha in the highlands of Antioquia.

All farmers depended on both annual and perennial crops for most of their income although it varied significantly across regions. In the Coffee-growing region, producers depended mostly on coffee for their income whereas in the Cundiboyacense altiplanicie they depended on annual crops, mainly potatoes and broad beans (*Vicia faba*). In the Piedmont and Caribbean regions producers depended mostly on maize, cassava, and rice. Fruits were the most important land use in Antioquia and a very important crop in the Piedmont and the Coffee-growing region.

Pastures were an integral part of land use in all farms, even though producers did not have cattle. This was specially the case in Antioquia, where 58% of farm size was under pastures. The reason for this was because most farmers interviewed were coffee growers which during the early 90's switched to fruits trees and fattening steers under intensive pasture rotation using high levels of N_2 fertilization. All interviewed farmers who had steers had sold them due to negative economic returns and were currently fattening pigs. Land under pastures was abandoned, as they did not want to go back to coffee or other agricultural land use at the time the surveys were executed.

With regards to land use, the largest proportion of farms under forest were found in the Piedmont and the lowest in the Cundiboyacense altiplanicie, which makes sense since the former is the agricultural frontier while the latter has been under agricultural production for the longest period of time.

Table 81. Farm size and land use by non-livestock owners in five regions of Colombia during 2002.

Parameter	Region				
	Piedmont (n=33)	Caribbean (n=33)	Coffee (n=23)	Antioquia (n=25)	Cundi-boyacense (n=29)
Farm size (ha)	11.7	9.6	9.9	13.4	5.7
Land use (ha)					
Annual crops					
- Maize	0.5	3.1	0.1	0	0.1
- Rice	1.0	0.3	0	0	0
- Beans	0.1	0.1	0.1	0.1	0.2
- Other [1]	0.8	2.2	0.3	0.9	3.2
Perennial crops					
Coffee	0	0	6.1	0	0.6
Sugarcane	0.1	0.1	0.2	0	0.1
Fruits	1.3	0.8	0.5	2.1	0.1
Other [2]	1.2	0.3	1.5	0	0
Pastures	3.3	1.9	0.4	7.8	1.1
Forest	3.2	0.9	0.9	2.3	0.4

[1] Cassava in the Piedmont and Caribbean; Onions and cassava in the Coffee region; Cassava, potatoes, and broad beans (*Vicia faba*) in the Cundiboyacense altiplanicie.
[2] Bananas and plantains.

The role of cattle in smallholder farms. In Table 82 we present the proportion of smallholders who had cattle in the past and the main reasons for selling or not owning cattle in 2002. Most smallholders interviewed in the Piedmont had cattle in the past (85%), followed by producers in Antioquia (60%) and least in the Coffee-growing region (39%).

The most important reason for selling their cattle in all regions was due to financial crisis and needed cash (i.e., from 27% of smallholders interviewed in the Caribbean and the Cundiboyacense altiplanicie to almost 50% in the Piedmont). Most smallholders surveyed used the money from the sale of animals to pay health bills of family members, to pay off debts, and/or to use the cash to survive due to crop failure from extreme weather conditions (i.e., drought or frost damage).

Table 82. Proportion of farmers who owned cattle in the past and reasons for selling or not owning livestock in five regions of Colombia during 2002.

Parameter	Region				
	Piedmont (n=33)	Caribbean (n=33)	Coffee Zone (n=23)	Antioquia (n=25)	Cundi-boyacense (n=29)
Non-livestock farmers who had cattle in the past (%)	84.8	42.4	39.1	60.0	52.2
Reason for selling or not having cattle today (%)					
Due to financial crisis	48.5	27.3	34.8	28.0	27.5
Not enough land to keep cattle	18.2	18.2	0	4.0	27.6
Owning cattle was a bad experience	9.1	6.1	4.3	24.0	0
Cattle died of disease	0	3.0	0	0	13.7
Cattle was robbed	0	3.0	0	4.0	6.9
Low profitability	6.0	0	0	8.0	0
Security problems	12.1	6.1	0	0	0
Other	6.0	0	0	4.0	3.4
Reasons for not owning cattle today but had it in the past (%)					
Not enough land to keep cattle	12.1	51.5	39.1	8.0	37.9
Not enough money to buy cattle	12.1	51.5	4.3	0	34.5
Requires high investment and agriculture is preferred	0	3.0	13.0	16.0	6.9
Does not like to own cattle	3.0	0	13.0	8.3	0
Not enough knowledge on how to manage cattle	0	6.1	0	0	3.4
Security problems	3.0	0	0	0	0
Other					
Producers who currently own livestock other than cattle (%)	72.7	100.0	52.2	36.0	69.0
Average inventory and species of livestock other than cattle (#)					
Laying hens / broilers	35.0	25.0	11.5	0	9.5
Pigs	3.0	10.0	4.0	30.0	0
Sheep	0	0.3	0	0	1.8
Goats	0	0	2.3	0	0
Ducks	0	4.4	0	0	0.1

The second most important reason for selling cattle in smallholder farms in Antioquia was due to low profitability (32%) when producers switched from coffee to fattening steers under intensive grazing during the 90's. In other regions the second most important reason was because of limited amount of land, especially in the Cundiboyacense altiplanicie, the region with the smallest farm size.

Other reasons for selling cattle was due to security problems (12% of smallholders in the Piedmont and 6% in the Caribbean regions), others because owning cattle was an unpleasant experience (9% in the Piedmont, 6% in the Caribbean, and 4% in the Coffee region), and another reason mentioned was due to cattle rustling (3% of smallholders in the Caribbean, 4% in Antioquia, and 7% in the Cundiboyacense altiplanicie).

Even though smallholders surveyed did not own cattle, most of them had in their farm other species of livestock, ranging from 36% of farmers in Antioquia to 100% in the Caribbean. The most common livestock specie owned was poultry (both laying hens and broilers), ranging between 9.5/farm in the Cundiboyacense altiplanicie to 35/farm in the Piedmont. The only region where poultry was not found on farms was in Antioquia.

The second most common specie found was pigs, ranging from 3/farm in the Piedmont to 30/farm in Antioquia, where farmers who had fattening steers switched to pigs. Other species found were sheep, goats, and ducks, but these were not common. These livestock assets provide smallholders with high quality protein (meat and eggs) for household consumption to complement the grain-based diet they have.

Farmer perception about cattle ownership. In Table 83 we show the perceptions on the role of cattle ownership by smallholder farmers who do not own cattle. The most striking result is that 76% of farmers in Antioquia to 97% of smallholders in the Cundiboyacense altiplanicie would like to own cattle if they had the opportunity. The most important reason for owning cattle in all regions was as a mechanism for savings and building capital. The second most important reason in all regions was to obtain milk and beef for family consumption, except in Antioquia where this issue

Table 83. Subjective perceptions about the role of cattle ownership by agricultural producers who did not own cattle in five regions of Colombia during 2002.

Parameter	Region				
	Piedmont (n=33)	Caribbean (n=33)	Coffee Zone (n=23)	Antioquia (n=25)	Cundi-boyacense (n=29)
Farmers who would like to own cattle today (%)	84.8	87.9	82.6	76.0	96.6
Reason for owning cattle (%)					
To obtain milk and beef for family consumption	51.5	87.8	30.4	0	58.6
A mechanism for savings and building capital	54.5	100.0	47.8	74.0	86.2
To reduce and diversify risk due to crop failure	15.2	3.0	8.7	8.0	51.7
To use manure as fertilizer	9.1	0	8.7	0	34.5
Preferred animal category to own (% of farmers)					
Milking cow	81.8	84.9	47.8	52.0	96.5
Female calf	6.1	0	8.7	24.0	31.0
Male calf	0	0	26.1	0	27.6
Steer	15.2	6.1	17.4	0	13.8
Bull	9.1	0	0	0	31.0
Desirable amount of animal category to own (#)					
Milking cow	8.4	13.2	2.3	9.4	5.8
Female calf	1.4	0	0.3	2.5	1.6
Male calf	0	0	1.8	0	1.5
Steer	0.6	0.6	7.4	0	0.3
Bull	0	0	0	0	0.4
Necessary conditions to own cattle (%)					
More land	21.2	78.8	39.1	4.0	75.9
Availability of credit	42.4	63.6	56.5	28.0	17.2
Security	18.2	6.1	0	8.0	0
Improve farm infrastructure	18.2	3.0	8.7	24.0	17.2

was irrelevant. Other reasons for owning cattle were to reduce and diversify risk due to crop failure and to utilize manure as fertilizer. This was specially the case in the Cundiboyacense altiplanicie (Table 83) where most farmers grow broad beans and potatoes and the risk of frost damage is significant and where manure from cattle can be an important source of fertilizer and organic matter.

The preferred animal category to own in all regions was by far the milking cow, ranging from 52% of smallholders in Antioquia to 96% in the Cundiboyacense altiplanicie. In addition, when smallholders were asked to express the desired amount of animals they would like to own, again the milking cow was the animal category with the highest number. When smallholders were elicited about the necessary conditions to own cattle, the most frequent answer was availability of credit in the Piedmont, the Coffee-growing region, and Antioquia, whereas the most frequent answer in the Cundiboyacense altiplanicie and the Caribbean was to have more land as a condition to own cattle. Other important conditions to own cattle was to have the adequate infrastructure and to a lesser extent, to improve the security conditions in rural areas.

Conclusions

Results from this study show that small farmers see cattle as a contribution to the improvement in the quality of life. The milking cow is one of the factors that contributes the most to the well-being due to the role she plays within the farm: utilization of labor with low opportunity cost, security against crop failure, liquidity against financial crisis, as collateral for informal credit, and as a protection against inflation (Estrada, 1995). Smallholders who had sold their cattle in the past were mainly for these same reasons. The challenge is to develop novel mechanisms to provide smallholders with livestock, either through credit loans or through the Fondos Ganaderos of Colombia, whose objective is to help small farmers who have production capacity but lack the resources to buy cattle.

4.6 Expert systems for targeting forages and extension materials for promoting adoption of forages

Highlights

- Beta version of SoFT (Selection of Forages for the Tropics) database completed, based on interaction of partners involved in project and expert consultation.
- Draft tool GEMS (Genotype x Environment x Management System) developed
- Developed extension materials of improved forages for smallholder farmers in Central America and Southeast Asia

4.6.1 Development of a database and retrieval system for the selection of tropical forages for farming systems in the tropics and subtropics

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Rationale

The demand for livestock products from both ruminants and monogastrics in the developing world is increasing at 6-8% per annum. This situation provides a unique opportunity for smallholder farmers in developing countries to increase income by satisfying that demand, and in some cases will enable them to advance to market-oriented production for the first time. Income obtained from market-oriented livestock production will impact on cash flow and purchasing power, and hence act as a driver for sustainable intensification.

The challenge facing all farmers is to develop strategies to meet the feed demand of their animals and the market demands for livestock products of specified quality. Sown tropical forages can provide part of the feed base that will support this market expansion. Forages can be used to provide improved feed quality and quantity in a range of farming systems, including those based on maize and rice cropping systems, as well as systems that depend almost entirely on continuous grazing of native grasslands. The benefits of including well-adapted sown forages in the diets of animals in these systems has been well documented, but adoption has been limited for a number of reasons, including poor access to appropriate information. These species can provide benefits beyond feeding livestock. In mixed cropping/livestock systems, forages can be integrated with cultivation cycles to improve soil fertility and structure, and in agro forestry and orchard systems, they can be used to suppress weeds as well as feed animals. Stoloniferous and rhizomatous species blanket the soil as live mulch, reducing erosion.

Farmers depend very heavily on advice from extension specialists, development agencies, researchers and seed companies, whose knowledge on forages is often limited because of inexperience in a region, the difficulty in harnessing the expertise of others, and poor access to information. Much of the important information is fragmented, unpublished or published in media of limited circulation. This project intends to synthesize and interpret this information and so overcome this limitation to the wider adoption of forages. This project is bringing together in one-knowledge system (SoFT – Selection of Forages for the Tropics) much of the accumulated information of the last 50 years from across the tropical world, on species adaptation, together with their use and management. Information will be sourced from scientific literature, the plethora of reports, and from unpublished information gleaned directly from agronomists with extensive tropical experience. The completed product will be a computer-based system that can be used to select “elite” forage accessions for a range of uses, farming systems and environments. This synthesis of information on the use (e.g. fish, pigs, mulch) and application (e.g. lay pasture, under trees, relay with rice) of forages, in addition to the conventional genotype x environment information, is unique and made possible by the development of new information management systems.

The specific objectives of the project are:

- To develop a knowledge system for the identification of forages suitable for specified niches within smallholder farming systems.
- To promote the system within the “communities” who are using tropical forages.
- To develop a strategy for maintenance and updating the knowledge system.

The knowledge system will provide users with fact sheets on the use and management of each accession, identify sources of seed and regional specialists, and contain a comprehensive bibliography. It will be available on CD/DVD or via the WEB and will initially be produced in English. Maintenance of the system will be carried out by CIAT, Colombia. The product is primarily intended for use by individuals and agencies providing advice to smallholder farmers.

This target audience in Africa, Asia and the Americas is likely to number in the thousands. Bringing together the partners in this project, CSIRO Sustainable Ecosystems, the Queensland Department of Primary Industries (DPI), Centro Internacional de Agricultura Tropical (CIAT), International Livestock Research Institute (ILRI) and the Food and Agriculture Organization of the United Nations (FAO), provides a unique opportunity to draw on the vast accumulated knowledge from Asia, Africa, the Americas and Australia. The knowledge system will be released at the International Grassland Congress in Dublin, Ireland in June 2005 and will be promoted through CGIAR and other international networks, through collaborating national programs and the Virtual Colombo Plan.

Results and Discussion

Tool development: Immediately after the approval of the project in July 2002 a draft Software tool based on the database Software LUCID was developed. This tool was presented to a range of forage experts from NARS, NGOs, development projects and international research organisations at a design workshop in Bangkok in October 2003. Based on the recommendations beta tools of the database software tool (SoFT-Selection of forages for the Tropics), with expert knowledge on a few well known species was developed until early 2003 (Figure 82). Forage species descriptors for the tool were categorised so that data could be used as an input into GEMS (working title), a GIS based targeting tool. An interface was developed to automatize data input from SoFT into GEMS. In addition, a tool for capturing data from experts was developed for utilization in expert workshops (Figure 83). The possibility to use the tool is via Internet is being tested, as one of the possibilities to access the database tool in addition to CD/DVD –ROM.

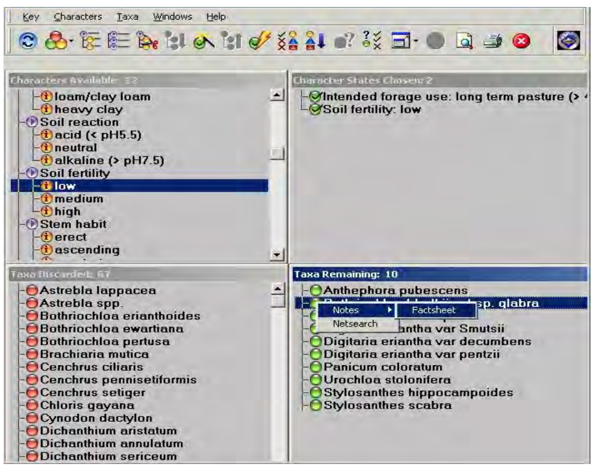

Figure 82. Result of a query to the database, with additional information, i.e. access to Fact sheets

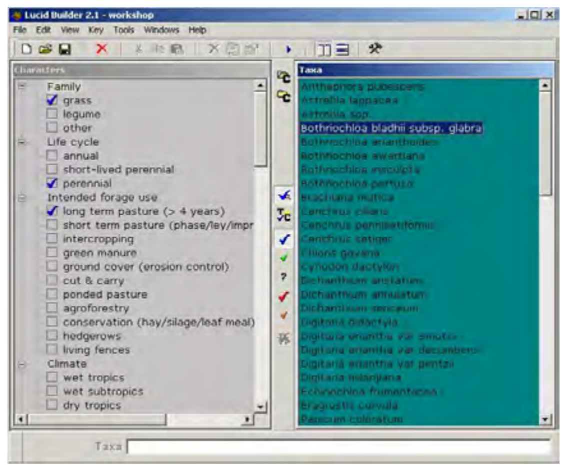

Figure 83. Data capturing interface for SoFT with list of characters and species.

Information gathering from forage experts: Parameters for forage description and range of species were defined in consultation with potential clients. To gather information from forage experts, workshops each with 15 – 30 participants were held in Addis Ababa (for African Forage Experts), and Brisbane (for Australian Forage Experts), Stuttgart Hohenheim (for tropical forage experts based in Europe) and Cali (for American experts), utilizing the above described tool for capturing expert data. Further workshops are planned in Honduras (November/December 2003) and Asia (February 2004). It is planned that by in the first half of 2004 information from all these expert workshops will be available in the tool for further revision.

4.6.2 Incorporating Socio-Economic Data and Expert knowledge in representation of complex Spatial Decision-Making

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Rationale

The GEMS (Genotype x Environment x Management System) project integrates biophysical, socioeconomic and management data together with expert knowledge to assist farmers' decision-making processes. The approach is based on the following two main assumptions:

- A wealth of information on the agro-ecological adaptation of tropical crop species is available in CIAT-held and other databases. However, the access and hence utilization of this information needs to be improved. In addition, data are often uncertain or missing, and methods are needed to combine existing data with expert knowledge to provide better analysis.
- In evaluations of species adaptation to environmental conditions, agro-ecological information is often separated from socioeconomic factors influencing species adoption.

The above assumptions are particularly the case for forage species, and this project grew out of the perceived need to address these issues for forages. Therefore, while the research is applicable to other crops, in the first instance it focuses on forages. Based on these assumptions, the targeting of forage germplasm is intended to enhance the utility of existing information, and in the future, to integrate environmental and socioeconomic adaptation of forage germplasm for multiple uses. It is anticipated that this approach will allow a more accurate and client-oriented prediction of possible entry points for forage germplasm.

One product of this research will be a fully functional Web-based or CD-ROM tool, primarily designed for targeting forage germplasm in Central America. The primary target users are NGOs, development agencies, national research institutes, and decision makers in government. In conjunction with farmers, these users will be able to more effectively target suitable locations for new forages, with the aid of the tool. This will result in more informed choices being made, thus allowing more effective use of public funds dedicated to agricultural development and natural resource conservation. Tools to better target forages will also help improve the well being of smallholders by assisting them to more effectively utilize their resources in sustainable ways. The addition of carefully selected forages to a farming system has a plethora of benefits both for the farmers and for the environment, as well as the wider community. These benefits derive both from the direct influence of forage planting, and the indirect increase in cattle production and cropping system improvements, and include for example improved sustainable intensification, reduced erosion, and alleviation of protein and micronutrient deficiencies in the community.

Materials and Methods

Review of literature and of existing similar models and software is ongoing, with existing tools currently being evaluated to determine their appropriateness in representing expert spatial decision-making and particularly in targeting forage germplasm. Bayesian modeling has been identified as the most appropriate method, especially for decisions involving sparse and uncertain data.

Case study–design and develop a tool

As a case study, a decision support tool to target forage germplasm is being designed and developed, using GIS technology. This targeting consists of identifying which forages would be suitable or successful in a particular location, given data and/or knowledge about the forages, and about the location in question.

Data used in this case study include the CIAT (Red Internacional de Evaluación de Pastos Tropicales) RIEPT database (linking forage adaptation, establishment, and production to climate and soil factors), and GIS surfaces of elevation, rainfall, temperature, soil, population density and distance to market for Central America, supplemented with expert and farmer knowledge. The tool is being implemented in Delphi 6.0 with Map Objects LT. It is envisaged that a future version of the tool will be Web-based.

Results and Discussion

Data: Although a large amount of data is available in the RIEPT database, it is characterized by uncertainties, biases, errors, and omissions. Similarly, much of the available data is correlated. Data analysis combined with expert opinion has defined the factors to be used in the modeling process (Table 84).

Table 84. Data used in model specification and model application for a decision support tool to target forage germplasm.

Data	Derived from	Source for model specification	Source for model application
Fine scale (1-4 km grid cells):			
Elevation (m)		RIEPT where available, GIS data	GIS data
Mean annual rainfall (mm)	Monthly rainfall	RIEPT where available, GIS data	GIS data
Consecutive dry months (< 60 mm)	Monthly rainfall	RIEPT where available, GIS data	GIS data
Holdridge life zones	Monthly rainfall, mean monthly temperature	RIEPT where available, GIS data	GIS data
Soil pH		RIEPT	Farmer
Soil texture	% sand, % clay, % silt	RIEPT	Farmer
Soil fertility	% organic matter, P	RIEPT	Farmer
Shade tolerance		Experts	Farmer
Drought tolerance		Experts	Farmer
Salinity tolerance		Experts	Farmer
Water logging tolerance		Experts	Farmer
Aluminum tolerance		Experts	Farmer
Frost tolerance		Experts	Farmer
Intended use		Experts	Farmer
Risk aversion		Confidence in data or knowledge	Farmer
Coarse scale (8-32 km grid cells):			
Population density		GIS data	GIS data
Distance to market	Roads, population centres	GIS data	GIS data
Soil pH	FAO classification	GIS data	GIS data
Soil texture	FAO classification	GIS data	GIS data

Models and algorithms: Literature on models and algorithms for habitat distribution prediction and spatial classification has been reviewed.

Commonly used methods include:
- Multiple linear regression,
- Generalized Linear Methods (GLM),
- Generalized Additive Models (GAM) and other statistical techniques,
- Rule-based systems,
- Habitat envelopes,
- Classification and Regression Trees (CART),
- Probability modeling, including Bayesian modeling and belief networks,
- Artificial intelligence approaches, including Artificial Neural Networks (ANN) and Multi-Agent Systems (MAS), and Cellular Automata.

Most of these methods are inappropriate for the nature of the problem. Lack of reliable and complete data precludes most statistical techniques. An additional requirement is that the model should be transparent, i.e., it should be logical and obvious to the user why a species is being suggested. The best candidates are rule-based systems, CART, and Bayesian modeling. Of these, Bayesian modeling has been selected because of its ability to deal with uncertainties and incorporate expert knowledge.

Tool development: Tool development is progressing for the case study of forages in Central America. The tool allows users to select a location of interest and define characteristics of the location and desired characteristics of the forage species. They are then presented with a selection of suitable forage species, which they can interrogate for more information (Figure 84).

The data and parameters for the tool are derived from RIEPT data updated with expert knowledge. This expert knowledge is either directly input by forage experts, or derived from SoFT (Selection of Forages for the Tropics) data. SoFT is an international project in which CIAT is involved. It began in 2002, and aims to collect and make accessible expert knowledge about tropical forages. Probabilities are updated graphically (Figure 85), and experts can examine maps to verify the parameterization of the model (Figure 86).

Figure 84. Suitable forage species with options for interrogation.

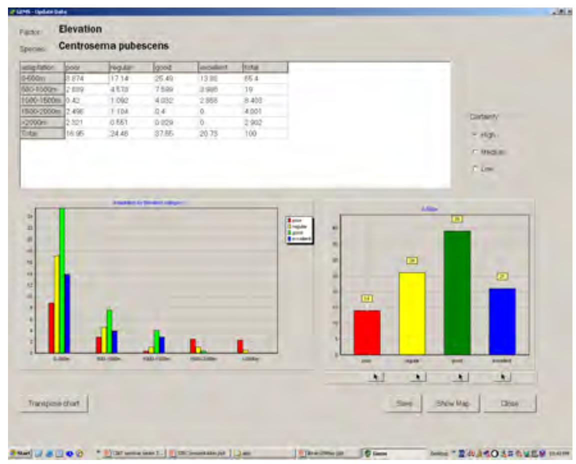

Figure 85. Graphically updated probabilities

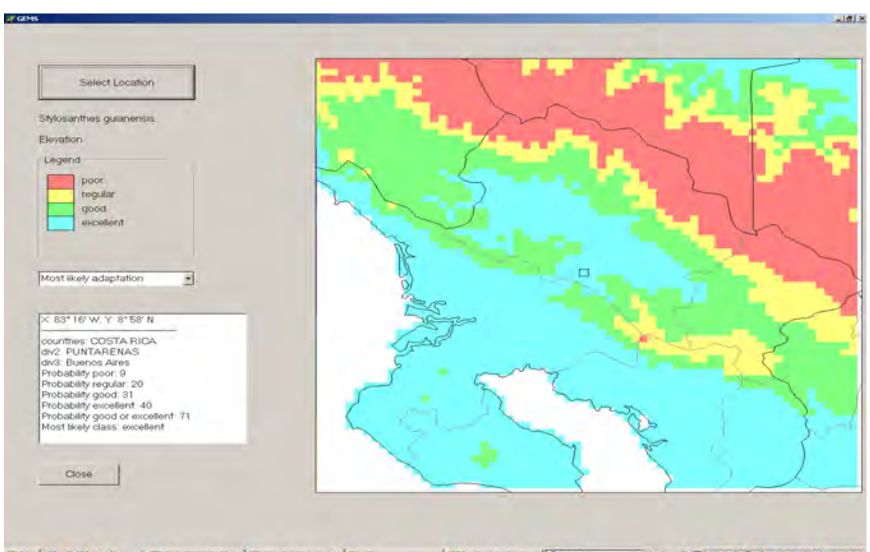

Figure 86. Map of adaptation related to elevation

4.6.3 Production of materials on forages for use in extension in Central America

Especies Forrajeras Multipropósito: Opciones para Productores de Centroamérica [Multi-purpose Forage Species: Options for Central American Producers]:

Contributors: M. Peters; L.H. Franco; A. Schmidt; B. Hincapié (CIAT)

Publication for smallholder farmers and technicians to select forage species depending on local climate and soil conditions, wit emphasis on Central American environments. The booklet contains easy-to-understand information on forage species. Information was compiled based on work of CIAT with farmers in Costa Rica, Honduras, and Nicaragua. The booklet was developed under the project " Farmer Participatory Research in Action: Selection and Strategic Use of Multipurpose Forage Germplasm by Smallholders in Production Systems in Hillsides of Central America", financed by BMZ/GTZ. The publication has five sections: (1) grasses, (2) herbaceous legumes, (3) cover legumes and green manures, (4) shrub legumes, and (5) other species of interest. Specific information is provided on the different forage species and their multiple uses over a broad range of production systems. The inclusion of color photographs facilitates the differentiation of species, and tables quickly provide the reader with a summary of the uses and characteristics of each species (Photo 29).

Producción Artesanal de Semillas de Pasto Toledo (Brachiaria brizantha, accesión CIAT 26110) [Artisanal Seed Production of Toledo Grass (*Brachiaria brizantha*, accession CIAT 26110)]

Contributors: P. J. Argel; G. Giraldo; M. Peters; C. E. Lascano

CIAT's Tropical Forage Project, together with several national research institutions, development projects and Non Governmental Organizations in Costa Rica, Honduras, Nicaragua and Colombia, is promoting Toledo grass (*Brachiaria brizantha* CIAT 26110. The good acceptance of Toledo in the region has translated into a high demand for seed. This handbook describes the experiences of technicians and of small- and medium-scale producers in Central America, and indicates the minimum management conditions necessary for artisanal seed production. The publication was developed under the project " Farmer Participatory Research in Action: Selection and Strategic Use of Multipurpose Forage Germplasm by Smallholders in Production Systems in Hillsides of Central America", financed by BMZ/GTZ. A summary of the most important information for artisanal production of Toledo seed is also available as a leaflet (Photo 29).

Producción Artesanal de Semillas de Cratylia *(Cratylia argentea* accesiones CIAT 18516 y 18668) [Artisanal Seed Production of Cratylia (*Cratylia argentea* accessions CIAT 18516 and 18668]

Contributors: P. J. Argel; G. Giraldo; M. Peters; C. E. Lascano

Recent studies carried out by CIAT's Tropical Forages Project, together with national research institutions in South America, Central America, and the Caribbean, have demonstrated the high potential of the shrub legume Cratylia *(Cratylia argentea*) in animal production systems. This handbook describes practices for the artisanal seed production of Cratylia to promote its use by small- and medium-scale livestock producers in those areas where drought is a limiting factor. The publication was developed under the project " Farmer Participatory Research in Action: Selection and Strategic Use of Multipurpose Forage Germplasm by Smallholders in Production Systems in Hillsides of Central America", financed by BMZ/GTZ. A summary of the most important information for artisanal production of Cratylia seed is also available as a leaflet (Photo 29).

Photo 29. Production of materials on forages for use in extension in Central America

4.6.4 Production of materials on forages for use in extension in Southeast Asia

Contributor: P. Horne (CIAT)

Throughout 2003, the FLSP worked with province and district staff to discuss the impacts that are emerging from forages, to discuss the important aspects of these impacts that need to be documented and to practice preparing case studies that can be used to drive extension and scaling-out. Case studies are starting to emerge that will excite other farmers, extension staff and extension managers. These cases are being developed into:

1. **Posters** which highlight maybe one to three cases in a single poster around a common theme
2. **Case study sheets** which highlight the main features of each case of impact in detail, to be used by the district staff for field days and cross visits
3. **Fliers** of each case study of impact that can be given to farmers in a small form and posted in villages in a large form

These extension materials developed from cases of impact will be complemented with ***"tech sheets"*** which highlight common technical problems and opportunities associated with each case. Ten case studies have been documented so far. An example case study (and a poster developed from it) are shown below:

Jong tried to escape from shifting cultivation by investing in a cow, heifer and calf for breeding but the management and feeding problems of free-range grazing in the highlands prevented his herd from increasing. All his labour was lost. Planting forages overcame these problems and his herd has now increased to 15 head. At the same time he has been able to reduce his area of shifting cultivation from 1.5 to less than 0.5 ha and wants to stop altogether

Swidden cultivation is the traditional system for cultivating upland rice in the highland areas of SE Asia. Farmers cut areas of forest, let it dry and then burn off the debris, leaving a cleared and fertilized soil, into which they directly seed upland rice. The following year farmers move to a new area, leaving forest to regenerate on the old field. As population pressures build up farmers are forced to leave less and less time for the forest to regenerate. Weeds become the predominate growth and farmers find themselves in a labour trap:- putting in increasing amounts of labour for weeding, on areas with declining soil fertility for reducing yields.

Swidden or slash and burn cultivation is highly destructive of the forest areas, reduces the bio-diversity and causes many downstream problems. Government policy in Laos is for the farmers to cease this system of cultivation to maintain the watersheds. But exploring and establishing a new livelihood does not come easily. Access of these remote highland villages to markets is difficult, and the farmers themselves are working from a declining resource base.

Jong Gor Her is a Hmong farmer with a young family of 4 children living in the village of Kieuwtalun Nyai on a high ridge south of Luang Prabang. Every year they would plant about 1.5 ha of swidden rice fields, beginning with cutting the forest in February and ending with harvest in November. Once the cycle began they could not stop, no matter the weather, their health or their energy. Even after childbirth, his wife would return to field work within a month, with the young baby strapped to her back. And at the end of this cycle they would have only just enough rice to eat. Opium had been grown as a cash crop, but this was no longer permitted. Jong realized that if they continued to rely on swidden cultivation of rice, the future of his family was not bright. In 1992 he put together enough money to buy a cow, a heifer and a young calf.

Addressing Immediate Problems. All the cattle in the village are kept in 4 areas of about 50 ha. each which are cordoned off between the steep limestone hills. Jong would visit his animals about once every 1-2 weeks. Nonetheless they would escape and damage other farmers' crops, resulting in arguments and in Jong paying compensation each year.

The highlands appear deceptively suitable for raising livestock, with large areas of forest and apparent natural feed sources. Feeding was, however, a serious problem when he tethered the cattle close to the house. This was done for different reasons: from May to July when the ticks and blood sucking flies were the worst; or when animals were injured; and when a cow would be calving, (a month before and then a month after birth). During these periods Jong collected native grasses to feed them, spending 2-3 hours each day. *"By the time this was done the whole morning would be gone with no other work done",* he said. This one load of feed (about 50 kg) was really *'just keeping the animals alive'*, and after a week on this diet they would start to grow thin. In the 4 years prior to having forages, the two calves that were born grew weak and both died from malnutrition. Thus in all this time, his herd had failed to increase, and he had only succeeded in adding an extra burden of labour to the family.

Jong joined in forage trials in 1997, planting a small area (about 250 m^2) of six varieties for evaluation. With these forages close by (only 5 minutes walk form the house) he was able to increase the feed for his cows from 1 to 2 loads per day, taking him just thirty minutes to cut and carry. When the children came home from school at midday, they added a third load. Thus, better accessibility to forage more than doubled the feed provided to the tethered cattle. The quality of feed was also better than from the native grasses, as indicated by all the feed being consumed without any residue being left.

After the initial success, it was clear to Jong that his small trial plot would not be enough and he purchased a second similar plot from a neighbour. He then expanded the original block further to 1250 m^2 giving a total area of more than 2000m^2, which he occasionally grazed as well as cut. He has been harvesting forages from these areas for more than 3 years now and they are still productive, regenerating within 2-3 weeks in the wet season. He has inter-cropped in to the forages the tree legume, calliandra, which he regularly adds to the cut forage grass when he feeds the cattle. The most important impact that Jong and his family have experienced is that all the calves born since they started planting forages were strong and have survived. Since 1997 the herd has increased to 15 (5 cows and 10 calves). Without planted forages, Jong said, he would never have been able to tether and feed more than two cattle at a time.

Management of Forages in an Extensive Environment. Jong noticed that the native grasses would die out after being cut more than 2-3 times or after being burned whereas the forage grasses survived all these stresses. He also noticed that if the area was grazed, the trampling of the plants and dropping of seed by feeding cattle was expanding the area of forage grasses wider. On the basis of this, he thought he could establish larger areas of improved forage for grazing by simply broadcasting seed in small plots and allowing the cattle to expand it further in due course. However, although this idea interests him, Jong still wants to keep the animals close to the house if he can. So, he plans to fence an area above his forage block for penning the whole herd there and hand feeding them. This would further reduce the time for carrying the forages to feed them and also allow collection of manure to re-fertilize the forage block (which is starting to show yellowing due to nutrient decline). He realises he will need to double the area of the forage block before he can corral the herd there.

Changing Livelihoods. Jong's family usually slashed and burnt 1.5 ha to grow upland rice each year. In 2001, as his herd began to increase, Jong began to cut back this area. This allowed his wife to put more time into handicraft work. With the income from this, and by selling off one calf, they purchased all the rice they needed. They repeated this again in 2002, selling off another calf. By 2003, they planted less than 0.5 ha. of upland rice and expect to this even further, planting cucumber on their upland fields instead.

This year Jong sold not just one calf but three, for a total of 5 million kip. As well as using this to buy the rice they need, Jong has also purchased some comforts for the family. While the family still maintain their traditional Hmong house with an earth floor and wood fired kitchen, they now enjoy their new TV and VCD player (with movies in Hmong). Jong also purchased one of the small Chinese motorcycles that have become common in Laos. With this he is now able to ride the 2 hours down the mountain road to the Provincial Livestock Department in Luang Phabang and purchase vaccine needed for the livestock in the village. The changes in livestock raising by Jong are quite profound. While he did have the vision to escape from swidden cultivation of rice by better livestock production, his initial use of forages was to solve the immediate problems of free-range grazing typical of most farmers in the highlands. As other benefits became apparent, with his ideas of corralling the animals and hand feeding them, Jong is moving towards an intensification of livestock. Along with this intensification he and other farmers in the village have begun to treat their livestock (pigs as well as cattle) for parasites and vaccinate them against common diseases. Through this intensification of livestock they have begun to stabilize their agricultural system. This has given them the means and the time to begin to diversify into other enterprises, such as handicrafts and vegetable crops. About half the households in the village now grow forages for their livestock (cattle and pigs). Four other farmers have also begun to reduce the swidden area they cultivate after improving their livestock production. As these farmers improve the lives of their families, so others will see to impacts and the trend of stabilization and diversification through livestock production will continue to gather speed in the village. On the following page is a sample poster, to be used in extension, based on this case study of impacts.

Publications. Three new publications were produced during this year, documenting (i) technical details of forages in smallholder farming systems, (ii) participatory research methods that have helped us in developing smallholder forage systems in Laos and (iii) the potential impacts that can come from forages in smallholder systems. The first two of these publications are part of the "CIAT in Asia Research for Development Series" (CARDS); a series of publications that is being produced to "provide research information to development workers in the region in a format and in languages that make the information more accessible to them".

Better access to feed means more calves surviving.

More calves surviving means more options for escaping poverty

An endless cycle of labour

The family of Jong Gor Her live in the mountainous village of Kieuw Talun Nyai in Xieng Ngeun district. They tried to raise cattle to escape the endless cycle of labour needed for shifting cultivation. When their only cow was calving they would keep her near the house and would spend 2-3 hours per day to collect 1 basket of grass to feed her. This was not enough and the calves were always born weak and died. It seemed impossible to increase the size of their herd

Forages save time

In 1997, Jong Gor Her grew a small plot of forages near the house. He was impressed that the forages continued to grow well even when cut many times. He was able to harvest 2 baskets of forage each day taking only 30 minutes of his time. He has since expanded the area to 2500m^2

More cattle and more income...

With good feed for his cows while calving, the herd has increased over the last six years from 3 to 15 animals. This released his wife from the endless cycle of field work and she was able to earn some cash from embroidery. With this extra money plus the sale of 3 cows, they bought a motorcycle and TV. Jong Gong Her now plans to double their forage area so he can keep his cattle by the house where he can watch over them. About half of the farmers in the village are now beginning to plant forages to get these benefits.

For more information contact NAFRI or CIAT
PO Box 6766, Vientiane Tel: (021) 222796/7

NAFRI CIAT AusAID

More details about the book series (and free downloads of the books) are available from the CIAT in Asia website (www.ciat.cgiar.org/asia). During the first half of 2003, work commenced on a new book in the series to document cases of impacts from forage technologies on smallholder farming systems in Southeast Asia. The book is being written by John Connell (a new CIAT employee) and will include cases of impacts from forages in Indonesia, Laos, Vietnam and the Philippines. It is expected the book will be published in the first half of 2004.

4.7 Facilitate communication through journals, workshops and the Internet

Highlights

- Tool for electronic access to 'Pasturas Tropicales' developed
- Spanish version of forage web page developed

4.7.1 Publications of Pasturas Tropicales

Contributors: A. Ramirez, C. E. Lascano, B. Hincapie, L. A. Franco and B. Arenas (CIAT)

In the 5 numbers of Pasturas Tropicales that correspond to Volumes 24 (3) and 25 (2) published as of August of 2003 there are 33 papers and research notes coming mainly from Brazil (25) followed by Colombia (7) and Chile (1) Table 85).

There are some reasons for the significant number of contributions from Brazil: (1) The large percentage of area in the ecosystems of tropical low lands dedicated to livestock, (2) the acceptance and wide diffusion of Pasturas Tropicales (distributed within several countries), which is attractive to the authors, (4) the interest of researcher for publishing their work, and (5) the fact that R& D institutions in Brazil give incentives to researcher that publish their work in international journals. These contrasts with to what happens in other countries where universities and other R &D institutions give the same credit to a research paper published in popular magazines as in specialized journal.

In the near future we will launch a campaign to invite forage researchers in the region to write up their work and submit for publication in Pasturas Tropicales. One of the most efficient ways of accomplishing these comes from the direct personal contact through visits to different institutions that collaborate with CIAT's Forage Program. We will also seek assistance from CIAT's Regional Coordinators since they are in close contact with a number of active forage researchers in their regions.

Table 85. Themes and total contributions published in Pasturas Tropicales in 2002 and 2003.

Themes	2002			2003		Country
	24(1)	24(2)	24(3)	25(1)	25(2)	
Pests in pastures	4			1		Colombia (4) Brazil (1)
Economic evaluation of Cratylia		1				Colombia
Simulation Models applied to dual-purpose cattle		1				Chile*
Germplasm Characterization		1				Brazil
Pasture Evaluation		1				Brazil
Pasture Establishment		2				Brazil
Pasture Renovation		1		1		Brazil
Seed Production			1			Brazil
Fertilization			2	1	2	Brazil
Root Systems			1			Brazil
Forage Quality			2	4	2	Colombia (1) Brazil (7)
Fodder Trees				1		Brazil
Forage and Animal Production					2	Brazil
Inoculation/Rhizobium					2	Brazil (1) Colombia (1)
Total	**4**	**7**	**6**	**8**	**8**	

4.7.2 Development of a CD of Pasturas Tropicales (Pasturas Tropicales: Unidas en un solo Volumen) [Tropical Pastures: United in one single volume]

Contributors: B. Hincapié; J. C. Calderón; A. Ramírez; B. Arenas; A. Franco; C. E. Lascano; M. Peters

The introduction of new communication technologies and the increasing access to the Internet allows development of novel web based electronic publications, information easily accessible to a large number of users. One of such publications is *Pasturas Tropicales: Unidas en un Solo Volumen*. This product compiles the complete set of articles published in *Pasturas Tropicales* from 1979 to 2002. Articles can be accessed in an HTML environment. Indexes to consult *Pasturas Tropicales* based on publication year, authors, species and themes are included. Selected articles can be downloaded as PDF files. With this product CIAT hopes to contribute to improved knowledge management, serving as institutional memory and improving efficiency (time, availability and cost) of information diffusion (Photo 29).

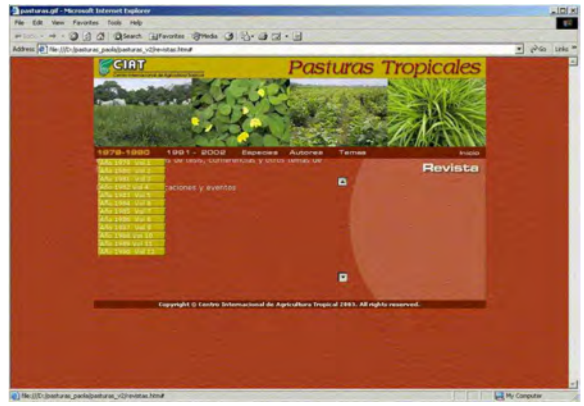

Photo 29. Opening screen of Pasturas Tropicales: Unidas en un solo volumen

4.8 Update of a Forage Web Site

Contributors: S. Staiger, C. Maya, B. Hincapié and M. Peters

Intensive work on the web site of CIAT's Tropical Forages Project commenced in January 2002; since August 2002 this web site is available to a wide range of external and internal users. The 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 Units.

The Web site has allowed to disseminate our research results extensively and to promptly communicate important news, for example the release of varieties and important new research findings.
The Web Site. The site, accessible in http://www.ciat.cgiar.org/forrajes/index.htm, allows users (universities, research institutes, partners, donors, and the scientific community in general) to:

- Consult the forages database.
- Consult databases on the extensive genetic resources of tropical forages conserved in the CIAT gene bank.
- Make on-line requests of samples of unimproved germplasm, which is available free to researchers and farmers.
- Contact CIAT to obtain samples of improved germplasm.
- Browse the easy-to-use catalog of electronic and printed products, many including useful tools and methods.
- Download PDF files containing the full text of recent publications and documents.
- Obtain specific information about each research theme: germplasm, highly nutritive grasses and legumes, genetic improvement of *Brachiaria*, pathology and endophytic fungi, spittlebug bioecology, host plant resistance to the spittlebug, and adaptation to abiotic stress.
- Access additional information resources, such as a publications list, the full text of

newsletters, background documents, annual reports, and general information about the project (project description, staff list, links to partners and donors).

- Keep up-to-date on the latest advances through Homepage's news section.

Advances 2002/2003: It is expected that by end of 2003 the Spanish version of the Forage web page will be launched (Photo 30). This is an important step forward to reach Spanish speaking users, especially as through new net connection points in CIATs areas of work such as the Llanos Orientales and Cauca, Colombia this information will be available to a large number of smallholder farmers. A webpage was developed to highlight the activities of the forages program in Southeast Asia and provide access to downloadable products on forages. The url is <http://www.ciat.cgiar.org/asia/forages.htm> . In the 13 months between launching in August 2002 and August 2003, 37,566 visits to the web site were recorded; in the same time frame 86, 120 downloads of products produced by the Forages projects such as manuals for evaluation of pastures with animals were made (Figure 87).

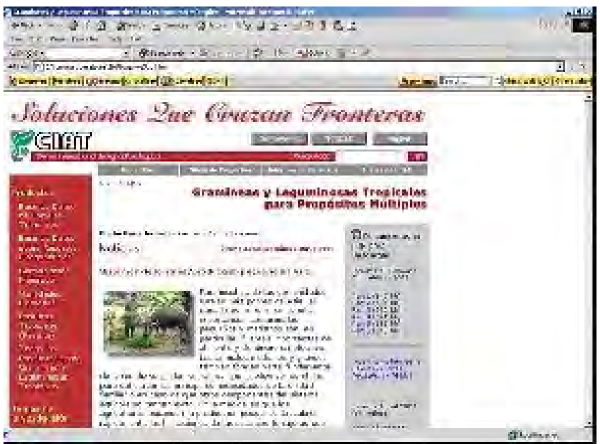

Photo 30. Development of Spanish version forages web page

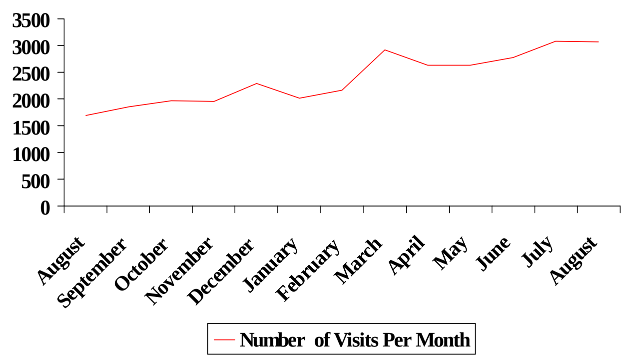

Figure 87. Number of visits to the forage web page between August 2002 and 2003

List of Publications

Refereed Journal (published, in press and submitted):

Barahona Rolando, Lascano Carlos E., Narvaez Nelmy, Owen Emir, Morris Phillip and Theodorou mike. 2003. In vitro degradability of mature and immature leaves of tropical forage legumes differing in condensed tannin and non-starch polysaccharide content and composition. Journal of the Science and Food and Agriculture 83:1256-1266.

Begum, H. H., M. Osaki, M. Nanamori, T. Watanabe, T. Shinano and I. M. Rao. 2003. Role of phosphoenolpyruvate carboxylase in the adaptation of a tropical forage grass, *Brachiaria* hybrid, to low phosphorus acid soils. Journal of Plant Nutrition (in review).

Cardona, C., P. Fory, G. Sotelo, A. Pabon, G. Díaz and J. W. Miles. 2003. Antibiosis and tolerance to five species of spittlebug (Homoptera: Cercopidae) in *Brachiaria* spp.: Implications for breeding for resistance. J. Econ. Entomol. (accepted with minor revisions September 25, 2003).

Chakraborty, S., Ghosh, R., Mukherjee, M., Fernandes, C. D., Charchar, M. J. and Kelemu, S**.** 2003. A comparison of artificial neural network and multiple regression analysis for weather-based prediction of anthracnose. (submitted).

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Hess, H.D., Monsalve, L.M., Lascano, C.E., Carulla, J.E., Díaz, T.E. and M. Kreuzer. 2003. Supplementation of a tropical grass diet with forage legumes and *Sapindus saponaria* fruits: effects on in vitro ruminal nitrogen turnover and methanogenesis. Australian Journal of Agricultural Research, 54:703-713.

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Kelemu, S., Mahuku, G., Fregene, M., Pachico, P., Johnson, N., Calvert, L., Rao, I., Buruchara, R., Amede, T., Kimani, P., Kirkby, P., Kaaria, S., and Ampofo, K. 2003. Harmonizing the agricultural biotechnology debate for the benefit of African farmers. African Biotechnology Journal (submitted).

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Muhr, L., Tarawali, S.A., Peters, M. and Schultze-Kraft, R. 2002 Soil mineral N dynamics and maize grain yields following *Centrosema macrocarpum* and *Stylosanthes guianensis*: effects of different improved fallow notations and varying levels of fertilizer to maize. Field Crops Research, 78(2-2), 197-209.

Nanamori, M., T. Shinano, T. Yamamura, I. M. Rao and M. Osaki. 2003. Low phosphorus tolerance mechanisms: Phosphorus recycling and photosynthate partitioning in tropical forage grass, *Brachiaria* hybrid cultivar Mulato compared with rice. Plant Physiology (in review).

O'Brien, R., Peters, M., Schmidt, A., Cook, S. and Corner, R. (2003) Helping farmers select forage species in Central America: the case for a decision support system. Agricultural Systems.(Submitted)

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Zhiping, Q., I. M. Rao, J. Ricaurte, E. Amézquita, J. Sanz and P. Kerridge. 2003. Root distribution effects on nutrient uptake and soil erosion in crop-forage systems on Andean hillsides. J. Sust. Agric. (In press).

Conference and Workshop Proceedings:

Andersson, M., Peters, M., Lascano, C. and Schultze-Kraft, R 2003 *Flemingia macrophylla*, a
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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 — 2001-2003
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 — 1999-2003
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

PRONATTA
Utilidad de la leguminosa semi-arbustiva *Cratylia argentea* en sistemas de ganado de doble propósito del piedemonte llanero: Validación y Difusión — 2001-2003

COLCIENCIAS (through FIDAR) — 2000-2003
Evaluación biofísica y económica de especies arbustivas y arbóreas del ciclo corto para el manejo de suelos erosionados en ladera

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) — 2000-2003
- Utilising multipurpose legume diversity to improve soil and feed quality including application in a watershed in the Central American hillsides – PostDoc program A. Schmidt — 2000-2003
- An integrated approach for genetic improvement of aluminium resistance of crops on low-fertility acid soils — 2001-2003
- Development of a knowledge system for the selection of forages for farming systems in the tropics (co-financed with ACIAR) — 2002-2005

Germany -DAAD (German Academic Exchange Service, University of Hohenheim) — 2001-2003
Genetic diversity and core collection approaches in the multipurpose shrub legumes *Flemingia macrophylla* and *Cratylia argentea*

Germany - Volkswagen Foundation
Research and development of multipurpose forage legumes for smallholders crop-livestock 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 – SDC- ZIL
Adaptation of Brachiaria to low P (with ETHZ) — 2003-2005
The forage potential of tanniferous legumes: The search for sustainable ways to cope with nutritional limitations on smallholder systems (with ETHZ) — 2004-2006

USAID-HGRP- Haiti Hillsides Agricultural Project — 2001-2003

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

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